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Journal of Chromatography A, 737 (1996) 171–180

JOURNAL OF CHROMATOGRAPHY A

In-column versus on-column fluorescence detection in capillary electrochromatography

Hans Rebscher, Ute Pyell*

Fachbereich Chemie der Philipps-Universität Marburg, Hans-Meerwein-Strasse, D-35032 Marburg, Germany

First received 15 September 1995; revised manuscript received 16 November 1995; accepted 27 December 1995

Abstract

Capillary electrochromatography (CEC) is a technique that uses electroosmotic flow for the propulsion of the mobile phase in liquid chromatography. Employing packed fused-silica capillaries, fluorescence detection is possible in a packed section of the separation capillary (in-column detection) or in an emptied section (on-column detection). These two detection modes were compared with each other with respect to detection limit, precision and instrumental band-broadening effects. By monitoring plug-like solute zones it is shown that with in-column detection the signal noise is dependent on the signal height, whereas with on-column detection the signal noise is independent of the signal height.

Keywords: Detection, LC; Fluorescence detection; Electrochromatography

1. Introduction

Capillary electrochromatography (CEC), a technique that employs electroosmotic flow for propulsion of the mobile phase [1], is attracting increasing interest. CEC can be performed with wall-coated open-tubular capillaries, with drawn-packed and with slurry-packed capillaries [2–14]. Recent studies have shown that CEC constitutes a possibility of creating a very efficient and rapid liquid chromatographic system.

With slurry-packed capillaries, photometric detection is mostly performed in an unpacked section behind a frit retaining the stationary phase (on-column detection) [4,7,9,11–14]. Rebscher and Pyell [10] reported photometric detection in a packed section of the capillary (in-column detection) as part of a method for the experimental determination of instrumental contributions to the band broadening in CEC. The packing (octadecylsilica gel) does not absorb at the detection wavelength (254 nm).

In contrast to photometric detection, fluorimetric detection in CEC was performed mostly in a packed section of the capillary [2,4]. It can be assumed that the preference for in-column fluorescence detection is due to experimental reasons. To our knowledge, there have been no studies comparing in- with on-column fluorescence detection in CEC with packed capillaries.

Guthrie and Jorgenson [15] reported in-column fluorescence detection (ICFD) for open-tubular liquid chromatography (OTLC). They

* Corresponding author.

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SSDI 0021-9673(95)01378-4

calculated that in OTLC an in-column detector (detection in a wall-coated section of the capillary) will have a factor of $(1 + k)$ better sensitivity than an on-column detector. The difference in sensitivity is attributed to the "general elution problem". The solute mass per section of capillary is reduced in a non-partitioning region compared with a region containing stationary phase, because the solute mass flow-rate must be equal in the two sections, whereas the solute linear velocity in the region containing stationary phase is reduced by factor of $(1 + k)$ compared with the linear velocity in a non-partitioning region. Guthrie and Jorgenson emphasized that the chemical environment of a fluorophore can be significant in determining the fluorescence excitation and emission wavelengths and the fluorescence quantum yield. Since in a region containing stationary phase part of the solute amount is sorbed on or in the stationary phase, solute zones may exhibit significantly different fluorescence characteristics depending on the presence of stationary phase.

Takeuchi and Yeung [16] monitored the separation of anthracene from salicylic acid by fluorescence detection in a wall-coated and in an uncoated section in OTLC. The fluorescence signal obtained by in-column detection was larger than that calculated by assuming that enhancement is due only to the focusing effect by the stationary phase. This environmental effect is due either to an increased fluorescence quantum yield or to a shift in the emission or excitation spectrum of the solutes investigated.

Verzele and Dewaele [17] reported that ICFD is also possible with micro-LC employing packed capillaries. The selectivity gain, compared with on-column fluorescence detection (OCFD), reaches a factor of $(1 + k)$ multiplied by a geometrical factor due to differences in the detection volume. Signal enhancement due to the focusing effect of the stationary phase has already been successfully used by Takeuchi and Miwa [18] in conventional HPLC, employing a detection cell filled with stationary phase.

In order to evaluate the advantages and disadvantages of the two detection modes in CEC, in this paper they are compared with each other with respect to detection limit, extra-column band broadening, signal noise and precision.

2. Experimental

2.1. Preparation of packed capillaries

The packing technique presented in a previous paper [10] was slightly modified. Fused-silica capillaries (75, 100 and 150 μm I.D., 363, 330 and 360 μm O.D.) (CeramOptec, Bonn, Germany) were used as columns. The inlet frit was prepared in the following manner. Native silica gel (KG 1000, $d_p = 5$–40 μm; Merck, Darmstadt, Germany) was wetted with water and the end of the capillary was tapped into the wetted silica gel and sintered in the flame of a bunsen burner.

After having burnt off the polyimide coating at the detection window, the columns were packed as described previously [10]. The slurry is pumped into the capillary at $p = 800$ bar using a pneumatic pump (DSTV-122; Armaturenbau, Wesel, Germany). During the packing process, the capillary and the slurry reservoir are immersed in an ultrasonic bath.

After packing, the slurry liquid in the capillary is replaced with water and a frit is sintered at the outlet end by heating the packing in the flame of a bunsen burner. During the sintering process under these conditions the reversed-phase material is pyrolysed. The white packing turns black in the heated zone. If detection is to be performed in an unpacked section of the capillary, the frit is fused 60 mm distant from the outlet end under conditions specified for each column. The packing material is then removed after the sintered frit by application of pressure or voltage. The packed capillary is installed in the chromatographic system and equilibrated with the separation buffer by pumping the buffer through the column ($p = 70$ bar) for at least 1 h.

2.2. Packing material and columns

Nucleosil 100-3-C_{18}, $d_p = 3$ μm, mean pore width = 10 nm (Macherey–Nagel, Düren, Germany) was used as the stationary phase. The

packed capillaries had a total length of 300–400 mm and an effective length of 260–365 mm. The dimensions of a packed capillary are given as follows: length of the packed bed (length of the column to the detection window, total length). If the column is completely filled, the total length of the column corresponds to the length of the packed bed. In this case the dimensions are given as follows: length of the column to the detector (total length).

2.3. Chromatographic system

Chromatographic runs were carried out with a laboratory-made apparatus. High voltage was generated with a CZE 1000 R system (Spellman, Plainview, NY, USA). Detection was performed with a Shimadzu (Kyoto, Japan) RF-551 spectrofluorimetric detector, modified for detection in capillaries. The samples were injected electrokinetically. The injection time was 5–10 s at a voltage of 5 kV. The separation voltage was 30 kV in all instances. The temperature of the separation capillary was not controlled. Mixtures of acetonitrile with a solution of sodium tetraborate in water (2 mmol/l) were employed as separation electrolytes. Data were recorded with either a Waters (Milford, MA, USA) Model 740 data processor or a Philips (Kassel, Germany) PM 8251 recorder. Resorcinol was used as a marker of the hold-up time.

2.4. Reagents

Sodium tetraborate was of analytical-reagent grade (Merck, Darmstadt, Germany). Fluorene and (1,2:5,6)-dibenzanthracene were obtained from Aldrich (Steinheim, Germany) and pyrene, acenaphthene and chrysene from Chem Service (West Chester, PA, USA). Naphthalene, anthracene and resorcinol were available at the Department of Chemistry, University of Marburg, Germany.

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

3. Results and discussion

3.1. Signal enhancement

In CEC with packed capillaries, the following considerations have to be made. The "general elution problem" taken into consideration by Guthrie and Jorgensen [15] will result in focusing of the analyte zone by a factor of $(1+k)$ in ICFD compared with OCFD. The fact that in a packed zone the mobile phase fills only part of the separation capillary produces focusing in the non-packed section counteracting the first one. In Fig. 1 the "geometrical focussing effect" is depicted schematically for a non-retarded solute.

Neglecting additional effects due to instrumental band broadening and differences in the chemical environment of the solute molecules, the sensitivity ratio (s_I/s_O = sensitivity in ICFD/sensitivity in OCFD) is given by the following equation, derived according to the method of Guthrie and Jorgenson [15]:

$$s_I/s_O = (k+1)\Phi_M \quad (1)$$

where k = retention factor and Φ_M = volume fraction of mobile phase in the packed column.

It can be shown that Eq. 1 is valid for peak-area ratios and peak-height ratios. If a rectangular peak shape is assumed (see Fig. 2) the solute concentrations in the solute zones will have a ratio corresponding to the ratio of the length of the solute zone before P_2, x_1, and the length of the solute zone after P_2, x_2 (mass of solute per zone and cross-sectional area of the capillary constant):

$$c_2/c_1 = x_1/x_2 = 1/[(1+k)\Phi_M] \quad (2)$$

Using a detector with a window, w ($w \ll x_1, x_2$), at either position P_1 or P_3, the time interval in which the analyte zone passes the window will be identical for the two positions, because at P_1 the velocity of the solute zone corresponds to $v_{eo}/(1+k)$, whereas at P_3 the velocity of the solute zone corresponds to $v_{eo}\Phi_M$ (v_{eo} = electroosmotic flow in the packed section of the capillary). The detection window in ICFD must be smaller than that in OCFD if a significant contribution of the detection to the instrumental band broadening is

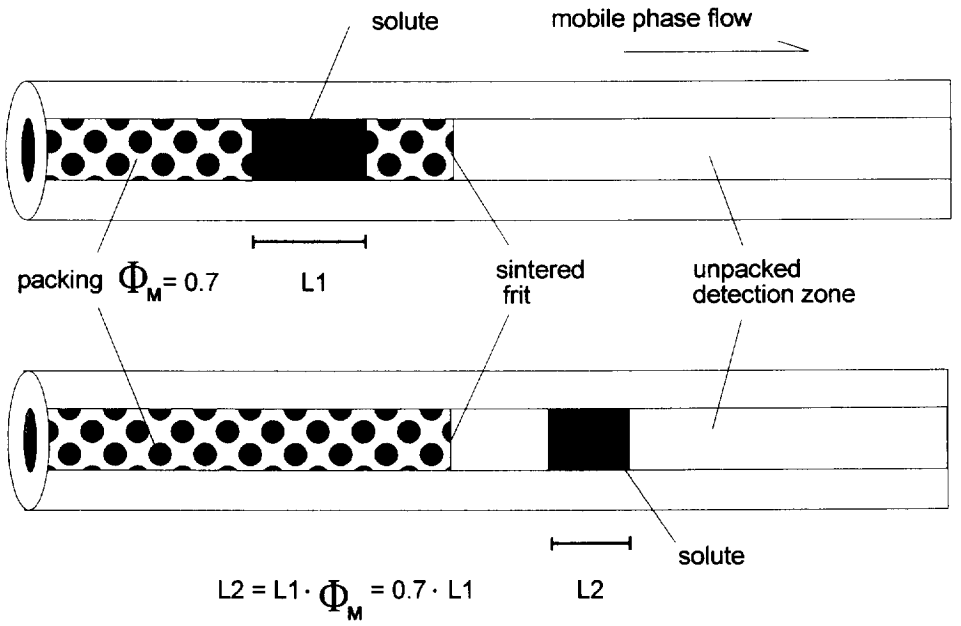

Fig. 1. Schematic diagram of the geometrical focusing effect (depicted for a non-retarded solute). Volume fraction of the mobile phase, $\Phi_M = 0.7$.

to be avoided. Jorgenson and Guthrie [15] have already stated that the spatial resolution of an in-column detector for OTLC must be a factor $(1 + k)$ better than the spatial resolution of a corresponding on-column detector. In CEC, the spatial resolution of an in-column detector must be a factor $(1 + k)\Phi_M$ better than the spatial resolution of a corresponding on-column detector.

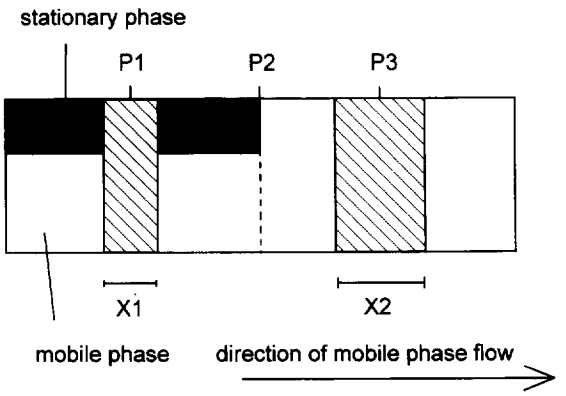

Fig. 2. Scheme of the dilution effect due to the elution of an analyte zone from the stationary phase.

Eq. 1 is valid only if the optical unit (the length of the detection cell) is the same for ICFD and OCFD, as is the case in our experiments. Φ_M can be assessed from the hold-up time of the chromatographic system and the mobile phase volume flow-rate. The mobile phase volume flow-rate, F_V, was measured using a 10-μl syringe connected to the outlet buffer reservoir [measurement conditions: capillary: 305(353) mm × 150 μm I.D., $U = 30$ kV]. In CEC, the hold-up time (the migration time of a non-retarded solute) serves as a measure of the electroosmotic velocity. For the packed capillary investigated, Φ_M was estimated to be 0.67 according to

$$\Phi_M = F_V/(v_{eo}\sigma_c) \quad (3)$$

where v_{eo} = electroosmotic velocity in the packed section and σ_c = cross-sectional area of the capillary, with $v_{eo} = 1.35$ mm/s, $F_V = 0.0160$ μl/s and $\sigma_c = 0.0177$ mm^2. v_{eo} was determined by ICFD, employing resorcinol as marker of the hold-up time. According to Mayer [19], Φ_M = 0.70–0.85 in a packed column with porous par-

ticles. Φ_M differs from packing to packing. In further calculations a rounded value of 0.7 was used.

With the assessed Φ_M, corrected values for v_{eo} and k can be calculated if OCDF is employed. It has to be taken into consideration that the velocity of the mobile phase is lower in the unpacked section than in the packed section and $k = 0$ in the unpacked section.

$$v_{eo}(\text{corrected}) = \frac{L_p + L_u/\Phi_M}{t_R} \quad (4)$$

where L_p = length of the packed section, L_u = length between frit and detection window and t_R = retention time.

$$k(\text{corrected}) = \frac{v_{eo} t_R}{L_p} - \frac{L_u}{L_p \Phi_M} - 1 \quad (5)$$

Rebscher and Pyell [10] reported a significant contribution of band broadening in the inlet frit to the overall band broadening in CEC. The assumption that extra-column band broadening in the second frit at the outlet end also contributes strongly to the overall band broadening is corroborated by results obtained by Behnke [20], imaging solute bands leaving the packed zone of a capillary by laser-induced fluorescence detection.

In order to eliminate instrumental band broadening effects and to assess whether s_1/s_0 corresponds to the theoretical value (Eq. 1), we chose the following approach. Solute zones of length 50–80 mm were injected (large volume injections), producing a non-Gaussian step-like signal (see Fig. 3). Band broadening effects only affect the shape of the detected steps and not the step height. For fluorene, a linear detection response (linear dependence of the step height on the solute concentration) was obtained for OCFD ($r^2 = 0.99998$) and ICFD ($r^2 = 0.994$) in the concentration range 0.26–16 mg/l.

The slope of the regression lines served as a measure of the detection sensitivity. s_1/s_0 was calculated by the ratio slope of regression line (ICFD)/slope of regression line (OCFD). For fluorene (with $k = 0.82$; for measurement conditions see Fig. 3) s_1/s_0 is 1.3. The theoretical value according to Eq. 1 for fluorene is also 1.3. Hence, in the studied case, Eq. 1 can be used to predict enhancement factors for ICFD.

It must be emphasized that a true enhance-

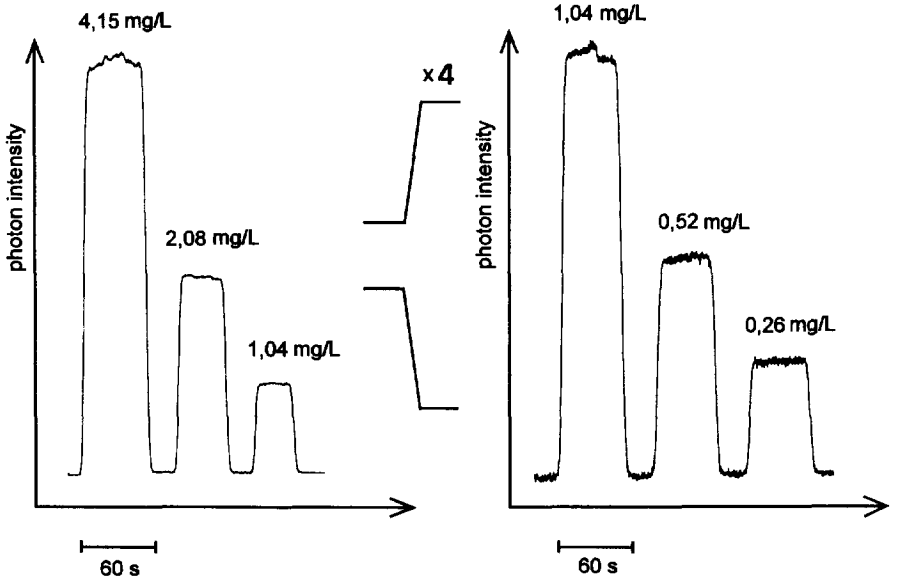

Fig. 3. Injected "plugs" of fluorene. Capillary, 313 (363) mm × 100 μm I.D.; fluorene concentration, 0.26–4.15 mg/l; electrokinetic sample injection, 30 kV for 60 s; in-column fluorescence detection, λ = 260 nm/320 nm.

ment has been obtained, not only an alteration of the peak-area ratios. In the case of low concentrations, the following equation can be given for the fluorescence intensity, I_F:

$$I_F = \text{constant} \cdot I_0 Q c \quad (6)$$

where I_0 = intensity of excitation radiation, Q = fluorescence quantum yield and c = concentration of fluorescent solute.

If the alteration of I_F can be put down nearly completely to alterations of c, it can be concluded that $I_0 Q$ remains basically constant. Although in a packed section of the capillary part of the excitation radiation is scattered away, decreasing I_0, on the other hand there is a multipath effect due to the process of diffuse scattering in the packing, so that I_0 can be increased locally. The experimental results suggest that in packed capillaries these two effects substantially compensate each other.

This conclusion is corroborated by the following experiment. The same synthetic sample was separated on a completely packed and a partly packed capillary (100 μm I.D.). As test solutes a mixture of four polycyclic aromatic hydrocarbons was used. The length to the detection window, the total length length of the capillary, injection, detection and separation parameters were kept constant. Detection was performed by ICFD with the completely packed capillary and by OCFD with the partly packed capillary. The capillary dimensions and other parameters are given in Tables 3 and 4. The inner frit in the partly packed capillary was fused according to the method of Boughtflower et al. [12] in a section of the chromatographic bed equilibrated with water under a pressure of 700 bar. The packing material did not turn black during the fusion process.

The ratio of the peak areas measured with ICFD and with OCFD is taken as a measurand of s_I/s_O. Theoretical values for s_I/s_O are calculated employing the retention factors determined by ICFD. In Table 1 theoretical enhancement factors are compared with the ratios of the peak areas. The experimentally obtained enhancement exceeds the predicted signal enhancement, supporting our assumption that the intensity of the excitation radiation in a packed section of the capillary employed as a detection window is not reduced compared with the intensity in an unpacked section of the capillary. The enhancement obtained by ICFD is in all instances higher than predicted, so that Eq. 1 allows an assessment of the actually achievable sensitivity gain by ICFD.

Table 1
Comparison of measured enhancement factors [s_I/s_O(m)] with predicted enhancement factors [s_I/s_O(th)]

Solute	k	s_I/s_O(th)	s_I/s_O(m)
Naphthalene	0.67	1.2	1.2
Fluorene	0.96	1.4	1.4
Pyrene	1.79	2.0	2.5
Chrysene	2.13	2.2	2.5

For measurement conditions, see Tables 3 and 4.

In Fig. 4, chromatograms obtained by ICFD and OCFD with a packed column (150 μm I.D.) connected to an unpacked column by a PTFE tube are compared. The PTFE tube had an I.D. corresponding to the O.D. of the fused-silica capillaries. A plug of glass-wool between the two fused-silica capillary ends served as a frit. As test solutes a mixture of six polycyclic aromatic hydrocarbons was used. All other experimental conditions except the length of the capillary and the location of the detection window were kept constant. The connection by a PTFE tube induces significant instrumental band broadening corresponding to a loss of plate numbers for the chromatographic signals. The retention factors (calculated with data obtained by ICFD) and theoretical enhancement factors for the solutes are given in Table 2. Because of the different lengths of the capillaries employed and consequently different electroosmotic velocities in the packing, peak areas determined with each of the two capillaries cannot be compared directly with each other. Therefore, the peak areas normalized to the peak area of the naphthalene peak (taking into account that the enhancement factor for naphthalene is 1.1) were taken as a measurand of the expected enhancement factor. In Table 2, theoretical enhancement factors are compared

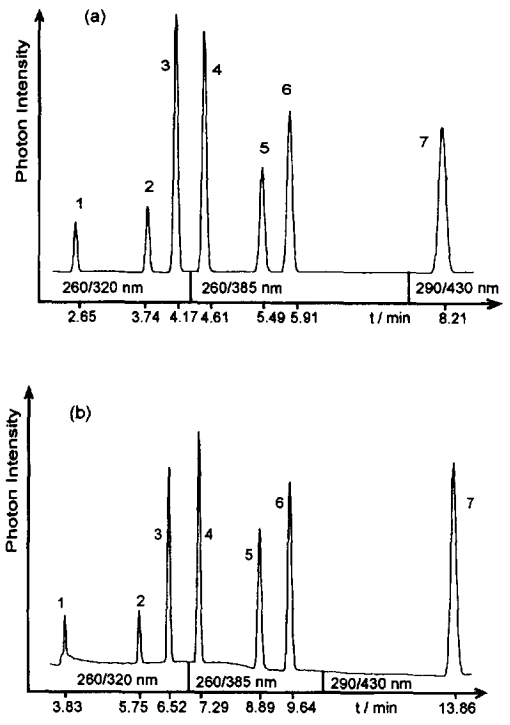

Fig. 4. Comparison of chromatograms obtained with (a) on-column and (b) in-column fluorescence detection (fluorescence intensity scales are the same). (a) Capillary, 245 (290, 335) mm × 150 μm I.D.; mobile phase velocity, 1.82 mm/s; (b) capillary, 335 (380) mm × 150 μm I.D.; mobile phase velocity, 1.46 mm/s. Peaks: 1 = resorcinol; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = pyrene; 6 = chrysene; 7 = (1,2:5,6)-dibenzanthracene. Mobile phase, acetonitrile–borate buffer (95:5, v/v); electrokinetic sample injection, 8 s at 5 kV.

Table 2
Comparison of measured enhancement factors [s_1/s_0(m)] with predicted enhancement factors [s_1/s_0(th)]

Solute	k	s_1/s_0(th)	s_1/s_0(m)
Naphthalene	0.50	1.1	
Fluorene	0.70	1.2	1.1
Anthracene	0.90	1.3	1.4
Pyrene	1.32	1.6	2.1
Chrysene	1.52	1.8	1.8
(1,2:5,6)-Dibenz-anthracene	2.60	2.5	2.4

For measurement conditions, see Fig. 4.

with the ratios of the normalized peak areas. With the exception of pyrene, there is excellent agreement of the theoretical enhancement factors with the measured data.

3.2. Precision

For ten consecutively repeated injections of the same sample separated on a completely packed (ICFD) and a partly packed (OCFD) capillary (I.D. 100 μm), the retention time, t_R, and the peak area, A, were determined. As test solutes a mixture of five polycyclic aromatic hydrocarbons was used. The capillary dimensions and other parameters are given in Tables 3 and 4. Data of the relative standard deviation (R.S.D.) of t_R, A and A normalized to t_R (A/t_R) are given for OCFD and ICFD in Tables 3 and 4.

With OCFD the R.S.D.(t_R) is very low, demonstrating that the flow of the mobile phase can be kept very constant in CEC under the measurement conditions employed. R.S.D.(A) is higher than R.S.D.(t_R). Normalization of the peak area to the retention time or the peak area of an internal standard does not improve R.S.D.(A) significantly. Hence the standard deviation of the peak area is mostly due to the detection and the data processing. Variations of the sample volume injected and of the mobile phase velocity have only a secondary influence on R.S.D.(A).

For ICFD, R.S.D.(t_R) is higher than for OCFD. This increase might be due to variations in the temperature of the capillary. The temperature of the capillary is not controlled with the apparatus employed for these experiments. With ICFD, R.S.D.(A) is about twice that obtained by OCFD. The increase in R.S.D.(A) with ICFD cannot be ascribed completely to the increase in R.S.D.(t_R) because with ICFD the R.S.D. of the peak areas normalized to t_R is also higher than R.S.D.(A/t_R) with OCFD. Normalization of the peak areas obtained with ICFD to the peak area of an internal standard did not decrease R.S.D.(A) significantly. It is interesting that the peak with the highest signal-to-baseline noise ratio, the fluorene peak, has the lowest R.S.D.(A) with OCFD but the highest R.S.D.(A)

Table 3
Precision of the determination of five PAHs (in-column fluorescence detection)

Solute	k	R.S.D.(t_R) (%)	R.S.D.(A) (%)	R.S.D.(A/t_R) (%)
Naphthalene	0.67	0.8	2.6	2.8
Fluorene	0.96	0.9	5.1	4.5
Anthracene	1.24	0.9	3.4	2.8
Pyrene	1.79	1.0	3.3	3.0
Chrysene	2.13	1.1	3.7	3.1

Ten repeated injections; column, 330 (377) mm × 100 μm I.D.; mobile phase, acetonitrile–borate buffer (9:1, v/v); in-column fluorescence detection, for wavelengths see Fig. 4; electrokinetic sample injection, 5 kV for 9 s.

Table 4
Precision of the determination of five PAHs (on-column fluorescence detection)

Solute	k	R.S.D.(t_R) (%)	R.S.D.(A) (%)	R.S.D.(A/t_R) (%)
Naphthalene	0.63	0.5	1.9	1.6
Fluorene	0.89	0.5	1.4	1.2
Anthracene	1.14	0.5	1.4	1.4
Pyrene	1.64	0.5	2.3	2.1
Chrysene	1.92	0.4	1.8	1.6

Ten repeated injections; column, 305 (330, 377) mm × 100 μm I.D.; mobile phase, acetonitrile–borate buffer (9:1, v/v); on-column fluorescence detection, for wavelengths see Fig. 4; electrokinetic sample injection, 5 kV for 9 s.

with ICFD. The data presented strongly supports our hypothesis (derived in the next section) that especially for signals with high signal-to-baseline noise ratios the precision with ICFD is lower than that with OCFD owing to the signal noise observed for ICDF.

Quantitative determinations are possible with ICFD and OCFD in connection with CEC for all solutes investigated under the measurement conditions employed.

3.3. Signal-to-noise ratio

Usually when determining the signal-to-noise ratio (S/N), the signal height is compared with the noise of the baseline assuming the noise to be independent of the signal height. This assumption is fulfilled with OCFD. With ICFD, however, there is a strong signal noise dependent on the signal height. Fig. 3 shows six recorded steps obtained by injection of the same large volume of fluorene at various concentrations. Whereas at low concentrations of the solute, resulting in a small signal height, the signal noise recorded in the upper plateau region corresponds to the baseline noise, at higher concentrations of the solute the signal noise increases with the signal height. In the signal noise, long-term fluctuations are superimposed over short-term fluctuations. The short-term fluctuations correspond to the fluctuations of the baseline noise.

For the calculation of S/N at the detection limit, only the baseline noise has to be taken into consideration, because the signal noise corresponds to the baseline noise at the detection limit. For a 100 μm I.D. capillary, with the detection parameters excitation wavelength = 260 nm, emission wavelength = 320 nm, electroosmotic velocity = 1–1.5 mm/s and mobile phase acetonitrile–borate buffer (90:10, v/v), the

baseline noise with ICFD is about twice that with OCFD. This increase in the baseline noise depends on the capillary employed. It can be attributed to the diffuse scattering in the packed bed increasing the background fluorescence level.

Consequently, if it is to be evaluated whether OCFD or ICFD is to be preferred with respect to the detection limit, not only the enhancement factors but also the baseline noise under the conditions of detection have to be taken into consideration. The ratio baseline noise (ICFD)/baseline noise (OCFD) is strongly dependent on the conditions of detection.

The characteristic of the signal noise in ICFD (proportional to the fluorescence signal, stepwise, long-term fluctuations) suggests that it might be due to minute movements of the chromatographic bed in the applied electric field. Further investigations are necessary in order to investigate whether the observed signal fluctuations can be avoided by using a different packing technique.

3.4. Tailing due to interaction with the frit

One problem, not completely solved in CEC, is the preparation of internal frits for on-column detection. Smith and Evans [9] and Boughtflower et al. [12] proposed the preparation of internal frits by fusing the actual reversed-phase packing. This approach is comparably simple in practice, avoiding tedious filling, emptying and refilling techniques.

It must be guaranteed, however, that pyrolysis of the bonded material is avoided. With the packing material employed in this study, blackening of the heated zone was observed during the process of fusing the inner frit due to pyrolysis of the alkyl groups if the conditions were as follows: capillary equilibrated with water, no pressure applied and heating with a hot wire for 10 s.

In Fig. 5, chromatograms of PAHs obtained by ICFD and OCFD on different parts of the same partly filled capillary with an inner frit are presented. The chromatographic bed at the inner frit of this capillary contains black material

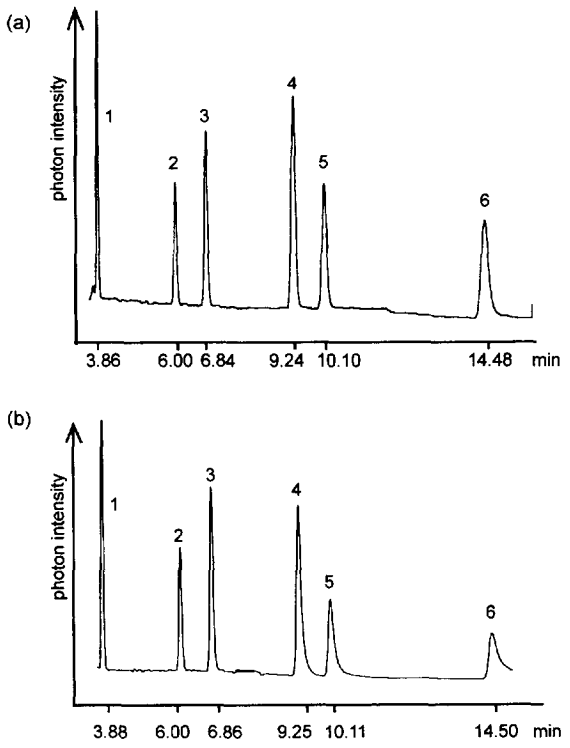

Fig. 5. Comparison of (a) in-column [345 (350, 390) mm × 150 μm I.D.] and (b) on-column [350 (390) mm × 150 μm I.D.] fluorescence detection in the same capillary. For wavelengths, see Fig. 4. Peaks: 1 = resorcinol; 2 = naphthalene; 3 = fluorene, 4 = pyrene; 5 = chrysene; 6 = (1,2:5,6)-dibenzanthracene. Mobile phase, acetonitrile–borate buffer (90:10, v/v); electrokinetic sample injection, 10 s at 5 kV.

produced by thermolysis. Whereas the signals recorded in front of the inner frit exhibit no asymmetry, they are strongly deformed after passing the inner frit. Comparison with Fig. 4 shows that the deformation of the signals cannot be attributed exclusively to instrumental band-broadening effects, but rather to strong interaction of the solutes with the thermolysed silica gel surface resulting in strong peak tailing. It is known that PAHs are strongly sorbed on graphitized carbon.

4. Conclusions

The advantages of in-column fluorescence detection, viz., signal enhancement, less instrumen-

tal band broadening and easier packing techniques, make this detection mode preferable to on-column fluorescence detection. The signal enhancement obtainable by ICFD compared with the signal in OCFD can be quantitatively estimated by employing Eq. 1. The detection limit is lower with ICFD than OCFD if the signal enhancement exceeds the factor by which the baseline noise in ICFD is increased compared with OCFD. However, with common capillary filling techniques currently applied in CEC, there are fluctuations in the fluorescence signal that render quantitative determinations with ICFD less precise than those with OCFD. The origin of the reported fluctuations needs further investigation.

If internal frits are prepared by fusing a section of the actual chromatographic packing, thermolysis of the packing material has to be avoided, because interactions between the solute and the thermolysed alkylated silica gel can cause peak tailing.

Acknowledgements

The authors gratefully acknowledge financial help from the Deutsche Forschungsgemeinschaft. They thank the workshops of the Chemistry Department of the University of Marburg for their kind cooperation. They also thank one of the referees for having pointed out that Eq. 1 is valid for both peak-height and peak-area ratios.

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