Optimization of detection sensitivity in the analysis of inorganic cations by capillary ion electrophoresis using indirect photometric detection

Andrea Weston[*] and Phyllis R. Brown

Department of Chemistry, University of Rhode Island, Kingston, RI 02881 (USA)

Petr Jandik, Allan L. Heckenberg and William R. Jones

Ion Analysis Department, Waters Chromatography Division of Millipore, 34 Maple Street, Milford, MA 01757 (USA)

ABSTRACT

Capillary ion electrophoresis (Waters' trade name Capillary Ion Analysis, or CIA) is the subdivision of capillary zone electrophoresis in which the conditions for the separation are optimized for the analysis of inorganic or low-molecular-mass ions. The first reported separation of alkali, alkaline earth and transition metals by capillary ion electrophoresis, published in 1991 [LC · GC, 9 (1991) 634], showed the separation of these cations in under 10 min with mid-ppb detection limits and separation efficiencies of more than 100 000 theoretical plates. Detection sensitivity has since been improved by altering both hardware and electrolyte components. These alterations have resulted in a decrease in detection limits of more than an order of magnitude, making trace level analyses practical for many applications. The good reproducibility and linearity of this technique are also discussed.

INTRODUCTION

Capillary zone electrophoresis (CZE) has developed into one of the more powerful separation techniques for the analysis of a wide variety of sample matrices. However, one of the major drawbacks in CZE is the lack of a sensitive and universal detection system. A number of variables may be manipulated to address these problems.

Indirect modes of detection provide a simple solution to the problem of universal detection, eliminating the need for pre- or post-column derivatization to convert the analyte of interest into a species that gives a response at the detector. Because the analytes are not chemically altered, fraction collection and further studies are facilitated. Several indirect modes have been described in the literature, including indirect UV absorbance [1–5], indirect fluorescence [6–8] and indirect amperometric detection [9]. Foret *et al.* [10] employed indirect photometric detection to analyze a group of low-molecular-mass anions. They found that the best sensitivities were obtained for sample ions having an effective mobility close to the mobility of the UV background-providing co-ion. Therefore, by choosing an electrolyte with the required mobility, sensitivity enhancements can be achieved.

Owing to its universal nature and its ease of use, UV detection (indirect of direct) is still the most popular method. Separations typically take place in capillaries of $\leqslant 100$ μm I.D., to provide efficient heat dissipation. As the capillary dimensions are in-

Correspondence to: Dr. P. R. Brown, Department of Chemistry, University of Rhode Island, Kingston, RI 02881, USA.
* Present address: Ion Analysis Department, Waters Chromatography Division of Millipore, 34 Maple Street, Milford, MA 01757, USA.

creased beyond 100 μm I.D., a dramatic decrease in separation efficiency is observed [1], due to Joule heating resulting from the ionic current carried between the electrodes. However, with capillaries of ≤ 100 μm I.D. considerable loss in sensitivity is encountered, consistent with the Lambert–Beer law, due to the shorter path lengths of the capillaries. Recently, several attempts have been made to extend the path length for UV detection in capillary electrophoresis (CE). Tsuda et al. [11] described the use of transparent, rectangular capillaries with dimensions ranging from 16 μm × 195 μm to 50 μm × 1000 μm. According to Tsuda et al. [11], rectangular capillaries are extremely efficient at dissipating heat compared with conventional circular capillaries. Thus, larger-volume rectangular capillaries can be used, resulting in more than an order of magnitude increase in concentration sensitivity. The authors noted that with the larger capillaries, however, normal hydrostatic or electromigration injection modes failed to give reproducible, high-efficiency separations and a split injection method had to be employed. Peak widths were wider than those normally obtained for CE separations employing circular cross-section capillaries.

Grant and Steuer [12] extended the path length of their capillary up to 3 mm, by axial illumination of the capillary using laser-induced fluorescence with indirect UV detection. Due to the presence of high background noise, however, the advantage of an increased path length by this method could not be fully exploited. Gordon [13] extended the path length of the capillary by employing an egg-shaped cell [13] "fabricated directly into the separations capillary, that increases both the flux and the path length of the ultraviolet light employed in an absorption measurement" [13]. Chervet and co-workers [14,15] constructed a Z-shaped flow cell in order to extend the path length of the capillary up to 3 mm for UV detection in CE. The loss in resolution caused by the extended path length was less pronounced than expected, while enhancements in signal-to-noise ratio of up to 6 times were observed.

In addition to optimizing the mobility of the electrolyte co-ion and increasing the capillary path length, the mode of sample introduction also plays a role in sensitivity. The high-efficiency capabilities of CE can be realized only when the injection system employed does not introduce significant zone broadening. CE systems are easily overloaded by large sample volumes, and hence it is important that the injection system employed is capable of delivering small volumes efficiently and reproducibly [16]. Sample introduction in CE may be accomplished in a number of ways. A rotary-type injector [17] has been employed, as have various sample splitters [18]. These injectors have been designed for capillaries with an internal diameter of 200–300 μm. For smaller capillaries with an internal diameter of 25–75 μm, low-dead-volume coupling of injectors to the separation capillary is difficult to achieve [19]. Therefore, sample introduction into smaller capillaries is accomplished by using electromigration or hydrostatic injection. It has been stated by Huang et al. [20] that the use of electromigration injection introduces biases and therefore care should be taken when comparing results from different sample solutions. The biases result both from the different mobilities of the ions migrating into the capillary, and from the differences in electrical resistance of the medium in which the species are dissolved. By performing the instrument calibration and the sample analyses from solutions of similar resistance, bias due to preferential migration of analyte ions should not be a problem. Dual calibration, however, such as constant conductivity with varying concentrations and constant concentration with varying conductivity, provides a more complete solution to the problem of preferential migration.

In this paper the effects on the detection limits of inorganic metal cations, of changing the detection wavelength, the mobility of the electrolyte co-ion, the length of the detection cell and the mode of injection are investigated.

EXPERIMENTAL

A Waters (Milford, MA, USA) Quanta 4000 CE system, equipped with a positive power supply, was used throughout this study. Indirect UV detection was achieved at 214 nm using a zinc lamp with a 214-nm optical filter, and at 185 nm using a mercury lamp with a 185-nm optical filter. Normal fused-silica capillaries, 60 cm in total length, 75 μm I.D. and 52 cm from the point of sample introduction to the detector window, were obtained from Waters (AccuSep capillaries). Capillaries with extended path lengths (60 cm × 75 μm I.D. with path lengths of

300 μm) were obtained from Waters. The samples were introduced into the capillary either by using a 30-s hydrostatic injection from a height of 10 cm, or by electromigration (10 kV) for 10 s. The separation voltage was set at 20 kV.

Standard 2-ml polyethylene sample vials (Sun Brokers, Wilmington, NC, USA) were used as containers for the carrier electrolyte and all the standards and samples. A Waters 860 data station and Waters SIM interface were used to record and evaluate the electropherograms at a sample rate of 20 points per s. The subsequent statistical processing was performed using CricketGraph (Cricket Software, Malvern, PA, USA) with a Macintosh SE personal computer (Apple Computers, Cupertino, CA, USA).

All solutions, electrolytes and standards were prepared using 18-MΩ water generated by Milli-Q laboratory water-purification system (Millipore, Bedford, MA, USA). The transition metal standards were prepared by dilution of atomic absorption standards obtained from Sigma (St. Louis, MO, USA), while the alkali and alkaline earth metal standards were prepared from salts obtained from Aldrich (Milwaukee, WI, USA). The UV background providing electrolytes, UVCat-1 and UVCat-2, were obtained from Waters. The analytical-grade complexing agents, α-hydroxyisobutyric acid (HIBA) and tropolone, were both obtained from Aldrich.

RESULTS AND DISCUSSION

The separation of alkali and alkaline earth metals in the presence of transition metals has been reported previously [4]. The electrolyte consisted of 5 mM UVCat-1 to provide the UV-absorbing background and 6.5 mM HIBA to selectively complex the metals for a better separation. Indirect UV detection at 214 nm was employed. Fig. 1a shows a separation of 11 metals under these previously reported conditions. Detection limits for the alkali and alkaline earth metals range from low- to mid-ppb levels. Fig. 1b shows the separation of the same group of metals under the same conditions as Fig. 1a, except the detection wavelength was changed from 214 to 185 nm. Although the baseline noise at 185 nm increased, the peak height is significantly larger. Thus, despite the increase in baseline noise, the de-
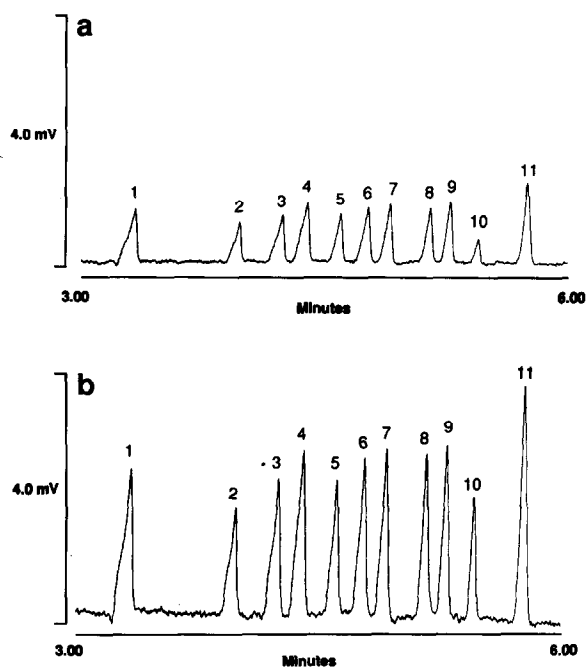

Fig. 1. (a) Capillary ion electrophoretic analysis of a standard solution of alkali and alkaline earth metals in the presence of transition metals. Carrier electrolyte: 5 mM UVCat-1, 6.5 mM HIBA, pH 4.4. Capillary: 60 cm × 75 μm I.D. fused silica. Applied voltage: 20 kV (positive). Hydrostatic injection: 10 cm for 30 s. Indirect UV detection at 214 nm. Sample: standards. Peaks: 1 = potassium (1.6 ppm); 2 = barium (2.0 ppm); 3 = strontium (1.6 ppm); 4 = calcium (0.8 ppm); 5 = sodium (0.6 ppm); 6 = magnesium (0.4 ppm); 7 = manganese (0.4 ppm); 8 = iron(II) (0.8 ppm); 9 = cobalt (0.8 ppm); 10 = lead (2.0 ppm); 11 = lithium (0.2 ppm). (b) Capillary ion electrophoretic analysis of a standard solution of alkali and alkaline earth metals in the presence of transition metals. Experimental conditions as in (a), except indirect UV detection at 185 nm. Peaks as in (a).

tection limits improve. Table I compares the detection limits obtained using indirect UV detection at 214 nm *versus* indirect UV detection at 185 nm.

Incorporating indirect UV detection at 185 nm into the separation protocol, the effect of altering the mobility of the UV background providing component of the electrolyte on the detection limits of the alkali and alkaline earth metals was investigated. Since the mobility of UVCat-1 is less than the mobilities of the alkali and alkaline earth metals, all the metal ion peaks show some degree of fronting. As explained by Foret *et al.* [10], in addition to the dispersion resulting from diffusion, initial sample width and Joule heat, dispersion due to sorption

TABLE I

COMPARISON OF THE DETECTION LIMITS OBTAINED WITH A 185-nm LAMP AND A 214-nm LAMP

Experimental conditions as described in Fig. 1a and b.

Cation	Detection limits (hydrostatic) (ppb)[a]	Detection limits (electromigration) (ppb)	Difference factor
Potassium	222	142	1.56
Barium	381	237	1.60
Strontium	258	145	1.78
Calcium	102	61	1.67
Sodium	96	57	1.68
Magnesium	55	33	1.66
Lithium	18	11	1.64

[a] Throughout the article the American billion (10^9) is meant.

phenomena and electromigration dispersion [21,22] can be expected to contribute to the dispersion of migrating zones. Electromigration dispersion always occurs during the migration of sample ions which possess effective mobilities different from that of the background electrolyte co-ion [10]. The higher the concentration of the sample component in its zone, the more pronounced the electromigration dispersion. Hence, one way of suppressing electromigration dispersion is by keeping the concentration of the analyte ions in their zones sufficiently below the concentration of the background electrolyte [10]. By keeping the concentration of the analyte ions low, however, greater detection sensitivity is required.
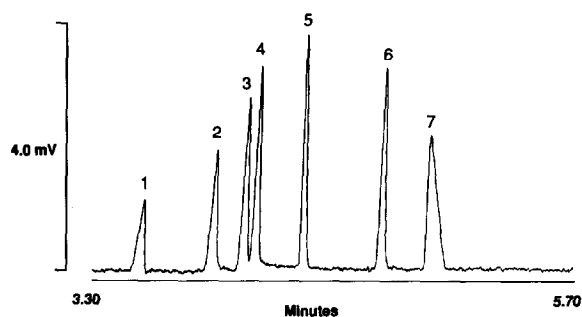

Fig. 2. Capillary ion electrophoretic analysis of a standard solution of alkali and alkaline earth metals. Experimental conditions as in Fig. 1b except using UVCat-2 and tropolone as the electrolyte system instead of UVCat-1 and HIBA. Peaks: 1 = potassium (1.0 ppm); 2 = barium (2.0 ppm); 3 = strontium (1.5 ppm); 4 = calcium (0.7 ppm); 5 = sodium (0.6 ppm); 6 = magnesium (0.4 ppm); 7 = lithium (0.2 ppm).

A more practical way of suppressing electromigration dispersion is to match the mobilities of the background electrolyte more closely with those of the analyte ions. UVCat-1 was therefore replaced by the more mobile UVCat-2. The natural pH of the UVCat-2–HIBA electrolyte was about pH 2. Since the complexing agents employed showed more significant complexation around pH 4, the pH of the electrolyte had to be raised. By raising the pH of the electrolyte, the possibility of an increased number of peaks was introduced, so HIBA was replaced by tropolone. The natural pH of the UV-Cat-2–tropolone electrolyte was pH 4. Fig. 2 shows the separation of the alkali and alkaline earth metals using UVCat-2 as the electrolyte. The mobility of UVCat-2 is similar to that of sodium, resulting in an almost symmetrical peak shape for sodium. The metals migrating faster than sodium all show a small degree of fronting, while the metals migrating slower than sodium show a little tailing. Under these conditions of more closely matched mobilities, the concentration of the analytes may even approach the concentration of the background electrolyte without electromigration dispersion producing a significant effect.

In Table II, the detection limits obtained using UVCat-2 and tropolone, in place of UVCat-1 and HIBA are compared, all other conditions being the same. While the sensitivity for all the metals has improved, the greatest improvement is seen for the peaks whose mobilities most closely match the mobility of UVCat-2, and less improvement is seen either side. Thus it can be seen that by matching the

TABLE II

COMPARISON OF THE DETECTION LIMITS OBTAINED WITH UVCat-1 AND UVCat-2 AS THE ELECTROLYTE

Experimental conditions as in Fig. 2.

Cation	Detection limits (hydrostatic) (ppb)	Detection limits (electromigration) (ppb)	Difference factor
Potassium	142	68	2.09
Barium	237	87	2.72
Strontium	145	46	3.15
Calcium	61	18	3.39
Sodium	57	13	4.38
Magnesium	33	10	3.30
Lithium	11	7	1.57

mobilities of the analyte ions with that of the background electrolyte, more efficient separations are obtained.

Incorporating UVCat-2 into the separation protocol, in addition to indirect UV detection at 185 nm, the effect on sensitivity of extending the capillary path length was investigated. A 75-μm I.D. capillary was used, and a "bubble" was blown at the detection end, approximately 8 cm from the end of the capillary, producing an extended path length of 300 μm. This resulted in an extended path length capillary similar to the "egg-shaped cell" described by Gordon [13]. From the Lambert–Beer law, it would be expected that by increasing the path
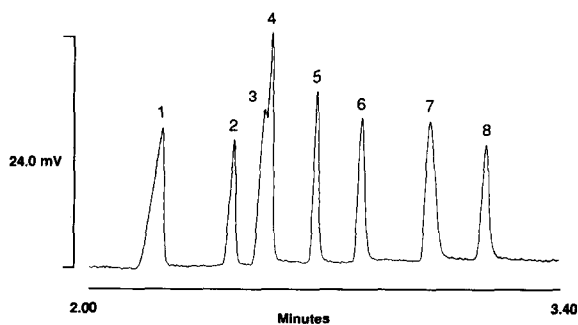

Fig. 3. Capillary ion electrophoretic analysis of a standard solution of alkali and alkaline earth metals in the presence of transition metals. Experimental conditions as in Fig. 2 except the conventional capillary was substituted for an extended path length capillary, with a path length of 300 μm. Peaks: 1 = potassium (1.6 ppm); 2 = barium (2.0 ppm); 3 = strontium (1.6 ppm); 4 = calcium (0.8 ppm); 5 = sodium (0.6 ppm); 6 = magnesium (0.4 ppm); 7 = lithium (0.2 ppm); 8 = manganese (0.8 ppm).

length, the sensitivity would increase. As can be seen from the electropherogram showing the separation of alkali and alkaline earth metals with the extended path length capillary (Fig. 3), the peak heights are significantly larger. Although detection limits have improved, the improvement is not as large as it would appear from the electropherogram, due to a concomitant increase in baseline noise. The detection limits obtained using the extended path length capillary versus a conventional capillary, are compared in Table III.

A significant enhancement in sensitivity for the analysis of inorganic metal cations is seen by altering the injection mode from hydrostatic to electromigration. The separation of 1–2 ppb of the alkali and alkaline earth metals using electromigration injection (10 kV) for 10 s is shown in Fig. 4. With electromigration injection, the sample introduction end of the capillary is placed into the sample vial and a voltage is briefly applied, causing a small band of sample to electromigrate into the capillary. Thus more analyte ions enter the capillary in a given volume of sample than is possible with hydrostatic injection. A comparison of the detection limits obtained using electromigration injection versus hydrostatic injection is shown in Table IV. Using electromigration injection, detection limits of the order of low ppt levels can be obtained.

The reproducibility of the technique for five consecutive injections, using electromigration injection with UVCat-2 as the background providing electrolyte, an extended path length capillary and indirect UV detection at 185 nm, is shown in Fig. 5. The

TABLE III

COMPARISON OF THE DETECTION LIMITS OBTAINED WITH A CONVENTIONAL AND AN EXTENDED PATH LENGTH CAPILLARY

Experimental conditions as in Fig. 3.

Cation	Detection limits (hydrostatic) (ppb)	Detection limits (electromigration) (ppb)	Difference factor
Potassium	68	27	2.5
Barium	87	40	2.2
Strontium	46	–	–
Calcium	18	–	–
Sodium	13	10	1.3
Magnesium	10	6	1.7
Lithium	7	4	1.8

TABLE IV

COMPARISON OF THE DETECTION LIMITS OBTAINED WITH HYDROSTATIC AND ELECTROMIGRATION INJECTION

Experimental conditions as in Fig. 4.

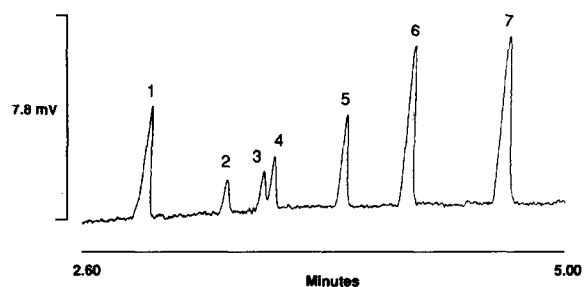| Cation	Detection limits (hydrostatic) (ppb)	Detection limits (electromigration) (ppb)	Difference factor
Potassium	27	52	520
Barium	40	267	150
Strontium	–	210	–
Calcium	–	91	–
Sodium	10	53	190
Magnesium	6	30	200
Lithium	4	31	130

Fig. 4. Capillary ion electrophoretic analysis of a standard solution of alkali and alkaline earth metals. Experimental conditions as in Fig. 3 except using electromigration injection (10 kV for 10 s) instead of hydrostatic injection. Peaks: 1 = potassium (1.0 ppb); 2 = barium (2.0 ppb); 3 = strontium (2.0 ppb); 4 = calcium (1.0 ppb); 5 = sodium (1.0 ppb); 6 = magnesium (1.0 ppb); 7 = lithium (1.0 ppb).

sample is a 1:800 dilution of a standard solution. Despite the fact that reproducibility decreases with increasing dilution, the reproducibility in terms of peak area was between 3 and 12% relative standard deviation (R.S.D.), depending on the cation; lithium determinations showed the best reproducibility with 3% R.S.D. and barium the worst with 12%. The reproducibility in terms of migration times was less than 2% R.S.D. At higher concentrations, reproducibility was generally less than 1% R.S.D. Typical calibration curves obtained for the technique are shown in Fig. 6. Valid calibration is demonstrated over 1.5 orders of magnitude.
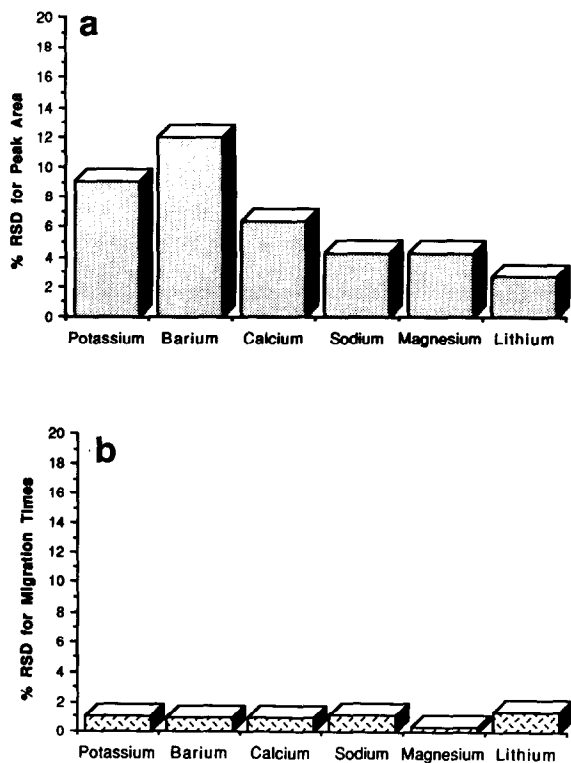
Fig. 5. (a) Graph showing the reproducibility of the technique in terms of peak area for five consecutive injections of a 1:800 dilution of the standard solution. (b) Graph showing the reproducibility of the technique in terms of migration times for 5 consecutive injections of a 1:800 dilution of the standard solution.
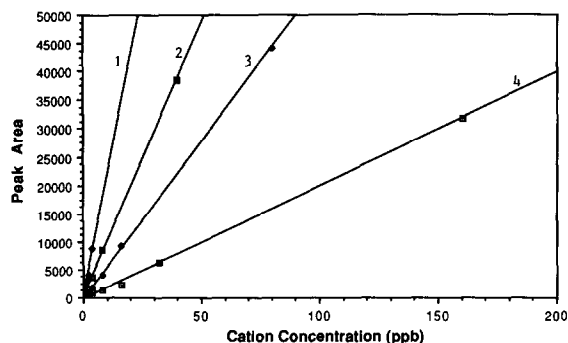

Fig. 6. Graph showing the linearity of the method over 1.5 orders of magnitude. 1 = Lithium ($R^2 = 0.986$); 2 = magnesium ($R^2 = 0.999$); 3 = calcium ($R^2 = 1.000$); 4 = barium ($R^2 = 0.999$).

CONCLUSIONS

There are a variety of ways to improve the detection limits of inorganic metal cations by capillary ion electrophoresis (Waters' trade name Capillary Ion Analysis, or CIA), the most significant of which is electromigration injection in lieu of hydrostatic injection. Using electromigration injection, an increase in sensitivity of between 150 and 500 times is possible. The next most significant method for the enhancement of sensitivity is optimization of the mobility of the UV background providing co-ion in the electrolyte to match the mobilities of the analyte ions. With this approach, an enhancement in sensitivity of between 1.5 and 4.5 times can be achieved. In the case of the inorganic metal cations, a significant improvement in sensitivity is not obtained by extending the capillary cell path length. However, an increase in sensitivity of between 1.3 and 2.5 times could make the difference between detecting and not detecting a peak. From these results, it is also apparent that when choosing a component to provide the UV absorbing background a compromise between its mobility and its UV absorbance is often necessary.

REFERENCES

1 A. Weston. P. R. Brown. P. Jandik. W. R. Jones and A. L. Heckenberg, J. Chromatogr., 593 (1992) 289.
2 A. Weston, P. R. Brown, A. L. Heckenberg, P. Jandik and W. R. Jones, J. Chromatogr., 602 (1992) 249.
3 P. Jandik, W. R. Jones, A. Weston and P. R. Brown, LC · GC, 9 (1991) 634.
4 W. J. Wildman, P. E. Jackson, W. R. Jones and P. G. Alden, J. Chromatogr., 546 (1991) 459.
5 F. Foret, S. Fanali, A. Nardi and P. Boček, Electrophoresis, 11 (1990) 780.
6 W. G. Kuhr and E. S. Yeung, Anal. Chem., 60 (1988) 1832.
7 W. G. Kuhr and E. S. Yeung, Anal. Chem., 60 (1988) 2642.
8 L. Gross and E. S. Yeung, J. Chromatogr., 480 (1989) 169.
9 T. M. Olefirowicz and A. G. Ewing, J. Chromatogr., 499 (1990) 713.
10 F. Foret, S. Fanali, L. Ossicini and P. Boček, J. Chromatogr., 470 (1989) 299.
11 T. Tsuda, J. V. Sweedler and R. N. Zare, Anal. Chem., 62 (1990) 2149.
12 I. H. Grant and W. Steuer, J. Microcol. Sep., 2 (1990) 74.
13 G. B. Gordon, US Pat., 5 061 361 (1991).
14 J.-P. Chervet, US Pat., 5 057 216 (1991).
15 J.-P. Chervet, R. E. J. van Soest and M. Ursem, J. Chromatogr., 543 (1991) 439.
16 R. A. Wallingford and A. G. Ewing, Adv. Chromatogr., 29 (1989) 1.

17 T. Tsuda, T. Mizuno and J. Akiyama, *Anal. Chem.*, 59 (1987) 799.
18 M. Deml, F. Foret and P. Boček, *J. Chromatogr.*, 320 (1985) 159.
19 D. J. Rose and J. W. Jorgenson, *Anal. Chem.*, 60 (1988) 642.
20 X. Huang, M. J. Gordon and R. N. Zare, *Anal. Chem.*, 60 (1988) 377.
21 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 1.
22 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.