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Influence of ionic strength and organic modifier on performance in capillary electrochromatography on phenyl silica stationary phase

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Abstract

The influence of three physicochemical parameters (temperature, ionic strength and organic modifier content of the hydro-organic buffer) upon electrophoretic (electroosmotic flow, EOF, chromatographic (retention factor) and separation (retention time, peak efficiency) performances has been carefully investigated in capillary electrochromatography (CEC) on a phenyl bonded silica column. Five benzodiazepines (diazepam, lorazepam, oxazepam, temazepam, tofisopam) have been selected as test solutes.

From our CEC results, an increase of the organic modifier content induces an increase of EOF and peak efficiency and a decrease of retention factor. Concerning the ionic strength parameter, an increase of the ionic strength undergoes a decrease of EOF and retention factor and an increase of peak efficiency. Finally, higher temperature of the column involves an increase of EOF and peak efficiency and a decrease of retention factor. So, the modification of ionic strength and temperature in CEC can mainly be interpreted as a CE-like behavior at the opposite of organic modifier content which acts as a LC-like behavior.

At last, the CEC separation of these benzodiazepines has been achieved in 18 min, using Tris·HCl (pH 8)–acetonitrile (60:40) mixture, ionic strength 5 m*M* as mobile phase, and a 3 μm phenyl-bonded silica as stationary phase. High peak efficiencies (200 000 theoretical plates/meter) and resolutions of 1.5 are easily obtained.

Keywords: Mobile phase composition; Electrochromatography; Benzodiazepines; Phenyl-bonded silica

1. Introduction

Benzodiazepines are frequently prescribed for pharmacotherapy of epilepsy, convulsions and many psychiatric disorders [1]. The analysis of such compounds is thus an important operation in many pharmaceutical analytical laboratories [2]. In general, the separation of benzodiazepines is performed by liquid chromatography (LC) using reversed-phase systems composed of silica support materials and chemically bonded alkyl chains [3] and by normal-phase chromatography with medium polarity stationary phases [4]. Separation on porous graphitic carbon (PGC) stationary phase have also been reported [5,6]. For neutral compounds (such as benzodiazepines) which are difficulty separated by capillary electrophoresis (CE), there may be a need for an alternative technique to LC which provides greater resolution. Micellar Electrokinetic Capillary Chromatography (MECC) was developed as such an alternative. MECC has been successfully used to separate benzodiazepines [7–11]. Recently, Renou-Gonnord and David [10] reported MECC separation of benzodiazepines using 50 m*M* borate–50 mM sodium dodecyl sulfate (SDS)–20 m*M* γ-cyclodextrin–2 *M* urea (pH 9.2) containing 1% of tetrahydro-

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furan as the running buffer. Imazawa et al. [11] have also developed MECC separation of benzodiazepine antiepileptics using a separation buffer composed of borate (pH 9.5)–18 m*M* SDS and 14% acetonitrile as an organic modifier. But, MECC has several drawbacks which limit its usefulness. Inherent problems in MECC are the limited elution range (or elution window), the coelution of hydrophobic compounds with the micelles resulting in a lack of separation and the insolubility of many classes of compounds in the surfactant containing MECC buffer. In order to solubilize and subsequently separate hydrophobic compounds, several buffer additives have been used in MECC including organic solvents, cyclodextrins or urea [12]. However, there is some incompatibility with mass spectrometry detection because of the high concentrations of surfactants used. These disadvantages preclude the utility of MECC as a routine analytical technique. Capillary electrochromatography (CEC) has become a valuable alternative to MECC for the separation of neutral molecules. CEC is an electrokinetic separation technique which received increasing attention over the past few years [13,14]. CEC is a hybrid technique that uses features of both LC and CE [13–18]. In CEC, the separation of solutes is based on their partitioning between phases and on their ratio of charge to friction coefficient (electrophoretic mobility). The mobile phase in CEC is driven via electroosmotic flow (EOF) induced by applying an electrical field over the column ; EOF originates from the electrical double layer generated at the surface of the capillary wall as well as at the surface of the packing material [19]. The flow profile of EOF in CEC is essentially flat as compared with the parabolic flow profile of pressure-driven. This flat flow profile is a contributing factor to the high efficiencies observed in CEC [20]. CEC has recently become more popular due to advances in CE techniques and equipment. Modern CE instruments offer sensitive on-column detectors and high capacity auto-samplers. The ability to repeatabilily inject low nanoliter volumes is also an important feature of modern CE equipment. The current range of solutes separated by CEC concerns neutral drugs and polycyclic aromatic hydrocarbons (PAHs) using generally C_{18} reversed-phase-packings [21–25]. The aim of our study was to develop CEC separation of benzodiazepines using another analytical system (phenyl-bonded silica) in order to obtain different selectivities and to achieve shorter analysis times and to understand the electrophoretic and chromatographic behaviors in CEP on a phenyl silica stationary phase.

2. Experimental

2.1. Capillary electrochromatography apparatus

CEC separation was performed on a P/ACE 2100 apparatus (Beckman-Coulter, Fullerton, CA, USA) equipped with a UV absorbance detector. Electropack phenyl column, EP-75-40-3-PH (40 cm packed (47 cm total)×75 μm I.D., 3 μm phenyl) was purchased from Unimicro Technologies, (Pleasanton, CA, USA). Packed capillary column was installed in a Beckman Model 2100 P/ACE capillary cartridge holder that was then inserted to into P/ACE instrument. A packed capillary column was positioned in the capillary cartridge holder and then pre-conditioned with the mobile phase by pressurization at around 500 p.s.i. with a syringe pressurized with a manual syringe pump (1 p.s.i.= 6894.76 Pa). The packed capillary column was installed in the P/ACE instrument and was further conditioned by driving the mobile phase through the capillary at an applied voltage of 5 kV for 60 min followed by 10 and 15 kV for 30 min. The mobile phase used in these conditioning was a Tris·HCl buffer (pH 8) containing 80% (v/v) acetonitrile (ionic strength in the final eluent: 5 m*M*).

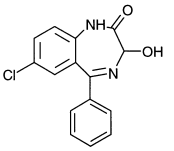
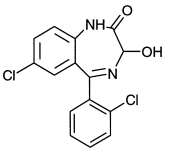
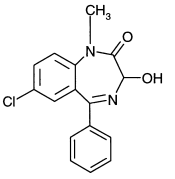
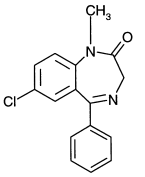
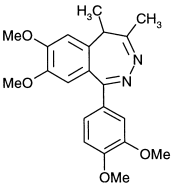
The pH of each buffer was checked on a Beckman pH meter (Model Φ10, Fullerton, CA, USA). The eluents were prepared by first adjusting the buffer to the desired pH then mixing with organic modifier. The analytes were electrokinetically injected into the packed capillary column at +10 kV for 4 s. The temperature of the separation was controlled by immersion of the capillary in a cooling liquid circulating in the cartridge (cooling liquid was purchased from Beckman-Coulter). The analytes were detected by monitoring their absorbance at 220 nm. Buffer preparation was achieved with the help of Phoebus software (Sedere, Franklin, MA, USA). Phoebus is a program that is designed to assist the scientist in creating and preparing buffers to be used

in capillary electrophoresis. This program can be used to determine a broad range of chemical, electrical and electrophoretic characteristics of buffer and can assist in the generation of the desired buffer using stock solutions.

2.2. Chemicals

Tris(hydroxymethyl)aminomethane (Tris) and thiourea were of analytical grade and obtained from Sigma (St. Louis, MO, USA). All buffers were adjusted to the desired pH using HCl (Sigma). The solvents used (acetonitrile, methanol, tetrahydrofuran) were of HPLC ultra gradient grade and were purchased from J.T. Baker (Noisy le Sec, France). The water used to the preparation of electrolytes was of HPLC quality obtained from Elgastat UHQ II system (Villeurbanne, France). The mobile phase was degassed by ultrasonication and filtered before use through a polypropylene filter with 0.7 μm porosity (Whatman, Clifton, NJ, USA).

Table 1
Structures of the studied benzodiazepines

	Solute	Structure
1	Oxazepam	
2	Lorazepam	
3	Temazepam	
4	Diazepam	
5	Tofisopam	

3. Results and discussion

In this study, a standard mixture of five benzodiazepines (diazepam, lorazepam, oxazepam, temazepam, tofisopam) was selected. Table 1 shows the chemical structures and numerical designations for each of the benzodiazepines. The main technical problem occurring in CEC was the presence of bubbles within the column, which lead to the breakdown of current and localized heating which induces drying out of the packed column. This would be a real problem because the packed capillary needs to be reconnected to a pumping system to remove air bubbles. Nevertheless, the formation of bubbles can be prevented by pressurizing the system [26,27]. Without any pressurization, only low buffer concentration can be used to limit Joule heating. However, the use of such diluted buffer results poor retention time reproducibility [25]. This reproducibility problem can be minimized by using zwitterionic buffers [e.g., 2-(N-morpholino)ethanesulfonic acid (MES) or Tris]. Indeed, the low conductivity of zwitterionic buffers allows higher concentrations to be used than for inorganic buffers. As in CE, increasing the pH of the mobile phase results in an increased EOF [27–29], which is caused by the dissociation of the surface silanol groups. This effect diminishes as the pH rises above 8 [29]. The use of high pH offered advantages both in terms of minimizing equilibration time when changing electrolyte and also minimizing analysis time in general. The selected electrolyte was composed of Tris and HCl at pH=8. Besides, Tris/HCl buffer has maximum buffering capacity around pH 8 and so allows to have a great stability of the electroosmotic flow.

3.1. Effect of the acetonitrile content

3.1.1. Electroosmotic flow

In CEC, the transport of mobile phase through the column is achieved by EOF. The origin of this flow is the electrical double layer that is formed at the solid–liquid interface of a charged surface in contact with an electrolyte solution. In a capillary packed with silica particles, both surfaces of the capillary wall and of the particles are negatively charged due to the dissociation of silanol groups. Consequently, the solution at the interface bears a net positive charge (caused by ions in solution). When an electrical field is applied to the column the ions migrate towards the cathode moving the bulk solution by viscous drag. The EOF velocity depends on the density of charges of the capillary wall and of the silica particles as well as on the properties of the eluent.

The relation between the electroosmotic velocity, ν_{eo}, and the zeta potential, ζ, has been given by Smoluchowski:

$$\nu_{eo} = -\frac{\varepsilon_{eo}\varepsilon\zeta}{\eta} \cdot E \quad (1)$$

where E is applied electric field strength, ϵ_o the dielectric constant of free space, ϵ the dielectric constant of the medium and η the viscosity of the medium.

The percentage of organic solvent influences the EOF velocity. Fig. 1 shows the variation of electroosmotic mobility versus organic modifier content on column packed with phenyl bonded silica. The EOF mobility was determined from the elution time of thiourea. In our set of experiments, the ionic strength of the aqueous buffers (Tris·HCl, pH 8) was kept constant (5 m*M*) in the hydroorganic buffer with variable acetonitrile concentrations.

On the contrary of CE, we observed a 17% increase in EOF velocity with an increase in acetoni-

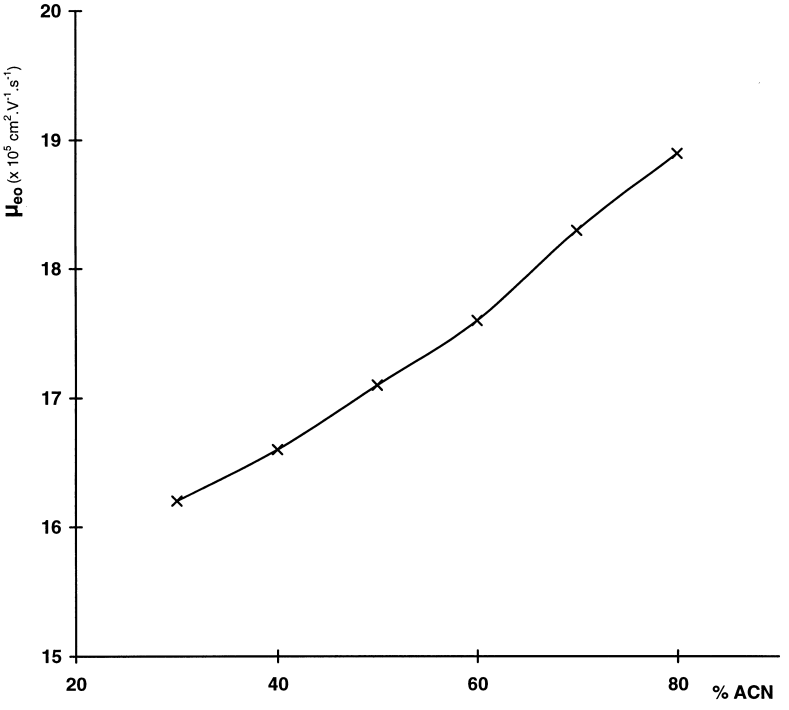


Fig. 1. Effect of the acetonitrile content on electroosmotic flow mobility in CEC. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total) 75 μm I.D.]; electrolyte : Tris·HCl (pH 8)–acetonitrile mixture (ionic strength 5 m*M*) ; temperature: 25°C ; applied voltage : +20 kV; UV detection: 220 nm ; electrokinetic injection: 4 s (+10 kV) ; EOF marker: thiourea.

trile concentration from 30 to 80% (EOF varies from $+16.2\cdot10^{-5}$ to $+18.9\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, respectively) on phenyl bonded silica stationary phase in CEC. Several contradictory reports have been published on this topic [20,30,31]. Firstly, the dependence of EOF and of zeta potential on the organic solvents has been studied by Schwer et al. [32] in fused silica capillaries ; the electroosmotic flow was found to decrease steadily with increasing fraction of organic solvent. At the opposite, Lelievre et al. [33] observed an increase in the electroosmotic mobility with acetonitrile content in 1 m*M* phosphate buffer (pH 6.5) in the range of 40 to 80% of acetonitrile with a capillary packed with 3 μm ODS particles. Similar behavior has been reported by Choudhary et al. [31] upon increasing acetonitrile concentration from 0 to 60% in CEC column packed with ODS silica.

3.1.2. Retention factor

In CEC, the separation of solute is based on their partitioning between phases and on their ratio of charge to friction coefficient (electrophoretic mobility). Then, the migration velocity of solute, ν, is given by the apparent velocity, ν_{app}, in the mobile phase and by the ratio of analyte quantity in the stationary phase to analyte quantity dissolved in the mobile phase:

$$\nu = \frac{1}{1+k'} \cdot \nu_{app} = \frac{1}{1+k'} \cdot (\nu_{eo} + \nu_{ep}) \quad (2)$$

where ν_{ep} is the electrophoretic velocity of the analyte and $\nu_{eo}$is the velocity of the electroosmotic flow and k' the retention factor.

For neutral solute, the electrophoretic velocity is equal to zero and k' reflects a purely chromato-

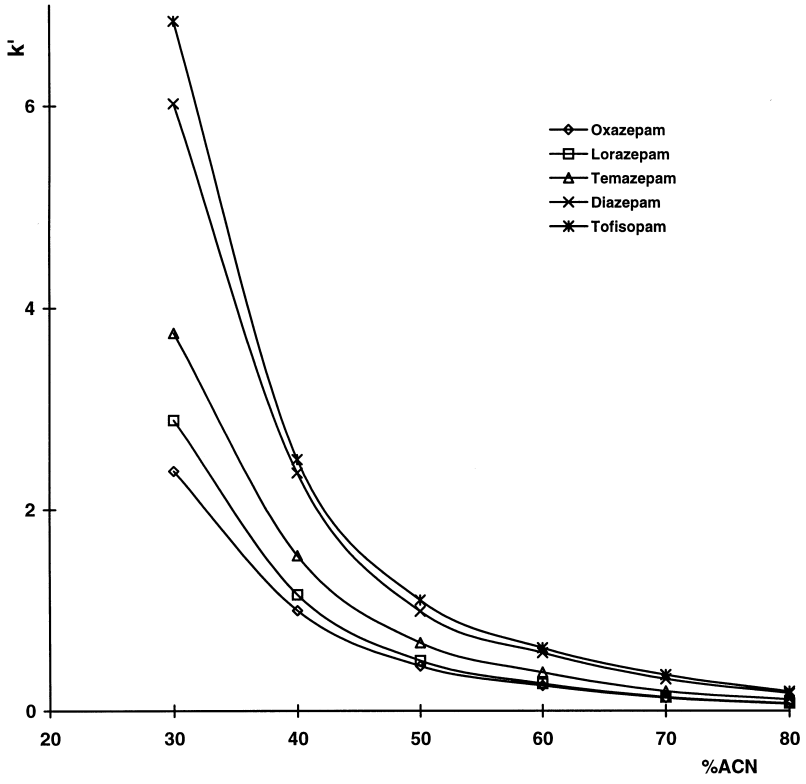


Fig. 2. Effect of the acetonitrile content on retention factors of benzodiazepines in CEC. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×75 μm I.D.] ; electrolyte : Tris·HCl (pH 8)–acetonitrile mixtures (ionic strength 5 m*M*) ; temperature : 25°C; applied voltage : +20 kV ; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV) ; benzodiazepine concentration : 100 ppm.

graphic process. Then, the retention factor can be expressed as [34]:

$$k' = \frac{t_M - t_{eo}}{t_{eo}} \quad (3)$$

where t_M is the retention time and t_{eo} the electro-osmotic time (thiourea).

The retention factor of each benzodiazepine was determined on phenyl bonded silica stationary phase at several acetonitrile percentages of Tris·HCl (pH 8)–acetonitrile mixtures (ionic strength 5 m*M*) and shown in Fig 2.

As expected in LC, the retention of solutes decrease with increasing in acetonitrile concentration. The log k' values of benzodiazepines are plotted versus acetonitrile concentration (Fig. 3). The results show that there are two concentration ranges in which the values of log k' vary proportionally with the acetonitrile content. In the first interval (30–50% of acetonitrile), log k' *vs.*% ACN decreases faster than in the range of the percentage varied from 50 to 80%. These results are in agreement with previous works concerning the analysis of aromatic hydrocarbons by reversed-phase liquid chromatography [35,36]. Indeed, log k'-values *vs.* acetonitrile content plots are directly related to the adsorption isotherm of acetonitrile. It should be stressed that the range where log k' vs.% MeCN of the benzodiazepines examined decreases more steeply should be related to the interval where the concentration of acetonitrile in the stationary phase increases rapidly.

To the first approximation, the retention mechanism of benzodiazepines on a silica phenyl stationary phase with an acetonitrile/water mobile phase was based on Horváth et al.'s solvophobic theory [37]. For a given mobile phase, benzodiazepine retention is determined by π–π interactions between the phenyl stationary phase and aromatic groups of benzodiazepines and by hydrophobic interactions

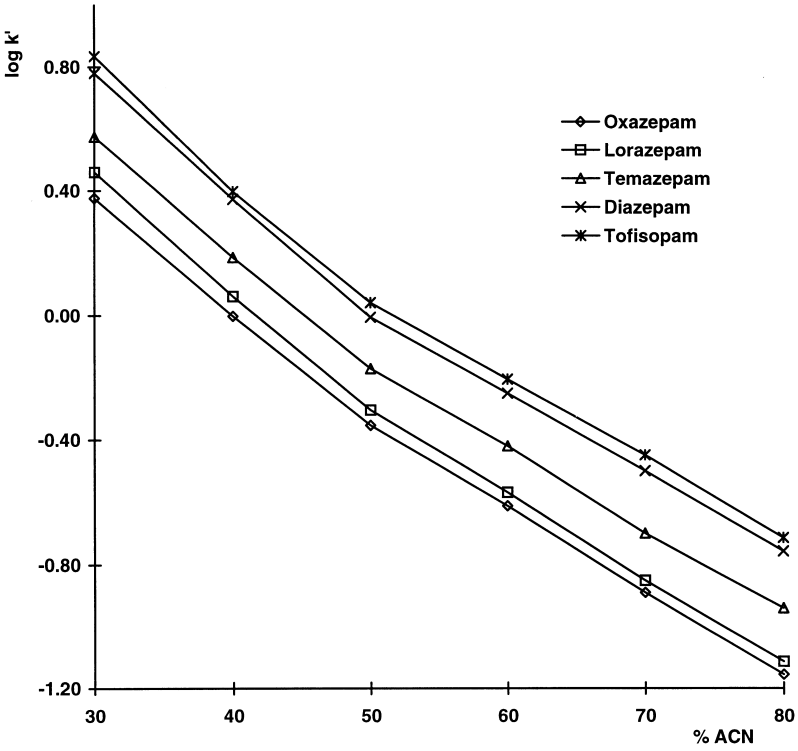


Fig. 3. Effect of the acetonitrile content on retention factor logarithm of benzodiazepines in CEC. Electropak phenyl column, EP-75-40-3-PH [×40 cm packed (47 cm total) 75 μm I.D.] ; electrolyte: Tris·HCl (pH 8)–acetonitrile mixture (ionic strength 5 m*M*); temperature: 25°C; applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm.

Table 2
Effect of the acetonitrile content on retention times of benzodiazepines in CEC. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×75 μm I.D.]; electrolyte: Tris·HCl (pH 8)–MeCN mixtures (ionic strength 5 m*M*); temperature: 25°C; applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm

ACN %	t_{eo} (min)	t_M (min)				
		Oxazepam	Lorazepam	Temazepam	Diazepam	Tofisopam
80	8.29	8.87	8.93	9.24	9.74	9.89
70	8.54	9.64	9.70	10.20	11.24	11.57
60	8.92	11.15	11.33	12.31	14.05	14.48
50	9.14	13.19	13.67	15.30	18.17	19.19
40	9.44	18.83	20.31	23.96	31.72	33.00
30	9.7	32.78	37.71	46.11	68.14	76.04

between the phenyl stationary phase and the nonpolar moiety of benzodiazepine molecule. Thus, the migration order of benzodiazepines is $t_{R\ Oxazepam} < t_{R\ Lorazepam} < t_{R\ Temazepam} < t_{R\ Diazepam} < t_{R\ Tofisopam}$.

3.1.3. Peak efficiency

The effect of the percentage of organic solvent on peak efficiency has been studied using Tris·HCl (pH 8)–acetonitrile mixtures (ionic strength 5 m*M*). We observed that an increasing percentage of water between 20 to 60% induced a 20% decrease in peak efficiency (Fig. 3). Indeed, as in LC, an increasing percentage of organic modifier (acetonitrile) induces a decrease in chromatographic retention factor (Fig. 2), retention time (Table 2), and consequently a smaller dispersion by mass transfer resistance.

3.2. Effect of the nature of organic modifier

The influence of the nature of organic solvent (methanol, acetonitrile, tetrahydrofuran) has been studied upon the resolution of benzodiazepine mixture. The organic solvent content has been fixed in order to get hydroorganic mobile phases having the same elution strength [38]. The aqueous buffer was Tris·HCl (pH 8) and the ionic strength of each hydroorganic mobile phase was constant (5 m*M*). Then, Fig. 4 compares the separation of benzodiazepines on phenyl silica stationary phase in acetonitrile–buffer (60:40), methanol–buffer

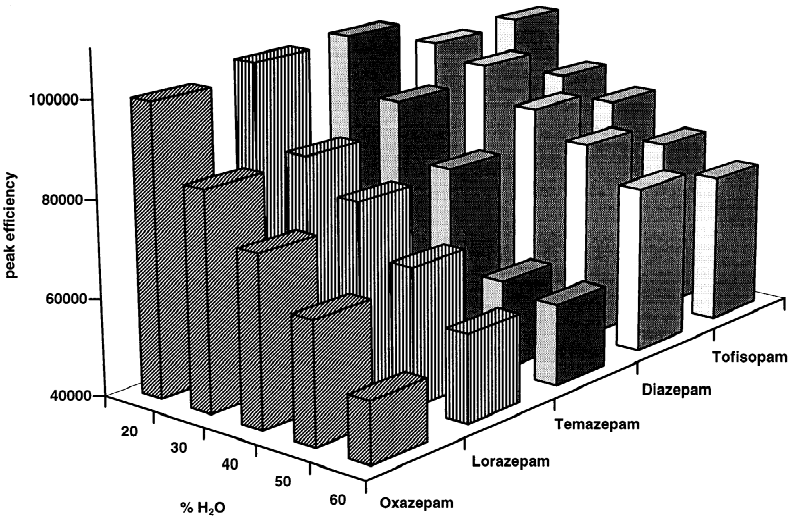


Fig. 4. Effect of the water content on peak efficiency (*N*). Experimental conditions as in Fig. 3.

(77.5:22.5) and finally tetrahydrofuran–buffer (48.2:51.8) mixtures. The mobility in the methanol–buffer system was $7.1 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} compared to $4.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} for the tetrahydrofuran–buffer system and $17.6 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} for the acetonitrile–buffer system. We observed a reduction in mobility by a factor of ca. 2.5 between acetonitrile–buffer and methanol–buffer mixtures and by a factor of ca. 4.5 between acetonitrile–buffer and tetrahydrofuran–buffer mixtures. This EOF reduction cannot be only caused by variation in dielectric constant or viscosity. For example, the ε/η ratio for the acetonitrile–water (60:40) mixture is about 60 cP^{-1} and for the methanol–water mixture (77.5:22.5) mixture about 40 cP^{-1} [32] ; this would involve an EOF reduction by a factor of 1.5 (and not 2.5). These behaviors agree with the result reported by Dittmann and Rozing [20].

This decreasing may be caused by the zeta potential change. Schwer and Kenndler [32] studied the effect of a series of organic solvents on the electrokinetic properties of fused silica, i.e., the electroosmotic velocity and zeta potential ; they observed that zeta potential decreases with increasing content of organic solvent and its value depends on solvent properties. In our case, acetonitrile–buffer mixture offered better selectivity between benzodiazepines compared to methanol–buffer (Fig. 5). The separation with tetrahydrofuran–buffer mixture is very disappointing due to low peak efficiencies.

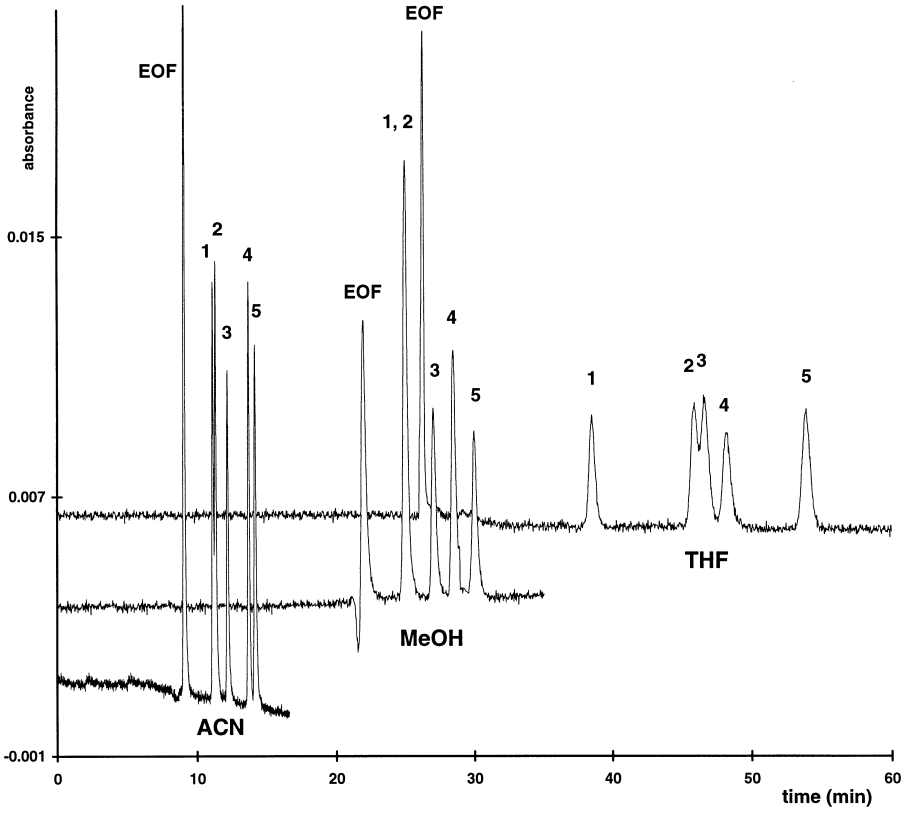


Fig. 5. Effect of the organic modifier nature upon the separation of benzodiazepines by CEC. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×75 μm I.D.]; temperature: 25°C; applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm. Ionic strength 5 m*M*. Electrolyte: ACN: Tris·HCl (pH 8)–acetonitrile (40:60) mixture, MeOH: Tris·HCl (pH 8)–methanol (22.5:77.5) mixture, THF: Tris·HCl (pH 8)–tetrahydrofuran (51.8:48.2) mixture.

3.3. Effect of the ionic strength of the buffer

3.3.1. Electroosmotic flow

The effect of increasing ionic strength of the mobile phase is well known for open fused-silica capillaries. The zeta potential depends on the buffer molar concentration C as following :

$$\zeta = \sigma\sqrt{\frac{RT}{2\varepsilon\varepsilon_0 CF^2}} \quad (4)$$

where R is the universal gas constant, T the absolute temperature, F the Faraday constant and σ the surface excess charge density. According to Eqs. (1) and (4), the EOF velocity is inversely proportional to the square root of salt concentration. We measured the effect of increasing ionic strength in phenyl silica packed capillary by using Tris·HCl (pH 8)–acetonitrile (40:60). As illustrated in Fig. 6, the EOF increases linearly with the reciprocal of the square root of ionic strength in the 2.5–25 ionic strength range ($R^2 = 0.9938$). For the capillary column packed with 3 μm phenyl particles, the EOF mobility decreases by 58% upon increasing ionic strength from 2.5 to 25 mM, as in CE. This EOF behavior versus ionic strength confirms results reported by Choudhary et al. [31]. The double layer thickness is confirmed to be proportional to the reciprocal of the square root of the buffer ionic strength.

3.3.2. Retention factor

The retention factors of the benzodiazepines were determined at different ionic strengths of electrolyte Tris·HCl (pH 8)–acetonitrile (40:60). At constant acetonitrile content (60%), as the ionic strength increases, the retention time of benzodiazepines

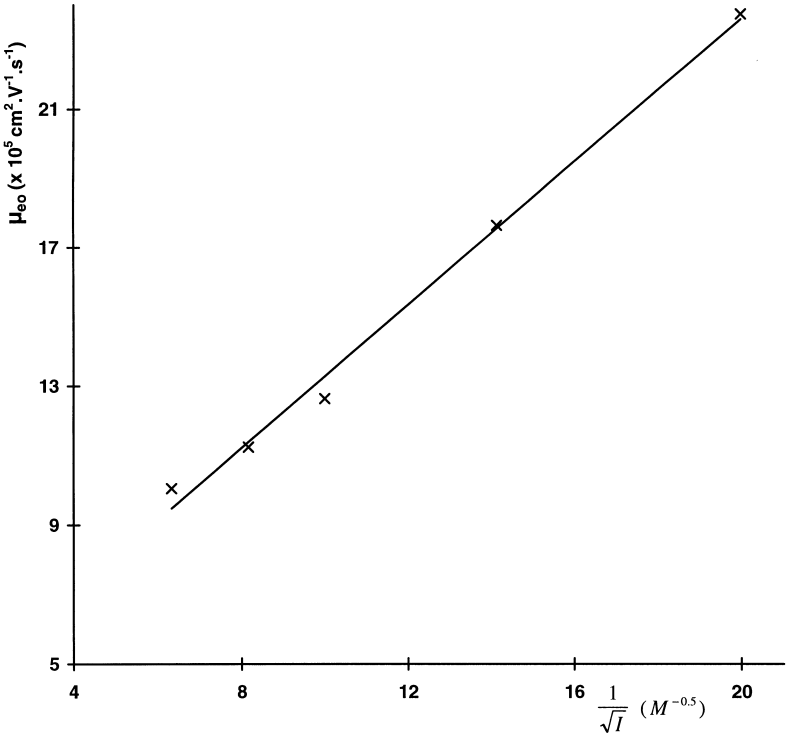


Fig. 6. Effect of the ionic strength (I) on electroosmotic flow in CEC. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×(75 μm I.D.]; electrolyte: Tris·HCl (pH 8)–acetonitrile (40:60) mixture; temperature: 25°C; applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm.

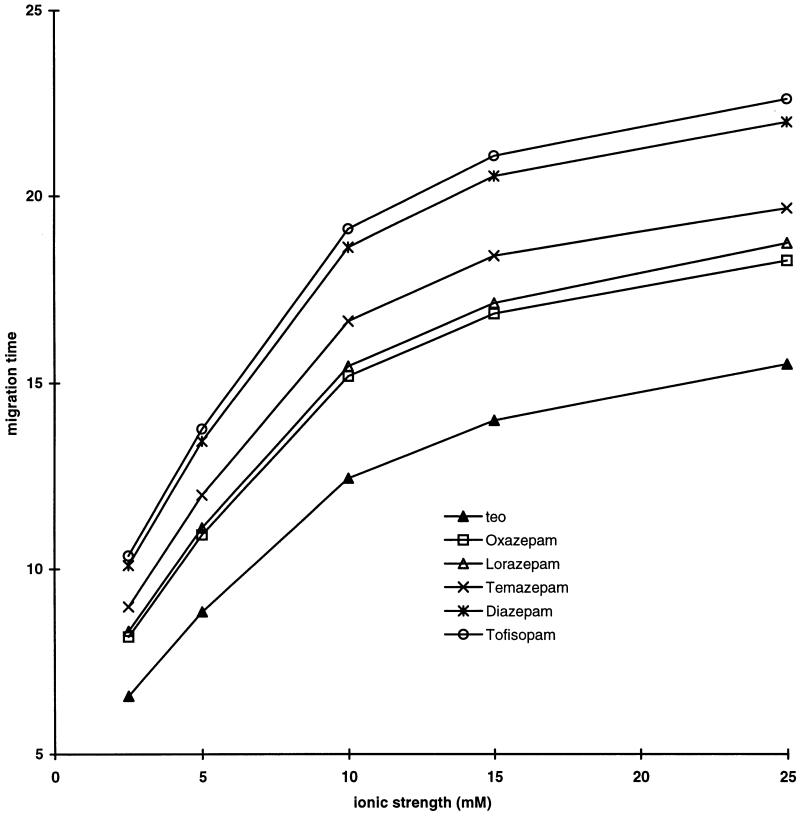


Fig. 7. Effect of the ionic strength (I) on retention times of benzodiazepines in CEC. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×75 μm I.D.)]; electrolyte: Tris·HCl (pH 8)–acetonitrile (40:60) mixture; temperature: 25°C; applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm.

increases and its retention factor decreases (Fig. 7 and Table 3) agree with result reported by Angus [39]. For example, as the ionic strength increases from 2.5 to 25 m*M*, the retention factor of the benzodiazepines decreases from 19% (tofisopam) to 27% (temazepam).

3.3.3. Efficiency

Peak efficiency of five benzodiazepines have been calculated at several ionic strengths [Tris·HCl (pH 8)–acetonitrile (40:60)]. The results are shown in Table 4. It is clear that ionic strength has a great impact on peak efficiency. Peak efficiency, in this

Table 3
Effect of the ionic strength on chromatographic retention factor of benzodiazepines. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×75 μm I.D.]; electrolyte: Tris·HCl (pH 8)–MeCN (40:60) mixture; temperature: 25°C; applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm

I (m*M*)	*k′*				
	Oxazepam	Lorazepam	Temazepam	Diazepam	Tofisopam
2.5	0.24	0.27	0.37	0.53	0.57
5	0.23	0.26	0.35	0.52	0.56
10	0.22	0.24	0.34	0.50	0.54
15	0.21	0.23	0.32	0.47	0.51
25	0.18	0.21	0.27	0.42	0.46

Table 4
Effect of the ionic strength (*I*) on peak efficiency of benzodiazepines in CEC. Experimental conditions as in Fig. 6

I (m*M*)	Efficiency				
	Oxazepam	Lorazepam	Temazepam	Diazepam	Tofisopam
2.5	64 500	67 100	70 900	79 300	70 000
5	75 000	81 500	80 000	87 500	84 000
10	84 500	78 700	81 000	86 800	88 200
15	82 500	77 900	79 100	85 300	86 300
25	80 900	76 200	78 600	83 700	83 400

packed column, has a maximum value at an intermediate ionic strength (ca. 10 m*M*). The mechanism by which the ionic strength affects the column efficiency in CEC seems to be rather complex [40]. Both stacking, thermal and double layer overlap effects may be suggested to explain this behavior. The double layer overlap effect may become appreciable at low ionic strength while thermal effect is most pronounced with high ionic strength due to a slower heat dissipation as in CE. Further studies seem necessary for a better understanding of the impact of ionic strength upon peak efficiency in CEC.

3.4. Effect of the temperature

3.4.1. Electroosmotic flow

Temperature variation may affect several physical

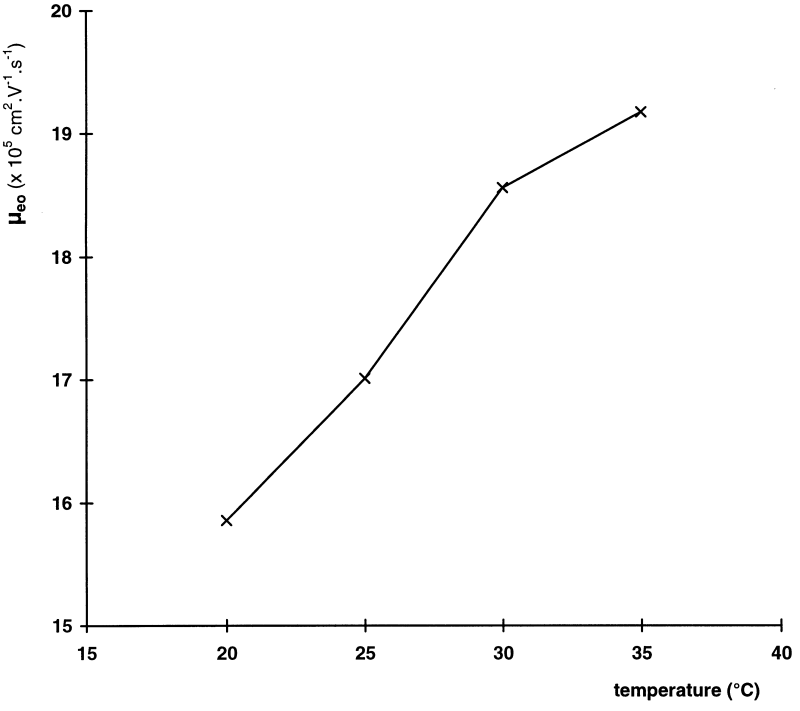


Fig. 8. Effect of the temperature on electroosmotic flow in CEC. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×75 μm I.D.]; electrolyte: Tris·HCl (pH 8)–acetonitrile (40:60) mixture (ionic strength 5 m*M*); applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm.

Table 5
Effect of the temperature on chromatographic retention factors of benzodiazepines in CEC. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×75 μm I.D.]; electrolyte: Tris·HCl (pH 8)–MeCN (40:60) (ionic strength 5 m*M*); applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm

T (C°)	*k′*				
	Oxazepam	Lorazepam	Temazepam	Diazepam	Tofisopam
20	0.27	0.29	0.41	0.62	0.67
25	0.25	0.27	0.38	0.58	0.62
30	0.23	0.26	0.36	0.54	0.59
35	0.22	0.24	0.34	0.52	0.56

parameters (electrolyte viscosity, dielectric constant, zeta potential, pH...) and consequently the electroosmotic mobility and the retention time of the analyte. Our study was carried out at four different temperatures (20, 25, 30, 35°C) with a Tris·HCl (pH 8)–acetonitrile (40:60) mixture. As in CE, the EOF increases with increasing temperature in CEC. Thus, using a capillary column packed with 3 μm phenyl particles, the EOF mobility increases by 21% upon increasing temperature from 20 to 35°C (Fig. 8).

3.4.2. Retention factor

In LC, an increase in temperature generally undergoes a decrease in retention factor and selectivity [41]. In our study, when the temperature increased from 20 to 35°C, the retention time and the retention factor of benzodiazepines decreased (Table 5). As expected in LC [42], linear relationships between the logarithm of the retention factor and the reciprocal of the column temperature were obtained in CEC ; the standard enthalpy ΔH° were deduced from the slope and given in Table 6.

Table 6
Regression parameters of linear equation between log *k′* *v*s. 1/*T*. Experimental conditions as in Table 5

	Slope	*y*-Intercept	ΔH (kJ)	Correlation coefficient
Oxazepam	480.40	−2.22	9.18	0.9993
Lorazepam	440.50	−2.04	8.42	0.9964
Temazepam	479.30	−2.02	9.16	0.9956
Diazepam	466.30	−1.80	8.91	0.9986
Tofisopam	488.60	−1.84	9.34	0.9989

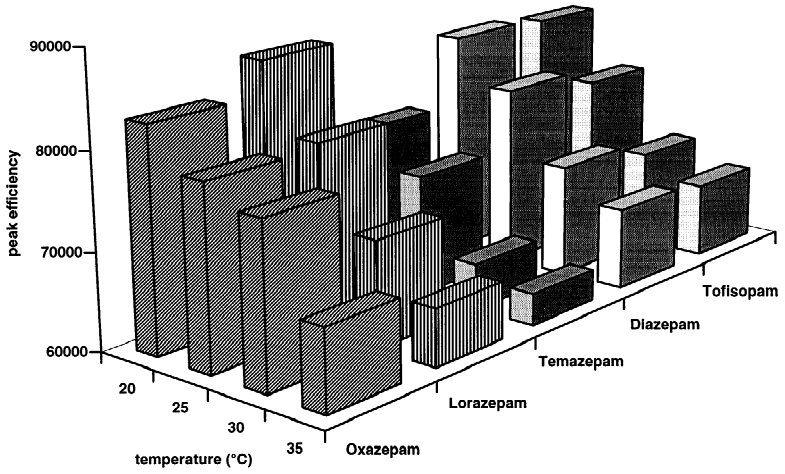


Fig. 9. Effect of the temperature on peak efficiency in CEC. Experimental conditions as in Fig. 8.

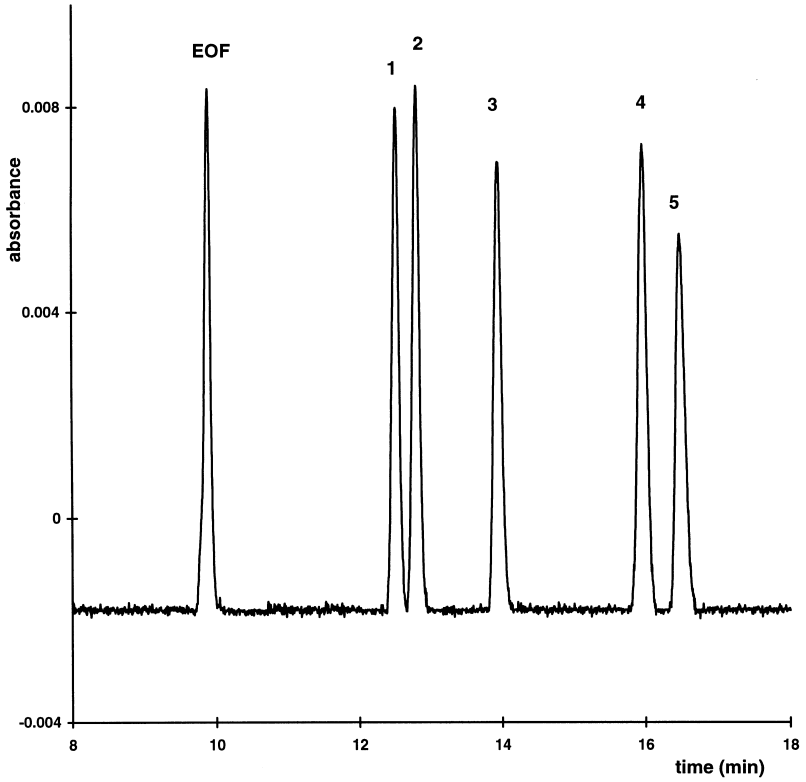


Fig. 10. Separation of benzodiazepines by CEC using a phenyl bonded silica stationary phase. Electropak phenyl column, EP-75-40-3-PH [40 cm packed (47 cm total)×75 μm I.D.]; electrolyte: Tris·HCl (pH 8)–acetonitrile (40:60) mixture (ionic strength 5 m*M*); temperature: 20°C; applied voltage: +20 kV; UV detection: 220 nm; electrokinetic injection: 4 s (+10 kV); benzodiazepine concentration: 100 ppm.

3.4.3. Efficiency

The effect of the temperature on peak efficiency has been studied using Tris·HCl (pH 8)–acetonitrile (40:60) mixture. As in CE, an increase in temperature from 20 to 35°C induces a 25% decrease of peak efficiency (Fig. 9).

3.5. Optimized separation

The separation of the benzodiazepine mixture in Tris·HCl (pH 8)–acetonitrile (40:60) mixture, ionic strength 5 m*M*, temperature 20°C) has been achieved in less than 18 min with peak efficiencies greater than 200 000 theoretical plates per meter and resolutions between two consecutive solutes greater than 1.5 (Fig. 10).

4. Conclusion

This study has shown the possibility of separating various benzodiazepines by CEC on phenyl silica stationary phase. Ionic strength and organic modifier influence the electrophoretic and chromatographic mechanisms. The decrease of the volume fraction of acetonitrile result in higher retention factors and better resolution. EOF and efficiency increase with acetonitrile content. The increase of ionic strength of electrolyte induces a decrease of EOF and retention factor and an increase of peak efficiency. Finally, higher temperature of the column involves an increase of EOF and peak efficiency and a decrease of retention factor. So, the modification of ionic strength and temperature in CEC can mainly be interpreted as a CE-like behavior at the opposite of

organic modifier content which acts as a LC-like behavior.

References

[1] L.H. Sternbach, L.O. Randall, H. Lehr, A. Burger (Eds.), Drugs Affecting the Central Nervous System, Vol. 2, M. Dekker, New York, 1968.
[2] O.H. Drummer, J. Chromatogr. B 713 (1998) 201.
[3] K. Valko, S. Olajos, T. Cserkati, J. Chromatogr. 499 (1990) 361.
[4] I. Choma, A. Dawidowicz, F. Lodkowski, J. Chromatogr. 600 (1992) 109.
[5] J. Mama, A.F. Fell, B.J. Clark, Anal. Proc. 26 (1989) 71.
[6] E.Y. Ting, M.D. Porter, J. Chromatogr. 793 (1998) 204.
[7] P. Wernly, W. Thormann, Anal. Chem. 64 (1992) 2155.
[8] M. Tomita, T. Okuyama, S. Sato, H. Ishizu, J. Chromatogr. 621 (1993) 249.
[9] M. Schafroth, W. Thormann, D. Allemann, Electrophoresis 15 (1994) 72.
[10] M.F. Renou-Gonnord, K. David, J. Chromatogr. 735 (1996) 249.
[11] M. Imazawa, Y. Hatanaka, J. Pharm. Biomed. Anal. 15 (1997) 1503.
[12] Y.F. Yik, C.P. Ong, S.B. Khoo, H.K. Lee, S.F. Li, J. Chromatogr. 589 (1992) 361.
[13] K.D. Altria, N.W. Smith, C.H. Turnbull, Chromatographia 46 (1997) 664.
[14] A.L. Greco, A. Gonzalez, M.L. Marine, Crit. Rev. Anal. Chem. 26 (1996) 261.
[15] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[16] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[17] T. Tsuda, K. Nomura, K. Nagagawa, J. Chromatogr. 248 (1982) 241.
[18] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[19] A.S. Rathore, C. Horvath, J. Chromatogr. 781 (1997) 185.
[20] M.M. Dittmann, G.P. Rozing, J. Chromatogr. 744 (1996) 63.
[21] C. Yan, R. Dadoo, R.N. Zare, D.J. Rakestraw, D.S. Anex, Anal. Chem. 68 (1996) 2726.
[22] P.B. Wright, A.S. Lister, J.G. Dorset, Anal. Chem. 69 (1997) 3251.
[23] R.M. Seifar, W.Th. Kok, J.C. Kraak, H. Poppe, Chromatographia 46 (1997) 131.
[24] M.R. Taylor, P. Teale, S.A. Westwood, D. Perrett, Anal. Chem. 69 (1997) 2554.
[25] M.R. Taylor, P. Teale, J. Chromatogr. 768 (1997) 89.
[26] N.W. Smith, M.B. Evans, Chromatographia 38 (1994) 649.
[27] R.J. Boughtflower, T. Underwood, C.J. Paterson, Chromatographia 40 (1995) 329.
[28] T. Zhu, Y. Sun, C. Zhang, D. Ling, Z. Sun, J. High Res. Chromatogr. 17 (1994) 563.
[29] H. Yammamoto, J. Baumann, F. Erni, J. Chromatogr. 593 (1992) 313.
[30] H. Rebscher, U. Pyell, Chromatographia 38 (1994) 737.
[31] G. Choudhary, C. Horvath, J. Chromatogr. 781 (1997) 161.
[32] C. Schwer, E. Kenndler, Anal. Chem. 63 (1991) 1801.
[33] F. Lelievre, C. Yan, R.N. Zare, P. Gareil, J. Chromatogr. 723 (1996) 145.
[34] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.
[35] A. Tchapla, S. Heron, E. Lesellier, H. Colin, J. Chromatogr. 656 (1993) 81.
[36] R. Nasuto, L. Kwietniewski, J.K. Rozylo, J. Chromatogr. 762 (1997) 27.
[37] C. Horvath, W. Melander, I. Molnar, J. Chromatogr. 125 (1976) 129.
[38] J.H. Hildebrand, R.L. Scott, Regular Solutions, Prentice-Hall, Englewood, NJ, 1962.
[39] P.D.A. Angus, E. Victorino, K.M. Payne, C.W. Demarest, T. Catalano, J.F. Stobaugh, Electrophoresis 19 (1998) 2073.
[40] Q.H. Wan, J. Chromatogr. 782 (1997) 181.
[41] S.R. Springton, P. David, J. Steger, M. Novotny, Anal. Chem. 58 (1986) 991.
[42] R. Rosset, M. Caude, A. Jardy, Chromatographies en Phases Liquide et Supercritique, Masson, Paris, 1991, pp. 312–314.