Short Communication

Separation of condensed phosphates using capillary zone electrophoresis with indirect UV detection

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ABSTRACT

A capillary zone electrophoresis (CZE) separation of ortho-, pyro- and tripolyphosphate anions using a phthalate buffer and indirect UV detection is described. Advantages of a CZE method for phosphates include speed, efficiency and unique selectivity. Quantitative parameters and application to the analysis of a commercial sample are presented.

INTRODUCTION

Orthophosphate and condensed phosphate compounds are important industrial chemicals used in many applications including cleaning formulations and as food acidulants. Instrumental analysis of ortho-, pyro- and tripolyphosphate species is commonly done using ion chromatography [1,2], paper chromatography [3] and ^{31}P NMR [4]. We and others have shown the utility of capillary isotachophoresis (ITP) for the separation and quantitation of condensed phosphate species [5,6]. A newer electrophoretic method, capillary zone electrophoresis (CZE), is gaining in popularity for the quantitative analysis of small ionic compounds [7,8] due to its high speed, resolution and the commercial availability of automated instruments. However, few applications of CZE for the analysis of polyvalent, inorganic anions have appeared.

We present here the initial separations of ortho-, pyro- and tripolyphosphate using CZE. The separation can be accomplished in 5 min using pH 4.2 phthalate buffer with indirect UV detection. Calculated mobilities of phosphate ions, example separations and the analysis of phosphate species in commercial food processing samples are presented.

EXPERIMENTAL

Apparatus

A Spectra-Physics (now Thermo Separation Products, San Jose, CA, USA) Spectra-PHORESIS 500 capillary electrophoresis instrument was used for all CZE experiments. A 45 cm long separation capillary was fabricated from 75 μm I.D. × 365 μm O.D. fused silica (Polymicro Technologies, Phoenix, AZ, USA). The capillary window was made by scraping off *ca.* 2 mm of polyimide coating prior to mounting in the cassette. The UV detector was operated at

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250 nm using a rise time of 1 s. Electrophoresis was performed at a constant applied voltage of -20 kV (resulting current = -13 μA) and at 25°C. An applied vacuum of 1.5 p.s.i. (1 p.s.i. = 6894.76 Pa) for 1 s was used for all injections.

Ion chromatography (IC) was performed on a Dionex (Sunnyvale, CA, USA) series 2000i IC equipped with an AS5 column, a CDM-II conductivity detector, an AMMS micromembrane suppressor with 25 mM H_2SO_4 regenerant, and a 50-μl injection loop. Data from both IC and CZE experiments were acquired and integrated using a Dionex AI-450 chromatography data system.

Chemicals

Potassium acid phthalate (KHP), ACS certified grade, monosodium phosphate monohydrate, reagent grade, and 50% sodium hydroxide were obtained from Fisher Scientific (Pittsburgh, PA, USA). Reagent-grade tetrasodium pyrophosphate decahydrate was obtained from Mallinckrodt (St. Louis, MO, USA) and technical-grade pentasodium tripolyphosphate, anhydrous, was obtained from Monsanto (St. Louis, MO, USA). Dodecyltrimethylammonium bromide (DTAB) was obtained from Sigma (St. Louis, MO, USA). IC regenerant was prepared from concentrate (Dionex, Sunnyvale, CA, USA). Water used to prepare buffers and dilute standards and samples was purified using a four-bowl Plus analytical Milli-Q system (Millipore, Bedford, MA, USA).

Methods

One mg/ml (as anion) stock phosphate standards were prepared by diluting appropriate masses of the sodium salts with deionized water. Mixed standards in the range 5–300 μg/ml for CZE or 5–100 μg/ml for IC were prepared by further diluting aliquots of the stock standards. Phthalate buffer was prepared by diluting 0.102 g KHP and 6.2 ml of 2.5 mg/ml DTAB in water to 100 ml with deionized water. The resulting buffer (5 mM KHP/0.5 mM DTAB) pH was found to be 4.2 as measured using a glass electrode. No pH adjustment of the buffer was performed.

The following daily washing procedure (using

vacuum) was found to give reproducible migration behavior: 15 min with 1 M NaOH, 5 min with 0.1 M NaOH, 5 min with water, 10 min with 0.25% DTAB and 10 min with running buffer. Between runs, a 2-min wash with buffer was performed.

IC was performed using 50 mM NaOH eluent pumped isocratically at 1 ml/min. The eluent was prepared by diluting 4 g 50% NaOH to 1 l. Potato bath samples were diluted 100- or 1000-fold in deionized water.

RESULTS AND DISCUSSION

Our previous experience with ITP separations of condensed phosphates showed that differences in electrophoretic mobilities are effective in providing separations of these species. Prior to attempting a CZE separation, effective mobilities of ortho-, pyro- and tripolyphosphate were calculated and the results are given in Fig. 1.

Calculations were based on the normal equations for effective mobility [9]. Values for pK_a and absolute mobilities were taken from the literature [10,11]. Absolute mobilities for tripolyphosphate were estimated from ITP data generated in our laboratories [5].

The plot of mobility *vs.* pH indicates that electrophoretic separations of ortho-, pyro- and tripolyphosphate are fairly robust, since mobility differences vary little with pH. The plot includes the calculated mobility curve for phthalate, a common buffer used for indirect UV detection of anions in CZE. To minimize electrodispersion
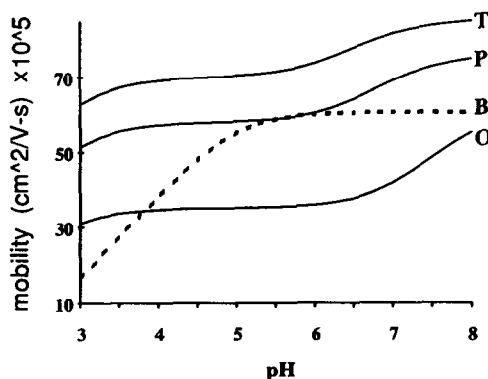

Fig. 1. Calculated mobilities of ortho- (O), pyro- (P), tripolyphosphate (T) and phthalate (B) *vs.* pH.
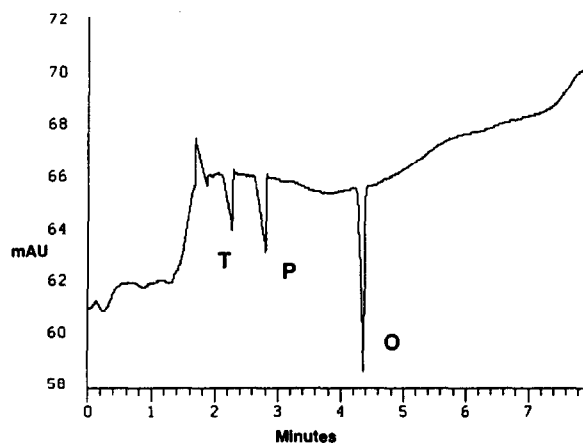
Fig. 2. Typical separation of ortho- (O), pyro- (P) and tripolyphosphate (T) in 5 m*M* KHP/0.5 m*M* DTAB, pH 4.2; 100 μg/ml of each anion injected. For details of the separation, see Experimental section.

[12], a buffer pH of 4.2 was chosen, which makes phthalate's mobility intermediate to those of the phosphates of interest. Another advantage of operating at this pH is that no pH adjustment of a 5 m*M* KHP solution is necessary. DTAB was added to the buffer to ensure anodic electroosmotic flow and rapid separations.

A typical separation of phosphates in 5 m*M* KHP/0.5 m*M* DTAB is shown in Fig. 2. The mobility order of the three phosphate species is consistent with calculated mobilities shown in Fig. 1. Significant baseline shifts are seen during the course of electrophoresis, a phenomenon we have observed consistently with indirect detection of phthalate buffers with different pH, concentration and additive content.

While the speed and efficiency of the separa-

TABLE I

CALIBRATION STATISTICS

Concentration (μg/ml)	Area relative standard deviation (%) $n = 3$		
	Ortho	Pyro	Tripoly
5	13	–	–
10	8	–	–
50	0.5	4	40
100	2	1	10
300	1	1	3

tion shown in Fig. 2 are acceptable, pyro- and tripolyphosphate peaks are small relative to that for orthophosphate. To investigate the quantitative potential of this system, calibration curves were constructed using triplicate injections of mixed standards over the concentration range 5–300 μg/ml. Results of the calibration are given in Table I. All precisions stated in Table I are within-day precisions.

Peak areas showed good reproducibility at 10–300, 50–300 and 100–300 μg/ml for ortho-, pyro- and tripolyphosphate, respectively. Calibration curves were linear as seen from the correlation coefficient. Of particular interest were the slopes and intercepts obtained for the three phosphates. From pK_a data, effective charges on the phosphates are *ca.* – 1, – 2 and – 3 at pH 4.2. Slopes can be corrected for migration time (t_m) and effective ionic charge by

corrected slope =

$$[\text{slope(area}\mu\text{g}^{-1}\text{ml)F.M.}]/[\text{charge} \cdot t_m] \quad (1)$$

where F.M. = formula mass of the anion and the term (F.M./charge) converts the slope from a mass-based to an equivalents-based measure. Normalization of areas using migration time corrects for differing velocities of ions past the detector. Corrected slopes are given in Table II, and they should be equal for 1:1 displacement of buffer ions using indirect detection [13]. The approximately equal slopes indicate that the low sensitivities seen with pyro- and tripolyphosphate arise primarily from the large negative intercepts. Possible causes of these large intercepts, including interaction with surfactant and/or on-line hydrolysis, are under investigation.

As a further check on quantitation using this method, commercial samples were analyzed by CZE and results compared with those obtained by IC. Samples were taken from solutions of sodium acid pyrophosphate used for the treatment of potatoes. Results of the analyses are given in Table III, and typical separations are shown in Fig. 3. Good agreement is seen between the two techniques. Somewhat higher migration times and different baselines seen in Fig. 3 *vs.* Fig. 2 are typical of day-to-day variations with this system.

TABLE II

SENSITIVITY AND REPRODUCIBILITY PARAMETERS

	Ortho	Pyro	Tripoly
Slope (area μg^{-1} ml)	300	190	154
Corrected slope	6360	5930	5690
Intercept (area)	−4520	−1746	−510
r^2	0.9995	0.9995	0.9960
Average t_m (min)	4.4	2.7	2.28
t_m R.S.D. (%)	1.8	3.3	3.0

TABLE III

CZE *VS.* IC ANALYSIS OF POTATO BATH SAMPLES

	% Pyro (S.D., n = 3)		% ortho (S.D., n = 3)	
	CZE	IC	CZE	IC
Sample A	6.98 (0.19)	6.92	0.79 (0.13)	0.76
Sample B	0.39 (0.002)	0.53	<0.1	<0.1

Analysis of phosphate in potato baths appears accurate despite the complex matrix. To check for interferences from common inorganic anions, sulfate, chloride and nitrate standards were analyzed in the present system. All three anions migrate ahead of tripolyphosphate, in agreement with previous ITP data [10]. Organic acids extracted from the potatoes should migrate well behind the phosphate species. The early-eluting peaks seen in Fig. 3b are likely organic acids (*e.g.* ascorbic) with migration times greater than 7 min in the CZE system.

The above results show that CZE is a viable alternative for quantitative determination of
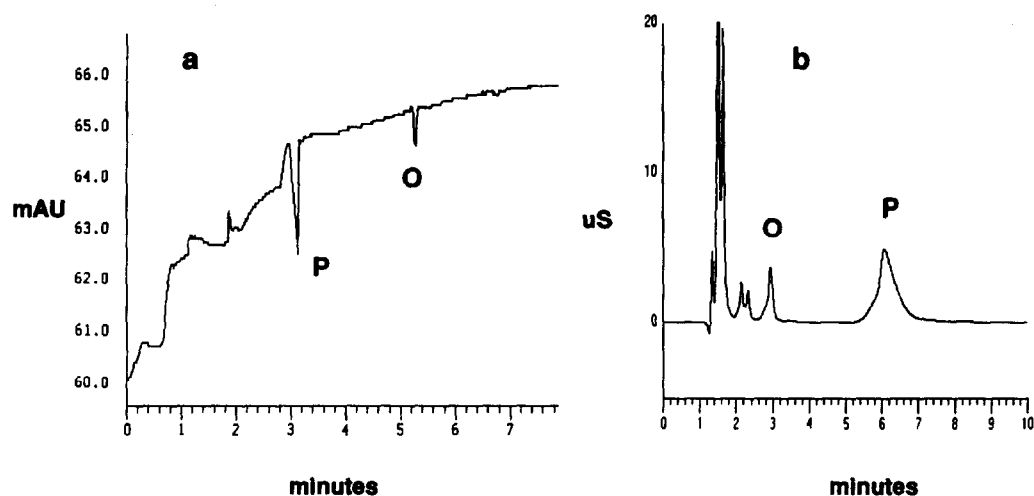

Fig. 3. Analysis of potato bath samples by (a) CZE and (b) IC. O = ortho- and P = pyrophosphate peaks.

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phosphate speciation. While the present system is not fully optimized in terms of baseline stability and day-to-day reproducibility, quantitative separations are demonstrated. Investigations into improved buffer systems for phosphate analyses, including improved stability, sensitivity and possible extension to other condensed phosphates, are continuing.

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