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Journal of Chromatography A, 787 (1997) 243–251

JOURNAL OF CHROMATOGRAPHY A

Sub-minute separations of organic and inorganic anions with co-electroosmotic capillary electrophoresis

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Received 10 April 1997; received in revised form 4 June 1997; accepted 4 June 1997

Abstract

Fast capillary electrophoretic separations of various classes of anionic compounds are performed by using very short effective separation lengths in addition to a similarly directed movement of the electroosmotic flow and the inherent electrophoretic mobilities of the analytes. A polycationic surfactant (hexadimethrine bromide) is added to the electrolyte, which dynamically coats the inner surface of the capillary and causes a fast anodic electroosmotic flow. Selective separations in the sub-minute range combined with high separation efficiencies of several hundred thousand theoretical plates per meter effective separation length can be achieved if organic solvents or mixtures thereof are added to the electrolytes. The methods are used for the high-speed separation of various anionic analytes, such as carbohydrates, carboxylic acids, phenolic compounds and inorganic anions. © 1997 Elsevier Science B.V.

Keywords: Reversed electroosmotic flow; Inorganic anions; Carbohydrates; Carboxylic acids; Phenolic compounds

1. Introduction

Capillary electrophoresis offers the possibility to separate ionic compounds with high separation efficiencies and short separation times [1–5]. Two fundamental effects contribute to the capillary electrophoretic separation of charged compounds in fused-silica capillaries. At first, ionic analytes migrate to the respective oppositely charged electrode when an electric field is applied due to the inherent electrophoretic mobility of the ions. Sign and magnitude of the inherent electrophoretic mobility mainly depend on the size-to-charge ratio of the solute. Secondly, in fused-silica capillaries the negatively charged inner surface of the capillary and the positively charged adjacent layers of liquid (inner and outer Helmholtz layer) cause a potential difference between the wall and the bulk liquid (ζ-potential) which results in a net flow of the liquid inside the capillary towards the cathode (electroosmotic flow, EOF). As a consequence, the observed electrophoretic mobility of a compound μ_{obs} can be calculated as the sum of the inherent electrophoretic mobilities μ_{ep} and the EOF μ_{eof} according to Eq. (1).

$$\mu_{obs} = \mu_{ep} + \mu_{eof} \quad (1)$$

In the case of cations the usual set-up of the detector being placed on the cathodic side of the capillary results in fast separations, as the positively charged ions migrate in the same direction as the electroosmotic flow. However, anionic compounds migrate against the direction of the EOF and thus pass the detector after the EOF (counter-electroosmotic migration).

PII S0021-9673(97)00645-6

In order to reduce the separation time, a higher separation voltage, a shorter separation length, or a high observed electrophoretic mobility have to be established according to Eq. (2):

$$t = \frac{L \cdot l}{\mu_{obs} \cdot U} \quad (2)$$

where L and l are the total and the effective length of the capillary, respectively.

The first attempt to reduce the analysis time is to increase the separation voltage. However, this often causes the production of Joule heat which, in turn, reduces the separation efficiency due to thermal dispersion.

The second attempt to achieve short separation times is to increase the observed electrophoretic mobilities of anionic analytes. This can, to a certain extent, be achieved by optimizing the composition of the separation buffer. However, the separation speed for anionic analytes is always limited by the mobility of the EOF. A dramatic increase of the observed mobilty is accomplished if the EOF is reversed. This is usually achieved by coating the inner surface of the capillary either by dynamic coatings based on hemimicelles [6–10] or using polycationic EOF modifiers [11–18] and reversing the polarity of the power supply. As a consequence, the movement of the anionic compounds and the EOF are then similarly directed (co-electroosmotic capillary electrophoresis). The ensuing high observed mobililties also result in an increase of separation efficiency N according to Eq. (3) [1,19]:

$$N = \frac{(\mu_{ep} + \mu_{eof})U}{2D} \quad (3)$$

where U is the applied separation voltage and D is the diffusion coefficient.

The third attempt, which also works with counter-electroosmotic methods to a limited extent, is to reduce the effective separation length. In addition to a co-electroosmotic movement of the analytes, this dramatically reduces separation times. Optimization of resolution for co-electroosmotic methods can be achieved by using electrolyte additives, including organic solvents. By this means the electrophoretic mobilities of various anionic analytes can be selectively altered [10,13,14,16,17].

2. Experimental

2.1. Apparatus

The analytical data were acquired with a Waters Quanta 4000 capillary electrophoresis system connected with a system interface module. Data processing was carried out with a commercial chromatography software (Maxima 820; Waters Chromatography, Milford, MA, USA) on a personal computer. Fused-silica capillaries (Composite Metal Services, Worchester, UK) with an inner diameter of 50 μm were used. Direct UV detection was performed at 214 nm for phenols, indirect UV detection was carried out at 254 nm for underivatized carbohydrates, and at 185 nm for carboxylic acids and inorganic anions.

A total capillary length of 32 cm was used throughout this investigation. An effective separation length of 24.5 cm was used for inorganic anions. For the other separations carried out with total capillary lengths shorter than 27 cm the Waters CE instrument had to be modified. The separation was then performed between the regular capillary outlet and the detector (7.5 cm) using a positive high voltage. Injection was carried out by dipping the regular capillary outlet end into the sample vial and either manually applying voltage for electromigrative injection or by fixing the injection block to the sample tray and lowering this part of the instrument. By this means a hydrostatic difference of 5 cm was established which enabled a hydrostatic introduction of the sample into the capillary.

2.2. Reagents

All compounds were of analytical grade if available. Standard solutions of carbohydrates, carboxylic acids and inorganic anions (Sigma–Aldrich, Vienna, Austria; Merck, Vienna, Austria) were prepared by dissolving the compounds in ultrapure water with a resistance of 18.2 MΩ (Barnstead/Thermolyne, Dubuque, IA, USA). Phenol standards were prepared in methanolic solution. 1,5-Dimethyl-1,5-diazaundecamethylene polymethobromide (polybrene; hexadimethrine bromide, HDB; Sigma–Aldrich) was used as EOF modifier at a concentration of 0.001% (w/v). Electrolyte solutions for the respective methods were

prepared from stock solutions of sodium chromate, potassium hydrogenphthalate, sodium sorbate, sodium tetraborate and phosphoric acid.

3. Results and discussion

In the present paper, separations of various classes of anionic analytes, such as underivatized carbohydrates, carboxylic acids, phenolic compounds and inorganic anions, are carried out by co-electroosmotic capillary electrophoresis, where the movement of anionic analytes and electroosmotic flow are similarly directed. As a consequence, an increase of separation speed is observed for the solutes.

By shortening the effective separation length, a further reduction of the separation time can be achieved, as the relation between separation time and separation length is quadratic, according to Eq. (2). Separation times below one minute are easily achieved if the composition of the separation buffer is optimized. For specific purposes, organic solvents are added to the buffers. In many cases this improves selectivity and resolution between specific analytes, which are otherwise difficult to separate and/or require long run times.

3.1. Carbohydrates

Because underivatized carbohydrates neither exhibit a UV absorptivity nor possess a sufficiently high electrophoretic mobility at moderate pH conditions, the capillary electrophoretic analysis is restricted to certain limits. Detection problems are usually overcome by pre-column derivatization, by using borate-containing electrolytes and detection wavelengths below 200 nm, or by indirect absorption methods with UV absorbing background electrolytes. Electrophoretic mobilities of underivatized carbohydrates are increased when high alkaline background electrolytes with pH values above the pK_a values of the carbohydrates are chosen [20–23].

Fig. 1 demonstrates the fast co-electroosmotic separation of underivatized carbohydrates with sorbate as background electrolyte. The separation is performed below 50 s using an effective separation length of 7.5 cm. Even though some analytes are not completely resolved, high separation efficiencies in the range of several hundred thousand theoretical plates per meter are achieved. With the chosen electrolyte system the underivatized carbohydrates separate according to their respective pK_a values (Table 1).

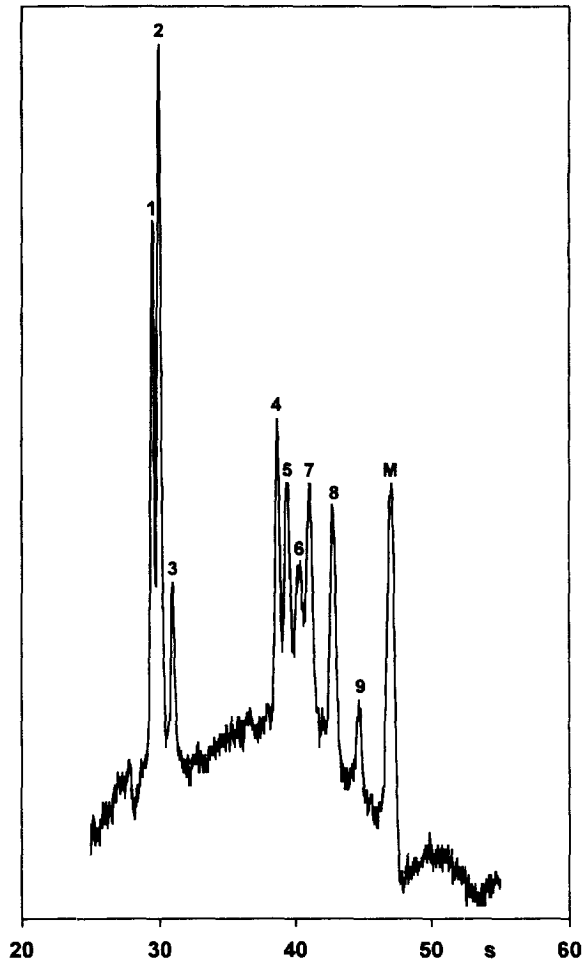

Fig. 1. Fast separation of underivatized carbohydrates. Electrophoretic conditions: 6 m*M* sorbate, 0.001% HDB, pH 12.2, indirect UV detection at 254 nm; $L=50$ cm, $l=7.5$ cm, $U=+20$ kV, $I=38$ μA, hydrostatic injection for 1 s at 5 cm. Peak assignment as in Table 1.

Sorbate serves as a suitable background electrolyte for the separation, because this compound exhibits some considerable advantages as compared to other background electrolytes [24,25]. It bears only a single charge which causes a theoretical transfer ratio of 1 for a maximum sensitivity. Furthermore, it has a

Table 1
pK_a values, migration times and separation efficiencies of underivatized carbohydrates [20–23]

	Compound	pK_a values	Migration times (s)	Theoretical plates (N/m)
1	Glucuronic acid	3.20	29.5	767 000
2	Galacturonic acid	3.48	29.9	588 000
3	Gluconic acid	3.76	30.9	847 000
4	Mannose	12.08	38.6	859 000
5	Rhamnose	n.a.	39.4	536 000
6	Glucose	12.35	40.3	249 000
7	Galactose	12.35	41.1	460 000
8	2-Deoxy-D-ribose	12.65	42.8	633 000
9	Raffinose	12.74	44.6	689 000

n.a.: not available.

high molar absorptivity coefficient at the wavelength of 254 nm which is important for widely distributed single-wavelength detectors.

By performing high-speed separations of underivatized carbohydrates with indirect UV detection, a fast monitoring of various sugars is possible without pre- or post-column derivatization.

3.2. Carboxylic acids

In industry, the monitoring of reaction products and process by-products is of great interest, because process control and regulation are indispensable for optimization in terms of low operational costs and high product yields. For a fast separation of organic acids, only few capillary electrophoretic methods using a fast co-directional migration of analytes and EOF have been presented [18,26].

Fig. 2 presents the separation of a mixture of 7 carboxylic and dicarboxylic acids with indirect UV detection below 40 s. Even though the effective separation length is only 7.5 cm, it is possible to separate the *cis* and *trans* isomers of 2-methyl-2-butendioic acid (citraconic and mesaconic acid) within 25 s. The migration order of the acids under these conditions corresponds to the respective pK_a values [27–29] which are listed in Table 2.

An effective reversal of the EOF and the subsequent separation of the solute mixture is carried out under acidic conditions. Although the inner surface of the fused-silica capillary should only be barely charged at pH values below 4, a fast anodic electroosmotic flow and short separation times are achieved. It is presumable that the reversal of the EOF only partly occurs by the attachment of the EOF modifiers due to electrostatic interaction of the negatively charged silanolate groups and the positively charged ammonium groups of the EOF. Moreover, non-electrostatic interactions, such as Van der Waals interactions of the methylene groups of the EOF modifier and the capillary wall also contribute to the binding of the EOF modifier to the capillary surface and the reversal of the EOF. This obviously seems to be a predominant mechanism of EOF reversal in acidic media.

Phthalate serves as the background electrolyte for the indirect UV detection of carboxylic acids in acidic media. With other commonly used background electrolyte systems, an effective separation of the analytes cannot be achieved. Sorbate, which effectively supports the separation of underivatized carbohydrates at high pH values is too insoluble at acidic conditions and exhibits only a low molar absobtivity. Also, chromate cannot be used at acidic pH values as it forms dichromate and causes the EOF modifier to precipitate.

One advantage of phthalate as a background electrolyte is that the pH value of a 5 m*M* potassium hydrogenphthalate solution represents the pH optimimum of 3.85 for the separation of the solutes. As a consequence, no pH adjustment has to be made for buffer preparation which further facilitates the method.

The turn-over time of a separation including reconditioning of the capillary can be further reduced by using a specific reconditioning protocol. It consists of purging and, at the same time, applying high voltage (30 kV) which results in separations with

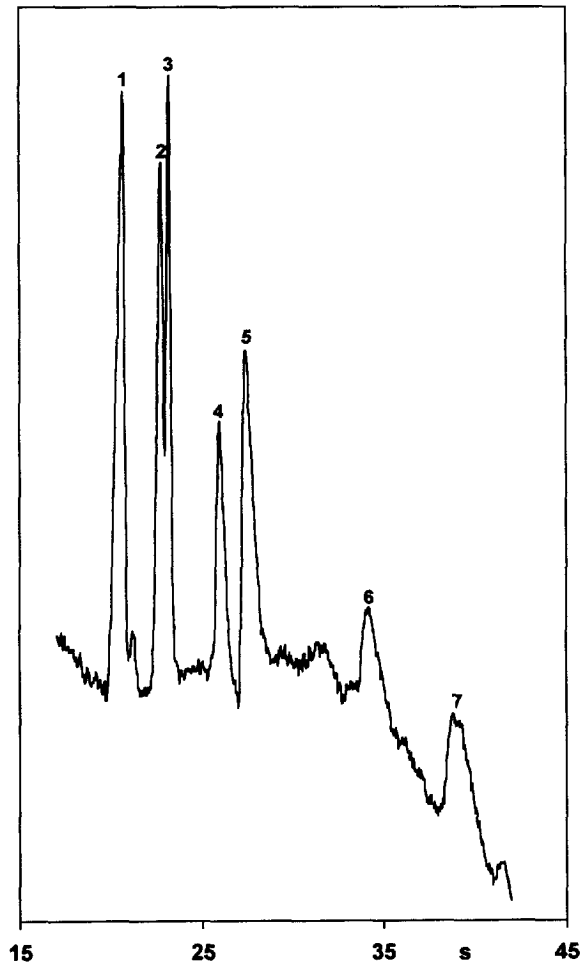

Fig. 2. Fast separation of carboxylic acids. Electrophoretic conditions: 5 m*M* phthalate, 0.001% HDB, pH 3.85, indirect UV detection at 185 nm; $L=32$ cm, $l=7.5$ cm, $U=+30$ kV, $I=12$ μA, hydrostatic injection for 10 s at 5 cm. Peak assignment as in Table 2.

Table 2
pK_a values, migration times and separation efficiencies of carboxylic acids [27–29]

	Compound	pK_a values	Migration times (s)	Theoretical plates (N/m)
1	Pyruvic acid	2.49	20.6	125 000
2	Citraconic acid	2.95; 5.98	22.7	257 000
3	Mesaconic acid	3.09; 4.75	23.2	634 000
4	Itaconic acid	3.85; 5.45	25.9	214 000
5	Hydroybutyric acid	3.98	27.3	141 000
6	Methacrylic acid	4.68	34.1	77 000
7	Crotonic acid	4.69	38.7	77 000

higher reproducibilities than by solely purging the capillary with electrolyte. The effect of purging and high voltage can be explained by a faster regeneration of the inner capillary surface and, as a consequence, a stable EOF.

The migration times and separation efficiencies of high-speed separations of carboxylic acids are listed in Table 2. The migration order is according to the respective pK_a values.

3.3. Phenolic compounds

Capillary electrophoretic separations of phenols are usually carried out with micellar electrokinetic capillary chromatography (MECC) [2,3,30,31] or with counter-electroosmotic methods [32,33]. In recent years co-electroosmotic methods also proved to be suitable for a fast separation of phenolic compounds [10,14–17].

In Fig. 3 the separation of 7 methylphenol isomers is shown. Fast separations of methylphenols have already been carried out by co-electroomsotic capillary electrophoresis [14,17] and the analysis time can be further reduced by using a short effective separation length of 7.5 cm which enables a fast separation of the present sample mixture. The migration order of the cresol and xylenol isomers is in accordance with the pK_a values [34], however, the xylenols exhibit a slightly lower electrophoretic mobility compared to the cresols which is due to the additional methyl group (Table 3).

To ensure a high inherent electrophoretic mobility of the phenols, an alkaline buffer with a pH value above the respective pK_a values of the analytes is used. However, the co-electroosmotic separation of hydrophobic analytes (e.g., aromatic compounds) bears some difficulties. Peak zones often become broad, asymmetric with low separation efficiencies and the solutes are sometimes strongly retained. This is attributed to hydrophobic interactions of phenolic compounds with the methylene groups of the EOF modifier. Alkyltrimethylammonium-based EOF modifiers, which are often used to reverse the EOF are only of limited use for the co-electroosmotic separation of phenols because these compounds carry long aliphatic chains (C_{14}–C_{16}), which strongly interact with the hydrophobic analytes. Electrostatic interactions can be excluded. With polycationic EOF

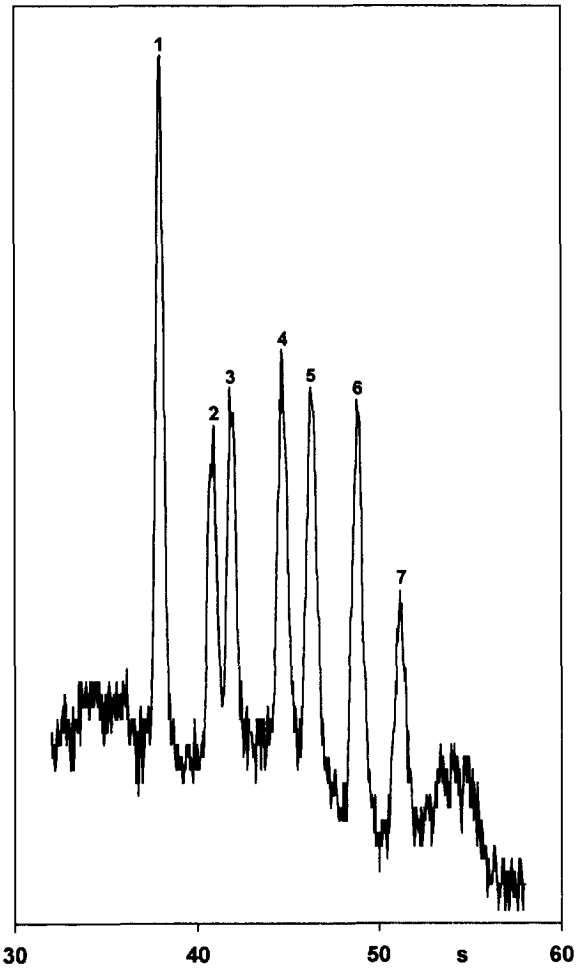

Fig. 3. Separation of phenolic compounds. Electrophoretic conditions: 15 m*M* phosphate, 10 m*M* borate, 0.001% HDB, 20% methanol, 20% 2-propanol, 5% acetonitrile, pH 11.5, detection at 214 nm; $L=32$ cm, $l=7.5$ cm, $U=+30$ kV, $I=48$ μA, hydrostatic injection for 3 s at 5 cm. Peak assignment as in Table 3.

modifiers, such as polybrene, the methylene groups are shorter and the concentration necessary to reverse the EOF is usually by the order of three magnitudes lower. In order to separate phenolic compounds with high separation efficiencies it is thus indispensable to use organic solvents as electrolyte additives [14,16,17]. However, the resolution of specific analytes is often not improved by simply using a higher solvent concentration. In addition, the separation time is often increased when high solvent concentrations are used which is due to a higher viscosity and a reduced ζ-potential.

For specific purposes it is necessary to work with mixed organic solvent systems [17]. This enables the variation of selectivity for a wide variety of phenolic analytes, such as the separation of cresol and xylenol isomers, which are not effectively separated with single solvent systems.

The alkaline buffer used for this separation consists of a mixed organic solvent electrolyte system (20% iso-propanol, 20% methanol and 5% acetonitrile) in order to increase both the electrophoretic mobilities and the separation efficiencies. On the one hand, the use of iso-propanol results in a higher resolution of the faster migrating phenols. On the other hand, methanol improves the selectivity of phenols with a lower electrophoretic mobility and, in addition, keeps a low viscosity of the buffer which enables a high separation speed. Acetonitrile also significantly affects the separation. Even though acetonitrile is only present at a concentration of 5% it increases the separation efficiency and reduces the separation time. Without acetonitrile, the separation efficiencies for the same phenolic would be as much as 30% lower and the separation time would be 20% longer. Acetonitrile reduces the hydrophobic interac-

Table 3
Migration times and separation efficiencies of methylphenols [34]

	Compound	pK_a values	Migration times (s)	Theoretical plates (N/m)
1	3-Methylphenol	10.09	38.5	666 000
2	4-Methylphenol	10.27	41.4	465 000
3	2-Methylphenol	10.32	42.4	467 000
4	3,4-Dimethylphenol	10.36	45.3	433 000
5	2,5-Dimethylphenol	10.41	46.8	482 000
6	2,3-Dimethylphenol	10.54	49.4	443 000
7	2,6-Dimethylphenol	10.63	51.7	485 000

tions of the phenolic solutes with the methylene groups of the EOF modifier. Separation efficiencies achieved with sub-minute separations of cresols and xylenols are in the range of 500 000 theoretical plates per meter (Table 3).

3.4. Inorganic anions

Usually, the separation of these compounds can be carried out easily by capillary electrophoretic methods. With this class of compounds the applicability of co-electroosmotic CE has been effectively demonstrated for the first time [8,35]. Due to the fast methods developed for CE analysis of anions in real samples [9,36] also a process monitoring for industrial purposes can be performed [13,37].

In Fig. 4 the fast separation of a mixture of inorganic anions is shown. An effective separation length of 24.5 cm is chosen for this method. The detection of inorganic ions is generally possible with indirect UV detection methods using backgound electrolytes containing a suitable chromophore. As a widely applicable background electrolyte for inorganic anions, chromate is often used for this purpose for various reasons. It exhibits a high molar absorbtivity and it matches the electrophoretic mobilities of many inorganic anions. The latter is important because otherwise the peak zones of the analytes become asymmetric and broad due to electrodispersion effects. Furthermore, at high pH values, which are used for the separation of inorganic anions, redox reactions with the analytes are not observed.

Inorganic anions usually do not exhibit any specific disadvantageous interactions with the EOF modifier which often lead to distorted or broad peaks in the case of hydrophobic anions. Ion pair formation can be excluded as long as electrolytes with a sufficiently high dielectric constant are used. This applies for aqueous electrolytes as well as for most of the organic solvent mixtures which are commonly used. Thus, pure aqueous electrolytes work considerably well with co-electroosmotic methods. However, the separation selectivity of inorganic anions can be improved when organic solvents are added to the electrolyte. Although the addition of organic solvents is not necessary for the method using a regular separation length of 24.5 cm, the addition of 20% acetonitrile to the background

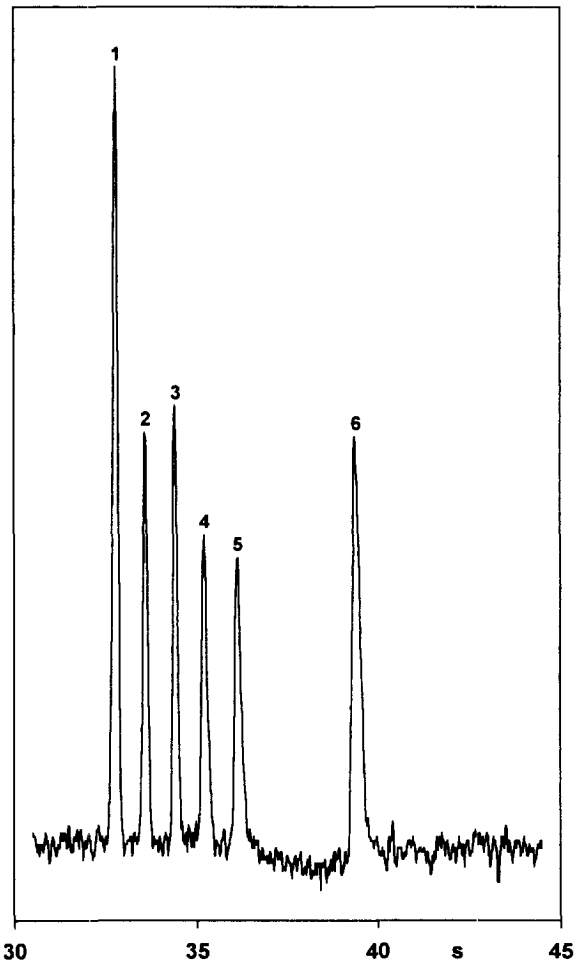

Fig. 4. Separation of inorganic anions using a regular separation length. Electrophoretic conditions: 5 m*M* chromate, 0.001% HDB, 20% acetonitrile, pH 11.0, indirect UV detection at 185 nm; $L = 32$ cm, $l = 24.5$ cm, $U = -30$ kV, $I = 20$ μA, hydrostatic injection for 10 s at 10 cm. Peak assignment as in Table 4.

electrolyte increases the resolution of some analytes, which becomes important for specific purposes, such as the high-speed separation of anions in the range of seconds when very short separation lengths are used.

For the method using a 24.5 cm effective separation length, the standard calibration of 5 dilutions injected for 3 repetitive times yields high correlation coefficients for the respective ions (Table 4). With this method the quantitative determination of inorganic ions in the sub-minute range is thus possible. By using a similar purging protocol as described for

Table 4
Migration times and separation efficiencies of inorganic anions

No.	Compound	Regular separation length (24.5 cm)			Short separation length (7.5 cm)	
		Migration times (s)	Theoretical plates (N/m)	r^2	Migration times (s)	Theoretical plates (N/m)
1	Thiosulfate	32.7	1 421 000	0.9993	17.5	998 000
2	Chloride	33.6	1 494 000	0.9990	17.9	1 045 000
3	Sulfate	34.4	1 435 000	0.9981	18.3	1 178 000
4	Oxalate	35.2	1 013 000	0.9970	18.6	700 000
5	Sulfite	36.1	818 000	0.9989	19.1	656 000
6	Carbonate	39.4	622 000	0.9920	20.7	467 000

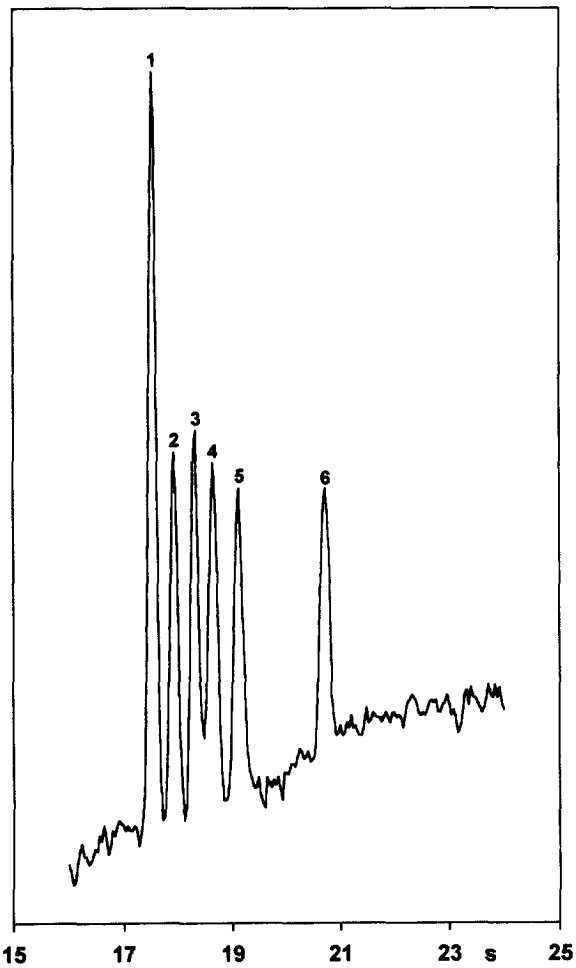

Fig. 5. Separation of inorganic anions using a short separation length. Electrophoretic conditions: 5 m*M* chromate, 0.001% HDB, 20% acetonitrile, pH 11.0, indirect UV detection at 185 nm; $L = 32$ cm, $l = 7.5$ cm, $U = +30$ kV, $I = 21$ μA, hydrostatic injection for 1 s at 5 cm. Peak assignment as in Table 4.

carboxylic acids (purging and, at the same time, applying high voltage) the total turn-over time for a reproducible analysis, including the capillary reconditioning, can be reduced to 90 s.

Fig. 5 depicts the electropherogram of the same mixture of 6 inorganic anions as in Fig. 4. A high-speed separation below 25 s can be accomplished. High separation efficiencies as well as high resolution can be achieved even though a very short effective separation length of 7.5 cm is used. The respective migration times and separation efficiencies of this electropherogram are also shown in Table 4.

4. Conclusion

High speed separations of anionic compounds below 1 minute can be performed by capillary electrophoresis with reversed electroosmotic flow conditions. Fast separations are not only for the sake of science but are important for process control purposes and the rapid analysis of large sample numbers. In the future the use of short separation lengths in miniaturized capillary electrophoresis instrumentation may play an important role for the on-line capillary electrophoretic control of industrial processes.

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