*Journal of Chromatography*, 218 (1981) 137–165
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,142

# MICROCAPILLARY LIQUID CHROMATOGRAPHY IN OPEN TUBULAR COLUMNS WITH DIAMETERS OF 10–50 μm

## POTENTIAL APPLICATION TO CHEMICAL IONIZATION MASS SPECTROMETRIC DETECTION

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SUMMARY

The theoretical separation efficiency of open microcapillary liquid chromatography (LC) columns, including peak-broadening effects resulting from interphase resistance to mass transfer, has been considered and an expression is derived for the plate height caused by interphase resistance.

The use of such columns with internal diameters down to 10 μm is explored for LC separations. The columns were prepared from soft glass tubing and coated with polar and non-polar stationary phases. Several applications in straight phase as well as reversed-phase systems demonstrate the high separation speed (up to 50 effective plates per second). Relatively wide (30–50 μm) and short (1–5 m) columns allow rapid analyses within minutes. Smaller (10–30 μm) and longer (5–25 m) columns yield extremely high plate numbers (up to $5 \cdot 10^6$), permitting very difficult separations in a reasonable time (2–5 h). The required pressure never exceeds the generally accepted value of 400 bar. Preliminary results obtained with fused silica columns are discussed.

Split-injection and addition of make-up mobile phase through the (UV) detector have been applied. In order to avoid undesirable dilution of the sample zones by the make-up liquid, the microcapillaries were directly coupled to a mass spectrometer (in the chemical ionization mode). This technique has yielded promising results.

## INTRODUCTION

Gas chromatography (GC) with capillary columns is a well-accepted method for gas analysis. This technique is becoming increasingly popular, as open tubular or capillary columns (with tube diameters of about 0.25 mm) offer more separation power than classical packed columns. In principle, the same applies to liquid chromatography (LC), but, in view of the much slower diffusion in liquids, much smaller column diameters are required (of the order of 10–50 μm)[1]. The practical difficulties accompanying this microcapillary LC technique have prevented its successful application until very recently. Apart from the work done in our laboratory, studies of microcapillary LC in columns with diameters down to about 30 μm have recently

0021-9673/81/0000–0000/$02.50 

been reported[2–7]. In the present work we describe the results obtained in columns with diameters down to 10 μm.

In earlier work we tried to enhance radial diffusion in open tubular columns by introduction of secondary flow effects[8–10], which turned out to be successful in GC and in flow injection analysis (FIA) but not in LC. Here the only way to obtain satisfactory results is to apply drastic miniaturization.

## EXPERIMENTAL

### *Apparatus*

Apart from the columns and their connections, only commercially available LC apparatus was used. The main pump was a Perkin-Elmer Series 2 liquid chromatography pump, a dual pump capable of delivering 60 ml/min at pressures up to 4000 p.s.i. A Tracor 950 pump, suitable for low flow-rates (< 1 ml/min), was used for delivering "make-up" or scavenger liquid to the detector. A Waters U6K injector was employed.

The main detectors used were UV absorption detectors, of three types: fixed wavelength (254 nm), Waters Model 440; variable wavelength, Jasco Uvidec 100-II; variable wavelength, Spectra-Physics SP 8400. For all three detectors it was necessary to reduce dead volumes by circumventing the inlet connecting tubes. The most simple way to achieve this is by using the detector cell outlet as the inlet, which can be done with the Waters and the SP detectors without any problems. The result is that the column exit actually protrudes into the measuring cell, thus minimizing dead volumes to the cell volume itself. With the Jasco detector some modifications were required to reach the same goal.

In this way the total dead volumes in the detectors were reduced to the order of 2–8 μl. This, however, is still too large[1,7] for direct application of the detectors in microcapillary LC, where mobile phase flow-rates through the column of 1–10 μl/min are common. When such flow-rates enter the detector cell the time constant will be about 1 min, far too large for the detection of peaks which are only a few tens of seconds wide, at most. Reduction of cell volumes to around 1 μl has recently been described[7,11], but even so scavenger ("make-up") liquid has to be added to the detector in order to reduce the time constant and peak broadening to acceptable levels. Using specially made T-pieces, each tailored to suit the particular column O.D., we added 0.3–1 ml/min of make-up liquid to the column effluent (just before it entered the detector cell) in order to eliminate most of the peak broadening in the detector. The Jasco detector, although having the lowest cell volume, required a flow-rate of about 1 ml/min, probably due to an unfavourable cell geometry.

The addition of make-up liquid to the column effluent has the major drawback that sample concentrations are diluted by at least a factor of 100. An alternative method of detection which avoids this dilution problem is chemical ionization mass spectrometry (CIMS), using the mobile phase as the ionizing reagent gas. As the column exit can be positioned directly into the ion source via a slightly modified hollow sample probe, no make-up liquid is necessary, thus ensuring far lower detection limits than are possible with UV detectors. A Finnigan 4000 mass spectrometer was used in this work.

*Sample introduction*

The split injection method was employed to introduce samples into the microcapillary LC columns. Specially made splitting T-pieces were connected to the outlet of the Waters U6K injector, carefully avoiding any additional dead volumes. The inside diameter of each T-piece was adapted to the outside diameter of the particular column used, such that the dead volume inside the splitter was minimized. Nonetheless, splitting ratios of 1:100 to 1:5000 had to be applied to avoid peak broadening from injection. The relatively large split flow (up to 30 ml/min) was recirculated to the mobile phase vessel, except for a short moment during injection, when this flow was directed to waste.

Injection with the U6K turned out to be most efficient when the injector was switched into the injection position only for a very short time (say 0.2–1 sec). Switching back into the "load" position ensures that the rear ends of sample zones are cut off. When this technique was used, especially the earlier peaks in the chromatograms were much more symmetrical and narrower than those found when the injector remains in the injection mode until the next injection.

Injection of samples, either pure or dissolved in some solvent, invariably leads to increased peak widths, especially for the earlier peaks. Obviously, mixing of the sample with the mobile phase requires a finite time, which may affect the efficiency of separations. Therefore samples were dissolved in the mobile phase.

*Columns*

With the aid of a modified Shimadzu GDM1 Glass Drawing Machine, soft-glass (AR) microcapillaries with internal diameters in the range of 10–100 $\mu$m (lengths up to 50 m) were drawn from either standard 6 × 0.3 mm glass tubing (different manufacturers) or thermometer glass capillaries. The fragile microcapillaries obtained in this way required very careful handling and mounting on a supporting frame onto which both splitting and make-up T-pieces were fastened. The ends of the columns were glued into these T-pieces using epoxy type resins; such connections can easily withstand pressures up to 600 bar. The low-pressure T-piece connection to the column exit can also be effected with a graphite ferrule.

With samples of high molecular weight (MW $>250$) and using a mass spectrometer as the detector, enhanced transfer of the column effluent into the ion source is found when a liquid jet is produced at the column outlet. As for packed LC columns[12], this requires that the column effluent should leave the column exit through a very narrow orifice of about 1–5 $\mu$m. This can be effected by melting the soft-glass column end and drawing out rapidly. Thus, a conically shaped tip is obtained as the column exit, which is polished to the required opening of a few $\mu$m (microscopic inspection).

Recently S.G.E. (Australia) kindly supplied us with fused silica microcapillaries with internal diameters in the range 7–40 $\mu$m. Manual handling of these flexible columns is so convenient that no supporting frame is needed.

*Coating of the columns*

As in capillary GC, it has been possible to coat the inner wall of the column with a thin layer of some polar stationary liquid phase, using the dynamic coating technique[13]. As the shear forces in LC with a liquid mobile phase are appreciably

greater than in GC with a gaseous mobile phase, the shear stability of film coatings has to be enhanced by some surface-roughening technique. We used a modified alkaline etching technique for the soft-glass column wall, originally proposed by Mohnke and Saffert[14]. Under somewhat milder conditions than proposed by these authors, *viz.*, 0.5–1.5 *N* KOH at 100°C for 15–30 min, we produced a very thin but highly adsorptive siliceous layer on the column wall. Nota *et al.*[15] and recently Tesařík and co-workers[16,17] have described analogous procedures.

This porous and active layer can be used either as an adsorptive stationary phase or supplied with a liquid phase by the dynamic coating technique; even the possibility of attaching a chemically bonded phase for reversed-phase LC has to be considered. The latter has been shown to be feasible[2,3,7], but in this work we only applied physically adhering coatings.

We also tried to attach a porous adsorptive layer to the column wall, as described by Schwartz and co-workers[18–20] and Cramers *et al.*[21] for GC capillaries. Recently Hibi *et al.*[22] applied the method to LC capillaries. In this method submicron silica particles are deposited onto the column wall from a stabilized suspension of the aerogel Cab-O-Sil (Cabot Corp., Boston, MA, U.S.A.) in water, again using the dynamic coating procedure. Like etched glass surfaces, Cab-O-Sil coatings can be used either as a stationary adsorbent or subsequently coated with a liquid phase.

For straight-phase LC we used oxydipropionitrile (ODPN) as the stationary liquid on both types of roughened and activated glass surfaces. The mobile phase was isooctane saturated with ODPN. For reversed-phase LC we first determined which non-polar materials were stable against aqueous mobile phases without droplet formation after some time. Using microscope slides we found that many silicon liquid phases (OV-101, SE-30, silicone oils) produced neat layers at first, but when immersed in aqueous mobile phases these layers disintegrated into separate droplets, even if the mobile phases contained a surfactant to reduce surface tension. Only very viscous greases like Apiezon L, M or N were stable for long periods of time. Therefore, for reversed-phase separations we coated the microcapillaries with Apiezon L.

We did not succeed in achieving any stable physically adhering coating on the fused silica columns. Probably, chemical bonding is to be preferred here as the coating technique; examples have been described for GC[23–25] and recently also in LC[7].

*Data handling*

Signals from the UV detectors were stored in digital form on a cassette tape, such that each peak was described by at least 100 data points. From these data the first statistical moment, $\mu_1$, and the second central moment (variance), $\mu_2$, were carefully obtained and used to calculate the plate height, $H$, from:

$$H = L\,\mu_2/\mu_1^2 \tag{1}$$

As has been discussed[26,27], the commonly used plate height in chromatography, $H = L(\sigma_t/t_R)^2$, may differ appreciably from the moment-based plate height of eqn. 1 for asymmetrical peaks. The mobile phase linear velocity, $u$, is obtained from the residence time, $\mu_1$, of non-retained solutes:

$$u = L/\mu_1 \tag{2}$$

## THEORETICAL

*Dispersion and separation speed*

According to the theory of Golay[28] for capillary chromatography in straight open tubular columns, the axial dispersion in terms of plate height, neglecting axial molecular diffusion, is given by:

$$H = (C_M + C_S)u \tag{3}$$

The convective dispersion term, $C_M$, represents the combined effect of velocity profile, radial diffusion and partitioning between mobile and stationary phase

$$C_M = \frac{1 + 6k + 11k^2}{(1 + k)^2} \cdot \frac{R^2}{24 D_M} \tag{4}$$

and the stationary phase term, $C_S$, represents the slowness of mass transfer by diffusion in the liquid phase film:

$$C_S = \frac{2}{3} \cdot \frac{k}{(1 + k)^2} \cdot \frac{\delta^2}{D_S} \tag{5}$$

For sufficiently thin films ($\delta \ll 1$ μm), $C_S$ has values of $10^{-4}$ sec or less, which are small in comparison with the $C_M$ term, values of which are in the range of $10^{-2}$–$10^{-3}$ sec. Therefore $C_S$ is negligible and eqn. 3 reduces to:

$$H = C_M u = \frac{1 + 6k + 11k^2}{(1 + k)^2} \cdot \frac{R^2 u}{24 D_M} \tag{6}$$

When comparing experimental plate heights reported in the literature (mainly GC) with the predicted values according to eqn. 6, one invariably finds that the theory underestimates the experimentally observed peak broadening. An obvious reason for this is the additional peak broadening in extra-column parts of the apparatus (injector, detector, connections)[26]. Even in carefully designed apparatus, however, observed plate heights are not in agreement with eqn. 6[9,29].

Several attempts have been made to correct the Golay equation for slow interphase mass transfer. Most notable are the theories by Aris[30,31], Khan[32] and Pethö[33], where an additional $C$ term in the plate height equation has been introduced, which contains a mass transfer coefficient, $k_M$. This coefficient describes the mass flux in the mobile phase perpendicular to the phase boundary, *i.e.*, the mass flux that is transferred between the two phases. The plate height equation in all three theories is:

$$H = C_M u + C_i u = \frac{1 + 6k + 11k^2}{(1 + k)^2} \cdot \frac{R^2 u}{24 D_M} + \left(\frac{k}{1 + k}\right)^2 \cdot \frac{Ru}{k_M} \tag{7}$$

The physical meaning of $k_M$ and its magnitude are still a matter of debate, however[29–34].

On the basis of the analogy between heat and mass transfer and using relations

given by Giddings' non-equilibrium theory for chromatographic dispersion[34], we have been able to find (see Appendix) an approximate expression for $k_M$ which leads to a simple relationship between $C_i$ and $C_M$:

$$C_i = kC_M/(1 + k) \tag{8}$$

Eqn. 8 is not exact, but gives an impression of the magnitude of the interphase resistance effect. Combining eqns. 7 and 8 leads to:

$$H = \frac{1 + 2k}{1 + k} \cdot C_M u = \frac{(1 + 2k)(1 + 6k + 11k^2)}{(1 + k)^3} \cdot \frac{R^2 u}{24 D_M} \tag{9}$$

Eqn. 9 shows that observed plate heights can indeed be well in excess of the Golay estimation, depending upon the capacity factor $k$ up to a factor of 2. In this work we investigate whether eqn. 9 is able to describe relative peak broadening in straight microcapillary columns for LC.

Eqn. 9 can be used to find an expression for the speed of separation in micro-

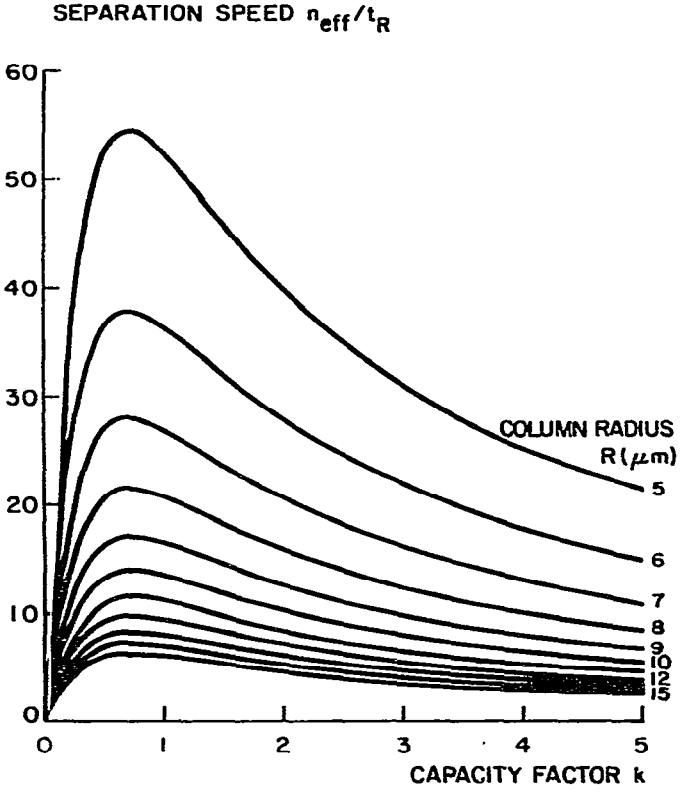



Fig. 1. Separation speed in microcapillary liquid chromatography according to eqn. 10, as a function of relative retention. The diffusion coefficient in eqn. 10 has been taken as $2.94 \cdot 10^{-5}$ cm$^2$/sec (aniline in isooctane).

capillary LC in terms of the number of effective plates generated per unit time. As $H = L/n$, $u = L/t_M$, $t_R = t_M(1 + k)$ and $n_{eff} = n(k/1 + k)^2$, we find that

$$\frac{n_{eff}}{t_R} = \frac{24\,D_M}{R^2} \cdot \frac{k^2}{(1 + 2k)(1 + 6k + 11k^2)} \tag{10}$$

which is plotted as a function of $k$ in Fig. 1, for different column radii, $R$. Differentiation of eqn. 10 with respect to $k$ shows that the maximum separation speed is obtained at $k = 0.72$; this value is somewhat lower than in packed LC columns, where the optimum is found in the range $k = 2$–4[35]. Fig. 1 clearly shows this maximum: values of more than five effective plates per second, which are of practical interest, can only be obtained in very narrow capillaries with diameters less than 30 $\mu$m. As the separation speed is inversely proportional to the square of the column diameter (eqn. 10), high separation speeds can be expected, especially in columns of diameter 10 $\mu$m or less. Fig. 1 shows that, *e.g.*, at $k = 1$ a 10-$\mu$m microcapillary yields about 50 effective plates per sec, which is much more than can be obtained with modern packed high-performance liquid chromatography (HPLC) columns, even with the smallest (2–5 $\mu$m) particles.

The high separation speed of microcapillary columns can be exploited in two ways: for rapid analyses of not too difficult separations (*e.g.*, $10^3$ effective plates are available in about 20 sec); or for a very high separation efficiency in the case of very difficult separations, at the cost of longer analysis times (of the order of $\geqslant 1$ h). For instance, in 1 h a 10-$\mu$m column at $k = 1$ yields $2 \cdot 10^5$ effective plates (*i.e.*, $7 \cdot 10^5$ theoretical plates). The number of plates obtained may be well above $10^6$, which indicates that very difficult separations which cannot be carried out in packed columns can potentially be performed.

An estimation of the pressure drop required for the operation of microcapillary columns can be based on the Poiseuille equation

$$u = R^2 \Delta p / 8\eta L \tag{11}$$

where the high permeability ($R^2/8$) of open tubular columns is an important advantage. The linear velocity, $u$, in eqn. 11 equals $L/t_M$ or $L(1 + k)/t_R$, where $t_R$ is the retention time required to obtain $n_{eff}$ effective plates from eqn. 10; eliminating $t_R$ from eqns. 10 and 11 yields:

$$\Delta p = \frac{192\,L^2}{R^4} \cdot \frac{\eta\,D_M}{n_{eff}} \cdot \frac{k^2(1 + k)}{(1 + 2k)(1 + 6k + 11k^2)} \tag{12}$$

For the near-optimum case $k = 1$, the pressure drop has been plotted in Fig. 2 as a function of the number of effective plates, $n_{eff}$, generated, for different column lengths, $L$, and for two retention times ($t_R = 1$ min and 1 h). For example, a 10 m × 20 $\mu$m column yields $3 \cdot 10^4$ effective plates in 1 h, requiring a pressure drop of only 22 bar. The same performance can be obtained in 1 min also in a 1 m × 2.5 $\mu$m column, requiring some 800 bar as the inlet pressure. Although Tesařík *et al.*[36] claimed to have prepared columns with such small diameters (down to 3 $\mu$m), it seems questionable whether these columns can be operated in an efficient way in view of the extra-column broadening. With respect to more practical column diameters of 10 $\mu$m or more, Fig.

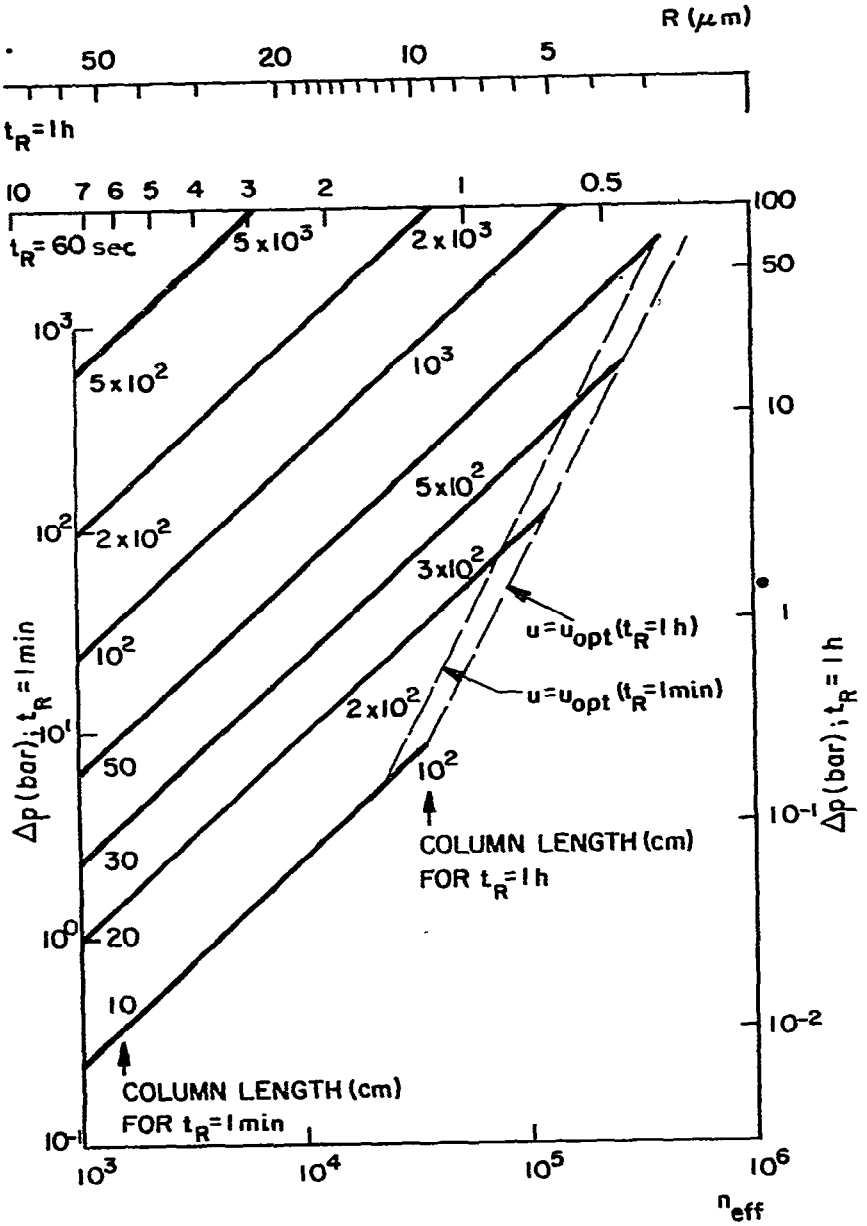



Fig. 2. Operational parameters for microcapillary LC for analysis times of 1 min and 1 h. $k = 1$; $D_M = 2 \cdot 10^{-5}$ cm²/sec; $\eta = 5 \cdot 10^{-3}$ P.

2 reveals that easy separations with several thousand effective plates can be performed within 1 min in columns of about 3 m in length, applying an inlet pressure of 300–600 bar. The required pressure sharply drops with decreasing column length (*cf.*, eqn. 12: $\Delta p$ is proportional to $L^2$): a 1-m column can be operated at the same performance as a 3-m column, but requires a tenth of the pressure.

The scope for optimization towards ever shorter columns is limited, however, for two reasons. First, the extra-column broadening should not become the major source of peak broadening, and secondly the length should be chosen such that the velocity $u = L/t_M$ does not drop below the chromatographic "optimum" velocity where axial diffusion becomes an important source of peak broadening ($H = 2\,D_M/u = B/u$). As the optimum velocity is given[1,13,34] by $u_{opt} = (B/C)^{\frac{1}{2}}$, we find with $C = C_i + C_M = (1 + 2k)\,C_M/(1 + k)$ that:

$$u_{opt} = \frac{D_M}{R}\left[\frac{48\,(1 + k)^3}{(1 + 6k + 11k^2)(1 + 2k)}\right]^{\frac{1}{2}} \tag{13a}$$

For $u \geqslant u_{opt}$ with $u = L/t_M = L(1 + k)/t_R$ we obtain:

$$L \geqslant \frac{D_M t_R}{R}\left[\frac{48\,(1 + k)}{(1 + 6k + 11k^2)(1 + 2k)}\right]^{\frac{1}{2}} \tag{13b}$$

In the near-optimum case where $k = 1$, and for $R = 10\ \mu m$ and $t \leqslant 10$ h, we have $L \geqslant 10$ m, for example. For $t = 1$ h and $t = 1$ min the dashed lines in Fig. 2 indicate the condition set by eqn. 13b, in the case where $k = 1$.

From Fig. 2 it may be concluded that rapid analysis by microcapillary LC should be performed in short ($L < 3$ m) and very narrow columns ($R < 10\ \mu m$). On the other hand, very difficult separation problems can be handled by wider ($R = 10$–$20\ \mu m$) and longer ($L = 2$–$20$ m) columns. In neither case are the required inlet pressures unacceptable.

*Direct LC–MS coupling with jet formation*

As stated above, the residence time and peak broadening in the commonly used LC detectors, with cell volumes in the range of 1–10 $\mu$l, should be kept within acceptable limits by the use of a scavenger liquid flow of the order of 1 ml/min. This flow causes dilution by a factor of 100–1000, such that only major components of sample mixtures can be detected (contents >0.01%).

A detector which avoids this severe dilution by accepting column effluent flows up to 20 $\mu$l/min is the mass spectrometer, when used in the chemical ionization (CI) mode. In fact, direct microcapillary LC flows of this magnitude ensure a nearly ideal compatibility with the requirement of ion source pressures of about 0.2–1 Torr for the CI process.

Interfacing of LC effluents from packed columns with MS in the CI mode has recently been studied intensively[12,37,38]. The main conclusion was that sufficiently rapid and effective vaporization of the column effluent can be obtained only by using additional vaporization techniques such as rapid heating[39], electrospraying[40], laser irradiation[38,41,42] or jet formation[12]. Especially for samples of high molecular weight (>250), for high concentrations and for thermally unstable or solid materials, where blocking of the microcapillary column exit can be envisaged, it is worthwhile to consider the liquid jet formation technique in connection with (soft-glass) microcapillary LC. A liquid jet created at the column exit avoids deposition of sample material there by ejecting the whole of the column effluent rapidly into the ion source. For packed HPLC columns this jet interface has already shown promise[12] (with per-

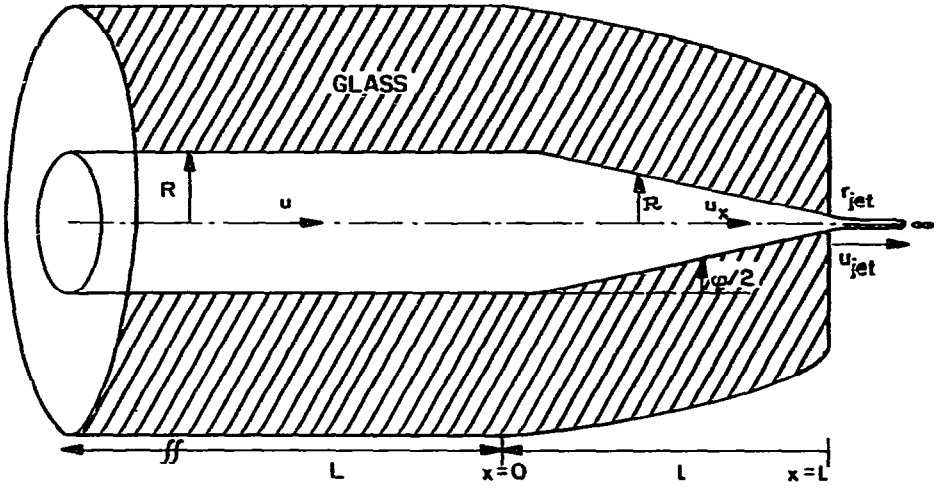



Fig. 3. Schematic representation of the liquid jet forming tip at the column exit.

forated membranes at the column outlet and an orifice of about 2–5 μm). Small diameters of that magnitude are necessary to create the high liquid velocities for jet formation.

Notably with soft-glass microcapillaries, it is possible (although tedious) to produce a conical tip at the column exit with an outlet diameter near the required value of 1 μm. Fig. 3 shows a schematic representation. In practice, the tip length, $l$, is typically 3 mm, and from this we can calculate the pressures needed for jet formation, on the basis of the viscous dissipation within the conical tip.

According to the literature[43,44], liquid jet formation occurs only above a certain critical liquid velocity in the orifice:

$$u_{jet} \geqslant 2\,(\gamma/\varrho)^{\frac{1}{2}}\, r_{jet}^{-\frac{1}{2}} \tag{14}$$

For most liquids $(\gamma/\varrho)^{\frac{1}{2}}$ lies in the range 4–10 dyne·cm$^2$·g$^{-1}$, and for estimating purposes eqn. 14 can be written as:

$$u_{jet} = 10\, r_{jet}^{-\frac{1}{2}} \tag{15}$$

For orifice radii smaller than 25 μm this implies very high velocities, in excess of 200 cm/sec, increasing with decreasing diameter. These velocities are too high for chromatographic purposes and so it will never be possible to reach the ideal situation where the column itself constitutes the jet forming channel. Hence, jet formation in combination with chromatographic separation implies that $r_{jet} \ll R$ in order to obtain sufficiently low column velocities for efficient separation as well as sufficiently high jet velocities.

As the flow-rate is the same in the column and in the jet, we have, with eqn. 15:

$$u R^2 = u_{jet}\, r_{jet}^2 = 10\, r_{jet}^{3/4} \tag{16}$$

At position $x$, where the local radius, $\mathscr{R}$, of the conical tip may be approximated by the linear relation

$$\mathscr{R} = R\left[1 - \frac{x}{l}\left(1 - \frac{r_{jet}}{R}\right)\right] \tag{17}$$

(*i.e.*, $\mathscr{R} = R$ at $x = 0$ and $\mathscr{R} = r_{jet}$ at $x = l$), the continuity of flow requires that:

$$u R^2 = u_x \mathscr{R}^2 \tag{18}$$

The average velocity, $u_x$, at location $x$ is now obtained from the Poiseuille equation as[45]

$$u_x = -(\partial p/\partial x)(\mathscr{R}^2/8\eta) \tag{19}$$

*i.e.*, the pressure gradient in the tip, taking eqn. 18 into account, is given by:

$$-(\partial p/\partial x) = 8\eta u R^2/\mathscr{R}^4 \tag{20}$$

If this is integrated over the tip length, $l$, using eqn. 17 for $\mathscr{R}$, we obtain the pressure drop, $\Delta p_l = p(x = 0) - p(x = 1)$, as:

$$\Delta p_l = \frac{8}{3} \cdot \frac{\eta u l R}{r_{jet}^3}\left[1 + \frac{r_{jet}}{R} + \left(\frac{r_{jet}}{R}\right)^2\right] \tag{21}$$

In eqn. 21 the terms in $(r_{jet}/R)$ can be neglected with respect to the term 1 between brackets, as $r_{jet} \ll R$. With the jet formation condition eqn. 16, we then find from eqn. 21:

$$\Delta p_l = \frac{80}{3} \cdot \frac{\eta l}{r_{jet}^{3/2} R} \tag{22}$$

As a typical example, $\Delta p_l$ for a 2-$\mu$m orifice at the end of a column of diameter 20 $\mu$m and a tip length, $l$, of 3 mm amounts to 80 bar. Such a high pressure differs considerably from the low pressures needed for pin hole jets in very thin membranes as discussed by Arpino *et al.*[12] for packed HPLC columns. The reason is that, in our case of a finite tip length, viscous dissipation dominates over kinetic energy losses, *i.e.*, the Poiseuille equation rather than the Bernouilli equation is valid.

The total pressure drop over the column of length, $L$($\Delta p_L$ after eqn. 11) and the jet forming tip of length, $l$ ($\Delta p_l$ after eqn. 22) now amounts to:

$$\Delta p = \frac{80\eta}{R^{5/2}}\left[\frac{l}{3}\left(\frac{R}{r_{jet}}\right)^{3/2} + L\left(\frac{r_{jet}}{R}\right)^{3/2}\right] \tag{23}$$

In Fig. 4, $\Delta p$ has been plotted as a function of $r_{jet}$ for a specified column radius $R$ = 16 $\mu$m and various column lengths, $L$. It is seen that the minimum pressure drop can be obtained for a rather narrow range of jet radii (between 1 and 2 $\mu$m) only. The

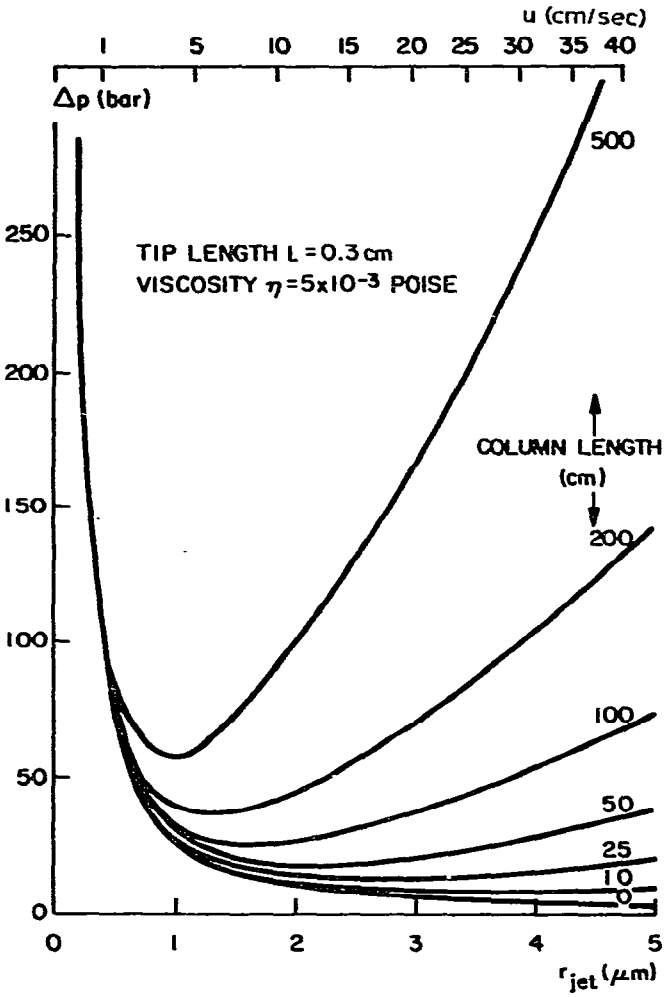



Fig. 4. Pressure drop in a microcapillary column (diameter 32 $\mu$m) with jet formation, as a function of the jet radius.

minimum pressure drop and accompanying jet radius are obtained from eqn. 23 by differentiation with respect to $r_{jet}$:

$$\Delta p_{min} = \frac{1}{R^{5/2}} \cdot \left(\frac{L\,l}{3}\right)^{\frac{1}{2}} \tag{24}$$

$$r_{jet,opt} = R\left(\frac{l}{3\,L}\right)^{1/3} \tag{25}$$

From this it follows that not too small column radii, $R$, should be used to keep $\Delta p_{min}$ within reasonable limits. As a typical example, for $R = 10$ $\mu$m, $L = 1$ m and $l = 0.3$ cm, we obtain $\Delta p_{min} = 100$ bar and $r_{jet,opt} = 1$ $\mu$m.

Now, the column radius, $R$, and length, $L$, are chosen such that a sufficient number of effective plates, $n_{eff}$, can be generated (eqn. 10 and Fig. 2). For the near-optimum case of $k = 1$ we obtain from eqn. 16 with $u = L/t_M = L(1 + k)/t_R = 2\,L/t_R$:

$$2\,L\,(R^2/t_R) = 10\,r^{3/2} \tag{26}$$

From eqn. 10 with $k = 1$ and $D_M = 2 \cdot 10^{-5}$ cm$^2$/sec the ratio $R^2/t_R$ in eqn. 26 can be derived:

$$R^2/t_R = 10^{-5}/n_{eff} \tag{27}$$

So, combining eqns. 26 and 27 yields the required length for a microcapillary column which produces $n_{eff}$ effective plates and forms a liquid jet as well:

$$L = 5 \times 10^5 \cdot n_{eff} \cdot r^{3/2} \qquad (k = 1) \tag{28}$$

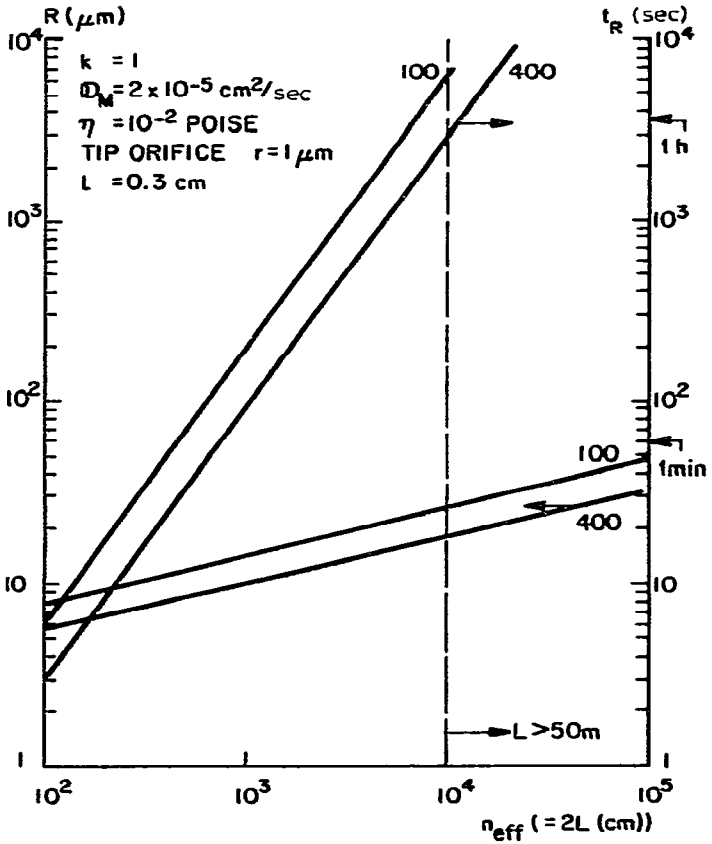



Fig. 5. Operating parameters for microcapillary LC with liquid jet formation. The data on the lines indicate the maximum allowable pressures (bar).

A standard jet radius of 1 μm then implies that:

$$L = \tfrac{1}{2}\, n_{\text{eff}}\ (r_{\text{jet}} = 1\ \mu\text{m},\ k = 1) \tag{28a}$$

As eqn. 16 results in $u = 10^{-5}/R^2$ for $r_{\text{jet}} = 1$ μm, the required column radius, $R$, that produces $n_{\text{eff}} = 2\ L$ effective plates from the Poiseuille equation for $u$, provided that $\Delta p_L$ dominates over $\Delta p_t$ (*i.e.*, $r_{\text{jet}}$ is slightly larger than $r_{\text{jet,opt}}$; *cf.*, Fig. 4):

$$R = (4\ \eta \cdot n_{\text{eff}}/\Delta p_L \cdot 10^{-5})^{\frac{1}{4}} \tag{29}$$

For an allowed pressure drop $\Delta p_L = 400$ bar, eqn. 29 yields approximately $R^4 = 10^{-15} \cdot n_{\text{eff}}$, *i.e.*, with eqn. 27:

$$t_R = 3 \cdot 10^{-3} \cdot n_{\text{eff}}{}^{3/2} \tag{30}$$

The required operating parameters $R$, $L$ and $t_R$ are plotted in Fig. 5 as a function of the required separation efficiency $n_{\text{eff}}$ for $\Delta p_L = 400$ and 100 bar.

Lengths in excess of about 50 m are not practical, which implies that the microcapillary LC–MS combination with jet interfacing will at most yield about $10^4$ effective plates (in about 1 h), a very useful number, although less than with conventional detection (*cf.*, Fig. 2).

## RESULTS AND DISCUSSION

### *UV-detection and non-retained solutes*

Non-retained solutes were used to obtain the amount of extra-column broadening resulting from injection, column connections and detection. Ideally, peak broadening only occurs in the column itself by convective dispersion, producing plate heights which are expected to follow the predictions of the well-established theories of Taylor[46–48], Aris[30,31] and Golay (for $k = 0$)[28]. From eqn. 3 with $k = 0$ it is seen that plate height depends on velocity as:

$$H = R^2\ u/24\ D_{\text{M}} \tag{31}$$

We measured $H$ *vs.* $u$ relationships for columns with diameters ranging from 100 to 10 μm and found little deviation from the theoretical relation eqn. 31, provided that make-up flows were in the range of 0.3–1 ml/min and splitting ratios well below 1:50. The most sensitive test for extra-column broadening is to measure plate heights in columns of very small diameters, where column broadening is only slight. Fig. 6 shows the $H$–$u$ curve we obtained from two of the smallest columns available at present, a soft-glass column (diameter 14 μm) and a fused silica column with a diameter of 10 μm (diameters obtained from microscopic measurement).

The peak shapes are nearly symmetrical, but the resulting plate heights deviate from the theoretical prediction, the more so for smaller make-up flows. With these smaller flows a distinct tailing occurs, accompanied by an increase in plate height. This is shown in Fig. 7.

The additional broadening observed for the best detector (Waters) can be

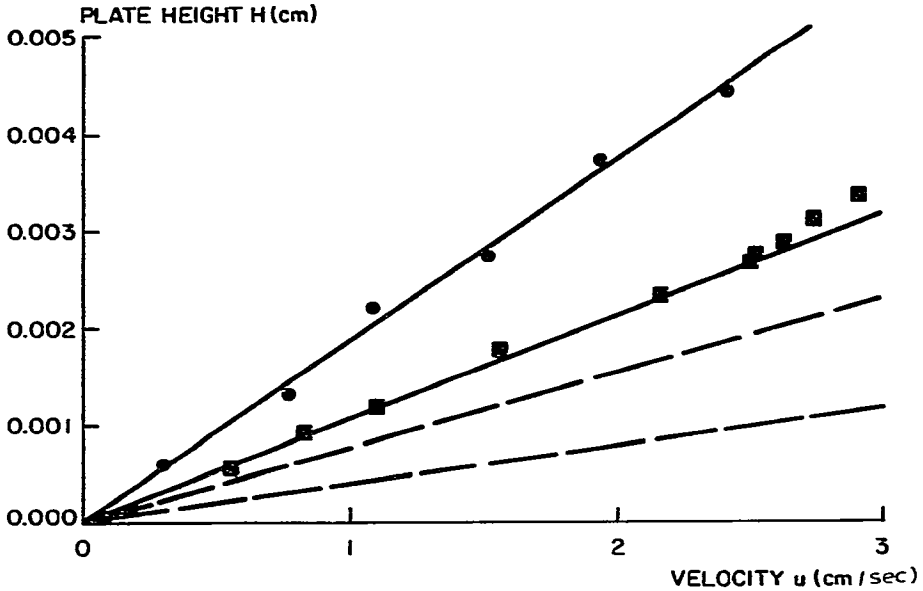



Fig. 6. Plate height *vs.* velocity for a non-retained solute in microcapillaries with diameters of 10 $\mu$m ($L$ = 291 cm; ■) and 14 $\mu$m ($L$ = 472 cm; ●) using UV detection. Solute: toluene. Mobile phase: isooctane. Detector: Waters 440 at 254 nm, with make-up flow of 1.3 ml/min. The dashed lines represent the theoretical predictions.

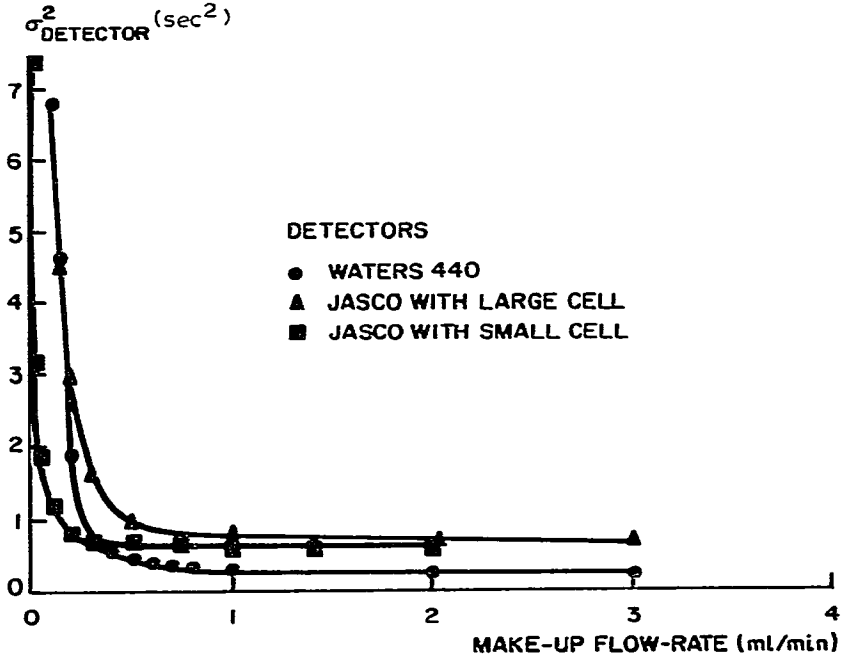



Fig. 7. Extra-column broadening in UV detectors as a function of the make-up flow-rate. Solute: toluene ($k$ = 0). Mobile phase: isooctane, $u$ = 3 cm/sec. Column: $R$ = 13.5 $\mu$m, $L$ = 325 cm. The theoretical column variance ($R^2\ t_R/24\ D_M$) = 0.32 sec$^2$ has been subtracted from measured variances.

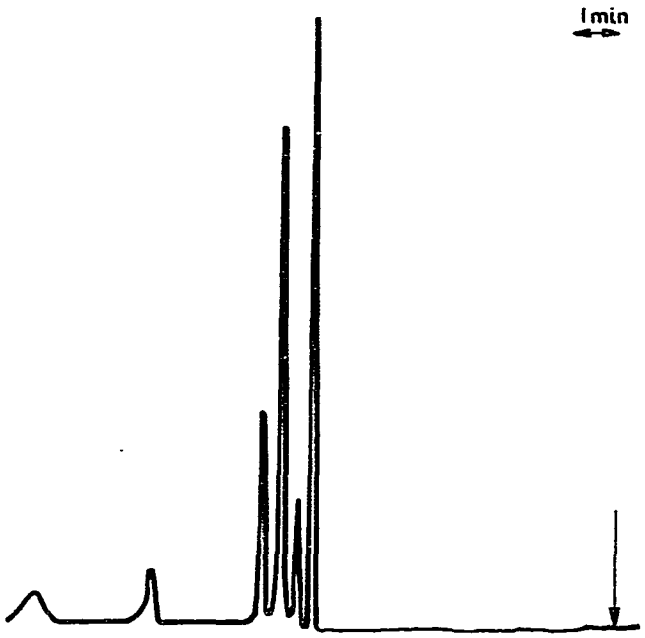



Fig. 8. Typical separation of polar aromatic compounds by microcapillary LSC. Column: etched soft glass, 355 cm × 32 μm. Mobile phase: isooctane. Detector: Waters UV, 254 nm, 0.01 a.u.f.s.; make-up flow 0.3 ml/min. Solutes (in order of elution): toluene; Sudan yellow; 2-ethylanthraquinone; 1,4-naphthoquinone; benzoquinone; *p*-aminoazobenzene.

characterized as the dispersion from an ideally mixed dead volume of 8 μl at a make-up flow-rate of 1 ml/min, which corresponds well with the detector cell volume. Recently, it has been shown[11] how this cell volume can be diminished.

*UV-detection and adsorption microcapillary LSC*

As described in the Experimental, alkaline etching of soft glass produces a

Fig. 9. Generation of very large plate numbers with microcapillary LC. Column: etched soft glass, 27.5 m × 32 μm. $u = u_{opt} = 0.12$ cm/sec, $n = 2.8 \cdot 10^6$ (!), $H = 9.8$ μm. Mobile phase: isooctane. Detector: Waters UV (254 nm, 0.01 a.u.f.s.). Solutes: toluene and N-propylaniline (relative retention 1.009). Analysis time: 6.5 h.

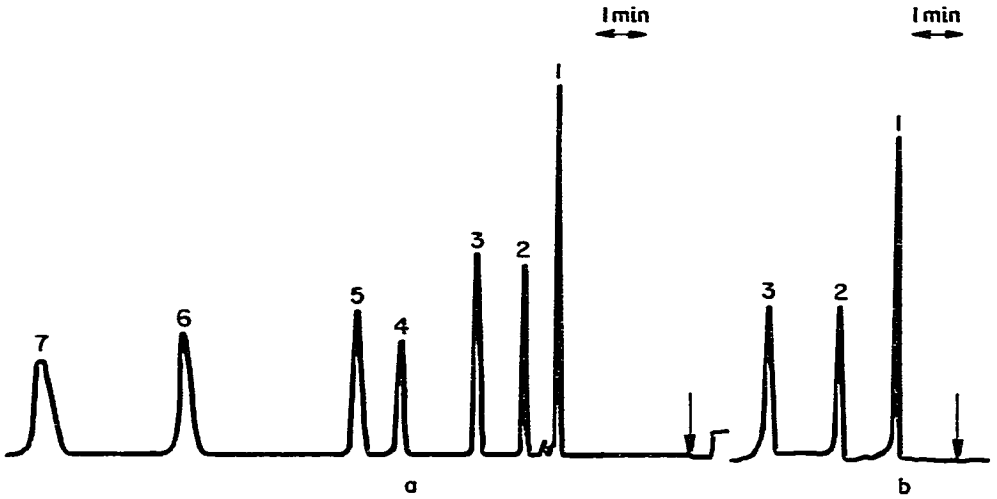



Fig. 10. Separation of aromatic compounds in a microcapillary (a) and a packed column (b). Columns: a, 325 cm × 27 μm; b, 10 cm Partisil 5 (particle size 5 μm). Stationary phase in both cases: ODPN. Mobile phase: isooctane (in b 1% methanol is added to reduce tailing); flow-rate 1 ml/min. Detection: UV absorption at 212 nm. Solutes: 1 = toluene; 2 = benzophenone; 3 = benzonitrile; 4 = *o*-toluidine; 5 = *m*-toluidine; 6 = aniline; 7 = benzyl alcohol.

highly adsorptive siliceous layer on the inner wall of the column, which layer can be used as the stationary phase.

Using isooctane as the mobile phase, saturated with water to suppress tailing, very useful chromatograms were obtained, examples of which are shown in Figs. 8 and 9. Fig. 9 demonstrates how several millions of plates were obtained in a separation in an etched 32-μm microcapillary column by using velocities near the optimum. The rather long time (6.5 h) required in this particular case may be drastically reduced by using smaller diameter columns. A 10-μm column of the same length would produce even more plates ($9 \cdot 10^6$!) in less time (2 h), operating at 80 bar only.

The present result illustrates the potential application of microcapillary LC to extremely difficult separations of closely related compounds, requiring separation

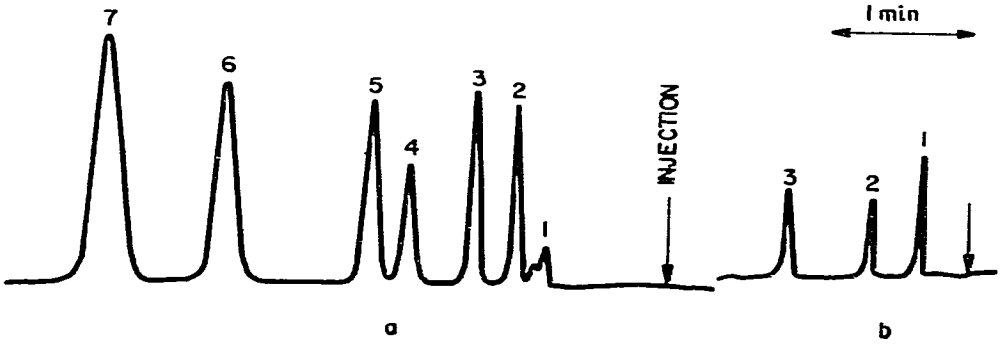



Fig. 11. Separation of aromatic compounds in a microcapillary (a) and a packed column (b). Conditions as in Fig. 10 except that velocity in b was 4 ml/min.

efficiencies of several million plates, which cannot be obtained with packed HPLC columns.

*UV-detection and straight phase microcapillary LLC*

Etched and Cab-O-Sil-treated microcapillaries could easily be coated by the dynamic coating technique with a polar stationary liquid phase like ODPN. Using isooctane (saturated with ODPN) as the mobile phase, these columns were stable for long operating periods (months).

Figs. 10–13 show separations of different samples at different velocities, illustrating that microcapillary LC can be used either as a rapid separation technique or as a more time-consuming but very efficient technique for more difficult separations. The presence of a small amount of ODPN in the mobile phase prohibited the use of a mass spectrometer as the detector, as this would lead to rapid contamination of the ion source.

*UV-detection and reversed-phase microcapillary LLC*

Reversed-phase LC in microcapillary columns has been achieved by coating etched capillaries with the non-polar phase Apiezon L. With methanol–water as the mobile phase, a 1 m × 32 μm column readily separated aromatic compounds within several minutes. When the retention time of the last peak is chosen to be about 20 min, as in the lower trace of Fig. 14, a comparison can be made with a separation of aromatics recently published by Yang[7]. This author used a chemically bonded ODS phase in a 2 m × 30 μm fused-silica column. The present separation compares very

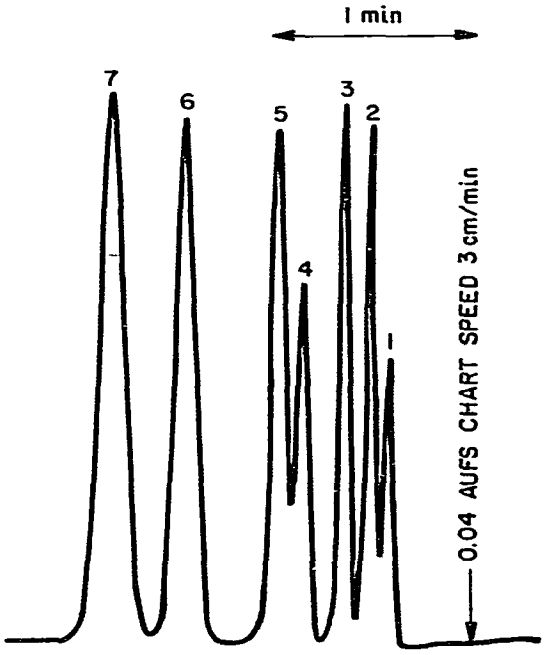



Fig. 12. Rapid separation of aromatic compounds in a microcapillary column. Conditions as in Fig. 10 except for velocity.

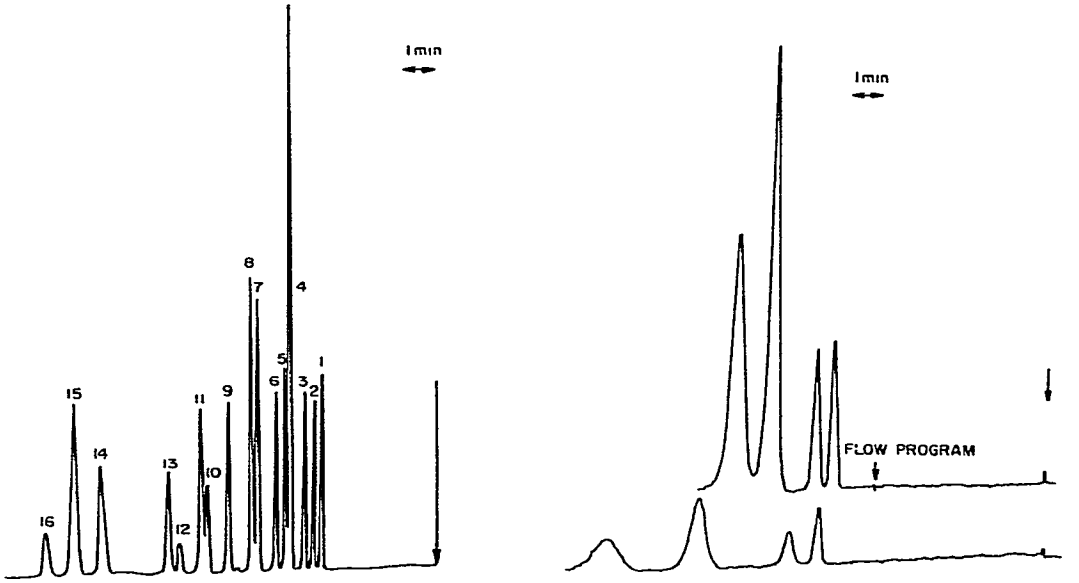



Fig. 13. Microcapillary LC separation of aromatic compounds by straight phase partitioning. Analysis time: 13 min. Inlet pressure: 200 bar. Separation speed: 16 effective plates per sec (last peak), in accordance with eqn. 10. Column: $R = 6$ μm, $L = 4.95$ m, etched soft glass, coated with ODPN as the stationary phase. Mobile phase: isooctane (saturated with ODPN at 23°C). Detector: UV absorption at 212 nm, 0.04 a.u.f.s.; make-up mobile phase 0.4 ml/min. Solutes: 1 = diisoheptyl phthalate; 2 = dibutyl phthalate; 3 = dipropyl phthalate; 4 = 2,4,5-trimethylaniline; 5 = N-methylaniline; 6 = diethyl phthalate; 7 = *o*-chloroaniline; 8 = 2,3-dimethylaniline; 9 = *o*-toluidine; 10 = *p*-toluidine; 11 = *m*-toluidine; 12 = benzoquinone; 13 = dimethyl phthalate; 14 = aniline; 15 = *m*-chloroaniline; 16 = *p*-chloroaniline.

Fig. 14. Separation of polynuclear aromatic compounds by reversed-phase microcapillary LC. Upper trace: programmed flow (after 6 min increase of flow at the rate of 27%/min). Lower trace: constant flow. Column: 108 cm × 32 μm I.D. Stationary phase: Apiezon L. Mobile phase: methanol–water (75:25). Detector: UV absorption at 254 nm, 0.01 a.u.f.s. Solutes (from right to left): benzene; naphthalene; anthracene and pyrene.

favourably with the cited work, as is evident from the plate numbers obtained for the pyrene peak [$n = 1600$ ($n_{eff} = 400$) on our 1-m column *vs.* $n = 200$ ($n_{eff} = 90$) in the 2-m (!) ODS column] as well as from the peak shapes (symmetrical on our column, see Fig. 14; several tailing peaks on the ODS column).

The same separation can be used to illustrate the profitable use of flow programming, provided that the chromatogram is not too crowded. By increasing the mobile phase flow-rate through the column during the analysis (or alternatively only during the elution of a peak), peak heights are appreciably enhanced, as is seen from the upper and lower traces in Fig. 14.

*Direct coupling of microcapillary LC with MS detection*

For samples of low molecular weight (MW $<250$) the direct inlet of column effluent from unmodified microcapillary columns is very well suited for practical qualitative as well as quantitative analysis. An example which illustrates this is the reversed-phase separation of polynuclear aromatics (PNAs), as discussed in the

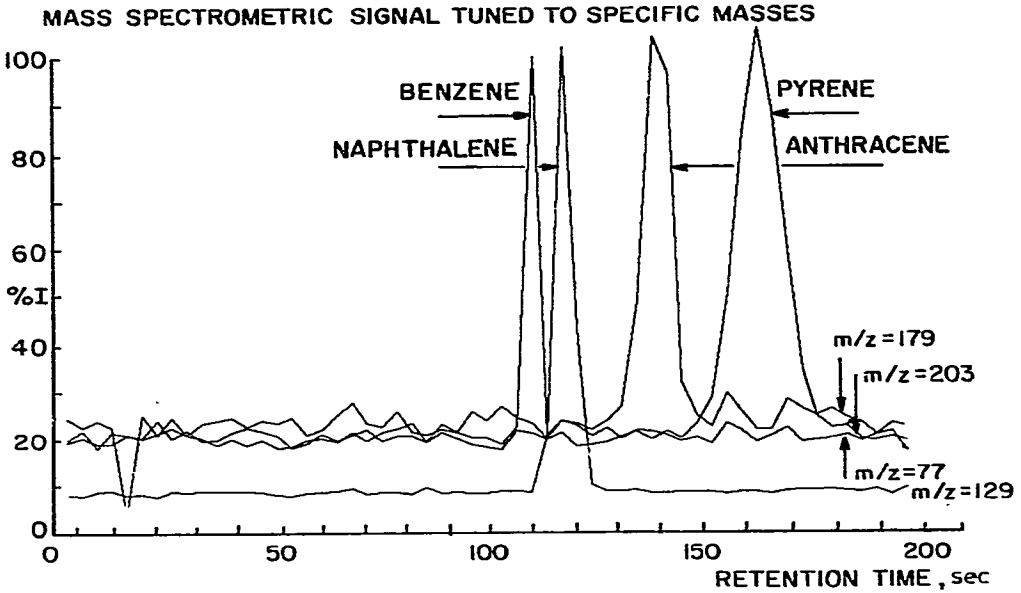



Fig. 15. Chromatogram showing the superimposed signals of four specific masses for polynuclear aromatics separated by reversed-phase microcapillary LC. (Benzene in CI-MS behaves differently from other PNAs and is detected at $m/z$ = MW − 1.) Peak heights (in counts): benzene, 383; naphthalene, 2939; anthracene, 158; pyrene, 539. Sample concentrations: $10^{-2}$ g/ml. Flow-rate: 2 μl/min. Splitting ratio: 1:5000. Each peak contains 10 ng of the compound.

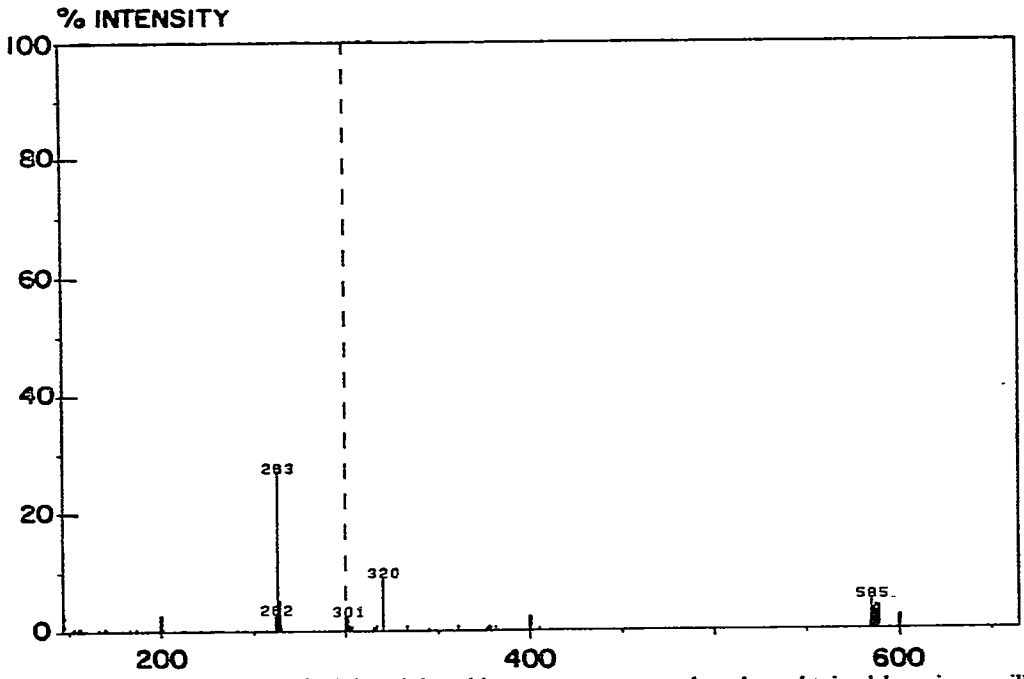
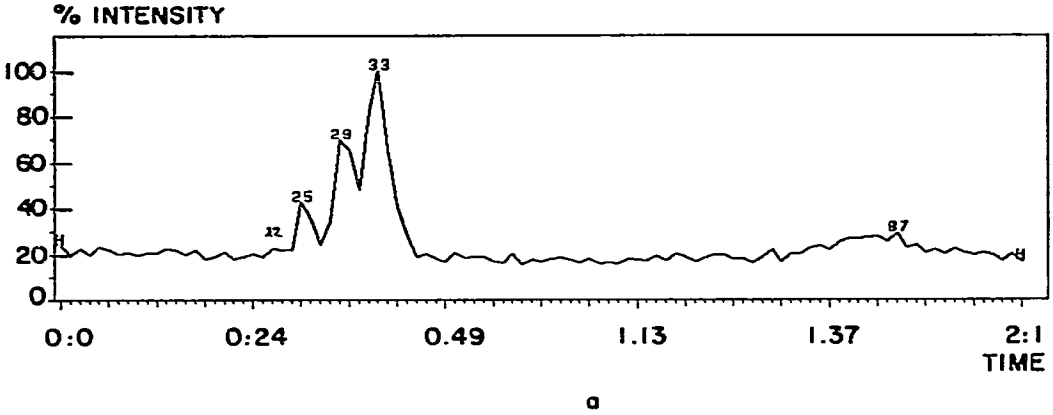



Fig. 16. CI mass spectrum of triphenylphosphinetungsten pentacarbonyl as obtained by microcapillary liquid jet introduction.



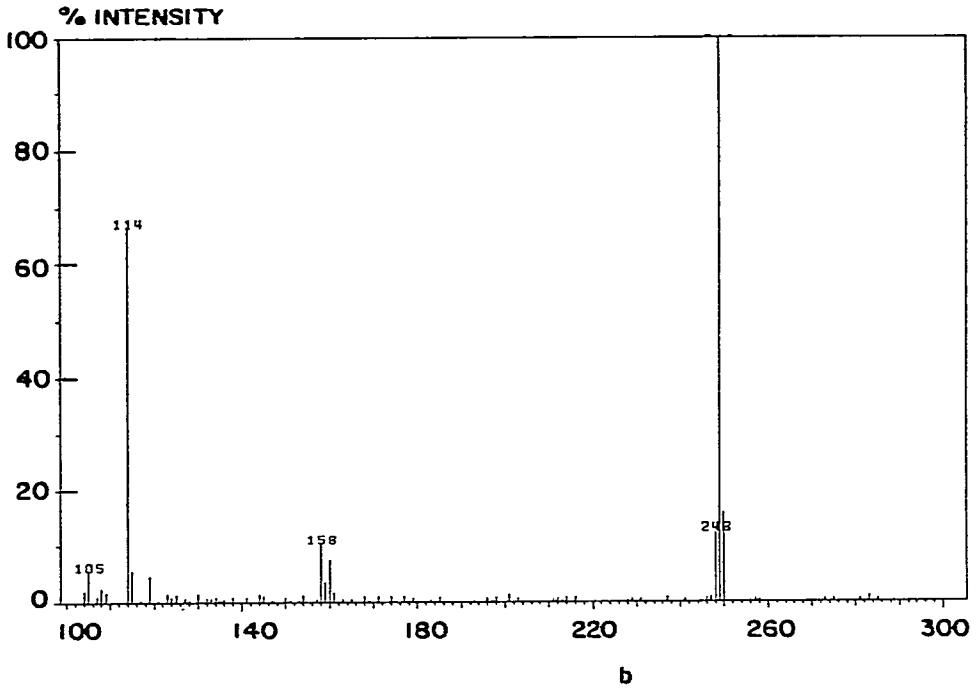




Fig. 17. Chromatogram obtained by microcapillary LC–MS with jet formation. a, Solutes: toluene (scan 22); Sudan yellow (scan 25); 2-ethylanthraquinone (scan 29); 1,4-naphthoquinone (scan 33) and *p*-aminoazobenzene (scan 87). Column: 322 cm × 32 $\mu$m, $r_{jet}$ = 2.5 $\mu$m, etched glass. Mobile phase: isooctane. b, Mass spectrum accompanying the Sudan yellow peak.

above paragraph, using Apiezon L as the stationary phase. This separation was performed on a 4.5 m × 32 $\mu$m column within 3 min, as is shown by Fig. 15. The peak shapes are quite symmetrical and the peak widths do not differ much from those obtained by UV detection. Samples of about 100 pg can still be detected, far smaller than with UV detectors.

For concentrated samples of high molecular weights (MW >250), irregular peak shapes as well as fluctuating ion source pressures are observed, however, indicating deposition and discontinuous evaporation of sample near the column outlet. Liquid jet formation could be realized by providing a conical tip with $r_{jet} = 2.5$ μm at the end of a 322 cm × 32 μm etched glass microcapillary. In this case, samples of high molecular weight could be introduced into the ion source without difficulty; symmetrical peaks were recorded and good CI spectra obtained, *e.g.*, for heavy hydrocarbons (squalane; MW = 422), undecane benzene sulphonic acid (MW = 312), sucrose (MW = 342), and Lubad J (4,4′-methylenebis-*tert.*-butylphenol; MW = 424).

Thermally unstable compounds such as triphenylphosphine tungsten pentacarbonyl (MW = 584) and volatile solids such as Ionol (2,6-di-*tert.*-butyl-*p*-cresol; MW = 220), notoriously problematic when other introduction techniques (*e.g.*, moving belt) are used, are flawlessly introduced into the ion source by the jet interface technique and yield good CI spectra, see Fig. 16. Fig. 17 shows an example of a rapid separation in this etched column with liquid jet formation, and an accompanying CI mass spectrum.

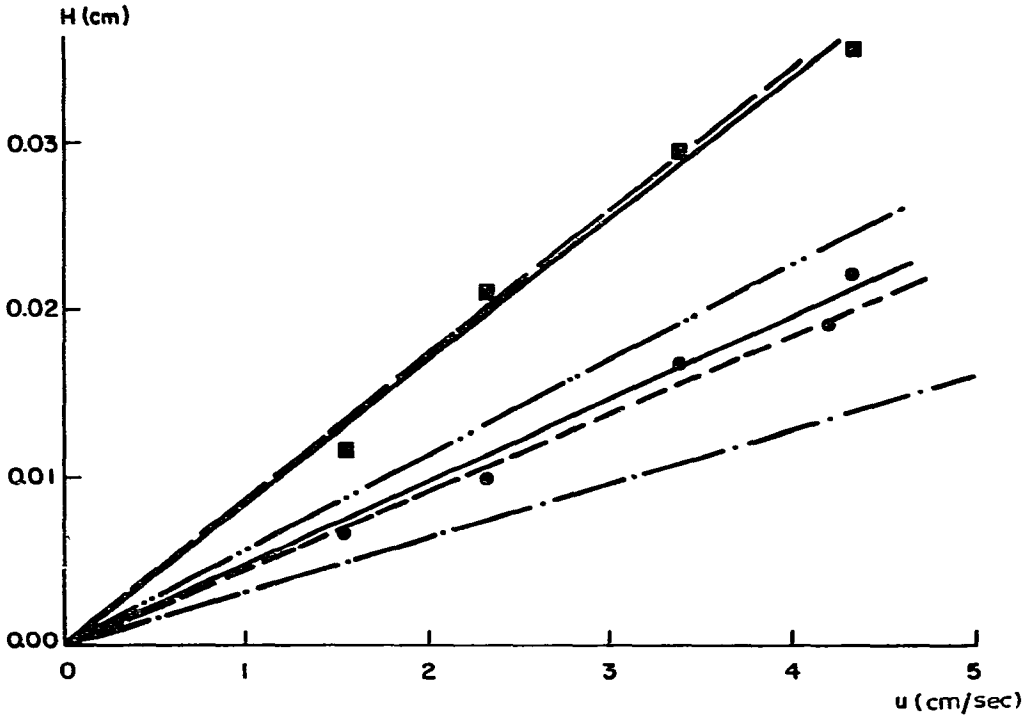



Fig. 18. Plate height *vs.* velocity for retained solutes in microcapillary LLC. Column: 472 cm × 14 μm, etched and coated with ODPN. Mobile phase: isooctane. Detector: Waters UV (254 nm) with make-up flow of 1.3 ml/min. ●, *o*-Toluidine, $k = 0.80$, $D_M = 2.53 \times 10^{-5}$ cm²/sec; ■, dimethyl phthalate, $k = 1.30$, $D_M = 1.88 \times 10^{-5}$ cm²/sec. ——, Golay theory for $k = 0.80$; ——, Golay theory for $k = 1.30$; — — —, present theory for $k = 0.80$; — — —, present theory for $k = 1.30$. In view of the non-linear partitioning isotherms, plate heights were determined from moments extrapolated to zero concentration.

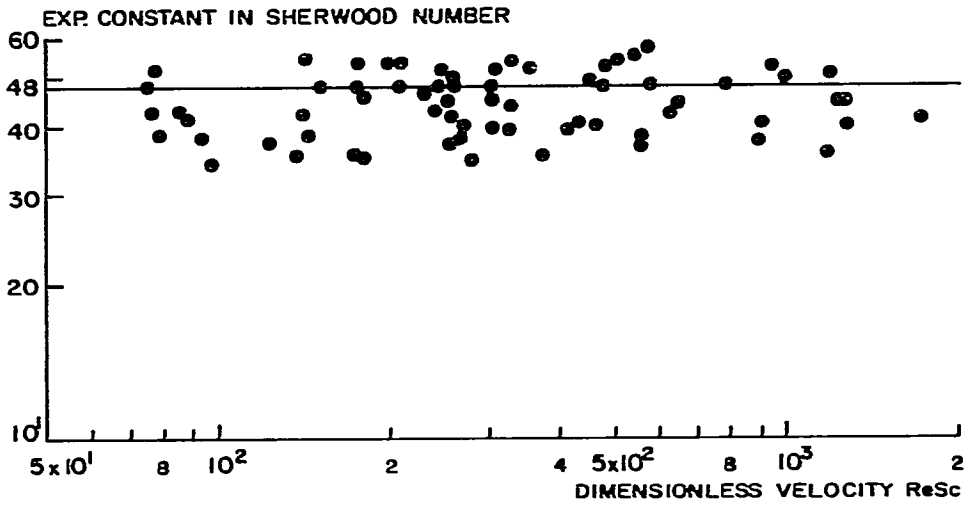



Fig. 19. Interphase resistance to mass transfer in microcapillary LC columns with diameters in the range of 11–100 μm. Capacity factors: 0.07–3.7. Velocities: 0.2–5 cm/sec. Adsorption as well as partitioning.

*Separation speed and axial dispersion*

Notwithstanding careful avoidance of extra-column broadening effects, and even after correction for these effects (by subtracting moments[26]), we observe that the experimental plate heights are invariably larger than those predicted by the Golay equation, up to a factor of about 2. A typical illustration of this can be seen in Fig. 18.

In the Theoretical we suggested that the additional broadening may originate from interphase resistance effects analogous to the resistance to heat transfer in tubular heat exchangers. If this is correct, the experimentally obtained coefficient $k_M$, which describes the additional broadening, should be in accordance with the approximation given in the Appendix (eqn. A7).

In other words, if we plot the experimentally observed $k_M$ in the dimensionless form of the Sherwood constant, given in the Appendix, as a function of (dimensionless) velocity (ReSc) under different experimental parameters ($R$, $k$, phase system), the theoretical value of 48 should be approached independently of the experimental parameters mentioned. Fig. 19 shows the resulting plot and it is observed that a tendency to approach the theoretical value of 48 is indeed present for column diameters in the range of 11–100 μm, for capacity factors in the range of 0.07–3.7, for velocities in the range of 0.2–5 cm/sec and for adsorption (etched glass and Cab-O-Sil) as well as partitioning (ODPN).

The spread observed around the value of 48 can largely be attributed to errors in the determination of the moments[26] as well as of the column diameters. In anticipation of more precise experiments and a more exact theoretical background for the mass transfer coefficient, on which we are working at present, we conclude that our approach of including interphase resistance in chromatographic dispersion is sound. At least it is clear that the mass transfer coefficient model for interphase resistance is able to describe the observed axial dispersion, which deviates from the Golay prediction by a factor between 1 and 2 in our experiments.

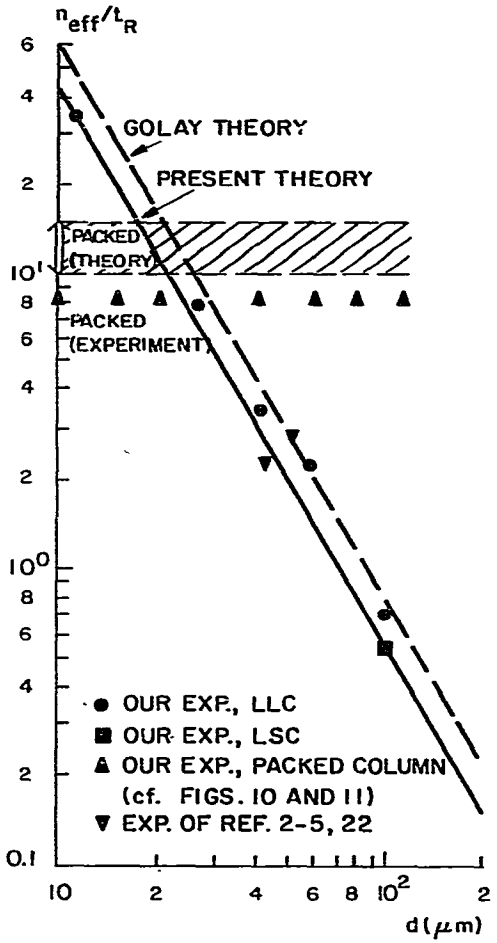



Fig. 20. Separation speed of microcapillary LC columns as a function of the column diameter. Retained solutes with capacity factors 0.6–0.9. The theoretical zone for the packed column is based on work by Snyder[35].

Although our theory is somewhat more conservative than Golay's, this does not imply that the technique of microcapillary LC will have less favourable prospects. This is illustrated by the separations shown, and is further supported by the observed separation speeds.

Fig. 20 shows the dependence of experimental separation speed on column diameter; it is clear that our theory describes these results very well. Evidently, columns with diameters of less than 30 μm will have the greatest future, as the separation speeds obtained are well above those of packed HPLC columns.

## CONCLUSIONS

(1) It has been shown experimentally that microcapillary columns for liquid

chromatography with diameters of 10–50 $\mu$m can be prepared and coated with polar as well as non-polar stationary phases. Both LSC (adsorption) and LLC (partitioning in straight- and reversed-phase modes) have proved to be of practical interest.

(2) The separation performance of a column of 30 $\mu$m I.D. compares very well with that of modern packed HPLC columns (5-$\mu$m particles) and yields about five effective plates per second as the separation speed. Smaller diameter columns have been found to have a much higher speed of separation, *e.g.*, 50 effective plates per sec for a 10-$\mu$m column.

(3) In small diameter (10–30 $\mu$m I.D.) and long (5–25 m) microcapillary columns operated at the optimum velocity, extremely high separation efficiencies (up to about $10^7$ plates) can be obtained within a few hours, which is still reasonable. Very difficult separations can thus be performed.

(4) Avoidance of extra-column peak broadening is a major problem in microcapillary LC. Especially detection with (UV-absorption) flow-through cells (relatively large volumes, 1–10 $\mu$l, and non-ideal cell geometry) requires the application of a scavenger liquid flow to reduce peak broadening. This make-up liquid flow amounts to 0.3–1 ml/min and causes dilution of the column effluent by a factor of 100–1000, such that only the main components can be detected.

(5) When a mass spectrometer is used as the detector and is operated in the chemical ionization mode, direct inlet of the total column effluent ($< 10$ $\mu$l/min) is possible. The dilution problem is absent, which implies that trace components can be determined (qualitatively and quantitatively) down to pg levels. Compounds of low molecular weight (MW $< 200$) can be separated and introduced; higher molecular weights require an additional transfer technique. Liquid jet formation at the column exit, under chromatographically still attractive velocity conditions, has been found to be of practical interest.

(6) The observed peak broadening, carefully corrected for extra-column effects, cannot be interpreted with the common Golay theory of capillary chromatography. Experimental plate heights are a factor of 1–2 larger than those predicted by that theory. By introducing interphase mass transfer in analogy with heat transfer to a solid pipe wall in tubular heat exchangers, we have expressed the additional peak broadening in terms of a mass transfer coefficient, using expressions from the theories of Aris, Khan, Pethö and Giddings. It is concluded that the observed peak broadening can be adequately described by a sum of convective dispersion (Golay) and interphase mass transfer effects.

## APPENDIX

### *Estimation of the axial dispersion from interphase resistance to mass transfer*

Golay's theory for peak broadening in open tubular columns for chromatography is based on a solution to the mass balance equations which are coupled to each other by the condition of equilibrium in the partitioning of solute molecules between the two phases. Upon removing this equilibrium condition and allowing for a resistance to interphase mass transfer to be described by linear kinetics with a transfer coefficient, $k_M$, several other theories[30–33] yield an additional $C$ term for peak broadening, which we call the interphase term, $C_i$:

$$H = (C_M + C_S + C_i)u \tag{A1}$$

$C_M$ and $C_S$ are the same as in the Golay theory (eqns. 4 and 5), while $C_i$ in all three theories is expressed as:

$$C_i = \frac{R}{k_M} \cdot \left(\frac{k}{1 + k}\right)^2 \tag{A2}$$

So far, a conclusive model for $k_M$ has not been presented and we propose that $k_M$ be found from the analogy of the mass transfer problem with the well-known solution to the heat transfer problem in tubular heat exchangers[49,50]. Earlier, Wong *et al.*[29] proposed the same, but they took an unrealistic extreme value for $k_M$, not recognizing the important dependency of $k_M$ on the partition ratio, $k$. This will be illustrated in the following approximate derivation, where use is made of the results obtained by Giddings[34] in his non-equilibrium theory.

The mass transfer coefficient, $k_M$, is defined as the proportionality constant between the mass flux, $\phi_i''$, at the phase boundary and the driving force for that flux (*i.e.*, concentration difference) in the mobile phase:

$$\phi_i'' = k_M (c_i - c_{bulk}) \tag{A3}$$

By continuity, this flux at the interface i equals those in both phases

$$\phi_i'' = \phi_M'' = \phi_S'' \tag{A4}$$

where both $\phi_M''$ and $\phi_S''$ can be expressed as diffusional fluxes by Fick's first law. Using the appropriate expressions in the non-equilibrium theory[34] for $\phi_M''$ and $\phi_S''$, from eqn. A4 we arrive at an expression for $k_M$:

$$k_M = \frac{u\,R}{2} \cdot \frac{k}{1 + k} \cdot \frac{\partial \overline{c_M}}{\partial z} \Big/ (c_{M,i} - c_{M,bulk}) \tag{A5}$$

In all dispersion theories[9,28–34], concentration differences in a cross-section of the column are so small that $c_{M,i}$ may be replaced by the average concentration $\overline{c_M}$ in eqn. A5.

The concentration difference $(\overline{c_M} - c_{M,bulk})$ plays an important rôle in Giddings' non-equilibrium theory[34] as total plate height is determined by this difference:

$$H = 2\,(\overline{c_M} - c_{M,bulk}) \Big/ \left(\frac{\partial \overline{c_M}}{\partial z}\right) \tag{A6}$$

We know already that $H = Cu$, where $C \approx C_M$ according to Golay, *i.e.*, from eqns. A5 and A6:

$$k_M \approx \frac{R}{C_M} \cdot \frac{k}{1 + k} \tag{A7}$$

Hence, when $k_M$ is substituted in eqn. A2:

$$C_i \approx k C_M/(1 + k) \tag{A8}$$

The dimensionless Sherwood number $Sh = 2Rk_M/D_M$ and we find from eqn. A7 and eqn. 4 for $C_M$ that

$$Sh = \frac{2}{D_M} \cdot \frac{R^2}{C_M} \cdot \frac{k}{1 + k} = 48 \cdot \frac{k\,(1 + k)}{(1 + 6\,k + 11\,k^2)} \tag{A9}$$

*i.e.*, the Sherwood number is a constant, which we will call the Sherwood constant (with the theoretical value of 48), times a function that depends on the retention, $k$. For infinite retention, $k \to \infty$ and $Sh = 48/11$, which is exactly the result reported for heat transfer[49] as well as for mass transfer[50] in tubes.

To test the usefulness of the present approximation for $k_M$ we plotted

$$\frac{2\,R^2\,u}{D_M\,(H_{exp} - C_M\,u)} \cdot \frac{(1 + 6\,k + 11\,k^2)\,k}{(1 + k)^3}$$

as a function of dimensionless velocity $ReSc = 2\,u\,R/D_M$ in Fig. 19. The figure shows that the theoretical value of 48 is approached. Although the present derivation of eqns. A7–A9 is only approximate, these equations seem to describe experimentally observed dispersion data quite well. A more exact derivation of mass transfer effects is under study.

## NOMENCLATURE

| | |
|---|---|
| $B$ | $2\,D_M$, plate height term ($cm^2$/sec) for axial diffusion |
| $c$ | Concentration (mole/$cm^3$) |
| $c_M$ | Concentration of solute in the mobile phase (mole/$cm^3$) |
| $c_S$ | Concentration of solute in the stationary phase (mole/$cm^3$) |
| $\bar{c}$ | Average concentration |
| $c_{bulk}$ | Mean cup concentration, $(1/u) \cdot \int c(r) \cdot u(r)\, r\, d\, r\, d\, \phi$ |
| $C$ | $H/u$, plate height term (sec) |
| $C_i$ | $H/u$, plate height term (sec) for interphase resistance |
| $C_M$ | $H/u$, plate height term (sec) for convective dispersion in the mobile phase |
| $C_S$ | $H/u$, plate height term (sec) for diffusion in the stationary phase |
| $D_M$ | Diffusion coefficient ($cm^2$/sec) in the mobile phase |
| $D_S$ | Diffusion coefficient ($cm^2$/sec) in the stationary phase |
| $H$ | Plate height (cm), $L\,\mu_2/\mu_1^2 \approx L\,(\sigma_t/t_R)^2$ |
| $k$ | Capacity factor, $(t_R - t_M)/t_M$ |
| $k_M$ | Mass transfer coefficient (cm/sec) |
| $L$ | Column length (cm) |
| $l$ | Length (cm) of the conical jet tip |
| MW | Molecular weight |
| $n$ | Number of plates, $L/H$ |
| $n_{eff}$ | Effective number of plates, $n\,(k/1 + k)^2$ |

| | |
|---|---|
| $\Delta p$ | Pressure drop (bar) |
| $r$ | Radial coordinate |
| $R$ | Column radius $R$ = I.D./2 (cm) |
| ReSc | Dimensionless velocity, $2\ u\ R/D_M$ |
| $\mathscr{R}$ | Radius (cm) in the jet tip |
| Sh | Dimensionless Sherwood number, $2\ R\ k_M/D_M$ |
| $t_M$ | Retention time (sec) of a non-retained solute = residence time of the mobile phase |
| $t_R$ | Retention time (sec) of a retained solute, $t_M\ (1 + k)$ |
| $u$ | Average linear velocity (cm/sec), $L/\mu_1 \approx L/t_M$ |
| $z$ | longitudinal coordinate along the column (cm) |
| $\gamma$ | Surface tension (dyne/cm) |
| $\delta$ | Film thickness (cm) of the stationary phase |
| $\eta$ | Dynamic viscosity (poise) |
| $\mu_1$ | First time moment (sec), $(1/c_0) \cdot \int c(t) \cdot t\ dt \approx t_R$ |
| $\mu_2$ | Second central moment (sec$^2$), $(1/c_o) \cdot \int c\ (t - \mu_1) \cdot t^2\ dt \approx \sigma_t^2$ |
| $\varrho$ | Density (g/cm$^3$) |
| $\sigma_t^2$ | Variance (sec$^2$) of symmetrical peaks |
| $\phi$ | Angle |
| $\phi''$ | Mass flux (mol/cm$^2$ · sec) |

ACKNOWLEDGEMENTS

The authors wish to thank Ms. A. van Dam for her collaboration in developing the LC–MS coupling technique. They are also indebted to Mr. E. F. Dawes of S.G.E. (Australia), who kindly supplied fused silica microcapillaries.

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* *Editor's Note:* A paper by T. Tsuda, K. Tsuboi and G. Nakagawa, *J. Chromatogr.*, 214 (1981) 283, received on May 18th, 1981 gives the same conclusions as this paper; Tsuda *et al.* give results with a 20-μm I.D. column. See also M. Krejči, K. Tesařík, M. Rusek and J. Pajurek, *J. Chromatogr.*, 218 (1981) 167. *Editor J. Chromatogr.*

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