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MODE OF OPERATION AND PERFORMANCE CHARACTERISTICS OF MICROBORE COLUMNS FOR USE IN LIQUID CHROMATOGRAPHY

R. P. W. SCOTT and P. KUCERA
Chemical Research Department, Hoffmann-La Roche Inc., Nutley, N.J. 07110 (U.S.A.)
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SUMMARY

The production and operating characteristics of microbore columns are described and details of the modification of the LDC UV monitor to give the necessary low dead volume detection cell for use with such columns are given. A method of connecting the Valco high-pressure sampling valve to microbore columns to provide the necessary small sample volume is described and a system given that permits the operation of the Waters Assoc. 6000 pump over the flow-rate range of 2 to 100 μl/min. The effect on column performance of column radius and particle diameter of the packing is examined in detail and height equivalent to a theoretical plate curves and reduced plate height curves are included; the effect of coiling the column on efficiency is also reported. Examples of the use of microbore columns for high-speed and high-efficiency separations are given and include results obtained from reversed-phase columns. The construction and performance of a column having 750,000 plates is described.

INTRODUCTION

Microbore columns are not novel to the field of liquid chromatography. At the beginning of the renaissance of liquid chromatography, Horváth and Lipsky[1] employed microbore columns with their pellicular packings. However, when methods for packing microparticulate silica were devised[2,3], microbore columns were replaced by 1/4 in. O.D. and 1/8 in. O.D. columns. There were several reasons for this. Firstly, microbore columns were difficult to pack even with the techniques devised by Majors[2] and Kirkland[3] and secondly, low cell volume detectors and appropriate injection devices that were necessary for the effective use of microbore columns were not available. More recently, Ishii[4,5] demonstrated the use of microbore columns to produce reasonable efficiencies and rapid analyses in conjunction with a specially designed low cell volume UV detector.

Microbore columns have several distinct advantages. They are extremely economical to use, both with respect to the column packing and the mobile phase flow-rates employed. Flow-rates of as little as 10 or 20 μl/min are frequently used reducing the solvent consumption to 1/20–1/50 of that required by the normal 1/4 in.

O.D. columns. If 20-μm particle diameter silica is employed, microbore columns can be packed to the limiting plate height of 40 μm to provide a corresponding efficiency of about 25,000 plates/m. A particularly valuable quality of microbore columns is their capability of producing extremely high intrinsic efficiencies, as they can be connected together in series to provide column efficiencies that increase linearly with column length[6]. If widebore columns are joined together, they soon reach a limiting efficiency above which further addition of column length does not increase the overall efficiency. There have been two reasons given for this. Firstly, as the column length increases, the pressure drop across the column also increases and thus the heat generated in the column at the flow-rates normally used, affect the mass transfer processes and produce poor column efficiencies. An alternative explanation is that the permeability across the column is not homogeneous and thus an aggravated multipath term occurs as the columns are joined together in series. Whichever the reason, the use of microbore columns obviates these problems. Firstly, owing to their geometry, there is rapid transfer of heat from the column and, owing to the low flow-rate, a relatively low production of heat, which virtually eliminates the thermal effect, and secondly, as the walls of the column are closer together, the possibility of an inhomogeneous permeability across the column becomes significantly less. Thus it has been possible to construct microbore columns having 750,000 plates.

In this paper, the design of suitable equipment for use with microbore columns is described and methods of packing and preparing them are discussed in detail. The characteristics of the columns with respect to the effect of particle diameter and column diameter on efficiency and loading capacity are also examined. Finally, examples of the use of these columns for high-speed analysis and to obtain very high efficiencies are also given.

EXPERIMENTAL

The modification of the LDC UV monitor for use with microbore columns

Columns of very high efficiency and columns of small cross-sectional area produce very narrow peaks in the sense that the total volume of mobile phase contained in them, may be only a few microliters. It follows that if such peaks pass through a detector connecting tube and cell of significant volume relative to that of the peaks, the peaks will spread owing to both the dispersion resulting from the parabolic velocity profile of the liquid through the tubes and cell, and the logarithmic dilution effect resulting from the finite volume of the cell. If peaks are broadened in the cell, efficiency and resolution will be lost. The effects of band dispersion in connecting tubes and detector cells have been studied by a number of workers[7–9] and specific equations have been developed for the calculation of the optimum dimensions of the connecting tube and detector cell for a column of a given geometry and efficiency[10]. In Table I the standard deviations of peaks eluted from a 25 cm $\times$ 1/4 in. O.D. column packed with particles of different sizes and realizing their maximum theoretical efficiency are shown; that is a height equivalent to a theoretical plate (HETP) of two particle diameters. Values are given for soluted at $k' = 0$ and $k' = 5$, the column dead volume being 3 ml. It is seen that for the columns packed with 10- or 5-μm particles, the detector volume must be less than 8 μl or the efficiency of the early peaks will not be realized. In Table II, the same data are given for a 1 m $\times$ 1/16

TABLE I

STANDARD DEVIATION (σ) OF MOBILE PHASE FOR PEAKS ELUTED FROM A 25 cm × 4.6 mm I.D. (1/4 in. O.D.) STANDARD COLUMN

Dead volume = 3 ml.

Particle diameter (μm)	*Limiting HETP (μm)*	*Maximum efficiency (theoretical plates)*	σ (μl)	
			$k' = 0$	$k' = 5$
35	70	3570	50	301
20	40	6250	38	228
10	20	12,500	27	161
5	10	25,000	19	114

TABLE II

STANDARD DEVIATION (σ) OF MOBILE PHASE FOR PEAKS ELUTED FROM A 100 cm × 1 mm I.D. (1/16 in. O.D.) COLUMN

Dead volume = 0.700 ml.

Particle diameter (μm)	*Limiting HETP (μm)*	*Maximum efficiency (theoretical plates)*	σ (μl)	
			$k' = 0$	$k' = 5$
35	70	14,285	5.86	35.1
20	40	25,000	4.43	26.6
10	20	50,000	3.13	18.8
5	10	100,000	2.21	13.3

in. O.D. column which has a dead volume of 700 μl. It is seen that for columns with 10- or 5-μm packing and for peaks eluted close to the dead volume, the volume of the detector cell system must be about 1 μl. The equations used to calculate the cell and connecting tube dimensions are as follows:

Cylindrical detector cell:

$$r_1 = \left(\frac{2.21\ D_M V_R^2}{\pi n Q l_2}\right)^{1/4}$$

Column detector connecting tube:

$$r_2 = \left(\frac{0.24\ D_M V_R^2}{\pi n Q l_2}\right)^{1/4}$$

where r_1 = radius of detector cell (cm), r_2 = radius of column connecting tube (cm), D_M = diffusivity of solute in mobile phase (cm²/sec), V_R = retention volume (ml), n = column efficiency (theoretical plates), Q = flow-rate (ml/sec), l_1 = length of detector cell, l_2 = length of column connecting tube.

These equations assume that 90% of the permitted dispersion is allowed to occur in the detector cell and the remaining 5% in the connecting tube.

The detector chosen for modification was the LDC UV monitor because its cell system is readily accessible and the geometry is of such a form that it could be easily changed. Two cells were designed having cell volumes of 3 and 1 μl, respectively.

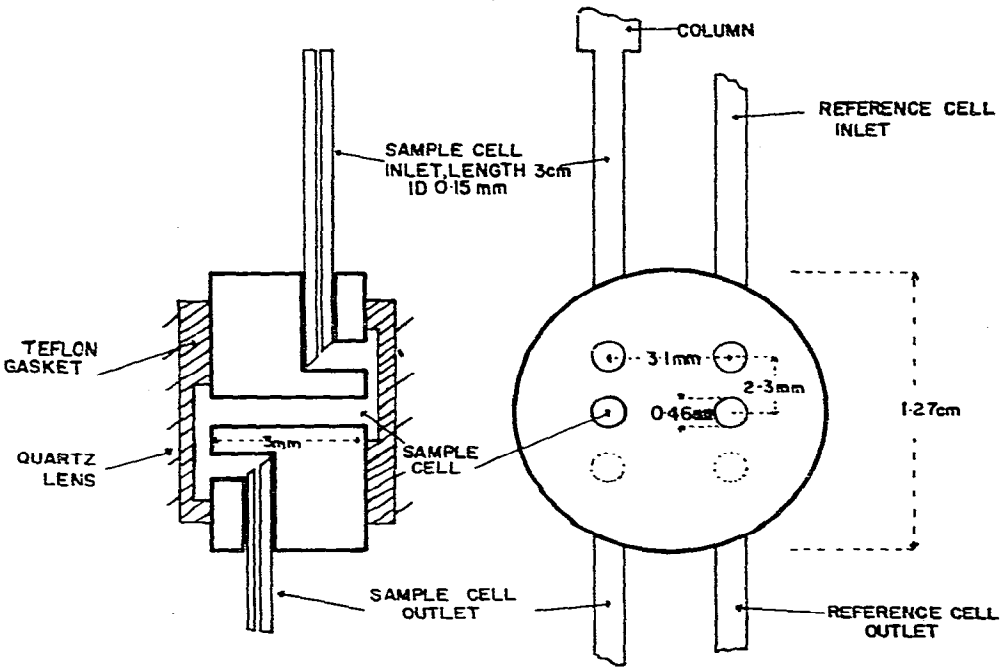

Fig. 1. Modification of the LDC UV monitor cell and tubes for use with microbore columns.

A diagram of the final, 1-μl volume cell is shown in Fig. 1. The cell consists of a disc of stainless steel, 1.27 cm in diameter and 3 mm thick. The cells themselves are holes in the stainless-steel disc 0.46 mm in diameter and have a volume of about 0.5 μl. The inlet tube has to be 3 cm long in order to project from the cell housing and allow the connection to the column to be made. The diameter of the connecting tube from the column to the cell was 0.15 mm, which was again equivalent to a volume of about 0.5 μl. Including the connecting slots in the gasket and the holes between the connecting tube and the edge of the cell, a total detector volume slightly in excess of 1 μl was obtained. A photograph of the cells used is shown in Fig. 2. The lower cell is the 8-μl cell with a 1-cm path length as supplied by LDC. The center cell has a volume of 3 μl and the top cell a volume of 1 μl.

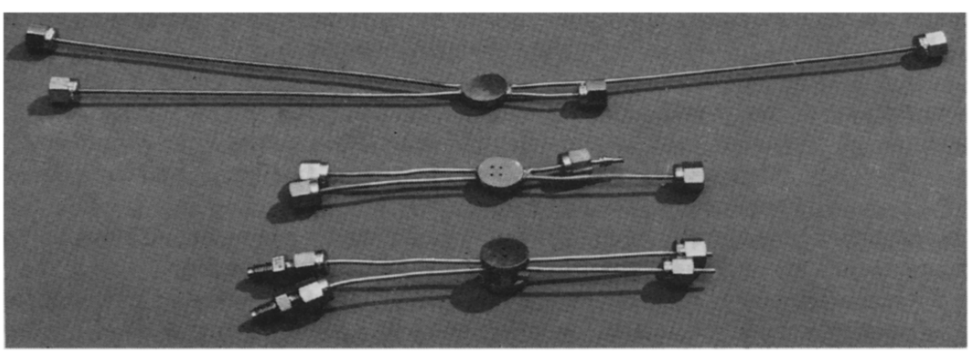

Fig. 2. Photograph of LDC UV monitor microcell.

The effect of reducing the cell volume is clearly shown in Figs. 3a and b. In Fig. 3a the elution curves obtained from benzene eluted at a flow-rate of 1 ml/min from a column 4.6 mm I.D. and 25 cm long are shown. The three curves are from detectors having cell volumes of 25, 8 and 3 μl, respectively. It is seen that the reduction in volume of the detector cell has a very great effect on the band width. In Fig. 3b,

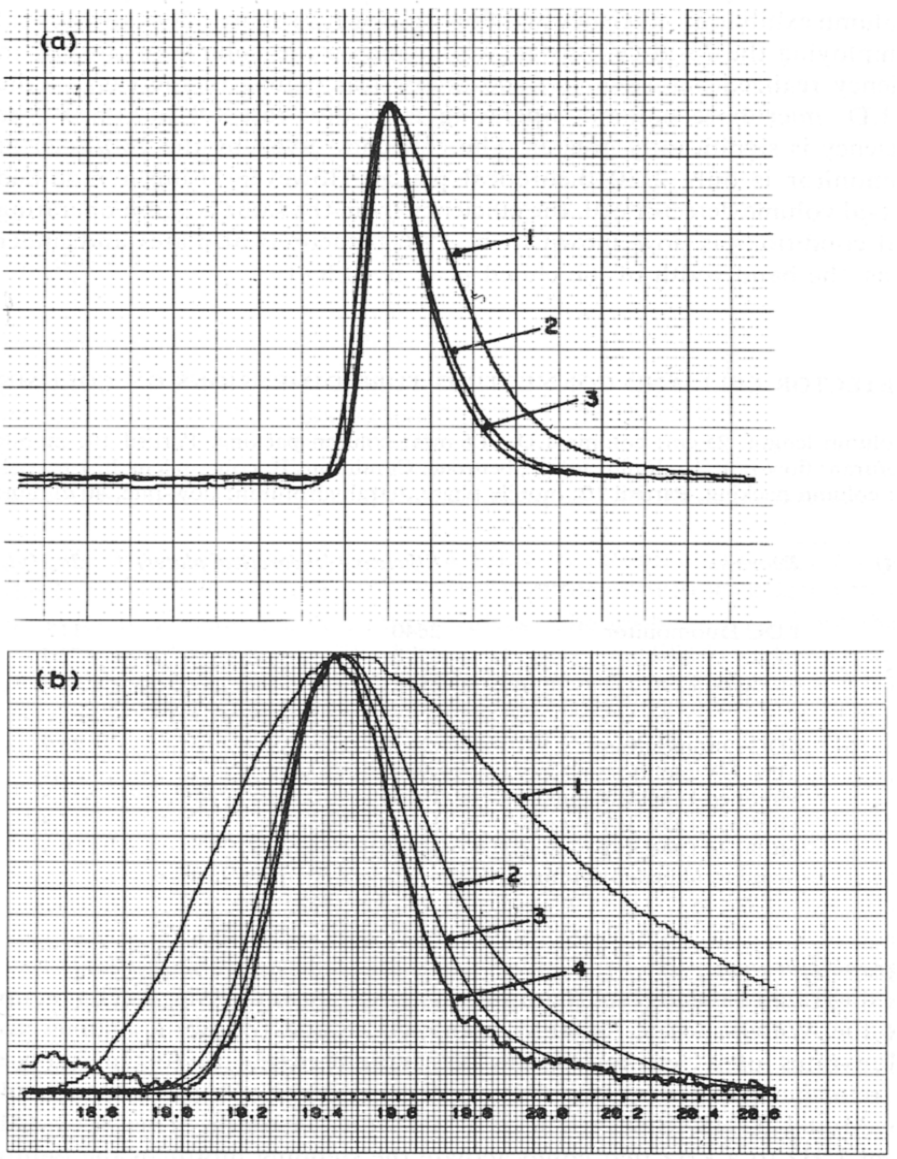

Fig. 3. Peak profiles from detectors having different cell dimensions. (a) Column, 25 cm × 4.6 mm I.D.; solvent, tetrahydrofuran; solute, benzene; flow-rate, 1 ml/min. Cell volume: 1, 25 μl; 2, 8 μl; 3, 3 μl. (b) Column, 1 m × 1 mm I.D.; packing, Partisil 20; solvent, tetrahydrofuran; solute, benzene; flow-rate, 40 μl/min. Cell volume: 1, 25 μl (LDC Duomonitor); 2, 8 μl (LDC UV monitor); 3, 3 μl (modified LDC UV monitor); 4, 1 μl (modified LDC UV monitor).

the elution curves obtained for benzene eluted from a microbore column, 1 m long, 1 mm I.D. having a flow-rate of 40 μl/min are shown. It is again seen that reducing the volume of the cell, very significantly reduces the band width. It should also be noted that with the 25-μl cell, the band is extremely asymmetric resulting from the logarithmic dilution effect being the major cause of band dispersion. The effect of the cell volume on the efficiency is shown in Table III. It is seen that an ordinary 25 cm $\times$ 4.6 mm I.D. column exhibits an efficiency at a flow-rate of 1 ml/min of 2640 theoretical plates when employing the 25-μl cell. By merely changing the volume of the cell to 3 μl, the efficiency realized has risen to 7800 theoretical plates. In the case of the 1 m $\times$ 1 mm I.D. microbore column, operating at a flow-rate of 40 μl/min, the change in efficiency is significantly greater. The efficiency given by the 25-μl cell of an LDC Duomonitor is only 2380 theoretical plates. However, using a modified injector and a 1-μl volume detector cell, the efficiency from the same column operating under identical conditions is now shown to be 17,600 theoretical plates and under these conditions, the band width of the eluted peak is 10.5 μl.

TABLE III

EFFECT OF DETECTOR CELL AND CONNECTING TUBE DIMENSIONS ON COLUMN EFFICIENCY

Conditions A: column length, 25 cm; column I.D., 4.6 mm; column packing, Partisil 10; mobile phase, tetrahydrofuran; flow-rate, 1 ml/min; solute, benzene. Conditions B: column length, 1 m; column I.D., 1 mm; column packing, Partisil 10; mobile phase, tetrahydrofuran; flow-rate, 40 μl/min; solute, benzene.

Volume of cell (μl)	*Remarks*	*Efficiency (theoretical plates)*	*$2\sigma_v$ (μl)*
Conditions A			
25	LDC Duomonitor	2640	117
8	LDC UV monitor	6740	73.1
3	Modified LDC UV monitor	7800	67.9
Conditions B			
25	LDC Duomonitor	2380	28.7
8	LDC UV monitor	9800	14.2
3	Modified LDC UV monitor	14100	11.8
1	Modified LDC UV monitor	15400	11.3
1	Modified LDC UV monitor and modified injection valve	17600	10.5

The chromatographic system

A block diagram of the chromatographic system is shown in Fig. 4. The pump employed was the Waters Assoc. 6000 M, modified in the following manner to provide the flow-rates of a few microliters per minute necessary for microbore columns. The frequency generator of the Waters Assoc. pump was disconnected and replaced by a Model 33118 Hewlett-Packard function generator. This provided frequencies from 1 Hz to a kHz that could actuate the stepping motor of the pump and provide a flow-rate range from about 1 μl/min to 1 ml/min. It was found that the Waters Assoc. pump functioned well down to a frequency of 2 Hz which corresponded to a flow-rate of about 2 μl/min, but below that, the pressure drop across the column could be so small that the non-return valves in the pump would not

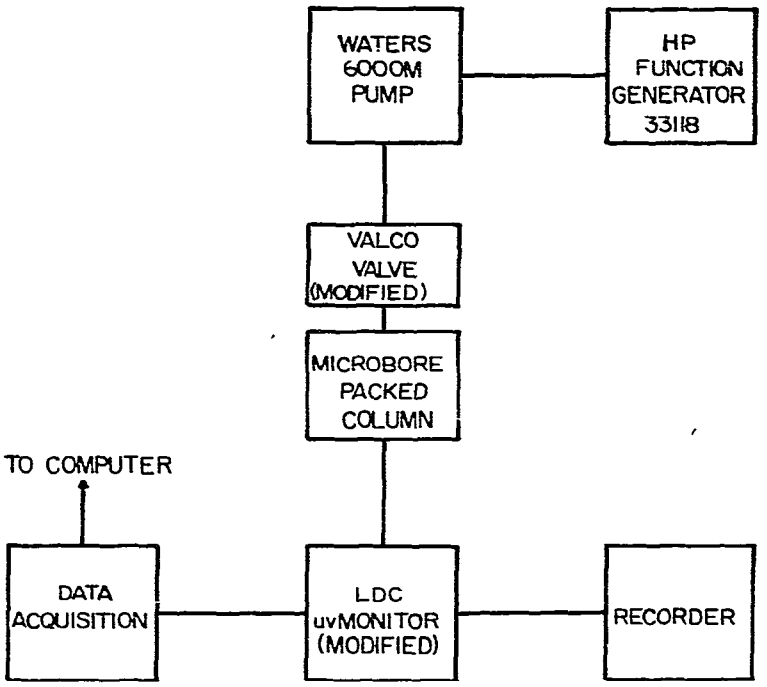

Fig. 4. Block diagram of liquid chromatograph for use with microbore columns.

actuate. When operating at low flow-rates, therefore, a resistance was placed between the pump and the column so that a back pressure of at least 250 p.s.i. was developed in order to actuate the non-return valves. The mobile phase from the pump passed directly to a Valco valve. The Valco valve was modified by the manufacturer to give a sample volume of 1/2 μl contained in the grooves of the valve's spigot itself. The valve was also designed to operate at a maximum of 10,000 p.s.i. The importance of eliminating any dead volume between the sample valve and the column in order to realize high column efficiency, has been discussed by a number of workers and a particularly detailed examination of this problem has been given by Kirkland *et al.*[8]. The connection of the microbore column to the Valco valve is shown in Fig. 5. It is seen that the column projects directly into the valve housing and between the end of the column and the valve's seat is situated a stainless-steel frit, the whole being retained by the sample valve union. The only volume existing between the column and the sample volume in the spigot of the valve itself is contained in the sintered frit and the small aperture in the valve wall between the valve spigot and the frit. The end of the microbore column is connected directly to the modified LDC UV monitor and the output fed to both a recorder and a data acquisition system and thence to a computer.

Column packing

Columns were constructed from 1-m lengths of 1/16 in. O.D., 1 mm I.D. stainless-steel tubing that had been previously washed with acetone and dried. The column end was connected to a 1/16 in. union fitted with a 1/16 in. stainless-steel 2-μm porosity frit.

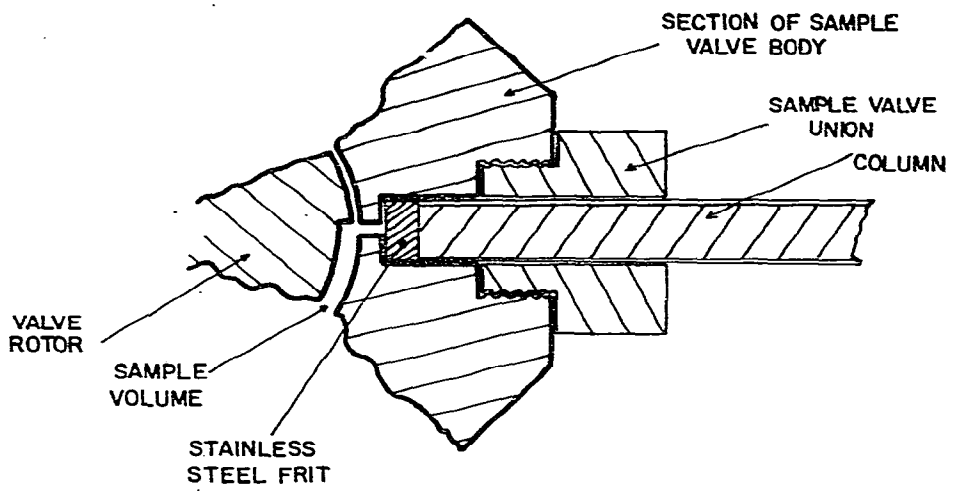

Fig. 5. Low dead volume connection between a Valco high-pressure valve and a microbore column.

The columns are packed at about 25,000 p.s.i. using an air driven fluid pump, manufactured by Haskell Engineering, Model ESX-HT-602-C. High-pressure stainless-steel tubing was employed in conjunction with "Sno-Trik" unions for connecting purposes. Columns were packed in 1 m lengths using about 1/2 g of packing material for each column. It was found that in order to pack silica gel effectively, a balanced density slurry method was necessary, but when using reversed-phase packings, a viscosity system was to be preferred. In general, the faster the column is packed, the better the column produces in terms of efficiency. It follows that a solvent suitable for balanced density packing must have a low viscosity as well as high density. In fact, a solvent mixture should be chosen that has the maximum density to viscosity ratio possible. For this reason, it was found that a methyl iodide–pentane mixture gave superior columns to the tetrachloroethylene–tetrabromoethylene mixtures. The packing was placed in a sonic bath for about 30 min and then poured into a small volume reservoir (10 ml), situated on top of the column. The pressure of the driving solvent, methanol, was raised to about 25,000 p.s.i. and then suddenly applied to the packing reservoir. The column packed in a few minutes and it was found the shorter the packing time, the higher the efficiency of the column produced. Reversed-phase columns were packed in a similar way and at the same pressures, but in their case the stationary phase was maintained in suspension by the use of a solution containing 25% of glycerol in methanol. Under these circumstances, the packing procedure took a little longer, possibly 8–10 min, but excellent columns were obtained. It is interesting to note that in order to obtain the highest efficiency, the polar packing required a non-polar solvent for packing purposes whereas the non-polar reversed-phase packing required a polar solvent for packing. After packing was complete, the silica gel columns were activated in the normal way by passing about 10–15 dead volumes of ethanol, acetone, ethyl acetate, 1,2-dichloroethane and heptane, respectively, through the column. In the case of the reversed-phase columns, the methanol was directly displaced with the appropriate methanol–water or acetonitrile–water solvent. Long columns were constructed from 1-m columns by connecting them together with 1/16-in. Swagelok unions. After packing, the terminal of the union was removed together

with the frit. The end of the column was then inserted into another 1/16 in. union that had been drilled through with a 1/16-in. drill and the union then tightened. The front of another column was then inserted in the other side of the union until the two columns were in close contact. While in close contact, the second nut of the union is firmly tightened. This procedure of joining columns together can be repeated, at will, until the required overall length is obtained.

The effect of column diameter on the efficiency obtained from microbore columns

To determine the effect of column diameter on efficiency, columns 0.02, 0.03, 0.04 and 0.05 in. I.D. and 1 m long were examined. Each column was packed with Partisil 20 silica gel using the method previously described. Subsequent to packing the columns were equilibrated with 5% isopropanol in heptane and the efficiency of the peak for the solute benzyl alcohol (eluted at k' of 2) was determined over a range of mobile phase linear velocities from about 0.02 mm per second to 2 cm/sec. Efficiencies were calculated using the band width at 0.6065 of the peak height and the following equation:

$$n = 4\left(\frac{y}{x}\right)^2$$

where y = the retention distance of peak and x = the peak width at 0.6065 of the peak height.

HETP curves were then constructed for each column relating HETP in mm to the linear velocity in cm/sec. The results are shown in Fig. 6. It is seen that the highest efficiencies were obtained from the column 0.04 in. in diameter. Furthermore, the slope of the HETP curve was smallest for this diameter column, indicating that the resistance to mass transfer terms were significantly smaller than those for the other columns. It is interesting to note that the smallest diameter column gives the poorest performance. It should be emphasized, however, that these curves do not necessarily mean that the best efficiencies under all circumstances will be obtained from 0.04 in. I.D. columns. The results, in fact, only indicate that, using the packing techniques described, the 0.04 in. column gave the best results.

The loading capacity of microbore columns of different diameters

The previously described columns of different diameter were used to determine the maximum sample mass that could be placed on each column before efficiency was significantly affected. This was carried out by injecting progressively larger quantities of benzyl alcohol onto each column and measuring the efficiency produced. From the efficiency, the HETP was calculated. The volume of the sample was kept constant at 0.5 μl and charges ranging from 0.1 to 100 μg of solute were injected onto each column. The results obtained are shown in Fig. 7 as curves relating the HETP against the mass of sample injected. All experiments were carried out using a linear solvent velocity of 2 mm/sec. The results showed that the largest charge that could be tolerated was by the largest diameter column, which was to be expected. The loading capacity of the column was taken as the largest charge that could be employed without significantly reducing the efficiency obtained from the column. It is seen that charges of 40 to 50 μg could be placed on the 0.05 in. I.D. column; 10 to 20 μg on the

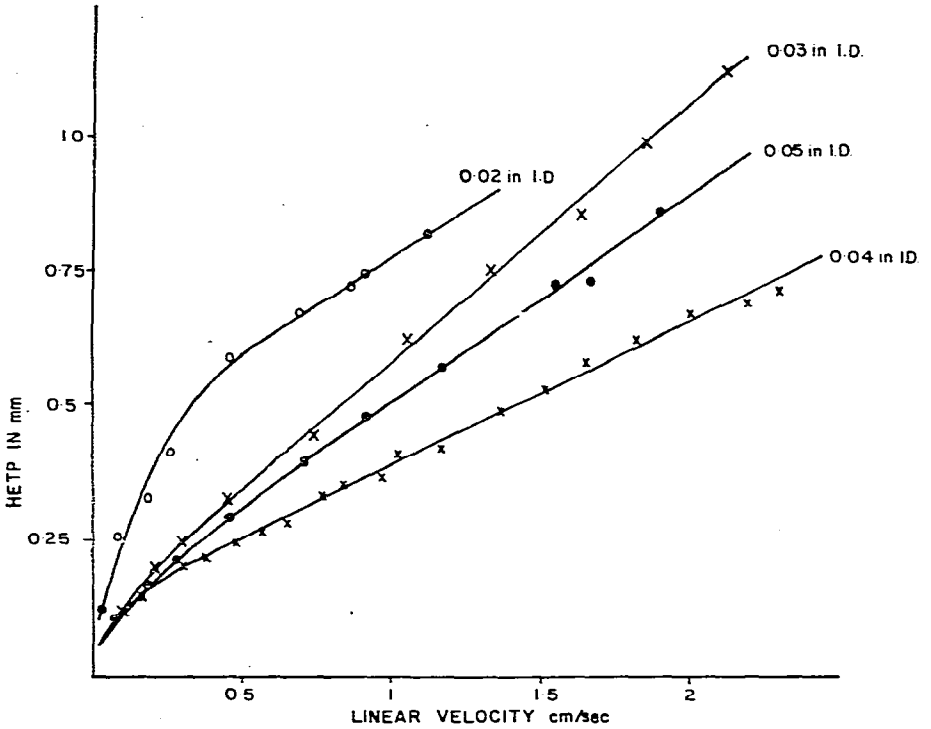

Fig. 6. Graphs of HETP against mobile phase velocity for columns of different diameter.

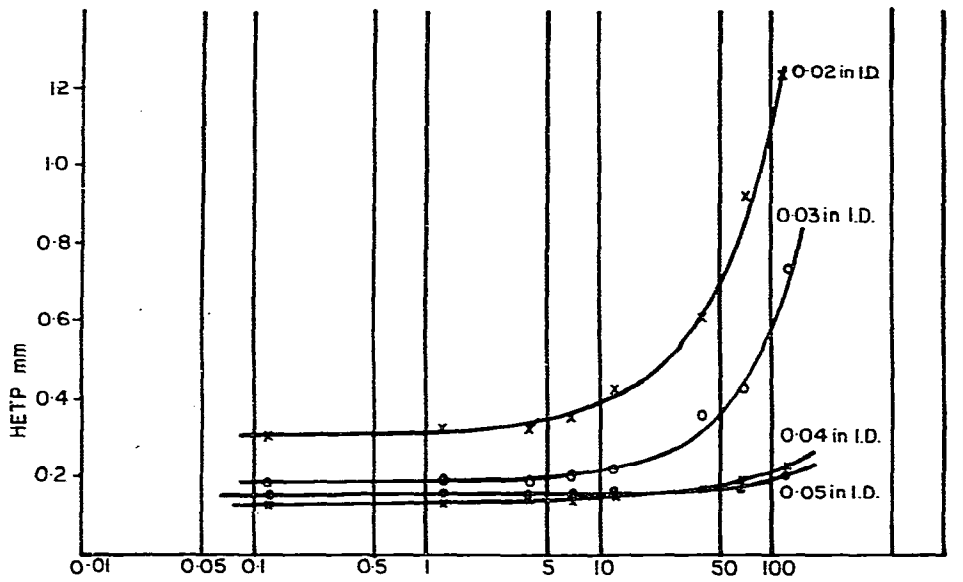

Fig. 7. Graphs of HETP against sample mass (μg, logarithmic scale) for columns of different diameter. Packing, Partisil 20; solvent, isopropanol–*n*-heptane (5:95); solvent velocity, 2 mm/sec; solute, benzyl alcohol; $k' = 2.0$.

0.04 in. I.D. column; 5 to 7 μg on the 0.03 in. I.D. column, whereas only 1 or 2 μg could be placed on the 0.02 in. I.D. column. Columns 0.04 in. I.D., which, in fact, are close to 1 mm in I.D., were used for subsequent investigations of microbore columns. Charges were employed between 5 and 10 μg of each solute present, thus insuring that the column, at no time, was overloaded.

The effect of coiling microbore columns on efficiency

Owing to the compact nature of the column system, it was of interest to know how small the microbore columns could be coiled before impairing their efficiency. A 1-m column, 1 mm I.D. packed with 20-μm Partisil silica gel and giving efficiencies of about 23,000 plates was sequentially formed into coils of different diameter. After forming each coil, the efficiency of the column for benzyl alcohol, eluted at $k' = 2$, and at a mobile phase linear velocity of 0.73 mm/sec was measured. The mobile phase used was a 5% solution of isopropanol in heptane. The results obtained are shown in Table IV. It is seen that there is a slight loss of efficiency in reducing the coil diameter from 23 to about 12 cm. Subsequent to that however, reduction in coil diameter caused a very significant reduction in the efficiency obtainable. The column coiled to a diameter of 23 cm which originally gave 23,000 theoretical plates, gave only 1100 theoretical plates when coiled in a spiral of 1 cm in diameter. It was interesting to determine whether the coiling procedure caused the reduction of efficiency or whether it was the result of the actual dimensions of the column coils themselves. For this reason, a similar column was formed into a coil 6 cm in diameter and packed in the coil form, using the procedure previously outlined. Again, Partisil 20 silica gel was used as the packing material. It is seen from Table IV that only 10,000 plates were obtained which would be about the value that would be obtained if it had been packed in a straight coil and subsequently coiled to the same diameter after packing. From this, it could be concluded that if a microbore column is formed in a coil the coil should be not less than 12 cm in diameter whether packed before or after coiling.

TABLE IV

THE EFFECT OF COILING MICROBORE COLUMNS ON EFFICIENCY

Coil diameter (cm)	*Efficiency (theoretical plates)*	*No. of coils*	*Linear velocity of mobile phase (cm/sec)*
23	23030	1.4	0.073
18	22180	1.8	0.072
12	21010	2.7	0.064
7	13390	4.55	0.069
3	5340	10.6	0.068
1	1160	31.8	0.084
6*	10080	5.3	0.08

* Packed in coiled form.

The effect of particle diameter on the efficiencies obtained from microbore columns

Three 1 m $\times$ 1 mm I.D. columns were packed with 5-, 10- and 20-μm Partisil respectively. The packing procedure, as outlined previously, was employed in conjunction with a balanced slurry solvent mixture of methyl iodide and pentane. After

packing, the columns were equilibriated to 5% isopropanol in heptane and then fitted to the Valco valve and the reduced volume detector as described previously. The solutes used were benzene and benzyl alcohol which were eluted at $k' = 0$ and 1.8, respectively. HETP curves were obtained for the two solutes over a range of linear velocities from 0.004 to 1 cm/sec. Efficiencies were calculated as stated previously and duplicate chromatograms were obtained at each velocity and the average taken for calculating the HETP. The HETP curves obtained are shown in Fig. 8. It is seen, as would be expected from theory, that the 5-μm packing gives the highest efficiencies and the 20-μm, the lowest efficiencies. It is also seen that the minimum HETP and therefore, the maximum efficiency occurs at the optimum velocity of about 0.04 cm/sec. It is also seen that the shapes of the curves are similar to those predicted by the Huber and Quaadgrass equation[11] and that the resistance to mass transfer term is increasing significantly with the k' value of the solute. However, the relative advantages of the three types of packings are concealed by the normal HETP equation and therefore the reduced plate height equation as suggested by Giddings[12] and employed ex-

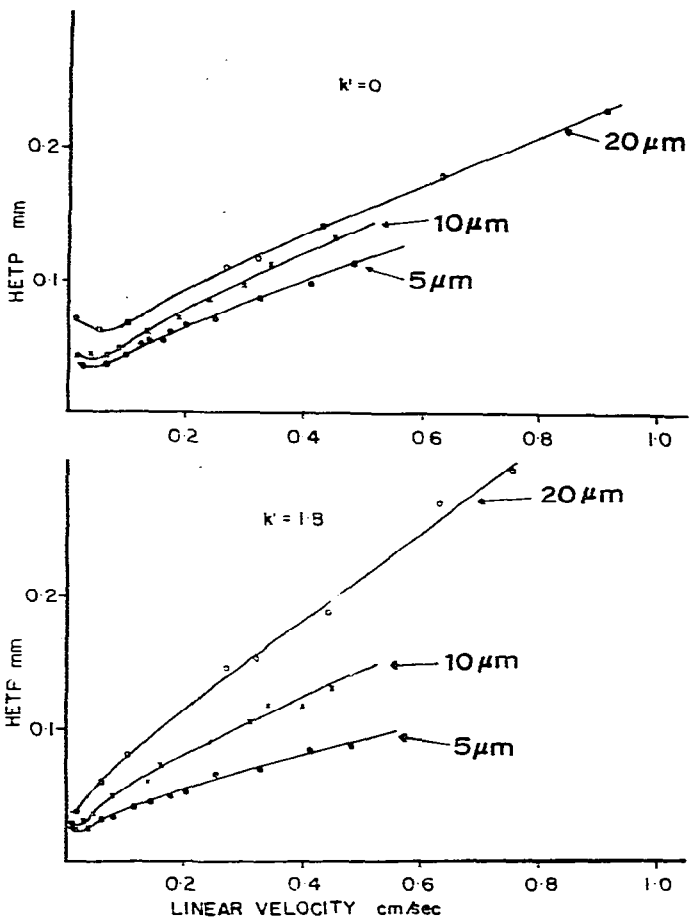

Fig. 8. HETP curves for microbore columns (1 m × 1 mm I.D.) packed with silica having different particle diameters.

tensively by Kennedy and Knox[13], was employed. Such curves give a much better indication of the column performance with respect to the relative particle diameters. Reduced plate height curves for the three different packings are shown in Fig. 9. The theoretical limit for the reduced plate height for any packing is two particle diameters and it is seen this is achieved only by the 20-μm packing and then, only for the solute eluted at $k' = 1.8$. It follows that almost ideal packing is obtained from the 20-μm material. The plate height, however, obtained for the solute eluted at k' of 0 is greater than that of solute eluted at $k' = 1.8$ and this means that although stringent precautions were taken to reduce the extra column effects in the microbore system, there was some significant band dispersion occurring outside the column. It is seen that the 10-μm material achieved a minimum plate height of about three particle diameters for the solute eluted at $k' = 1.8$. Thus only 2/3 of the theoretical maximum efficiency could be achieved by the 10-μm material. Finally, for the 5-μm material the minimum reduced plate height achieved was greater than four particle diameters, indicating that for this material less than 50% of the theoretical maximum efficiency was obtained. It follows that although higher efficiencies were obtained from the 5-μm material, this type of material should not be used for producing high efficiency

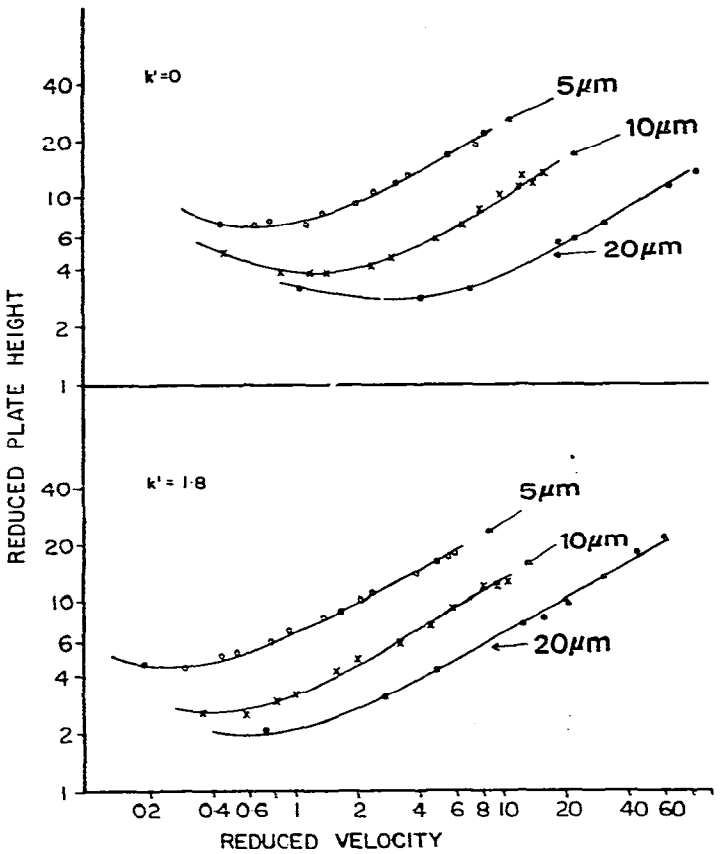

Fig. 9. Reduced plate height curves for microbore columns (1 m × 1 mm I.D.) packed with silica having different particle diameters.

columns. The 20-μm material is far more attractive because the maximum efficiency can be obtained from it and at the same time, at significantly lower pressures. From the results, it was uncertain whether the poor efficiency obtained from the 5-μm material was due to the nature of the material itself or to the fact that 5-μm particles could not be packed into a microbore column as effectively.

Applications of microbore columns

The great advantage of microbore columns, from a purely chromatographic point of view, is the ease by which it is possible to alternate between very high efficiencies and very high speeds while maintaining reasonable pressure drops across the column. An example of this is in the use of a 20-μm particle diameter packing in a 1 m $\times$ 1 mm I.D. column to provide rapid analysis by operating the column at 6000 p.s.i. and at very high efficiencies by operating at a pressure of 60 p.s.i. An example of the versatile use of a 1-m column packed with 20-μm silica gel in this way is shown in Fig. 10. The mobile phase used was 5% isopropanol in heptane and the solutes were a number of aromatic alcohols. Operating at 6000 p.s.i., the 1-m column provided 2000 to 3000 theoretical plates and separated the aromatic alcohols in about 2.4 min. Employing the same column at an inlet pressure of only 600 p.s.i., efficiencies of 6000 to 7000 theoretical plates were available and the elution time has now increased to 23 min. The separation is obviously very much improved and the small impurity at the tail end of the first peak is beginning to show. Finally, one can trade in time still further by operating at a pressure of 60 p.s.i. and having an efficiency of 10,000 to 12,000 theoretical plates. Under these conditions, the elution time is 260 min and

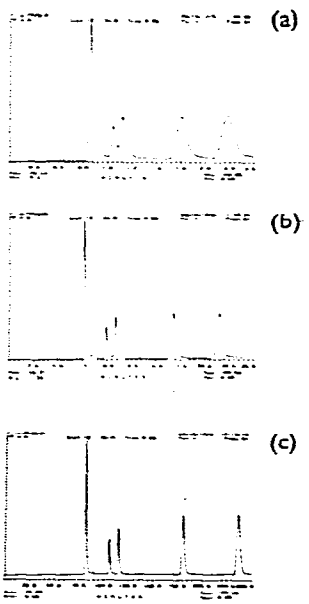

Fig. 10. Chromatograms demonstrating the versatility of microbore columns (1 m $\times$ 1 mm I.D.). Packing, Partisil 20; solvent, isopropanol–*n*-heptane (5:95); solutes, aromatic alcohols. Efficiency: (a) 2000–3000 plates; (b) 6000–7000 plates; (c) 10,000–12,000 plates. Pressure: (a) *ca.* 6000 p.s.i.; (b) *ca.* 600 p.s.i.; (c) *ca.* 60 p.s.i.

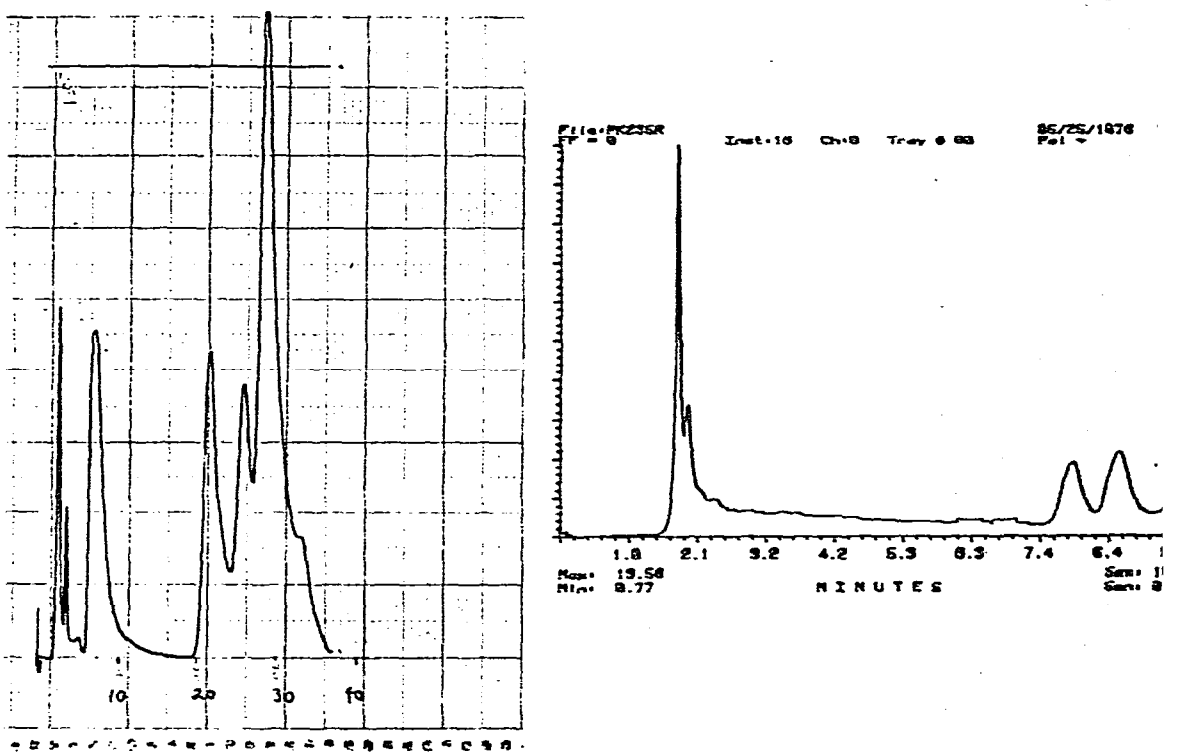

Fig. 11. Chromatogram of the separation of organic acids from a fermentation broth. Operating conditions: (a) column, 25 cm × 4.6 mm I.D.; (b) Column, 50 cm × 1 mm I.D.; packing, RP-18 (10 μm); flow-rate, 50 ml/min; solvent, methanol–water (15:85); pressure, 6200 p.s.i.

the impurity after the second peak is clearly separated. If a column packed with 10 μm silica gel was employed, the same advantages could be realized and much higher efficiencies would be obtained, but the inlet pressure would have to be increased to produce high speed separations. A practical example of the use of microbore columns to reduce analysis time is shown in Fig. 11. On the left-hand side of this figure is the separation of three organic acids obtained from a fermentation broth. The three peaks are the ones eluted between 20 and 40 min and it is seen that there is an impurity on the tail of the last peak. This is the best separation that could be obtained from a commercially available 25-cm long reversed-phase column. On the right-hand side, the same separation is carried out using a microbore reversed-phase column and it is seen that the organic acids are completely separated in less than 11 min. The impurity is eluted a little later but occurs in the dead volume of the next analysis. The use of the microbore column has permitted the number of analyses per day to be increased by nearly a factor of 4, providing a far more effective and frequent monitoring procedure for the fermentation process.

Microbore columns packed with reversed-phase materials offer the same advantages as the silica gel columns. Very high efficiencies are readily obtainable and an example of the type of chromatograms obtained from a Partisil ODS-2 reversed-phase microbore column is shown in Fig. 12. Efficiencies of nearly 30,000 plates are being realized from the 1-m column, but it should be noted the chromatogram extends

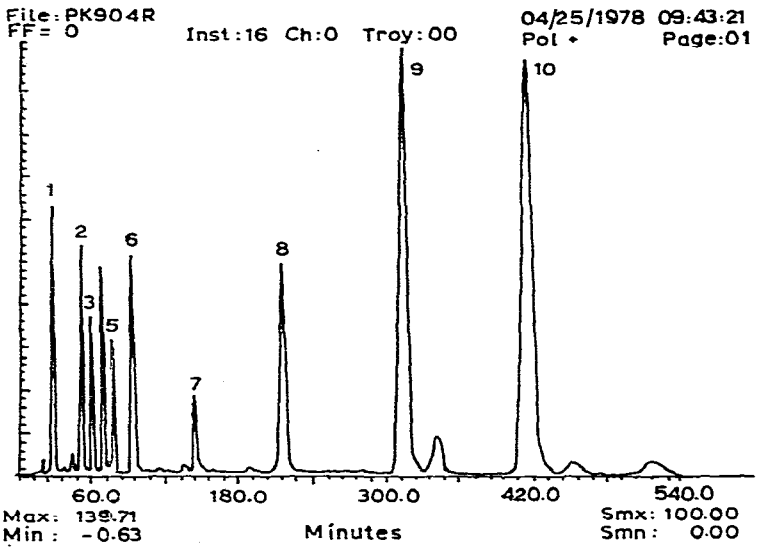

Fig. 12. Chromatogram from a reversed-phase microbore column. Column, 1 m × 1 mm I.D.; packing, Partisil ODS-2; solvent, acetonitrile–water (65:35); flow-rate, 4 μl/min; sample volume, 0.5 μl; maximum k' value, 24. 1 = Sodium nitroprusside; 2 = phenol; 3 = *m*-cresol; 4 = 3,4-xylenol 5 = *p*-ethylphenol; 6 = 2-isopropylphenol; 7 = benzene; 8 = anthraquinone; 9 = 2-methylanthraquinone; 10 = 2-ethylanthraquinone.

for nearly 10 h. Unfortunately it is not possible to have speed and resolution at the same time and if the sample is sufficiently complex, it is necessary to wait a period of time in order to realize the separation required. A practical example of the kind of separation this column can be used for is the separation of an extract of polynuclear aromatic hydrocarbons from coal (Fig. 13). In this case, the chromatogram has been developed for a period in excess of 20 h. However, there are over 120 discernible peaks in the chromatogram. It also should be pointed out that the k' of the last eluted peak is about 60 and the peaks obtained are still sharp, well separated and easily detected. One of the advantages of high efficiency is the increased scope of the system and the high peak capacity of the chromatograms. As the peaks remain narrow, the chromatogram can be developed to much higher k' values and at the same time, ensure trace components are readily detectable[14]. The chromatogram shown in Fig. 13 strongly resembles the chromatograms obtained from capillary columns in gas chromatography (GC). Thus, liquid chromatography, using microbore columns, can now take on the same type of separation problem involving multi-component mixtures as GC can using capillary columns.

The use of microbore columns to produce ultra high efficiencies

One of the exciting possibilities of microbore columns lies in their capability of providing efficiencies that are linearly related to their length. Microbore columns (1 m long) can be joined together providing efficiencies that are the sum of the individual efficiencies of each column. Wide-bore columns cannot be joined together to provide efficiencies that are linearly related to length. There have been two reasons proposed that explain this non-linearity between efficiency and column length for wide-

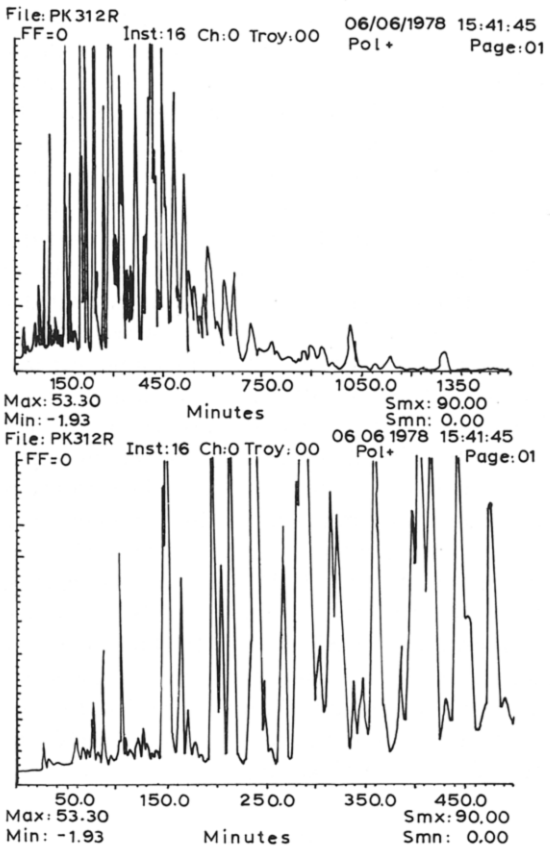

Fig. 13. Chromatogram of a sample of polynuclear aromatics extracted from coal. Column, 1 m × 1 mm I.D.; packing, Partisil ODS-2 (10 μm; solvent, acetonitrile–water (65:35); flow-rate, 5 μl/min. The lower chromatogram is an amplified section of the upper, over the period 0 to 450 min.

bore columns. The first explanation involves the heat generated by the high-pressure drop that develops across long columns and the flow of liquid through them. As liquids have significant viscosity, a considerable amount of work is done in forcing the liquid through the long column and this work is converted into heat and the temperature of the mobile phase and the stationary phase rises. It is considered that this rise in temperature changes the transfer properties of the solute between the two phases producing peak dispersion and, further, possibly results in a non-linear adsorption isotherm between solute and the stationary phase producing asymmetric peaks. The second explanation involves the non-homogeneity of the permeability of the column across its radius. Columns of wide bore can be packed in such a manner that the permeability of parts of the column cross-sectional area is in some parts greater than in others. This results in channelling in the stationary phase and the mobile phase flows more rapidly through one part of the column than in others. This phenomenon is certainly known in preparative chromatography where columns of an inch or greater in diameter are employed. A very badly packed preparative column can provide two peaks for a single substance injected into the column. The first peak

results from a solute band moving down the walls of the column and the second peak results from the solute moving down the center of the column. As the permeability is different, the retention time can be different and two distinct peaks can be perceived. In fact, the non-homogeneity of the column permeability causes an exaggerated multi-path term and as this will become greater as the column length increases, particularly if the column length is made up of a series of individual columns, then the expected efficiency will not be realized. Whichever the explanation, the microbore column obviates both these problems. Considering the thermal effect, the heat generated is more easily conducted away from the column due to its small dimensions. At the flow-rates involved, which are just a few microliters per min, the heat generated is relatively small even though the pressure drop may be quite high. In the second case, as the packing has a much lower cross-sectional area, the likelihood of a homogeneous permeability is much greater and there will be much less dispersion resulting from the multi-path effect. The first high-efficiency column prepared consisted of ten 1-m columns packed with Partisil 20 and joined together to form a column 10 m in length, coiled in a circle 30 cm in diameter Each column was tested after packing to insure that it was adequately packed before being incorporated in the long column This column operated at about 5 μl/min was shown to have an efficiency of 250,000 plates, indicating that the column had been packed to the HETP limit of two particle diameters. The pressure drop across this column when operating at 38 μl/min was only 900 p.s.i. and under these conditions produced an efficiency of 160,000 theoretical plates. A chromatogram of bergamot oil, separated on the column, using a 3% solution of ethyl acetate in heptane as the mobile phase is shown in Fig. 14. It is seen that the chromatogram resembles that obtained from a capillary column in GC and the individual components of the bergamot oil are well separated.

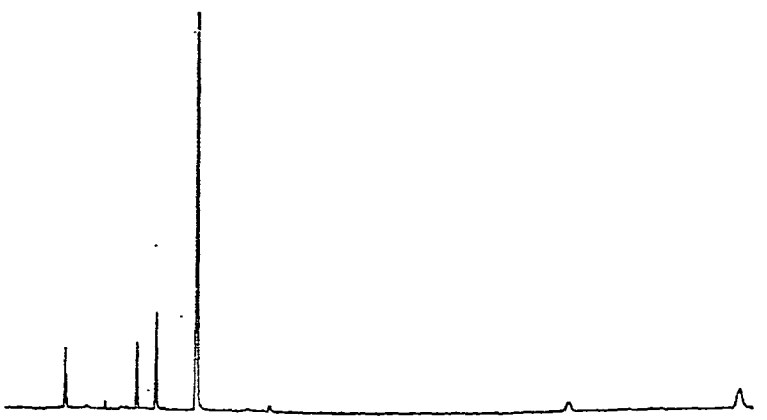

Fig. 14. Chromatogram of bergamot oil (n = 160,000). Column, 10 m × 1 mm I.D.; packing, Partisil 20; mobile phase, ethyl acetate–n-heptane (3:97); sample volume, 0.5 μl; flow-rate, 38 μl/min.

The second column was constructed 14 m long in an attempt to obtain an efficiency in excess of one million theoretical plates. The packing selected was 5-μm Spherasorb and this was used, as opposed to an irregular packing in an attempt to reduce the pressure drop across the column. The columns were packed by the methods previously described, the columns checked and joined together in the same manner

as the 10-m column. The column was first used to separate the C_2 to C_8 alkylbenzenes in tetrahydrofuran on the basis of exclusion. The chromatogram obtained is shown in Fig. 15 and the last eluted peak at the dead volume of the column, which was benzene, had an efficiency of 650,000 theoretical plates. This was far short of the efficiency expected and indicated that the Spherasorb did not pack to provide the minimum HETP of two particle diameters. In fact, slightly less than half the expected efficiency

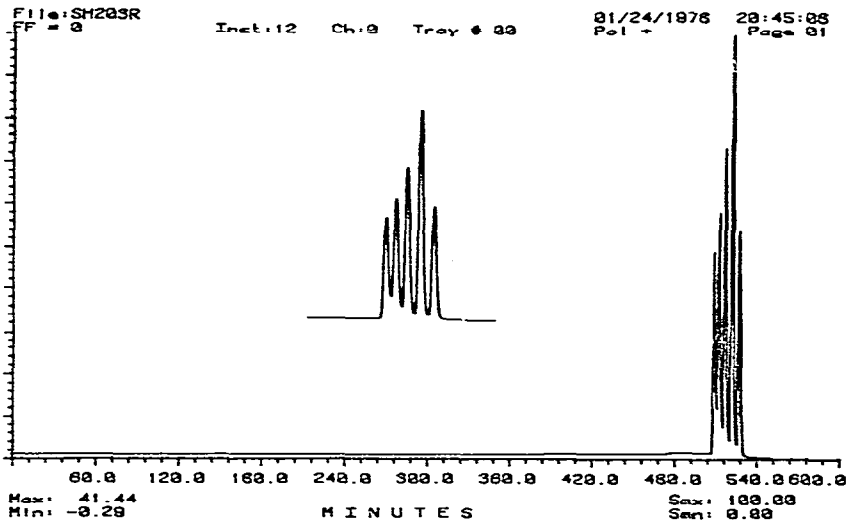

Fig. 15. Exclusion chromatogram from a column having an efficiency of 650,000 theoretical plates. Column, 14 m × 1 mm I.D.; packing, Spherasorb (5 μm); flow-rate, 25 μl/min; sample, 0.5 μl of a 10% (v/v) solution of C_2–C_8 alkylbenzenes in tetrahydrofuran.

was obtained. The chromatogram, however, does show how well high-efficiency columns can separate closely similar substances and it is seen that the benzenes with only two carbon number difference are separated almost to the baseline, which is achieved purely on the basis of molecular size. A chromatogram from this column used in the normal elution mode is shown in Fig. 16 where a flow-rate of 35 μl/min was employed, providing an efficiency of 510,000 theoretical plates. The sample separated was the essential oil cinnamon and it is seen that excellent separations are obtained from this column, although the total elution time is over 20 h. The chromatograms, indeed, are very similar to the chromatograms obtained from high-efficiency capillary columns in GC. However, the analysis times are 10 to 20 times longer and this unfortunately reflects the higher resistance to mass transfer in liquid chromatography as opposed to GC columns, which results from the use of a liquid as a mobile phase as opposed to a gas. Using very small particle diameters in liquid chromatography helps to offset the high resistance to mass transfer in the liquid mobile phase, but so far, it has not achieved performance comparable to GC. However, high efficiencies in liquid chromatography in terms of many hundreds of thousands of theoretical plates are now possible, but at present we can only achieve high efficiencies by accepting long analysis times.

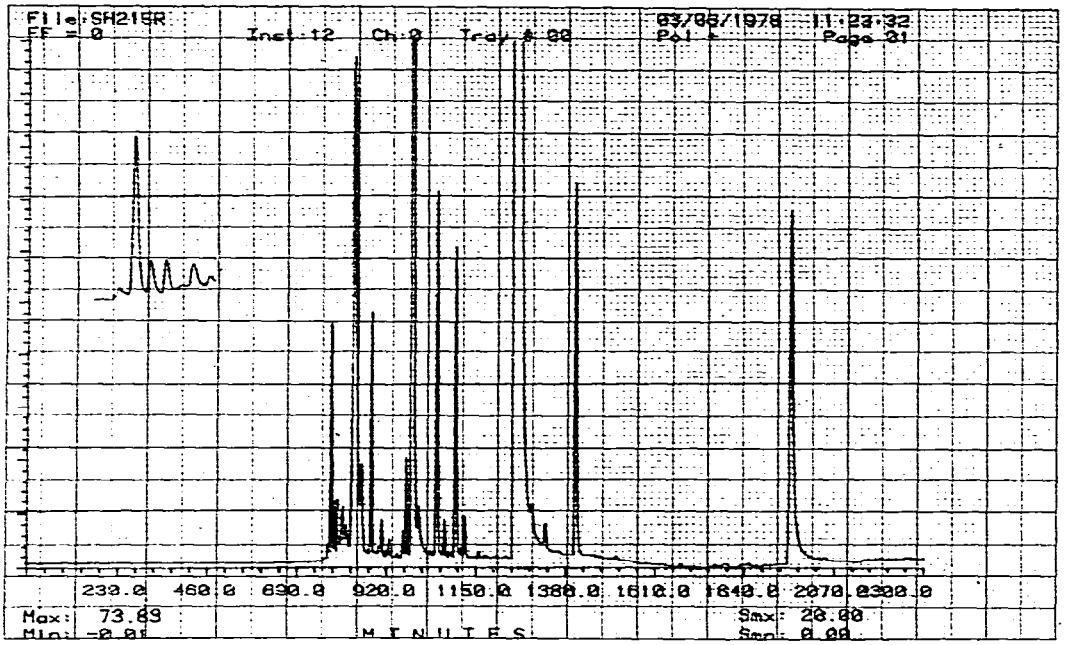

Fig. 16. Chromatogram of an essential oil. Column, 14 m × 1 mm I.D.; solvent, ethyl acetate–*n*-heptane (5:95); efficiency, 510,000 theoretical plates.

CONCLUSIONS

The results shown in this paper indicate that the microbore column is an attractive alternative to the normal 1/4 in. O.D. or 1/8 in. O.D. column. Microbore columns, if packed with a relatively large particle diameter adsorbent, *e.g.* 20-μm silica gel, can attain the theoretical limiting efficiency and the column has a relatively low pressure drop across it. As a result, the column can be used to provide rapid separations at a relatively low efficiency or difficult separations over an extended period of time. Microbore columns require specially modified detectors and injection systems, together with pumps that provide flow-rates down to 1 or 2 μl/min. At present, such devices have to be made in the laboratory, but already two instrument companies are in the process of designing detectors that have appropriate low dead volumes for use with microbore columns. The sample valves of low dead volume are already available from Valco and a number of currently available pumps could be modified to provide low flow-rates. It follows that equipment suitable for use with microbore columns will be available in the near future. Microbore columns have the distinct advantage of using small quantities of packing material and are very economical to operate. The relative cost of solvents used in liquid chromatographic equipment is shown in Table V. It is seen that most normal 4.6 mm I.D. (1/4 in. O.D.) columns use about 480 ml of solvent in an 8-h day and 121 l a year. Taking the present cost of the solvent heptane at $28.00 per gallon, the cost of operating a chromatograph for a 250-day year is $769.00. It follows that a laboratory operating 13 liquid chromatographs is going to spend at least $10,000.00 a year on solvent. In contrast, a 1 mm I.D. (1/16 in. O.D.) column will use about 24 ml in an 8-h day,

TABLE V

SOLVENT CONSUMPTION FOR COLUMNS OF DIFFERENT DIAMETERS

Column diameters (I.D.) *	*Flow-rate for a linear velocity of 0.14 cm/sec (ml/min)*	*Volume used in an 8-h day (ml)*	*Volume used in a 250-day year (l)*	*Cost/year/ chromato-graph (distilled in glass heptane at $28/3.79 l) ($)*
0.51 mm (0.020 in.) 1/16 in. O.D.	0.012	6.9	1.5	15
0.76 mm (0.030 in.) 1/16 in. O.D.	0.027	13	3.3	21
1.02 mm (0.040 in.) 1/16 in. O.D.	0.044	24	6.1	39
1.29 mm (0 050 in.) 1/16 in. O.D.	0.079	38	9.5	60
1.59 mm (0.063 in.) 1/8 in. O.D.	0.12	57	14	89
4.6 mm (0.181 in.) 1/4 in. O.D.	1	480	121	769

* A linear velocity of 0.14 cm/sec corresponds to a flow-rate of 1 ml/min through a 25-in.-long column having a dead volume of 3 ml.

operating at the same mobile phase linear velocity which will be equivalent to only 6.1 l a year and will cost only $39.00. It follows that the solvent cost for 13 liquid chromatographs employing 1 mm I.D. microbore columns would cost only $500.00 a year.

Microbore columns also lend themselves to obtaining extremely high efficiencies. Because of the linear relationship between column length and efficiency, columns of several hundred thousand theoretical plates can be constructed and operated at relatively low inlet pressures, for example, less than 1000 p.s.i. From the work that is described in this paper, liquid chromatographic columns of a million theoretical plates are certain, columns of 10 million theoretical plates highly likely and, at this time, it remains to be seen whether columns of 100 million plates are feasible or not. Although very high efficiencies are attainable from microbore columns, the basic limitations of the liquid chromatographic system still hold. Solutes diffuse slowly in liquids and as liquids are an essential part of the liquid chromatography system, the resistance to mass transfer factors will always be relatively high and therefore, very high efficiencies can only be attained at the expense of long analysis times. It is, however, a significant step forward in the technique to be able to attain such high efficiencies and where extremely complex and difficult mixtures require to be resolved, separations can now be achieved, even though at the expense of considerable time.

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