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## EFFECT OF PRESSURE ON SOLUTE DIFFUSIVITY, SOLVENT VISCOSITY AND COLUMN TEMPERATURE IN LIQUID CHROMATOGRAPHY

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### SUMMARY

The interrelationship between column pressure, solvent viscosity, solute diffusivity and column temperature is complex. Any increase in inlet pressure to provide a higher flow-rate and consequently a faster analysis increases solvent viscosity and column temperature and decreases solute diffusivity. However, a higher column temperature resulting from increased operating pressure reduces the solvent viscosity and increases the solute diffusivity, thereby masking the direct effect of pressure on these variables. *Ipso facto*, the net effect of pressure and temperature on solute diffusivity for an unthermostated column can be relatively small; consequently, the effect of pressure on column efficiency and column resolution can be minimal for unthermostated columns. However, the effect of this temperature increase on solute retention is very significant for unthermostated columns and leads to a 5% change in the value of  $k'$  for a solute by merely changing the flow-rate from 0.5 to 5 ml/min. Hence, as the heat generated in the column is directly related to the flow-rate and further as the heat transfer through the packed bed of the column is very poor, the use of well thermostated small-bore columns could be mandatory for the precise measurement of solute retention.

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### INTRODUCTION

The demand for higher resolution and faster separations to cope with more complex mixtures and to provide a more economic analytical service has resulted in the use of much higher inlet pressures in liquid chromatography (LC). More chromatographers are operating their chromatographs at pressures close to 40 MPa as opposed to about 15 MPa which was previously adequate for low-efficiency columns or columns packed with particles 10  $\mu\text{m}$  or more in diameter. However, the viscosity of the mobile phase and the diffusion coefficient of the solute (solute diffusivity) in the mobile phase are possibly significantly different at pressures of 40 MPa<sup>1</sup>. The compressibility of a liquid can change the measured retention time of a solute and this effect has been studied by Martin *et al.*<sup>1</sup>. On the other hand, a change in mobile phase viscosity and solute diffusivity resulting from an increase in pressure would manifest itself as a change in column dispersion due to a change in the resistance-

to-mass transfer in the column and consequently a change in column efficiency. The situation is further complicated by the fact that there will be a concurrent generation of heat in the column at high pressures<sup>2-4</sup> which will have the opposite effect of pressure on the solvent viscosity and solute diffusivity, and which will also effect solute retention as well as band dispersion.

The work described in this paper was carried out to determine the net effect of pressure on mobile phase viscosity, solute diffusivity and column temperature. The overall effect of pressure on solute retention and the solute dispersion is also considered. A forward phase LC system was used in conjunction with a mobile phase that had an *n*-alkane as the major component and which exhibits the greatest change of viscosity and diffusivity with pressure. The relationship between solute diffusivity and solvent viscosity is also empirically identified.

#### EFFECT OF PRESSURE ON SOLVENT VISCOSITY

The effect of pressure on viscosity can be determined by measuring how the flow-rate changes through a column with pressure. However, as the mobile phase is compressible the flow-rate through the column will not be exactly that measured volumetrically at the exit. Nevertheless, if it is assumed that the compressibility of mobile phase is relatively small, the mean pressure in the column can be taken as half the inlet pressure and the true mean volume flow through the column determined from the retention time of an unretained peak and the column dead volume.

#### Theory

The viscosity ( $\eta$ ) of most liquids increases with increasing pressure ( $P$ ). Over the pressure range used in liquid chromatography, this increase in viscosity is linear with pressure<sup>1</sup> and can be described by the following equation:

$$\eta = \eta_0 (1 + \alpha P) \quad (1)$$

where the viscosity at atmospheric pressure is  $\eta_0$  and  $\alpha$  is a constant. Values for  $\alpha$  are in the range of  $5 \cdot 10^{-3}$ – $1.2 \cdot 10^{-2}$  per MPa for typical organic solvents (but is an order of magnitude smaller for water)<sup>5</sup>. To a first approximation, the pressure drop can be taken as linear across the length of the column, so the average pressure is half the inlet pressure. Thus, for an inlet pressure of 40 MPa the percentage increase in viscosity ranges from about 10% for methanol to about 20% for hexane.

The equation describing the dependence of viscosity on pressure (eqn. 1) can be combined with the well known Darcy equation, *viz.*

$$u = K_0 d_p^2 \Delta P / \eta L \quad (2)$$

where  $K_0$  is the specific permeability of the column of length  $L$ , packed with particles of diameter  $d_p$ . Substituting for  $\eta$  from eqn. 1 in eqn. 2 yields the ultimate expression relating pressure and linear velocity:

$$u = K_0 d_p^2 \Delta P / L \eta_0 (1 + \alpha P) \quad (3)$$

From eqn. 3 it is seen that the linear velocity  $u$ , and hence the flow-rate ( $F$ ), should be non-linear functions of the column pressure (assuming all other variables are held constant). However, as heat generated in the column at high flow-rates and consequently high pressure could reduce the solvent viscosity, pressure-flow relationships were obtained for unthermostated and thermostated standard columns together with a thermostated microbore column.

#### *Flow-pressure relationship for an unthermostated standard column*

A column 25 cm  $\times$  4.6 mm I.D. packed with silica gel (Partisil 10) having a nominal particle diameter of 10  $\mu\text{m}$  and an actual particle diameter of 7.8  $\mu\text{m}$  was employed in conjunction with a Valco sampling valve (having a sample volume of 0.2  $\mu\text{l}$ ) and a Perkin-Elmer LC-75 UV detector. In this first experiment the column was not thermostated. The LC-75 detector was used rather than a low-dispersion LC-85B as the former contributed negligible resistance to mobile phase flow and thus the pressure drop in the system could be taken as solely that across the column. The pump was a Perkin-Elmer Series 3B which had a flow-rate range of 0.1–30 ml/min. The maximum operating pressure was restricted by the pressure limit of the injection valve to 36 MPa. The mobile phase was a 5% (v/v) solution of ethyl acetate in *n*-hexane and the unretained solute *p*-xylene was injected on to the column as a 1.0% (w/v) solution in the mobile phase. The dead volume of the column was determined at a flow-rate of 0.5 ml/min (inlet pressure 1.5 MPa, to ensure any thermal effects were minimized) and was taken as the product of the retention time of *p*-xylene (dead time) and the flow-rate. The actual flow-rate (column dead volume divided by retention time of *p*-xylene) was measured over a range of pressures and the results obtained are shown in Table I. It should be pointed out that throughout all the experimental work no column deterioration was observed. *Ipsa facto*, the columns were well packed and stable.

The data given in Table I are shown as a graph relating flow-rate with pressure in Fig. 1A. The data were fitted to linear and second-order polynomial functions, the results of which are also included in Table I. It was found that the second-order coefficient was not statistically different from zero. Consequently, the relationship between flow and pressure was demonstrated to be linear and there appeared to be little change in viscosity with respect to pressure, which is contrary to theoretical predictions.

However, on measuring the inlet and outlet temperatures of the mobile phase, it was found that, whereas the inlet temperature was 25°C, the exit temperature was over 30°C owing to the heat generated in the column. This is in accordance with the results reported by Halász *et al.*<sup>2</sup>, Lin and Horváth<sup>3</sup> and Poppe *et al.*<sup>4</sup> and is caused by the work done by the pump forcing the mobile phase through the column and thus generating heat. The increase in temperature, however, reduces the viscosity of the solvent, in opposition to the increase in viscosity due directly to pressure.

#### *Flow-pressure relationship for a water-thermostated standard column*

The above experiment was repeated with the column thermostated in a water-bath at 25°C. The results obtained are shown in Table II and Fig. 1B and were also curve fitted to a second-order polynomial equation and the results included in Table II. The quadratic coefficient is statistically significant, reflecting the definite curvature

TABLE I

FLOW-RATES AT DIFFERENT PRESSURES FOR AN UNTHERMOSTATED PACKED COLUMN

Column length 25 cm; I.D. 4.6 mm.

Pressure ( <i>P</i> ) (MPa)	Flow-rate ( <i>Q</i> ) (ml/min)
1.1	0.48
4.8	2.37
8.7	4.66
12.3	6.89
15.3	9.06
19.0	11.04
21.9	13.20
25.8	15.08
29.6	17.05

Result of curve fit to function  $Q = A + BP$ :

Index of determination 0.998:

Constant  $A = -0.323$ Constant  $B = 0.5974$ .Result of curve fit to function  $Q = A + BP + CP^2$ :

Index of determination 0.998:

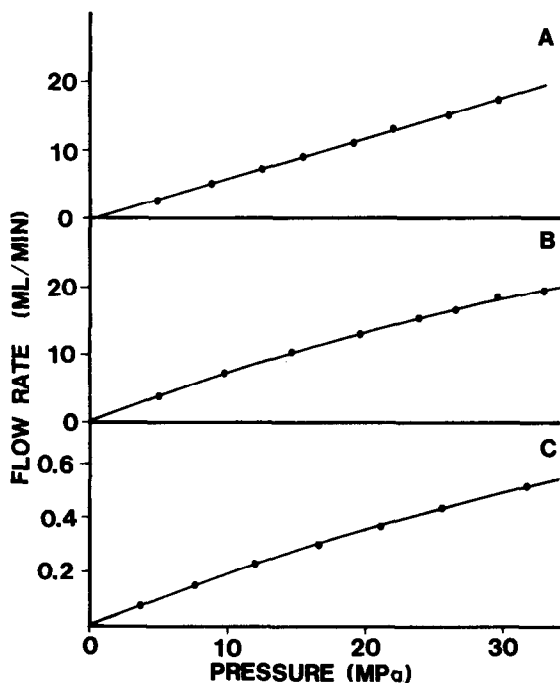
Constant  $A = -0.460$ Constant  $B = 0.625$ Constant  $C = -8.96 \cdot 10^{-4}$ .

Fig. 1. Graphs of column flow against inlet pressure. A, Column not thermostated, 25 cm  $\times$  4.6 mm I.D., Partisil 10; B, water-thermostated column, 25 cm  $\times$  4.6 mm I.D., Partisil 10; C, water-thermostated micro-bore column, 59.8 cm  $\times$  0.76 mm I.D., Partisil 20.

in the plot in Fig. 1B. The ratio of the quadratic coefficient to the linear coefficient is now 0.49% per MPa, equivalent to 14.7% for an inlet pressure of 30 MPa (average column pressure 15 MPa). This value of 0.49% is much closer to the predicted value of 0.565% MPa.

*Flow-pressure relationship for a water-thermostated microbore column*

The experiment was next repeated using a microbore column, 59.8 cm  $\times$  0.76 mm I.D., packed with 20  $\mu$ m particles in an attempt to eliminate all temperature effects. This column was also thermostated at 25°C in a water-bath. Owing to the physical shape of the column, a much lower flow-rate was employed and thus much less heat was generated. Further, any heat would be dissipated rapidly and the water-bath could control the overall temperature in the column more precisely. The results obtained are shown in Table III.

TABLE II

FLOW-RATES AT DIFFERENT PRESSURES FOR A WATER-THERMOSTATED PACKED COLUMN

Column length 25 cm; I.D. 4.6 mm.

Pressure ( <i>P</i> ) (MPa)	Flow-rate ( <i>Q</i> ) (ml/min)
5	3.78
9.8	6.99
14.5	9.93
19.6	12.82
23.7	14.99
26.4	16.57
29.5	18.18
33.2	19.93

Result of curve fit to function  $Q = A + BP + CP^2$ :

Index of determination 1.000:

Constant *A* = 0.402

Constant *B* = 0.702

Constant *C* =  $-3.41 \cdot 10^{-3}$ .

The results given in Table III were curve fitted to a second-order polynomial and the calculated data are shown as the curve in Fig. 1C. The curve is not linear and thus the viscosity of the mobile phase is significantly pressure dependent. From the constants of the second-order polynomial curve it is seen that the change in viscosity with pressure was 0.58% per MPa or about 17.3% at an inlet pressure of 30 MPa (average column pressure 15 MPa). This is far more in keeping with published values of 1.15% per MPa<sup>1</sup>, bearing in mind that the mean pressure in the column is half the inlet pressure and thus the true change in viscosity with pressure would be double the value given, namely about 1.16% per MPa.

It is fairly obvious that in the first experiment the effect of temperature on viscosity compensated for the direct effect of pressure on viscosity, and it is therefore of interest to estimate the net temperature change in a packed column with pressure

TABLE III

## FLOW-RATES AT DIFFERENT PRESSURES FOR A WATER-THERMOSTATED MICROBORE COLUMN

Column length 59.8 cm; I.D. 0.76 mm.

Pressure ( <i>P</i> ) (MPa)	Flow-rate ( <i>Q</i> ) (ml/min)
3.6	0.07
7.5	0.145
11.9	0.225
16.4	0.295
21.1	0.365
25.3	0.430
31.7	0.515

Result of curve fit to function  $Q = A + BP + CP^2$ :  
 Index of determination 1.000:  
 Constant  $A = 2.18 \cdot 10^{-3}$   
 Constant  $B = 1.98 \cdot 10^{-2}$   
 Constant  $C = -1.14 \cdot 10^{-4}$ .

to determine the effect of high pressure and high flow-rate on retention measurements.

## EFFECT OF PRESSURE AND FLOW-RATE ON COLUMN TEMPERATURE

The average column temperature cannot be measured in a simple manner. Heat is generated at a constant rate over the whole length of the column. However, as the thermal conductivity of the packed bed is small most of the heat is carried away by the mobile phase. Thus there is a negative temperature gradient from the center of the column to the wall and a positive temperature gradient from the column inlet to the column outlet. It would be impossible to introduce a sufficient number of temperature sensors into the column without disturbing the normal flow characteristics of the column to obtain an accurate value for the mean column temperature, so the mean temperature was determined indirectly by a chromatographic procedure.

The retention volume and thus the capacity factor ( $k'$ ) of a solute is dependent on the distribution coefficient of the solute between the two phases, which in turn is dependent on the column temperature. Therefore, if the  $k'$  value of a solute is measured at a series of temperatures using a well thermostated column (operated at low pressure and flow-rate to eliminate thermal effects), a relationship between  $k'$  and temperature can be obtained. This relationship can then be used as a calibration curve to determine the mean column temperature of an unthermostated column operated at different pressures and flow-rates, from the  $k'$  values of the same solute.

The same apparatus and conventional column were used as that described previously except that the column and inlet tube were immersed in a thermostating bath. The sample consisted of a 1% (v/v) mixture of *p*-xylene and benzyl acetate dissolved in the mobile phase. The  $k'$  of benzyl acetate was measured over a temperature range from 20 to about 40°C and the results are given in Table IV. The

TABLE IV

RETENTION ( $k'$ ) OF BENZYL ACETATE AT DIFFERENT TEMPERATURES ON A WATER-THERMOSTATED PACKED COLUMN

Column length 25 cm; I.D. 4.6 mm.

Temperature ( $T$ ) ( $^{\circ}\text{C}$ )	$k'$
20.1	1.612
25.1	1.573
30.0	1.547
35.0	1.509
40.0	1.480

Result of curve fit to function  $k' = A + BT$ :  
 Index of determination 0.997:  
 Constant  $A = 1.741$   
 Constant  $B = -6.6 \cdot 10^{-3}$ .

results were fitted to a linear function and the constants obtained are included in Table IV. In Fig. 2 the values of  $k'$  are plotted against temperature; the straight line is the theoretical linear curve fit to the data. The correct relationship between  $k'$  and temperature (from the Van 't Hoff equation) is logarithmic. However, over a small temperature range the relationship can be treated as linear for calibration purposes and this can be confirmed from the data in Table IV.

Employing the same apparatus but with the thermostat removed the  $k'$  of benzyl acetate was then measured over a range of flow-rates and pressures. The

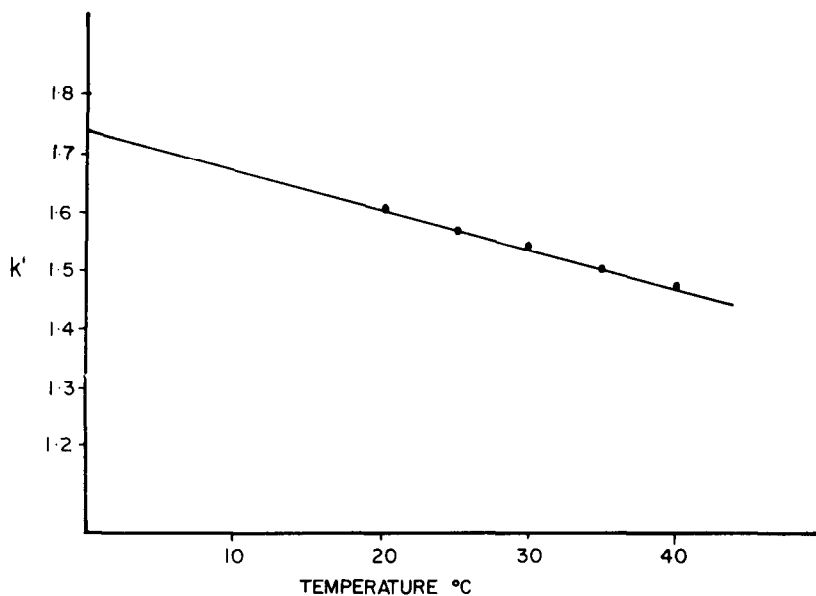


Fig. 2. Graph of  $k'$  for benzyl acetate against temperature chromatographed on a water-thermostated packed column. Column, 25 cm  $\times$  4.6 mm I.D., Partisil 10. Note that  $k' \neq 0$  at the origin.

TABLE V

RETENTION DATA ( $k'$ ) FOR BENZYL ACETATE ELUTED FROM AN UNTHERMOSTATED PACKED COLUMN AT DIFFERENT INLET PRESSURES AND CONSEQUENTLY DIFFERENT FLOW-RATES

Column length 25 cm; I.D. 4.6 mm.

Pressure ( $P$ ) (MPa)	$k'$
5.2	1.535
10.6	1.495
16.3	1.459
22.5	1.444
27.8	1.429
31.5	1.430

Result of curve fit to function  $k' = A + BP$ :

Index of determination 0.911:

Constant  $A = 1.54$

Constant  $B = -3.95 \cdot 10^{-3}$ .

column was allowed to come into thermal equilibrium over a period of 10 min after each pressure change and before the  $k'$  was measured which was taken as the average of three replicates. The values of  $k'$  for benzyl acetate were also measured at different column inlet pressures for the column water thermostated. The results obtained are shown in Tables V and VI, respectively, and were fitted to a linear function and the results of the curve fits included in Tables V and VI. The results are also shown as graphs relating  $k'$  to column pressure in Fig. 3A and B.

Finally, the relationship between  $k'$  and pressure was determined for the wa-

TABLE VI

RETENTION DATA ( $k'$ ) FOR BENZYL ACETATE ELUTED FROM A WATER-THERMOSTATED PACKED COLUMN AT DIFFERENT INLET PRESSURES AND CONSEQUENTLY DIFFERENT FLOW-RATES

Column length 25 cm; I.D. 4.6 mm.

Pressure ( $P$ ) (MPa)	$k'$
5.0	1.519
9.8	1.484
14.5	1.472
19.6	1.453
23.7	1.436
26.4	1.450
29.5	1.438
33.2	1.401

Result of curve fit to function  $k' = A + BP$ :

Index of determination 0.908:

Constant  $A = 1.526$

Constant  $B = -3.43 \cdot 10^{-3}$ .



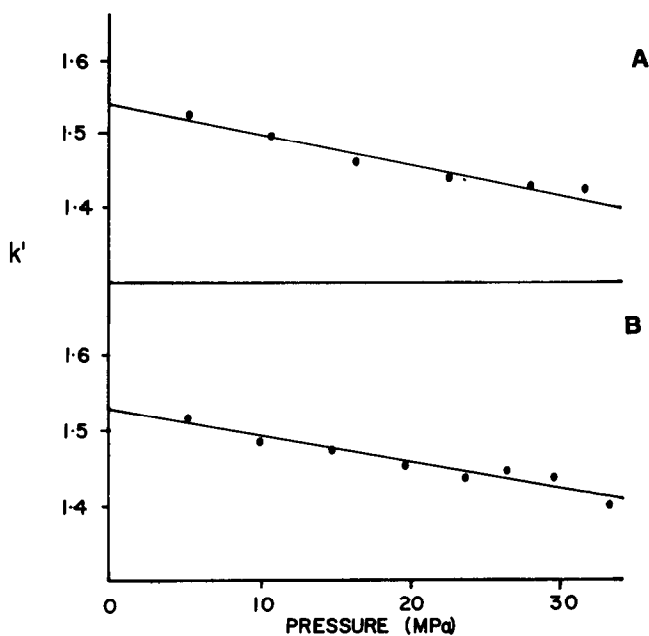


Fig. 3. Graphs of  $k'$  for benzyl acetate against inlet pressure for (A) unthermostated and (B) water-thermostated columns. Columns, 25 cm  $\times$  4.6 mm I.D., Partisil 10. Note that  $k' \neq 0$  at the origin.

ter-thermostated microbore column, 156 cm  $\times$  0.76 mm I.D. The temperature calibration data and the results relating  $k'$  values with inlet pressure are given in Tables VII and VIII, respectively, and in Fig. 4.

It can be seen from Fig. 3A and B that the value of  $k'$  changes very significantly with column pressure and consequently column flow-rate. It is also seen that even thermostating the column in a water-bath does not reduce the change in  $k'$  suffi-

TABLE VII

RETENTION ( $k'$ ) OF BENZYL ACETATE AT DIFFERENT TEMPERATURES ON A WATER THERMOSTATED MICROBORE COLUMN

Column length 156 cm; I.D. 0.76 mm.

Temperature ( $T$ ) ( $^{\circ}\text{C}$ )	$k'$
19.0	1.843
25.0	1.809
29.1	1.773
35.0	1.752
41.7	1.743

Result of curve fit to function  $k' = A + BT$ :

Index of determination 0.921:

Constant  $A = 1.92$

Constant  $B = -4.55 \cdot 10^{-3}$ .

TABLE VIII

RETENTION DATA ( $k'$ ) FOR BENZYL ACETATE ELUTED FROM A WATER-THERMOSTATED MICROBORE COLUMN AT DIFFERENT INLET PRESSURES AND CONSEQUENTLY DIFFERENT FLOW-RATES

Column length 156 cm; I.D. 0.76 mm.

Pressure ( $P$ ) (MPa)	$k'$
7.2	1.817
11.6	1.817
14.9	1.815
22.5	1.798
30.6	1.791

Result of curve fit to function  $k' = A + BP$ :

Index of determination 0.926:

Constant  $A = 1.830$

Constant  $B = 1.27 \cdot 10^{-3}$ .

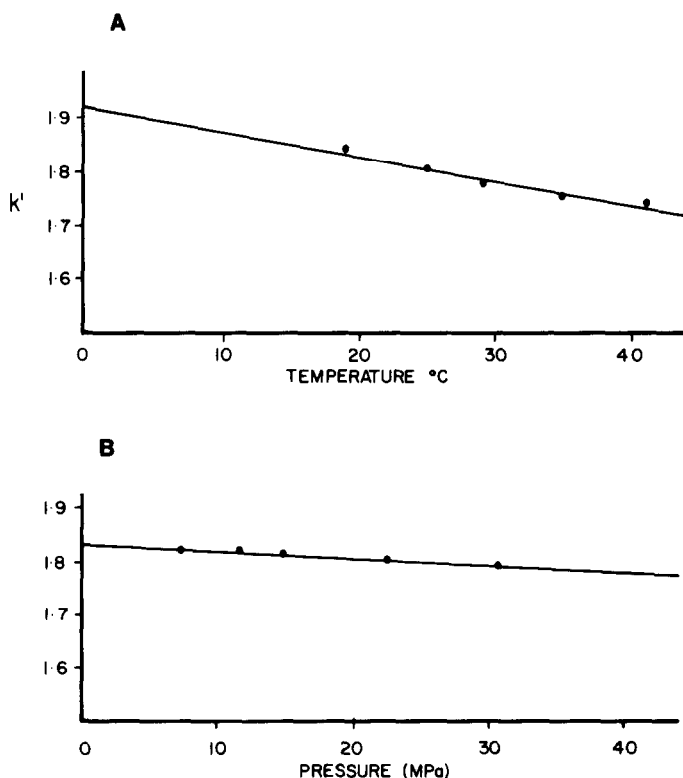


Fig. 4. Graphs of  $k'$  for benzyl acetate against (A) temperature and (B) inlet pressure for a microbore column. Water-thermostated column, 156 cm  $\times$  0.76 mm I.D., Partisil 20. Note that  $k' \neq 0$  at the origin.

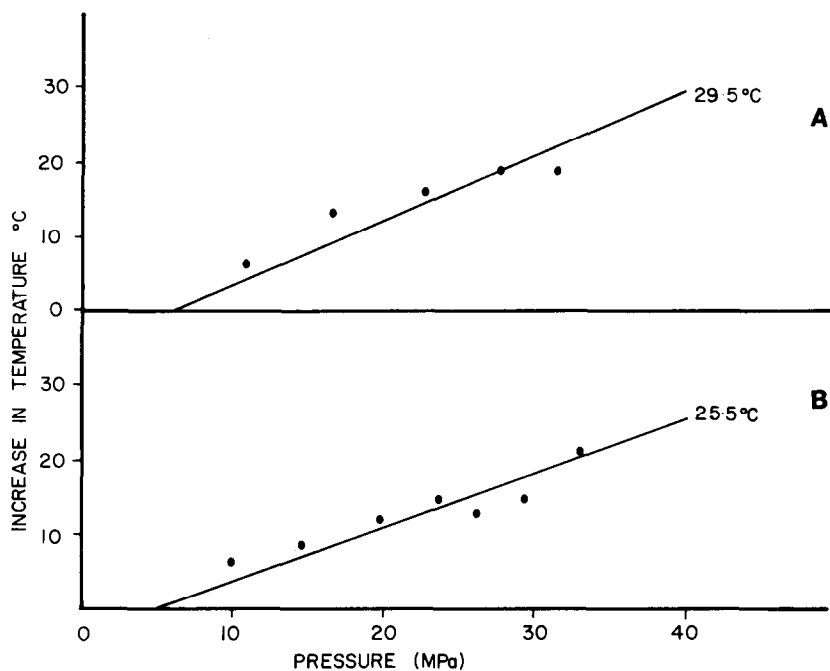


Fig. 5. Graphs relating mean effective column temperature to inlet pressure. A, Unthermostated column, 25 cm  $\times$  4.6 mm I.D.; B, water-thermostated column, 25 cm  $\times$  4.6 mm I.D.

ciently for accurate retention measurements. Changing the column pressure from 5 to 30 MPa still results in a 5.7% change in  $k'$ .

The changes in  $k'$  with pressure can be expressed as a change in temperature with pressure by employing the calibration curve shown in Fig. 2. In Fig. 5 the mean column temperature is shown plotted against column inlet pressure for the unthermostated and water-thermostated column. It is seen that the column temperature changes very significantly with column pressure even when the column is thermostated with a water-bath. Raising the column inlet pressure from 5 MPa at a flow-rate of 3.8 ml/min to 30 MPa at a flow-rate of 18.5 ml/min results in a temperature increase of 18°C.

The change in  $k'$  as a function of column inlet pressure, shown in Tables V and VI, reflects the change in  $k'$  occurring as a consequence of the temperature change in the center of the column caused by viscous dissipation of heat (the column wall was held at 25°C). The axial column temperature is not uniform, but varies continuously from inlet to outlet. The results in Tables V and VI thus represent the effect of a mean temperature change and can be taken as roughly half the temperature change at the outlet. Thus, for an inlet pressure of 40 MPa on an unthermostated column, the coefficients in Tables V and VI give a change in  $k'$  of 0.158. From the calibration data given in Table IV, this is equivalent to a temperature increase of about 24°C. Moreover, this is the temperature change averaged across the full column radius; the temperature at the center of the outlet stream could be as much as double this value. From the data in Tables VII and VIII and Fig. 6 it is seen that the slope of the  $k'$  vs. pressure plot is much flatter for the microbore column than it is for the

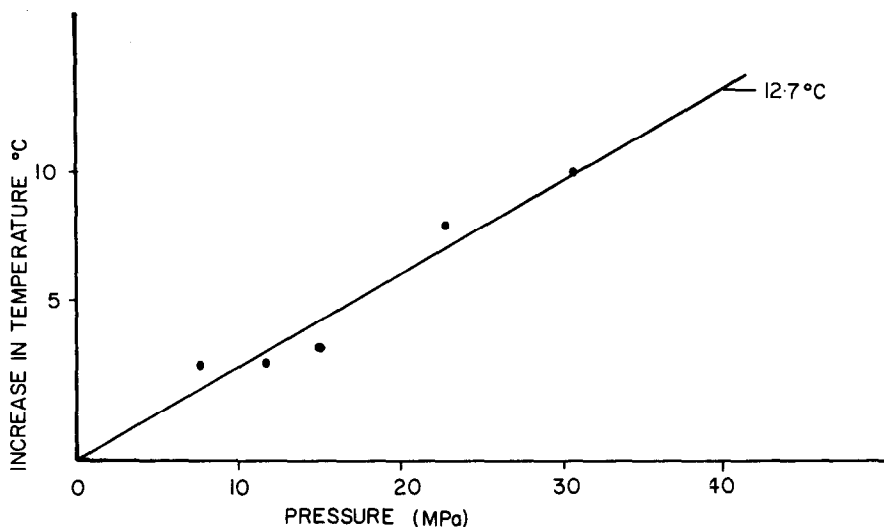


Fig. 6. Graph of temperature against inlet pressure for a water-thermostated microbore column. Column, 156 cm  $\times$  0.76 mm I.D.

larger bore analytical column reflecting a much smaller temperature change due to viscous dissipation of heat; indeed, the slope is just barely statistically significant. The predominant factor in the reduction of the temperature change was the reduction of the column diameter from 0.46 to 0.076 cm. Generally, reduction in column diameter has three consequences. First, although the heat capacity of the column is reduced, a much lower flow-rate was employed to achieve the same linear mobile phase velocity (consequently the same column efficiency) and thus much less heat is generated. Second, the reduced diameter permits heat to be conducted from it far more readily. Hence, for accurate retention measurements, the column should have the minimum radius, be constructed of a material with high thermal conductivity and be thermostated using a high thermal capacity liquid such as water.

#### RELATIONSHIP BETWEEN VISCOSITY AND DIFFUSIVITY

In general, solute diffusivity in a given solvent is inversely proportional to the viscosity of the solvent but the precise form of the relationship for specific solvents is uncertain. It is therefore of interest to determine experimentally the diffusivity of a given solute in a series of solvents of known but different viscosities. From such data, the true relationship between viscosity and diffusivity could be identified. If it is found that there is indeed a change in both viscosity and diffusivity with pressure the relative change in these variables should therefore be in accordance with the above relationship.

#### *Experimental*

The solvents employed were solutions (*ca.* 5%, w/v) of ethyl acetate in a series of *n*-alkanes from *n*-pentane to *n*-decane. The viscosity of each solution was measured employing the Ostwald viscometer thermostated at 25°C. The calibration solvent mixture was pure *n*-hexane. The results obtained are given in Table IX.

TABLE IX

DIFFUSIVITY VALUES OF BENZYL ACETATE IN SOLVENT MIXTURES OF DIFFERENT VISCOSITIES

<i>Solvent composition</i> [% (w/v) ethyl acetate]	<i>Alkane</i>	<i>Viscosity (<math>\eta</math>)</i> (cP)	<i>Diffusivity (<math>D</math>)</i> (cm <sup>2</sup> /sec)
4.7	<i>n</i> -Pentane	0.225	$3.61 \cdot 10^{-5}$
4.9	<i>n</i> -Hexane	0.297	$3.06 \cdot 10^{-5}$
4.3	<i>n</i> -Heptane	0.386	$2.45 \cdot 10^{-5}$
4.5	<i>n</i> -Octane	0.509	$2.01 \cdot 10^{-5}$
4.4	<i>n</i> -Nonane	0.626	$1.65 \cdot 10^{-5}$
4.8	<i>n</i> -Decane	0.792	$1.46 \cdot 10^{-5}$

Result of curve fit to function  $D = A\eta^B$ :  
 Index of determination 0.995:  
 Constant  $A = 1.205 \cdot 10^{-5}$   
 Constant  $B = -0.746$ .

The diffusivity of benzyl acetate was measured in each of the above solvents employing an LC-85B low-dispersion detector, a Valco valve (sample volume 0.2  $\mu$ l) and a Series 3 pump.

From the Golay equation<sup>6</sup> describing the dispersion in a capillary tube with no retentive phase:

$$H = \frac{2D}{u} + \frac{r^2 u}{24 D}$$

where  $H$  is the height equivalent to a theoretical plate of the tube,  $r$  is the radius of the tube,  $D$  is the diffusivity of the solute in the mobile phase and  $u$  is the linear velocity of the mobile phase. As in general  $D$  is about  $10^{-5}$  cm<sup>2</sup>/sec and  $u$  about  $10^{-1}$  cm/sec,

$$\frac{2D}{u} \ll \frac{r^2 u}{24 D}$$

Further noting that the flow-rate  $Q = \pi r^2 u$  and rearranging, we obtain

$$D = Q/24 \pi H \quad (4)$$

A straight stainless-steel capillary tube 365 cm long and 0.015 cm I.D. was connected between the Valco valve and the LC-85B detector. It was extremely important to ensure that there was no secondary flow which would aid diffusion and this would take place if the tube is coiled. In fact, a significant change in band dispersion was observed even when the tube was formed in a coil 50 cm in diameter. Thus, to obviate any likelihood of secondary flow occurring, the tube was formed into a straight length from injection valve to detector. The mobile phase flow-rate was kept constant at 0.5 ml/min and the value of  $H$  for benzyl acetate (taken as the average of three replicates) was measured for each mobile phase. The results obtained are also given in Table IX.

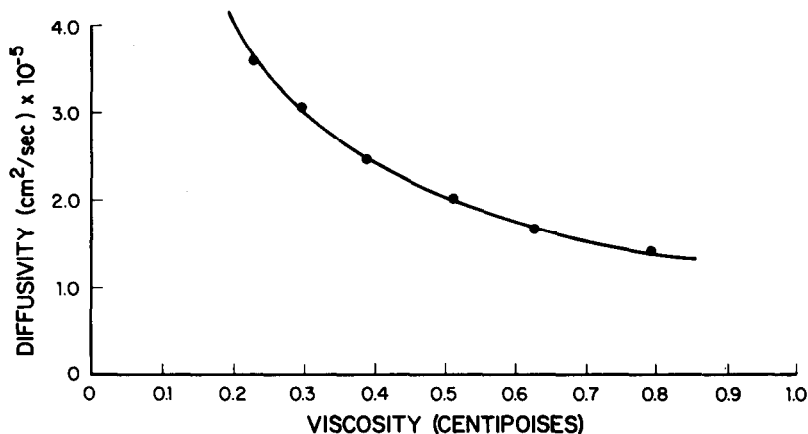


Fig. 7. Graph of solute diffusivity against viscosity (benzyl acetate in *n*-alkanes).

The values of the solute diffusivity and the solvent viscosity from Table IX were fitted to a power function and the results are shown as a graph relating the diffusivity with viscosity in Fig. 7. From the fit, it is seen that the following equation empirically describes the relationship between  $D$  and  $\eta$ :

$$D = 1.205 \cdot \eta^{0.746} \quad (5)$$

It can be seen from eqn. 5 that a change in viscosity of the ethyl acetate solution in *n*-hexane ( $\eta \approx 0.3$  cP) of 10% would result in a change in the diffusivity of benzyl acetate of about 6.9% but the magnitude of the change will depend on the physical properties of solute and solvent. It follows that the change in diffusivity of benzyl acetate in a solution of 5% (w/v) ethyl acetate in an *n*-alkane will be less than the corresponding change in viscosity. Furthermore, as the experimentally observed change in viscosity of the *n*-hexane + 5% ethyl acetate solvent mixture was 1.16% per MPa, then a change of about 0.80% in diffusivity would be expected. The curve fit of the data in Table IX to the power function gives a strong indication that the solute diffusivity varies as the viscosity of the solvent to the power 0.75 the power relationship is in keeping with the equation of Arnold<sup>7</sup> and Hiss and Cussler<sup>8</sup>.

#### EFFECT OF PRESSURE ON SOLUTE DIFFUSIVITY

Changes in diffusivity with pressure can also be determined by observing peak dispersion in a chromatographic system with pressure. However, if the effect of pressure on diffusivity is small, its effect on dispersion is likely to be slight. Thus, to observe accurately the change in band dispersion, the dispersion arising from the chromatographic system has to be made large compared with the dispersion from the instrument employed. *Ipsa facto*, a standard packed column of 4.6 mm I.D. needs to be used for such measurements. It is also necessary to operate the column under conditions where the band dispersion is predominantly dependent on the solute diffusivity, *i.e.*, where the major factor controlling the value of  $H$  is the resistance to

mass transfer in the mobile phase. Over the practical range of mobile phase linear velocities, the HETP of a column is best described by the Van Deemter equation<sup>9-11</sup>.

$$H = \lambda d_p + \frac{2\gamma D}{u} + \frac{f(k')d_p^2 u}{D}$$

where  $\lambda$  and  $\gamma$  are constants,  $d_p$  is the particle diameter of the packing and  $k'$  is the capacity factor of the solute. Again, for  $D \approx 10^{-5}$  cm<sup>2</sup>/sec, at high velocities, the equation reduces to

$$H = A + \frac{C'}{D} \cdot u$$

where  $A$  and  $C'$  are constants. Thus, if  $H$  is determined over a range of values for  $u$  and the results are fitted to a linear function, from which  $A$  is determined as the intercept. Then

$$\frac{C'}{D} \cdot u = H - A \quad (6)$$

### Experimental

The same apparatus was used as for the measurement of change of viscosity with pressure except that the LC-75 detector was replaced with the low-dispersion LC-85B detector to ensure that extra column dispersion was negligible compared with that from a column of 4.6 mm I.D. The detector output was monitored by a Bascom-Turner recorder. The column exit was connected to the detector by means of a 5 cm length of 0.25 mm I.D. tube: this tube could be successively crimped by means of a pair of pliers in order to raise the mobile phase pressure in the column without changing the flow-rate. The column was thermostated at 25°C.

First, the HETP curve for benzyl acetate was determined over a linear velocity range of 0.05–0.5 cm/sec. The efficiency of the benzyl acetate was taken as four times the square of the ratio of retention distance to the peak width measured at 0.6065 of the peak height.  $H$  was calculated as the ratio of the column length to the column efficiency. The results obtained are given in Table X.

Second, the effect of pressure on HETP was measured. The tube connecting the column and detector was then crimped to increase the inlet pressure by about 5 MPa and the exit flow and the retention time of benzyl acetate were measured. The pump flow-rate was then increased until the retention time of *p*-xylene was the same as in the first experiment. The exit flow, the retention time of *p*-xylene and the efficiency of the benzyl acetate peak were then measured. This procedure was repeated until the maximum pump inlet pressure (determined by the maximum operating pressure of the Valco valve) was reached, namely 36 MPa. The results obtained are given in Table XI. The above experiment was repeated with the column removed from the thermostated bath and the column temperature controlled only by ambient air. The results are shown in Table XII.

The data given in Table X were fitted to a linear function and the coefficients

TABLE X

## HETP VALUES OF DIFFERENT MOBILE PHASE VELOCITIES FOR A CONVENTIONAL PACKED COLUMN NOT THERMOSTATED

Column length 25 cm; I.D. 4.6 mm.

Linear velocity ( $u$ ) (cm/sec)	HETP ( $H$ ) (cm)
0.250	0.00261
0.376	0.00301
0.497	0.00377
0.616	0.00442
0.736	0.00531
0.849	0.00565

Result of curve fit to function  $H = A + Bu$ :

Index of determination 0.988:

Constant  $A = 1.12 \cdot 10^{-3}$ Constant  $B = 5.42 \cdot 10^{-3}$ .

TABLE XI

## HETP AND RESISTANCE TO MASS TRANSFER FACTORS FOR A PACKED WATER-THERMOSTATED COLUMN OPERATED AT A CONSTANT LINEAR VELOCITY BUT DIFFERENT ABSOLUTE PRESSURES

Column length 25 cm; I.D. 4.6 mm; mobile phase velocity 0.497 cm/sec.

Mean pressure ( $P$ ) (MPa)	HETP ( $H$ ) (cm)	Resistance to mass transfer factor ( $H - A$ ) = $Cu$ (cm)
3.8	$3.77 \cdot 10^{-3}$	$2.65 \cdot 10^{-3}$
3.9	$3.77 \cdot 10^{-3}$	$2.65 \cdot 10^{-3}$
7.4	$3.69 \cdot 10^{-3}$	$2.57 \cdot 10^{-3}$
7.8	$3.67 \cdot 10^{-3}$	$2.55 \cdot 10^{-3}$
11.9	$3.79 \cdot 10^{-3}$	$2.67 \cdot 10^{-3}$
12.2	$3.93 \cdot 10^{-3}$	$2.81 \cdot 10^{-3}$
18.2	$3.96 \cdot 10^{-3}$	$2.83 \cdot 10^{-3}$
18.6	$3.91 \cdot 10^{-3}$	$2.79 \cdot 10^{-3}$
21.7	$3.93 \cdot 10^{-3}$	$2.82 \cdot 10^{-3}$
22.8	$4.04 \cdot 10^{-3}$	$2.92 \cdot 10^{-3}$
26.5	$4.13 \cdot 10^{-3}$	$3.01 \cdot 10^{-3}$
28.7	$4.04 \cdot 10^{-3}$	$2.93 \cdot 10^{-3}$
31.5	$4.05 \cdot 10^{-3}$	$2.92 \cdot 10^{-3}$
33.5	$4.14 \cdot 10^{-3}$	$3.02 \cdot 10^{-3}$

Result of curve fit to function  $Cu = A + BP$ :

Index of determination = 0.838:

Constant  $A = 2.55 \cdot 10^{-3}$ Constant  $B = 1.41 \cdot 10^{-5}$ .



TABLE XII

HETP AND RESISTANCE TO MASS TRANSFER FACTORS FOR A PACKED UNTHERMOSTATED COLUMN OPERATED AT A CONSTANT LINEAR VELOCITY BUT DIFFERENT ABSOLUTE PRESSURES

Column length 25 cm; I.D. 4.6 mm; mobile phase velocity 0.615 cm/sec.

Mean pressure $P$ (MPa)	HETP ( $H$ ) (cm)	Resistance to mass transfer factor ( $H - A$ ) = $Cu$ (cm)
5.8	$4.27 \cdot 10^{-3}$	$3.15 \cdot 10^{-3}$
10.2	$4.31 \cdot 10^{-3}$	$3.19 \cdot 10^{-3}$
15.3	$4.37 \cdot 10^{-3}$	$3.25 \cdot 10^{-3}$
20.9	$4.27 \cdot 10^{-3}$	$3.15 \cdot 10^{-3}$
24.8	$4.43 \cdot 10^{-3}$	$3.31 \cdot 10^{-3}$
33.2	$4.52 \cdot 10^{-3}$	$3.40 \cdot 10^{-3}$

Result of curve fit to function  $Cu = A + BP$ :

Index of determination = 0.681:

Constant  $A = 3.09 \cdot 10^{-3}$

Constant  $B = 8.18 \cdot 10^{-6}$ .

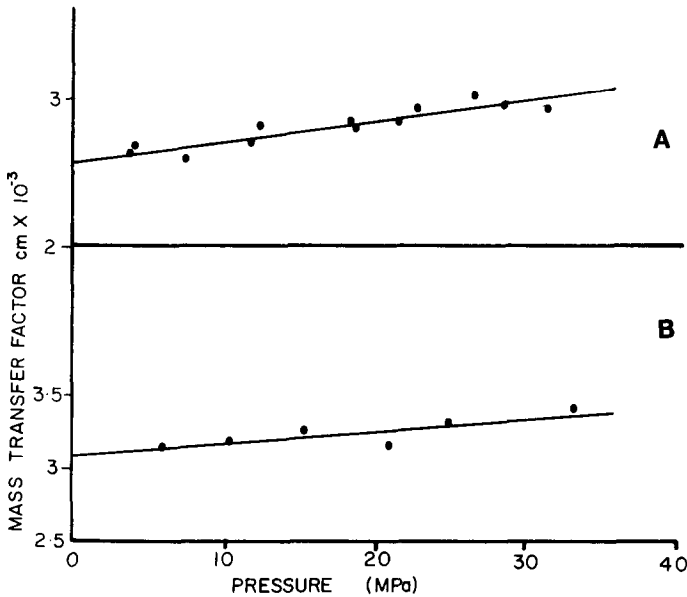


Fig. 8. Graphs of the resistance to mass transfer factor against inlet pressure for (A) water-thermostated and (B) unthermostated columns. Columns, 25 cm  $\times$  4.6 mm I.D. Note that mass transfer factor  $\neq 0$  at the origin.

are also included in Tabel X. From the intercept given as  $A$  in Table X, the multipath term was found to be  $1.12 \cdot 10^{-3}$  cm.

The values for  $H$  obtained for the thermostated and unthermostated column were corrected for the multipath contribution and the values of  $H - A$  and the corresponding values of pressure were fitted to a linear function and the results are included in Tables XI and XII. The values of  $H - A$  are shown plotted against the pressure for the thermostated and unthermostated columns in Fig. 8. It is seen that the solute dispersion changes very little for the unthermostated column but the results from the thermostated column show a 0.26% change per MPa. Thus, for an inlet pressure of 30 MPa, which would be equivalent to an average pressure of 15 MPa, a change of 4.0% could be expected in the resistance-to-mass transfer term in the HETP equation. However, as the change in diffusivity would also be linearly related to the inlet pressure, the overall resistance-to-mass transfer term would still be sensibly linear with velocity.

Thus, for wide-bore unthermostated conventional columns, pressure-induced effects on the solute diffusivity are small, smaller than would be expected from the direct effect of pressure alone. This is evidently due to the counteracting effect of temperature increases due to viscous dissipation of heat. For the water-thermostated column, the change in the resistance-to-mass transfer term, at a linear velocity of 0.5 cm/sec, is 0.55% per MPa. This value compares reasonably well with the value of 0.80% predicted from the change in the viscosity with pressure of 1.16% and the relationship between viscosity and diffusivity described by eqn. 5. At a column inlet pressure of 40 MPa, equivalent to an average pressure of 20 MPa, this results in a 11.1% change in the  $C$  term. The net effect on the measurements of dispersion curves, or on the measurement of resistance-to-mass transfer coefficients, cannot be readily determined insofar as the temperature changes due to viscous dissipation are very much a function of the specific geometry of the system, as well as strongly dependent on the flow velocity.

The effect will range from virtually zero for an unthermostated conventional column to a maximum of 10% for a well thermostated column of appropriate geometry operated at an inlet pressure of 40 MPa. For reversed-phase columns employing methanol-water as the mobile phase the change in diffusivity should be less than 4% even with a well thermostated column because the effect of pressure on the viscosity of the solute diffusivity in aqueous solvent mixtures is much less than that of the normal paraffins.

## CONCLUSION

The effect of inlet pressure on column performance is very complex. An increase in column pressure directly increases the viscosity of the mobile phase and decreases the diffusivity of the solute and can increase the overall column temperature. However, an increase in temperature will also decrease the viscosity of the mobile phase and increase the diffusivity of the solute, thereby tending to compensate for the primary effect of pressure on these variables. Owing to this compensation effect, increasing the pressure of an unthermostated conventional column has a minimal effect on column efficiency. At the other extreme, for a well thermostated microbore column employing a mobile phase, the major component of which is an  $n$ -

alkane, then an inlet pressure of 40 MPa could change the solute diffusivity by as much as 11%. This would be the maximum change because, of the typical solvents used for LC, the *n*-alkanes exhibit the greatest change in viscosity and solute diffusivity with pressure. Consequently, a change of 11% in column efficiency could result. Well thermostated analytical reversed-phase columns employing methanol-water as the mobile phase would show little change in viscosity and solute diffusivity with pressure and the column efficiency would be virtually independent of pressure for inlet pressures up to 40 MPa. However, retention data can be seriously effected at high column inlet pressures. The heat evolved on changing the flow-rate from about 4 to 18 ml/min, with a corresponding pressure change from 5 to 30 MPa, can produce an 18°C rise in effective column temperature and a consequent 6% reduction in capacity ratio. This effect can be extremely serious for short columns packed with small particles and operated at high pressure such as those used by Katz and Scott<sup>12</sup> and DiCesare *et al.*<sup>13</sup>. The results indicate that, for general chromatographic analysis involving accurate retention measurements, microbore columns should be employed. Further, the thermostating medium should have high thermal capacity and the microbore column should be constructed from material that has a high thermal conductivity compatible with appropriate inertness and mechanical strength.

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