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OPEN TUBULAR COLUMNS IN LIQUID CHROMATOGRAPHY

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SUMMARY

Capillary liquid chromatography was studied in liquid–solid and liquid–liquid systems. It was proved that selectivity data obtained with an analytical column are the same as those obtained with a capillary column having a layer of adsorbent on its wall. In the liquid–liquid system, up to 1,250,000 theoretical plates were obtained for an unretained solute with an effectiveness of 50 theoretical plates per second. Possibilities of applying commercial pumps and detectors to the work with capillary columns were demonstrated.

INTRODUCTION

In recent years the separation efficiency of packed columns in liquid chromatography (LC) has reached about 5000 theoretical plates, and the selectivity of such systems means that it may ultimately be possible to solve a number of technically important analytical problems. However, it appears that neither gradient elution nor controlled selectivity of the stationary phase will solve the problems associated with the necessity of an extremely high number of theoretical plates for the separation of some components. This is especially relevant to the analysis of complex mixtures of biological materials or of environmental samples.

A number of authors have developed micropacked columns[1–11] which will make it possible to obtain up to 650,000 theoretical plates. Recently, papers have appeared dealing with the use of capillary columns[12–17], where the main advantages besides high efficiency are the small sample volume and flow-rate of the mobile phase which permit a simpler liquid chromatography–mass spectrometry (LC–MS) technique[18,19].

These papers follow theoretical analyses which have shown that high separation efficiencies can be reached[14] in LC, and have summarized the technical parameters that will have to be realized in capillary LC[20]. At present it is not possible to carry out capillary chromatography in the range of turbulent flow, owing to the demands on the inlet pressures[21], sampling and detection. However, capillary LC in the laminar range of flow makes it possible to attain reasonable efficiencies at the expense of prolonged analysis times[22].

EXPERIMENTAL AND DISCUSSION

Despite the advantages mentioned above the application of capillary LC presents indisputable problems. One of the fundamental problems is that the required number of theoretical plates for a given separation is higher than that required for the analytical column. n_{req} is frequently expressed as a function of the phase ratio, β

$$n_{req} = 16\,R^2\left(\frac{\alpha}{\alpha-1}\right)^2\left(\frac{\beta}{K}+1\right)^2 \tag{1}$$

where K denotes the distribution constant, α the separation ratio and R the resolution. If the same chromatographic system is used in the analytical and in the capillary columns, then the distribution constant for the packed and capillary columns will be $K_P = K_T$. At the same time, the capacity ratio, $k = \beta K$, may be smaller for the capillary column in the ratio β_T/β_P up to two orders of magnitude. With the required resolution, R, in packed (subscript P) and capillary (subscript T) columns, the ratio of the required number of theoretical plates[23], n_T/n_P, is given as

$$\frac{n_T}{n_P} = \left(\frac{k_P}{k_T}\cdot\frac{k_T+1}{k_P+1}\right)^2 \tag{2}$$

If under the above conditions

$$K_P = K_T = \beta_P k_P = \beta_T k_T \tag{3}$$

and

$$F = \frac{k_P}{k_T}\cdot\frac{k_T+1}{k_P+1} = \frac{\beta_T}{\beta_P}\cdot\frac{k_T+1}{k_P+1} \tag{4}$$

then we obtain:

$$n_T = F^2 n_P \tag{5}$$

It is obvious that n_T is dependent on the phase ratio β.

Since it is not easy to satisfy the assumption that $K_T = K_P$ in gas chromatography (GC), the identity of the selectivity data measured with packed and capillary columns was verified in liquid–solid chromatography (LSC).

A packed column (50 cm × 2 mm I.D.) filled with Corasil II (Waters Assoc., Milford, Mass., U.S.A.), particle diameter 37–50 μm, was used in the experiment. The mobile phase (was) hexane–isopropanol (97:3). The same chromatographic system was used for the glass capillary column (1 m × 120 μm I.D.) which had a layer of silica gel on the wall. The layer was prepared by etching[24] with ammonia. A capillary chromatograph was assembled as shown in Fig. 1. A pneumatic pump, designed in the Institute of Analytical Chemistry of the Czechoslovak Academy of Sciences, and an Orlita pump (Orlita, Giessen, G.F.R.) were used. To the capillary column inlet was attacted a splitter incorporating the glass capillary column, as described earlier[25]. Injection was carried out with a Hamilton syringe. An LCD 254 detector (Laboratory Instruments, Prague, Czechoslovakia) was placed at the outlet.

It was also connected to the capillary via a splitter, designed from a glass T-piece through which scavenger liquid was introduced. Using the scavenger liquid in the detector, the value of the minimal detectable concentration is increased, but it enables cell volumes of 8 μl to be used for detection of peaks having a total volume less than 1 μl without any noticeable distortion of the peak.

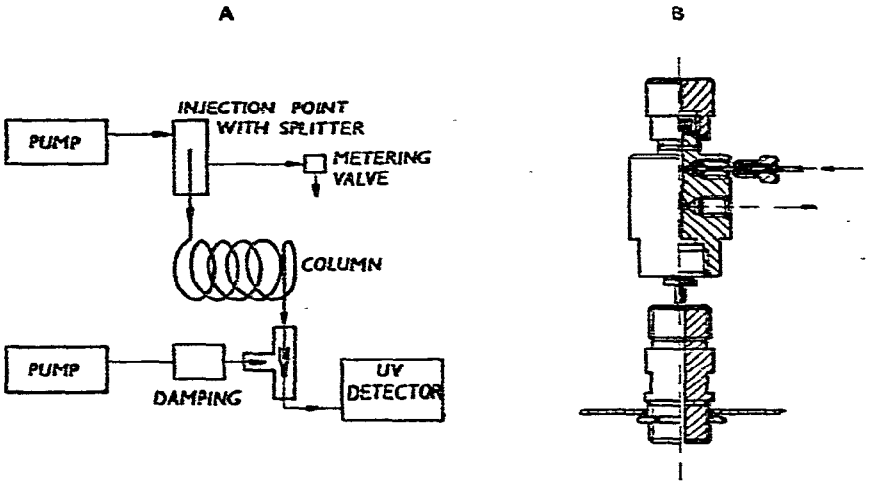

Fig. 1. A, Chromatograph with open tubular column; B, injection splitter.

The results are listed in Table I. The distribution constant for the studied nitroanilines is the same both on Corasil II and on the silica gel layer created on the capillary wall since the relative retentions, r, in Table I are identical both for the packed and the capillary column. The column capacity ratio is, however, less for the capillary column, in accord with the phase ratio given in eqn. 4. The volume of the capillary column was 450 μl and the surface area of the silica gel layer was 0.35 m^2, corresponding to a value $\beta_T/\beta_P = k_P/k_T = 71$.

TABLE I

COMPARISON OF CAPACITY RATIOS, k, AND RELATIVE RETENTIONS, r, FOR PACKED AND OPEN TUBULAR COLUMNS

Compound	k_P	r_P	k_T	r_T	F^2
o-Nitroaniline	0.44	0.23	0.0061	0.23	2580
m-Nitroaniline	1.88	1.00	0.0265	1.00	640
p-Nitroaniline	4.36	2.32	0.0612	2.31	200

In accord with eqns. 1 and 3, the number of theoretical plates required for the separation increases with decreasing capacity ratio. The ratio of the number of theoretical plates required for the separation to that for the packed column is represented by the factor F^2 in eqn. 5, see Table I.

In LC, as in GC, higher numbers of theoretical plates can apparently be reached for capillary columns than for packed ones. The well-known expression for

the most significant contribution to the height equivalent to a theoretical plate, H, *i.e.*, the coefficient of mass transfer in the mobile phase, C_M

$$C_M = \frac{1 + 6k + 11k^2}{24(1 + k)^2} \cdot \frac{r^2}{D_M} \qquad (6)$$

was tested with experimental data for the liquid–liquid system using the values $D_M = 2 \cdot 10^{-5}$ cm²/sec and $r = 30 \cdot 10^{-4}$–60.10^{-4} cm. 1,2,3-Tris(2-cyanoethoxy)-propane (Tris III) served as the stationary phase, hexane saturated with Tris III as the mobile phase. The column was coated with the stationary phase by forcing a measured volume of 30% Tris III in methanol through the column. The coating solution was expelled from the column with the mobile phase.

The value of H decreases with decreasing diameter of the column. Mobile phase velocity, u_{opt}, corresponding to H_{min}, increases with decreasing radius of the capillary column. Whereas when $r = 60\,\mu$m, u_{opt} is 0.02 cm/sec, when $r = 15\,\mu$m, u_{opt} is 0.1 cm/sec. However, in practice, the increase in efficiency does not correspond to the theoretical dependence, eqn. 6. This has been found previously with columns of 30-μm radius, particularly at high mobile phase flow-rates. However, the highest values of n were obtained by using the column with $d_c = 60\,\mu$m and length 21 m. A value of $n = 1.250{,}000$ was measured for the non-sorbed solute at a mobile phase flow-rate of 0.18 cm/s. The analysis time required was 6 h 54 min which corresponds to a performance of *ca.* 50 theoretical plates per sec. An example of a chromatogram obtained with the column mentioned above at a mobile phase flow-rate $u = 0.30$ cm/sec is shown in Fig. 2.

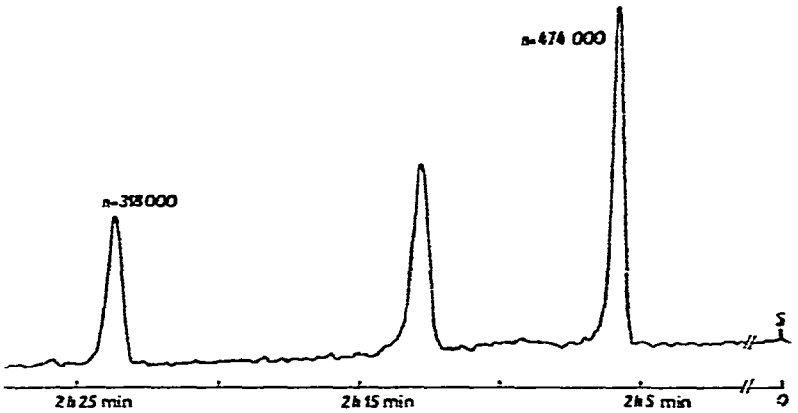

Fig. 2. Example of a chromatogram obtained with the capillary column (21 m × 60 μm I.D.). Mobile phase, hexane saturated by stationary phase. Stationary phase, 1,2,3-tris(2-cyanoethoxy)-propane. Linear velocity, 0.30 cm/sec.

In order to be able to evaluate the influence of the instrumental arrangement on the efficiency of the capillary columns, the dependence of the height equivalent to a theoretical plate on the mobile phase velocity was plotted in the system of reduced coordinates $h = H/d_c$ and $v = ud_c/D_M$. In the dependence shown in Fig. 3, the points measured with columns having $r = 60\,\mu$m ($d_c = 120$) and 40 μm ($d_c = 80$) are

obviously closest to the theoretical values. The fact that these points lie below the calculated dependence can be explained in terms of inaccuracies in the values of d_c and D_M used for the theoretical callulation. Despite this, the experimental arrangement is considered to be acceptable.

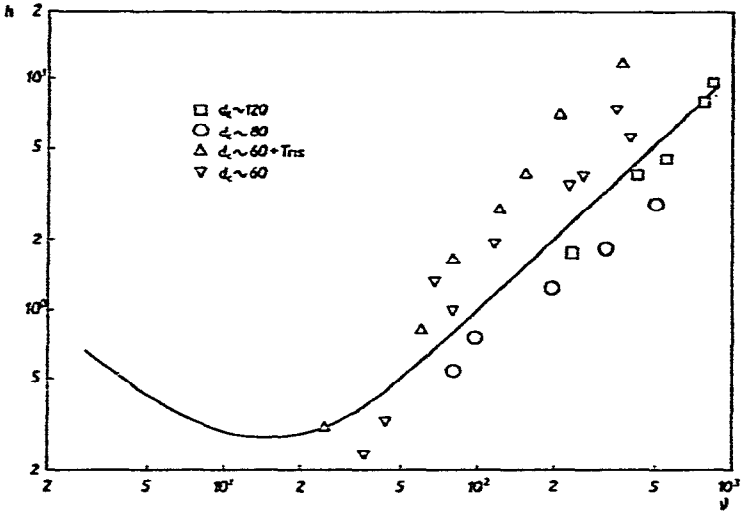

Fig. 3. Dependence of the reduced height equivalent to a theoretical plate, h, on the mobile phase velocity, ν, in reduced coordinates.

Table II gives the ratio between the calculated values of the mass transfer coefficients, C_M (calc.), and the experimentally measured values, C_M, which depend on the capacity ratio. Even here the values measured with the capillary columns with smaller d_c deviate more from the theoretical values.

TABLE II

COMPARISON OF THEORETICAL AND EXPERIMENTAL MASS TRANSFER COEFFICIENTS

d_c	k	C_M	C_M (calc.)	C_M/C_M (calc.)
60	0.061	0.067	0.024	2.79
	0.157	0.046	0.031	1.48
80	0.029	0.037	0.037	1.00
	0.191	0.111	0.059	1.88

In applying columns having $r = 15\ \mu$m a number of technical difficulties were experienced. The flow-rate dependence shown in Fig. 4 was measured in the column coated with Tris III (length 30 m) and with hexane saturated with Tris III as mobile phase. On removing Tris III from the column, a linear dependence of the flow-rate on pressure was obtained even for low-pressure values. Difficulties associated with sampling and also with detection at low column loadings must also be taken into account.

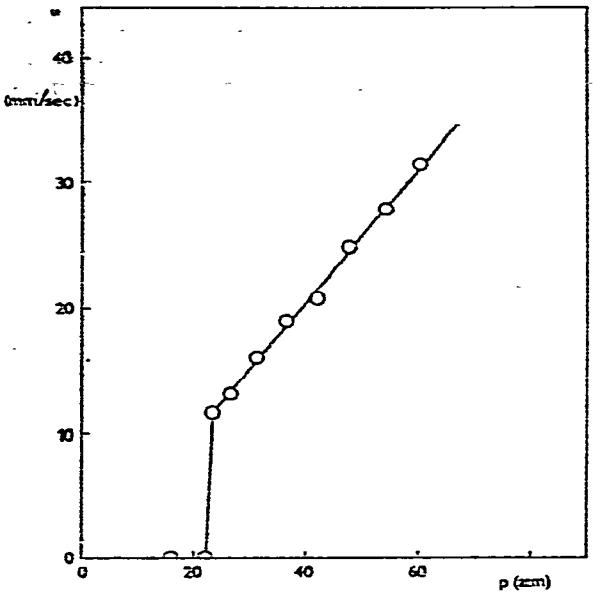

Fig. 4. Dependence of the linear velocity of the mobile phase on the pressure. Column, 30 m × 30 μm I.D.

CONCLUSIONS

The present study has shown:

(1) The possibility of using capillary columns with commercial LC instrumentation

(2) The values of the height equivalent to a theoretical plate almost reach the theoretical ones, but in some instances they are much worse, by a factor of 3

(3) The possibility of reaching a high number of theoretical plates (1,250,000) at an acceptable column efficiency (50 plates per sec).

(4) The possibility of applying LSC and LLC to capillary columns.

REFERENCES

1 C. Horvath and S. R. Lipsky, *Anal. Chem.*, 41 (1969) 1227.
2 D. Ishii, K. Asai, K. Hibi, T. Jonokuchi and M. Nagya, *J. Chromatogr.*, 144 (1977) 157.
3 D. Ishii, K. Hibi, K. Asai and T. Jonokuchi, *J. Chromatogr.*, 151 (1978) 147.
4 D. Ishii, K. Hibi, K. Asai and M. Nagaya, *J. Chromatogr.*, 152 (1978) 341.
5 D. Ishii, K. Hibi, K. Asai, M. Nagaya, K. Mochizuki and Y. Mochida, *J. Chromatogr.*, 156 (1978) 173.
6 D. Ishii, A. Hirose, K. Hibi and Y. Iwasaki, *J. Chromatogr.*, 157 (1978) 43.
7 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 271.
8 R. P. W. Scott, *Analyst (London)*, 103 (1978) 37.
9 K. Iriyama, M. Yoshiura and M. Shiraki, *J. Chromatogr.*, 154 (1978) 302.
10 K. Miyake and H. Terada, *J. Chromatogr.*, 157 (1978) 386.
11 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 169 (1979) 51.
12 R. Tijssen, *Separ. Sci. Technol.*, 13 (1978) 681.
13 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 632.

14 K. Hibi, D. Ishii, I. Fujishima, T. Takeuchi and T. Nakanishi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 21.
15 T. Tsuda, K. Hibi, T. Nakanishi, T. Takeuchi and D. Ishii, *J. Chromatogr.*, 158 (1978) 227.
16 C. Dewaele and M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 174.
17 M. Krejčí, K. Šlais and K. Tesařík, *J. Chromatogr.*, 149 (1978) 645.
18 T. Takeuchi, Y. Hirata and Y. Okumura, *Anal. Chem.*, 50 (1978) 659.
19 S. Tsuge, Y. Hirata and T. Takeuchi, *Anal. Chem.*, 51 (1979) 166.
20 T. W. Smuts, F. A. van Niekerk and V. Pretorius, *J. Gas Chromatogr.*, 5 (1967) 190.
21 V. Pretorius and T. W. Smuts, *Anal. Chem.*, 38 (1966) 274.
22 G. Guiochon, *Anal. Chem.*, 50 (1978) 1812.
23 L. S. Ettre, *Open Tubular Columns in Gas Chromatography*, Plenum, New York, 1965.
24 K. Tesařík, *J. Chromatogr.*, 191 (1980) 25.
25 J. F. K. Huber, *J. Chromatogr. Sci.*, 7 (1969) 85.