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Separation of polyphosphates and polycarboxylates by capillary electrophoresis in a carrier electrolyte containing adenosine 5'-triphosphate and cetyltrimethylammonium bromide with indirect UV detection

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Abstract

A capillary electrophoresis method was developed for the separation of polyanions of environmental concern, such as polyphosphates [ortho- (P_1), pyro- (P_2) and tripoly- (P_3)] and polycarboxylic acids (nitrilotriacetic acid, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid and citrate) which are used as builders in detergents. A carrier electrolyte consisting of adenosine 5'-triphosphate as the UV chromophore for indirect detection at 260 nm and cetyltrimethylammonium bromide as modifier to reverse the electroosmotic flow was used. The changes in the effective mobilities of some solutes and common UV chromophores with pH were either predicted theoretically or determined experimentally. Differences in effective mobilities of the solutes were used to optimize the pH for their separation. Baseline separation for all of the solutes was achieved. Detection limits for all of the solutes were in the range $2 \cdot 10^{-5}$ – $5 \cdot 10^{-5}$ M. Application of the developed method to real samples was demonstrated by separation and determination of polyphosphates (P_1 , P_2 and P_3) in a commercial detergent. For sample preparation, only simple dilution with water and filtration were needed.

1. Introduction

Detergent builders play a central role in the course of the washing process [1]. Builders are components in a formulated detergent which enhance the cleaning effectiveness of the surfactant. Chemically, they perform a number of functions, the principal one being the removal of calcium and magnesium ions from the solution where they interfere with the action of the surfactants. These metal ions are removed by

builders either in a soluble form by complexation or in an insoluble form by precipitation [2].

Among several components in builders, one major type are complexing agents such as inorganic polyphosphates and polycarboxylates [2]. The separation and determination of builders in a formulated detergent are necessary not only for production monitoring and product quality control to guarantee cleaning effectiveness, but also for environmental protection because most of the environmental problems recently associated with detergents have arisen from builders rather than surfactants [3].

It is known that many complexing agents can

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lead to heavy-metal remobilization and that the release of phosphates from sewage effluent into water results in eutrophication [3,4]. Much attention has been paid to developing non-phosphate builders owing to such environmental concern. In addition, it has been suggested that aminopolycarboxylic acids may constitute a source of nutrient for aquatic algae, although this has been questioned [5–7].

Ion chromatography (IC) has been one of the most commonly used methods for the separation and determination of builders of polyphosphates and polycarboxylates [8–11]. However, there are two factors which make it not entirely satisfactory to separate and determine builders in detergents by IC. One is the strong affinities of the builders to ion exchangers. Gradient elution with harsh eluents is often needed to separate them in a reasonable time range. The other is their poor ability to absorb UV radiation when an absorbance detector is used. Postcolumn reaction with Fe^{3+} as a detection reagent is commonly necessary for their detection. There is therefore a need for the development of a fast and reliable instrumental method for the determination of these polyanions.

Capillary electrophoresis (CE) separates components inside fused-silica capillaries on the basis of their differences in migration velocity in a suitable electrophoretic medium under the influence of an electric field of high strength. Since Jorgenson and Lukacs' pioneering work on CE [12–14], it has become one of the fastest growing separation techniques in analytical chemistry. CE has the advantages of high separation efficiency, short separation time and low consumption of samples and reagents compared with conventional separation techniques [15]. Among many successful applications of CE [15,16] are the separation and determination of low-molecular-mass anions [17–19]. For example, Jones and Jandik [20] obtained a remarkably successful separation of 30 anions, most of them being singly charged, with a carrier electrolyte consisting of chromate as UV chromophore and a hydrophobic quaternary ammonium ion as electroosmotic flow (EOF) modifier to reverse the EOF. However, so far only a few reports have

appeared on the application of CE with indirect UV detection to the separation and determination of poor UV-absorbing polyanions, such as polyphosphates and polycarboxylates [21,22]. Stover and Keffer [21] separated ortho-, pyro- and tripolyphosphates with phthalate as UV chromophore with significant baseline shifts. Wiley [22] used direct UV detection with a copper(II) salt added to enhance both the detectability and selectivity for polycarboxylic acids, but the resolution was still limited.

In this work, a CE method was developed for the separation of polyanions, such as polyphosphates and polycarboxylates used in some builders of detergents. A polyanion, adenosine 5'-phosphate (ATP), was used as a UV chromophore for indirect UV detection at 260 nm and a hydrophobic quaternary ammonium, cetyltrimethylammonium bromide (CTAB), as an EOF modifier to reverse the EOF. The migration behaviour of the polyanions and some common UV chromophores used in CE to detect anions as a function of pH was studied. Optimum CE conditions for the separation of the above compounds were obtained. Baseline separation of all the solutes tested was achieved. The application of the CE method to real samples was demonstrated by the separation and determination of polyphosphates [ortho- (P_1), pyro- (P_2), and tripoly- (P_3)] in a commercial detergent.

2. Experimental

2.1. Chemicals

CTAB and potassium chromate were purchased from Aldrich (Milwaukee, WI, USA). ATP (disodium salt) and tripolyphosphate (pentasodium salt hexahydrate) (P_3) were products of Sigma (St. Louis, MO, USA). Pyrophosphate (tetrasodium salt decahydrate) (P_2) was obtained from Riedel-de Haën (Seelze, Germany), anhydrous disodium hydrogenphosphate (P_1) from Fluka (Buchs, Switzerland), nitrilotriacetic acid (NTA) from Merck (Darmstadt, Germany), ethylenediaminetetraacetic acid (EDTA) from J.T. Baker (Phillipsburg, NJ,

USA) and diethylenetriaminepentaacetic acid (DTPA) (technical grade) as a gift from Dr. Peck of Singapore Institute of Standard and Industrial Research (SISIR). All other chemicals were of analytical-reagent grade. Deionized water used throughout the experiments was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. pH adjustment in carrier electrolyte and measurement of EOF

Buffer solutions composed of 10 mM ATP, which served as the carrier electrolyte and background absorber for indirect UV detection, and 0.05 mM CTAB, which served as the EOF modifier, were used unless stated otherwise. pH adjustment was accomplished by either adding 0.5 M nitric acid or 0.5 M sodium hydroxide until the required pH was obtained. NO_3^- , an anion with known electrophoretic mobility, was used to determine the EOF.

To determine the effective mobilities of common UV chromophores, 250 mM acetic acid–acetate was employed as the electrophoresis buffer. When this buffer was used, the EOF was measured with benzyl alcohol as a neutral marker at 254 nm.

2.3. Apparatus

CE was performed with a laboratory-built system, equipped with a Spellman (Plainview, NY, USA) CZE1000R power supply and a Linear Instruments (Reno, NV, USA) UVIS 200 detector. Electropherograms were recorded with an HP 3394A integrator (Hewlett-Packard, Avondale, PA, USA) connected with a switch for changing the polarity of the input signal. Polyimide-coated fused-silica capillaries of I.D. 75 μm and O.D. 362 μm (Polymicro Technologies, Phoenix, AZ, USA) were used. The total length was 50.0 cm and the effective length was 40.0 cm. On-column indirect UV detection was conducted at 260 nm unless stated otherwise. The hydrostatic sample injection mode was employed for sample introduction into the capillary at the cathodic side, with an injection time of 15 s at a height difference of 4.5 cm between

the liquid levels of the sample vial and the buffer reservoir at the grounded electrode. The field strength applied for separation was 300 V/cm and the voltage was 15 kV. The temperature was maintained at $25 \pm 1^\circ\text{C}$.

2.4. CE procedure

The capillary was first flushed with 0.1 M NaOH for 10 min, then with water for 5 min and finally with buffer for 10 min every day before CE experiments. Between two successive runs, the capillary was flushed with buffer for 1 min. When not in use, the capillary was flushed with water for at least 10 min before being kept in storage.

4. Results and discussion

4.1. Effect of pH on effective mobility

The effective mobility of a solute which can dissociate in multiple steps is the weighted sum of the mobilities of all the ionic and non-ionic forms in which the solute can exist at equilibrium. As all of the polyanionic solutes in this study are partially dissociated polyanions in common pH ranges used in CE, it is reasonable to assume that the effective mobilities of some solutes change significantly with buffer pH and hence pH is expected to be a critical parameter to affect their separation.

Fig. 1 shows the changes of effective mobilities with pH of some solutes and common UV chromophores used for indirect detection of anions in CE. Data on mobilities and dissociation constants obtained from the literature [23] were used for the calculation of the effective mobilities in Fig. 1 using the following equation:

$$U^{\text{eff}} = \sum \delta_i U_i \quad (1)$$

where U^{eff} is the effective mobility, δ_i is the mole fraction of species i and U_i is the mobility of species i . Depending on pH, the mole fractions (δ_i s), together with the intrinsic differences in the mobilities (U_i s) of different species i , dominate the effective mobility (U^{eff}). The dis-

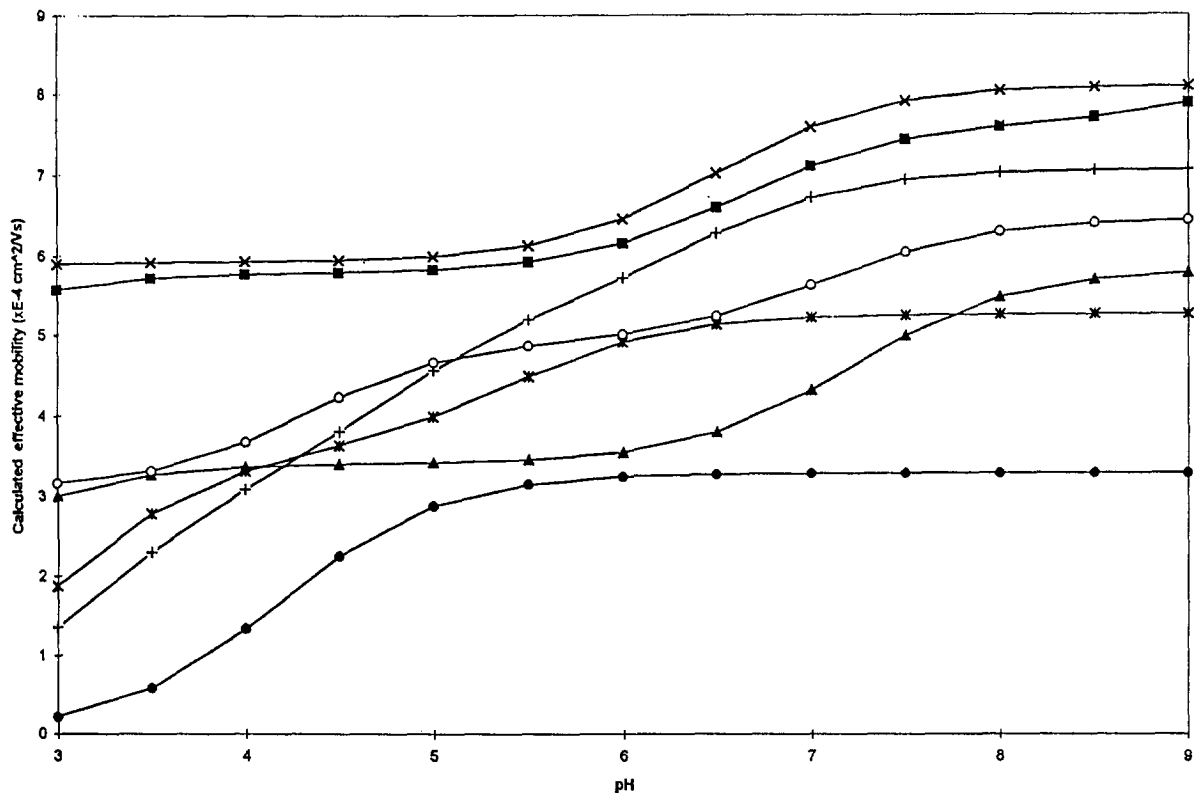


Fig. 1. Theoretical prediction of effect of pH on the changes in effective mobilities of some solutes, ATP and common carrier electrolytes used in CE for indirect UV detection of anions. ■ = pyrophosphate; ▲ = orthophosphate; + = citrate; × = chromate; * = phthalate; ● = benzoate; ○ = ATP.

sociation constants of ATP were found to be different in different sources [24–26] as listed in Table 1. To calculate the effective mobility of ATP, only data on dissociation constants which are given in bold type in Table 1 were used because it is common that the more negatively charged a species is, the greater is its pK_a owing

Table 1
Dissociation constants of ATP from different sources

pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}	Ref.
		4.418	7.056	[24]
	4.00	6.48		[25]
0.9	6.05			[26]

The data used to calculate the effective mobility of ATP and to construct the corresponding curve in Fig. 1 are given in bold type.

to the stronger electrostatic attraction between the negatively charged species and protons. As we could not find the mobility of ATP^- in the literature, its value was calculated from our experimental CE results. Briefly, the effective mobility of ATP was determined in 250 mM acetic acid–acetate buffer of pH 3.50, then the mobility of ATP^- was calculated by using Eq. 1 with known data on the dissociation constants and mobilities of ATP^{2-} , ATP^{3-} and ATP^{4-} . The calculated value of $3.12 \cdot 10^{-4} \text{ cm}^2/\text{V} \cdot \text{s}$ of the mobility of ATP^- was used to construct the curve (○) in Fig. 1. Because of the lack of data on mobilities in the literature, the other solutes could not be included in Fig. 1. It is noted that the effective mobility of citrate changes most significantly with pH in the range 3–7. This is due to the fact that the three pK_a values of

citrate lie within the range 3–7 but their magnitudes are significantly different from each other, and there are appreciable differences in the mobilities of the three charged forms of citrate. At pH 3, neutral molecules of citric acid and singly charged citrate ions exist predominantly, since $\delta_0 = 0.592$ and $\delta_1 = 0.402$. As the pH increases, polycharged citrate ions increase. At pH 7, polycharged citrate ions become predominant, since $\delta_2 = 0.437$ and $\delta_3 = 0.555$.

Fig. 2 shows the experimentally determined effective mobilities of the solutes studied in a carrier electrolyte of 10 mM ATP and 0.05 mM CTAB at different pH values (data for pH higher than 6.5 were not obtained because the peaks of the solutes started to merge appreciably and peak identification was difficult). A concentration of 0.05 mM CTAB in the carrier electrolyte

was chosen because a lower concentration would have led to a prolonged separation time [27] and a higher concentration would have led to its precipitation in the carrier electrolyte.

To correct the effective mobilities of the solutes for the EOF, a charged solute with known electrophoretic mobility, which we refer to as an internal standard for EOF here, was employed. It was considered that the use of a charged internal standard instead of a neutral marker would be advantageous when the EOF is extremely low or the stability of the buffer is poor.

NO_3^- was employed as the internal standard for EOF in this study. We assume that the following equations are valid:

$$U_{\text{IS}}^{\text{eff}} = U_{\text{IS}}^{\text{app}} - U_{\text{EOF}} \quad (2)$$

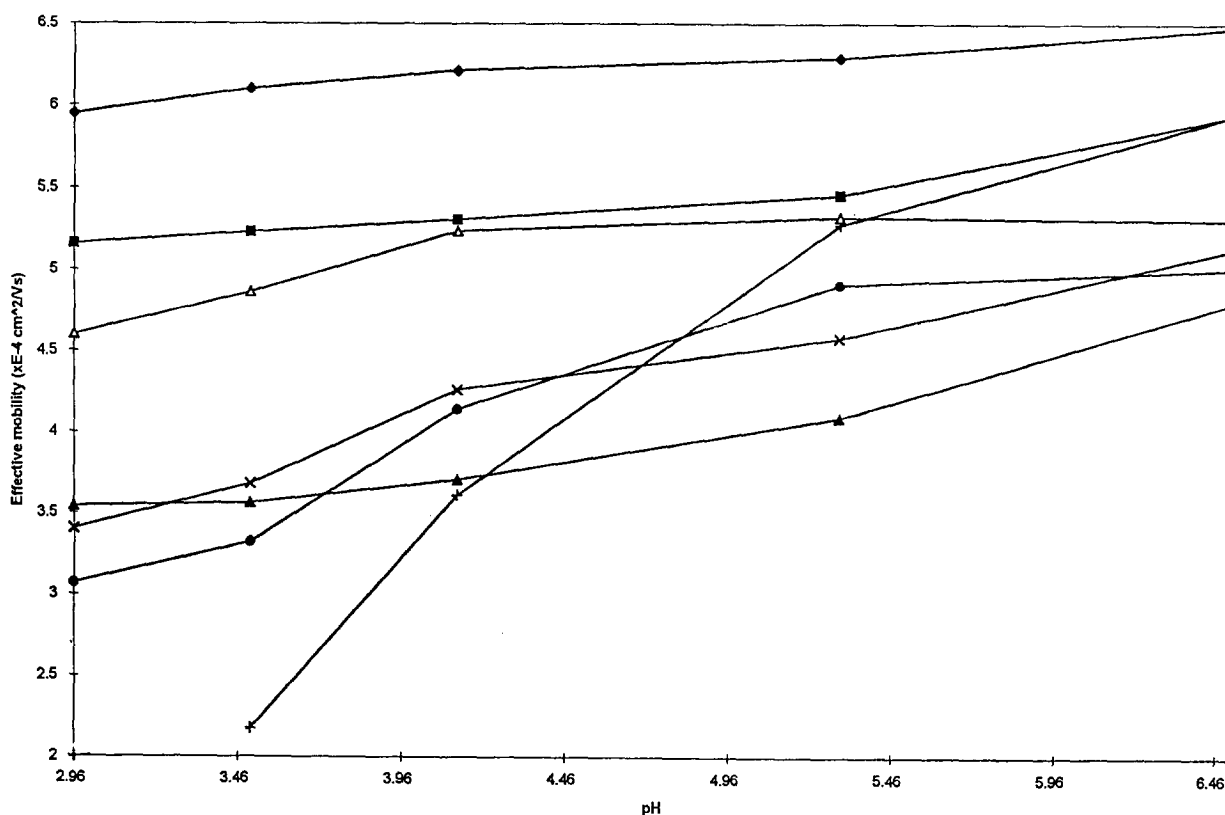


Fig. 2. Experimental results on the effect of pH on effective mobilities of the solutes in a carrier electrolyte containing 10 mM ATP and 0.05 mM CTAB. ◆ = tripolyphosphate; ■ = pyrophosphate; △ = NTA; × = EDTA; ▲ = orthophosphate; ● = DTPA; + = citrate.

$$U_j^{\text{eff}} = U_j^{\text{app}} - U_{\text{EOF}} \quad (3)$$

where $U_{\text{IS}}^{\text{eff}}$ and U_j^{eff} represent the effective mobility of the internal standard for EOF and the effective mobility of a solute j , respectively, and U^{app} denotes apparent mobility. To eliminate the electroosmotic mobility, U_{EOF} , in Eq. 3, Eq. 2 can be substituted into Eq. 3 to give

$$\begin{aligned} U_j^{\text{eff}} &= U_{\text{IS}}^{\text{eff}} + U_j^{\text{app}} - U_{\text{IS}}^{\text{app}} \\ &= U_{\text{IS}}^{\text{eff}} + (L_t L_e / V)(1/t_j - 1/t_{\text{IS}}) \end{aligned} \quad (4)$$

where L_t and L_e are total length and effective length of capillary, respectively, V is applied voltage and t is migration time. As an approximation, the limiting ionic mobility of NO_3^- , $7.40 \cdot 10^{-4} \text{ cm}^2/\text{V}\cdot\text{s}$ [28], was employed instead of its effective mobility. Although the above approximation could be corrected for the effect of ionic strength [29], this was not considered necessary as the above approximation was found to be adequate for pH optimization in the present work.

The theoretical prediction and experimental results for the effect of pH on effective mobilities are shown in Figs. 1 and 2, respectively. The trends of the changes in the effective mobility of the solutes with pH in Figs. 1 and 2 agree fairly well with each other. However, there are still some discrepancies in their absolute values. This might to a large extent be due to discrepancies in the data for mobilities and dissociation constants in the literature. Dissociation constants and mobilities from different sources vary appreciably. Other possible reasons include differences in ionic strength and fluctuations in temperature in different studies. Nevertheless, relative values of effective mobilities of the solutes and UV chromophores could still be useful for determining optimum conditions for separation. In spite of the slight discrepancies in some of the values, both Figs. 1 and 2 show that separation of all of the solutes could be achieved around pH 3.5.

It is worth noting that the effective mobilities of polycarboxylates are sensitive to pH, but the effective mobilities of polyphosphates are relatively less affected by pH changes in the range 3–6.5. Generally, the effective mobilities of the

solutes studied are closer to each other at high pH, mainly owing to large increases in the effective mobilities of most of the polycarboxylates in this study with increase in pH.

4.2. Selection of electrophoretic medium for separation

Significant differences in the effective mobilities of all the solutes around pH 3.5 indicate a potential for their separation by CE. However, to realize the separation of the solutes, a good electrophoretic medium must be chosen.

An electrolyte consisting of chromate and a hydrophobic quaternary ammonium ion for reversing the EOF introduced by Jones and Jandik [20] is now widely used for the separation of inorganic anions by CE with indirect UV detection. Previous studies in our laboratory have shown that many anions in water samples can be separated in a carrier electrolyte consisting of ca. 5 mM chromate and 0.05 mM CTAB [27], but it could not completely separate all of the solutes in this study, such as EDTA, orthophosphate and DTPA, as shown in Fig. 3. Incomplete separation might be due to the much higher effective mobility of chromate than most of the solutes in this study. The effective mobility of chromate was determined to be $5.34 \cdot 10^{-4} \text{ cm}^2/\text{V}\cdot\text{s}$ (R.S.D. = 0.5%, $n = 3$) in 250 mM acetic acid–acetate buffer (pH 3.50) after correcting for the EOF using the migration time of a neutral marker (benzyl alcohol). The reason for using 250 mM acetic acid–acetate buffer (pH 3.50) was that it gave the same pH and current as the carrier electrolyte of 10 mM ATP–0.05 mM CTAB, which gave the optimum separation for all the solutes. In Fig. 1 it can be seen that the effective mobility of chromate is higher than those of all the others. In general, it is difficult to lower the effective mobility of chromate by only adjusting the pH because the mobilities of both chromate(I) and chromate(II) have relatively large values and $\text{p}K_{\text{a}1}$ is relatively small. Mismatched mobilities between the carrier electrolyte and the solute can lead to either fronting or tailing of the peak, depending on the relative

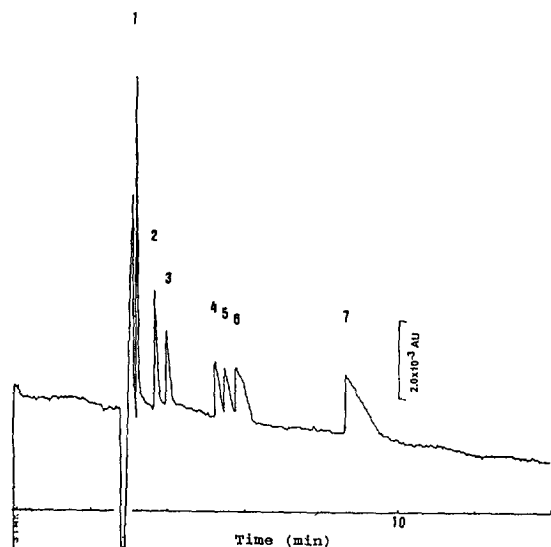


Fig. 3. Separation of the solutes in a carrier electrolyte of 5 mM K_2CrO_4 -0.05 mM CTAB at pH 3.50. Peaks: 1 = P_3 ; 2 = P_2 ; 3 = NTA; 4 = ETDA; 5 = P_1 ; 6 = DTPA; 7 = Cit. Sample concentrations: citrate = 1 mM and the other solutes = 0.5 mM. For other experimental conditions, see Experimental.

magnitude of their effective mobilities [30]. The fact that the effective mobility of chromate was significantly higher than those of the four late-eluting solutes was consistent with the observation of the four tailing peaks in Fig. 3. Other commonly used carrier electrolytes, such as benzoate and phthalate [18], could not give satisfactory results for the separation of the solutes in this study because their effective mobilities are lower than and do not match well those of the solutes, as seen from Fig. 1. In addition, Stover and Keffer [21] reported that there was a significant baseline shift when phthalate was used as the carrier electrolyte to separate P_1 , P_2 and P_3 .

ATP is a polycharged anion with several dissociable groups as in the cases of polyphosphates and polycarboxylates, and has strong UV absorption at 260 nm. Its effective mobility was determined to be $3.31 \cdot 10^{-4} \text{ cm}^2/\text{V} \cdot \text{s}$ (R.S.D. = 1.4%, $n = 3$) in the same way as the effective mobility of chromate was determined. Unlike chromate, whose effective mobility is higher than those of most of the solutes, ATP has an effec-

tive mobility which lies in the middle of the range where the effective mobilities of the solutes spread. Therefore, ATP was considered to be a good carrier electrolyte for the separation of polyphosphates and polycarboxylates in terms of effective mobility and UV-absorbing properties. An electropherogram of all seven solutes in a carrier electrolyte of 10 mM ATP and 0.05 mM CTAB is shown in Fig. 4. The resolutions between all adjacent peaks were much greater than unity. The theoretical plate number for the orthophosphate peak, which showed the best peak shape in terms of symmetry, was ca. $9.5 \cdot 10^4$. In both Figs. 3 and 4, the peak for DTPA (peak 6) exhibits a slight distortion of its shape. The most likely reason is that the DTPA used was impure, since only technical-grade DTPA was available in this study. In addition, it was noted that the apparent mobilities of all the solutes in Fig. 4 were lower than those in Fig. 3, i.e., the migration times were longer in Fig. 4 than those in Fig. 3. This might be due to the adsorption of negatively charged ATP on to the positively charged adsorbed layer of cetyltrimethylammonium ions on the internal surface of the capillary. This could lower the charge density in the double layer and consequently slow the reversed EOF. In the two electrophoretic media

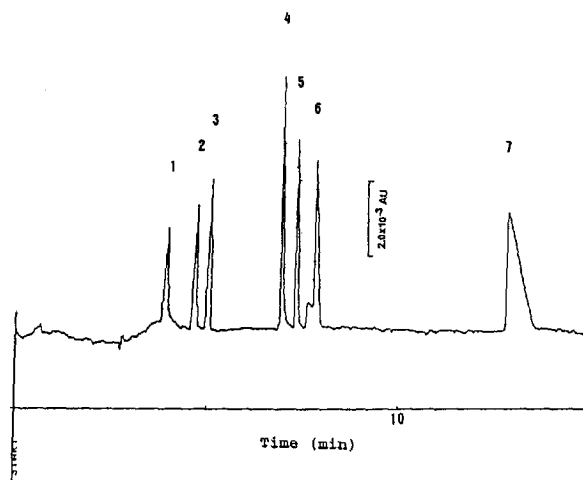


Fig. 4. Separation of the solutes in a carrier electrolyte of 10 mM ATP-0.05 mM CTAB. Other experimental conditions and peak identifications as in Fig. 3.

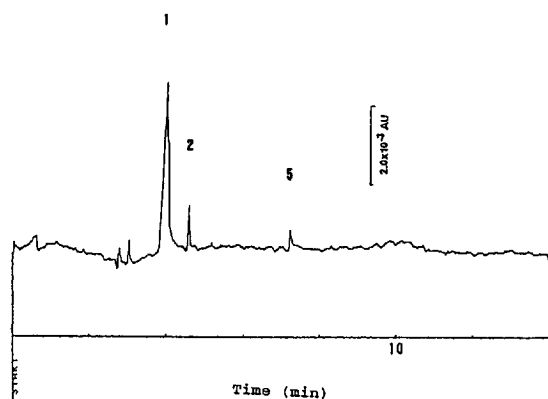


Fig. 5. Electropherogram obtained for a commercial detergent. Other conditions and peak identifications as in Fig. 3.

used in the experiments in Figs. 3 and 4, no significant differences in current and viscosity were observed.

4.3. Application to the separation and determination of polyphosphates in a commercial detergent

The method developed was applied to the determination of polyphosphates (P_1 , P_2 and P_3) in a commercial detergent. A 75–100-mg amount of detergent was accurately weighed and dissolved in water to prepare 25.00 ml of sample solution. Apart from simply passing the sample solution through a 0.45- μ m filter, no other

sample pretreatment was necessary. Fig. 5 illustrates a typical electropherogram obtained. Peak identification was carried out by comparing the migration times with those of the standards and by spiking the sample with standards. Table 2 gives quantitative results obtained by CE for the above sample.

Detection limits for all of the solutes were in a range $2.0 \cdot 10^{-5}$ – $5.0 \cdot 10^{-5}$ M ($S/N \geq 2$) (triphosphate, $5.0 \cdot 10^{-5}$ M; pyrophosphate, $2.0 \cdot 10^{-5}$ M; orthophosphate, $2.5 \cdot 10^{-5}$ M; NTA, $2.0 \cdot 10^{-5}$ M; EDTA, $2.5 \cdot 10^{-5}$ M; DTPA, $5 \cdot 10^{-5}$ M; citrate, $5.0 \cdot 10^{-5}$ M). It was noted that the reproducibilities of citrate in terms of both migration time (R.S.D. = 5.7%, $n = 3$) and area (R.S.D. = 14%, $n = 3$) were not as good as those for the other solutes (generally, in terms of migration time, R.S.D. < 2% and in terms of area, R.S.D. < 7%). The problem might be due to the greater change in its effective mobility with pH (the highest slope) at pH 3.50 as shown in Fig. 2.

Acknowledgements

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Table 2

CE results for the determination of polyphosphates [ortho- (P_1), pyro- (P_2) and triply- (P_3)] in a commercial detergent

Parameter	Triphosphate	Pyrophosphate	Orthophosphate
Concentration (%, w/w)	17.6	1.52	0.674
Reproducibility	3.2 ^a , 0.96 ^b ($n = 3$)	5.9 ^a , 1.0 ^b ($n = 3$)	5.6 ^a , 1.4 ^b ($n = 3$)
Recovery	91.8%	91.5%	106%
Linearity	$y = 365125x - 13873$ ($r^2 = 0.995$)	$y = 315701x - 2573$ ($r^2 = 0.999$)	$y = 302081x + 6975$ ($r^2 = 0.996$)

^a Values are the relative standard deviations (%) in terms of peak area.

^b Values are the relative standard deviations (%) in terms of migration time.

^c Number of experimental replicates.

Note

After this paper had been submitted for publication, S.A. Shamsi and N.D. Danielson published a paper entitled "Ribonucleotide Electrolytes for Capillary Electrophoresis of Polyphosphates and Polyphosphonates with Indirect Photometric Detection" [*Anal. Chem.*, 67 (1995) 1845]. In their paper the use of other ribonucleotide electrolytes was evaluated and the effects of modifiers on separation and of pH on detection limits in CE were studied for phosphorus-containing polyanions, but not for both polyphosphates and polycarboxylates.

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