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Analysis of halides, oxyhalides and metal oxoacids by capillary electrophoresis with suppressed electroosmotic flow

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Abstract

A rapid and easy method for the determination of halides, oxyhalides and metal oxoacids by capillary electrophoresis was developed. This is the first paper to report their simultaneous analysis. Electroosmotic flow was suppressed more than 40-fold by using a poly(ethyleneglycol)-coated capillary, compared with that in a bare fused-silica capillary under similar conditions. Using this capillary, the separation of eleven anions was accomplished. Anion migration was dependent only on their mobility and detection was carried out directly with a diode-array detector. The R.S.D.s were better than 0.6% for migration time and between 1.0% and 3.4% for peak area. Calibration graphs for all the anions were linear, with correlation coefficients better than 0.9995. Using stacking sample preconcentration, the achievable lower detection limits were found to be in the range 14–260 $\mu g\ l^{-1}$. This represents a 33–43-fold increase in sensitivity over the results obtained by using 200 mbar·s pressure injection.

Keywords: Capillary electrophoresis; Electroosmotic flow; Capillary columns; Halides; Oxyhalides; Metal oxoacids; Oxoacids

1. Introduction

The demand for a reliable and rapid method for the determination of inorganic anions has increased, because of the toxicity of some of these compounds to humans [1–3]. Inorganic anions are usually analyzed using ion chromatography (IC); however, it is difficult to separate halides, oxyhalides and metal oxoacids in a short analysis time. In a conventional anion-exchange column, halides and oxyhalides elute early and tend to be poorly resolved, while metal oxoacids are strongly retained on the column since they are polyvalent anions and they also behave like hydrophobic species by interacting with packing materials.

Capillary electrophoresis (CE) is a new and powerful technique that can provide high separation efficiency. The mechanisms responsible for separation in CE are different from those in chromatography. In CE ionic species are sepa-

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rated based on their charge and size. Application to anion analysis, however, has one drawback. Typically, in CE a fused-silica capillary is used and the direction of electroosmotic flow (EOF) is toward the negative (detection) electrode, whereas anions migrate toward the positive electrode. Therefore, anions have excessive migration times or are not observed at all.

This problem can be overcome by reversing or suppressing the EOF. Tsuda [4] described a method for EOF reversal by adding cetyltrimethylammonium bromide (CTAB) to the buffer. Several reports on anion analysis have used this technique [5–8]. In another approach, Foret et al. [9] suppressed the EOF by adding Triton X-100 to the buffer for organic and inorganic anion analysis.

Gross and Yeung [10] analyzed inorganic anions at low pH, which has an associated low EOF. However, both in the EOF reversal and suppression techniques, complicated buffers have to be used and the EOF depends on the concentration and pH.

A number of polymer-coated capillaries have been developed to suppress the EOF and to avoid wall adsorption of charged molecules [11–14]. In previous work, these polymer-coated capillaries were generally used to analyze biomolecules (i.e. peptides, protein). In this paper we utilized a polymer-coated capillary for inorganic anion analysis. Halides, oxyhalides and metal oxoacids were separated using a coated capillary with a highly suppressed EOF. Given the higher concentration limits of detection associated with CE compared to liquid chromatography (LC), sample stacking was used to increase the sensitivity of the analysis.

2. Experimental

2.1. Chemicals

Anion standards were prepared from their sodium salts, except for chromate (potassium salt). Phosphate buffer (HPCE grade) was obtained from Fluka (Tokyo, Japan). All other chemicals were from Wako (Osaka, Japan). The reagents used were of analytical grade. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Apparatus

All CE experiments were performed using a Hewlett-Packard ^{3D}Capillary Electrophoresis System from Hewlett-Packard (Waldbronn, Germany). The system consists of a CE unit with built-in diode-array detector and an HP ^{3D}CE ChemStation for system control, data collection and data analysis.

2.3. Capillaries

Different types of polymer-coated fused-silica capillaries were tested to study possible interaction between anions and the polymer materials. Three commercially available GC capillaries, polyethyleneglycol (DB-WAX), dimethylpolysiloxane (DB-1) and (50%-cyanopropylphenyl)methylpolysiloxane (DB-225), were purchased from J&W Scientific (Folsom, CA, USA). All capillaries used were 50 μm I.D. (350 μm O.D.) $\times$ 64.5 cm total length (56 cm effective length).

2.4. Electrophoretic procedures

Prior to injection, the capillary was preconditioned for 4 min by flushing with the run buffer. A 20 m*M* phosphate buffer, pH 8.0, was used as the electrolyte. The sample was injected with a pressure of 50 mbar for 4.0 s. The applied voltage was set at -15 kV and the capillary temperature was thermostatted to 20°C. Anions were detected at 200 nm with a spectral bandwidth of 10 nm.

3. Results and discussion

3.1. Comparison of polymer-coated capillaries

The influence of coated phases on the separation of anions was investigated (Fig. 1). When separated using IC, anions such as bromide,

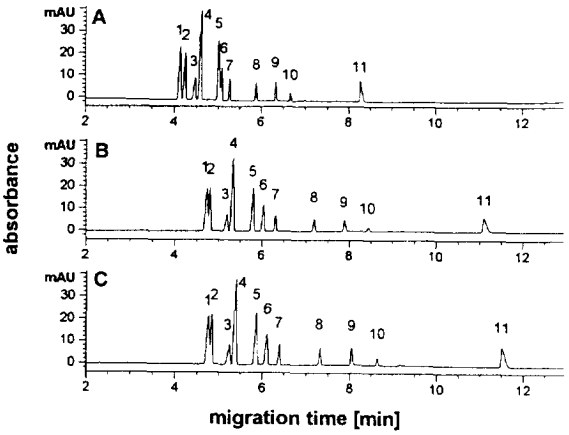

Fig. 1. Influence of capillary phase on anion separation. (A) DB-WAX, (B) DB-1 and (C) DB-225. Experimental conditions: capillary, 50 μm × 64.5 cm (l = 56 cm); buffer, 20 mM phosphate buffer, pH 8.0; applied potential, −20 kV; injection pressure, 4 s at 50 mbar; capillary temperature, 20°C; detection, 200 nm.

iodide and thiocyanate show peak tailing and their elution times tend to increase when using hydrophobic packing materials. In the case of CE, although the migration times of the anions are different, their migration order is the same in all three capillary types. These results indicated that anions exhibit no or minimal interaction with the capillary coatings tested.

With respect to detection sensitivity, the peak heights of the anions were the same in the different capillaries, but the baseline noise in DB-WAX was half that in DB-1 and one-third that in DB-225. In addition, DB-WAX also provided good migration time reproducibility. When using the DB-225 capillary and especially the DB-1 capillary, migration times of anions decreased with repeated runs. Therefore, the DB-WAX capillary was used for all subsequent experiments.

3.2. Electroosmotic mobility determination

The electroosmotic mobility, u_{eo}, was calculated using the following equation

$$u_{eo} = lL/tV (cm^2 V^{-1} s^{-1}),$$

where l and L are the length of the capillary to the detector and the total length of the capillary, respectively, V is the applied potential and t is the migration time of the neutral marker (both benzyl alcohol [15] and acetone). After 300 min, no peak was observed, therefore the calculated electroosmotic mobility was $<0.1 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}.

This indicated that the EOF in the DB-WAX capillary was <2.5% of the EOF typically associated with uncoated capillaries under the same conditions [16].

3.3. Separation of inorganic anions

Effect of buffer pH

Separations of thirteen inorganic anions were studied over the pH range 5.0–9.0 using 20 mM phosphate buffer (Fig. 2). Migration times, electroosmotic mobilities and equivalent ionic conductivities [17] are shown in Table 1. The electrophoretic mobilities were calculated using migration time data at a pH value of 8.0. Jones and Jandik [8] reported that plotting the migration times of anions against equivalent ionic conductivities gives a second-order polynomial curve. However, in this study plots of electrophoretic mobilities against equivalent ionic conductivities were linear, with a correlation coefficient of 0.917 (Fig. 3).

As shown in Fig. 2, selectivity changes were observed by varying the pH. In particular the migration times of chromate, arsenite and arsenate changed markedly with pH. The migration time of chromate ($pK_{a2} = 6.49$) decreased between pH 6 and 7. At pH values higher than 6.49, chromate is a divalent anion and it will therefore have a larger charge-to-mass ratio, higher mobility and will migrate faster. At a pH below 6.49 chromate is a monovalent anion. Similarly, the migration time of arsenate (pK_{a2} = 7.08) decreased as its ionic charge increased. Below pH 8.0 arsenite ($pK_{a1} = 9.05$) was not observed even after 20 min. This is because it is neutral at pH values less than its pK_{a1} and it will move in the direction and with the velocity of the EOF, which means that in this case, since the

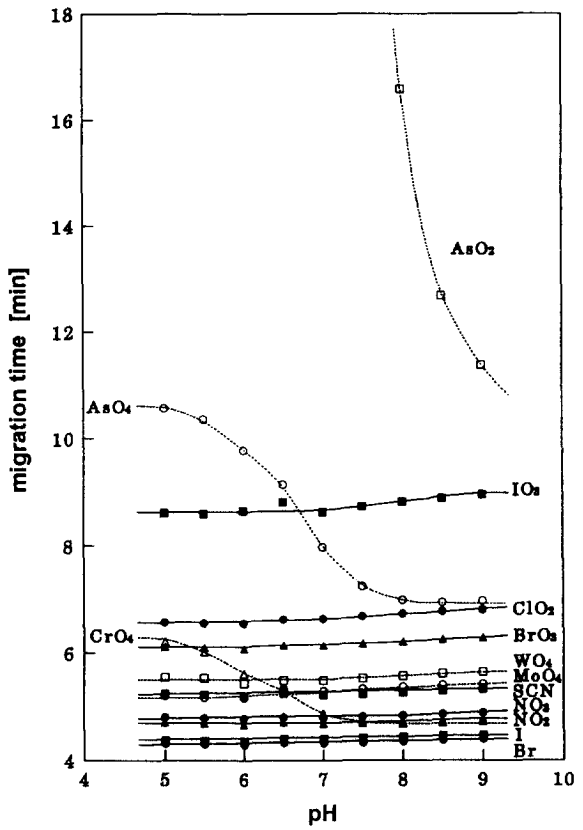

Fig. 2. Influence of buffer pH on anion separation. Experimental conditions: capillary, DB-WAX 50 μm × 64.5 cm (l = 56 cm); buffer, 20 mM phosphate buffer; applied potential, −20 kV; injection pressure, 4 s at 50 mbar; capillary temperature, 20°C; detection, 200 nm.

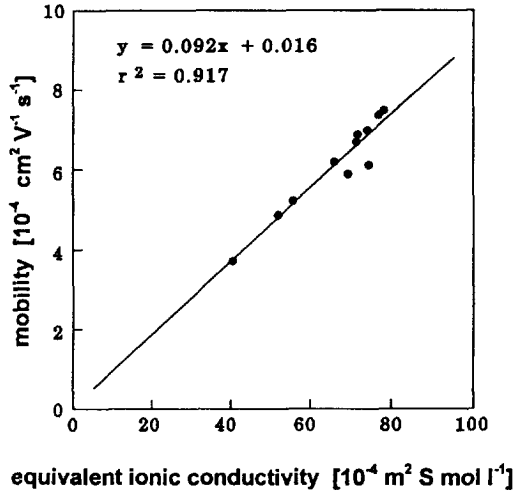

Fig. 3. Relationship between equivalent ionic conductivity and electrophoretic mobility. Experimental conditions: buffer, 20 mM phosphate, pH 8.0. Other conditions as in Fig. 2.

EOF was fully dimished, the arsenite did not migrate.

Effect of buffer concentration

The effect of the buffer concentration on the anion separation was investigated. Using a phosphate buffer at pH 8, the buffer concentration was varied from 5 to 50 mM (Fig. 4). Changing the buffer concentration can have a significant effect on the effective mobility, and subsequent

Table 1
Comparison of migration times, calculated mobilities and equivalent ionic conductivities

Anion	Species	Migration time (min)	Mobility (10^{-4} cm^2 V^{-1} s^{-1})	Equivalent ionic conductivity (10^{-4} m^2 S mol l^{-1})
Bromide	Br	4.313	6.98	74.8
Iodide	I	4.387	6.86	73.6
Chromate	CrO_4^{2-}	4.862	6.19	69.8
Nitrite	NO_2^-	4.680	6.43	68.8
Nitrate	NO_3^-	4.789	6.29	67.0
Thiocyanate	SCN^-	5.216	5.77	62.0
Molybdate	MoO_4^{2-}	5.284	5.70	61.2
Tungstate	WO_4^{2-}	5.483	5.49	58.9
Bromate	BO_3^-	6.144	4.90	52.3
Chlorite	ClO_2^-	6.689	4.50	48.8
Iodate	IO_3^-	8.623	3.49	37.2

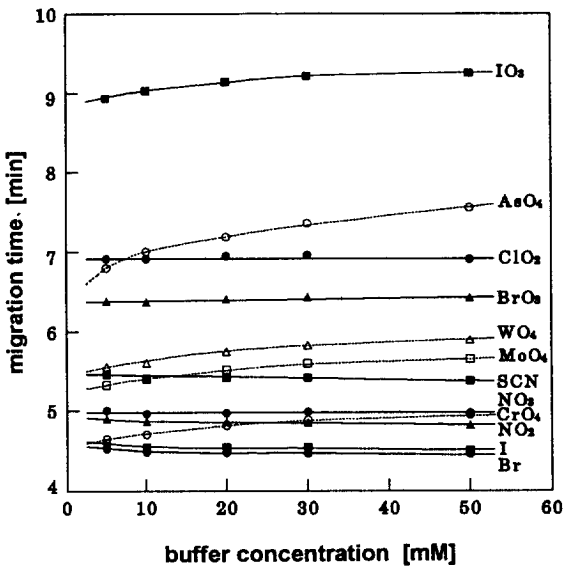

Fig. 4. Influence of buffer concentration on anion separation. Experimental conditions: buffer, phosphate buffer. Other conditions as in Fig. 2.

migration time, of an analyte when this mobility contains a significant proportion of electroosmotic mobility [11]. This is because increasing the buffer concentration will decrease the electroosmotic mobility. However, in these experiments, changes in buffer concentration had a negligible effect on migration time, because the EOF was effectively eliminated in this study. Only metal oxoacids showed increasing migration times with increasing buffer concentration. This result suggests that divalent anions are more sensitive to buffer concentration than monovalent anions. This effect of buffer concentration is expected in light of the well-known dependence of mobility on ionic strength, which has a larger effect on the mobility of higher charged ions ($3^- > 2^- > 1^-$) [18].

The peak shape was considerably influenced by the buffer concentration. At a buffer concentration of 5 m*M*, all peaks were broadened and poorly shaped. Increasing the buffer concentration improved the peak shape; however, an increase in baseline drift was observed above 30 m*M*, therefore 20 m*M* was chosen as the optimum buffer concentration.

Influence of applied potential

Increasing the applied potential above −25 kV resulted in baseline drift and a loss of resolution. Although the analysis time was increased at the lower potential of −15 kV, the resolution was much improved.

Influence of capillary temperature

The influence of capillary temperature was studied at 15, 20, 25 and 30°C. Resolution between thiocyanate and molybdate became poor at 25°C and these peaks merged above 30°C. Although the resolution was much better at 15°C, this resulted in a long analysis time. Optimum resolution with minimal analysis time was obtained at 20°C.

3.4. Detection

Ion analysis by CE has generally utilized indirect detection methods. However, in the present study direct UV absorbance detection was employed. The detection wavelength was optimized by acquiring the spectrum of each inorganic anion using a diode-array detector. Values of λ_{max} and the calculated molar absorptivities from the spectrum of the anions are shown in Table 2. Detection was carried out at

Table 2
Values of λ_{max} value and molar absorptivity of inorganic anions

Species	λ_{max} (nm)	Molar absorptivity ($cm^{-1}\ mol^{-1}\ l$)
Bromide	<190	$6.4 \cdot 10^3$ [a]
Iodide	195	$1.1 \cdot 10^4$
Chromate	<190	$7.4 \cdot 10^3$ [a]
Nitrite	210	$3.1 \cdot 10^3$
Nitrate	203	$5.8 \cdot 10^3$
Thiocyanate	<190	$8.1 \cdot 10^3$ [a]
Molybdate	209	$1.1 \cdot 10^4$
Tungstate	197	$8.1 \cdot 10^3$
Bromate	<190	$5.1 \cdot 10^3$ [a]
Chlorite	<190	$1.4 \cdot 10^3$ [a]
Arsenate	<190	$4.4 \cdot 10^3$ [a]
Iodate	197	$4.6 \cdot 10^3$

[a] Molar absorptivity at 190 nm.

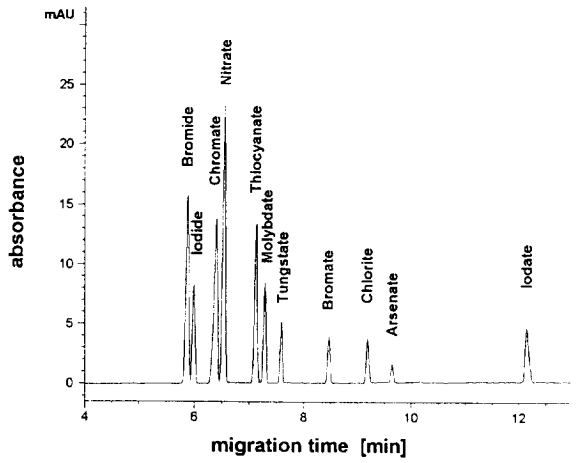

Fig. 5. Electropherogram of 50 mg l^{-1} each of inorganic anions. Experimental conditions: buffer, 20 m*M* phosphate buffer, pH 8.0; applied potential, −15 kV. Other conditions as in Fig. 2.

200 nm because most anions had an absorption maximum around this wavelength or below 190 nm.

3.5. Method validation

Reproducibility, linearity, sensitivity and stability of the method were tested. Fig. 5 shows an electropherogram of 50 mg l^{-1} of eleven inorganic anion standards, demonstrating that halides, oxyhalides and metal oxoacids were well resolved in a short analysis time. Table 3 shows the excellent reproducibilities obtained for migration time and peak area as reflected by the %R.S.D. This high degree of reproducibility is a direct result of the highly suppressed and stable EOF. The calibration graphs for all inorganic anions were linear over the range 10–200 mg l^{-1}, with correlation coefficients better than 0.9995. The detection limits for all inorganic anions were in the range 0.5–11 mg l^{-1} at a signal-to-noise ratio of 3. The lifetime of the DB-WAX exceeded more than 200 injections without any noticeable impairment of the separation efficiency.

3.6. Sample stacking

In order to obtain a lower minimum detection level, sample stacking was investigated. Vinther and Soeberg [19,20] reported that sample stacking occurs when the conductivity of the injected sample is lower than that of the surrounding buffer. By increasing the injection time, ranging from 20 to 500 s with a pressure of 50 mbar, the influence of injection volume on the anion separation was studied. With an injection volume

Table 3
Reproducibility, linearity and sensitivity

Species	R.S.D. (n = 10) (%)		Linearity correlation	Detection limit (mg l^{-1})
	Migration time	Peak area		
Bromide	0.5	1.0	0.9998	0.8
Iodide	0.4	1.2	0.9995	1.1
Chromate	0.4	1.2	0.9999	1.8
Nitrate	0.5	1.0	0.9999	0.5
Thiocyanate	0.5	1.4	0.9999	0.9
Molybdate	0.5	1.2	0.9999	1.3
Tungstate	0.5	1.3	0.9999	2.9
Bromate	0.5	2.9	0.9998	3.5
Chlorite	0.5	2.2	0.9999	3.5
Arsenate	0.2	3.4	0.9998	11.1
Iodate	0.6	1.8	0.9999	3.0

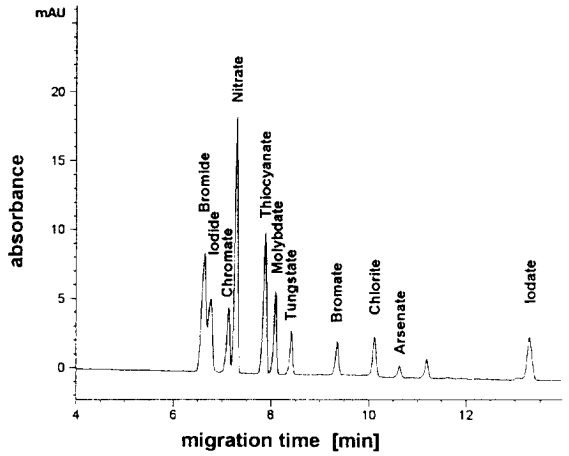

Fig. 6. Electropherogram of 1 mg l^{-1} each of inorganic anions by using sample stacking. Experimental conditions: injection pressure, 200 s at 50 mbar. Other conditions as in Fig. 5.

from lower than or equal to 10 000 mbar · s, good resolution was maintained. However, at an injection volume from 25 000 mbar · s, bromide and iodide could not be separated. Therefore, using the injection volume from 10 000 mbar · s, reproducibility, linearity and sensitivity were evaluated. Fig. 6 shows an electropherogram of 1 mg l^{-1} of anion standards using sample stacking. The R.S.D.s ($n = 5$) were better than 0.6% for the migration times and 1.5–5.3% for the peak areas. Over the anion concentration range 0.2–5 mg l^{-1}, satisfactory linearity ($r = 0.9993$–0.9999) could be obtained. However, above a concentration of 10 mg l^{-1}, peak broadening was observed. As shown in Table 4, the detection limits for anions ranged from 14 to 260 $\mu g\ l^{-1}$ at a signal-to-noise ratio of 3. The use of stacking sample injection resulted in a 33–43 fold increase in sensitivity over a 200 mbar · s pressure injection. Isotachophoretic preconcentration [21] was also studied using 10 m*M* sodium pyrophosphate as the leading electrolyte and 30 m*M* octane sulfonic acid sodium salt as the terminating electrolyte. Although reproducibility and linearity of isotachophoretic preconcentration were the same as for sample stacking, the maximum injection volume was less than for sample stacking. Furthermore, sample stacking was much easier. These results indicate that sample stacking is useful for trace-anion analysis.

4. Conclusions

A reliable and easy method for the determination of halides, oxyhalides and metal oxoacids has been developed. The DB-WAX capillary provided a highly suppressed EOF, enabling a separation of anions in less than 13 min. This method demonstrated excellent reproducibility, good linearity and stable capillary life time. By using sample stacking, a more than 33-fold increase in sensitivity was obtained. These results demonstrate that the use of a coated capillary with an associated suppressed EOF is useful for anion analysis. Further applications of this technique are now being studied.

Table 4
Detection limit of anions by using sample stacking

Specie	Detection limit ($\mu g\ l^{-1}$)	Species	Detection limit ($\mu g\ l^{-1}$)
Bromide	24	Tungstate	75
Iodide	33	Bromate	99
Chromate	53	Chlorite	82
Nitrate	14	Arsenate	260
Thiocyanate	23	Iodate	74
Molybdate	40		

References

[1] A.M. Dietrich, T.D. Ledder, D.L. Gallagher, M.N. Grabeel and R.C. Hoehn, Anal. Chem., 64 (1992) 496.
[2] W.R. Haag, Water Res., 27 (1993) 521.
[3] M. Martinez and M. Aguilar, J. Chromatogr. A, 676 (1994) 443.
[4] T. Tsuda, J. High Resolut. Chromatogr. Chromatogr. Commun., 10 (1987) 622.
[5] X. Huang, J.A. Luckey, M.J. Gordon and R.N. Zare, Anal. Chem., 61 (1989) 766.
[6] J. Romano, P. Jandik, W.R. Jones and P.E. Jackson, J. Chromatogr., 546 (1991) 411.
[7] B.F. Kenney, J. Chromatogr., 546 (1991) 423.
[8] W.R. Jones and P. Jandik, J. Chromatogr., 546 (1991) 445.
[9] F. Foret, S. Fanali, L. Ossicini and P. Bocek, J. Chromatogr., 470 (1989) 299.
[10] L. Gross and E.S. Yeung, J. Chromatogr., 480 (1989) 169.
[11] S.F.Y. Li, Capillary Electrophoresis–Principles, Practice and Applications, J. Chromatogr. Library Ser., Vol. 52, Elsevier, Amsterdam, 1993.
[12] B.J. Herren, S.G. Shafer, J. van Alstine, J.M. Harris and R.S. Snyder, J. Colloid Interface Sci., 115 (1987) 46.
[13] A.M. Dougherty, C.L. Woolley, D.L. Williams, D.F. Swaile, R.O. Cole and M.J. Sepaniak, J. Liq. Chromatogr., 14 (1991) 907.
[14] J.A. Lux, H. Yin and G. Schomburg, J. High Resolut. Chromatogr., 13 (1990) 145.
[15] Y.-H. Lee and T.-I. Lin, J. Chromatogr. A, 675 (1994) 227.
[16] K.D. Lukacs and J.W. Jorgenson, J. High Resolut. Chromatogr. Chromatogr. Commun., 8 (1985) 407.
[17] R.C. Weast, M.J. Astle and W.H. Beyer, Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 69th ed., 1988.
[18] D. Kaniansky, V. Madajova, I. Zelensky and S. Stankoviansky, J. Chromatogr., 194 (1980) 11.
[19] A. Vinther and H. Soeberg, J. Chromatogr., 559 (1991) 3.
[20] A. Vinther and H. Soeberg, J. Chromatogr., 559 (1991) 27.
[21] D.S. Stegehuis, H. Irth, U.R. Tjaden and J. van der Greef, J. Chromatogr., 538 (1991) 393.