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Influence of sample plug width in capillary electrochromatography

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Abstract

Capillary electrochromatography (CEC) is a highly efficient miniaturized chromatographic technique that is very sensitive to extra-column band broadening effects. It is investigated whether band broadening due to the sample injection process is within a tolerable range for the standard sample injection procedures usually employed in CEC. Equations are derived that permit the user of CEC to calculate optimum injection parameters. These equations operate with magnitudes easily accessible in CEC (dimensions of column, plate number and electroosmotic mobility). © 1997 Elsevier Science B.V.

Keywords: Electrochromatography; Band broadening; Injection parameters; Computer simulation; Benzoates; Polynuclear aromatic hydrocarbons

1. Introduction

In capillary electrochromatography (CEC) the mobile phase is electroosmotically driven through the chromatographic bed [1–5]. In the case of packed capillaries, particles with very small diameters are possible as packing material due to the fact that the linear electroosmotic velocity produced in the liquid-filled channels of a capillary system is not dependent on the channel diameter as long as the volume fraction of the volume of the electric double layer is negligible [6]. Knox and Grant [4] calculated that under conditions ordinarily employed in CEC packings with particles down to 0.4 μm will not considerably reduce the electroosmotic velocity. Recently, capillaries filled with continuous beds have been described as separation columns for CEC [7].

The unique features of the electroosmotic flow (EOF) make it possible to employ packings or continuous beds that exhibit in conventional pressure driven chromatography a permeability which is too low and produces a back pressure that is too high for conventional equipment. CEC performed with capillaries packed with particles with a mean diameter $d_p < 5$ μm is a highly efficient and rapid liquid chromatographic technique. Knox and Grant [8] and Dittmann and Rozing [9] report plate numbers per column of 100 000. Due to the flat flow profile of the EOF very low reduced plate numbers have been reported [8,10]

In order to generate systems with a very high efficiency (200 000 plate numbers per column), chromatographic packings with particles $d_p = 1.5–1.8$ μm have been used [11–13]. In accordance to the theoretical considerations of Knox and Grant [4] the electroosmotic velocity is not significantly reduced by diminishing d_p from 3 μm to 1.5 μm. Recently, Lüdtke et al. [14] reported the successful use of capillaries packed with octadecylated monodisperse porous silica beads with a mean particle diameter of

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0.5 μm. Plate heights of 5 μm and less are reported for CEC.

It is obvious that the problem of extra-column band broadening will be greater with increasing efficiency and increasing degree of miniaturisation of the chromatographic system. Rebscher and Pyell [15] developed a method that permits the experimental determination of extra-column band broadening contributions in CEC. They have been able to show that under conditions of their work extra-column band broadening has a measurable influence on the efficiency of the chromatographic system.

One major parameter influencing the extra-column band broadening is the injected sample plug length. In the present paper, theoretical and experimental investigations on the influence of the injected plug length on the extra-column band broadening are presented that permit to decide whether injection parameters that are commonly employed in CEC, result in a tolerable extra-column band broadening contribution. A computer program is presented that permits modeling of the detected peak shape in dependence on the injection parameters. An equation is developed that helps the user of CEC to select suitable injection parameters.

2. Experimental

2.1. Preparation of packed capillaries

The packing technique presented in our previous paper [15] was slightly modified. Fused-silica capillaries (100, 180 μm I.D.; 337 μm O.D) (CeramOptec, Bonn, Germany) were used as column material. The inlet frit was prepared in the following manner. Native silica gel (Nucleosil 100-3, Macherey–Nagel, Düren, Germany) was wetted with a solution of sodium silicate in water. The end of the capillary was tapped into the wetted silica gel and sintered with a hot resistance wire (t=3 min, P=2.5 W). The coating is not pyrolyzed during this process.

The columns were packed as described in [15]. The slurry is pumped into the capillary at p=600 bar using a pneumatic pump (DSTV-122, Armaturenbau, Wesel, Germany). During the packing process the capillary and the slurry reservoir are immersed into an ultrasonic bath and the slurry is agitated by an in-house made stirrer bar and a magnetic stirrer.

After packing, the slurry liquid in the capillary is replaced by water and a frit is sintered at the outlet end by heating the packing with a hot resistance wire (t=3 min, P=2.5 W, p=800 bar). During the sintering process, under these conditions the reversed-phase material is not pyrolyzed. Detection is performed by photometric detection in a packed section of the capillary. In the detection zone the coating is removed by application of hot sulphuric acid. The packed capillary is installed in the chromatographic apparatus and equilibrated with the mobile phase by pumping this phase through the column (p=50–100 bar) for at least 30 min.

2.2. Packing material, columns

The following two columns have been employed:

Column 1: packing material, Nucleosil 100-3-C_{18}; d_p=3 μm; mean pore width=10 nm; specific surface area=300 m^2 g^{-1} (Macherey–Nagel); inner diameter, 100 μm; total length, 270 mm; length to the detector, 200 mm.

Column 2: packing material, YMC-ODS-1.5; d_p= 1.5 μm; mean pore width, 10 nm; specific surface area=200 m^2g^{-1} (YMC Europe, Schermbeck, Germany); inner diameter, 180 μm; total length, 310 mm; length to the detector, 258 mm.

Column 3: packing material, Nucleosil 100-3-C_{18}; inner diameter, 180 μm; total length, 395 mm; length to the detector, 345 mm.

2.3. Sample preparation

Due to the low solubility of polycyclic aromatic hydrocarbons, alkyl, and aryl benzoates in polar solvents the samples were prepared with pure acetonitrile. The concentration of each solute in the sample was 0.2–0.3 g l^{-1}.

2.4. Chromatographic system

Chromatographic runs with Column 1 were carried out with a Beckman (Fullerton, CA, USA) P/ACE capillary electrophoresis system equipped with a UV absorbance detector. The temperature of the capillary was controlled by liquid cooling and was maintained

at 25°C. Samples were injected electrokinetically by application of voltage for a defined time interval. Detection was at 254 nm. All separations were carried out at 20 kV. Data were recorded with Beckman System Gold software.

Chromatographic runs with Columns 2 and 3 were carried out with a laboratory-made apparatus. High voltage was generated with a HCN 35-35000 (FUG, Rosenheim, Germany). Detection was performed with a Spectra 100 variable-wavelength UV–Vis CE detector (Thermo Separation Products, San Jose, USA). The samples were injected electrokinetically. The separation voltage was set at 15 or 20 kV. The temperature of the separation capillary was not controlled. Data were recorded with EZChrom (Scientific Software, San Ramon, USA) software.

Mixtures of acetonitrile with a solution of sodium tetraborate in water (c=2 mmol/l) (Column 1) and mixtures of acetonitrile with a solution of NaH_2PO_4 (c=1 mmol/l) and NaH_2PO_4 (c=1 mmol/l) in water (Column 2 and 3) were employed as mobile phase. Thiourea and acetonitrile were used as marker of the hold-up time.

2.5. Reagents

Sodium tetraborate was analytical grade (Merck, Darmstadt, Germany). Fluorene and tolyl benzoate were obtained from Aldrich, Steinheim, Germany. Pyrene and chrysene were obtained from Chem Service (West Chester, UK). Phenyl benzoate, propyl benzoate, benzyl benzoate, butyl benzoate, and isopentyl benzoate were from Merck. Methyl benzoate, ethyl benzoate, and resorcinol were available at the department of chemistry, university of Marburg, Germany.

Acetonitrile was distilled before use as a component of the mobile phase. Water was doubly distilled. The mobile phases were degassed by ultrasonication and filtered through a membrane filter (pore size=0.2 μm).

2.6. Software

The simulation software was written in Pascal employing Turbo Pascal 6.0 (Borland International, CA, USA).

3. Theoretical considerations

In 1966 Sternberg [16] investigated in detail extra-column contributions to chromatographic band broadening. He showed that the second moment (variance) for the total distribution can be derived from the second moments for the partial distributions. He also discussed the second-moment distributions for various input functions. The input function is the distribution function of the analyte concentration in the sample zone due to the injection process. Early workers in gas chromatography [17,18] characterized sample injection as corresponding to either plug or exponential introduction. In liquid chromatography (LC) generally a rectangular distribution function of the analyte concentration in the sample zone at the beginning of the chromatographic process is assumed [19,20].

Due to the use of injection valves with constant injected sample volume and pumps with constant volume flow, in HPLC and Microscale HPLC [21] the maximum sample volume given in volume units is of interest relative to the peak volume. These magnitudes are rather inconvenient in capillary electrochromatography (CEC). In CEC the sample is directly injected on the column via the controlled application of a voltage (electrokinetic injection) and the velocity of the mobile phase is not given as flow-rate (volume/time) but as linear velocity (distance/time) determined via a non-retarded marker. It is therefore useful to transfer the considerations made in LC for injection and retention volumes and flow-rates in equations that operate with magnitudes easily accessible in CEC.

In CEC the following extra-column band broadening contributions have to be taken into consideration: sample injection, detection, and data processing. With instrumentation designed for capillary electrophoresis (CE) no significant band broadening due to detection and data processing is expected for CEC. Because of on-column injection and on-column (or in-column [22]) detection no band broadening contributions due to transfer lines have to be taken into consideration. According to Belenkii [23], on-column detection is defined to be detection in an unpacked section in the column, whereas in-column detection is used for detection in the chromatographic bed itself. With ideal plug flow the dis-

tribution function for injection and detection can be considered as rectangular.

If it is assumed that the contribution due to detection and data processing can be neglected, the tolerable injection plug length for a column of given length and efficiency for an analyte can be easily calculated. Although the maximum increase in band width that can be tolerated due to any extra-column band broadening process is a matter of choice, a 10% increase in peak variance (total second moment) is generally accepted as decision criterion [19].

The partial second moment (variance, σ_I^2) for the injection plug (rectangular distribution) can be calculated from the injection plug length L_I [16].

$$\sigma_I^2 = L_I^2/12 \quad (1)$$

The partial second moment (variance, σ_C^2) for the distribution exclusively due to the column is given by:

$$\sigma_C^2 = L^2/N \quad (2)$$

where L=length of the column to the detection window, N=plate number.

The total second moment (variance of the recorded peak, σ_T^2) can be calculated by addition of the partial second moments.

$$\sigma_T^2 = \sigma_I^2 + \sigma_C^2 \quad (3)$$

If the reduction of the recorded plate numbers N_R is considered as decisive criterion for the tolerable injection plug length and if N_R is given by ($N_R = N(1-\delta)$) (δ=reduction parameter), the maximum injection plug length is given by Eq. (4).

$$L_I = \sqrt{\frac{12\delta}{1-\delta}} \cdot \frac{L}{\sqrt{N}}, \quad 0 < \delta < 1 \quad (4)$$

A reduction parameter δ of 0.091 corresponds to a 10% increase in peak variance. With electrokinetic injection L_I can be determined employing Eq. (5), if zone compression during the injection process due to enrichment of the solutes in the stationary phase can be neglected.

$$L_I = t_I \mu_{eo} U_I L_T^{-1} \quad (5)$$

where t_I=injection time, μ_{eo}=electroosmotic mobility, U_I=injection voltage, L_T=total column length. If zone compression during the injection process cannot be neglected, the retention factors for the solutes investigated with the sample solvent as mobile phase, k_s, have to be taken into consideration (Eq. (6)).

$$L_I = t_I \mu_{eo} U_I L_T^{-1} (1 + k_s)^{-1} \quad (6)$$

It must be emphasized that k_s is different from k, the retention factor obtained for the chromatographic run itself.

4. Results and discussion

4.1. Dependence of the total peak variance on the injected plug length

For a given column length and plate number the square root of the total variance σ_T in dependence on the injection plug can be modeled. In Fig. 1 σ_T is plotted versus L_I for a column with a length of 50 cm having plate numbers of 50 000, 100 000, 200 000 (Eq. (7)).

$$\sigma_T = \sqrt{\frac{L_I^2}{12} + \frac{L^2}{N}} \quad (7)$$

At small L_I, σ_T is almost independent of the injection parameters, whereas a dramatic decrease of the efficiency can be observed when exceeding a thres-

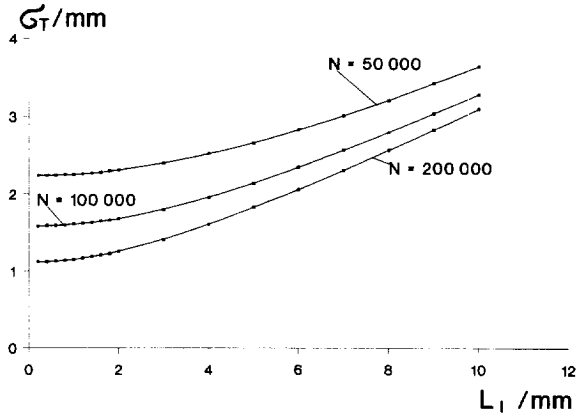

Fig. 1. Calculated dependence of the square root of the total peak variance on the injected plug length for a column of 500 mm length for different plate numbers.

hold value. The threshold value is at constant column length decreasing with increasing N. With large L_I the square root of the total variance is proportional to L_I. In this case σ_T is dominantly given by the injection process.

σ_T cannot easily be transferred into the peak width w or the peak width at half height w_h, as the distribution function describing the peak shape with L_I exceeding a threshold value is no longer purely Gaussian but partly rectangular [24].

4.2. Modelling of the peak shape

In 1941, Martin and Synge [25] have shown that the chromatographic process can be modelled by a series of distribution steps. Although the assumptions made are extremely simplifying, the resulting band profiles correspond to those that are expected for a chromatographic process, if the number of theoretical plates is sufficiently high. The model of Martin and Synge permits variation of the starting conditions. The number of plates filled at the beginning of the chromatographic process represents the injected plug length.

The chromatographic process was modelled by software written in-house (SimChrom) [26] that generates the peak profile in arbitrary units in dependence on the number of plates of the column and the number of plates that are filled at the beginning of the chromatographic process.

Fig. 2 shows the simulated peak profiles for a column of 10 000 plates for an analyte with $k=1$ and different starting conditions. SimChrom permits to calculate the peak width and the peak width at half height for conditions producing strong deviations of the peak profile from the Gaussian distribution. Considering the influence of the injection parameters on the resolution of the adjacent peaks, w is the magnitude of interest, not the peak variance.

Fig. 3 shows for a simulated chromatographic process with $N=100\,000$ for an analyte with $k=1$ the dependence of w_h on the injected plug length (number of filled theoretical plates at beginning of the process). The second trace (crosses) represents the values that are obtained employing Eq. (7) assuming a Gaussian distribution function ($w_h = 2.355\ \sigma_T$, $L=N/2$).

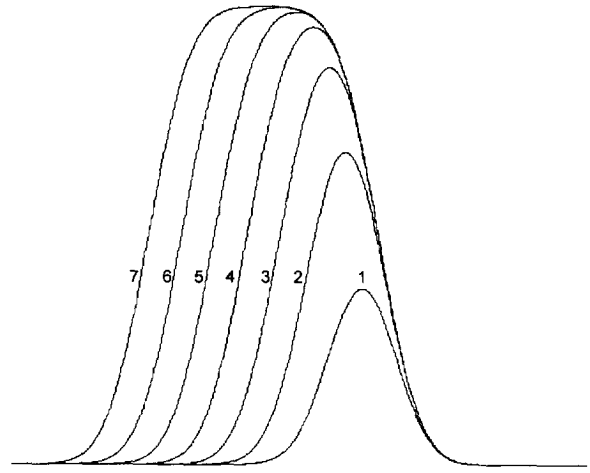

Fig. 2. Simulated peak profiles ($N=10\,000$; $k=1$) with varied number of initially filled cells (1=50 cells; 2=100 cells, 3=150 cells; 4=200 cells; 5=250 cells; 6=300 cells, 7=350 cells).

4.3. Experimental studies

For two columns w_h was recorded with varied injection parameters. It was assumed that Eq. (5) is valid. Zone compression during injection due to enrichment of the solutes in the stationary phase was not taken into consideration, because k_s with acetonitrile as sample solvent was very low (k_s (methyl

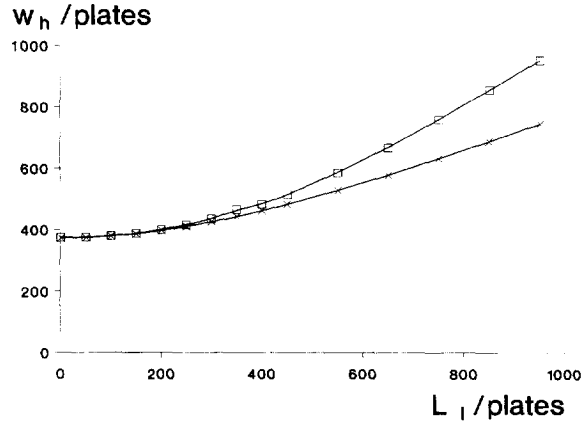

Fig. 3. Calculated dependence of the peak width at half height on the injected plug length (in cell units) for a column with $N=100\,000$; □ = data calculated with SimChrom; × = data calculated with assuming a Gaussian peak shape.

benzoate) = 0.23, k_s (butyl benzoate) = 0.43, extrapolated data). The mobile phase velocity was in all studies in the optimum range. Preliminary studies have shown that the concentration of the solutes in the sample did not produce column overloading.

In Fig. 4 the dependence of the peak width in length units on the injected plug length for polycyclic aromatic hydrocarbons (PAHs) as test solutes (k = 0.88–1.85) ($N(L_I$ = minimum) = 27 000–28 000, length to the detector = 200 mm) is presented for Column 1. The data show that in the parameter range investigated w_h is strongly dependent on L_I. The repeatability of the injection conditions with the fully automated CE-apparatus is not sufficient for quantitative evaluation.

In Fig. 5 the dependence of the peak width in length units on the injected plug length for alkyl- and arylbenzoates as test solutes (k = 0.52–1.97) is presented for Column 2 ($N(L_I$ = minimum) = 43 000–52 000, length to the detector = 258 mm). The experimentally determined dependence of the peak width in length units on the injected plug length corresponds to the curve predicted with SimChrom (Fig. 3). At low injected plug length ($L_I \leq 1$ mm) w_h is independent of L_I, while at high volume overload w_h is linearly dependent on L_I. The assumption that with acetonitrile as sample solvent zone compression

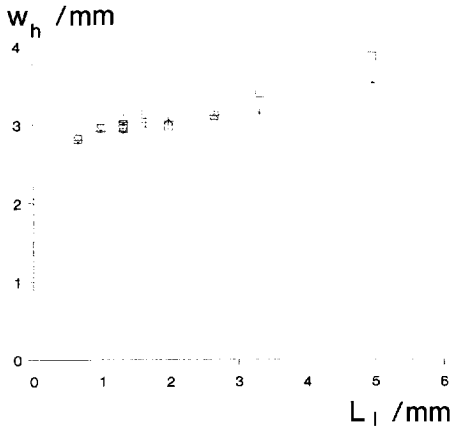

Fig. 4. Dependence of the peak width at half height on the injected plug length (injection: electrokinetic; column: 200 mm (270 mm) × 100 μm; packing octadecylsilica gel, d_p = 3.0 μm; mobile phase: acetonitrile–borate buffer (90:10, v/v); in-column photometric detection, 254 nm; assignment: □ = fluorene, + = pyrene, ◇ = chrysene).

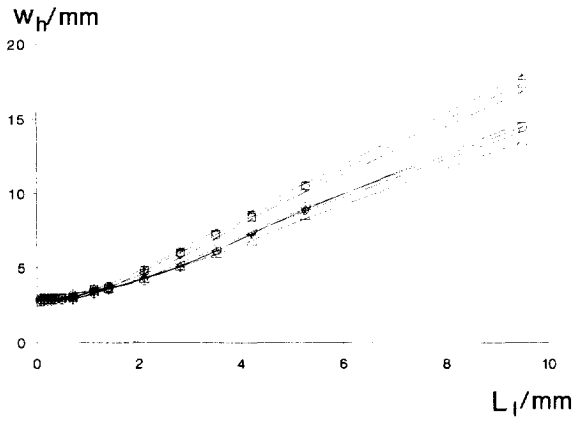

Fig. 5. Dependence of the peak width at half height on the injected plug length (injection: electrokinetic; column: 258 mm (310 mm) × 180 μm; packing octadecylsilica gel, d_p = 1.5 μm; mobile phase: acetonitrile–phosphate buffer (80:20, v/v), pH = 7.3; in-column photometric detection, 230 nm; assignment: ○ = methyl benzoate, + = ethyl benzoate, * = phenyl benzoate, □ = benzyl benzoate, × = *p*-tolyl benzoate, ◇ = butyl benzoate, △ = isopentyl benzoate).

effects can be neglected is strongly corroborated by the fact that the range where w_h is independent of L_I is not dependent on the solute, hence not dependent on the retention factor.

4.4. Discussion of the decision criterion

As the magnitude, that is easily obtainable from any chromatogram, is the peak width at half height, we propose to use w_h directly to trace the peak broadening in dependence on the injection parameters. An increase of w_h of about 5% due to extra-column band broadening effects is generally excepted by chromatographers as maximum allowable value [19].

If the distribution function of the recorded peak is approximated as Gaussian, Eq. (8) can be obtained employing Eqs. (1)–(3). Eq. (8) gives the maximum tolerable injection plug length L_{max}, for which the decision criterion is fulfilled.

$$L_{max} = 1.11 \cdot \frac{L}{\sqrt{N}} \tag{8}$$

If w_h is modeled in dependence on L_I with SimChrom, the same equation is obtained, indicating that

the resulting peak shape with $L_I = L_{max}$ deviates only marginally from the Gaussian distribution function.

4.5. The prediction of optimum injection parameters

The maximum allowable L_I for Column 2 taken from the data presented in Fig. 5 employing the decision criterion described in the previous section is 0.9 mm. With Eq. (5) ($N = 45\,000$; $L = 258$ mm) L_{max} is 1.35 mm. This bias can be understood as deviation of the input function (the distribution function of the concentration in the injected sample zone) from the assumed rectangular distribution function. Inhomogeneities in the flow in the zone of the frit keeping the packing in place can be responsible for the difference observed. The deviation of the input function from the rectangular distribution function must be taken into account by an experimental factor F_I.

According to the data material presented F_I equals 1.5. Eq. (8) modified with F_I results in Eq. (9).

$$L_{max} = 1.1 \cdot \frac{L}{F_I\sqrt{N}} = 0.7 \cdot \frac{L}{\sqrt{N}} \quad (9)$$

In 1989, Terabe et al. [27] have calculated L_{max} according to the method resulting in Eq. (8) for micellar electrokinetic chromatography (MEKC). They obtained $L_{max} = 0.79$ mm for a capillary of 500 mm effective length and a plate height of 2 μm. The calculated L_{max} is in good agreement with the experimental data, indicating that in case of open capillaries the input function is approximately rectangular and no focusing effects have to be taken into account.

In case of continuous beds [7], no frits have to be placed at the ends of the chromatographic bed. Hence, it can be expected that with continuous beds the input function will be closer to a rectangular function than with CEC with packed capillaries.

With Eqs. (5) and (9) tolerable injection parameters (electrokinetic injection) for columns packed with material of different mean particle diameters can be calculated. For a fixed injection time Eq. (10) can be applied, predicting the suitable injection voltage, while for a fixed injection voltage Eq. (11) can be applied, predicting the suitable injection time.

Table 1
Predicted optimum injection time t_{max} in dependence on the mean particle diameter d_p of the packing with electrokinetic injection ($U_I = 3$ kV) assuming following conditions: $h = 2$, $L = 200$ mm, $L_T = 250$ mm, $\mu_{eo} = 0.25$ cm^2 s^{-1} kV^{-1}

d_p/μm	N	t_{max}/s
10.0	10 000	4.7
5.0	20 000	3.3
3.0	33 000	2.6
2.0	50 000	2.1
1.5	67 000	1.8
1.0	100 000	1.5
0.5	200 000	1.0

$$U_{max} = 0.7 \cdot \frac{LL_T}{\mu_{eo} t_I \sqrt{N}} \quad (10)$$

$$t_{max} = 0.7 \cdot \frac{LL_T}{\mu_{eo} U_I \sqrt{N}} \quad (11)$$

Table 1 shows calculated injection times for electrokinetic injection with an applied voltage of 3 kV during injection, a total length of the capillary of 250 mm, and a length of the packing to the detector of 200 mm for different mean particle diameters. The following data are assumed: $h = 2.0$, electroosmotic mobility = 0.25 cm^2 s^{-1} kV^{-1}. As in practice the sample solution is often prepared with a solvent having a higher elution strength than the mobile phase (for solubility reasons), in Eqs. (10) and (11) zone compression due to enrichment of the solutes in the stationary phase has not been taken into account.

4.6. Zone sharpening

If the elution strength of the sample solvent is comparable to the elution strength of the mobile phase, or even lower, zone sharpening effects can be used for improvement of the detection sensitivity. In Fig. 6 the peak width at half height for several alkyl and aryl benzoates as test solutes is plotted against the volume fraction of water in the sample solution (Column 3). Mixtures of water with acetonitrile were used as sample solvents. The concentration of the solutes in the sample ($\beta = 0.20$–0.25 g l^{-1}), sample injection parameters (23 kV, 7 s) and other parameters were kept constant. In the range of volume overload, the peak width at half height is strongly

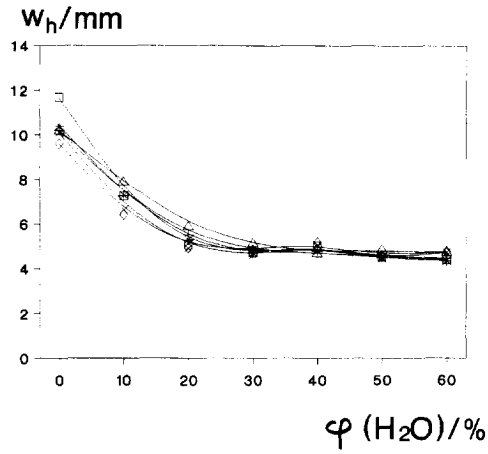

Fig. 6. Dependence of the peak width at half height on the volume fraction of water in the sample solution (injection: electrokinetic, 23 kV, 7 s; column: 345 mm (395 mm)×180 μm; packing octadecylsilica gel, d_p=3.0 μm; mobile phase: acetonitrile–phosphate buffer (80:20, v/v), pH=7.3; in-column photometric detection, 230 nm; assignment: △=methyl benzoate, +=ethyl benzoate, *=propyl benzoate, □=benzyl benzoate, ×=butyl benzoate, ◇=isopentyl benzoate).

dependent on the composition of the sample solution. At high volume fraction of water in the sample ($\varphi(H_2O$, sample)≥30%) w_h is independent of the composition of the sample solution. In this range band broadening is dominated by the chromatographic process.

In Fig. 7 chromatograms obtained with $\varphi(H_2O$, sample)=0% and $\varphi(H_2O$, sample)=50% are compared to each other. In Fig. 7a an extreme volume overload reduces strongly the measurable efficiency of the chromatographic system, while in Fig. 7b due to focusing effects band broadening resulting from the injection process has no major impact on the measurable efficiency. The peak height-to-noise-ratio is improved with $\varphi(H_2O$, sample)=50% compared to $\varphi(H_2O$, sample)=0% by factor 5.7 (taking differences in the solute concentration into account).

This zone sharpening process can be taken into account with the following equations. If the retention factors k_s for the solutes with the sample solvent as mobile phase are known, the suitable injection time and the suitable injection voltage can be calculated with Eqs. (12) and (13) (derived from Eqs. (6) and (9)).

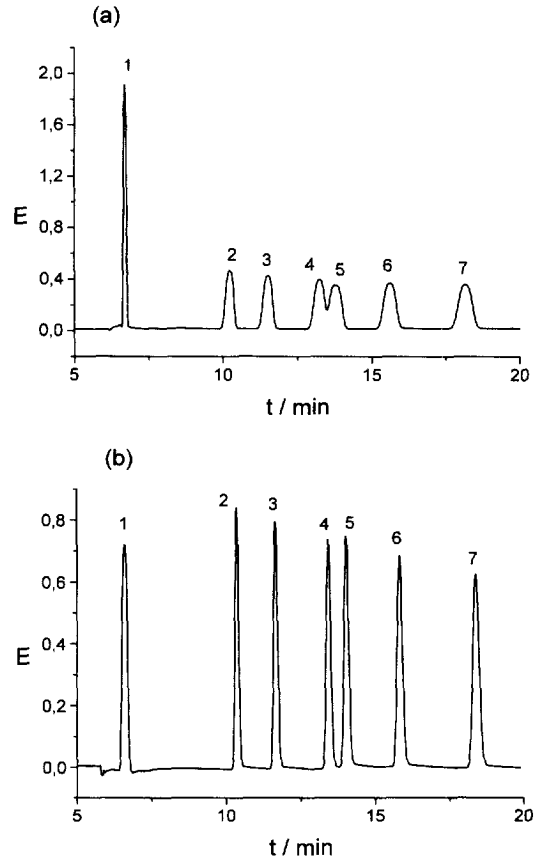

Fig. 7. Comparison of chromatograms obtained with different volume fractions of water in the sample solution, (a) $\varphi(H_2O$, sample)=0%, (b) $\varphi(H_2O$, sample)=50% (sample preparation: β(solute): 0.25 g l^{-1} (a), 0.20 g l^{-1} (b), solvent=acetonitrile–water; peak identification: 1=thiourea, 2=methyl benzoate, 3=ethyl benzoate, 4=propyl benzoate, 5=benzyl benzoate, 6=butyl benzoate, 7=isopentyl benzoate; other parameters see Fig. 6).

$$U_{max} = 0.7 \cdot \frac{LL_T(1+k_s)}{\mu_{eo} t_I \sqrt{N}} \quad (12)$$

$$t_{max} = 0.7 \cdot \frac{LL_T(1+k_s)}{\mu_{eo} U_I \sqrt{N}} \quad (13)$$

5. Conclusions

Capillary electrochromatography as a miniaturized highly efficient technique is very sensitive to extra-column band broadening. The presented equations

allow the user of CEC to select suitable injection conditions without the need to measure the volume flow. All magnitudes necessary for the calculations can be derived from geometrical parameters, the electroosmotic mobility and retention data.

The calculations (Table 1) show that with standard injection procedures (electrokinetic injection, $t=5$ s, $U=5$ kV) in CEC with octadecylsilica gel as stationary phase and columns with a total length <250 mm the criterion for the tolerable extra-column band broadening (an increase in w_h by 5%) is not fulfilled, if focusing effects due to enrichment of the solutes in the stationary phase are not taken into account.

Focusing effects (depending on the solubility of the solutes in the sample solvent) can be employed in order to greatly improve the detection sensitivity, thus lowering the detection limits when CEC is used as part of a validated method.

Acknowledgments

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