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PERFORMANCE OF MICRO-LIQUID CHROMATOGRAPHIC COLUMNS IN AN INDUSTRIAL ENVIRONMENT: A CASE HISTORY

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SUMMARY

Packed fused-silica capillary liquid chromatography (micro-LC) columns, with an inner wall polymer coating stabilizing the packed column bed, were evaluated in an industrial laboratory environment. Data on their performance and long-term stability in "real-world practice" are reported. The results indicate that micro-LC is a viable proposition for routine application.

INTRODUCTION

Micro-liquid chromatography (micro-LC) is that form of liquid chromatography (LC) carried out on packed fused-silica (or other capillary) columns of I.D. 50–500 μm. Initially, the most interesting aspect of micro-LC was thought to be the reduced amounts of solvent and packing material needed. Hirose *et al.*[1] showed, however, that micro-LC is a relatively easy route to LC with high plate numbers. Another important feature of packed fused-silica capillary LC is that the columns are more permeable than conventional columns[2]. This high column permeability is the result of the "direct wall effect", which leads to a less densely packed column bed in columns of capillary dimensions[3]. Possible column instability can be avoided by using an elastic polymeric inner-wall coating which "holds" the packing. This higher permeability with good stability of micro-LC columns made from polymeric inner-wall-coated tubing, leads to a number of important advantages of micro-LC[4–10].

This contribution to the miniaturization of LC emphasizes the excellent column stability of micro-LC. It is shown that this is available in routine application in an industrial environment. Packed fused-silica capillary LC combined with low-dispersion chemiluminescence detection[11] proved to be a sensitive and suitable method for analyses where only a small sample volume is available. Currently a method is being developed to permit neurotransmitter release studies *in vitro* (brain slices) and *in vivo* (brain dialysis) in which fmol/μl sensitivity is required. Chemiluminescence and micro-LC are used for this purpose. During the development of this method, the characteristics of the packed fused-silica capillary columns were measured for a period of

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nearly 3 years. The reduced plate height, h, was monitored at several linear velocities, u, and the column resistance parameter, φ, peak asymmetry, A_S, and retention ratio, (RR)[a], were determined for the dansyl derivatives of β-histine {2-[2-(methylamino)-ethyl]pyridine dihydrochloride} relative to a by-product of the dansylation. The linear velocity was calculated assuming a total porosity of 0.50.

EXPERIMENTAL

The columns were a 500 × 0.5 mm I.D. column packed with 5-μm RoSiL-C_{18} (a spherical octadecylated silica gel from RSL, Eke, Belgium) and a 270 × 0.32 mm fused-silica capillary packed with 10-μm RSiL-C_{18} (a highly loaded irregularly shaped octadecylated silica gel phase from RSL). The instrument consisted of an MCP 36 pump (Haskel, Burbank, CA, U.S.A.), a Type 7520 or 7010 six-port injection valve (Rheodyne, Berkeley, CA, U.S.A.), a Perfusor ED2 syringe pump (Braun, Melsungen, F.R.G.), a 25-μl glass-coil flow cell of 0.4 mm I.D., a mixing cross (Valco Instruments, Houston, TX, U.S.A.), a Model PR-305 photomultiplier (Products for Research, St. Danvers, MA, U.S.A.), a Type PM 28B power supply (Thorn EMI, Middlesex, U.K.), a Type C-10 photon counter (Thorn EMI) and an HP 3392A integrator (Hewlett-Packard, Waldbronn, F.R.G.).

Further chromatographic conditions are given in the legends to the figures. A detailed description of the instrumental set-up for chemiluminescence LC has been published elsewhere[11]. Eltoprazine [1-(2,3-dihydro-1,4-benzodioxin-5yl)piperazine hydrochloride], a drug under development for the indication of pathological destructive behaviour, was used.

RESULTS AND DISCUSSION

Immediately after production, the columns were tested with polycyclic aromatic hydrocarbons as samples and with acetonitrile–water (75:25) as the mobile phase at the optimum linear velocity. The smaller column had a reduced plate height of 2–2.5 whereas for the larger I.D. column it was *ca.* 3–4 (compound and k' dependent). Under these chromatographic conditions the optimum linear velocity, or the minimum in the Van Deemter plot, is observed at u values between 1 and 2 mm/s. With the probes and eluting solvents studied here a minimum in the h/u curve is not observed (even down to 0.3 mm/s) and the efficiencies were generally not as good, although the smaller I.D. column shows astonishingly low h values at very low flow-rate (Tables I and II). That the h/u relationship and the h values are strongly solvent and compound dependent is well recognized and is illustrated again here.

The first column was used to analyse dansylated amino acids and dansylated drugs; the other column was used to analyse catecholamines. Because the concentra-

[a] The retention ratio (RR) is measured instead of the α value or selectivity factor. This is unusual and needs some clarification. Measuring "dead time" is awkward for two reasons: it was too difficult to find a chemiluminescence active compound that really showed no interaction with the column packing, and in some instances large volumes were injected with deliberately different composition to the eluent. This creates gradient effects which render "dead time" measurement nearly impossible. For these reasons RR values are used to express the degree of separation in the analysis being studied. The aim was to control the RR reproducibility.

TABLE I

SEPARATIONS OF DANSYLATED DRUGS AND AMINO ACIDS ON THE 0.5 mm I.D. COLUMN

Column, 500 × 0.5 mm I.D., 5-μm RoSiL-C_{18}. Eluent acetonitrile–water (65:35) with 10 m*M* imidazole. Samples, dansylated drugs and amino acids (see legend to Fig. 1). RR values for the dansyl derivatives of β-histine relative to a byproduct of the dansylation. Detection, chemiluminescence.

No. of injections	*u (mm/s)*[a]	*h*	*RR*	A_s	φ
50	0.39	5.6	2.20	1.05	520
	0.70	9.4			
	1.49	17			
	2.40	*24*			
	3.27	28			
125	0.27	6.4	2.20	1.30	540
	0.51	9.4			
	1.38	18			
	2.19	23			
	3.20	28			
200	1.04	13	2.20	1.27	580
	1.40	18			
	2.14	19			
	2.95	27			
300[b]	0.47	6.0	2.19	0.96	590
	0.83	13			
	1.10	*14*			
	1.66	19			
	2.40	22			
	3.08	26			
400	0.49	7.9	2.19	1.02	772
	0.93	10			
	1.53	14			
	2.31	18			
500	0.40	6.9	2.20	1.04	1050
	0.95	9.8			
	1.70	18			

[a] *u* measured with ε_t (total porosity) = 0.50.
[b] Column shortened to 480 mm.

tion of the buffer influences the rate of the chemiluminescence reaction, the concentration was kept constant at 10 mmol/dm^3 (ref. 11). This resulted in a relaxation time of the reaction of less than 1 s, which has only a small influence on the peak width and peak shape. The peak width of the first eluting compound was about 6 s at the indicated flow-rate.

Some column chromatographic characteristics for the 0.5 mm I.D. column as a function of the number of injections are presented in Table I. It can be concluded that the column is stable with respect to asymmetry and selectivity of the peaks. However, it suffers from clogging owing to dirty sample injection, which results in a gradually increasing back-pressure and a higher resistance parameter. A doubled φ value was observed after 4 months of regular use.

Similar experience over a period of nearly 3 years is tabulated for the 0.32 mm I.D. column in Table II. Whenever the back-pressure became excessive, a 1-cm piece

TABLE II

SEPARATION OF DRUGS AND AMINO ACIDS ON THE 0.32 mm I.D. COLUMN

Column, 270 × 0.32 mm I.D., 10-μm RSiL-C_{18}. Eluent, acetonitrile–water (65:35) with 10 m*M* imidazole.

No. of injections	*u (mm/s)*	*h*	A_s	φ	*Date*[a]
10	0.30	1.3	1.47	870	March 86 (1)
	0.42	1.6			
	0.60	1.9			
	1.52	2.4			
	1.82	4.0			
	3.8	8.0			
	6.4	10.7			
	10.2	14.8			
50	1.4	1.4	1.51	860	March 86 (1)
	2.9	2.7			
	4.3	2.8			
	4.9	3.1			
	11.8	10.7			
250[d]	1.6	2.1	1.52	1060	Aug. 86
	10.3	5.8	1.05[b]		(2)
500[e]	1.1	2.2		2600[c]	Oct. 86
1000[f]	4.6	6.2	1.98	1550	(2)
1250[g]	2.2	2.9	1.23	2960	Apr. 87 (3)
1500	2.3	3.0		2830	May 87 (3)
1600[h]	8.2	6.2	1.33	1000	Apr. 88 (2)
1900[i]	4.0	4.0	1.29	1230	Aug. 88 (2)
2000[j]	2.5	3.5	1.40	1550	Jan. 89 (2)

[a] Measurements with (1) dansylated drugs and amino acids; (2) dansylated catecholamines [acetonitrile–water (85:15) with 10 m*M* imidazole]; (3) dansylated Eltoprazine in plasma.

[b] Measured under optimum chemiluminescent conditions.

[c] High φ reached, leading to shortening of column.

[d–j] Column shortened to 250, 240, 230; 210, 170, 145 and 130 mm, respectively.

of the column top was removed, which restored the performance to acceptable levels. Breaking off a piece of the column has to be carried out carefully so that an even, clean break is produced. We do this with a capillary scoring tool (Supelco, Bellefonte, PA, U.S.A.).

Restoring the column in this way was necessary, with our sample, after about every 250 injections and was kept up for 2000 injections. The peak asymmetry appears high for this column, but the chemiluminescence was not performed under ideal conditions. As soon as these conditions were further optimized[11] a more favourable value for A_s was observed. As Tables I and II show, the columns were shortened by 1–2 cm every time the back-pressure or the reduced plate height increased excessively. Even after 2000 analyses the reduced plate height had hardly changed. The back-

pressure of the column was higher than at the start, however, probably because clogging occurs not only at the injector site but also at the end frit site. Clogging of the top end is removed by cutting off a piece of the column, but clogging of the bottom frit cannot be repaired so easily. However, the results are aceptable.

The catecholamines were derivatized (in some instances "on-line") with dansyl chloride. For the plasma analysis of Eltoprazine, derivatization was carried out off-line. Examples of this chemiluminescence analysis on both columns are shown in Figs. 1 and 2. In Fig. 1 for the 0.5 mm I.D. column, the separations after 50 and after 300 runs are shown at eluent flow-rates far above the optimum. In Fig. 2 similar chromatograms are shown for the 0.32 mm I.D. column of length 250, 230 and 210 mm. The detection limits for the compounds analysed are in the femtomole range.

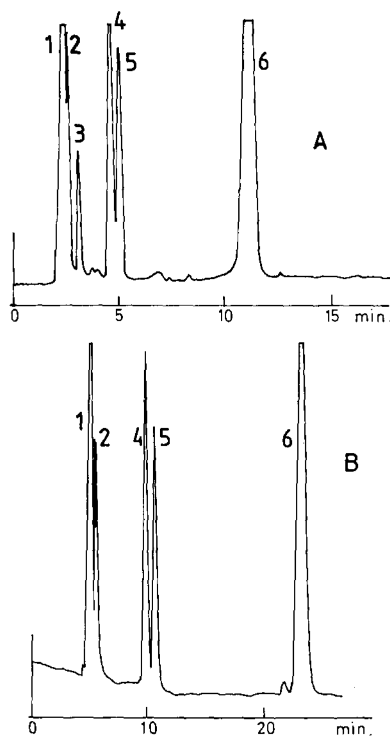

Fig. 1. Separation of dansylated amino acids. Column, 500 × 0.5 mm I.D., packed with 5-μm RoSiL-C_{18}. Eluent, acetonitrile–water (65:35) with 10 m*M* imidazole. Sample, 0.2 μl of dansyl derivatives of the compounds mentioned. Dansyl derivative of β-histine at 1.6 ng/μl. Peaks in order of appearance: 1, DNS-OH; 2, methionine; 3, tryptophan; 4, mebeverinic acid; 5, byproduct of the dansylation of β-histine; 6, β-histine. Detection, chemiluminescence. Trace A after 50 analyses and at 15 μl/min (2.4 mm/s or far above the optimum). Back-pressure, 19 MPa. Trace B after 300 analyses on a 480-mm column and at 7 μl/min (1.1 mm/s). Back-pressure, 10 MPa.

In Tables I and II, entries which are derived from the chromatograms in Figs. 1 and 2 are given in italics. The chromatograms in Fig. 2B, C and D do not appear in Tables I and II. Table II shows that the 0.32 mm I.D. column, finally shortened to nearly half its original length (130 cm), was still performing well.

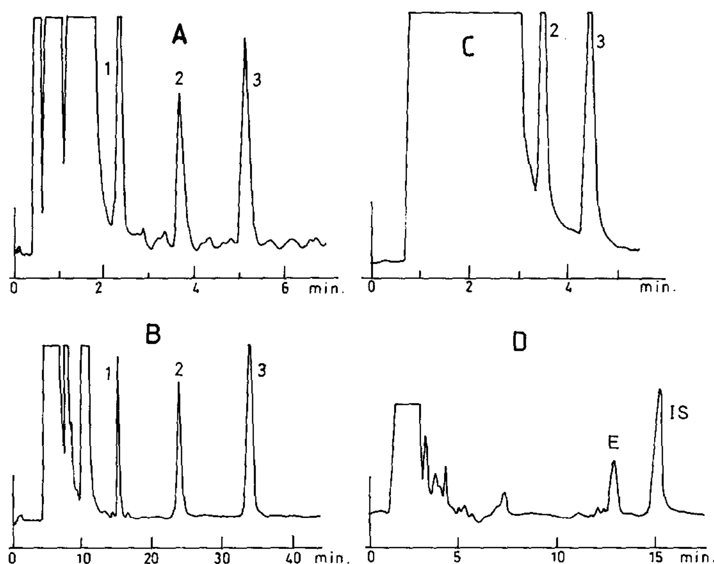

Fig. 2. Separation of dansylated drugs and amino acids. Column, 250 × 0.32 mm I.D. packed with 10-μm RSiL-C_{18}. Eluent, acetonitrile–water (85:15) with 10 m*M* imidazole. Chemiluminescence detection. Peaks in order of appearance: 1, serotonin (5-HT); 2, noradrenaline (NA); 3, dopamine (DA). (A) Sample, 5 μl of catecholamines each at 1 pg/μl; flow-rate, 15 μl/min (u = 6.4 mm/s); back-pressure, 15 MPa. (B) Same as under A but with 0.5 μl of a catecholamine solution at 20 pg/μl and with a flow-rate of 2.5 μl/min ($u \doteq$ 1.0 mm/s); back-pressure, 2.5 MPa. (C) After 1000 analyses with a column shortened to 230 mm with a flow-rate of 20 μl/min (u = 8.3 mm/s), 20 μl of catecholamine solution and on-line derivatization. (D) After 1500 analyses with a column shortened to 210 mm. Eluent, acetonitrile–water (65:35) with 10 m*M* imidazole; 0.5 μl of plasma extract. Sample, Eltoprazine at 25 pg/μl; internal standard (an analogue of Elkoprazine) at 75 pg/μl. Flow-rate, 10 μl/min (u = 4.1 mm/s); back pressure, 13 MPa.

CONCLUSION

This case study shows that in a practical environment, micro-LC columns behave remarkably well. An increasingly interesting aspect of micro-LC is the low volume of spent solvent.

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