REDUCTION OF THEORETICAL PLATE HEIGHT IN GAS CHROMATO-GRAPHY BY INCREASE OF PRESSURE AND DECREASE OF PARTICLE SIZE

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SUMMARY

The influence of particle size and pressure on the column efficiency was investigated. The experimental results are explained by means of the theory of the theoretical plate height in chromatography. Columns of small diameter (0.75–1.5 mm) were prepared from porous solid supports of small particle diameter (down to 30 μm) which were coated with low liquid loadings (3–4% w/w) and operated at elevated pressure (inlet pressure up to 50 bar). As a result, a maximum of more than 10,000 theoretical plates per meter of column length were obtained for 30–35-μm particles at an inlet pressure of 25 bar at a mobile phase velocity of 2.5 cm/sec.

INTRODUCTION

In 1958 Scott[1] already paid attention to the use of small-diameter columns (d_c = 2.2 mm) operated at elevated pressure (up to 14 bar). A minimum theoretical plate height of 0.3 mm was reported. The injection system used in this work was rather complicated and could only afford an inlet pressure up to 14 bar.

In 1965 Myers and Giddings[2,3] described a column system with long and narrow columns (length up to 1200 m, diameter 1.5 mm) which was operated at high inlet pressure (up to 150 bar). The apparatus was designed for gaseous samples only. Very high numbers of theoretical plates, in the order of 10^6, could be obtained with theoretical plate heights of about 1 mm. This approach has limited value in practice, however, since the apparatus can only be used for gases, the sample is rather strongly diluted and the flow-rate at the column outlet is quite high.

Another approach to achieve high theoretical plate numbers in gas chromatography (GC) with packed columns was reported by Cramers *et al.*[4,5]. Small-diameter columns of 0.8-mm diameter were used and theoretical plate numbers in the order of 40,000 were obtained with 15-m long columns corresponding to a theoretical plate height of about 0.3 mm. A relatively low inlet pressure of about 4 bar was needed. This approach has the drawback of requiring rather long columns in order to obtain high theoretical plate numbers resulting in a significant dilution of the sample.

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In the present paper an attempt is made to investigate the possibility of achieving: (1) high theoretical plate numbers with relatively short columns in order to reduce the dilution of the sample; (2) sufficiently small flow-rates of the carrier gas at the column outlet to allow direct coupling to a mass spectrometer; and (3) relatively low flow velocity without loss in resolution in order to obtain an appropriate long scan time for high-resolution mass spectrometry.

THEORY

The dependence of the theoretical plate height in chromatography on the different process parameters can be described by theory[6-9] assuming constant flow velocity and constant diffusion coefficients in the column.

$$H_i = a_d \frac{D_{im}}{v} + \frac{a_c' d_p}{1 + a_c'' (D_{im}/v d_p)^{1/2}} + a_f \left(\frac{v}{D_{im}}\right)^{1/2} d_p^{3/2} \left(\frac{\kappa_i}{\kappa_i + 1}\right)^2 +$$

$$+ a_b \frac{v}{D_{ip}} d_p^2 \frac{\kappa_i}{(\kappa_i + 1)^2} \qquad (1)$$

where H_i = theoretical plate height for component i; v = flow velocity; D_{im} = diffusion coefficient of component i in the mobile phase m; d_p = particle diameter of the column packing material; κ_i = capacity ratio = mass distribution ratio of component i; D_{ip} = overall diffusion coefficient of component i in a particle; and a_d, a_c' and a_c'', a_f, a_b = factors expressing the effect of the column geometry on diffusional mixing, convective mixing, dispersion caused by the partial mass exchange process in the flowing part of the mobile phase, dispersion caused by the partial mass exchange process in the fixed bed, i.e. in both the stagnant part of the mobile phase and the stationary phase.

According to eqn. 1 the theoretical plate height has a minimum value at a given flow velocity. At lower values of the flow velocity it is increasingly dominated by the diffusion term, at higher flow velocities by the mass exchange term in the fixed bed.

The theoretical plate height in GC depends on pressure since the diffusion coefficient in the mobile phase, being a gas, depends on pressure according to the following relationship.

$$D_{im} p = \text{constant} \qquad (2)$$

where p is the pressure.

From eqns. 1 and 2 it follows that the value of the theoretical plate height in GC decreases with increasing pressure at low flow velocities and increases at high flow velocities. Furthermore, the minimum value decreases and shifts to lower values of the flow velocity with increasing pressure.

In a chromatographic column a pressure gradient exists. With a gas as mobile phase, this pressure gradient causes gradients of the flow velocity as well as of the diffusion coefficient in the mobile phase because of the high gas compressibility. The first three terms in the theoretical plate height equation remain constant, however, since the ratio D_{im}/v does not change because of eqn. 2 and the relationship

$$v_z p_z = \text{constant} \qquad (3)$$

which is valid for an ideal gas: where z = length coordinate of the column with z = 0 at the beginning; v_z = flow velocity at position z; and p_z = pressure at position z.

The influence of the pressure gradient on the theoretical plate height is exclusively determined.by the fixed-bed mass exchange term, the fourth term in eqn. 1. This term increases with flow velocity which rises along the column. depending on the ratio of the inlet and outlet pressure[10].

$$v_z = v_L \left\{ \left(\frac{p_0}{p_L}\right)^2 - \left[\left(\frac{p_0}{p_L}\right)^2 - 1\right] \frac{z}{L} \right\}^{-\frac{1}{2}} \tag{4}$$

where L = length of the column; v_L = flow velocity at the end of the column; p_0 = inlet pressure; and p_L = outlet pressure.

From eqns. 1 and 4 it follows that the theoretical plate height increases from the beginning to the end of the column. This gradient of the theoretical plate height in the column can be described by an expression which is obtained by combination of eqns. 1 and 4.

$$H_{lz} = \text{constant} + a_b \cdot \frac{v_L}{D_{ip} \left\{ \left(\frac{p_0}{p_L}\right)^2 - \left[\left(\frac{p_0}{p_L}\right)^2 - 1\right] \frac{z}{L} \right\}^{\frac{1}{2}}} \cdot d_p^2 \cdot \frac{\kappa_i}{(\kappa_i + 1)^2} \tag{5}$$

where H_{lz} = theoretical plate height at position z.

In practice, average values of the theoretical plate height in GC are measured at average values of the flow velocity.

.. Summarizing, it can be pointed out that the influence of the pressure on the theoretical plate height in GC is described by eqn. 1 in connection with eqn. 2 whereas the effect of the pressure drop is described by eqn. 5.

EXPERIMENTAL

Apparatus

The GC system was custom-made and assembled from parts of different origin. It consists of a quickly interchangeable column. a high-pressure control unit for the carrier gas, a high-pressure sampling device, a micro thermal conductivity detector and a thermostatted air bath for column, sampling device and detector.

The columns are constructed from stainless-steel tubing (1.6 mm O.D., 1.0 mm I.D., length 1500 mm) equipped with end fittings (Serto couplings). The tubing is filled with chromatographic packing material, which is locked up on each end of the tubing by means of a stainless-steel filter of 10-μm opening diameter enclosed in a copper ring.

The flow scheme of the GC system is shown in Fig. 1.

The pressure is controlled in front of the sampling device by means of a dome-type pressure controller (GHR-H2). After the pressure controller the carrier gas is split into two streams which are led to the measuring and the reference cell of the detector, respectively. The first stream leads via a stainless-steel high-pressure Bourdon-tube manometer (Wika), the sampling valve, and the chromatographic column. The second stream passes a fine metering valve (Nupro SS-2SG) which is used as variable flow resistor in order to balance in the reference stream the flow resistance
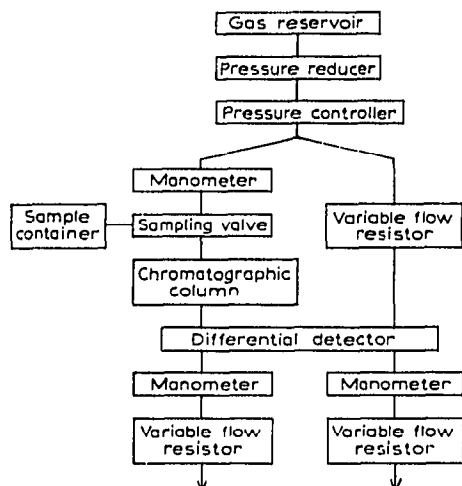
Fig. 1. Flow-scheme of the gas chromatographic apparatus.

of the chromatographic column. The pressures at the two detector outlets are adjusted to a given value by means of fine metering valves (Nupro SS-2SG) acting as flow resistors.

The sampling device consists of a high-pressure sampling valve (Valco CV-6-UHPa) with an exchangeable sample loop. The valve can withstand pressures up to 100 bar at temperatures up to 100°. A sample loop with a volume of 7 μl was used. The loop is connected to a pressure vessel of 3 ml volume containing the sample mixture. The pressure in the sample container is adjusted to the inlet pressure of the column by means of the sampling valve. The sample container is filled with the sample by means of a high-pressure syringe (Hamilton) via an injection port. The sample is present in the container and applied to the column as vapour mixed with carrier gas. In accordance with the volume ratio of the sample loop and the container, a static split of the sample with a constant ratio is performed.

A micro thermal conductivity detector (Taylor Servomex MK 158) is used for monitoring the column effluent. The volume of each cell is extremely low and amounts to only 3 μl, which allows measurement of very narrow concentration profiles. In order to allow detection at elevated pressure, the detector block is included in a pressure vessel, which can withstand pressures up to 100 bar. During the measurement, the pressure in the vessel is held about 0.5 bar above the pressure in the detector cells. In this manner leakage of the thermal conductivity cells is overcome.

The flow-rate was measured at atmospheric pressure at the outlet of the apparatus by means of a precision flow meter (Brooks Vol-U-Meter®).

Packing of the column

The column tubing is coiled before filling with the packing material. The solid support is coated with the stationary liquid in the usual way and the coated material is placed in a chamber connected at one end to the column tubing via a narrow hole of 0.5 mm and at the other end to the carrier-gas source. The coiled tubing is placed in an ultrasonically vibrated water-bath and the carrier gas is allowed to flow, carrying

the packing material into the tubing. In order to recognize the end of the filling procedure, a short glass tube (1 mm I.D.) is placed in front of the column inlet. After the column is filled completely, the gas flow is switched off and the column is allowed to depressurize via the column outlet.

Chemicals and materials

Two types of materials were used as solid supports: Spherosil® XOC-005 (Rhone-Poulence) and Chromosorb® G AW-DMCS (Johns-Manville). Appropriate particle-size ranges were prepared by sieving.

Squalane (GC-grade, E. Merck) was used as stationary liquid in the chromatographic experiments. The sample compounds for the test of the columns were: krypton (high-purity grade, L'air liquide N47), n-pentane and n-hexane (both GC-grade, Merck). The carrier gas was nitrogen GC-grade (L'air liquide).

RESULTS AND DISCUSSION

As already mentioned it was the intention to develop packed GC columns with a high number of theoretical plates at relatively small length and flow-rate. Such columns would be very useful in trace analysis and in coupling with a mass spectrometer. In order to find the conditions for the design and operation of columns with these characteristics, the dependence of the relationship between the theoretical plate height and the flow velocity on the column diameter, the operating pressure and the particle size of the packing material was investigated. Because of the difficulty to measure the flow velocity in a packed column, the flow velocity v was replaced by the migration velocity u of the mobile phase, which is proportional to v^{7-9}.

Theoretical plate–column diameter

The average theoretical plate height was measured as function of the average mobile phase velocity for columns of different diameter. The reproducibility was determined by preparing several columns of the same diameter from 120–140-μm . Chromosorb G, AW-DMCS, coated with 3% (w/w) squalane. Columns with diameters of 0.75, 1.0 and 1.5 mm were prepared and measured. No significant influence of the column diameter on the theoretical plate height could be observed in this range.

Theoretical plate height–pressure

A number of columns was prepared using different particle sizes, d_p, of the same type of solid support coated with about the same amount of a given stationary liquid. For each column, the average theoretical plate height, $\bar{H}$, was determined as function of the average mobile phase velocity, $\bar{u}$, at different outlet pressures, p_L, for compounds with different capacity ratios, κ_i. The corresponding inlet pressures were measured too. Representative results are plotted in Figs. 2–4. From the comparison of the curves measured on the same column at different outlet pressures it can be concluded that the following changes in $\bar{H}_i$ occur if p_L is increased: (1) $\bar{H}_i$ decreases at low values of $\bar{u}$; (2) the limiting value of $\bar{H}$ which is approached at high values of $\bar{u}$ for unretarded compounds ($\kappa_i = 0$) remains unchanged; (3) the minimum value of $\bar{H}_i$ which is found for retarded compounds ($\kappa_i > 0$) shifts to lower values of $\bar{u}$ and decreases slightly; and (4) $\bar{H}_i$ for retarded compounds increases at high values of $\bar{u}$. (This effect
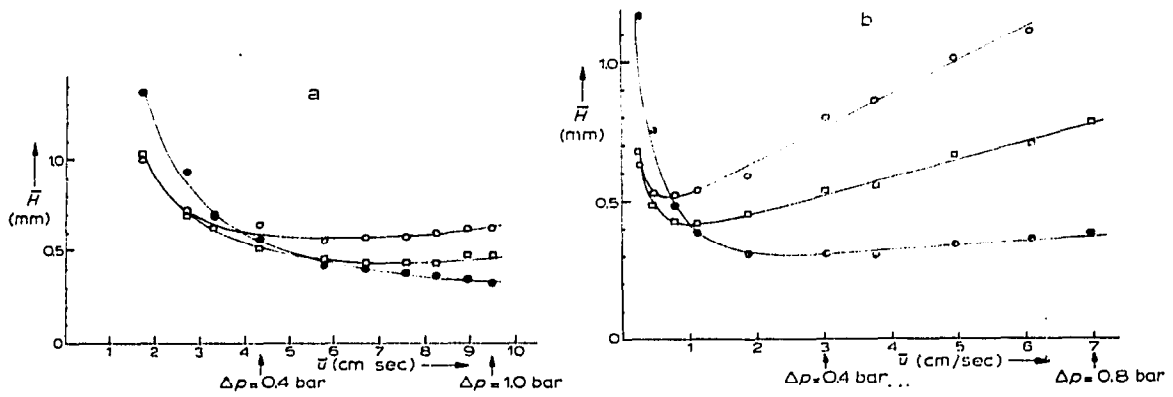
Fig. 2. *H versus ū* curves for a packed column with 200-250 μm particles at different pressures. Column: 1.0 mm I.D., length 1500 mm; packing Spherosil® XOC-005, 200-250 μm, coated with 3.0% (w/w) squalane; carrier gas, nitrogen; outlet pressure, (a) 1 bar; (b) 10 bar; temperature, 78.3°. External dead volume, 57.3 μl. Sample: (●) krypton, κ ≈ 0; (□) pentane, κ = 0.7; (○) hexane, κ = 1.5; sample pressure equals column inlet pressure.
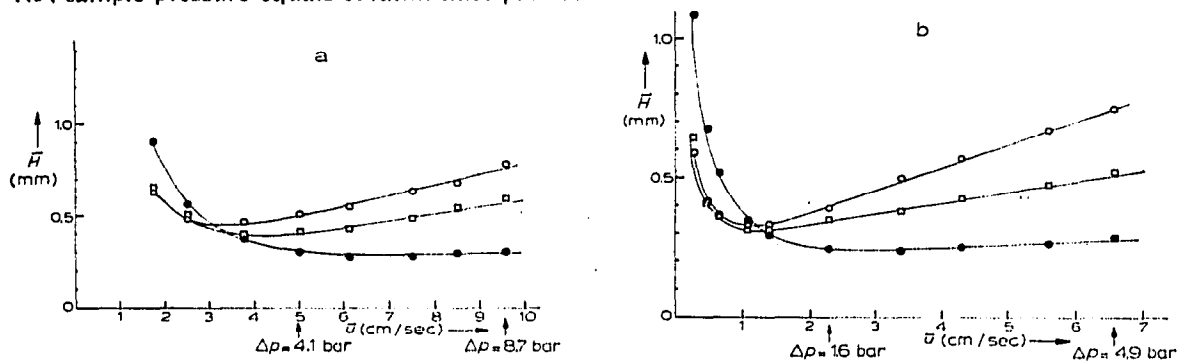

Fig. 3. *H versus ū* curves for a packed column with 120-140 μm particles at different pressures. Column: packing Spherosil® XOC-005, 120-140 μm, coated with 4.1% (w/w) squalane; outlet pressure, (a) 1 bar; (b) 10 bar; temperature, 78.2°. Sample: (●) krypton, κ ≈ 0; (□) pentane, κ = 0.9; (○) hexane, κ = 2.1. Other conditions as in Fig. 2.

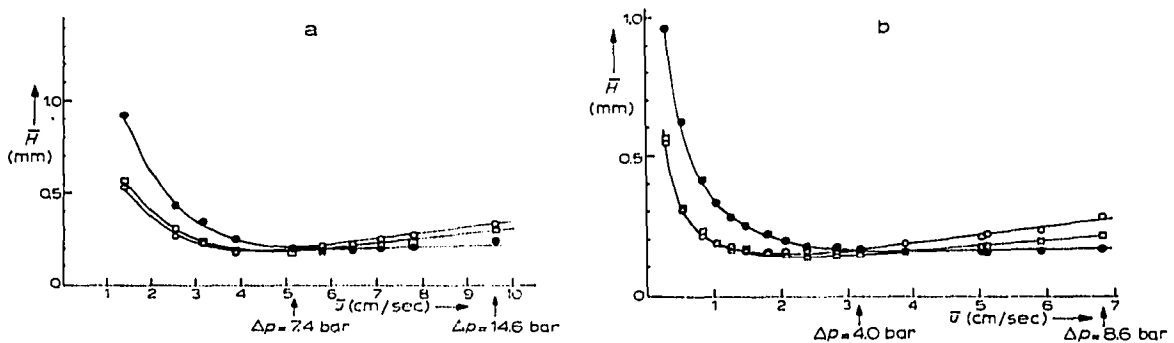Fig. 4. *H versus ū* curves for a packed column with 63-71 μm particles at different pressures. Column: packing Spherosil® XOC-005, 63-71 μm, coated with 3.1% (w/w) squalane; outlet pressure, (a) 1 bar; (b) 10 bar; temperature, 79.0°. Sample: (●) krypton, κ ≈ 0; (□) pentane, κ = 1.0; (○) hexane, κ = 2.3. Other conditions as in Fig. 2.

diminishes with decreasing d_p and decreasing κ_i.)

These experimental observations can be theoretically explained by means of eqn. 1 or 5 by which the effect of the pressure on the theoretical plate height is described.

In a first approach the influence of the pressure drop is neglected and eqn. 1 is used for the interpretation of the experimental results. In GC D_{im} and eventually D_{ip} in eqn. 1 depend on pressure. The influence of the pressure on D_{im} is described for an ideal gas as mobile phase by eqn. 2. As a consequence, the first term in eqn. 1 decreases, the second term remains virtually unchanged in the usual flow-velocity range and the third term increases if the pressure increases. The influence of the pressure on the fourth term depends on its effect on D_{ip}, which describes the overall diffusion within the particles of the fixed bed of the column. The effect of pressure on D_{ip} depends on the filling of the pore space of the particles. In the simplest case, the pores of the particles are totally filled by a single phase in which case D_{ip} is proportional to the diffusion coefficient in this phase[6-9]. If the phase is a liquid, D_{ip} does not depend on pressure; if the phase is an ideal gas, D_{ip} depends on pressure analogous to eqn. 2. In gas–liquid chromatography the particles are usually filled by stationary liquid as well as stagnant carrier gas. In this case, the effect of pressure on D_{ip} is determined by the volume ratio of the stationary liquid and the stagnant gas phase within a particle and their geometrical distribution. As a result, D_{ip} will decrease and the fourth term in eqn. 1 will increase with pressure, its magnitude depending on the degree of filling of the particles with stagnant carrier gas. Applying eqn. 1 to the average values $\bar{H}_i$, $\bar{v}$ and $\bar{P}$ and taking into account the pressure dependence of D_{im} and D_{ip}, it describes correctly the trend in the change of $\bar{H}_i$ which is observed at a given value of $\bar{v}$ as result of a pressure change in practice and was summarized previously in this section. The average values of the pressure and the mobile phase velocity were calculated according to the following expressions.

$$\bar{p} = p_L/j$$

$$\bar{u} = L/t_{R0} \tag{6}$$

where $j = 3/2 \cdot [(p_0/p_L)^2 - 1]/[(p_0/p_L)^3 - 1] =$ compressibility factor[10] and $t_{R0} =$ average residence time of a non-retarded component in the column.

In a more thorough treatment of the influence of the pressure on H_i, the existence of a pressure drop in the column should be considered. As a result of the pressure drop, a rise of H_i in the column occurs which is described by eqn. 5. In gas–liquid chromatography eqn. 5 does not allow accurate prediction of the gradient of H_i in the column since D_{ip} is an unknown function of z. This function is determined by the pressure dependence of D_{ip} which cannot be described if the particles are filled partially by gas and partially by liquid. It follows, however, that D_{ip} increases with decreasing pressure. The drop in the pressure factor, $\{(p_0/p_L)^2 - [(p_0/p_L)^2 - 1] z/L\}^{\frac{1}{2}}$, and the unknown rise of D_{ip} with z in eqn. 5 determine the increase of H_i from the beginning to the end of the column. Since the exact gradient of D_{ip} and the magnitude of its pressure dependence are not known, it is not possible at the present time to derive a complete expression for the average theoretical plate height, $\bar{H}_i$, and its pressure dependence and to discuss the experimental results in the view of an exact theory taking into account the pressure drop.

In order to get some impression regarding the significance of the pressure drop influence compared with the influence of the average pressure, the following experiment was carried out. For two virtually identical columns, $\bar{H}_t$ was measured as a function of $\bar{u}$, after which the columns were coupled and the measurement was repeated at the same pressure ratio p_0/p_L. In this manner the same pressure gradient was present in all columns so that, exclusively, the average pressure, which was twice as high in the case of the coupled column, could affect the value of $\bar{H}_t$. The results are shown in Fig. 5. Comparison with Figs. 2–4 shows the same effect of the pressure change on the value of $\bar{H}_t$. This result suggests that the pressure drop has only a minor effect compared with the average pressure.
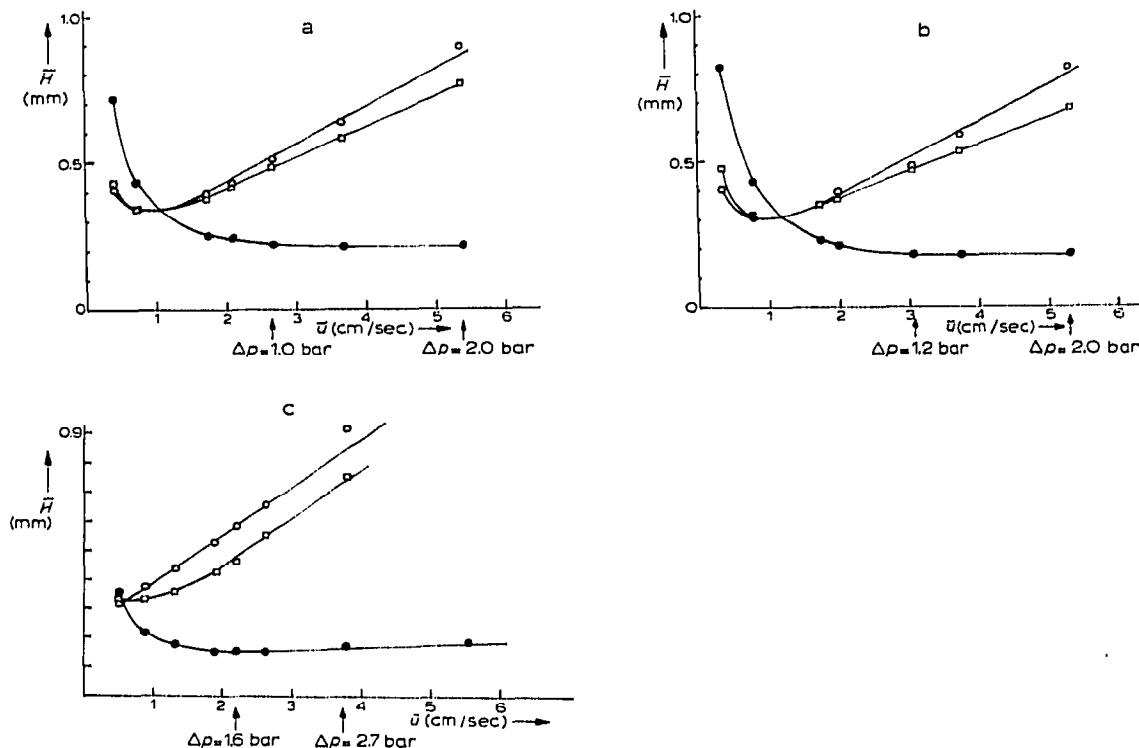

Fig. 5. $\bar{H}$ versus $\bar{u}$ curves for single and coupled columns. (a) Column 1, (b) column 2, (c) columns 1 + 2: packing Chromosorb G AW DMCS, 100–120 μm, coated with 3.6 % (w/w) squalane; outlet pressure, (a, b) 10 bar, (c) 20 bar; temperature, 79.0°. Sample: (●)krypton, $\kappa \approx 0$; (□) pentane, $\kappa =$ 1.4; (○) hexane, $\kappa = 3.3$. Other conditions as in Fig. 2, except external dead volume 65.3 μl for coupled columns (c).

Theoretical plate height–particle size

Particle diameters of 200–250, 100–120 and 63–71 μm were used in the columns the characteristics of which are shown in Figs. 2–4. Comparing the experimental results with different particle sizes, we can draw a number of conclusions concerning the influence of the particle size on the average value of the theoretical plate height. At atmospheric outlet pressure (Figs. 2a–4a), a reduction of d_p has the following

consequences: (1) $\bar{H}_t$ decreases at low values of $\bar{u}$; (2) the limiting value of $\bar{H}_t$ approached at high values of $\bar{u}$ for non-retarded compounds decreases; (3) the minimum value of $\bar{H}_t$ which is found for retarded compounds shifts to smaller values of $\bar{u}$ and decreases significantly in value; and (4) the slope of the rise of $\bar{H}_t$ for retarded compounds at high values of $\bar{u}$ decreases.

At elevated outlet pressure (Figs. 2b–4b), a reduction of d_p has the following consequences: (1) $\bar{H}_t$ remains virtually unchanged at low values of $\bar{u}$; (2) the limiting value of $\bar{H}_t$ approached at high values of $\bar{u}$ for non-retarded compounds decreases; (3) the minimum value of $\bar{H}_t$ for retarded compounds decreases significantly and is found at about the same value of $\bar{u}$; and (4) the slope of the rise of $\bar{H}_t$ for retarded compounds at high values of $\bar{u}$ decreases.

These experimental results can be explained by means of eqn. 1. With atmospheric outlet pressure, the changes in $\bar{H}_t$ are caused partially by the direct effect of the change of d_p in eqn. 1 and partially by the indirect effect due to the dependence of the pressure drop on the particle size by which $\bar{P}$ increases for a given value of $\bar{u}$ with decreasing d_p. At elevated outlet pressure, $\bar{P}$ increases only slightly with decreasing d_p for a given value of $\bar{u}$. In this case, the changes of $\bar{H}_t$ are mainly caused by the change of d_p in eqn. 1. It can be seen especially that the significant decrease of the minimum value of $\bar{H}_t$ for retarded compounds and the decrease of the limiting value at high values of $\bar{u}$ for non-retarded compounds, as well as the decrease of the slope of the $\bar{H}_t$–$\bar{u}$ curves of retarded compounds at high values of $\bar{u}$, are caused by the direct effect of the reduction of d_p in eqn. 1.

In order to support the conclusions reached on the pressure influence and as a reference for the effect of the column packing, measurements with the empty column tubing were carried out. The results are represented in Fig. 6.
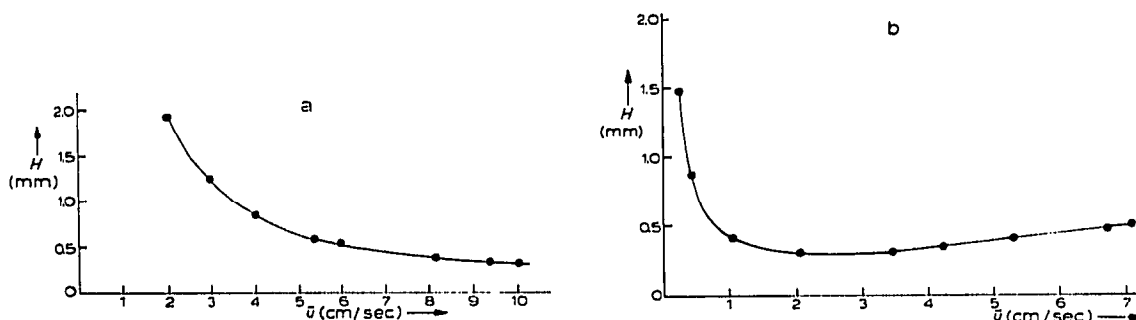

Fig. 6. H versus $\bar{u}$ curves for an open tubular column at different pressures. Internal diameter, 1.0 mm; length, 1495 mm; carrier gas, nitrogen; outlet pressure, (a) 1 bar, (b) 12.6 bar. Sample: krypton. Theoretical plate heights are corrected for the increments of injection, dead volume (57.3 μl) and detection. Temperature, 78.4°.

In an open tubing the value of the theoretical plate height is given by the following equation[11,12]:

$$H_t = 2\frac{D_{l\,m}}{v} + \frac{r^2 v}{24 D_{l\,m}}$$ (7)

where r = internal tubing radius.

As can be seen, the influence of the pressure on H_t is described correctly by eqn. 7 taking into account the pressure dependence of D_{im} according to eqn. 2. The pressure drop in this case was very small and could not be measured.

By extrapolation of the results obtained so far it seemed promising to reduce the particle size further. A particle size of 30–35 μm was assumed to be a good compromise with respect to theoretical plate height and pressure drop. Such a column was prepared and tested. The results are shown in Fig. 7.
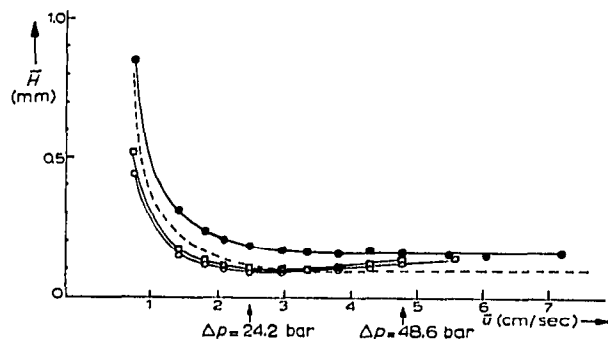

Fig. 7. $\bar{H}$ versus $\bar{u}$ curve for a packed column with 30–35 μm particles. Column: packing Spherosil XOC-005, 30–35 μm, coated with 3.0% (w/w) squalane; outlet pressure 1 bar; temperature 79.0°. Sample: (●) krypton, $\kappa \approx 0$; (□) pentane, $\kappa = 1.0$; (○) hexane, $\kappa = 2.2$. Dashed curve indicates theoretical plate height for krypton corrected for the injection plug according to eqn. 11. Other conditions as in Fig. 2.

It can be seen that the column follows the extrapolation and fits in the line of the other columns (Figs. 2a–4a, 7). More than 15,000 theoretical plates were achieved with a column of 1.50 m length at a mobile phase velocity of 2.5 cm/sec corresponding to a flow-rate at atmospheric pressure of 14 ml/min. An inlet pressure of 25 bar was needed. The results shown in Fig. 7 allow the conclusion that packed columns with more than 50,000 theoretical plates are feasible at a length of 6 m, a pressure of 50 bar and an outlet flow-rate of 5 ml/min.

Accuracy of the measurement of the theoretical plate height

The average theoretical plate height is calculated from the recorded signal peak according to

$$\bar{H} = \frac{\sigma_t^2}{t_R^2} \cdot L \tag{8}$$

where σ_t^2 = variance of the recorded peak in time units and t_R = retention time.

Eqn. 8 is correct only if σ_t^2 is exclusively produced in the chromatographic column. In general, the measured overall peak variance also contains contributions from outside the column owing to sampling and detection.

$$\sigma_t^2 = \sigma_t^2 \, _{\text{column}} + \sigma_t^2 \, _{\text{external}} \tag{9}$$

where $\sigma_{t\ column}^2$ = variance caused by the chromatographic process and $\sigma_{t\ external}^2$ = variance caused by effects outside the column. In principle, H must be calculated correctly from the part of the variance which arises from the column.

With a well-designed apparatus and small sample volumes $\sigma_t^2 \gg \sigma_{t\ external}^2$ the correction for the external effects can be omitted. At increasing pressure ratio, however, the amount of sample introduced into the column at constant sample volume increases. This results finally in a significant contribution of the feed volume to the measured peak variance. For a sampling valve, it can be assumed that the sample is introduced into the stream of mobile phase in a rectangular distribution function. Considering the expansion of the feed volume, because of the pressure drop in the column, we obtain the following expression for the external contribution to the peak variance at the column outlet:

$$\sigma_{t\ external}^2 = \frac{V_0^2}{12} \cdot \left(\frac{p_0}{p_L}\right)^2 \cdot \frac{1}{w_L^2} \tag{10}$$

where V_0 = feed volume of the sample at the inlet pressure and w_L = flow-rate at the column outlet with the outlet pressure, p_L.

In order to obtain the true value of H, the measured apparent value has to be corrected by the increment $-\Delta H$ caused by the feed volume.

$$H_{true} = H_{apparent} - \Delta H_{feed\ volume} \tag{11}$$

with $\Delta H_{feed\ volume} = V_0^2/12 \cdot L/V_{RL}^2 \cdot (p_0/p_L)^2$ and $V_{RL} = t_R \cdot w_L$ = retention volume at the pressure p_L at the column outlet.

As can be seen, the magnitude of the correction is proportional to $(p_0/p_L)^2$.

An example of the correction is shown in Fig. 7 where it was carried out for the non-retarded compound. The corrected curve is indicated by the dotted line. In this case, the correction is extremely large because of the high pressure ratio p_0/p_L and the low value of V_{RL}. It can be seen that, in this case, the accuracy of the measurement is very low.

The results for the other compounds in Fig. 7 are also significantly too high, although to a lesser extent, and the same is true for the results represented in Fig. 4a, especially the non-retarded component. They were not corrected, however, since in practice this contribution of the feed volume to the measured peak variance cannot be avoided at high values of p_0/p_L since the smallest possible sample loop was used in all cases. In order to exclude this contribution, the construction of a new type of sample valve will be required.

ACKNOWLEDGEMENTS

REFERENCES

1 R. P. W. Scott, in D. H. Desty (Editor), *Gas Chromatography 1958*, Butterworths, London, 1958, p. 189.

2 M. N. Myers and J. C. Giddings, *Separ. Sci.*, 1 (1966) 761.
3 M. N. Myers and J. C. Giddings, *Anal. Chem.*, 37 (1965) 1453.
4 C. A. Cramers, J. A. Rijks and P. Boček, *J. Chromatogr.*, 65 (1972) 29.
5 C. A. Cramers, J. A. Rijks and P. Boček, *Clin. Chim. Acta*, 34 (1971) 159.
6 J. F. K. Huber and J. A. R. J. Hulsman, *Anal. Chim. Acta*, 38 (1967) 305.
7 J. F. K. Huber, *J. Chromatogr. Sci.*, 7 (1969) 85.
8 J. F. K. Huber, in E. Kováts (Editor), *Column Chromatography, Chimia Supplementum*, Sauer-länder, Aarau, 1970, p. 24.
9 J. F. K. Huber, *Ber. Bunsenges. Phys. Chem.*, 77 (1973) 159.
10 A. T. James and A. J. P. Martin, *Biochem. J.*, 50 (1952) 679.
11 G. Taylor, *Proc. Roy. Soc., London*, A219 (1953) 186.
12 R. Aris, *Proc. Roy. Soc., London*, A235 (1956) 67.