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# DESIGN AND USE OF SHORT MICROBORE COLUMNS IN LIQUID CHROMATOGRAPHY

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## SUMMARY

The various aspects of design and performance of short microbore columns for use in liquid chromatography are discussed. The basic hydrodynamic factors important in column design are considered. Consideration is given to instrumental time constant and solute band broadening due to the injection system, the column and the detector. The design and construction of a chromatographic system using commercially available components permitting the use of short microbore columns is described and experimental details for operation in isocratic and gradient elution modes are given. It is shown that a column 25 cm in length with 1 mm internal diameter can be packed with a 10-$\mu$m particle diameter silica gel to the limiting plate height of 20 $\mu$m to achieve 50,000 plates per meter. Examples of the use of the system for separations of compounds of pharmaceutical interest are given and advantages of the system are discussed.

## INTRODUCTION

Reducing the size of a liquid chromatographic column offers a number of practical and theoretical advantages. The use of microbore columns by various workers[1–4] has demonstrated that very high linear mobile phase velocities can be obtained using relatively low flow-rates and low solvent consumption. Very high efficiency separations (up to 750,000 theoretical plates) which could not be achieved previously were obtained[2]. However, in order to obtain high efficiencies, it was necessary to couple 1-m columns together and to use long column lengths, which led to excessive analysis times. Due to the difficulty in packing small bore columns, it was not possible to prepare columns of 10-$\mu$m silica gel particles which would give a limiting plate height of 20 $\mu$m (ref. 1).

Most chromatographic separations today can be handled with about 10,000 theoretical plates, and thus the development of short and efficient microbore columns would significantly improve the speed and economy of analysis. The use of long microbore columns in liquid chromatography can be substantiated only in the

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case of very complex mixtures where high efficiency and high peak capacity of the system is desired and long analysis times can be tolerated.

This paper deals with theoretical and experimental aspects of the design and performance of short microbore columns. The associated chromatographic system for use in isocratic and gradient elution, or flow programming modes is described.

## FACTORS AFFECTING THE DESIGN OF A MICROBORE LIQUID CHROMATOGRAPH

What are the important parameters to consider when changing the geometry of a liquid chromatographic column?

The column length ($L$), column diameter ($D$), particle diameter ($d$), pressure drop ($\Delta P$), linear velocity ($U$) and column porosity ($\varepsilon$) are the basic hydrodynamic parameters important in column design. It will be seen that some of these parameters are interrelated with parameters, such as standard deviation of the elution curve ($\sigma$), as well as parameters dependent on $\sigma$, such as column efficiency ($N$), plate height (HETP), limiting sample mass ($M_s$), limiting sample volume ($V_s$), peak volume ($V_p$), maximum solute concentration ($X_m$) and detector time constant ($T_D$). Column temperature is also an important operating parameter. When designing a liquid chromatographic system for best performance, all of these parameters have to be considered. In general, a limiting plate height of two particle diameters is expected from a well-designed system. This requires a careful evaluation of the detector and injector compatibility with the overall system and elimination of all unnecessary dead volume.

Decreasing the column diameter at constant column length represents a change in the total column volume. This volume can be expressed as the sum of the total column interstitial volume resulting from the space between the particles, the internal volume originating from the pores of the particles, and the total volume of the solid packing.

Dividing these volumes by the total column volume, we obtain dimensionless quantities called porosities; the interstitial porosity, the internal porosity, and the volume fraction of the solid packing. The sum of the interstitial and the internal porosity is the total column porosity $\varepsilon$.

If a mass balance is applied to the system, the volumetric flow-rate ($Q$) in ml/sec will be a function of the linear velocity of the mobile phase ($U$) in cm/sec, the column diameter and the total column porosity.

$$Q = \frac{U \cdot \pi D^2 \cdot \varepsilon}{4} \tag{1}$$

Any analytical chromatographic system can be described in terms of three basic attributes —namely, resolution, speed and scope— the resolution being the most important for the analyst. The resolution depends on column efficiency, which is a function of the linear velocity of the mobile phase. In order to operate two columns of the same length and different column diameters under the same chromatographic conditions, it is necessary to have the same linear velocity. The volumetric flow-rate has to be scaled down in proportion to the square of the column diameter, assuming that the porosities of the respective columns are the same.

The retention time of a solute eluted at the end of a column can be expressed as the retention time of an unretained solute ($t_0$) times ($1 + k'$), where $k'$ is the solute capacity ratio and $t_R$ is a function of column length, linear velocity, solute distribution coefficient $K$ and the phase ratio $V_s/V_0$. We assume here that the particle diameter does not change and the phase ratio remains constant.

$$t_R = t_0(1 + k') = \frac{L}{U}\left(1 + K \cdot \frac{V_s}{V_0}\right) = N \cdot \frac{H}{U}(1 + k') \tag{2}$$

Thus, two columns of the same length and different column diameter operated at the same linear velocity will yield the same solute retention time. Also, it is interesting to note that the speed of the analysis is inversely proportional to the ratio $H/U$ at given $N$. However, the absolute retention volume of the solute, $V_R$, is dependent on the volumetric flow-rate and is a function of the square of the column diameter. Therefore, decreasing the column diameter results in considerable savings of the mobile phase.

$$V_R = Qt_R = \frac{U \cdot \pi D^2 \cdot \varepsilon \cdot t_R}{4} \tag{3}$$

The relative consumption of solvent per unit mass of separated solute will remain constant; it is shown later that the loading capacity of a column also decreases with the square of the column diameter.

The column pressure drop, $\Delta P$, or the difference between the inlet and outlet column pressure, is proportional to the linear velocity of the mobile phase and the mobile phase viscosity ($\eta$), according to the Darcy equation

$$\Delta P = \frac{U \cdot \eta \cdot L}{K_0} \tag{4}$$

where $K_0$ is a constant. Thus, operating two different columns at the same $U$ will give the same column pressure drop, keeping all other parameters constant. It was suggested by Snyder[5] that, at faster linear velocities where most analyses are carried out,

$$\text{HETP} = C \cdot U^{0.4} \cdot d^{1.8} \tag{5}$$

where C is a constant for a given solute.

The combination of eqns. 3, 4 and 5 gives the number of plates per unit time, a parameter which should be as high as possible for fast, efficient analytical separations.

$$\frac{N}{t_R} = \frac{K_0 \cdot \Delta P}{\eta \cdot L \cdot (1 + K') \cdot C \cdot U^{0.4} \cdot d^{1.8}} \tag{6}$$

From eqn. 6, it is evident that this can be achieved by using short columns packed with small particles and operated at high pressures with low viscosity mobile phase systems, and under linear velocities, which will ensure a desired number of theoretical plates and resolution. Thus, the use of short microbore columns packed with small particles is an attractive and economical way to maximize the number of plates per unit time.

## VOLUME OF SOLUTE PEAK

Assuming only Gaussian elution peaks, the volume of solute eluted at the end of the column, corresponding to four standard deviations of the elution curve, can be calculated using plate theory[6]. If the column dead volume is expressed as the product of the column cross-sectional area, the column length and the column total porosity

$$V_p = \frac{\pi D^2 \cdot L \cdot \varepsilon \cdot (1 + k')}{\sqrt{N}} = 4\sigma \qquad (7)$$

It follows that peak volume ($V_p$) decreases with the square of column diameter ($D$) and directly with column length ($L$). In order to design a system which can be used with short microbore columns to take advantage of the small band widths, one has to use a detector with low cell volume which is compatible with the small peak volume. Detector cell volume has been discussed by Kirkland *et al.*[7], who showed that, if the detector cell volume is smaller than 1/10 of the peak volume, the extra column band broadening due to the detector cell is insignificant. For example, in order to achieve about 10,000 theoretical plates with a 50 cm × 1 mm I.D. column ($k' = 0$), the volume of the detector cell has to be about 1.1 $\mu$l and the peak volume obtained will be about 11 $\mu$l, as shown in Fig. 1. Under these conditions, all extra column dead volumes became very important and have to be reduced to a minimum. It is necessary to eliminate any connecting tubing between the column and the detector and the column and the injector. Experimentally, this can be done by connecting the microbore column directly to the detector cell, as shown in Fig. 2, and

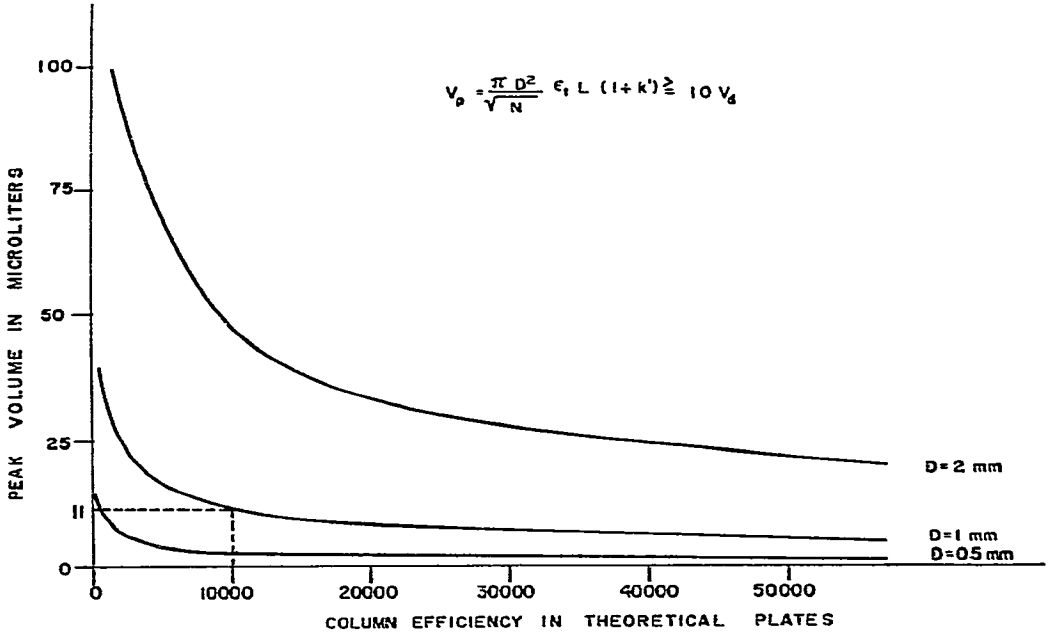


Fig. 1. Graphs relating peak volume to efficiency for columns of different diameters ($k' = 0$, $L =$ 50 cm).

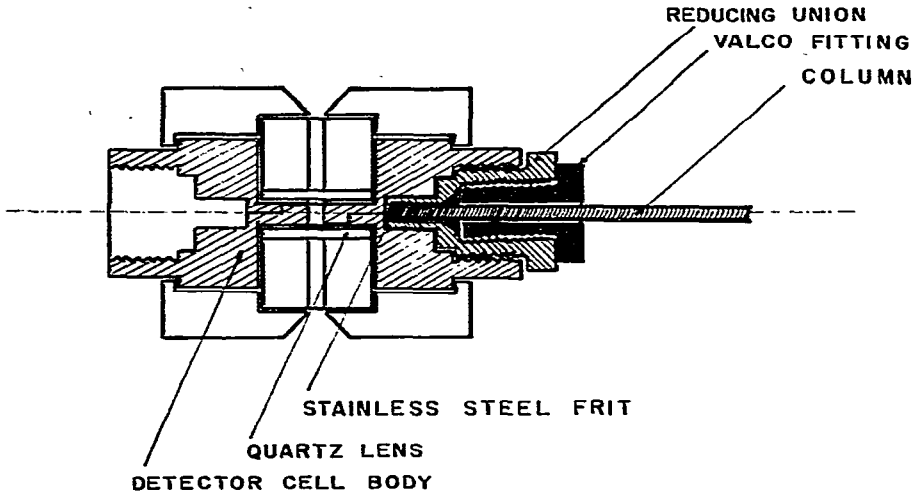


Fig. 2. Connection between a microbore column and a Schoeffel detector cell.

bringing the column directly to the injection sample valve (Fig. 3). Most components necessary for this design are commercially available now. Only microbore columns are not currently available but it is hoped that, in the future, they will be on the market. Assuming a maximum of 5% increase in solute band width due to the detector cell volume, the dimensions of the cylindrical detector cell ($L$, $D$) can be calculated from column efficiency, flow-rate, solute diffusivity and retention volume[8].

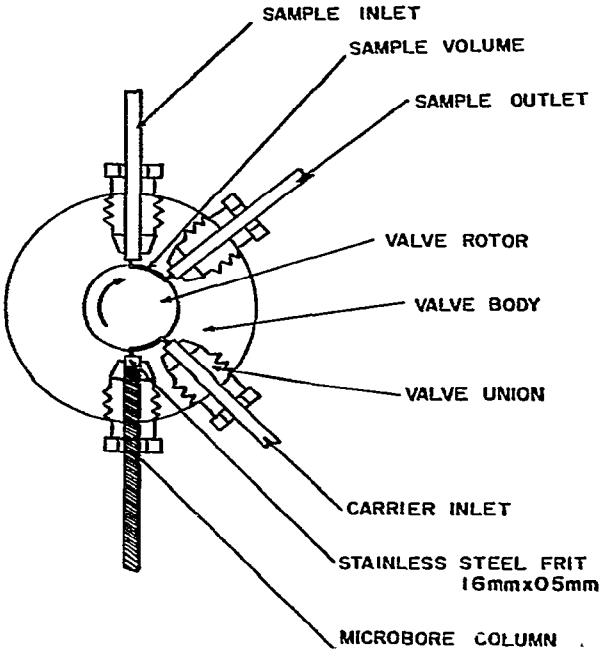


Fig. 3. Connection between a Valco valve and a microbore column.

The effect of detector cell volume on apparent column efficiency can be seen in Fig. 4. The column conditions were kept constant during the experiment and only the detector cell volume has changed. It can be seen that the efficiency for benzene increased from about 7000 to about 15,000 theoretical plates as a result of changing only the detector cell volume from 25 to 1 $\mu$l.

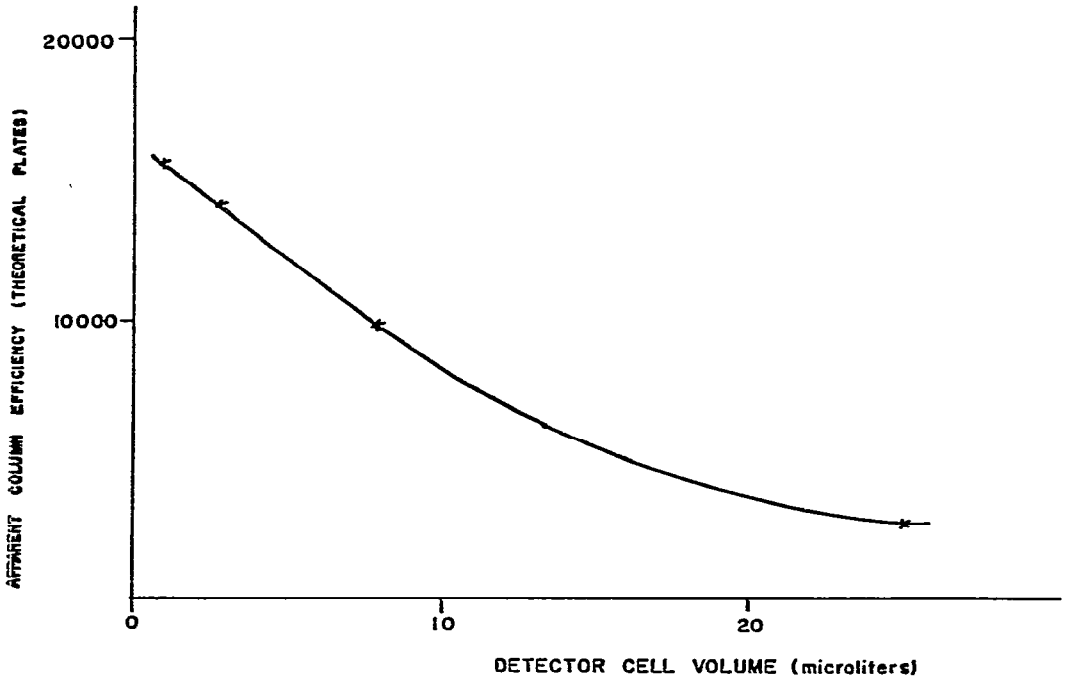


Fig. 4. Graph relating column efficiency to detector cell volume for solute benzene. Condition: column, 1 m × 1 mm I.D.; packing, Partisil 10; mobile phase, THF; $U = 0.083$ cm/sec.

## LIMITING SAMPLE MASS

Decreasing the column diameter decreases the sample mass that can be placed on a microbore column without seriously impairing apparent column efficiency. It was shown by Klinkenberg[9] that the maximum concentration ($X_m$) of a solute peak eluted from a column is proportional to the maximum solute mass ($M_s$) that can be placed on the column and reciprocal of the solute band width ($V_p$). Modifying this treatment for columns of different diameters

$$M_s = \frac{X_m \cdot \pi D^2 \cdot L \cdot \varepsilon \cdot (1 + k')}{2 \cdot \sqrt{N}} \tag{8}$$

A similar equation is obtained in the case of peak volume. Thus, for two columns of the same length and different diameter operated under the same conditions, the ratio of the limiting masses which can be placed on each column to give the same solute maximum concentration, $X_m$, in the mobile phase will be equal to the square of the

ratio of respective column diameters. This can be verified experimentally by determining loading capacities of microbore columns of different diameters and calculating $M_2/M_1$ ratios.

The loading capacity is taken here as the solute mass that can be placed on the column to limit the fall in efficiency to 10% (ref. 10). It can be seen in Fig. 5 that the ratio of limiting masses is equal to the square of the ratio of column diameters, within experimental error. For example, a 25 cm × 1 mm I.D. column, packed with Partisil 20 silica gel will have a loading capacity of only 5 $\mu$g (solute phenylcarbinol $k' = 2$).

Substituting $N$ from eqn. 5 into eqn. 8 extends this argument to columns packed with particles of various diameters.

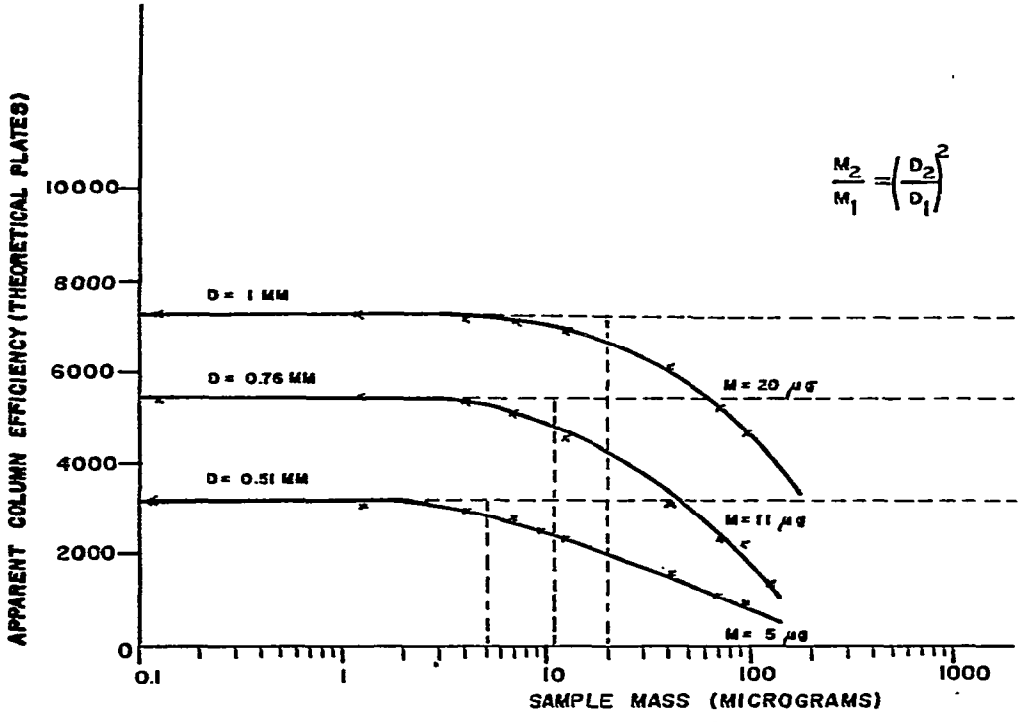


Fig. 5. Graphs of efficiency against sample mass for columns of different diameter. Conditions: solute, phenylcarbinol ($k' = 2$); column, 1 m × 1 mm I.D.; packing, Partisil 20; mobile phase, isopropyl alcohol–heptane (5:95); flow-rate, 100 $\mu$l/min.

## LIMITING SAMPLE VOLUME

The original treatment of Klinkenberg[9] can also be extended to the case of microbore columns of various diameters. Assuming that any band broadening process produces a Gaussian elution curve of variance $\sigma^2$, the total variance, $\sigma_t^2$, can be expressed as the summation of individual variances[11],

$$\sigma_t^2 = \sigma_s^2 + \sigma_c^2 = \sum_{i=1}^{m} \sigma_i^2$$

This procedure is generally valid in chromatography, assuming that the elution peaks are symmetrical, a condition that is well fulfilled with microbore columns. The sample variance ($\sigma_s^2$) can be calculated from the second statistical moment of rectangular distribution:

$$\sigma_s^2 = \frac{\int_{-\frac{V_s}{2}}^{+\frac{V_s}{2}} X_0 \cdot y \cdot dy}{\int_{-\frac{V_s}{2}}^{+\frac{V_s}{2}} X_0 \cdot dy} = \frac{V_s^2}{12}$$

where $V_s$ is the volume of injected sample. From plate theory, the column variance $\sigma_c^2 = V_R^2/N$. If we assume that the sample injection causes an arbitrary 5% increase in band width,

$$\frac{V_s^2}{12} + \frac{V_R^2}{N} = \left[\left(\frac{V_R}{\sqrt{N}}\right) + \left(\frac{5}{100}\frac{(V_R)}{\sqrt{N}}\right)\right]^2$$

and

$$V_s \leqslant \frac{0.278 \cdot \pi D^2 \cdot \varepsilon \cdot L \cdot (1 + k')}{\sqrt{N}} = 1.11 \cdot \sigma_c \qquad (9)$$

At constant $N$ and $\varepsilon$, maximum sample volume can be seen to decrease again with the square of column diameter and directly with column length. A typical analytical column, 25 cm × 4.6 mm I.D., has a dead volume of about 3 ml and $N$ of about 10,000 plates, from which it can be calculated that the maximum sample volume should not exceed 30 $\mu$l. A similar calculation for a microbore column of the same length and 1 mm I.D. will give 1-$\mu$l sample volume, assuming similar column efficiencies; typically, 0.2 to 0.5 $\mu$l is injected. Injection valves on the market (Valco) with an internal injection loop volume of 0.2 $\mu$l and a pressure rating of 7000 p.s.i. (Fig. 3) can be used without any modification to accept 1/16 in. O.D. microbore columns. Direct sample injection can be employed at low column pressures.

## COLUMN GEOMETRY

When designing a column for best performance, the goal is to obtain best column efficiency at given linear velocity, particle diameter, solute, solvent and stationary phase conditions. The column efficiency is a function of interstitial porosity which depends on packing density, and therefore the packing method is of great importance in column design. Two slurry packing procedures frequently used are the constant pressure and the constant flow method. Unfortunately, in most studies, the pressure is held constant during the slurry packing, and, as a result, the

column permeability is not a linear function of the column length. Because the linear velocity slows down considerably at the end of the packing operation, there is a difference in interstitial porosity between the top and the bottom of the column, and HETP is not independent of column length as is usually assumed. This can be demonstrated by an experiment. A microbore column, 1 m × 1 mm I.D., was packed with 10-$\mu$m ODS-2 reversed-phase at 25,000 p.s.i. pressure using 55% cyclohexanol in methylene chloride as the packing liquid. The column was equilibrated with 65% acetonitrile in water and a HETP *versus* linear velocity plot was determined for solutes benzene and phenol in the manner previously described[2]. The column was then carefully cut to 50-cm length and the bottom part of the column was used to determine HETP/$U$ curves under the same chromatographic conditions. The results obtained are shown in Fig. 6. It can be seen that, if the microbore column length is greater than 50 cm, the HETP becomes dependent on column length. A similar effect was observed for larger diameter columns by Majors[12] but has not been demonstrated for microbore columns yet. In spite of the work of Knox[13] and Knox and Parcher[14], the effect of column diameter on plate height is not well understood. An attempt was made to pack two columns of the same length and different column diameters, 4.6 mm and 1 mm I.D., with 10-$\mu$m silica gel using identical packing conditions. At 10,000 p.s.i. packing pressure, the 4.6 mm I.D. column gave satisfactory results, whereas the 25-cm microbore column had reproducibly poor efficiency. When the short microbore column was packed at 25,000 p.s.i., satisfactory performance was obtained. It is shown in Fig. 7 that, although both columns exhibit an optimal plate height of 20 $\mu$m, the slope of the HETP curve for a 1 mm I.D. column,

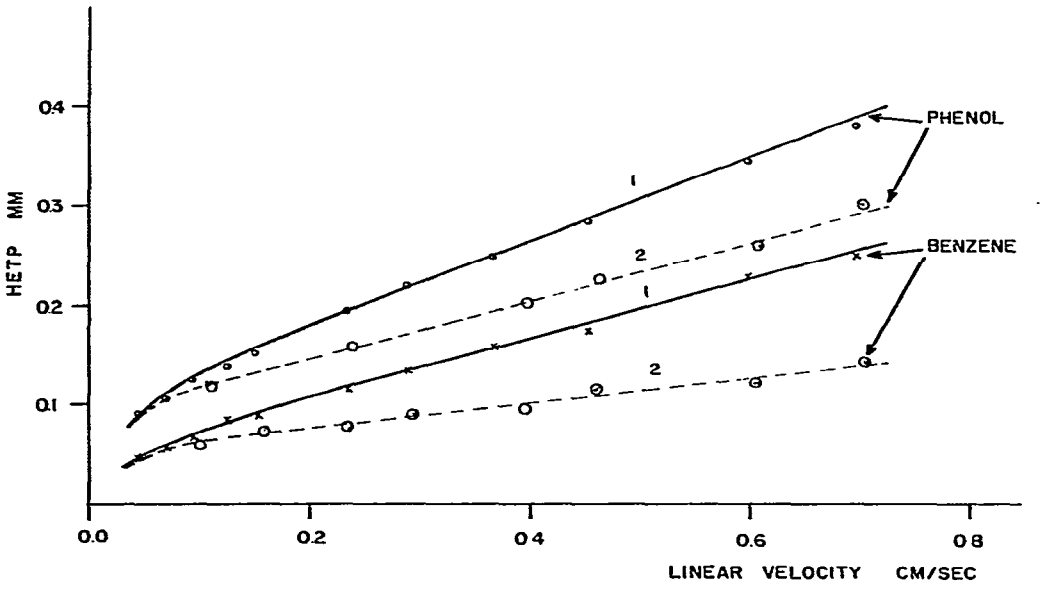


Fig. 6. Curves relating HETP to linear velocity for reversed-phase columns. Conditions 1: column, 1 m × 1 mm I.D.; packing, ODS-2, 10 $\mu$m; mobile phase, acetonitrile–water (65:35). Conditions 2: as in 1, except column, 50 cm × 1 mm I.D.

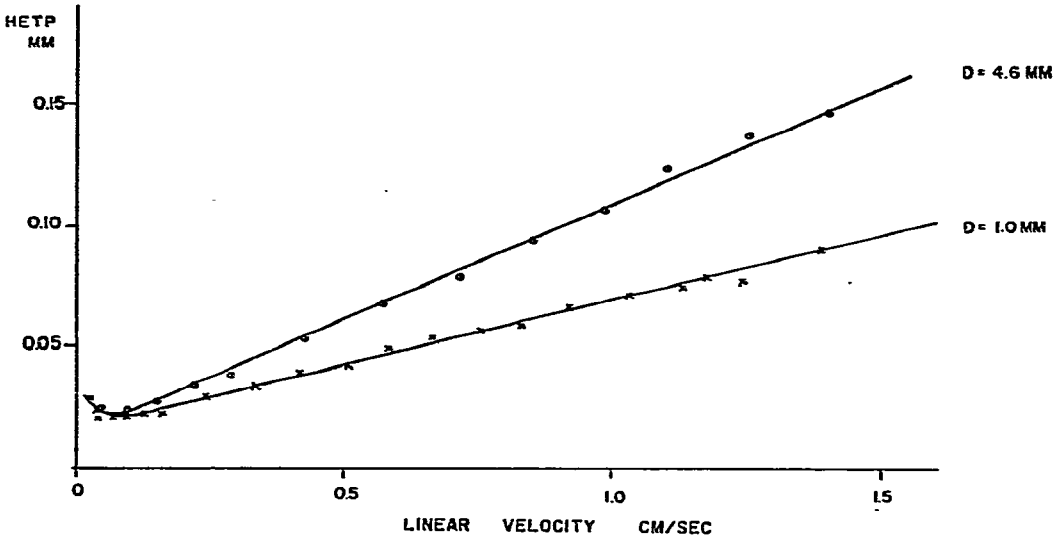


Fig. 7. Graph of HETP against mobile phase velocity for columns of different diameter and solute diazepam. Conditions: columns, 25 cm; packing, Partisil 10, 10 $\mu$m; temperature, 20°C; mobile phase, methanol–ethyl acetate–heptane (2:10:88).

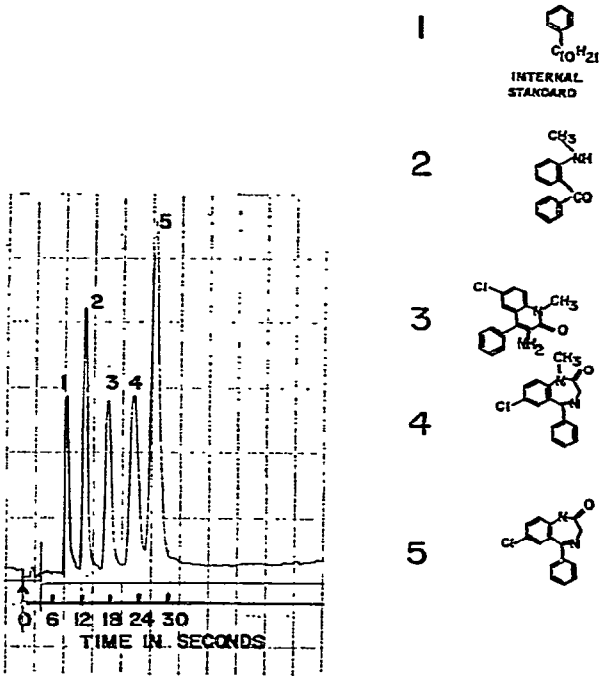


Fig. 8. The separation of diazepam and its metabolites on a microbore column in 30 sec. Conditions: column, 15 cm × 1 mm I.D.; packing, Partisil 10; mobile phase, methanol–ethyl acetate–heptane (8:10:82); flow-rate, 1.5 ml/min.

corresponding to the resistance to mass transfer in the stationary phase, is smaller. Since the speed of analysis is proportional to the $H/U$ ratio according to eqn. 2, there is an advantage in operating short microbore columns at high speeds. Trading efficiency for speed, it was possible to separate diazepam from its major metabolites, a pharmaceutically important assay, in 30 sec. The result can be seen in Fig. 8. In this separation, 70 plates per second were obtained on a 15 cm × 1 mm I.D. column packed with 10 $\mu$m silica gel for diazepam ($k' = 1.8$).

## LIMITING DETECTOR TIME CONSTANT

When using short microbore columns for high-speed analyses, the question of maximum permitted detector time constant arises. The limiting detector time constant ($t_D$) will be a function of the standard time deviation of the solute band ($\sigma_t$).

$$t_D = 0.32 \frac{t_R}{\sqrt{N}} = 0.32\sigma_t \tag{10}$$

The constant 0.32 can be calculated assuming again a 5% increase in band width due to the detector electronics. Thus, the detector time constant that can be tolerated is about 32% of the standard deviation in seconds. For example, for the high-speed separation shown in Fig. 8, a time constant of about 0.2 sec is needed. The detector time constants of current liquid chromatographic detectors are in many instances greater than 0.4 sec, which limits the speed of the analysis and the resolution that can be attained. Thus, current detector technology lags seriously behind column technology.

## COLUMN TEMPERATURE

The use of column temperature as an operating parameter in liquid chromatography has largely been neglected. Increase in temperature decreases the viscosity of the mobile phase, increases the solute diffusivity which is largely dependent on the mobile phase, and, in most cases, decreases the solute retention volume. The solute diffusion coefficient ($D_m$), solvent viscosity ($\eta$) and the retention volume have similar exponential dependence on temperature. The rate of change of $D_m$ and $\eta$ with temperature depends on the activation energies of diffusion and viscous processes.

From eqns. 4 and 6, a decrease in solvent viscosity decreases the column pressure drop that is necessary to achieve desired velocity and increases plates per unit time. This is advantageous when operating short microbore columns at high speed since pressure drop is a limiting factor. The effect of temperature on efficiency results from the combination of temperature effects of $D_m$, $\eta$, and $V_R$[15]. At higher mobile phase velocity where the longitudinal diffusion does not contribute significantly to band broadening of the solute and the convective mixing term can be considered as negligible, the two major temperature-dependent terms of the HETP/$U$ function are the resistance to mass transfer in the mobile phase, coefficient $a$ (ref. 16), and the resistance to mass transfer in the stationary phase, coefficient $b$.

$$a = C_1 \left(\frac{k'}{1 + k'}\right)^2 \frac{\eta^{1/6} \cdot U^{1/2} \cdot d^{3/2}}{D_m^{2/3}} \quad (11)$$

$$b = C_2 \frac{k'}{(1 + k')^2} \frac{Ud^2}{D_m} \quad (12)$$

where $C_1$ and $C_2$ are constants.

For a well-retained solute, $k' \gg 1$ and the term $a$ always decreases with increase in temperature but the term $b$ may increase or decrease, depending on the enthalpy and entropy of the solute transfer between the mobile and the stationary phase.

The activation energy of both the molecular displacement and the viscous shear are greater for polar solvents than for the non-polar ones and thus, regardless of column diameter, a reversed-phase system operated with a polar mobile phase, such as methanol–water, should exhibit an increase in efficiency with temperature because of the predominant effect of smaller solute resistance to mass transfer in the mobile phase at the higher temperature.

Experimentally, this was confirmed using a 25 cm × 1 mm I.D. column packed with Zorbax ODS reversed phase and equilibrated with 70% methanol–water mobile phase. The temperature increase had a marked effect on efficiency. At 0.2 cm/sec linear velocity of the mobile phase, the number of theoretical plates increased from 6050 to 9050, pressure drop decreased from 3200 to 2300 p.s.i., and $k'$ of toluene changed from 7.0 to 4.9, increasing the plates per unit time. Thus, in general, a change in column temperature can be used to effect column performance drastically in reversed-phase liquid chromatography.

## CHROMATOGRAPHIC APPARATUS

The basic design of the chromatographic system which can be used with microbore columns in the isocratic elution mode was reported previously[1]. The system utilized a Waters M6000A pump driven by a Hewlett-Packard 3311A function generator, a Valco sample valve, microbore column, detector and computer data acquisition system. The relationship between the flow-rate and the frequency of the function generator is linear between 2 and 800 $\mu$l per min and for a calibrated pump 10 Hz is equal to 38.5 $\mu$l/min.

For the purpose of this work, a more versatile system was designed. Using two Waters pumps, controlled by the M660 solvent programmer modified for low flow-rates by Waters Assoc., allowed operation in isocratic elution, gradient elution, or flow programming mode (Fig. 9). A simple drilled-through Swagelok T was used as a mixing chamber with 0.01 in. I.D. stainless-steel tubes entering and leaving the T; the internal volume of the T was about 10 $\mu$l.

The system differs from the one mentioned in a previous publication in several points. A commercially available Schoeffel SF770 UV/VIS detector equipped with a wavelength drive and 0.5-$\mu$l cell was employed; any connecting tubing between the column, the detector, and the Valco sample valve was eliminated, as shown in Figs. 2 and 3, and the column ends were designed to use standard Waters low dead volume

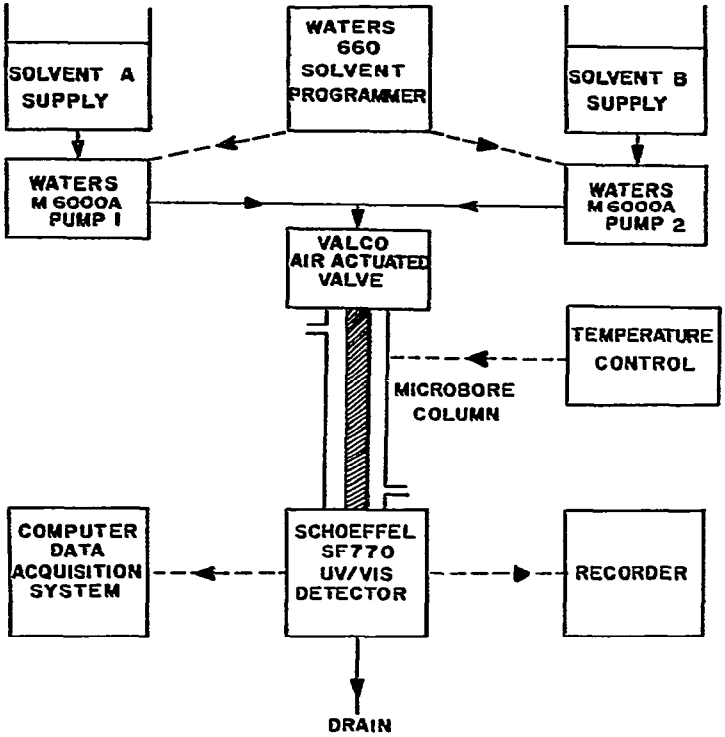


Fig. 9. Schematic diagram of a microbore column liquid chromatograph for use with gradient elution.

fittings. The columns were thermostatted to $\pm 0.01$°C with a Techne TE-14 temperature control system.

The function generator and the Waters solvent programmer of the previously mentioned system can be substituted by the new Schoeffel Klic 1 interactive controller, which can be programmed to drive Waters pumps and to generate various concentration/time functions. Evaluation of this instrument gave satisfactory performance in isocratic and gradient elution operation.

## APPLICATIONS

Examples of the use of short microbore columns in separations of benzodiazepines and vitamin derivatives are shown in Figs. 10 and 11. A 50 cm $\times$ 1 mm I.D. column packed with 10 $\mu$m silica gel and operated at 0.08 cm/sec linear velocity was employed. The column exhibited the optimal plate height of two particle diameters yielding 25,000 theoretical plates for well-retained solutes ($k' = 6$ and $U = 0.03$ cm/sec).

The Waters programmer, capable of generating twelve different concentration profiles, was used to study the effect of concentration shape and time in gradient elution of gasoline. In this separation of aromatic hydrocarbons, a logarithmic concentration shape produced best resolution; increasing the time period to 8 h did not

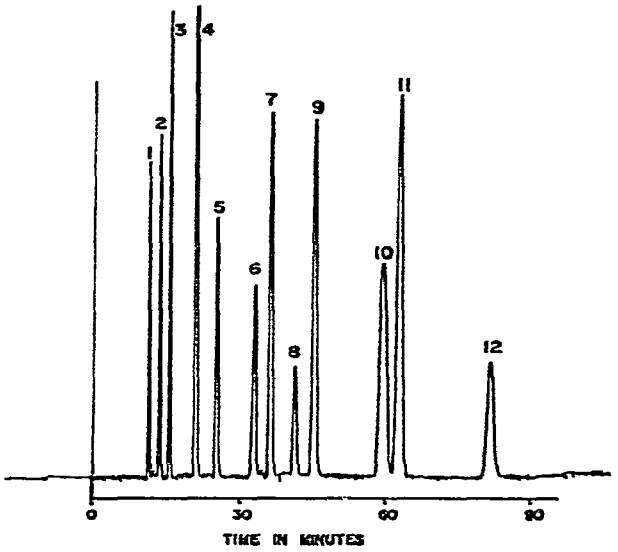


Fig. 10. The separation of a synthetic mixture of benzodiazepines on a microbore column. Conditions: column, 50 cm × 1 mm I.D.; packing, Partisil 10, 10 μm; mobile phase, methanol–ethyl acetate–heptane (5:10:85); flow-rate, 15 μl/min. Peaks: 1 = 1-phenyldecane, 2 = naphthalene, 3 = 2-methylamino-5-chlorobenzophenone, 4 = *o*-ethylphenol, 5 = 3-amino-6-chloro-1-methyl-4-phenylcarbostyril, 6 = benzyl alcohol, 7 = diazepam, 8 = impurity, 9 = nordiazepam, 10 = flunitrazepam, 11 = nitrazepam, 12 = clonazepam.

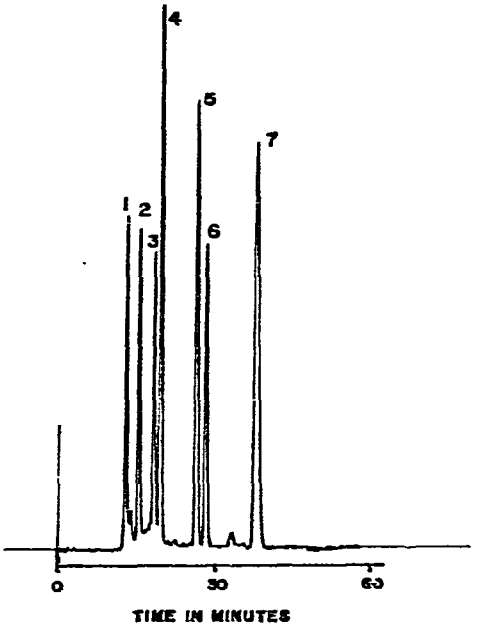


Fig. 11. The separation of synthetic mixture of vitamin derivatives on a microbore column. Conditions: column, 50 cm × 1 mm I.D.; packing, Partisil 10, 10 μm; mobile phase, methanol–ethyl acetate–heptane (2:10:88); flow-rate, 25 μl/min. Peaks: 1 = *trans*-β-carotene, 2 = α-tocopheryl acetate, 3 = α-tocopherol, 4 = 2-methylamino-5-chlorobenzophenone, 5 = *o*-ethylphenol, 6 = *o*-methylphenol, 7 = *trans*-vitamin A alcohol.

improve the resolution significantly, as shown in Fig. 12. An optimal resolution of major components of sandalwood essential oil chromatographed on a 50 cm × 1 mm I.D. column using a linear gradient and different conditions was obtained in 15 min, as shown in Fig. 13. It can be seen that microbore columns 25 or 50 cm long and 1 mm I.D. can very successfully compete with current analytical liquid chromatographic columns. High efficiencies can be easily obtained and operation at high speed can produce a high number of plates per unit time.

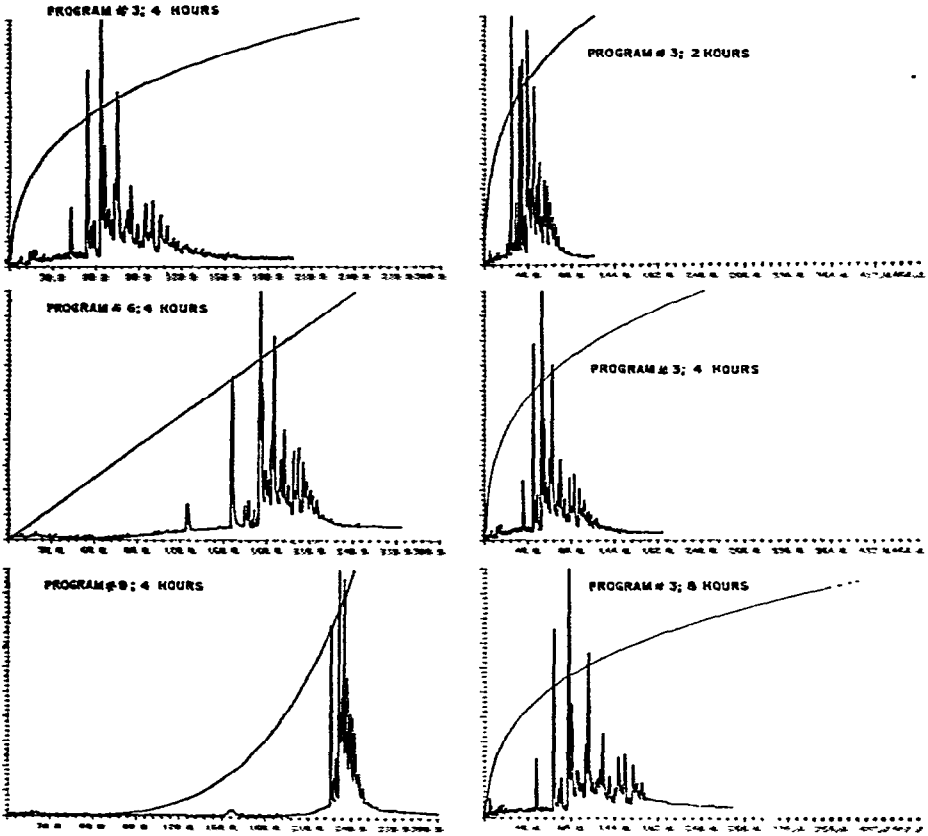


Fig. 12. Gradient elution of Exxon unleaded gasoline. Conditions: column, 50 cm × 1 mm I.D.; packing, RP-18, 10 μm; initial solvent, methanol–water (20:80); final solvent, 100% methanol. Waters solvent programmer, 100 μl/min.

## CONCLUSIONS

Factors important in the design of a microbore column system have been examined. It is shown that, in order to achieve the same chromatographic results with columns of the same length, packed with the same adsorbent, and employing the same mobile phase but different column diameters, it is necessary to decrease the flow-rate, sample mass and volume, and detector cell volume in proportion to the square of column diameter. However, the retention time and the column pressure drop remain unchanged.

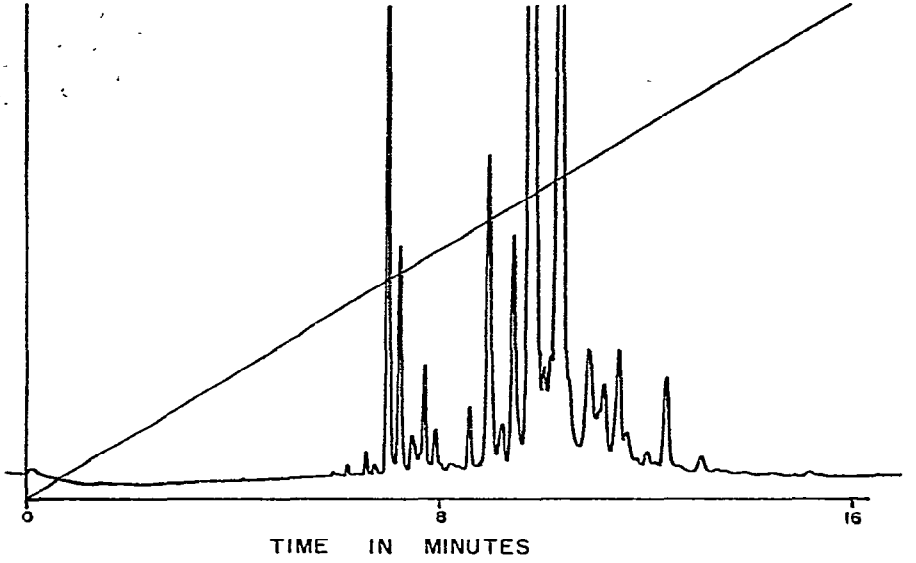


Fig. 13. Gradient elution of sandalwood essential oil. Conditions: column, 50 cm × 1 mm I.D.; packing, RP-18, 10 μm; mobile phase, 50–100% methanol, linear program; flow-rate, 100 μl/min.

Allowing 5% increase in band width due to the detector electronics, the limiting time constant of the detector can be calculated to be 32% of the standard time deviation of the band width.

The development of microbore column systems has advanced to the state where all components necessary for construction and operation of a liquid chromatograph are commercially available except for the columns. Several manufacturers already expressed interest in producing microbore columns according to our specifications, and it is hoped that the columns will appear on the market soon.

The system has several distinct advantages when compared with 1/4 in. O.D. analytical columns. There is a possibility that it can replace these columns in the near future. The operation of the system is very economical with respect to mobile phase consumption, amount of column packing, the sample injected, and associated column hardware used. The use of short columns packed with small particles represents a most economical way to maximize plates per unit time. Band broadening study of columns of different diameters shows an advantage in operating short microbore columns at high speed because of smaller HETP/$U$ ratio. This is true, especially at elevated temperatures, when the resistance to mass transfer in the mobile phase decreases with decreasing solvent viscosity. From all of the components of the microbore column liquid chromatograph, the detector appears to be the weakest link, predominantly because of the limited time constant, detector sensitivity and the cell volume.

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