ELSEVIER

Journal of Chromatography A, 834 (1999) 243–256

JOURNAL OF CHROMATOGRAPHY A

Review

Capillary electrophoresis of phosphorus oxo anions

Frederick S. Stover*

Technology and Commercial Development, Solutia Inc., 10300 Olive Boulevard, P.O. Box 66760, St. Louis, MO 63166-6760, USA

Abstract

Capillary electrophoresis (CE) is a useful technique for separating and quantitating the various inorganic phosphorus oxo anions (phosphates, phosphites and their oligomers). This paper reviews work to date on analyses for the oxo anions of phosphorus using CE, with an emphasis on electrolyte systems and their performance in phosphate separations. Applications of CE for quantitative speciation of phosphates in real samples are highlighted. Recent separations of polyphosphates, and the challenges remaining for the CE characterization of longer-chain polyphosphates, are discussed.

Keywords: Reviews; Phosphorus oxo anions; Inorganic anions; Phosphate; Phosphite; Hypophosphite

Contents

1. Introduction

Phosphorus oxo acids and their salts (i.e., inorganic phosphates, phosphites, condensed phosphates and polyphosphate glasses) are important compounds with biological, agricultural and industrial chemical significance [1]. Phosphorus oxo anions also are chemically interesting in that they form oligomeric species, exist in several oxidation states, and can undergo multiple protonation and metal–ligand reactions [2]. Due to this complex nature of inorganic phosphorus oxo anions, speciation of these anions in aqueous solution has been studied by a variety of analytical techniques. These range from wet chemi-

*Fax: +1-314-6745-466.

0021-9673/99/$ – see front matter
PII: S0021-9673(98)00857-7

cal sample preparations and the well-known colorimetric orthophosphate detection, through earlier separation methods such as paper or thin-layer chromatography and paper or gel electrophoresis, to high resolution phosphorus-31 nuclear magnetic resonance spectroscopy or gradient elution ion chromatography (IC) [3–5].

Capillary electrophoresis (CE) is one of the latest additions to methods for analysis of inorganic (and organic) ions in solution [6]. Traditional electrophoretic separations are among the most powerful techniques for phosphate speciation, particularly an early commercial form of CE: capillary isotachophoresis (ITP) with conductivity detection [7]. This review summarizes work to date on phosphorus oxo anion analysis using modern CE instrumentation.

2. Phosphorus oxo anion chemistry

Table 1 gives a summary of the nomenclature, structure, and pK_a values for phosphorus oxo anions analyzed by CE. A feature of these anions is that most contain both strong and weak acid groups, and the weak acid groups are generally similar in strength with pK_a~7 for formation of a -2 charge on each phosphorus oxo group. For non-cyclic anions containing P^V or P^{III}, effective charge can be varied by changing pH in the neutral region 6–8. However, since their pK_a values are similar but their effective charges or hydrodynamic sizes are reasonably different, most phosphorus oxo anions display good electrophoretic separations across a wide pH range. This is illustrated in Fig. 1, where relative mobilities vs. hypophosphite are plotted vs. pH. Much of the information regarding phosphorus oxo anion electrophoretic properties can be found in the literature on capillary ITP measurements of phosphate species. Results in Fig. 1 were calculated from the mobility data of Hirokawa et al. [8] for phosphorus oxo anions, and from the stability constant (pK_a) data of Martell and Smith [9], using simple equations for effective mobility as a function of species distribution [10].

For the oligomeric series of condensed phosphates, the migration order $P_3O_{10}<P_2O_7<PO_4$ is consistent across the pH range. For the oxidation state series, hypophosphite has no weak acid group and remains essentially -1 charged at pH 3–11. This leads to two migration orders: $H_2PO_2^-<H_2PO_3^-<H_2PO_4^-$ at pH<7 and $HPO_3^{2-}<HPO_4^{2-}<H_2PO_2^-$ at pH>>7. It is apparent that care in selecting buffer conditions is needed when a separation of phosphorus oxo anions including hypophosphite is under development. Conversely, the consistent mobility differences across a wide pH range allows considerable flexibility in tailoring CE for phosphorus oxo anions when separations from other inorganic or organic ions is required.

From Fig. 1 it appears that separation of tri- and tetrameta- from tripoly- and pyrophosphate should be problematical at pH~7 and above. However, bear in mind that the plots shown in Fig. 1 are derived from data at infinite dilution. Finite ionic strength will affect both pK_a values and mobility values of polyvalent ions to a greater extent than for ions of lower charge [11]. In our experience with ITP [12] and CE [13], and from other data [8], trimetaphosphate easily remains the faster migrating anion vs. tripolyphosphate in the pH range 3–8. Thus, Fig. 1 should be used only as a guide to mobility trends, since we currently lack a complete model of phosphorus oxo anion mobility dependence on pH, ionic strength, and specific interactions with electrolyte components.

3. CE of phosphorus oxo anions

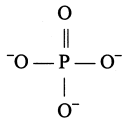
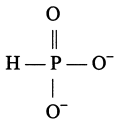
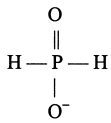
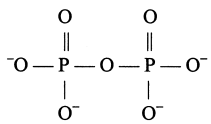
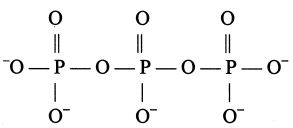
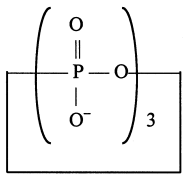
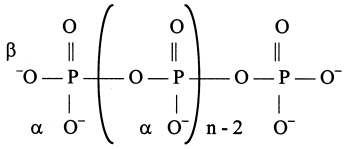
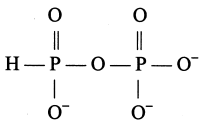
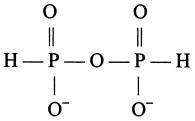
3.1. Orthophosphate

The majority of work to date on CE determinations of inorganic phosphorus-containing anions involves analyses for orthophosphate. The simplest and most abundant form of phosphorus as an oxo anion, phosphoric acid and phosphate salts play a major role in a variety of commercial uses. Orthophosphate, like many other inorganic anions, has no chromophore. Indirect UV detection, developed for small ion analysis [14], has been the major detection mode in CE analyses for orthophosphate (and phosphorus oxo anions in general).

3.1.1. Buffers for PO_4 with indirect UV detection

Table 2 gives a compilation of buffers which have

Table 1
Phosphorus oxo anions studied by CE

Anion name (acid)	Formulas or abbreviations	Structure[a]	pK_a[b]
Simple anions			
Orthophosphate, (phosphoric acid)	PO_4^{3-}, P^5, P_1		2.21 7.20 12.20
Phosphite (phosphorous acid)	HPO_3^{2-}, P^3		1.5 6.78
Hypophosphite (hypophosphorous acid)	$H_2PO_2^-$, P^1		1.3
Condensed anions			
Pyrophosphate	$P_2O_7^{4-}$, P_2		0.9 2.3 6.7 9.4
Tri(poly)phosphate	$P_3O_{10}^{5-}$, P_3		0.5[c] 1.0[c] 2.4 6.54 9.40
Trimetaphosphate	$P_3O_9^{3-}$, P_{3m}		pK_3=2.05
Tetra-, penta-, hexa-metaphosphate, etc.	$P_4O_{12}^{4-}$, P_{4m}		pK_4=2.77 (n=4)
Polyphosphate	$P_nO_{3n+1}^{(n+2)-}$, P_n		K [α]~2[d] pK [β]~7
Isohypophosphate	$HP_2O_6^{3-}$, P^3–O–P^5		–
Pyrophosphite	$H_2P_2O_5^{2-}$, P^3–O–P^3		–

[a] Anions shown in their fully ionized form.
[b] pK values, at infinite dilution (ionic strength μ=0) unless otherwise noted, from Ref. [9].
[c] pK value at μ=0.1 M.
[d] pK values for polyphosphate estimated from pH titrations, this laboratory.

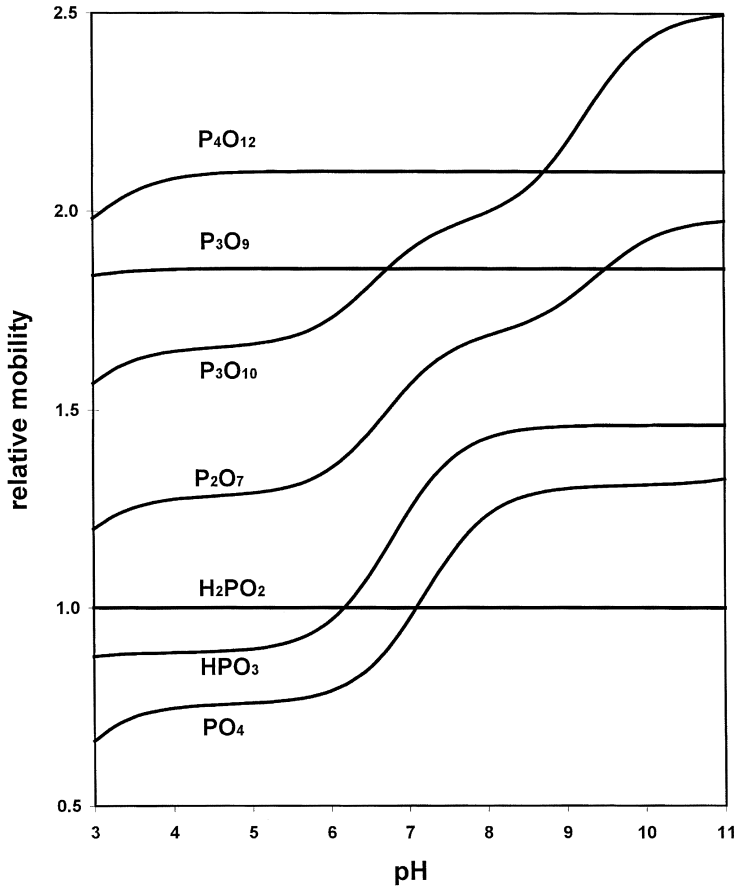


Fig. 1. Calculated relative mobilities vs. hypophosphite ($=\mu_x/\mu_{H2PO2-}$) for different phosphorus oxo anions at infinite dilution. Calculated from equations in Ref. [10] with absolute mobility data from Ref. [8] and pK_a from Ref. [9].

been used for the CE of orthophosphate (and, in some cases, other phosphorus oxo anions). The common electrolyte for inorganic ions, chromate at pH~8 with a variety of electroosmotic flow (EOF) modifiers, dominates published separations. The basic electropherogram of phosphate and common inorganic anions with this electrolyte is shown in Fig. 2. Although chromate gives efficient separations for many inorganics (e.g., Cl^-, Br^-, NO_3^-, SO_4^{2-}), mobility mismatches with F^- and HPO_4^{2-} lead to distinct tailing of these peaks. In addition, the pH range of chromate is restricted by changes in its chemical composition at lower pH values. A second popular co-ion for inorganics and small organics with a wider pH range is pyromellitate, and numerous examples of separations using this buffer have been given. Doble et al. [48] compared mobilities of buffer co-ions with various analytes at pH 9. From these electropherograms, it appears that the most efficient separations for HPO_4^{2-} should use phthalate or trimesate as co-ions. Fig. 3 shows the peak efficiency for phosphate obtained with a phthalate buffer. Other co-ions with mobilities closely matched to orthophosphate have been employed, as seen in Table 2, including salicylate, adenosine monophosphate (AMP), and pyridine dicarboxylate (PDC). The latter was claimed to improve separation efficiency by complexing metals adsorbed on the capillary surface [51].

As the buffer pH decreases, orthophosphate mobility decreases due to formation of monovalent $H_2PO_4^-$. At lower pH, orthophosphate's mobility is

Table 2
Electrolytes used for orthophosphate analysis by CE with indirect UV detection

No.	Co-ion (m*M*)	EOF modifier (m*M*)	pH buffer/other modifier (m*M*)	pH	Ref.
1	Chromate (4.5–10)	OFM Anion-BT (0.3–0.5)	–	8–12	[14–17] [18–21]
1a	Chromate	OFM Anion-BT	Butanol	7–8	[22]
1b	Chromate (7–10)	OFM in OH form (0.5–1.5)	Borate	8, 11	[23]
1c	Chromate (2–15)	CTAB (0.05–0.7)	–	7.9–9.5	[24–27]
1d	Chromate (6)	CTAB (0.032)	Boric acid (3)	8	[28]
1e	Chromate (2.7)	CTAB (0.03)	Aminopyridine (6)	8	[29]
1f	Chromate (5)	TTAB (0.01–2.6)	–	8.1, 8.8	[30,31]
1g	Chromate (5)	TTAB (0.5)	Boric acid (5)	8	[32]
1h	Chromate (5–7.5)	TTAB+DTAB (ca. 6)	–	8.8, 9.1	[33,34]
1i	Chromate (7)	DTAH (1)	Tris (17)	7.9	[35]
1j	Chromate (5)	DETA (2)	–	7.5	[33]
1k	Chromate (2)	HDMH/CTAH/TTAH/HMH	–	7.5–9	[36]
1m	Chromate (10)	HDM rinse	DEB (1)	8	[37]
1n	Chromate (5)	PDDPi chromate (0.14%)	–	8	[38]
1o	Chromate (10)	Polybrene (0.5%)	–	9.1	[39]
1p	Dichromate (5)	DM (0.1)	TEA (1.6)	8	[40]
1q	Dichromate (1.8)	18-Crown-6 (4)	Boric acid, K tetraborate	7.2	[41]
2	PMA (2.25)	HMH (0.75)	TEA (1.6)	7.7	[19,32,42–44]
2a	PMA (1.13)	HMH (2.1)	TEA (0.8)	5.5–8.5	[45]
2b	PMA (3)	DETA (3)	–	7.5	[46]
3	Phthalate (5)	OFM	–	5.6	[20]
3a	Phthalate (5)	DTAB (0.5)	–	4.2	[13]
3b	Phthalate (25)	CTAB (0.2)	–	5.5	[47]
3c	Phthalate (10)	CTAH (0.5)	DEA (40)	9.3	[48]
4	Salicylate (7.5)	TTAH (0.1)	–	8	[35]
4a	Salicylate (7.5)	DTAH (0.5)	Tris (15), CaOH (0.18)	8.3	[49]
5	Trimesic (10)	CTAH (0.5)	DEA (60)	9.3	[48]
5a	Trimesic (5)	HMB (0.5)	–	8	[50]
6	PDC(5)	CTAB (0.5)	–	5.6	[51,52]
7	Benzoate (10)	OFM Anion-BT (0.5)	–	6	[20]
8	AMP (5)	DETA (0.2)	Boric acid, tetraborate, Mg (2)	7.1, 7.8	[53]
9	ATP (5–10)	CTAB (0.02–0.05)	–	3.5, 3.6	[54,55]
10	EDTA (5)	OFM Anion-BT (0.15)	–	10.3	[56]
11	Mandelate (10)	Coated capillary	6-Aminocaproic acid (10)	3.8	[57]
12	Molybdate (5)	CTAH (0.15)	Tris (5), PVA (0.01%)	7.9	[58]
13	NDS (8.3)	DETA (2)	Boric acid (100), borate (5)	8	[59]
14	Nitroso-R (0.5)	Counter-EOF	–	8	[60]
15	ITS (0.5)	(Bis-Tris)	Bis-Tris (2.67)	6.8	[61]

OFM Anion BT=Proprietary product of Waters Corp., CTAB/H=cetyltrimethylammonium bromide/hydroxide, TTAB/H=tetradecyltrimethylammonium bromide/hydroxide, DTAB/H=dodecyltrimethylammonium bromide/hydroxide, Tris=tris(hydroxymethyl)aminomethane, DETA=diethylenetriamine, HDM/H=hexadimethrine/hydroxide (=polybrene), PPDPi=poly(1,1-dimethyl-3,5-dimethylene pipiridinium), DM=decamethonium, HMB/H=hexamethonium bromide/hydroxide. PDC=pyridine dicarboxylic acid, AMP=adenosine monophosphate, ATP=adenosine triphosphate, EDTA=ethylenediaminetetraacetic acid, NDS=naphthalene disulfonic acid, Nitroso-R=1-nitroso-2-naphthol-3,6-disulfonic acid, DEB=5,5-diethylbarbituric acid, DEA=diethanolamine, TEA=triethanolamine, PVA=poly(vinyl alcohol), ITS=indigo tetrasulfonate, Bis-Tris=1,3-bis[tris-(hydroxymethyl)-methylamino]propane.

matched better using monocarboxylates such as benzoate or mandelate (buffers 7 and 12 in Table 2). Conversely, at higher pH polyvalent co-ions such as trimesate and EDTA can be used effectively.

Common EOF modifiers include the commercial OFM Anion BT [15], quaternary ammonium surfactants such as hexadecyl-, tetradecyl- and dodecyltrimethyl ammonium bromides or hydroxides

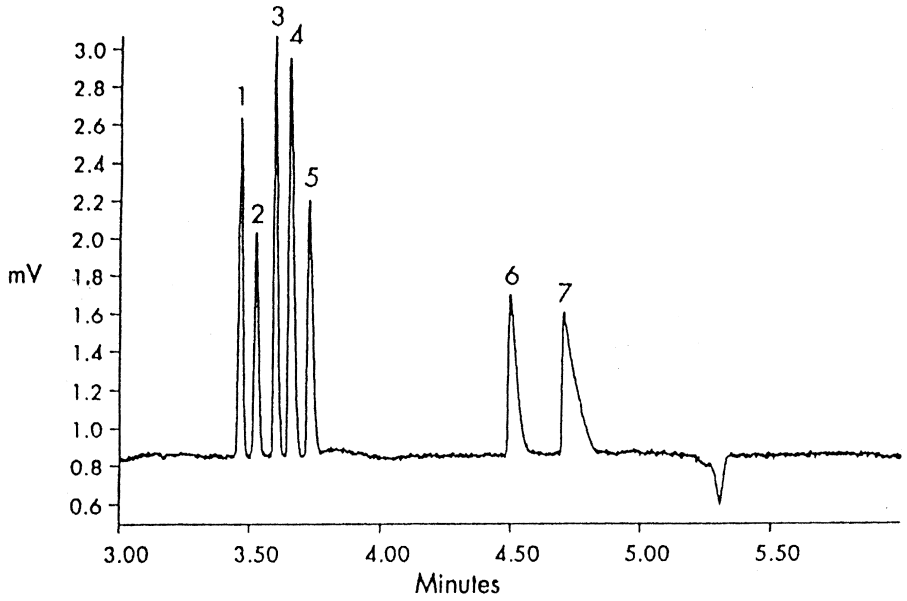


Fig. 2. Electropherogram of a seven anion standard showing 4 ppm phosphate (peak 7) using electrolyte No. 1, Table 2. Voltage −15 kV; 60 cm×75 μm capillary; 4 m*M* chromate–0.3 m*M* OFM Anion BT at pH 8.1; detection wavelength 254 nm; hydrostatic injection 30 s at 10 cm. Other peaks in order are bromide, chloride, sulfate, nitrite, nitrate and fluoride. Reprinted with permission from Ref. [15].

(CTAB/H, TTAB/H and DTAB/H, respectively), and polyamines ranging from small (diethylenetriamine, DETA; hexamethonium bromide or hydroxide, HMB/H) to large (hexadimethrine, HDM).

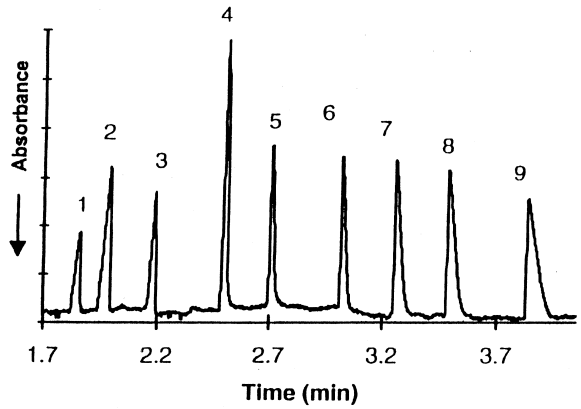


Fig. 3. Electropherogram of phosphate (peak 4) using electrolyte No. 3c, Table 2. Voltage −25 kV, 50 cm×75 μm capillary; hydrostatic injection at 10 cm for 10 s, detection wavelength 254 nm; temperature 25°C; 0.6 m*M* of each anion. Other peaks in order are chloride, sulfate, chlorate, carbonate, ethanesulfonate, propanesulfonate, butanesulfonate and pentanesulfonate. Reprinted from Ref. [48] with permission.

While selection of EOF modifier rests mainly with finding an acceptable migration window, studies have shown that the EOF modifier type and concentration [18,26,34,36,37] can alter the selectivity toward orthophosphate through presumed ion pairing. Also, orthophosphate can adsorb on the capillary surface [30], and the EOF modifier has a role in the dynamics of capillary equilibration to optimize phosphate separation reproducibility and sensitivity. The use of the hydroxide form of EOF modifiers is recommended to avoid reducing the co-ion transfer ratio [48]; however, many successful separations have employed EOF modifiers in the bromide form.

Since the introduction of the earliest indirect UV electrolytes, increasing attention has been paid to addition of buffering reagents to these systems [62,63]. Although several electrolytes in Table 2 use anionic buffers, counterions (cations) are generally used to provide pH buffering in order to avoid the loss of sensitivity seen when adding a second non-absorbing anion. Common reagents include tris-(hydroxymethyl)aminomethane (Tris), diethanolamine (DEA) and triethanolamine (TEA). Whether buffering counterions substantially influence separation selectivity through interaction with

phosphates has not been extensively investigated. Interactions with metals (electrolytes 4a and 8 in Table 2) have also been employed to modify CE phosphate separations.

3.1.2. Other detection methods

Several other detection methods have been employed for orthophosphate determinations, most commonly conductivity detection. Table 3 lists non-UV CE detectors used for orthophosphate analysis. Applications of other detectors generally use either counter-electroosmotic separations (bare fused-silica without EOF modifiers) or coated capillaries. One approach to co-electroosmotic separations with fused-silica is pre-rinsing of the capillary with CTAB [67]. Another application of particular interest is the use of fluorescence tagging of organic and biochemical phosphates [78,79], which shows some promise for sensitive and specific direct fluorescence detection of inorganic phosphate species [80].

3.1.3. Interferences

A list of ions with migration times similar to orthophosphate is given in Table 4. In this case, ''similar'' is defined as within 5–10% of phosphate's migration time. It should be noted that ions listed in Table 4 are, for the most part, fully resolved from the orthophosphate peak when similar concentrations are analyzed. However, it is appropriate to note species which migrate closely, since large differences in sample concentrations, matrix effects, or differences in EOF modifier type, pH or capillary preparation may result in lost resolution.

The main concern in the CE separation of orthophosphate from other inorganics is separation from pre-migrating fluoride and post-migrating carbonate at pH 8. At pH>9, increased phosphate mobility causes close migration with fluoride. Improved F/PO_4 separations have been reported by operating at the lower end of the chromate pH range [18,22] and through use of specific EOF modifier interactions (CTAB [37] and polybrene [39]). Other inorganics which pose potential problems are listed in Table 4. For organic anions, some divalent anions are problematical at higher pH, while certain monocarboxylates can interfere at lower pH.

3.1.4. Application to orthophosphate determinations

Analysis of samples for orthophosphate dominates the application of CE for phosphate species determinations in the literature. Table 5 summarizes references to using CE for method development and actual sample analyses. Several applications required optimization of electrolyte systems. These include pH adjustment for toothpaste analysis [22,55] and optimizing mixed surfactant systems for Bayer liquor analysis [33]. The studies in Table 5 show that CE is

Table 3
Other detection modes for orthophosphate analysis by CE

Detection	No.	Electrolyte	Ref.
Conductivity	16	50 m*M* CHES, 20 m*M* LiOH, 0.03% Triton X-100, pH 9.2	[64,65]
	16a	100 m*M* CHES, 40 m*M* LiOH, 8% 2-propanol, 0.08 m*M* spermine, pH 9.3	[66]
	16b	Same as No. 16, but with 1 m*M* CTAB pre-rinse	[67]
	16c	50 m*M* CHES, 30 m*M* arginine, pH 9.0, 0.1 m*M* CTAB pre-rinse	[68]
	17	60 m*M* His, 60 m*M* MES, 0.7 m*M* TTAH, 0.03% Triton X-100, pH 6	[69]
	18	10 m*M* Aspartic acid+BALA to pH 3.4, 0.2% MHEC	[70]
	19	7 m*M* Succinic acid+Bis-Tris to pH 3.55, 0.2% MHEC, 5% PVP	[71]
Suppressed conductivity	20	2 m*M* Tetraborate, pH~10	[72,73]
Indirect	21	0.5 m*M* Salicylate, pH 4.0	[74]
fluorescence	22	2 m*M* Tetraborate+0.32 m*M* fluorescein	[75]
MS	23	2.5 m*M* PMA, pH 7.8, 20% methanol	[76]
FPD	24	50 m*M* Ammonium acetate, pH 9.3	[77]

CHES=cyclohexylethanesulfonic acid, MHEC=methylhydroxyethyl cellulose, BALA=β-alanine, MES=morpholinoethanesulfonic acid, His=L-histidine, PVP=polyvinylpyrrolidone, FPD=flame photometric detection, MS=mass spectrometry. Other abbreviations, see Table 2.

Table 4
Summary of ions with migration times similar (±~10%) to orthophosphate

Interferent	pH	Electrolyte No.	Ref.
Fluoride	7–9.2	1, 1a, 1c, 1h, 1m, 1n, 14, 16	[18,22,27,34,33,16,20,37,38,60,64,65]
Phosphite	7.7–8	1, 1a, 1c, 2	[18,22,27,44]
Arsenate	7.7–8	1, 2	[81,82]
Selenite	9.2	16	[64,65]
Bromate	7.5–7.7	1h, 2	[33,82]
Perchlorate	9.1	10	[39]
Thiocyanate	9.1	10	[39]
Formate	7.7–9.1	1m, 1h, 2	[37,34,82]
Glutarate	8–9.2	1, 1m, 4a, 16	[37,16,64,65,49]
Phthalate	8–9.2	1, 16	[16,64,65]
Succinate	9.1	1h	[34]
Tartarate	8–10	1h, 13, 16, 20	[34,59,64,65,72]
Citraconic	9.2	16	[64,65]
Fumarate	8	1	[21]
Malate	8.3, 10	4a, 20	[49,72]
Iodate	6.8	15	[61]
Lactate	5.5–5.6	3, 3b, 6	[20,47,51,52]
Propionate	5.6–6	3, 17	[20,69]
Acetate	5.6–6	6, 7	[51,52,20]

competitive with IC for phosphate analysis. Validation studies have included favorable comparison of results with IC and colorimetric tests. Quantitation ranges are generally near 0.5–10 μg/ml, although this range can be lowered significantly using electrokinetic injection methods [83], including iso-

Table 5
Applications of CE to the determination of orthophosphate

Matrix	Electrolyte No.	Ref.
Ground, surface, environmental waters	18, 2a, 1, 1f	[71,45,83,15,70,84]
Water treatment (on-line)	1j	[85]
Detergent	9	[54]
Toothpaste	1c, 1a, 8, 9	[25,22,53,55]
Soap	8	[53]
Ionic matrices	1f, 1c, 3a	[35,27,13]
Boric acid	1f	[31]
Bayer liquor	1h	[34,33]
N-Methylpyrrolidone	2	[43]
Dyes	1	[86]
Electrodeposition coatings	16c	[68]
Beverages	2b, 1c, 16	[46,24,64]
Wine, juices	2b	[87]
Coffee	3b	[47]
Beer	6, 1j	[51,52]
Milk	1	[88]
Plant materials	4a	[49]
Serum	11	[57]
Urine	1	[81]
Rat lung	16a	[66]
Drugs	5a, 1	[89,90]
Vitamins	1	[21]
Dental plaque, saliva	3	[20]

tachophoretic stacking and cutting [70], matrix enhanced stacking [35], highly absorbing dyes as coions [61], or more sensitive detection schemes [64]. Overall CE methods for orthophosphate are well-established.

3.2. Lower oxo anions

In contrast to the large database on applications for orthophosphate, less work has been done applying CE to separations of phosphorus oxo anions in an oxidation state lower than +5, i.e., phosphorous acid and phosphite salts (+3), and hypophosphorous acid and hypophosphite salts (+1). Capillary ITP with conductivity detection has been applied to the separation of phosphorus anion species, including phosphite and hypophosphite [8,91,92] using electrolytes with pH 3–6. Modern capillary zone electrophoresis separations include use of chromate electrolyte, which showed marginal PO_3/PO_4 separation [16], and the work of Dasgupta and Bao [93], who separated HPO_3^{2-} from other anions using suppressed conductivity detection.

Several papers have focused on separating the important series of $P^1/P^3/P^5$ anions. Shamsi and Danielson [53] employed AMP (buffer 8 in Table 2) for this separation. The migration time order $P^3 < P^5 < P^1$ was consistent with predictions from Fig. 1 for a pH 7.8 buffer. Phosphate/phosphite separation also was shown using N-nitroso-R (buffer 14, Table 2) in a counter-electroosmotic separation, with migration times $P^5 < P^3$. A recent application to lower oxo acid analysis was described by Soga [94] for electroless plating baths, where hypophosphite is an active ingredient. This separation of hypophosphite from phosphite, orthophosphate, and various organic anions is shown in Fig. 4, and the migration order is as expected at pH 5.6 and consistent with ITP results. While IC does a reasonable job in phosphite analysis, hypophosphite often is poorly retained, and CE may be preferred for its ability to separate these phosphorus oxo anions and organics simultaneously.

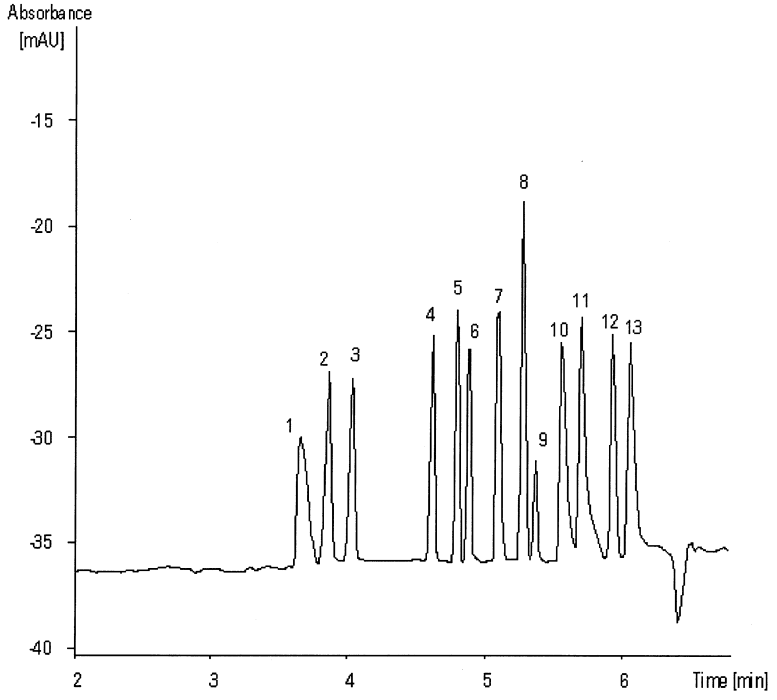


Fig. 4. Separation of (8) hypophosphite, (10) phosphite and (13) orthophosphate from other inorganic and organic ions using electrolyte No. 6, Table 2. Voltage −25 kV, 72 cm×75 μm capillary, detection wavelength 200 nm, temperature 20°C. Other peaks: 1=chloride, 2=sulfate, 3=oxalate, 4=tartarate, 5=malate, 6=citrate, 7=succinate, 9=EDTA, 11=acetate and 12=lactate. Reprinted with permission from Ref. [94].

3.3. Condensed phosphates

A class of phosphate oligomers, generally referred to as condensed phosphates, are important compounds that are commercially produced for a variety of uses (e.g., detergent builders, preservatives, sequestering agents, acidulents). These include pyrophosphate, tripolyphosphate and trimetaphosphate salts. Separation of these species from orthophosphate and other inorganics is often performed using suppressed-conductivity IC [4] or ion-exchange chromatography with post-column reaction detection [95,96].

As with the lower oxo anions, capillary ITP has been used extensively for condensed phosphate separations and analysis [7,8,12,92,97,98]. ITP also is the only capillary electrophoretic technique demonstrated to separate condensed species of lower oxidation state [91], P^3–O–P^3 and P^3–O–P^5 (see Table 1). Paper electrophoresis has also been a standard technique for phosphate speciation [3,99]. Nevertheless, there are few reports of the use of modern CE for condensed phosphate analysis.

Shamsi and Danielson [53] described the addition of Mg^{2+} to an AMP buffer to separate phosphate complexes as cations, and demonstrated the analysis of pyrophosphate in toothpaste. Pyrophosphate added to a general anion mixture was separated with chromate electrolyte [37]. In our laboratory, we have shown that a phthalate buffer containing DTAB modifier can be used for separating ortho-, pyro- and tripolyphosphate [13]. Wang and Li have used ATP (buffer 9 in Table 2) at a lower pH to achieve separation of these ions, as shown in Fig. 5. Applications of this buffer include determining condensed phosphates in detergents [54] and toothpaste [55].

An interesting aspect of these separations is that significant differences from expected migration order can be seen depending on the electrolyte composition. Reported migration orders include the expected $P_3<P_2<P_1$ at pH 4.2 with phthalate–DTAB [13], $P_{3m}<P_1<P_2<P_3$ at pH 7.1 with AMP–DETA–borate

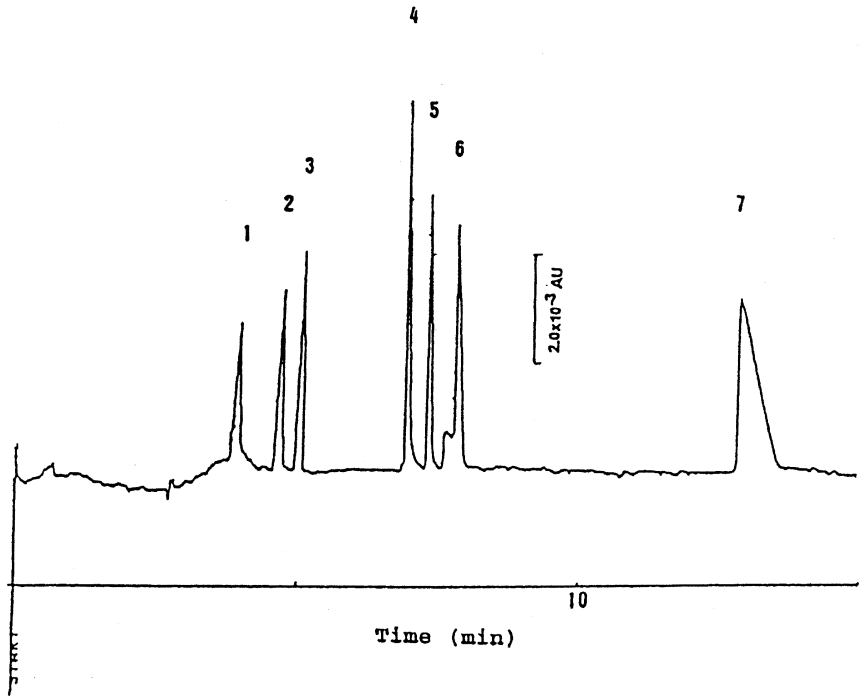


Fig. 5. Separation of (1) tripolyphosphate, (2) pyrophosphate and (5) orthophosphate at 0.5 m*M* using electrolyte No. 9, Table 2. Voltage −15 kV; hydrostatic injection at 4.5 cm for 15 s; 40 cm× x 75 μm capillary; detection wavelength 260 nm; temperature 25°C. Other peaks: 3=0.5 m*M* NTA, 4=0.5 m*M* EDTA, 6=0.5 m*M* DTPA and 7=1 m*M* citrate. Reprinted with permission from Ref. [54].

[53], and $P_2<P_3<P_1$ at pH 10.3 with EDTA–OFM Anion BT [56] and at pH 7.7 with PMA–HMH [42]. It is apparent that significant interactions may be occurring between condensed phosphates and CE system components (electrolyte and/or capillary) which are not adequately explained by simple pK_a and mobility considerations.

3.4. Polyphosphates

The largest challenge in determining phosphorus species distributions is in characterizing the complex polymeric mixture of phosphate compounds known as glassy polyphosphates (also called sodium hexametaphosphate, sodium phosphate glasses, or Calgon). A glassy polyphosphate sample can be characterized by its number-average chain length, n_{ave}, and materials may be encountered with n_{ave} from ~5 through at least 1000. Distributions are relatively broad, so that a phosphate glass with $n_{ave}=10$ will contain significant amounts of species with individual chain lengths (n) from *ortho* to >50 [2].

Capillary ITP separations [100] have shown that polyphosphates ($n\geq4$) at pH 5.5 have an unresolved, but fairly narrow, mobility distribution near 0.00055 $cm^2\ V^{-1}\ s^{-1}$, similar to pyrophosphate. Determination of distinct species concentrations (other than P_1 and P_{3m}) are not possible by ITP. However, average chain lengths can be estimated from the quantitative response per amount injected. Using polyacrylamide gel electrophoresis [101], rather impressive separations of individual polyphosphates have been obtained with nearly complete unit resolution for chain lengths approaching 100. Gel electrophoresis practitioners have used enzymatic reactions with polyphosphate glucokinase [102] to reduce chain lengths into a range which allows characterization of materials with average chain lengths approaching 750.

Recently, Wang and Li [103] have shown the utility of gel electrophoresis in the capillary format for separating polyphosphate oligomers with indirect UV detection. While several examples of free solution separations were given, the best separation of polyphosphates up to n~25 was found using a 12% linear polyacrylamide gel. Another polyphosphate separation using free solution CE was shown using EDTA buffer [56], where metaphosphates and other peaks are seen for a polyphosphate injection. Our laboratory has investigated the use of PMA buffer (No. 2 in Table 2) for polyphosphate separations [42], and the results are shown in Fig. 6. Numbers correspond to phosphate species chain length with "*m*"=*meta*. Bold numbers indicate peaks whose identity was confirmed by spiking with standard materials. Other species identification is inferred

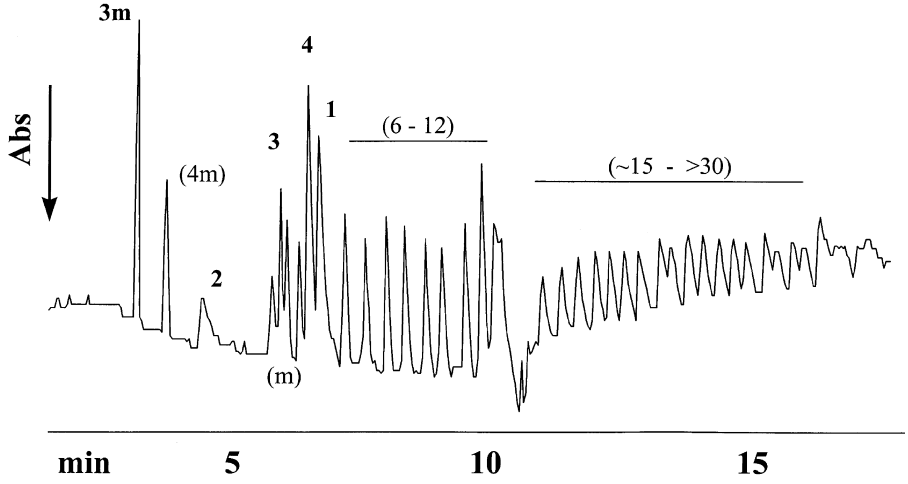


Fig. 6. Separation of species in a polyphosphate sample using electrolyte No. 2, Table 2. For peak identification, see Section 3.4. Voltage −20 kV; hydrostatic injection at 1.5 p.s.i.g. for 2 s; 36 cm×75 μm capillary; detection wavelength 254 nm; temperature 25°C; total polyphosphate sample concentration 0.2 mg/ml (1 p.s.i.=6894.76 Pa). From Ref. [42] with permission.

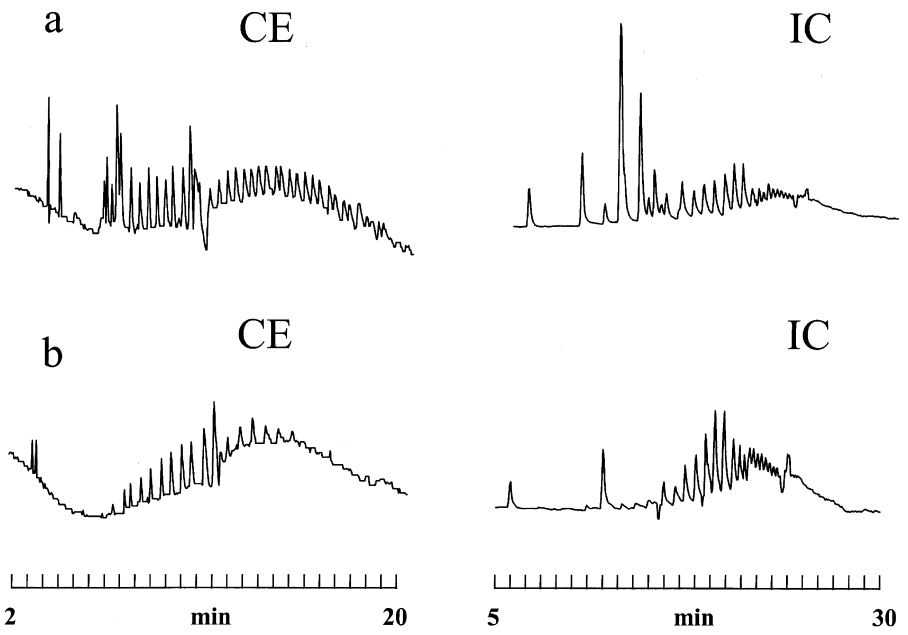


Fig. 7. Comparison of CE and IC separations of (a) polyphosphate standard with n_{ave}=22, and (b) a polyphosphate–protein mixture enriched in smaller chain polyphosphates. CE separation conditions as in Fig. 6. For IC conditions, see Ref. [4].

from migration times, standard material n_{ave}, and comparison with IC results.

The use of CE vs. IC for characterizing polyphosphates is illustrated in Fig. 7. Comparisons of (a) n_{ave}=22 standard, and (b) a polyphosphate–protein mixture, run by IC and CE show similar performance. Somewhat better relative sensitivity to longer-chain polyphosphates is apparent with CE, and run times are similar. For the IC separation, the polyphosphate–protein mixture was passed through a C_{18} solid-phase extraction cartridge to remove protein, while for CE direct injection was used. Advantages of CE for polyphosphate analysis can include simpler sample preparation and smaller sample size. While CE is a promising tool for polyphosphate characterization, more work remains to be done in assessing the origin of the free solution separations and defining the ultimate limits of the method.

4. Conclusions

CE as a method for determining orthophosphate in solution is well established. Numerous buffers are available with which to perform this analysis, and optimized methods for analyzing various sample matrices have been developed. Less progress has been made in analyzing other oxo anions of phosphorus. Several areas need to be investigated in order to more uniformly establish CE as a technique for phosphorus oxo anion analysis:

(1) Development, optimization and validation of methods for condensed phosphates and lower oxo anions of phosphorus in a variety of samples.

(2) New methods for use in difficult matrices which employ more sensitive or selective detection of phosphates, such as flame photometric detection [104] or fluorescence tagging [78].

(3) Development of robust methods for characterizing polyphosphate mixtures, including further translation of traditional gel methods, application of learnings from DNA analysis using CE, and quantitative aspects of phosphate oligomer separations.

(4) Understanding the role of buffer components, ionic strength, and other interactions on the effective mobility of condensed and polyphosphates.

CE has already achieved significance as a valuable technique for separating and quantitating phosphorus oxo anions. Future advances in methodology, instrumentation, and the physical chemistry basis of the

separations are expected to enhance the utility of CE in these applications.

References

[1] D.E.C. Corbridge, Phosphorus – An Outline of its Chemistry, Biochemistry and Technology, Elsevier, Amsterdam, 4th ed., 1990.
[2] J.R. Van Wazer, Phosphorus and its Compounds, Vol. I, Interscience, New York, 1958, pp. 345–770.
[3] M. Halmann (Ed.), Analytical Chemistry of Phosphorus Compounds, Wiley-Interscience, New York, 1972.
[4] F.S. Stover, J.A. Bulmahn, J.K. Gard, J. Chromatogr. A 688 (1994) 89.
[5] D.R. Gard, J.C. Burquin, J.K. Gard, Anal. Chem. 64 (1992) 557.
[6] H. Shintani, J. Polonsky (Eds.), Handbook of Capillary Electrophoresis Applications, Blackie, London, 1997.
[7] F.M. Everaerts, J.L. Beckers, Th.P.E.M. Verheggen, Isotachophoresis – Theory, Instrumentation and Applications, Elsevier, Amsterdam, 1976.
[8] T. Hirokawa, S. Kobayashi, Y. Kiso, J. Chromatogr. 318 (1985) 195.
[9] NIST Standard Reference Database 46, version 4.0, NIST Critically Selected Stability Constants of Metal Complexes Database, US Department of Commerce, Gaithersburg, MD, 1997.
[10] J. Pospichal, P. Gebauer, P. Bocek, Chem. Rev. 89 (1989) 419.
[11] R.A. Robinson, R.H. Stokes, Electrolyte Solutions, Butterworths Scientific, London, 2nd ed., 1959.
[12] F.S. Stover, Electrophoresis 11 (1990) 750.
[13] F.S. Stover, S.S. Keffer, J. Chromatogr. A 657 (1993) 450.
[14] P. Jandik, W.R. Jones, J. Chromatogr. 546 (1991) 431.
[15] J.P. Romano, J. Krol, J. Chromatogr. 640 (1993) 403.
[16] W.R. Jones, P. Jandik, J. Chromatogr. 608 (1992) 385.
[17] G. Bondoux, P. Jandik, W.R. Jones, J. Chromatogr. 602 (1992) 79.
[18] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[19] V. Verhelst, J.-P. Mollie, F. Campeol, J. Chromatogr. A 770 (1997) 337.
[20] J. Romano, P. Jandik, W.R. Jones, P.E. Jackson, J. Chromatogr. 546 (1991) 411.
[21] M.E. Swartz, J. Chromatogr. 640 (1993) 441.
[22] A.H. Harakuwe, P.R. Haddad, J. Chromatogr. A 734 (1996) 416.
[23] S.A. Oehrle, J. Chromatogr. A 745 (1996) 81.
[24] K. Li, S.F.Y. Li, J. Liq. Chromatogr. 17 (1994) 3889.
[25] P. Wang, S.F.Y. Li, H.K. Lee, J. Chromatogr. A 765 (1997) 353.
[26] M. Jimidar, D.L. Massart, Anal. Chim. Acta 294 (1994) 165.
[27] R. Saari-Nordhaus, J.M. Anderson, J. Chromatogr. A 706 (1995) 563.
[28] P. Kuban, B. Karlberg, Anal. Chem. 69 (1997) 1169.
[29] P. Kuban, B. Karlberg, Anal. Chem. 70 (1998) 360.
[30] A.H. Harakuwe, P.R. Haddad, Anal. Commun. 33 (1996) 103.
[31] J. Boden, M. Darius, K. Baechmann, J. Chromatogr. A 716 (1995) 311.
[32] M.M. Rhemrev-Boom, J. Chromatogr. A 680 (1994) 675.
[33] A.H. Harakuwe, P.R. Haddad, W. Buchberger, J. Chromatogr. A 685 (1994) 161.
[34] P.R. Haddad, A.H. Harakuwe, J. Chromatogr. A 706 (1995) 571.
[35] J. Boden, K. Baechmann, J. Chromatogr. A 734 (1996) 319.
[36] M.T. Galceran, L. Puignou, M. Diez, J. Chromatogr. A 732 (1996) 167.
[37] P.J. Oefner, Electrophoresis 16 (1995) 46.
[38] C. Stathakis, R.M. Cassidy, Anal. Chem. 66 (1994) 2110.
[39] O.V. Krokhin, H. Hoshino, O.A. Shpigun, T. Yotsuyanagi, J. Chromatogr. A 776 (1997) 329.
[40] C. Francois, Ph. Morin, M. Dreux, J. High Resolut. Chromatogr. 19 (1996) 5.
[41] J.D. Lamb, B.R. Edwards, R.G. Smith, R. Garrick, Talanta 42 (1995) 109.
[42] F.S. Stover, J. Chromatogr. A 769 (1997) 349.
[43] R.J. Geise, N.I. Machnicki, R.M. Ianniello, J. Liq. Chromatogr. 16 (1993) 3699.
[44] M.P. Harrold, M.J. Wojtusik, J. Riviello, P. Henson, J. Chromatogr. 640 (1993) 463.
[45] M.A. van den Hoop, J.J. van Staden, J. Chromatogr. A 770 (1997) 321.
[46] M. Arellano, J. Andrianary, F. Dedieu, F. Couderc, Ph. Puig, J. Chromatogr. A 765 (1997) 321.
[47] M. Weers, H. Balzer, A. Bradbury, O.G. Vitzthum, Colloq. Sci. Int. Cafe 16 (1995) 218.
[48] P. Doble, P. Andersson, P.R. Haddad, J. Chromatogr. A 770 (1997) 291.
[49] A. Bazanella, H. Lochmann, A. Mainka, K. Baechmann, Chromatographia 45 (1997) 59.
[50] S.M. Cousins, P.R. Haddad, W. Buchberger, J. Chromatogr. A 671 (1994) 397.
[51] T. Soga, G.A. Ross, J. Chromatogr. A 767 (1997) 223.
[52] T. Soga, M. Wakaura, J. Am. Soc. Brew. Chem. 55 (1997) 44.
[53] S.A. Shamsi, N.D. Danielson, Anal. Chem. 67 (1995) 1845.
[54] T. Wang, S.F.Y. Li, J. Chromatogr. A 723 (1996) 197.
[55] T. Wang, S.F.Y. Li, J. Chromatogr. A 781 (1997) 457.
[56] K.-W. Lee, J.-Y. Jeon, K.-P. Lee, S.-H. Lee, B. Chiswell, Anal. Sci. 13(Suppl.) (1997) 247.
[57] V. Dolnik, J. Dolnikova, J. Chromatogr. A 716 (1995) 269.
[58] Y. Fung, K. Lau, Talanta 45 (1998) 641.
[59] S.A. Shamsi, N.D. Danielson, Anal. Chem. 66 (1994) 3757.
[60] H. Siren, A. Maattanen, M.-L. Riekkola, J. Chromatogr. A 767 (1997) 293.
[61] P. Doble, M. Macka, P.R. Haddad, J. Chromatogr. A 804 (1998) 327.
[62] M.E.P. Hows, L.N. Alfazema, D. Perrett, LC·GC 15 (1997) 1034.
[63] P. Doble, M. Macka, P. Andersson, P.R. Haddad, Analyst 122 (1997) 351.

[64] C. Haber, W.R. Jones, J. Soglia, M.A. Surve, M. McGlynn, A. Caplan, J.R. Reineck, C. Krstanovic, J. Cap. Electrophoresis 3 (1996) 1.
[65] W.R. Jones, J. Soglia, M. McGlynn, C. Haber, J.R. Reineck, C. Krstanovic, Am. Lab. 28 (1996) 25.
[66] K. Govindaraju, E.A. Cowley, D.H. Eidelman, D.K. Lloyd, Anal. Chem. 67 (1997) 2793.
[67] R.C. Williams, R. Boucher, J. Brown, J.R. Scull, J. Walker, D. Paolini, J. Pharm. Biomed. Anal. 16 (1997) 469.
[68] C.W. Kampfl, M.U. Katzmayr, W. Buchberger, N. Basener, J. Chromatogr. A 804 (1998) 357.
[69] W.R. Jones, Pittsburgh Conference, New Orleans, LA, 4 March, 1998, Paper 1014.
[70] D. Kaniansky, I. Zelensky, A. Hybenova, F.I. Onuska, Anal. Chem. 66 (1994) 4258.
[71] D. Kaniansky, V. Zelenska, D. Baluchova, Electophoresis 17 (1996) 1890.
[72] N. Avdalovic, C.A. Pohl, R.D. Rocklin, J.R. Stillian, Anal. Chem. 65 (1993) 1470.
[73] M. Harrold, J. Stillian, L. Bao, R. Rocklin, N. Avdalovic, J. Chromatogr. A 717 (1995) 371.
[74] L. Gross, E.S. Yeung, J. Chromatogr. 480 (1989) 169.
[75] H. Liu, P.K. Dasgupta, Anal. Chem. 69 (1997) 1211.
[76] J.J. Corr, J.F. Anacleto, Anal. Chem. 68 (1996) 2155.
[77] C.E. Sanger-van de Griend, C.E. Kientz, U.A.Th. Brinkman, J. Chromatogr. A 673 (1994) 299.
[78] P. Wang, R.W. Geise, Anal. Chem. 65 (1993) 3518.
[79] P. Wang, R.W. Geise, Anal. Biochem. 230 (1995) 329.
[80] R.W. Giese, P. Wang, personal communication.
[81] B.J. Wildman, P.E. Jackson, W.R. Jones, P.G. Alden, J. Chromatogr. 546 (1991) 459.
[82] Th. Ehmann, K. Baechmann, L. Fabry, H. Ruefer, S. Pahlke, L. Kotz, Chromatographia 45 (1997) 301.
[83] P.E. Jackson, P.R. Haddad, J. Chromatogr. 640 (1993) 481.
[84] M. Panstar-Kallio, P.K.G. Manninen, Chemosphere 31 (1995) 3699.
[85] L. Arce, A. Rios, M. Valcarcel, J. Chromatogr. A 791 (1997) 279.
[86] S.A. Oehrle, J. Chromatogr. A 671 (1994) 383.
[87] M. Arellano, F. Couderc, Ph. Puig, Am. J. Enol. Vitic. 48 (1997) 408.
[88] M. Schmitt, F. Saulnier, L. Malhautier, G. Linden, J. Chromatogr. 640 (1993) 419.
[89] I.S. Lurie, J. Cap. Electrophoresis 3 (1996) 237.
[90] J.B. Nair, C.G. Izzo, J. Chromatogr. 640 (1993) 445.
[91] H. Nariai, K. Nakazaki, M. Tsuhako, I. Motooka, J. Chromatogr. 248 (1982) 135.
[92] T. Yagi, K. Kojima, H. Nariai, I. Motooka, Bull. Chem. Soc. Jpn. 55 (1982) 1831.
[93] P.K. Dasgupta, L. Bao, Anal. Chem. 65 (1993) 1003.
[94] T. Soga, HP Application Note, Capillary Zone Electrophoresis for Electroless Plating Bath Samples, Publication No. 12-5965-8938E, Hewlett-Packard, 1997.
[95] Y. Baba, N. Yoza, S. Ohashi, J. Chromatogr. 350 (1985) 119.
[96] ASTM Standard Test Method D 2761-89, American Society for Testing Materials Committee on Standards, Philadelphia, PA, 1989.
[97] I. Motooka, H. Nariai, K. Nakazaki, M. Tsuhako, J. Chromatogr. 295 (1984) 533.
[98] P. Janos, J. Chromatogr. 516 (1990) 473.
[99] J. Fenger, P.B. Pagsberg, J. Inorg. Nucl. Chem. 35 (1973) 31.
[100] I. Motooka, H. Nariai, K. Nakazaki, M. Tsuhako, J. Chromatogr. 260 (1983) 377.
[101] N.A. Robinson, N.H. Goss, H.G. Wood, Biochem. Int. 8 (1984) 757.
[102] C.A. Pepin, H.G. Wood, N.A. Robinson, Biochem. Int. 12 (1986) 111.
[103] T. Wang, S.F.Y. Li, J. Chromatogr. A 802 (1998) 159.
[104] C.E. Kientz, E.W.J. Hooijschuur, U.A.Th. Brinkman, J. Microcol. Sep. 9 (1997) 253.