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Review

Indirect photometric detection of anions in capillary electrophoresis

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Abstract

Indirect photometric detection (absorbance and fluorescence) of anions by capillary electrophoresis is reviewed. Factors which influence the displacement process of analyte ions in background electrolytes containing one or more co-ions are discussed and factors which influence detection sensitivity in indirect detection modes are outlined. Procedures are presented for buffering background electrolytes when indirect absorbance detection is to be used in the determination of anions, and approaches to the design and optimization of the composition of background electrolytes for indirect detection of anions are discussed. The application of indirect detection to the separation of major groups of analytes including inorganic anions, organic acids, carbohydrates, phosphates, phosphonates and miscellaneous species is then reviewed. Detailed information on the physical properties of species used as probes for indirect detection of anions is provided.

Keywords: Indirect detection; Capillary electrophoresis; Anions; Probes

Contents

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1. Introduction and scope of the review

Indirect photometric detection in capillary electrophoresis (CE) is often employed for detection of cations and anions that lack suitable chromophores. In this form of detection, an absorbing co-ion (an ion with the same charge as the analyte) called the probe is added to the background electrolyte (BGE). Detection is accomplished by displacement of the co-ion leading to a quantifiable decrease in the background absorbance. The indirect detection method is universal in its applicability, and the instrumentation required is the same as for direct photometric detection, which is simple and commercially available.

Indirect absorbance detection was first introduced as a detection mode for CE by Hjerten et al. [1] in 1987. With the introduction of a sensitive universal detection scheme, the utility of CE has increased significantly. Indeed, the number of publications detailing applications of indirect photometric detection in CE has grown substantially in the past few years. Accordingly, the aim of this review is to offer a critical summary of all publications dealing with the separation and indirect photometric detection (indirect absorbance and fluorescence) of anions by capillary electrophoresis that have appeared in abstracted journals up until the end of 1997. The discussion in this review is structured according to the following sequence:

1. Factors which influence the displacement process of analyte ions in BGEs containing one or more co-ions.
2. Procedures for buffering BGEs when indirect absorbance detection is to be used in the determination of anions.
3. Factors which influence detection sensitivity in indirect detection modes.
4. Design and optimization of the composition of BGEs for indirect detection of anions.
5. Discussion of separations of major groups of analytes including inorganic anions, organic acids, carbohydrates, phosphates and phosphonates.

2. Kohlrausch's regulating function and the transfer ratio

The degree of displacement of the probe (co-ion) by the analyte is known as the transfer ratio (TR) [2]. The TR is defined as the number of moles of the probe displaced by one mole of analyte ions. As the detector response is proportional to the TR, a higher value of the TR results in a larger analyte peak area. On an intuitive level, one would expect displacement on an equivalent-per-equivalent basis so that, for example, the TR between a singly charged solute and a singly charged probe would be expected to be unity. Consequently, the peak areas for analytes of the same charge and concentration should be the same, allowing the theoretical possibility of calibration of each component without the need for separate calibration curves. However, Ackermans et al. [3] showed that a non-linear relationship existed between peak area and the effective mobilities of the ionic species for an equimolar sample composition. This behavior can be explained by consideration of the electrophoretic separation mechanism for fully ionized ionic constituents, which can approximately be described by Kohlrausch's Regulating Function (ω) [4]:

$$\omega = \sum_i \frac{c_i . z_i}{\mu_i} = \text{constant} \quad (1)$$

where c_i, z_i and μ_i represent the ionic concentrations, absolute values of the charge, and absolute values of the effective mobilities of all ionic constituents, respectively.

Migration of ions through a capillary filled with a uniform electrolyte will be governed by one single ω function. If a sample containing a single analyte is introduced, the migration of ions is governed by two ω functions, the first associated with the sample plug, and the second with the bulk electrolyte. The ω function for each must be constant, so that the concentration distributions of the ions for the bulk electrolyte and the sample plug remain as they were before the voltage was applied. That is, the flux of ions into the sample plug is exactly equal to the flux out.

A consequence of the ω functions is that the TR is

dependent on the mobility of the probe, the analyte, and the counter ion. The relationship between these can be derived directly from the ω function [5,6] or consideration of the migration of ions using an eigenvalue approach [6–8].

Consider an electrolyte consisting of a single ion A, and its corresponding counter-ion, C, then from Eq. (1)

$$\omega_1 = \frac{c_A z_A}{\mu_A} + \frac{c_C z_C}{\mu_C} \tag{2}$$

where c_A and c_C are the concentrations of A and C in the background electrolyte.

For the electroneutrality condition: $c_A z_A = c_C z_C$

$$\therefore \quad \omega_1 = c_A z_A \left(\frac{1}{\mu_A} + \frac{1}{\mu_C}\right) = \frac{c_A z_A}{\mu_A \mu_C}(\mu_A + \mu_C) \tag{3}$$

Now consider injection of an ionic analyte, BC, dissociated into a co-ion B, and counter-ion C. After an appropriate time the sample zone consists of A, B, and C. Then from Eq. (1):

$$\omega_2 = \frac{c'_A z_A}{\mu_A} + \frac{c_B z_B}{\mu_B} + \frac{c'_C z_C}{\mu_C} \tag{4}$$

where c'_A, c'_C are the concentrations of A, and C in the sample zone.

For the electroneutrality condition: $c'_A z_A + c_B z_B = c'_C z_C$

$$\therefore \quad \omega_2 = \frac{c'_A z_A}{\mu_A} + \frac{c_B z_B}{\mu_B} + \frac{c'_A z_A}{\mu_C} + \frac{c_B z_B}{\mu_C} \tag{5}$$

$$= c'_A z_A \left(\frac{1}{\mu_A} + \frac{1}{\mu_C}\right) + c_B z_B \left(\frac{1}{\mu_B} + \frac{1}{\mu_C}\right) \tag{6}$$

Now $\omega_1 = \omega_2$

$$\therefore \quad c_A z_A \left(\frac{1}{\mu_A} + \frac{1}{\mu_C}\right) = c'_A z_A \left(\frac{1}{\mu_A} + \frac{1}{\mu_C}\right) + c_B z_B \left(\frac{1}{\mu_B} + \frac{1}{\mu_C}\right) \tag{7}$$

$$(c_A - c'_A) z_A \left(\frac{1}{\mu_A} + \frac{1}{\mu_C}\right) = c_B z_B \left(\frac{1}{\mu_B} + \frac{1}{\mu_C}\right) \tag{8}$$

Let $\Delta c_A = c_A - c'_A$

$$\therefore \quad \frac{\Delta c_A}{c_B} = \frac{z_B}{z_A} \frac{\left(\frac{1}{\mu_A} + \frac{1}{\mu_C}\right)}{\left(\frac{1}{\mu_A} + \frac{1}{\mu_C}\right)} \tag{9}$$

$$= \frac{z_B}{z_A} \frac{(\mu_B + \mu_C)}{(\mu_A + \mu_C)} \cdot \frac{\mu_A \mu_C}{\mu_B \mu_C} \tag{10}$$

$$= \frac{z_B}{z_A} \cdot \frac{\mu_A}{\mu_B} \frac{(\mu_B + \mu_C)}{(\mu_A + \mu_C)} = \mathrm{TR} \tag{11}$$

Several publications have attempted to validate the applicability of Eq. (11) in practical situations and to samples that contain more than one analyte, i.e. electrophoresis systems that consist of more than three components. Nielen [9] showed that the response factors of alkylsulfate surfactants, analyzed with veronal as the probe, fitted well with predictions made from Eq. (11). Cousins et al. [2,10] experimentally determined the TR values for a series of anions using a number of different probes and showed that the experimental values of TR followed the general trend predicted by Eq. (11), but agreement between the theoretical and observed values was poor. Doble et al. [11] showed that the responses of analytes were indeed described accurately by Eq. (11) when the BGE was free of any potentially interfering co-ions, which are often introduced by EOF modifiers such as tetradecyltrimethylammonium bromide (TTAB). The authors overcame the problem of co-anion introduction due to the EOF modifier by employing an EOF modifier in the hydroxide form, with the added hydroxide co-anions being largely consumed by the buffer.

3. Peak shapes

Peak shapes have been the subject of a number of papers [8,12–15]. Mikkers et al. [7,16] first described the effect of electrophoretic migration on analyte zone concentration distributions using a non-diffusional mathematical model derived from Kohlrausch's Regulating Function. The concentration distributions of the analyte bands were found to be dependent upon the relative mobility of the analyte and the BGE carrier co-ion. Analytes that have a

higher mobility than the BGE co-ion migrate with a concentration distribution that is diffuse at the front and sharp at the rear of the zone, resulting in a fronting peak. The reverse holds true for analytes that are less mobile than the BGE co-ion, resulting in tailing peaks. Symmetrical peaks are obtained when the mobility of the co-ion and analyte are the same.

4. Multiple co-ion electrolytes

Currently, most BGEs designed for indirect absorbance detection consist of a single co-ion because under these conditions the displacement process is relatively simple and well understood. In the previous discussions of the Kohlrausch Regulating Function, transfer ratios and peak shapes, BGEs containing only a single co-ion were considered. The question of what happens with the displacement process when the BGE contains two co-ions has been the subject of several publications. Wang and Hartwick [17] derived a theoretical model that indicated that when the mobility of an analyte was intermediate between the mobilities of two BGE co-ions, the analyte ion would mainly displace the co-ion to which its mobility was closest. When the analyte ion mobility was very different to either of the mobilities of the BGE co-ions, competitive displacement occurred, i.e. no obvious discrimination of displacement of either of the BGE co-ions took place. A consequence of this behavior was that peak shapes for analytes of different mobilities could be improved by choosing two probe co-ions that matched the mobilities of the analytes. However, if one of the BGE co-ions was a UV-transparent species, detection sensitivity was severely compromised for analytes that had a similar mobility to that of the transparent species. The authors also reported inducement of a system peak that had the potential to interfere with possible analytes of interest.

Beckers in a series of publications [14,15,18] examined the system characteristics of BGEs that contain two co-ions for the analysis of cations. Initially, a two co-cation BGE [18] consisting of an UV-transparent co-ion (potassium) and a single probe (histidine) was considered, with potassium having a higher mobility than histidine. The induced system peak always migrated with a mobility which was between those of the two co-anions, with the exact mobility of the system peak being dependent upon the relative concentrations of each co-ion. UV-transparent sample components that migrated before the system peak always migrated as a positive peak (i.e. increased absorbance) and analytes that migrated after the system peak migrated as a negative peak. Analytes that migrated near the system peak had distorted peak shapes and were enlarged, losing all quantitative properties. In a subsequent publication [14] the reverse situation was considered, i.e. a two co-cation BGE with a lower mobility UV-transparent co-ion (Tris), and a fast probe (imidazole). Analytes that migrated before the system peak were negative (lowered absorbance) and peaks that migrated after the system peak were positive. The system peak behaved as it did in the former situation.

Macka et al. [19] developed some practical rules for predicting the existence of system peaks for the analysis of anions. These rules were based on qualitative descriptions of transient isotachophoresis of the analyte species and of the co-ion to which its mobility was closest. Two cases were considered, the first being when the analyte had a higher mobility than either of the BGE co-ions and the second when the mobility of the analyte was slower than the co-ions. For both cases, it was demonstrated that the system peak was created by a vacancy of one component of the BGE that had the greatest difference in mobility relative to that of the analyte species. The authors also reported that a practical transition existed in which the BGE changed in behavior from a single co-ion BGE to a two co-ion BGE when the concentration of the second co-ion was approximately 5% of the concentration of the first (major) co-ion.

Doble et al. [20] utilized multiple probes to improve peak shapes by matching the mobilities of the probe co-anions in the BGE with as broad a range of analyte mobility as possible. When n absorbing probe co-anions were added to the BGE, $n-1$ system peaks were produced. Multiple probe BGEs produced mobility zones between the system peaks in which the co-anions were displaced preferentially by analytes with the closest mobility. Analytes migrating in each zone gave similar peak symmetry to that obtained with the corresponding single probe BGE. The authors demonstrated that the

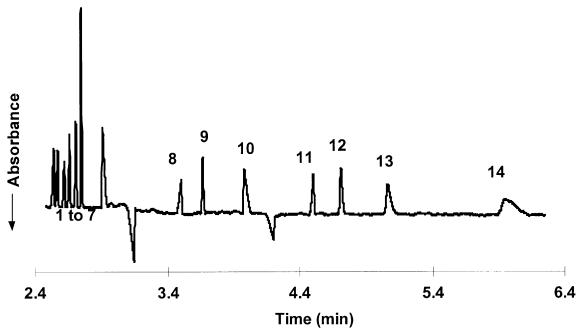

Fig. 1. Electropherogram obtained using a BGE with three probes. Conditions: BGE 5.0 m*M* chromic acid, 5.0 m*M* phthalic acid, 10 m*M* benzoic acid, 60 m*M* DEA, 0.5 m*M* TTAOH, pH 9.20. Separation voltage −20 kV, hydrostatic injection at 10 cm for 10 s, detection wavelength 254 nm, temperature 25°C. Key: 1=bromide, 2=chloride, 3=iodide, 4=nitrite, 5=nitrate, 6=sulfate, 7=chlorate, 8=phosphate, 9=carbonate, 10=ethanesulfonate, 11=butanesulfonate, 12=pentanesulfonate, 13=hexanesulfonate, 14=heptanesulfonate. The sample mixture contained 0.3 m*M* of each anion. Reprinted from Ref. [20] with permission.

mobility of system peaks for BGEs, comprising two probes, could be calculated from the mobility of each of the probes and their corresponding concentrations according to a simple linear function. Fig. 1 gives an example of an electropherogram obtained with a BGE comprising three probes.

5. Buffering of BGEs when indirect detection is used for the determination of anions

Buffering of BGEs is essential for reproducible and rugged separations. One common method of buffering BGEs when indirect detection is used for the analysis of anions is by utilizing the probe itself as buffer. A weak acid is chosen as the probe and the pH of the BGE is maintained near the pK_a of the probe. Benzoate [21] and phthalate [22] are typical weak acid probes used for this method of buffering. The obvious disadvantages are: (i) the pH buffering range is limited to narrow regions approximately one pH unit either side of the pK_a of the probe; (ii) the probe is partially ionized and therefore has low mobility and is useful only for the analysis of anions of intermediate to low mobility and; (iii) the buffering capacity is limited to the concentration of the probe.

Another method of buffering is the use of co-anionic buffers such as acetic acid [23], borate [22,24–28], carbonate [29], and phosphate [30]. This approach would seem to overcome some of the disadvantages of the former method. However, the BGEs no longer contain a single co-anion, leading to potentially interfering system peaks and the probability of reduced detection sensitivity due to competitive displacement of the added co-anion (buffer). In studies where co-anionic buffers have been used, little emphasis has been placed on the appearance of system peaks and reduced sensitivity. The success of these separations has most likely been due to mobility-selective displacement of the probe, because the authors have intuitively chosen probes with mobilities close to the analytes of interest, which provides the best peak shapes, maximizes the transfer ratio, and minimizes competitive displacement.

A further approach for buffering electrolytes for the analysis and indirect detection of anions is by use of a buffering counter-cation such as Tris [17,31–39], and triethanolamine (TEA) [40–44]. These electrolytes are typically prepared by titration of the acid form of the probe with the buffering base to the pK_a of the base. The advantage of this approach is that the BGE consists of a single co-anion (the probe), eliminating problems associated with multiple co-anion BGEs. However, the buffering capacity is still limited to the concentration of the probe since the buffering counter-cation is added in equal concentration to that of the probe anion.

Despite the desirability of buffering BGEs, a review of the literature shows that most of the BGEs utilized are unbuffered. As capillary electrophoresis is a relatively new technique, most published studies have been concerned with the feasibility of various separations, and, as a consequence, few methods have been validated by rigorous attention to reproducibility and ruggedness.

6. Optimizing limits of detection in indirect detection methods

The limit of detection (LOD) for a non-absorbing analyte detected using indirect absorbance detection is given by [45]:

$$C_{lod} = \frac{C_p}{TR\, D_r} = \frac{N_{BL}}{TR\, \varepsilon l} \quad (12)$$

where C_{lod} is the concentration limit of detection of the analyte, C_p is the concentration of the probe, TR is the transfer ratio (the number of moles of the probe displaced by one mole of the analyte), and D_r is the dynamic reserve (i.e., the ratio of the background absorbance to the noise), N_{BL} is the baseline noise, ε is the molar absorptivity of the probe, and l is the pathlength of the detection cell.

According to Eq. (12) C_{lod} can be optimized by reducing C_p or by increasing D_r. However for indirect absorption detection, D_r is also related to C_p so decreasing the probe concentration will not necessarily improve the detection limit, because D_r is simultaneously reduced [39]. Consequently, minimizing C_{lod} often involves maximizing D_r by either reducing the noise or increasing the BGE background absorbance.

Detection limits for indirect absorbance detection around 10^{-4} to 10^{-5} *M* are routinely achieved [46].

6.1. Noise

Conventional optical arrangements consist of UV lamps such as deuterium, cadmium and zinc etc. Optical systems based on light emitting diodes have been investigated as a means of improving optical baseline noise over UV lamp sources [47]. These systems typically reduce noise levels by two to 10 times. A potential drawback is that most of these light sources emit in the visible region, which limits choice of the BGE.

6.2. Pathlength

Increasing the pathlength, l, of the detection cell in order to increase the background absorbance has been the subject of several papers. Ma and Zhang [48] increased the pathlength by utilizing capillaries of increasing diameter. They reported that for a narrow range of capillary diameters from 25 to 75 μm, the diameter was not a critical contribution to LODs. They speculated that the increase in joule heating with increasing capillary diameter increased the noise, negating any advantage obtained with the higher BGE background absorbance. This result was in disagreement with that reported by Steiner et al. [49], who calculated signal to noise ratios (S/N) for capillaries from 10 to 10 000 μm and showed that the S/N increased with capillary diameter. The discrepancy may be due to the fact that the contribution of joule heating to noise levels was neglected in the latter study by measuring the background absorbance of the BGE in each capillary without the voltage on.

Weston et al. [50] extended the pathlength by use of a 'bubble cell'. A 75-μm capillary pathlength was extended to 300 μm by blowing a 'bubble' at the detection end. For such an arrangement the influence of increased joule heating on noise is absent because the capillary diameter is unchanged over most of its length. They reported that the detection sensitivity improved by approximately a factor of two, a result somewhat less than expected due to a concomitant increase in baseline noise.

6.3. Molar absorptivity

Most publications report optimized detection limits by consideration of the molar absorptivity (ε) of the probe [2,31,43,48,50–56]. Increasing the molar absorptivity of the probe increases the dynamic reserve, and simultaneously reduces the necessary probe concentration, resulting in a lowering of detection limit. However, the probe must have a similar mobility to the analytes for the full benefit of high ε to be realized. Foret et al. [53] report a 50-times improvement in the detection limits of anions when the probe was changed from benzoate (low ε) to sorbate (high ε). Beck and Engelhardt [55] investigated a series of cationic probes for the analysis of inorganic and organic cations. Optimum conditions consisted of the probe with the closest mobility to the analytes and highest ε. Weston et al. [50] improved detection limits of inorganic cations by two to four times when changing the probe from UV Cat1 to UV Cat2. The improvement in detection was again due to the higher molar absorptivity of UV Cat2 and its closer mobility to the analytes.

Mala et al. [54] utilized the highly absorbing dyes, chlorophenol red and methyl green for the analysis of inorganic cations, and indigo carmine for the analysis of inorganic anions. These dyes have molar absorptivities an order of magnitude higher than

common probes such as phthalate and chromate, and were expected to decrease the detection limits by a similar degree. However, the reported detection limits were no better than those obtained with probes that are more conventional. The likely reason was due the presence of competing co-ions used as buffering agents.

6.4. Choice of the probe

The most crucial aspects for the success of separation and indirect detection of any analyte are: (i) mobility of probe, (ii) mobility range of the analytes, (iii) and sensitivity of detection. For optimum peak shapes, the mobility of the probe must be as close as possible to the mobility of the analytes. Sensitivity of detection is greatest when the molar absorptivity of the probe is high. Table 1 lists the probes which have been reported for indirect detection of anions and shows some of the important physical data (molar absorptivity, pK_a, mobility, and the pH at which the mobility was measured) relating to these probes.

The probes can be grouped as shown below and Tables listing some typical applications of these probes are also provided:

1. Chromate as a probe for indirect absorbance detection (Table 2)
2. Aromatic carboxylates as probes for indirect absorbance detection (Table 3)
3. Aromatic sulfonates as probes for indirect absorbance detection (Table 4)
4. Miscellaneous probes for indirect absorbance detection (Table 5)
5. Probes for indirect fluorescence detection (Table 6)

The use of these probes for the determination of inorganic anions, organic acids, carbohydrates, phosphates and phosphonates, and other miscellaneous species is discussed below.

7. Determination of inorganic anions

Sodium chromate has been the most commonly used carrier electrolyte for the analysis of inorganic anions. It has been applied to the analysis of anionic constituents in urine [57], Bayer liquors [58–60], Kraft black liquors [61,62], drinking water [63], brewed coffee [62], fine chemicals [62] and many other samples (see Table 2).

Jones and Jandik [64] first used chromate for the determination of eight common inorganic anions: fluoride, carbonate, chloride, nitrite, bromide, nitrate, phosphate and sulfate. In a following study, [65] they investigated factors that controlled the selectivity of separation and extended the number of analytes separated (Fig. 2). The ionic strength of the BGE was found to have a limited influence on selectivity. Increasing the ionic strength increased the migration times of all the anions due to an accompanying decrease in EOF velocity. The migration order remained essentially the same with the exception of co-migration of sulfate and nitrite anion when the chromate concentration exceeded 7 m*M*. The pH of the BGE (pH 8 to 11) had little effect on the more strongly acidic anions (i.e. pK_a values below 8). Weaker acids such as borate, carbonate and phosphate decreased in migration time with increasing pH due to a corresponding increase in ionization, and hence mobility. The concentration of the EOF modifier (TTAB) had pronounced effects on the relative migration times for bromide, sulfate and nitrate, but little influence for the rest of the anions. The authors speculated that ion-pairing of these anions with the EOF modifier caused this effect.

Buchberger and Haddad [66] have reported that the migration order of inorganic anions was strongly influenced by the addition of organic solvents to the chromate BGE. A general increase in the migration time of all anions occurred due to a decrease in the electrical conductivity of the BGE, as well as slower electro-osmotic velocity because less of the EOF modifier was adsorbed onto the capillary wall. The resolution of the highly mobile ions thiosulfate, bromide and chloride decreased with increasing organic solvent concentration. The relative migration time of nitrite also increased with higher organic solvent concentrations, reversing the order of migration of nitrate and nitrite. The same authors [66] also investigated the effect of the alkyl chain length of the EOF modifier. Changes in the peak order were observed for the ions thiosulfate, iodide and thiocyanate when the alkyl chain length was sequentially increased from C12 to C16. The mechanism for this behavior was unclear, although the authors specu-

Table 1
Physical data for probes

Probe	Mobility			Molar Absorptivity				pK_a	
	10^{-9} m^2 V^{-1} s^{-1}	pH	Ref.	l mol^{-1} cm^{-1}	nm	pH		pK_a	Ref.
Adenosine diphosphate	n/a	n/a	n/a	9 200	259	7.8	[25]	n/a	n/a
Adenosine monophosphate	n/a	n/a	n/a	9 335	n/a	n/a	[25]	n/a	n/a
p-Aminobenzoate	−28.5	11	[97]	n/a	n/a	n/a	n/a	4.94	[97]
p-Aminosalicylate	−30.1	n/a	[97]	n/a	n/a	n/a	n/a	3.25	[97]
Anisate	−30	Inf Dil	[108]	n/a	n/a	n/a	n/a	n/a	n/a
Benzoate	−26.7	6.5	[79]	44 480	194	6.5	[79]	4.21	[79]
	−33.6	Inf Dil	[21]	11 900	228	6.5	[48]		
	−29.0	11	[97]	809	254	8.0	[2]		
1,3-Benzenedisulfonate	−54.3	8.05	[31]	9 950	214	8.05	[31]	n/a	n/a
o-Benzylbenzoic acid	n/a	n/a	n/a	19 000	228	6.5	[48]	n/a	n/a
Chlorophenol red	−22.1	n/a	[54]	28 000	578	6.5	[54]	n/a	n/a
				33 000	578	7.3	[54]		
Chromate	−56.7	6.5	[79]	2 640	254	8.1	[31]	(1) −0.98	[79]
	−81	Inf Dil	[109]	3 180	254	8.0	[2]	(2) 6.5	
p-Cresol	−28.5	12.1	[91]	8 320	236	12.1	[91]	10.26	[91]
Cytidine monophosphate	n/a	n/a	n/a	5 640	271	7.8	[25]	n/a	n/a
1,3-Dihydroxynaphthalene	−29.3	12.1	[91]	14 680	256	12.1	[91]	(1) 9.37	[91]
								(2) 10.9	
2,3-Dihydroxybenzoate	−33	Inf Dil	[33]	n/a	n/a	n/a	n/a	n/a	n/a
3,4-Dimethoxycinnamic acid	n/a	n/a	n/a	27 000	310	n/a	[110]	n/a	n/a
(*N*,*N*′-Dimethylamino)benzoate	−2.49	11	[97]	n/a	n/a	n/a	n/a	6.03	[97]
Guanosine monophosphate	n/a	n/a	n/a	8 600	254	7.8	[25]	n/a	n/a
p-Hydroxybenzoate	n/a	n/a	n/a	10 299	254	8	[2]	(1) 4.48	[86]
								(2) 9.32	
Indigo carmine	−40	n/a	[54]	12 000	620	4.2–7.3	[54]	n/a	n/a
2,6-Naphthalenedicarboxylate	n/a	n/a	n/a	7 667	254	n/a	[111]	n/a	n/a
				10 020	280				
1,5-Naphthalenedisulfonate	−48.1	8.1	[31]	31 000	214	8.1	[31]	n/a	n/a
2-Naphthalenesulfonate	−31.3	Inf Dil	[33]	11 520	206	6	[26]	n/a	n/a
1,3,6-Naphthalenetrisulfonate	−62	8	[31]	31 600	214	8	[31]	n/a	n/a
1-Naphthylacetic acid	−26.3	12.1	[91]	81 100	222	12.1	[91]	4.24	[91]
	−24.8	12.1	[92]						
	−25.5	7.25	[17]						
Nicotinoate	−30.9	11	[97]	n/a	n/a	n/a	n/a	4.82	[97]
Phenylacetic acid	−30	12.1	[91]	7 600	209	12.1	[91]	4.31	[91]
Phthalate	−48	8.1	[31]	9 950	214	8.1	[31]	(1) 2.95	[79]
	−41.2	6.5	[79]	37 160	196	6.5	[79]	(2) 5.41	
	−55	Inf Dil	[109]	1 357	254	8.0	[2]		
Picrate	−31.5	Inf Dil	[33]	n/a	n/a	n/a	n/a	n/a	n/a
2,6-Pyridinedicarboxylate	−41.5	6.5	[79]	43 680	192	6.5	[79]	(1) 2.16	[79]
								(2) 6.92	
Pyromellitate	−55.1	8.0	[31]	26 200	214	8.0	[31]	(1) 1.92	[79]
	−52.8	6.5	[79]	23 900	214	6.5	[79]	(2) 2.87	
				7 062	254	8.0	[2]	(3) 4.49	
								(4) 5.63	
Riboflavin	n/a	n/a	n/a	30 000	267	n/a	[110]	n/a	n/a
Salicylate	−37	Inf Dil	[109]	n/a	n/a	n/a	n/a	2.94	[97]
	−32.9	11	[97]						

Table 1 (*continued*)

Probe	Mobility			Molar Absorptivity				pK_a	
	10^{-9} m^2 V^{-1} s^{-1}	pH	Ref.	l mol^{-1} cm^{-1}	nm	pH		pK_a	Ref.
Sorbate	−29.6	12.1	[91]	24 120	254	12.1	[91]	4.77	[91]
	−28.9	12.1	[92]	28 800	254	12.1	[92]		
	−28.1	11	[97]						
2-Sulfobenzoic acid	n/a	n/a	n/a	40 000	228	6.5	[48]	n/a	n/a
p-Toluenesulfonate	−18.9	6	[26]	7 520	221	6.0	[26]	n/a	n/a
				344	254	8.0	[2]		
Trimellitate	−47.3	6.5	[79]	7 147	254	8	[2]	(1) 2.52	[79]
	−63.9	7.25	[17]					(2) 3.84	
								(3) 5.20	
Tryptophan	−21.8	12.1	[92]	5 630	280	12.1	[92]	n/a	n/a
Uridine monophosphate	n/a	n/a	n/a	7 240	261	7.8	[25]	n/a	n/a

Mobilities marked as Inf Dil are limiting mobilities, and mobilities at a given pH are effective mobilities.

Table 2
Typical examples of chromate as a probe for indirect absorbance detection[a]

Analytes	BGE	Detection wavelength (nm)	LOD	Matrix	Ref.
Acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	5 m*M* chromate, 0.5 m*M* TTAB, pH=8.0	220, 240, 265	n/a	Beverages	[86]
C2–C14 fatty acids	5 m*M* chromate, Tris, 30% THF, pH=8.2	254	1–2.5 μ*M*	Cocoa oilextract	[33]
Arsenate, arsenite, ascorbate, bromide, carbonate, chloride, citrate, fluoride, nitrate, nitrite, oxalate, phosphate, sulfate	5 m*M* chromate, 0.5 m*M* TTAB, pH=8.10	254	n/a	Urine	[57]
Acetate, carbonate, chlorate, citrate, fluoride, formate, malonate, oxalate, phosphate, succinate, sulfate, tartrate	5.5–7.5 m*M* chromate, 3–5 m*M* TTAB, 1–3 m*M* DTAB, pH=9.1	254	0.1–0.4 μg/ml	Bayer liquor	[59]
Ethyl methylphosphonic acid, isopropyl methylphosphonic acid, methylphosphonic acid, pinacolyl methylphosphonic acid	4.5 m*M* chromate, 1.0 m*M* sodium hydrogencarbonate, 0.5 m*M* TTAB, pH=9.2	254	n/a	Groundwater	[29]
Citrate, diethylenetriaminepentacetic acid, ethylenediaminetetraacetic acid, nitrilotriacetic acid, phosphate, pyrophosphate, tripolyphosphate	5.0 m*M* potassium chromate, 0.05 m*M* CTAB, pH=3.5	260	20–50 μ*M*	Commercial detergent	[112]
Bromide, chlorate, chloride, fluoride, nitrate, sulfate	7.5 m*M* potassium chromate, 0.2 m*M* TTAOH, pH=8.5	211, 254	n/a	Silicon wafer surfaces	[109]
Acetate, chloride, citrate, fumarate, nitrate, phosphate, sulfate	5.0 m*M* chromate, 0.4 m*M* TTAB, pH=8	254, 185	150–320 ng/ml	Vitamin tablet	[113]
Bromide, chloride, fluoride, nitrate, nitrite, phosphate, sulfate	4 m*M* chromate, 0.3 m*M* TTAB, pH=8.1	254	n/a	Tapwater, wellwater, industrial wastewate, power plant waste water	[63]
Chloride, fluoride, monofluorophosphate, nitrate, phosphate, sulfate, tungstate	10 m*M* sodium chromate, 0.1 m*M* CTAB, pH=9.37	254	0.1–0.4 μg/ml	Toothpaste	[114]
Acetate, bromide, butyrate, chloride, formate, nitrate, nitrite, oxalate, propionate, sulfate	5.0 m*M* chromate, 0.5 m*M* TTAB pH=8	254	100–200 ng/ml	Atmospheric aerosols	[40]

CTAB=cetyltrimethylammonium bromide, THF=tetrahydrofuran, TTAB=tetradecyltrimethylammonium bromide, TTAOH=tetradecyltrimethylammonium hydroxide.

[a] Other references where chromate has been used as the probe: [2,22,24,31,36,37,41,58,60–62,65–69,74–76,99,101,115–133].

Table 3
Aromatic carboxylate probes

Probe	Analytes	BGE	Detection wavelength (nm)	LOD	Matrix	Ref.
p-Aminobenzoate	Acetate, chloride, nitrate, oxalate, succinate	7.5 m*M* *p*-aminobenzoate, 750 μ*M* barium hydroxide, 100 μ*M* TTAOH, pH=9.4	264, 220	n/a	Rain drops	[134,97]
p-Aminosalicylate	Alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, seine, threonine, tryptophan, tyrosine, valine	5–10 m*M* *p*-aminosalicylate, pH=10–11.2	222–288	n/a	Standard solutions	[97]
Anisate	C2–C14 fatty acids	10 m*M* anisate, 20 m*M* Tris, 0.75 m*M* trimethyl-β-cyclodextrin, pH=8.2	254, 270, 300	1–2.5 μ*M*	Cocoa oil extract	[33]
Benzoate	Inorganic anions, alkyl sulfates, organic acids	10 m*M* benzoate, 0.5 m*M* TTAB, pH=6.0	254	n/a	Kraft black liquor, dental plaque extract, human saliva, butyric acid extract of air filter	[2,3,21,74] [32–34] [48,53,86,97] [135–137]
O-Benzylbenzoic acid	Bromide, chloride, nitrate, sulfate	0.02 M *o*-benzylbenzoic acid, pH=6.5	228	0.3–1.1 fmol	Standard solutions	[48]
2,3-Dihydroxy benzoic acid	C2–C14 fatty acids	10 m*M* 2,3-dihydroxybenzoate, Tris, 30% THF, pH=8.2	254, 270, 300	1–2.5 μ*M*	Cocoa oil extract	[33]
2,4-Dihydroxy benzoic acid	Dodecyl sulfate	5 m*M* 2,5-dihydroxybenzoic acid, 5% methanol, pH=8.1 with NaOH	250	<0.8 mg/l	Stream water	[95]
2,5-Dihydroxy benzoic acid	Chlorate, chloride, perchlorate	0.5 m*M* cerium(III) sulfate, 0.5 m*M* 2,5-dihydroxybenzoic acid, pH=3.4	251, 345	n/a	Standard solutions	[138]
3,5-Dimethoxy benzoate	α-Cyclodextrin, β-cyclodextrin, γ-cyclodextrin	100 m*M* 3,5-dimethoxybenzoate, 100 m*M* Tris, pH=7	254	50 μ*M*	Fermentation broth, urine, plasma, pharmaceutical preparations	[32]
3,4-Dimethoxy cinnamic acid	Fructose, glucose, maltose, sucrose	12 m*M* 3,4-dimethoxycinnamic acid, 63 m*M* lithium hydroxide	310, 267, 256	0.01–0.04 m*M*	Culture media	[110]
(*N*,*N*′-Dimethylamino)-benzoate	Alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5–10 m*M* (*N*,*N*′-dimethylamino)benzoate, pH=10–11.2	222–288	n/a	Standard solutions	[97]
2,4-Dimethyl benzoate	α-Cyclodextrin, β-cyclodextrin, γ–cyclodextrin	100 m*M* 2,4-dimethylbenzoate, 100 m*M* Tris, pH=7	254	50 μ*M*	Fermentation broth, urine, plasma, pharmaceutical preparations	[32]
2,5-Dimethyl benzoate	α-Cyclodextrin, β-cyclodextrin, γ-cyclodextrin	100 m*M* 2,5-dimethyl benzoate, 100 m*M* Tris, pH=7	254	50 μ*M*	Fermentation broth, urine, plasma, pharmaceutical preparations	[32]

(*continued on next page*)

Table 3 (*continued*)

Probe	Analytes	BGE	Detection wavelength (nm)	LOD	Matrix	Ref.
3,5-Dimethyl benzoate	α-Cyclodextrin, β-cyclodextrin, γ-cyclodextrin	100 m*M* 3,5-dimethyl benzoate, 100 m*M* Tris, pH=7	254	50 μ*M*	Fermentation broth, urine, plasma, pharmaceutical preparations	[32]
3,5-Dinitrobenzoate	Caprylate, laurate, myristate, oleate, palmitate, stearate	5 m*M* 3,5-dinitrobenzoate, 10 m*M* phosphate, pH=8.0, 50% *n*-propanol	200, 254	6 μM	Human stratum corneum	[30,139]
p-Hydroxybenzoate	Bromide, butanesulfonate, carbonate, chloride, citrate, ethanesulfonate, hexanesulfonate, molybdate, nitrate, nitrite, pentanesulfonate, phthalate, propanesulfonate, sulfate, tungstate	5.0 m*M* *p*-hydroxybenzoate, 0.5 m*M* TTAB, pH=9.37	254	n/a	Standard solutions	[133,140]
2,6-Naphthalenedicarboxylate	Acetate, adipate, azelate, benzoate, butyrate, carbonate, chloroacetate, dichloroacetate, formate, fumarate, glutarate, methanesulfonate, phthalate, pimelate, propionate, sebacate, suberate	2 m*M* 2,6-naphthalenedicarboxylate, 0.5 m*M* TTAB, pH=8.1 1 with NaOH	280	20 μg/ml	Atmospheric aerosols	[111,40,141]
Naphthoate	C_2–C_{14} fatty acids	10 m*M* naphthoate, Tris, 30% THF, pH=8.2	254, 270, 300	1–2.5 μ*M*	Cocoa oil extract	[33]
1-Naphthylacetic acid	Carbohydrates	0.1-2 m*M* naphthylacetic acid, pH=12.2	222	0.1 m*M*	Fruit juice, dairy products	[91,17,142]
Nicotinoate	Alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	5-10 m*M* nicotinoate, pH=10-11.2	222-288	n/a	Standard solutions	[97]
Phenylacetic acid	Carbohydrates pH=12.2	0.1 m*M* phenylacetic acid,	222	0.1 m*M*	Fruit juice, dairy products	[91]
Phthalate	Adipate, alpha-ketoglutarate, citrate, ethylmalonate, glutarate, lactate, malonate, methylmalonate, methylsuccinate, oxalate, pyruvate, succinate, tartrate	5 m*M* carbonate, 1.5 m*M* phthalate, 0.15m*M* MTAB, pH9.0	230	60–360 pg	Human serum and urine	[2,22,78] [28,31,40] [48,71,74] [82,86] [102,104,107] [143,144] [145–147]
2,6-Pyridine-dicarboxylate	Acetate, bromide, butyrate, citrate, formate, heptanoate, hexanoate, iodide, lactate, malate, nitrate, octanoate, oxalate, pyruvate, succinate, tartrate, valerate	5 m*M* 2,6-pyridinedicarboxylate, 0.5 m*M* CTAB, pH=5.65	214	1–2.5 mg/l	Beer	[79]

Table 3 (*continued*)

Probe	Analytes	BGE	Detection wavelength (nm)	LOD	Matrix	Ref.
Pyromellitate	Arsenate, bromate, bromide, chlorate, chloride, citrate, fluoride, formate, malate, malonate, molybdate, nitrate, nitrite, oxalate, perchlorate, phosphate, sulfate, sulfosuccinate, tartrate, tetrafluoroborate, thiocyanate, thiosulfate, tungstate	2.25 m*M* pyromellitate, 6.5 m*M* sodium hydroxide, 1.6 m*M* TEA, 0.75 m*M* HMB, pH=7.7	250	0.2–1.0 ng/ml emi	Silicon wafer surfaces	[42,2,31] [40,41,43] [44,71,72] [77,81,86]
Salicylate	Ascorbate, carbonate, chloride, citrate, formiate, fumarate, glutarate, lactate, malate, nitrate, oxalate, phosphate, pyruvate, succinate, sulfate	7.5 m*M* salicylic acid, 15 m*M* Tris, 1 m*M* calcium hydroxide, 0.5m*M* DoTAOH	232	0.2–2 μM	Plants	[6] [38,94] [97,142]
2-Sulfobenzoic acid	Bromide, chloride, nitrate, sulfate	0.02 *M* 2-sulfobenzoic acid, pH=6.5	228	0.3–1.1 fmol	Standard solutions	[48]
5-Sulfosalicylic acid	Heparin fragments	5 m*M* 5-sulfosalicylic acid pH=3.0	214	5 fmol	Synthetic heparin fragments	[148]
Terephthalic acid	Acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	5 m*M* terephthalate, 0.25 m*M* TTAB, pH=9.0	220, 240, 265	n/a	Beverages	[86]
1,2,4-Tricarboxybenzoic acid	Heparin fragments	5 m*M* 1,2,4-tricarboxybenzoic acid, pH=3.5	214	5 fmol	Synthetic heparin fragments	[148]
Trimellitate	Acetate, ascorbate, citrate, glucanate, glutamate, lactate, malate, oxalate, tartrate	5 m*M* trimellitate, 1 m*M* TTAB, pH=9.0	220, 240, 265	n/a	Beverages	[86,2,17] [39,98,149]

The experimental conditions are those listed for the first cited reference.
DoTAOH=dodecyltrimethylammonium hydroxide, HMB=hexamethonium bromide, MTAB=myristyltrimethylammonium bromide, TEA=triethanolamine, Tris=tris(hydroxymethyl)aminoethane. Other abbreviations given in Table 2.

lated that the most probable cause was an ion interaction phenomenon between these anions and the EOF modifier. A further observation was that the average migration time of the anions decreased with increasing chain length of the EOF modifier.

Benz and Fritz [67] added 1-butanol to the chromate BGE to aid in the reversal of the EOF. In previous studies [64–66] concentrations of the EOF modifier of 0.3 m*M* or more were found to be required to reverse the EOF. However, addition of 1-butanol up to 5% (v/v) reduced the required amount of modifier by a factor of 10. The authors report that separations using this approach exhibited less noise and greater reproducibility.

Harakuwe et al. [68] adjusted the selectivity of separation of inorganic anions with the chromate BGE by utilizing binary surfactant mixtures, namely TTAB and dodecyltrimethylammonium bromide (DTAB). Adjusting the ratios of TTAB:DTAB was found to be a useful means to fine tune the separation. In a following study Haddad et al. [59] optimized the separation of inorganic and organic anions present in Bayer liquors. They reported that two optimal TTAB:DTAB ratios existed in which most of the components of the Bayer liquor were separated, a result which was not achievable with the use of a single EOF modifier.

Although the separation selectivity has been studied extensively, most studies using the chromate electrolyte have involved the electrolyte being prepared from the sodium salt and therefore unbuffered. A number of publications have attempted to buffer the chromate electrolyte by the addition of a co-anionic buffer such as borate [24,41,69], sodium carbonate [29], and 2-(cyclohexylamino)-ethanesulfonic acid (CHES) [70]. Additions of such buffering

Table 4
Aromatic sulfonate probes

Probe	Analytes	BGE	Detection wavelength (nm)	LOD	Matrix	Ref.
1,3-Benzene-disulfonate	Sulfate, sulfide, tetrathionate, thiosulfate	3 m*M* 1,3-benzenedisulfonate, 10 m*M* Tris, 0.5 m*M* DETA, pH=8.15	254, 214	2–4 μ*M*	Natural water	[31]
1,5-Naphthalene-disulfonate	Sulfate, sulfide, tetrathionate, thiosulfate	3 m*M* 1,5-naphthalenedisulfonate, 10 m*M* Tris, 0.5m*M* DETA, pH=8.15	254, 214	2–4 μ*M*	Natural water	[31,23,73]
2-Naphthalene-sulfonate	Inorganic anions, alkyl sulfates, organic acids	10 m*M* naphthalenesulfonate, 30% acetonitrile, pH=10.0	254	n/a	Kraft black liquor, dental plaque extract, human saliva, butyric acid extract of air filter	[74,26] [33,73,91]
1,3,6-Naphthalene-trisulfonate	Sulfate, sulfide, tetrathionate, thiosulfate	2 m*M* 1,3,6-naphthalenetrisulfonate, 10 m*M* Tris, 0.5 m*M* DETA, pH=8.15	254, 214	2–4 μ*M*	Natural water	[31,73]
1-Naphthol-3,6-disulfonic acid	Inositol phosphates	0.5 m*M* 1-naphthol-3,6-disulfonic acid, 30 m*M* acetic acid, 0.01% hydroxypropylmethylcelluose	214	4–22 μ*M*	Fermentation broths	[93]
1-Nitroso-2-naphthol-3,6-disulfonate	Acetate, butyrate, chloride, clodronate, fluoride, hippurate, isovalerate, malonate, nitrate, nitrite, oxalate, phosphate, phosphite, propionate, pyruvate, sulfate	0.5 m*M* 1-nitroso-2-naphthol-3,6-disulfonate, pH=8	254	$2\cdot10^{-8}$–$9\cdot10^{-7}$ *M*	Standard solutions	[150]
1-Nitroso-2-naphthol-6-sulfonate	Acetate, butyrate, chloride, clodronate, fluoride, hippurate, isovalerate, malonate, nitrate, nitrite, oxalate, phosphate, phosphite, propionate, pyruvate, sulfate	0.5 m*M* 1-nitroso-2-naphthol-6-sulfonate, pH=8	254	$2\cdot10^{-8}$–$9\cdot10^{-7}$ *M*	Standard solutions	[150]
2-Nitroso-1-naphthol-6-sulfonate	Acetate, butyrate, chloride, clodronate, fluoride, hippurate, isovalerate, malonate, nitrate, nitrite, oxalate, phosphate, phosphite, propionate, pyruvate, sulfate	0.5 m*M* 2-nitroso-1-naphthol-6-sulfonate, pH=8	254	$2\cdot10^{-8}$–$9\cdot10^{-7}$ *M*	Standard solutions	[150]
p-Toluenesulfonate	Anion and cation surfactants	5 m*M* pyridine, 5 m*M* *p*-toluenesulfonate, 0% and 50% methanol, 100 m*M* borate, pH=6.0	220	n/a	Standard solutions	[26]
Trinitrobenzene-sulfonic acid	C_1–C_{18} fatty acids	10 m*M* trinitrobenzenesulfonic acid, 60% acetonitrile, pH=9.0	214, 254	n/a	Fat hydrolysates of butter and palm oil	[139]

The experimental conditions are those listed for the first cited reference. DETA = diethylenetriamine. Other abbreviations as for Tables 2 and 3.

agents have the potential to interfere with analytes of interest due to inducement of system peaks and competitive displacement of the probe and the buffering co-anion. A far superior approach to buffer the chromate BGE is to utilize a buffering counter-cation such as Tris or diethanolamine (DEA) [11,70].

Pyromellitic acid (PMA) has been used as an alternative probe for the analysis of fast inorganic anions [2,31,41–43,71,72]. Evaluations of PMA have concluded that the sensitivity is about four times lower than when chromate is used as the probe [41]. Furthermore, many EOF modifiers form insolu-

Table 5
Miscellaneous probes

Probe	Analytes	BGE	Detection wavelength (nm)	LOD	Matrix	Ref.
Adenosine monophosphate	Polyphosphates and polyphosphonates	5 m*M* adenosine monophosphate, 5 m*M* sodium tetraborate, 100 m*M* borate, 2 m*M* DETA, pH 7.8	259, 261	1.2–11 μ*M*	Toothpaste, commercial herbicide	[25,96]
Bromocresol green	Pyruvate	0.5 m*M* bromocresol green, pH=8.0	633	$2\cdot10^{-16}$ mol	Standard solutions	[47]
Chlorophenol red	Bromide, chloride, citrate, fumarate, glutamate, malonate, nitrate, sulfate	0.5 m*M* chlorophenol red, 5 m*M* Tris, pH=6.0 with acetic acid	578, 620, 635	$2\cdot10^{-13}$–$8\cdot10^{-16}$ mol	Standard solutions	[54]
p-Cresol	Carbohydrates	0.1 m*M* *p*-cresol, pH=12.2	222	0.1 m*M*	Fruit juice, dairy products	[91]
Diethylbarbiturate	Acetate, fatty acids, butyrate, formate, lactate, malonate, propionate	5 m*M* diethylbarbiturate, 0.5 *M* Z1-Methyl, 70% ethyleneglycol monomethyl ether	240, 254	n/a	Butter, standard solutions	[80,87]
1,3-Dihydroxy-naphthalene	Carbohydrates	0.1 m*M* 1,3-dihydroxynaphthalene, pH=12.2	222	0.1 m*M*	Fruit juice, dairy products	[91]
Histidine	Acetate, benzoate, chloride formate nitrate	20 m*M* histidine, 0.5 m*M* butyric acid, 0.5 m*M* hydrochloric acid, pH=6.1	214	n/a	Standard solutions	[15]
Indigo carmine	Bromide, chloride, citrate, fumarate, glutamate, malonate, nitrate, sulfate	0.5 m*M* indigo carmine, 5 m*M* Tris, pH=6.0 with acetic acid	578, 620, 635	$2\cdot10^{-13}$–$8\cdot10^{-16}$ mol	Standard solutions	[54]
Iodide	Bromide, chloride, nitrate, nitrite, sulfate, thiosulfate	7.5 m*M* sodium iodide	254, 226, 270	n/a	Standard solutions	[75]
Permanganate	Chlorate, chloride, fluoride, nitrate, sulfate	20 m*M* permanganate, pH=7.0	635, 670	3.5 μ*M*	Standard solutions	[162]
Phenylphosphonic acid	Butylphosphonic acid, ethylphosphonic acid, methylphosphonic acid, propylphosphonic acid	10 m*M* phenylphosphonic acid, 200 m*M* sodium borate, pH=6.0	200	0.15–0.21 pmol	Standard solutions	[27]
Riboflavin	Fructose, glucose, maltose, sucrose	12 m*M* riboflavin acid, 63 m*M* lithium hydroxide	310, 267, 256	0.01–0.04 m*M*	Culture media	[110]
Sorbate	D-Fucose, D-qalactose, D-glucose, D-glucuronic acid, galacturonate, *N*-acetyl-galactosamine, *N*-acetyl-glucosamine, *N*-acetyl-neuraminic acid	6 m*M* sorbate, pH=12.2	256	0.23–0.29 m*M*	Fruit juice	[103,53,89,90,97] [110,133,142,163]
Tiron	Chloride, fluoride, iodide, nitrate, perchlorate, sulfate	5 m*M* tiron	293	n/a	Sea water, standard solutions	[164]
Tryptophan	Carbohydrates	1.0 m*M* tryptophan, 50 m*M* NaOH	280	n/a	Standard solutions	[92]
Uridine monophosphate	Polyphosphates and polyphosphonates	5 m*M* uridine monophosphate, 5 m*M* sodium tetraborate, 100 m*M* borate, 2 m*M* DETA, pH 7.8	259, 261	1.2–11 μ*M*	Toothpaste, commercial herbicide	[25]
Vanadate	Bromide, chloride, nitrate, nitrite, sulfate, thiosulfate	7.5 m*M* sodium vanadate	254, 226, 270	n/a	Standard solutions	[75]

The experimental conditions are those listed for the first cited reference.
Z1-methyl=Waters proprietary zwitterion. Other abbreviations as for Tables 2–4.

Table 6
Fluorescent probes

Probe	Analytes	BGE	Detection wavelength (nm)	LOD	Matrix	Ref.
Coumarin	D-Arabinose, D-fructose, D-glucose, D-xylose, lactose, maltose, raffinose, sucrose	1 m*M* coumarin, pH=11.65	Ex: 488 Em: 640	2 fmol	Standard solutions	[151]
2,5-Dihydroxybenzoic acid	Bromate, carbonate, chloride, methanesulfonate, nitrate, oxalate, sulfate	1 m*M* 2,5-dihydroxybenzoic acid, 0.5 m*M* lead acetate	Ex: 314 Em: 389	0.2–0.5 μ*M*	Standard solutions	[152]
Fluorescein	2,4,6-Trichlorophenol, 2,4-chlorophenol, 2,4-dimethylphenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-chloro-3-methylphenol, 4-nitrophenol, pentachlorophenol	1 m*M* fluorescein, 15 m*M* sodium borate, pH=9.9	Ex: 488 Em: 520	<1 μ*M*	Industrial waste waters	[153–155] [156,157]
Salicylate	Farnesyl pyrophosphate, geranyl pyrophosphate, geranylgeranyl pyrophosphate, isopentenyl pyrophosphate	1 m*M* salicylic acid, pH=4.0, 5.0, 6.5	Ex: 325 Em:–	0.5 μ*M*	Standard solutions, rat liver	[107,105,106,158,159]
Terbium(lll)-acetylacetone	Chromate, hexacyanoferrate(III), nitrite	0.8 m*M* acetylacetone, 0.35 m*M* terbium (lll) chloride, 6 m*M* sodium chloride, pH=8.3	Ex: 295 Em: 545	0.1 μ*M*	Standard solutions	[160]
2-*p*-Toluidino-naphthalene-6-sulfonate	Dodecyl sulfate	0.1 m*M* 2-*p*-toluidino-naphthalene-6-sulfonate, 10 m*M* sodium hydrogen phosphate, 6.0 m*M* sodium tetraborate	Ex: 325 Em: 450	n/a	Soap	[161]

The experimental conditions are those listed for the first cited reference.

ble precipitates with PMA, so it is necessary to use agents that only suppress rather than reverse the EOF, such as hexamethonium bromide and diethylenetriamine. This results in generally longer migration times. Shamsi and Danielson [73] investigated naphthalene sulfonates as probes for the analysis of inorganic anions. They concluded that naphthalenedisulfonic acid was applicable for the analysis of inorganic anions, and had the advantage over the chromate probe of larger analyte mobility ranges. The probe was suitable for the analysis of organic acids as well as alkyl sulfonic acids, and attained similar detection sensitivities as chromate. However, the peak shapes of the very fast inorganic anions were not as good as those attained with chromate. Other probes used successfully for the analysis of inorganic anions include phthalate [74], and trimellitate [2], however these probes are more suited for the analysis of lower mobility organic acids. Therefore, at the present time, chromate remains the most suitable probe for the analysis of inorganic anions.

A number of publications have described the successful separation of highly mobile inorganic anions without the addition of EOF modifiers. The absence of an EOF modifier in the BGE is advantageous as precipitates can occur between the cationic surfactant and the probe, especially at lower pH [11]. Tindall and Perry [75] utilized commercially available silane-coated capillaries. They found that column life was limited by the stability of the coating, which was poor at high or low pH. Lamb et al. [76] evaluated polypropylene capillaries. The EOF of these capillaries was much lower than bare fused-silica capillaries. They concluded that the migration times of inorganic anions at high pH were low enough for practical use. Burt et al. [77], used a reactive polyamide resin to provide a permanent positive coating inside the capillary. Although the

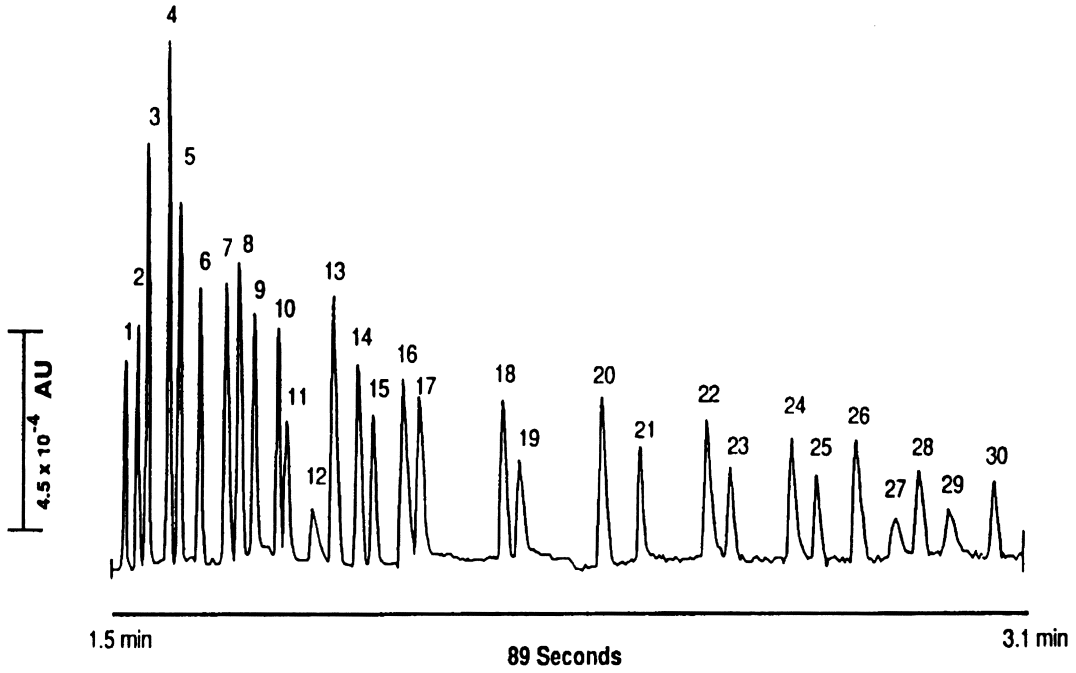

Fig. 2. Separation of anions. Conditions: BGE 5m*M* chromate, 0.5 m*M* TTAB, pH=8.0. Key: 1=thiosulfate, 2=bromide, 3=chloride, 4=sulfate, 5=nitrite, 6=nitrate, 7=molybdate, 8=azide, 9=tungstate, 10=monofluorophosphate, 11=chlorate, 12=citrate, 13=fluoride, 14=formate, 15=phosphate, 16=phosphite, 17=chlorite, 18=galactarate, 19=carbonate, 20=acetate, 21=ethanesulfonate, 22=propionate, 23=propanesulfonate, 24=butyrate, 25=butanesulfonate, 26=valerate, 27=benzoate, 28=L-glutamate, 29=pentanesulfonate, 30=D-glucanate. Reprinted from Ref. [65] with permission.

authors could successfully analyze inorganic anions, the stability of the coating was a problem. Furthermore, the reproducibility of migration time of the analytes from capillary to capillary was poor. Therefore, the addition of EOF modifiers to the BGE and the use of bare silica capillaries is still the method of choice.

8. Determination of organic acids

Organic acids are another class of anions that are often determined by CE and detected indirectly. Applications of this method include the analysis of Kraft black liquor [74], Bayer liquor [6], urine [78], and beverages [79]. The following discussion outlines some of the more important aspects, as organic acid analysis by CE has been reviewed elsewhere [80].

Manipulation of BGE pH is one method for the control of separation selectivity. A typical example was performed by Arellano et al. [81] for the determination of oxalate, tartrate, malate, succinate, citrate, acetate and lactate using pyromellitate as the probe. Baseline resolution of all of the organic acids was achieved by finding an optimum pH. The concentration of the EOF modifier had little effect on the selectivity of separation between 1 and 4 m*M*, and above this concentration caused the co-migration of tartrate, succinate and malate.

Another method for manipulation of separation selectivity is the addition of complexing agents to the BGE [38,82–85]. Lalljie et al. [82] added calcium to the BGE to monitor formate, tartrate, succinate, malate, glycolate, and acetate levels during sugar refinement processes. Manipulation of the BGE pH achieved the separation of all of the organic acids with the exception of formate and tartrate. The addition of calcium allowed resolution of these two analytes due to complex formation, which altered the mobility of the organic acids.

Wu et al. [86] developed methods for the de-

termination of oxalic, citric, tartaric, malic, succinic, lactic, aspartic, glutamic, ascorbic and gluconic acids using indirect absorbance detection. Chromate, pyromellitate, trimellitate, phthalate, terephthalate and benzoate were investigated as potential probes for this analysis, of which trimellitate was found to be the most suitable. This probe provided the best mobility match to the analytes and the highest molar absorptivity. The selectivity of the separation was found to be strongly dependent on the pH of the BGE, particularly for the diprotic and triprotic acids. Migration time relative standard deviations (RSDs) of less than 1%, and peaks area RSDs of less than 3% were obtained with the exception of citrate, which had a peak area RSD of around 4%. The authors gave no explanation for the higher RSD of citrate, but a study by Soga and Ross [79] reported that when phthalate or trimellitate were used as probes, neither citrate nor oxalate could be observed below a concentration of 50 mg/l (see Fig. 3). Furthermore, there was a reduction in the signal of malate. The authors demonstrated that the reduction in signal was due to adsorption of the organic acids onto the capillary wall, which contained metal

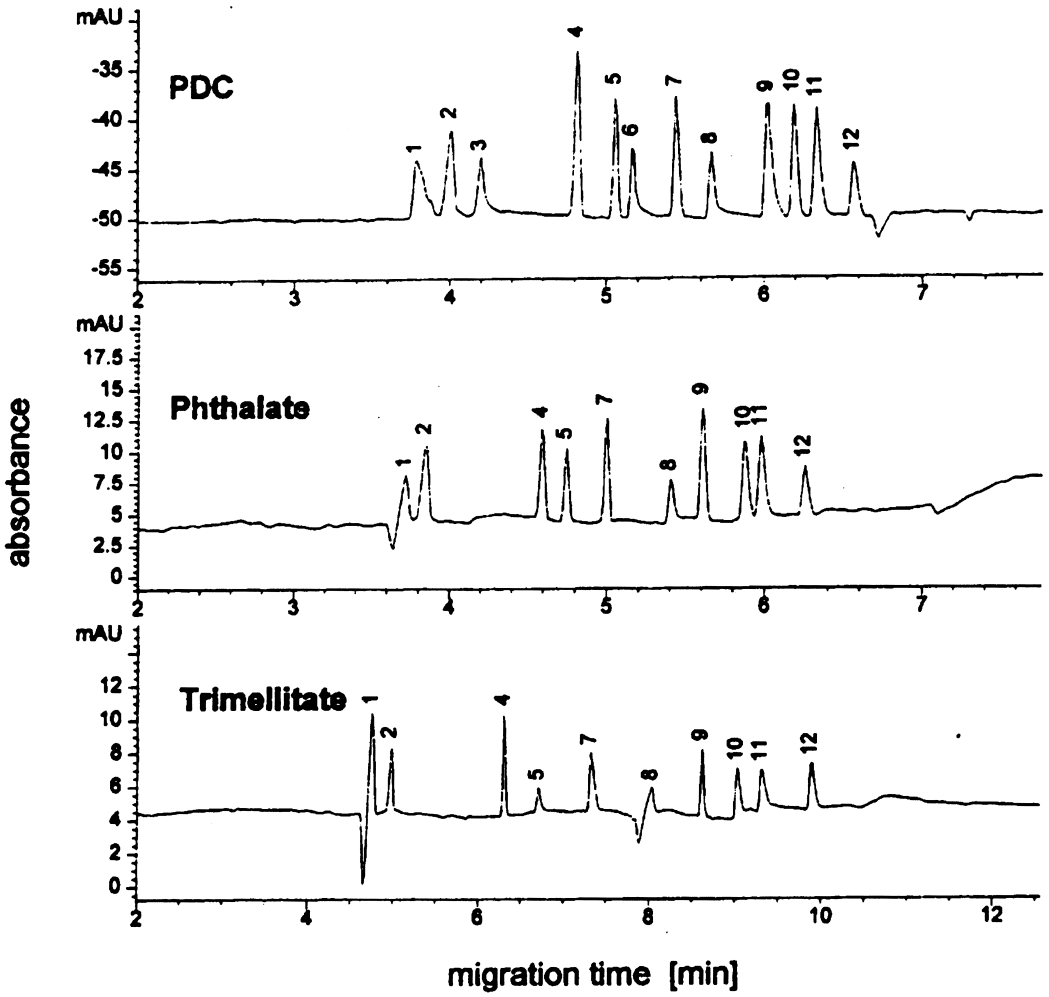

Fig. 3. Comparison of electropherograms obtained with 2,6-pyridendicarboxylic acid (PDC), phthalate and trimellitate. Key: 1=chloride, 2=sulfate, 3=oxalate, 4=formate, 5=malate, 6=citrate, 7=succinate, 8=pyruvate, 9=acetate, 10=lactate, 11=phosphate, 12=pyroglutamate. Reprinted from Ref. [79] with permission.

impurities. The adsorption problem was overcome by flushing the capillary with phosphoric acid before each run, however a large phosphate peak appeared in the electropherograms and interfered with detection of phosphate and lactate. A better alternative was to use 2,6-pyridendicarboxylic acid as the probe, which had the ability to complex with the metal impurities on the capillary wall, thereby preventing adsorption of the organic acids.

Fatty acids are another class of compounds that have been determined by CE and detected indirectly as an alternative to gas chromatographic techniques. Buchberger and Winna [87] determined short chain and long chain free fatty acids in a counter electroosmotic mode (different directions of electroosmotic and electrophoretic mobility). Solubility problems of the fatty acids were overcome by the addition of up to 70% ethyleneglycolmonomethylether in the BGE. The fatty acids were noted to adsorb to the capillary wall under the conditions investigated, which was overcome by addition of 0.5 *M* trimethylammonium propanesulfonate. This zwitterionic agent is well known to avoid adsorption of proteins to the capillary wall [88], so presumably a similar mechanism of suppression of adsorption was occurring for the fatty acids. The developed method was applied to the analysis of free fatty acids in butter, but lacked the resolving power that can be achieved by gas chromatography, making it applicable only to relatively simple fatty acid mixtures. However, the method has the advantage of very simple sample preparation.

Roldan-Assad and Gareil [165] successfully determined C2–C14 fatty acids using a purely aqueous BGE and *p*-anisate as the probe. The solubility problems of the higher chain length fatty acids were overcome by addition of trimethyl-β-cylcodextrin to the BGE. The range of fatty acids was increased to up to a C18 homologue with addition of 60% methanol. The analyte solubility was enhanced through inclusion of the alkyl chain of the acid in the cyclodextrin cavity. Furthermore, addition of the cyclodextrin derivative to the BGE enhanced the separation selectivity since the stability constant for the inclusion reaction increased with increasing chain length of the acid. The researchers did not mention adsorption difficulties, so presumably the inclusion complexation was responsible for the minimization of adsorption effects.

9. Determination of carbohydrates

The determination of carbohydrates has always been hampered by their lack of UV absorbance above 200 nm. As far as CE is concerned this problem has been overcome by derivatization of the carbohydrates with regents such as 2-amino-pyridine and *p*-aminobenzoic acid [89], or by indirect detection. Carbohydrates are generally weakly acidic, and therefore require very basic conditions to attain any appreciable ionization, usually around pH 12. Vorndran et al. [90] used indirect detection with sorbate as the probe for the analysis of 11 carbohydrates. They investigated the effect of the BGE pH and found that although resolution of the carbohydrates increased with increasing pH, the sensitivity of detection was severely compromised above pH 12.1 due to displacement of hydroxide, rather than sorbate, by the analytes. Therefore, the optimum pH for separation considering resolution and detection sensitivity was 12.1. Klockow et al. [103] used sorbate as the probe for the analysis of carbohydrates in fruit juices and again reported that the optimum was at pH 12.2.

Lee and Lin [91] investigated the performance of six probes, 1-naphthylacetic acid, 2-naphthalenesulfonic acid, 1,3 dihydroxynaphthalene, phenylacetic acid, *p*-cresol and sorbate for the analysis of carbohydrates. They reported that 1-naphthylacetic acid was the best suited probe at pH 12.2, and provided a 3–6 fold improvement in resolution, and 2–5 times improvement in detection sensitivity (0.1 m*M*), when compared to sorbate. Fig. 4 is a typical electropherogram obtained using 1-naphthylacetic acid as the probe. Lu and Westerlund [92], investigated tryptophan as an alternative probe, resulting in an approximate two times improvement in detection sensitivity when compared to sorbate.

10. Determination of phosphates and phosphonates

Phosphates and phosphonates have been determined by CE using indirect detection. Henshall et al. [22] explored the feasibility of utilizing CE for the determination of inositol phosphates in physiological samples without the limitations of long analysis

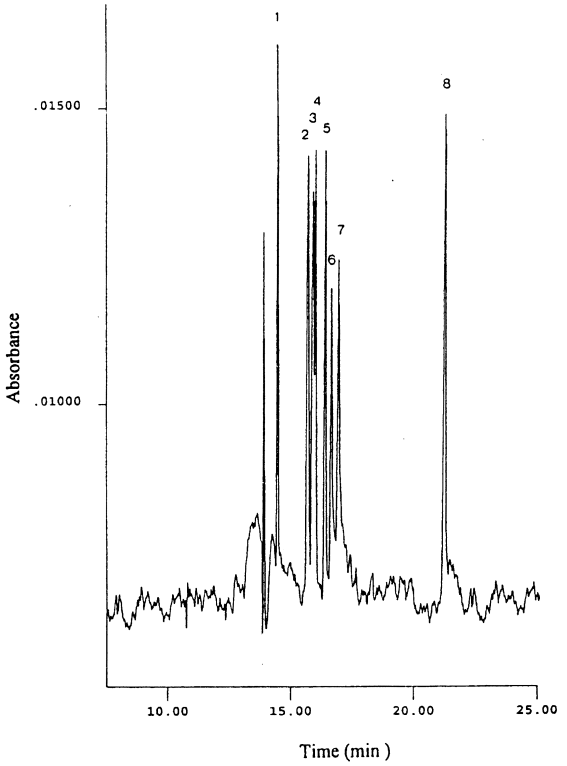

Fig. 4. Electropherogram of a mixture of eight selected monosaccharides. Conditions: BGE 2 m*M* 1-naphthyl acetic acid, pH = 12.2. Separation voltage +25 kV, detection wavelength 222 nm, temperature 25°C. Key: 1 = sucrose, 2 = lactose, 3 = maltose, 4 = glucose, 5 = rhamnose, 6 = fructose, 7 = ribose, 8 = gluconic acid. Reprinted from Ref. [91] with permission.

times and pre- or post-column derivatization. The authors used phthalate and dichromate as probes. The dichromate probe was unsuitable due to shifts in migration time, but acceptable results were obtained with phthalate. Buscher and coworkers utilized 1-napthol-3,6-disulfonic acid as the probe for the analysis of six inositol phosphates in a fermentation broth [93] and inositol triphosphate derivatives in plasma [23] as an alternative method to ion chromatography and gas chromatography.

Alkylphosphonic acids represent a class of compounds that are widely used as herbicides and insecticides. Therefore determination of these compounds is of environmental importance. Pianetti et al. [27] developed a CE method for the determination of C1–C4 alkylphosphonic acids utilizing phenylphosphonic acid as the probe. The BGE contained 200

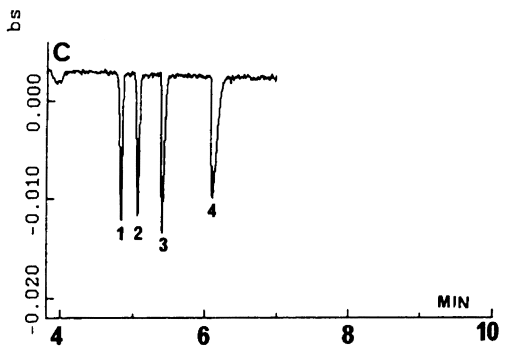

Fig. 5. Electropherogram of alkylphosphonic acids. Conditions: BGE 10 m*M* phenylphosphonic acid, 100 m*M* sodium borate pH = 6.0. Separation voltage +30 kV, detection wavelength 200 nm, temperature 30°C. Key: 1 = butylphosphonic acid, 2 = propylphosphonic acid, 3 = ethylphosphonic acid, 4 = methylphosphonic acid. Reprinted from Ref. [27] with permission.

m*M* sodium borate at pH = 6.0 as the buffering agent. Inspection of the electropherogram (Fig. 5) reveals the apparent absence of system peaks or interference in the detection process by the added co-anion (borate). The most likely explanation for this was the low mobility of borate and the selective displacement of the probe by the analytes. Other phosphonate compounds of environmental concern are glyphosphate and its major metabolite aminomethylphosphonic acid. Glyphosphate is used as a non-selective herbicide. Cikalo et al. [145] developed a method for the analysis of these two compounds using phthalate as the probe. The required detection limits of ng/ml were achieved by electrokinetic injection for standards. However, application of the method to a wheat sample was unsuccessful due to the high conductivity of the sample which inhibited electrokinetic injection.

Shamsi and Danielson [25] investigated ribonucleotides (adenosine, cytidine, guanosine, and uridine monophosphates) as probes for the analysis of polyphosphates and polyphosphonates. Adenosine monophosphate (AMP) was the most suitable probe mainly due to its higher molar absorptivity. Analysis of orthophosphate, pyrophosphate, tripolyphosphate and tetrapolyphosphate was achieved using AMP as the probe and diethylenetriamine to suppress the EOF. The selectivity of separation was manipulated by addition of magnesium to the BGE, which formed complexes with the phosphates and reduced their

mobilities. The developed methods were applied to the analysis of phosphates in toothpaste, and phosphonates in a commercial herbicide.

11. Miscellaneous applications using indirect absorbance detection

Other applications of indirect absorbance detection include the analysis of alkylsulfates and alkylsulfonates [26,94–96], amino acids [97], metallo-cyanide complexes [98], arsonates [99] and cyclodextrins [100,32]. Chen and Pietrzyk [94] separated alkyl sulfonates and sulfates utilizing salicylate as the probe and various BGE counter cations to manipulate selectivity. In general, the use of divalent cations increased the migration times of the analytes to a greater extent than monovalent cations. This effect was due to changes in EOF caused by cation-exchange of the cation BGE additive with the silanol surface of the capillary. Magnesium was found to provide the best overall resolution of the analytes. Shamsi and Danielson again demonstrated the versatility of AMP by utilizing it as the probe for the analysis of anionic surfactants [96]. In another study [26] the same authors determined cationic and anionic surfactants simultaneously with BGEs that contained cationic and anionic probes.

Lee and Lin [97] investigated a number of probes for the analysis of underivatized amino acids, with *p*-aminosalicyclic acid, and 4-(*N*,*N*′-dimethylamino)benzoic acid proving to be the most suitable at high pH (10–11.1). Divalent metal ions were investigated as buffer additives to suppress the EOF, and as in the previous example [94], magnesium provided the greatest gain in resolution of the amino acids. Cationic surfactants (DTAB and TTAB) were also investigated for resolution improvements. DTAB was used to suppress the EOF, allowing more time for the amino acids to separate. TTAB completely reversed the EOF, which had the major advantage of decreasing the analysis time. A typical electropherogram is shown in Fig. 6.

Buchberger and Haddad [98] investigated pyromellitate as probe for the analysis of metallo-cyanide complexes. Although most metallo-cyanide complexes have strong molar absorptivities from 200 to 220 nm and can be detected directly, chromium cyanide has a moderate molar absorptivity. Indirect detection was employed to overcome this problem, but the detection limits for most of the metallo-cyanide complexes were not improved. Therefore in this instance direct detection was a better choice for the analysis of these compounds.

12. Method validation

Although most studies have been concerned mainly with the feasibility of using CE and indirect detection, a number of publications have validated methods for routine use. Grocott et al. [58] validated a CE method for the analysis of oxalate in Bayer liquor using chromate as the probe. Typical RSD values were around 0.3% for replicate analysis and around 1% for batch sampling. These results compared favorably with gas chromatographic procedures.

Dabek-Zlotorzynska and Dlouhy [40] compared the analysis of sulfate in atmospheric aerosols by CE and Ion Chromatography (IC). There was a high degree of correlation between the two methods. The authors concluded that CE is a complementary technique to the recognized method of ion analysis, by providing unique selectivities, which aids in peak confirmation. Oehrle [101] compared the analysis of chloride and sulfate using IC and CE for trace analysis in power plant water, and in sulfonated dyes. The results again indicated good agreement between the two methods, with typical RSD around 1% for CE and less than 0.7% for IC. The CE method had the advantage of shorter run times, and overcame an adsorption problem of the sulfonated dyes on the IC column.

Lagoutte et al. [102] compared the analysis of low molecular weight organic acids in cigarette smoke using IC and HPLC. The HPLC method required derivatization of the organic acids with *p*-bromophenacyl bromide. The RSDs ranged from 6 to 12% for both methods, with the authors concluding that the results from both methods were almost identical. The CE method had distinct advantages over the HPLC method, including simple sample preparation (derivatization was not required for the CE method), speed, low costs due to small solvent consumption, and the use of non-hazardous solvents.

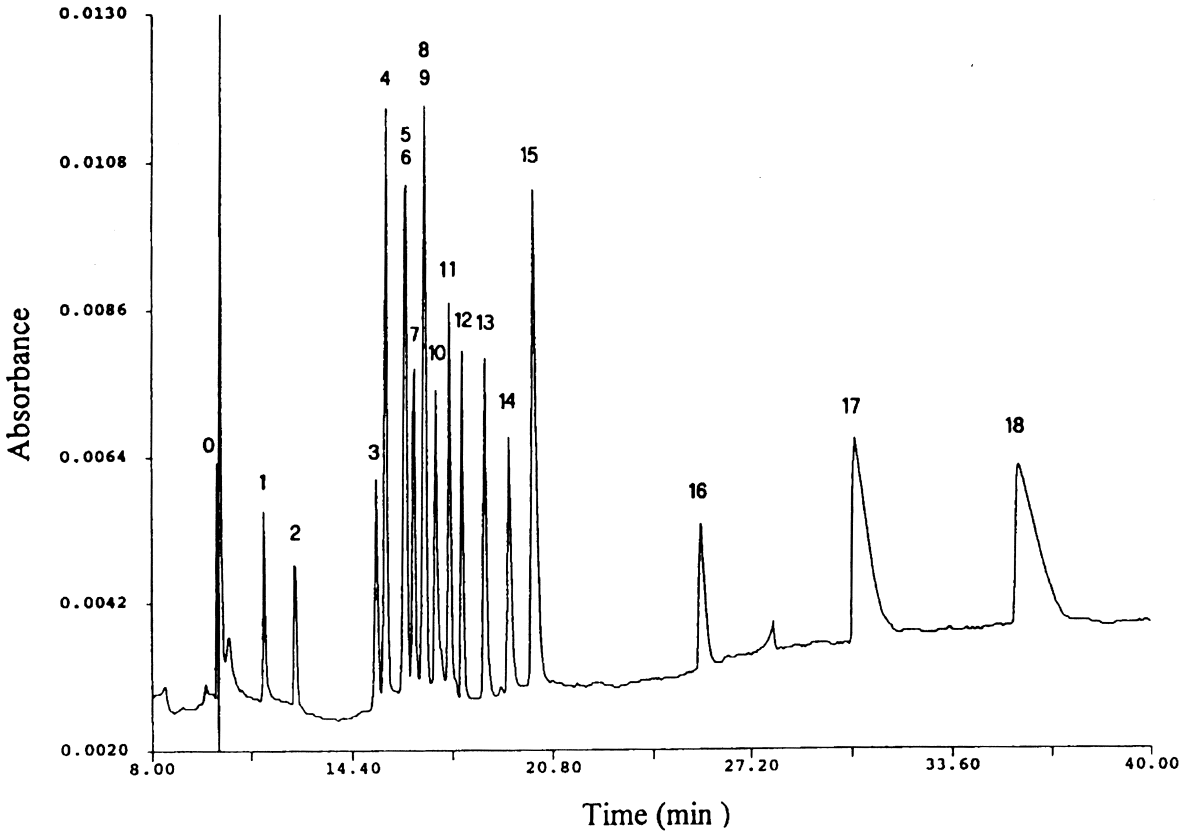

Fig. 6. Electropherograms of common amino acids. Conditions: BGE 10 m*M* *p*-aminosalicyclic acid, 0.25 m*M* DTAB, pH = 10.9. Separation voltage +20 kV, detection wavelength 266 nm, temperature 25°C. Key: 0 = Arg, 1 = Lys, 2 = Pro, 3 = Trp, 4 = Leu, 5 = Phe, 6 = Val, 7 = His, 8 = Met, 9 = Gln, 10 = Ala, 11 = Thr, 12 = Asn, 13 = Ser, 14 = Gly, 15 = Tyr, 16 = Cys, 17 = Glu, 18 = Asp. Reprinted from Ref. [97] with permission.

Klockow et al. [103] compared the analysis of carbohydrates in fruit juices by CE with those from a routine HPLC method. Statistical analysis of the results (*t*-test) demonstrated no significant differences between the two methods, provided internal calibration was used for the CE method. Further, the authors suggested that CE allows the possibility of validating HPLC methods with a second independent analytical method.

Altria et al. [104] validated a method for the analysis of organic acid drug counter-ions. The method provided suitable sensitivity, (l mg l^{-1} LOD), injection precision (0.5% internal standard), robustness, repeatability and accuracy. The method is now in routine use for quality control testing.

13. Indirect fluorescence

Kuhr and Yeung [105,106] first introduced indirect fluorescence detection for the analysis of native amino acids. In this form of detection, a fluorescent species rather than an absorbing species is used as the probe. Detection is accomplished by on-column laser excitation of the fluorophore. Rather than the BGE having a background absorbance, as is the case with indirect absorbance detection, the BGE has background fluorescence. Displacement of the fluorophore by an analyte ion leads to a quantifiable decrease in background fluorescence. The major advantages of this detection method are that, like indirect absorbance detection, it is universal in its

applicability, but is at least an order of magnitude more sensitive than indirect absorbance detection [46,107]. However, there are few fluorophores available. Probes suitable for indirect fluorescence detection are listed in Table 6.

14. Conclusions

Some general conclusions can be drawn in regard to indirect detection of anions by capillary electrophoresis. In particular the displacement process occurring between analytes and a single probe is very well understood, so the prediction and modeling of electropherograms has become a fairly straightforward process. Therefore, like retention modeling of HPLC and IC, it would be expected that commercially available software applications for predicting electropherograms should become available. Obvious advantages of this are the possibility of optimizing separations from previously known analyte and probe data (mobility, molar absorptivity, etc) without performing experiments, or at least minimizing the number of experiments. The displacement processes of BGEs that contain two or more co-ions are somewhat more complicated. Nevertheless, some problems associated with mobility mismatch of the probe and analytes can be overcome with BGEs that contain multiple probes. Therefore it would be expected that further research would be performed in this area.

Further research in buffering electrolytes for indirect detection with ampholytes is required. It is expected that efforts would be directed towards the design of ampholytes with favorable buffering capacities at their isoelectric points. Currently, very few ampholytes are available that fit these criteria, so the range of accessible pH values for the BGE is limited.

Acknowledgements

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References

[1] S. Hjerten, K. Elenbring, F. Kilar, J. Liao, A.J.C. Chen, C.J. Siebert, M. Zhu, J. Chromatogr. 403 (1987) 47.
[2] S.M. Cousins, P.R. Haddad, W. Buchberger, J. Chromatogr. A 671 (1994) 397.
[3] M.T. Ackermans, F.M. Everaerts, J.L. Beckers, J. Chromatogr. 549 (1991) 345.
[4] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer Laboratory, 1993.
[5] T. Rabilloud, Electrophoresis 15 (1994) 278.
[6] G.J.M. Bruin, A.C. Van Asten, X. Xu, H. Poppe, J. Chromatogr. 608 (1992) 97.
[7] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 1.
[8] H. Poppe, Anal. Chem. 64 (1992) 1908.
[9] M.W.F. Nielen, J. Chromatogr. 588 (1991) 321.
[10] W. Buchberger, S.M. Cousins, P.R. Haddad, Trends Anal. Chem. 13 (1994) 313.
[11] P.A. Doble, M. Macka, P.R. Haddad, Anal. Commun. 34 (1997) 351.
[12] X. Xu, W.T. Kok, H. Poppe, J. Chromatogr. A 742 (1996) 211.
[13] J.L. Beckers, J. Chromatogr. A 693 (1995) 347.
[14] J.L. Beckers, J. Chromatogr. A 741 (1996) 266.
[15] J.L. Beckers, J. Chromatogr. A 764 (1997) 111.
[16] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[17] T. Wang, R.A. Hartwick, J. Chromatogr. 589 (1992) 307.
[18] J.L. Beckers, J. Chromatogr. A 679 (1994) 153.
[19] M. Macka, P.R. Haddad, P. Gebauer, P. Bocek, Electrophoresis 18 (1997) 1998.
[20] P.A. Doble, P.R. Haddad, Anal. Chem. (1997) in press.
[21] C.O. Thompson, V.C. Trenerry, B. Kemmery, J. Chromatogr. A 704 (1995) 203.
[22] A. Henshall, M.P. Harrold, J.M.Y. Tso, J. Chromatogr. 608 (1992) 413.
[23] B.A.P. Buscher, U.R. Tjaden, H. Irth, E.M. Andersson, J. van der Greef, J. Chromatogr. A 718 (1995) 413.
[24] L.K. Goebel, H.M. McNair, H.T. Rasmussen, B.P. McPherson, J. Microcol. Sep. 5 (1993) 47.
[25] S.A. Shamsi, N.D. Danielson, Anal. Chem. 67 (1995) 1845.
[26] S.A. Shamsi, N.D. Danielson, Anal. Chem. 67 (1995) 4210.
[27] G.A. Pianetti, M. Taverna, A. Baillet, G. Mahuzier, D. Baylocq Ferrier, J. Chromatogr. 630 (1993) 371.
[28] R.W. Hepler, C.C. Yu Ip, J. Chromatogr. A 680 (1994) 201.
[29] S.A. Oehrle, P.C. Bossle, J. Chromatogr. A 692 (1995) 247.
[30] R. Neubert, K. Raith, J. Schiewe, Pharmazie 52 (1997) 212.
[31] S. Motellier, K. Gurdale, H. Pitsch, J. Chromatogr. A 770 (1997) 311.
[32] K.L. Larsen, F. Mathiesen, W. Zimmermann, Carbohydr. Res. 298 (1997) 59.
[33] R. Roldan Assad, P. Gareil, J. Chromatogr. A 708 (1995) 339.
[34] A. Nardi, S. Fanali, F. Foret, Electrophoresis 11 (1990) 774.

[35] E.V. Dose, G.A. Guiochon, Anal. Chem. 63 (1991) 1063.
[36] C. Vogt, J. Vogt, H. Wittrisch, J. Chromatogr. A 727 (1996) 301.
[37] C. Vogt, G. Werner, J. Chromatogr. A 686 (1994) 325.
[38] A. Bazzanella, H. Lochmann, A. Mainka, K. Bachmann, Chromatographia 45 (1997) 59.
[39] T. Wang, R.A. Hartwick, J. Chromatogr. 607 (1992) 119.
[40] E. Dabek Zlotorzynska, J.F. Dlouhy, J. Chromatogr. A 671 (1994) 389.
[41] M.M. Rhemrev Boom, J. Chromatogr. A 680 (1994) 675.
[42] T. Ehmann, K. Bachmann, L. Fabry, H. Rufer, S. Pahlke, L. Kotz, Chromatographia 45 (1997) 301.
[43] M.P. Harrold, M.J. Wojtusik, J. Riviello, P. Henson, J. Chromatogr. 640 (1993) 463.
[44] M.J. Wojtusik, M.P. Harrold, J. Chromatogr. A 671 (1994) 411.
[45] M. Macka, P. Haddad, Electrophoresis 18 (1997) 2482.
[46] M. Albin, P.D. Grossman, S.E. Moring, Anal. Chem. 65 (1993) 489a.
[47] Y.J. Xue, E.S. Yeung, Anal. Chem. 65 (1993) 2923.
[48] Y.F. Ma, R.L. Zhang, J. Chromatogr. 625 (1992) 341.
[49] E.S. Yeung, W.G. Kuhr, Anal. Chem. 63 (1991) 280a.
[50] A. Weston, P.R. Brown, P. Jandik, A.L. Heckenberg, W.R. Jones, J. Chromatogr. 608 (1992) 395.
[51] F. Steiner, W. Beck, H. Engelhardt, J. Chromatogr. A 738 (1996) 11.
[52] P. Jandik, W.R. Jones, J. Chromatogr. 546 (1991) 431.
[53] F. Foret, S. Fanali, L. Ossicini, P. Bocek, J. Chromatogr. 470 (1989) 299.
[54] Z. Mala, R. Vespalec, P. Bocek, Electrophoresis 15 (1994) 1526.
[55] W. Beck, H. Engelhardt, Chromatographia 33 (1992) 313.
[56] J. Aupiais, Chromatographia 44 (1997) 303.
[57] B.J. Wildman, P.E. Jackson, W.R. Jones, P.G. Alden, J. Chromatogr. 546 (1991) 459.
[58] S.C. Grocott, L.P. Jefferies, T. Bowser, J. Carnevale, P.E. Jackson, J. Chromatogr. 602 (1992) 257.
[59] P.R. Haddad, A.H. Harakuwe, W.W. Buchberger, J. Chromatogr. A 706 (1995) 571.
[60] A.H. Harakuwe, P.R. Haddad, P.E. Jackson, J. Chromatogr. A 739 (1996) 399.
[61] D.R. Salomon, J. Romano, J. Chromatogr. 602 (1992) 219.
[62] W.R. Jones, P. Jandik, J. Chromatogr. 608 (1992) 385.
[63] J.P. Romano, J. Krol, J. Chromatogr. 640 (1993) 403.
[64] W.R. Jones, P. Jandik, Am. Lab. 22 (1990) 51.
[65] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[66] W. Buchberger, P.R. Haddad, J. Chromatogr. 608 (1992) 59.
[67] N.J. Benz, J.S. Fritz, J. Chromatogr. A 671 (1994) 437.
[68] A.H. Harakuwe, P.R. Haddad, W. Buchberger, J. Chromatogr. A 685 (1994) 161.
[69] C.A. Lucy, T.L. McDonald, Anal. Chem. 67 (1995) 1074.
[70] P. Doble, M. Macka, P.R. Haddad, Electrophoresis 19 (1998) 2257.
[71] L. Kelly, R.J. Nelson, J. Liq. Chromatogr. 16 (1993) 2103.
[72] E. Dabek Zlotorzynska, J.F. Dlouhy, N. Houle, M. Piechowski, S. Ritchie, J. Chromatogr. A 706 (1995) 469.
[73] S.A. Shamsi, N.D. Danielson, Anal. Chem. 66 (1994) 3757.
[74] J. Romano, P. Jandik, W.R. Jones, P.E. Jackson, J. Chromatogr. 546 (1991) 411.
[75] G.W. Tindall, R.L. Perry, J. Chromatogr. A 696 (1995) 349.
[76] J.D. Lamb, T.L. Huxford, K.B. Czirr, J. Chromatogr. A 739 (1996) 373.
[77] H. Burt, D.M. Lewis, K.N. Tapley, J. Chromatogr. A 739 (1996) 367.
[78] H. Chen, Y. Xu, F. Van Lente, M.P.C. Ip, J. Chromatogr. B 679 (1996) 49.
[79] T. Soga, G.A. Ross, J. Chromatogr. 767 (1997) 223.
[80] C.W. Klampfl, W. Buchberger, Trends Anal. Chem. 16 (1997) 221.
[81] M. Arellano, J. Andrianary, F. Dedieu, F. Couderc, P. Puig, J. Chromatogr. A 765 (1997) 321.
[82] S.P.D. Lalljie, J. Vindevogel, P. Sandra, J. Chromatogr. A 652 (1993) 563.
[83] O. Devevre, D.P. Putra, B. Botton, J. Garbaye, J. Chromatogr. A 679 (1994) 349.
[84] W. Buchberger, K. Winna, J. Chromatogr. A 739 (1996) 389.
[85] M. Chiari, E. Kenndler, J. Chromatogr. A 716 (1995) 303.
[86] C.H. Wu, Y.S. Lo, Y.H. Lee, T.I. Lin, J. Chromatogr. A 716 (1995) 291.
[87] W. Buchberger, K. Winna, Mikrochim. Acta 122 (1996) 45.
[88] M.M. Bushey, J.W. Jorgenson, J. Chromatogr. 480 (1989) 301.
[89] P.J. Oefner, A.E. Vorndran, E. Grill, C. Huber, G.K. Bonn, Chromatographia 34 (1992) 308.
[90] A.E. Vorndran, P.J. Oefner, H. Scherz, G.K. Bonn, Chromatographia 33 (1992) 163.
[91] Y.H. Lee, T.I. Lin, J. Chromatogr. B 681 (1996) 87.
[92] B. Lu, D. Westerlund, Electrophoresis 17 (1996) 325.
[93] B.A.P. Buscher, H. Irth, E. Andersson, U.R. Tjaden, J. van der Greef, J. Chromatogr. A 678 (1994) 149.
[94] S. Chen, D.J. Pietrzyk, Anal. Chem. 65 (1993) 2770.
[95] J.M. Gibbons, S.H. Hoke, J. High Resolut. Chromatogr. 17 (1994) 665.
[96] S.A. Shamsi, N.D. Danielson, J. Chromatogr. A 739 (1996) 405.
[97] Y.H. Lee, T.I. Lin, J. Chromatogr. A 680 (1994) 287.
[98] W. Buchberger, P.R. Haddad, J. Chromatogr. A 687 (1994) 343.
[99] L. Lin, J. Wang, J. Caruso, J. Chromatogr. Sci. 33 (1995) 177.
[100] A.T. Timperman, K.E. Oldenburg, J.V. Sweedler, Anal. Chem. 67 (1995) 3421.
[101] S.A. Oehrle, J. Chromatogr. A 671 (1994) 383.
[102] D. Lagoutte, G. Lombard, S. Nisseron, M.P. Papet, Y. Saint Jalm, J. Chromatogr. A 684 (1994) 251.
[103] A. Klockow, A. Paulus, V. Figueiredo, R. Amado, H.M. Widmer, J. Chromatogr. A 680 (1994) 187.
[104] K.D. Altria, K.H. Assi, S.M. Bryant, B.J. Clark, Chromatographia 44 (1997) 367.
[105] W.G. Kuhr, E.S. Yeung, Anal. Chem. 60 (1988) 1832.
[106] W.G. Kuhr, E.S. Yeung, Anal. Chem. 60 (1988) 2642.
[107] P.E. Andersson, W.D. Pfeffer, L.G. Blomberg, J. Chromatogr. A 699 (1995) 323.

[108] J. Collet, P. Gareil, J. Chromatogr. A 716 (1995) 115.
[109] J. Boden, K. Bachmann, L. Kotz, L. Fabry, S. Pahlke, J. Chromatogr. A 696 (1995) 321.
[110] X. Xu, W.T. Kok, H. Poppe, J. Chromatogr. A 716 (1995) 231.
[111] E. Dabek Zlotorzynska, J.F. Dlouhy, J. Chromatogr. A 685 (1994) 145.
[112] T.L. Wang, S.F.Y. Li, J. Chromatogr. A 723 (1996) 197.
[113] M.E. Swartz, J. Chromatogr. 640 (1993) 441.
[114] P. Wang, S.F.Y. Li, H.K. Lee, J. Chromatogr. A 765 (1997) 353.
[115] G. Bondoux, P. Jandik, W.R. Jones, J. Chromatogr. 602 (1992) 79.
[116] S.A. Oehrle, J. Chromatogr. A 733 (1996) 101.
[117] S.A. Oehrle, J. Chromatogr. A 745 (1996) 81.
[118] K.D. Altria, D.M. Goodall, M.M. Rogan, Chromatographia 38 (1994) 637.
[119] J.C. Transfiguracion, C. Dolman, D.H. Eidelman, D.K. Lloyd, Anal. Chem. 67 (1995) 2937.
[120] R. Saari Nordhaus, J.M. Anderson Jr., J. Chromatogr. A 706 (1995) 563.
[121] M. Jimidar, C. Harmann, N. Cousement, D.L. Massart, J. Chromatogr. A 706 (1995) 479.
[122] M. Jimidar, B. Bourguignon, D.L. Massart, Anal. Chim. Acta 310 (1995) 27.
[123] K. Bachmann, I. Haag, T. Prokop, A. Roeder, P. Wagner, J. Chromatogr. 643 (1993) 181.
[124] C. Stathakis, R.M. Cassidy, Anal. Chem. 66 (1994) 2110.
[125] M.T. Galceran, L. Puignou, M. Diez, J. Chromatogr. A 732 (1996) 167.
[126] A.H. Harakuwe, P.R. Haddad, J. Chromatogr. A 734 (1996) 416.
[127] P. Morin, C. Francois, M. Dreux, Analusis 22 (1994) 178.
[128] C. Francois, P. Morin, M. Dreux, J. High Resolut. Chromatogr. 14 (1996) 5.
[129] K. Li, S.F.Y. Li, J. Liq. Chromatogr. 17 (1994) 3889.
[130] M. Jimidar, Q. Yang, J. Smeyers Verbeke, D.L. Massart, Trends Anal. Chem. 15 (1996) 91.
[131] G.Y. Jung, T.H. Kim, H.B. Lim, Anal. Sci. 12 (1996) 367.
[132] E.S. Kwak, F.A. Gomez, Chromatographia 43 (1996) 659.
[133] W.R. Jones, J. Chromatogr. 640 (1993) 387.
[134] B. Tenberken, P. Ebert, M. Hartmann, M. Kibler, A. Mainka, T. Prokop, A. Roder, K. Bachmann, J. Chromatogr. A 745 (1996) 209.
[135] R.J. Tait, D.J. Skanchy, D.P. Thompson, N.C. Chetwyn, D.A. Dunshee, R.A. Rajewski, V.J. Stella, J.F. Stobaugh, J. Pharm. Biomed. Anal. 10 (1992) 615.
[136] J.L. Beckers, J. Chromatogr. A 696 (1995) 285.
[137] E.A. Luna, E.R.N. Bornancini, D.O. Thompson, R.A. Rajewski, V.J. Stella, Carbohydr. Res. 299 (1997) 103.
[138] K. Bachmann, I. Haumann, T. Groh, Fresenius' J. Anal. Chem. 343 (1992) 901.
[139] G. Gutnikov, W. Beck, H. Engelhardt, J. Microcol. Sep. 6 (1994) 565.
[140] L. Zhou, A. Dovletoglou, J. Chromatogr. A 763 (1997) 279.
[141] G.W. Tindall, D.R. Wilder, R.L. Perry, J. Chromatogr. 641 (1993) 163.
[142] Y.H. Lee, T.I. Lin, Electrophoresis 17 (1996) 333.
[143] F.Y. Guan, H.F. Wu, Y. Luo, J. Chromatogr. A 719 (1996) 421.
[144] D. Volgger, A.J. Zemann, G.K. Bonn, M.J. Antal, J. Chromatogr. A 758 (1997) 263.
[145] M.G. Cikalo, D.M. Goodall, W. Matthews, J. Chromatogr. A 745 (1996) 189.
[146] F.S. Stover, S.S. Keffer, J. Chromatogr. A 657 (1993) 450.
[147] H. Salimi Moosavi, R.M. Cassidy, Anal. Chem. 67 (1995) 1067.
[148] J.B.L. Damm, G.T. Overklift, J. Chromatogr. A 678 (1994) 151.
[149] M. Van Holderbeke, H. Vanhoe, L. Moens, R. Dams, Biomed. Chromatogr. 9 (1995) 281.
[150] H. Siren, A. Maattanen, M.L. Riekkola, J. Chromatogr. A 767 (1997) 293.
[151] T.W. Garner, E.S. Yeung, J. Chromatogr. 515 (1990) 639.
[152] T. Groh, K. Bachmann, Electrophoresis 13 (1992) 458.
[153] Y.C. Chao, C.W. Whang, J. Chromatogr. A 663 (1994) 229.
[154] A.M. Desbene, C.J. Morin, N.L. Mofaddel, R.S. Groult, J. Chromatogr. A 716 (1995) 279.
[155] I.H. Grant, W. Steuer, J. Microcol. Sep. 2 (1990) 74.
[156] V. Marti, M. Aguilar, E.S. Yeung, J. Chromatogr. A 709 (1995) 367.
[157] Q. Xue, E.S. Yeung, J. Chromatogr. A 661 (1994) 287.
[158] L. Gross, E.S. Yeung, J. Chromatogr. 480 (1989) 169.
[159] B.L. Hogan, E.S. Yeung, J. Chromatogr. Sci. 28 (1990) 15.
[160] M.W.F. Nielen, J. Chromatogr. 608 (1992) 85.
[161] D.F. Swaile, C.L. Copper, M.J. Sepaniak, D.E. Burton, L.L. Powell, Talanta 41 (1994) 1499.
[162] W. Tong, E.S. Yeung, J. Chromatogr. A 718 (1995) 177.
[163] W.H. Robins, B.W. Wright, J. Chromatogr. A 680 (1994) 667.
[164] J. Boden, T. Ehmann, T. Groh, I. Haumann, K. Bachmann, Fresenius' J. Anal. Chem. 348 (1994) 572.
[165] R. Roldan-Assad, P. Gareil, J. Chromatogr. A 708 (1995) 339.