Journal of Chromatography, 363 (1986) 187–198
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 720

POST-COLUMN REACTION DETECTION FOR OPEN-TUBULAR LIQUID CHROMATOGRAPHY USING LASER-INDUCED FLUORESCENCE*

H. P. M. VAN VLIET, G. J. M. BRUIN, J. C. KRAAK and H. POPPE*
University of Amsterdam, Analytical Department, Nieuwe Achtergracht 166, 1018 WV Amsterdam (The Netherlands)
(First received December 24th, 1985; revised manuscript received April 5th, 1986)

SUMMARY

The applicability of post-column reaction detection to open-tubular liquid chromatography is investigated, using the *o*-phthalaldehyde–amino acid reaction and laser-induced fluorescence detection. Two mixing devices were designed and evaluated in terms of external peak broadening on the nanolitre scale. It was found that mixing devices having a volume of about 10 nl resulted in an acceptable loss in plate numbers for unretained peaks using 25 μm I.D. fused-silica columns. A method was developed for determining the flow-rates and residence times in the various parts of the system, based on the correlation of observed total residence times and the pressures on the column and the reagent delivery system.

INTRODUCTION

The favourable features of open-tubular liquid chromatography (OTLC), *i.e.*, separation speed and efficiency, can only be exploited when the external contribution to the peak broadening is kept in the order of 1–10 nl[1,2]. It has been shown that an external broadening of less than 1 nl can be realized by using split injection and laser-induced fluorescence detection (LIF)[3,4].

That work was carried out with a relatively simple and cheap helium–cadmium laser which might be considered for more general application in the not too distant future. However, the availability of only two lines, 325 and 442 nm, limits the number of fluorophores that can be detected. This disadvantage can be overcome by using pre- or post-column derivatization reactions. Many derivatization agents, such as dansyl chloride, bansyl chloride, *o*-phthalaldehyde[5–7] and coumarin-based reagents[8], are suitable for excitation at 325 nm and their use has been described.

Batchwise pre-column derivatization has the advantage of introducing no extra-column peak broadening and a relatively free choice of reaction conditions. On

* The main part of this paper was presented at the *9th International Symposium on Column Liquid Chromatography, Edinburgh, July 1–5, 1985*. The majority of the papers presented at this symposium have been published in *J. Chromatogr.*, Vols. 352 and 353 (1986).

0021-9673/86/$03.50

the other hand, on-line post-column derivatization techniques require less sample pretreatment, do not lead to interfering artefacts and are easier to automate. The main drawback is the loss in resolution, due to the extra broadening caused by the mixing devices and reactors[9]. The requirements in terms of the external peak broadening become more stringent for very efficient columns, for compounds with small capacity factors, k', and for miniaturized columns. For OTLC these requirements are rather extreme because the volume standard deviations of the peaks are at the nl to pl level.

At the low flow-rates used in OTLC, open-tubular reactors of small diameters appear to be best. The dispersion in that type of reactor is low due to the small I.D. and the capillaries are easy to manipulate. However, the mixing devices are problematic. Two designs were investigated with respect to their contribution to the external broadening, using two different methods. Given a certain extra broadening, the demands on the remaining parts of the chromatographic system can be discussed, and one can estimate the minimum plate number required to keep the loss in resolution within preset limits.

No pumps are available that can meter the required flow-rates in the range of nl/s. Therefore, in OTLC, the solvents are always delivered in a constant pressure mode. Also the flow-rates are too low for measurement by volume or by weight. Further, the pressure drop over the reactor, *i.e.*, between the mixing point and outlet, cannot be measured independently due to the low volume scale. Therefore, we have investigated a method to determine the residence time of a sample in the column and the reactor as a function of the pressure on the column and on the reagent delivery capillary. Amino acids were used as model compounds, while *o*-phthalaldehyde (OPA) was used as the post-column reagent.

MATERIALS AND METHODS

Chemicals

The solvents used were analytical and Uvasol grade methanol, purchased from Baker (Deventer, The Netherlands) and Merck (Darmstadt, F.R.G.) respectively, HPLC-grade acetonitrile (Rathburn, Walkerburn, U.K.) and deionized water, filtered through a PSC filter assembly (Barnstead, Boston, MA, U.S.A.). Prior to use, all solvents were degassed by vacuum suction over 0.5-μm acetate filters (Millipore, Bedford, MA, U.S.A.).

The sample compounds used were fluoranthene and D-alanine (P-L Biochemicals, Milwaukee, WI, U.S.A.). Stock solutions of fluoranthene were prepared in methanol and acetonitrile, and the amino acid was dissolved in acetonitrile–0.05 *M* borate buffer, pH 9 (1:1).

For the post-column reaction, 100 mg of OPA (Merck) and 0.4 ml of 2-mercaptoethanol (Aldrich, Beerse, Belgium) were dissolved in 4 ml ethanol (Baker) and diluted to 100 ml in the borate buffer.

Apparatus

Fig. 1 shows the general experimental set-up. The column flow was delivered by a syringe pump (Series 4100; Varian, Walnut Creek, CA, U.S.A.), the reagent by a reciprocating pump (100-S-2; Eldex, Menlo Park, CA, U.S.A.), equipped with a

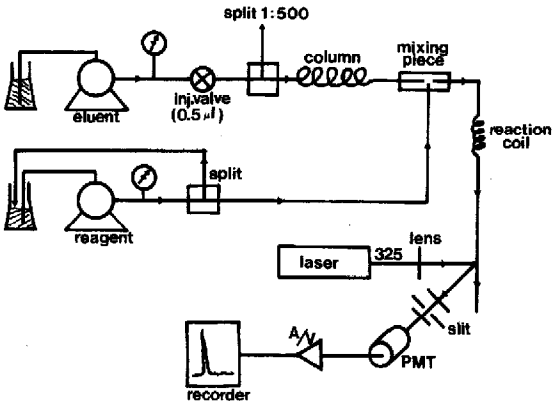


Fig. 1. The chromatographic system. At the emission side a lens, cut-off filter and diaphragm were inserted.

Bourdon-type pulse damper. A Model 7520 0.5-µl injection valve (Rheodyne, Berkeley, CA, U.S.A.), laboratory-made splitting devices[4] and fused-silica capillaries of different lengths and internal diameters (SGE, Ringwood, Victoria, Australia) were used. The various laboratory-made mixing devices will be described below.

Optics and detection

The light source was a Model 4110 H-uv helium–cadmium laser (Liconix, Sunnyvale, CA, U.S.A.), operated at its 325-nm line. The optical system and its alignment have been described elsewhere[4]. A 420-nm cut-off filter and a diaphragm were inserted at the emission side.

Detection was done "on column". In this detection mode the laser beam is focused on the end of the fused-silica reaction capillary, the protective polymer layer of which has been burnt away over a short distance.

Column and capillaries

Since for the characterization of a detection system the most stringent requirements on external peak broadening apply to unretained peaks, the column used did not contain a stationary phase. The dimensions of the fused-silica column were 425 cm × 25 µm I.D. The reaction capillary (25 µm I.D.) had a length of 72 cm and the reagent was delivered through a 20 cm × 10 µm I.D. capillary.

Mixing devices

Two mixing designs were investigated. The first was based on a modified 1/16-in. zero dead volume connector (Valco, Houston, TX, U.S.A.), shown schematically in Fig. 2A. A hole was drilled into the side of the connector in which a stainless-steel tube (0.5 mm I.D.) was soldered. The connector was drilled through and a stainless-steel liner (5 mm × 1 mm O.D.) was inserted. The I.D. of the liners investigated were 0.3 and 0.2 mm.

The column and the reaction capillary were inserted in the liner and fixed by using appropriately drilled no-hole PTFE ferrules (Chrompack, Middelburg, The

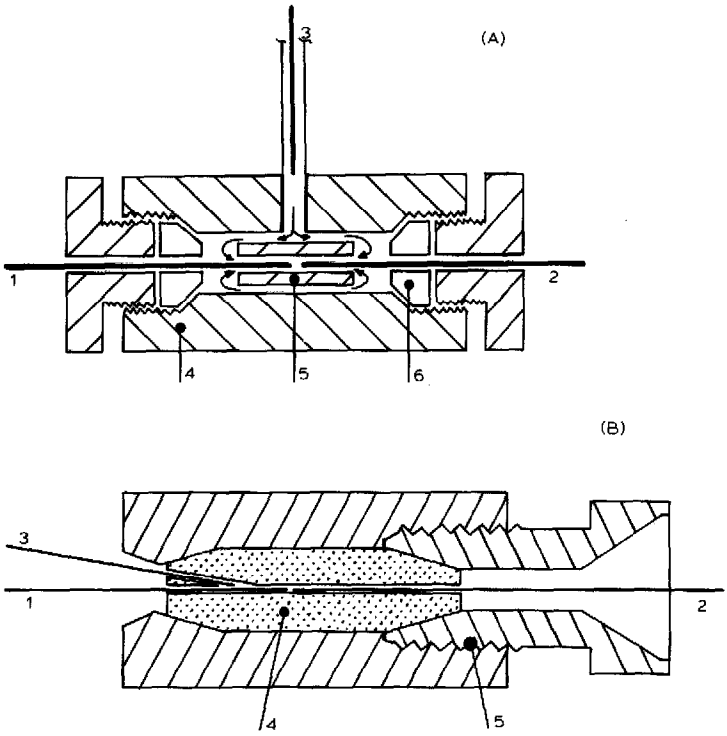


Fig. 2. Design of the mixing devices. 1 = Open-tubular column; 2 = reaction capillary; 3 = reagent delivery capillary; (A) based on a Valco 1/16-in. zero dead volume connector, 4 = connector body, 5 = stainless-steel liner, 6 = PTFE ferrules, arrows indicate reagent flow; (B) based on a Supelco butt-connector, 4 = vespel ferrule, 5 = connector body.

Netherlands). Care has to be taken that the ferrules do not block the reagent flow, as indicated by the arrows in Fig. 2A. The reagent delivery capillary was connected to the stainless-steel tubing by means of a common 1/16-in. zero dead volume connector.

The second design was based on a low dead volume butt-connector (Supelco, Bellefonte, PA, U.S.A.), as shown in Fig. 2B. The double tapered ferrule was replaced by a laboratory-made vespel ferrule, drilled through so as to have an I.D. of 0.2 mm. Then another 0.2-mm hole was drilled at an angle of 10–15° to accommodate the reagent delivery capillary. The column was inserted about half-way into the ferrule and fixed by means of a short piece of adhesive tape. Then the reagent delivery capillary and the reaction capillary were inserted and the connector fastened more than hand tight.

Determination of the residence time in the column and the reactor

As stated before, both the mobile phase and the reagent are delivered under constant pressure. If the pressure on the reagent line is increased the flow-rate through the reaction capillary will increase, resulting in an increase in the pressure drop over this capillary. Since the pressure at the beginning of the column is constant, the

pressure drop over the column will decrease. The overall result is an increase in the residence time in the column and a decrease in the residence time in the reactor.

The residence times in the column and the reactor cannot be measured and have to be calculated from the flow-rates. However, the latter also cannot be measured directly and have to be determined from the pressure drop over the column and the reactor, using the Hagen–Poiseuille equation. Once the flow-rates are known, the residence time of a sample in the column and in the reactor can be calculated. Unfortunately, due to the low volume scale, it was not possible to monitor the pressure in the mixing device. However, with capillaries of known diameters and lengths and known mobile phase and reagent compositions, the various flows-rates and the composition of the flow through the reactor can be calculated. This requires an iterative algorithm, as the viscosity of the reagent mixture changes with its composition. Of course the viscosity must be known as a function of the composition, either from the literature or from measurements, using a viscosity meter. Then calculation of the flows through the three capillaries is possible without taking the system apart.

The nominal diameters of the capillaries are not accurate, therefore it is preferable to determine these experimentally. This can be done in a preliminary experiment, in which the mobile phase and the reagent comprise the same solvent. In order to simplify the calculations it is preferable to carry them out in terms of "resistances" rather than in terms of diameters.

The resistance, R, of a capillary i can be defined as

$$R_i = \Delta P_i / F_i \tag{1a}$$

in which ΔP is the pressure drop and F is the flow-rate. Using the Hagen–Poiseuille equation, the resistance can be written as

$$R_i = 128\, L_i \eta_i / \pi d_i^4 \tag{1b}$$

in which L is the length, η is the viscosity and d is the diameter. As shown in the Appendix, the expression $t_0 = t_c + t_m$ can be written as

$$t_0 = V_c \cdot \frac{R_r R_c + R_c R_m + R_r R_m}{P_c (R_r + R_m) - P_r R_m} + V_m \cdot \frac{R_r R_c + R_c R_m + R_r R_m}{P_c R_r + P_r R_c} \tag{2}$$

in which c, r and m represent the column, reagent and mixing, *i.e.*, reaction, capillary, respectively.

In the preliminary experiments, eqn. 2 was used to determine the diameters of the three capillaries. This was done using methanol as the only solvent in the system, yielding a constant and known viscosity in all the capillaries. The t_0 was measured at different column and reagent pressures. The diameters were calculated by use of eqn. 2 using an iterative multiple non-linear fitting program, based on the Marquardt algorithm[10].

Next the column and reagent solvents were changed to the required compositions. R_c and R_r can be calculated, taking account of the viscosity of the new solvents. Since the composition and the viscosity of the reactor mixture is unknown, it is not possible to calculate R_m. However, given P_c and P_r and the resulting t_0, the resistance of the reactor, R_m, is the only unknown in eqn. 2 and can be determined.

Then t_c, t_m, F_c, F_m and the ratio of the mobile phase to reagent flow-rates in the reactor can be calculated.

From the calculated flow-rate ratio the viscosity of the fluid in the reactor is calculated. This viscosity can be checked against the known viscosity at the calculated ratio, and provides an indication of the accuracy of the method. Calculations were done using Fortran 77 programs on an HP-1000 minicomputer and using BASIC programs on a Sinclair ZX Spectrum.

External broadening due to the mixing devices

When evaluating the dispersion in the mixing devices it was assumed that they behave as ideal, exponential mixing chambers. Two methods were used, both based on the exponential modified gaussian peak model[1].

The first method has been used previously to determine the volume of a low dead volume connector, and results in an average value for the velocity range measured[3,4]. From the volume the extra peak broadening can be calculated. The reagent capillary is closed and peaks are recorded at various velocities. By using the method of Foley and Dorsey[11] or that of Anderson and Walters[12], the peaks are deconvoluted into a gaussian part with a standard deviation σ and an exponential part with a time constant τ. The σ value is checked against the one obtained by calculation using the Golay equation. The τ value is corrected for the injection volume (1 nl), as described[3]. Next a plot is made of τ^2 against $1/F_c^2$. In the case of an ideal mixing chamber this plot results in a straight line, the volume of the chamber being equal to the square root of the slope[3]. Assuming that the apparent volume does not change with the reagent flow-rate, the external broadening due to the mixing device can then be calculated from:

$$\tau_{v,extra} = \frac{F_c}{F_c + F_r} \cdot V_m \tag{3}$$

The second method does not depend on this assumption and is carried out with a non-zero reagent flow-rate. It directly gives a value for the extra column broadening at a specified column and reagent flow-rate. Again the obtained peak is deconvoluted. Using the procedure described above, the linear velocities in the column and the reaction capillary are calculated and the gaussian σ checked against the calculated one. In order to determine the external broadening caused by the mixing device alone, the τ value in time units is corrected for the overall electronic time constant, T_{RC}, converted into a τ value in volume units by multiplication with the column flow-rate and corrected for the injection volume.

Detection limits

Mass detection limits were defined as the mass required for a peak height equal to three times the standard deviation of the noise, σ_N. Concentration detection limits were calculated from chromatograms using the equation

$$c = \frac{m}{\sigma_v\sqrt{2\pi}} \tag{4}$$

where c is the concentration detection limit, m is the mass detection limit and σ_v is the peak volume standard deviation.

Noise levels were calculated by recording the baseline signal over 1-min intervals and taking the average of at least seven measurements of the peak-to-peak signal as $4\sigma_N$.

RESULTS AND DISCUSSION

Residence time

Using the described method, plots like the one shown in Fig. 3 can be made of the overall residence time, t_0, and the reactor residence time, t_m, for a given column pressure, P_c, at a varying reagent pressure, P_r. An increase in P_r results in an increase in the pressure drop over the reactor. Since the pressure on the column is constant, the pressure drop over the column decreases, resulting in an increase in the residence time in the column. On the other hand the residence time in the reactor decreases. In general the resistance of the reactor will be smaller than that of the column. The overall result is an increase in the observed t_0 and an increase in the reagent-to-column solvent ratio.

The measured viscosity of the solvent in the reactor never differed by more than 9% from the value calculated using eqn. 2. It can be concluded that the method yields acceptable results for the residence time of the sample in the various parts of the system. During all experiments the temperature was assumed to remain constant, therefore no corrections of the viscosities were made in this respect.

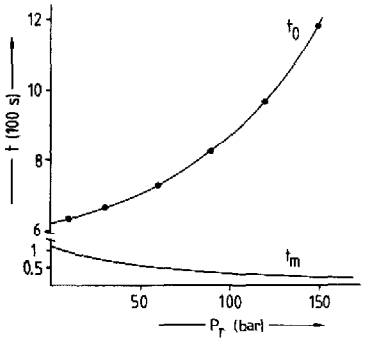
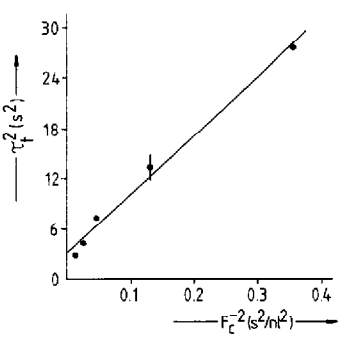


Fig. 3. Dependence of the column and reaction capillary residence times on reagent pressure. Column: 425 cm × 25 μm I.D. Reaction capillary: 72 cm × 25 μm I.D. Solvents: methanol. Solute: fluoranthene. Column pressure: 21 bar.

External peak broadening

In Fig. 4 a plot is shown of τ_t^2 *vs.* $1/F_c^2$ for the 0.2 mm I.D. Supelco-based mixing device. As is seen, the slope and thus the apparent volume decreases somewhat at higher values of $1/F_c^2$, *i.e.*, at lower flow-rates. The shape of the curve was the same for all the mixing devices. Therefore, for comparison purposes each mixing device can be assigned an apparent volume over the investigated velocity range (3–20 mm/s).



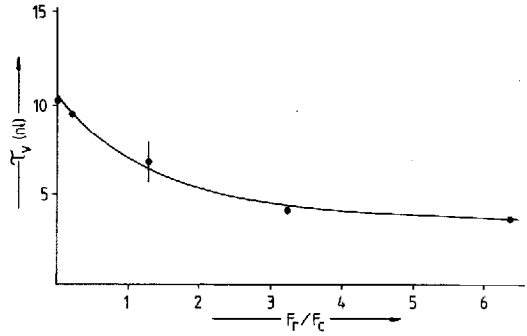


Fig. 4. Dependence of the external broadening on the reciprocal of the column flow-rate for the 0.2 mm I.D. Supelco-based mixing device (Fig. 2B). Details as in Fig. 3.

Fig. 5. Dependence of the external broadening on the ratio of the column and reagent flow-rates for the 0.3 mm I.D. Valco based-mixing device (Fig. 2A). Details as in Fig. 3.

This volume is equal to the square root of the average slope of the plot. The results for all the mixing devices investigated are shown in Table I.

In Fig. 5 a plot is shown of the resulting extra peak broadening in volume units, τ_v, against the ratio of the volumn and reagent flow-rates for the 0.3-mm bore Valco mixing device. Similar plots were obtained for the other designs.

Using either method and assuming a 1:1 ratio of the column to reagent flow-rates, both these methods show that the external broadening due to the mixing device is about 4 nl for 0.2 mm I.D. and about 7 nl for 0.3 mm I.D.

The contribution of the reaction capillary was calculated to be 5–15% of the total external broadening by means of the Golay equation. This contribution can be considered negligible relative to that of the mixing devices alone.

The use of more elementary methods, based on the assumption that the peaks remain gaussian, resulted in lower values for the calculated extra broadening. Some results were obtained by taking the peak width at 10% of the peak height as 4.29 times the standard deviation of the peak. Then the extra broadening can be obtained by subtracting the calculated column variance. The values obtained in this way are about 25% smaller. When the peak width at 60% is taken as twice the standard

TABLE I

APPARENT VOLUMES OF THE MIXING DEVICES

Mixing device	*Internal diameter (mm)*	*Volume (nl)*
Modified Valco connector	0.3	13 ± 3
	0.2	8.3 ± 0.3
Modified Supelco connector	0.2	8 ± 2

deviation an even lower extra broadening is found (40%). However, in this study the assumption of a gaussian peak shape is less realistic than the assumption of an exponential modified gaussian peak shape.

As expected, care has to be taken to cut the column end and the beginning of the reaction capillary as straight as possible. The fused silica has to be scored through the polyimide layer before breaking. Simply breaking the capillaries by hand resulted in apparent volumes of 20 nl or more. Before inserting the capillaries in the mixing device, the end faces were observed under a microscope.

The external peak broadening determined is large compared to that found for a connector used to couple 25 μm I.D. fused-silica tubes. This was determined to be about 2 nl, using the Valco 1/32-in. "through"-type connector. In this connector the capillaries are completely surrounded by the adaptors. This results in a good alignment of the capillaries[3,4]. In the present mixing devices leakage through the gap between the liner and the capillaries is essential. Therefore the bore of the liner has to be somewhat wider then the O.D. of the capillaries. This makes a rather bad alignment inevitable, as shown in Fig. 6A. Therefore a porous liner as shown in Fig. 6B is suggested as optimal. In this design the liner is drilled to a close fit around the capillaries, eliminating their free movement. However, we have not yet been able to drill a precise hole in a metal frit.

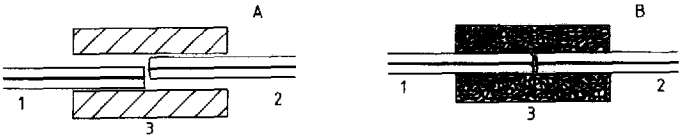


Fig. 6. Design of the liner used in the Valco-based mixing device. 1 = Open-tubular column; 2 = reaction capillary; 3 = liner. (A) Present liner design; (B) proposed porous liner design.

Loss in efficiency

In order to evaluate the extra broadening in terms of the loss in efficiency, calculations were done using the Golay equation. Assuming a fixed internal column diameter of 25 μm and a rather high extra broadening of 6 nl, the theoretical results for two columns are as shown in Table II. For an unretained peak the loss in efficiency will be less than 10% for a column length of greater than 25 m. For a compound with a k' of 0.1 the loss in efficiency is less than 3%. When using short columns the decrease in efficiency is larger. In the case of a 2.5-m column the loss at $k' = 0.5$ is still about 5%.

Alternatively the approach of Knox and Gilbert[2] can be followed. They assume a fixed extra broadening and then calculate optimum system parameters. Assuming an extra broadening of 0.5 nl, it can be calculated that OTLC gives a faster separation than high-performance liquid chromatography (HPLC) under optimum conditions in packed columns when plate numbers of > 25 000 are required. This plate number increases to 88 000 when a fixed extra broadening of 6 nl is assumed. The optimum column I.D. is then 15 μm. However, since the apparent volume of the mixing devices depends on the flow-rate, the external broadening will decrease when

TABLE II

INFLUENCE OF EXTERNAL BROADENING ON THE EFFICIENCY OF OPEN-TUBULAR COLUMNS WITH AN INTERNAL DIAMETER OF 25 μm

System: OTLC, on-column detection; stationary phase film thickness 1 μm; diffusion coefficient in stationary phase $5 \cdot 10^{-12}$ m²/s; diffusion coefficient in mobile phase $1 \cdot 10^{-9}$ m²/s; time constant 0.16 s; injection volume 1 nl; linear velocity 10 mm/s. Post-column reactor: extra broadening taken as 6 nl.

Column length (m)	*k'*	*Number of plates, N*		*Loss in N (%)*
		System	*System + post-column reactor*	
25	0	382 000	346 000	9.5
	0.1	123 500	120 200	2.5
	0.5	50 800	50 600	0.5
	1	39 900	39 850	0.1
	3	34 250	34 230	< 0.1
2.5	0	37 510	18 520	52
	0.1	12 300	9670	21
	0.5	5080	4830	5.0
	1	3990	3930	1.5
	3	3430	3430	< 0.1

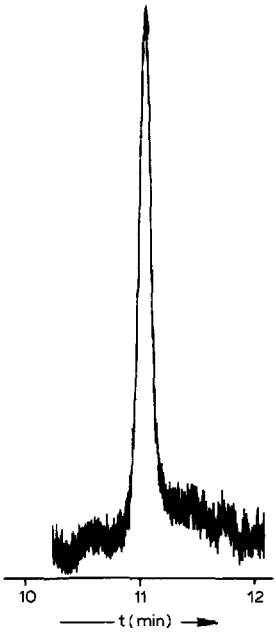


Fig. 7. Chromatogram of 12.4 pg alanine. Column flow-rate: 3.5 nl/s. Reagent flow-rate: 1.8 nl/s. Column: 425 cm × 25 μm I.D. Reaction capillary: 72 cm × 25 μm I.D. Residence time in reaction capillary: 71 s. Mobile phase: acetonitrile–water (60:40, v/v). Reagent solvent: 0.05 *M* borate buffer, pH 9, in water.

changing from 25 to 15 μm I.D. capillaries. Therefore the 15-μm capillary is a conservative estimate and the break-even point probably lies at a lower plate number.

It is clear that the use of a post-column reactor results in a highly significant extra peak broadening. The potential advantage of OTLC can no longer be exploited at plate numbers up to about 80 000, when compared to HPLC in packed columns under optimum conditions, *i.e.*, without extra broadening due to injection, post-column reaction detection, etc. On the other hand, the increased applicability of the LIF system is of great importance. Since OTLC will be used especially when high plate numbers are needed, where the loss in efficiency is relatively small, it can be concluded that the extra broadening caused by the post-column reactor is acceptable.

Detection limit

The performance of the post-column reactor is demonstrated in Fig. 7 which shows a peak obtained by injection of 12 pg of alanine. The mass detection limit is 0.8 pg, using an electronic time constant, T_{RC}, of 0.16 s. The concentration detection limit was calculated[9] to be $2 \cdot 10^{-7}$ mol/l. No attempts were made to optimize further the system for a better detection limit.

CONCLUSION

It has been shown that post-column reaction techniques are feasible in OTLC when using 25 μm I.D. columns. Since the apparent volume of the chamber in the mixing device decreases with decreasing flow-rate, it is expected that post-column reactions will also be feasible with smaller I.D. columns. Work on post-column reaction detection, including chemiluminescence reactions, using 10 μm I.D. columns is in progress[13].

APPENDIX

Using the Hagen–Poiseuille equation the resistance, R, of a capillary i can be defined as

$$R_i = \frac{\Delta P_i}{F_i} = \frac{128\ L_i \eta_i}{\pi d_i^4} \tag{A1}$$

in which ΔP is the pressure drop, F is the flow-rate, d is the diameter, η is the viscosity and L is the length.

The t_0 in the system is given by

$$t_0 = t_c + t_m = \frac{V_c}{F_c} + \frac{V_m}{F_c + F_r} \tag{A2}$$

where t_c is the residence time in the column and t_m is that in the reaction capillary; V_c and V_m are the respective volumes of the column and reaction capillary. The flow-rate in the reaction capillary, F_m, is equal to the sum of the column flow-rate, F_c, and the reagent flow-rate, F_r.

F_c can be rewritten as

$$F_c = \frac{P_c - P_m}{R_c} = \frac{P_c - R_m F_m}{R_c} = \frac{P_c - R_m (F_c + F_r)}{R_c} \quad \text{(A3)}$$

and F_r likewise as:

$$F_r = \frac{P_r - R_m (F_c + F_r)}{R_r} \quad \text{(A4)}$$

Here P_c and P_r are the pressures on the column and the reagent capillary. The combination of eqns. A3 and A4 leads to

$$F_c = \frac{P_c (R_r + R_m) - P_r R_m}{R_r R_c + R_c R_m + R_r R_m} \quad \text{(A5)}$$

and

$$F_r + F_c = \frac{P_c R_r + P_r R_c}{R_r R_c + R_c R_m + R_r R_m} \quad \text{(A6)}$$

in which the subscripts c, r and m again represent the column, reagent and reaction capillary, respectively. Combination of eqns. A2, A5 and A6 leads to eqn. 2.

ACKNOWLEDGEMENTS

P. H. Scheeren and E. M. M. Roosendaal are acknowledged for providing the non-linear multi-parameter regression program "MENU". The latter is also thanked for his invaluable assistance in the use of the program. The mixing devices were made highly precisely by H. Luyten and B. H. Klein Meulenkamp.

REFERENCES

1 J. C. Sternberg, *Adv. Chromatogr. (N.Y.)*, 2 (1966) 205.
2 J. H. Knox and M. T. Gilbert, *J. Chromatogr.*, 186 (1979) 405.
3 W. M. A. Niessen, H. P. M. van Vliet and H. Poppe, *Chromatographia*, 20 (1985) 357.
4 H. P. M. van Vliet and H. Poppe, *J. Chromatogr.*, 346 (1985) 149.
5 H. Lingeman, W. J. M. Underberg, A. Takadata and A. Hulshoff, *J. Liq. Chromatogr.*, 8 (1985) 789.
6 K. Imai, T. Toyo'oka and H. Miyano, *Analyst (London)*, 109 (1985) 1365.
7 W. R. Seitz, *CRC Crit. Rev. Anal. Chem.*, 8 (1980) 367.
8 J. Gluckman, D. Shelly and M. Novotny, *J. Chromatogr.*, 317 (1984) 443.
9 R. S. Deelder, M. G. F. Kroll and J. H. M. van den Berg, *J. Chromatogr.*, 125 (1976) 307.
10 P. H. Scheeren and E. M. M. Roosendaal, in preparation.
11 J. P. Foley and J. G. Dorsey, *Anal. Chem.*, 55 (1983) 730.
12 D. J. Anderson and R. R. Walters, *J. Chromatogr. Sci.*, 22 (1984) 353.
13 H. P. M. van Vliet, W. Frankema, J. C. Kraak and H. Poppe, in preparation.