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THE USE OF SYRINGE-TYPE PUMPS IN LIQUID CHROMATOGRAPHY IN ORDER TO ACHIEVE A CONSTANT FLOW-RATE

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SUMMARY

The variation of the flow-rate of mobile phase pumped through a chromatographic column by a screw-driven syringe-type pump is discussed. It is shown that because of the compressibility of liquids, a steady-state flow is achieved only after a long period, which depends on the characteristics of the pump, the flow resistance of the column and the nature of the liquid. This period is normally between 15 and 60 min if liquid pumping starts from zero pressure at a constant piston speed.

The retention times observed for compounds injected during the transitory period can be up to several times greater than those observed for the same compounds under steady-state conditions. All retention data and efficiencies measured during the transitory period are not reproducible and are meaningless. Quantitative results also are not reproducible.

Instructions are given for the better use of this type of pump, which, in spite of some well-known advantages, has been found to be very difficult to use for any measurements.

INTRODUCTION

Single-stroke displacement pumps are widely used in liquid chromatography and are available from several manufacturers¹. It seems to be generally accepted that these pumps offer several important advantages², such as a very constant flow-rate and inlet pressure, with no pulsations, independent of the value of the downstream back-pressure and of its possible variations due to column switching, thus offering better baseline stability and a lower detection limit than are possible with reciprocating pumps. Moreover, a constant flow-rate is particularly necessary in high-precision quantitative analysis, because with the most commonly used concentration-sensitive liquid chromatographic (LC) detectors (UV absorptiometers, differential refractometers, etc.) the peak area is inversely proportional to the flow-rate of the mobile phase³. In spite of the mechanical problems, which are mainly associated with the design of a leak-proof system that will permit the use of a wide variety of aggressive solvents,

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pumps are commercially available that operate at pressures above 500 atm and their only disadvantages seem to be the impossibility of recycling the column effluent through the pump and the limited volume of solvent held in the cylinder. This volume is usually 250–500 cm³, while the flow-rate through LC columns is usually from 0.2 to a few cm³/min, so that the latter drawback is not serious (in special cases, pumps with flow-rates as low as 0.05 cm³/min or as high as 16 cm³/min are available and as these pumps are silent, reliable and easy to operate, it seems that only their price prevents them having a monopoly).

This idealistic situation is, in fact, far from the truth. In this paper, we show that because of the compressibility of liquids, the period necessary to achieve a steady-state flow-rate is very long (typically 15–60 min) and the variations in retention data measured during this period are very important, so that if very careful attention is not paid to operating the pump under well defined conditions, unreliable results are achieved, with a very large spread of retention times and relative retentions even for closely eluted compounds, of efficiencies and of quantitative results. The situation is so serious as to cast doubt on the reliability and even the validity of HETP curves and retention data tables published in the literature by workers who have used this type of pump. The situation could even be catastrophic in gradient elution. The problem is made all the more important as a result of the increasing application of stop-flow injection techniques. Fortunately, some rules, if they are strictly adhered to, may help in improving the reproducibility of the results, but syringe-type pumps will remain difficult to use.

COMPRESSIBILITY OF LIQUIDS

The compressibility of liquids is not zero, as is usually assumed by chemists performing LC analysis, but it is very small (*cf.* Table I). It has been demonstrated

TABLE I
PHYSICAL CHARACTERISTICS OF LIQUIDS USED AS SOLVENTS IN CHROMATOGRAPHY

The compressibilities (χ) are given at 25° and the viscosities (η) at 30°, so the $\chi\eta$ values are only approximate.

<i>Liquid</i>	<i>Compressibility under atmospheric pressure, $\chi \cdot 10^4$ (atm⁻¹)</i>	<i>Viscosity under atmospheric pressure, D (cP)</i>	<i>Pressure dependence of viscosity (eqn. 5), $0 \cdot 10^3$ (atm⁻¹)</i>	<i>Product $\chi\eta$ under atmospheric pressure $\cdot 10^4$ (cP · atm)</i>
<i>n</i> -Pentane	3.14	0.220	1.06	0.691
<i>n</i> -Hexane	1.61	0.296	1.15	0.477
<i>n</i> -Heptane	1.42	0.355	1.09	0.504
<i>n</i> -Octane	1.20	0.483	1.12	0.580
Benzene	0.96	0.566	1.22	0.543
Chloroform	0.97	0.519	0.625	0.503
Carbon tetrachloride	1.07	0.845	1.25	0.904
Diethyl ether	1.87	0.212	1.11	0.396
Acetone	1.24	0.285	0.684	0.353
Methanol	1.23	0.520	0.470	0.640
Ethanol	1.11	1.003	0.585	1.113
Water	0.46	0.80	0.053	0.368

that this compressibility has negligible effects on the retention time of an inert or a retained compound, provided that the inlet pressure is below 200–300 atm⁴. In the future, many analyses may be carried out at pressures much lower than is usual today in high performance LC⁵, so that this effect can justifiably be neglected, and will be henceforth in this paper.

This is not, however, the only effect of compressibility on the operation of LC equipment using a positive displacement pump, because (a) the pump cylinder has a much larger volume than that of the column and (b) the pressure in the pump is twice the average pressure along the column. An order of magnitude change in liquid volume can be obtained from Table 1, which shows that χ is of the order of 10^{-4} atm⁻¹. For $\Delta P = 100$ atm and $V_0 = 500$ cm³ under atmospheric pressure, the reduction in liquid volume is 5 cm³.

In a screw-driven syringe-type pump, the carrier liquid is pushed through the column by a piston that is moved at a constant speed under isocratic conditions. The corresponding geometrical displacement is called the piston flow. In gradient elution, the movements of the pistons of two syringe-type pumps are programmed. If there is no flow resistance downstream from the pump (infinite column permeability), a constant flow-rate is obtained. However, because the compressibility, χ , of the liquid is finite, the flow-rate small and the cylinder volume large, a long period is required for a steady-state flow-rate to be reached. Consequently, the liquid flow-rate at the outlet of the pump, and hence through the column, is not equal to the piston flow.

If the cylinder is closed by a valve, the pressure and volume are related by the equation

$$V = V_0 e^{-\chi(P-P_0)} \quad (1)$$

If Q_0 is the piston flow, which is set by the analyst, the apparent volume at time t is

$$V = V_0 - Q_0 t \quad (2)$$

The time necessary to reach a pressure P in such a closed pump is thus

$$t = \frac{V_0}{Q_0} [1 - e^{-\chi(P-P_0)}] \quad (3)$$

In this derivation, we have assumed that χ is constant, which is true only in a limited pressure range (1–500 atm)⁴. Further, the dilatation of the cylinder itself has been neglected. From Hooke's law:

$$\frac{S}{S_0} = \left\{ 1 + \frac{2(P-P_0)}{E \left[1 - \left(\frac{r_0}{R_0} \right)^2 \right]} \right\} \quad (4)$$

where r_0 and R_0 are the inner and outer diameters of the cylinder under normal pressure, S and S_0 its section under pressures P and P_0 , respectively, and E the Young's modulus of the metal. For example, for a stainless-steel ($E = 2 \cdot 10^6$ atm) cylinder, 5 cm I.D., 6 cm O.D., at 500 atm pressure, $S/S_0 = 3 \cdot 10^{-3}$. The increase in the length of the cylinder is of the same order of magnitude. The variation of the liquid volume

itself is thus more than 10 times greater than the variation of the geometrical volume of the cylinder, which supports our assumption. Whenever the variation of the volume of the cylinder is not negligible, the time needed in order to reach the steady-state conditions is increased. Because of the low value of χ , in most practical cases in LC, the curves $P(t)$ given by eqn. 3 are nearly straight lines. An example is given in Fig. 1, which shows the results for different liquids in a typical case of a 500-cm³ pump compressed with a piston flow of 1 cm³/min. More than 15 min are necessary in order to reach 100 atm with *n*-pentane and 200 atm with *n*-heptane. Other results are given in Table II.

Fig. 2 shows the reduction of the apparent volume of the closed cylinder as a function of pressure. The period during which the equipment can operate before a refill of the pump is necessary is reduced by the same amount.

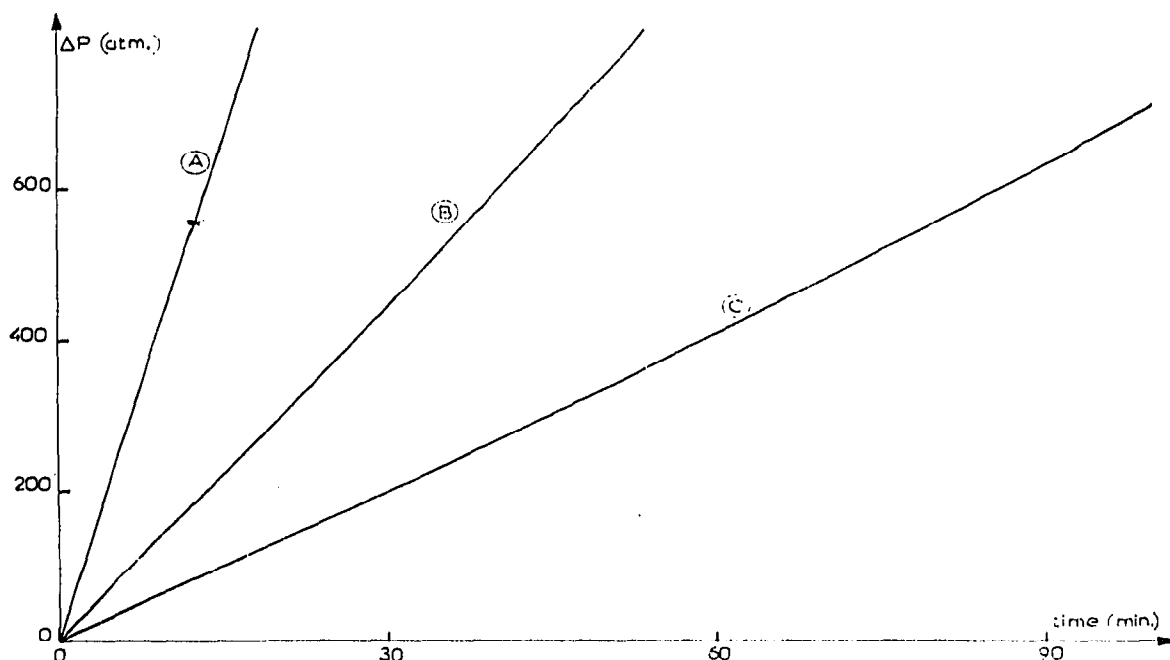


Fig. 1. Variation of pressure with time in a 500 cm³ cylinder full of liquid compressed with a 1 cm³/min piston flow. A, Water; B, *n*-heptane; C, *n*-pentane.

VARIATION OF FLOW-RATE WITH TIME DURING THE TRANSITORY STATE

In this case, the pump is not closed by a valve but is connected to a column of length L , cross-section area S and permeability k . These parameters are virtually independent of pressure⁴. The viscosity of the carrier liquid, η , is given by

$$\eta = \eta_0 [1 + \theta (P - P_0)] \quad (5)$$

Consequently, when the liquid begins to be pumped through the column, its apparent

TABLE II

TIME NECESSARY TO COMPRESS TO A PRESSURE OF 100 ATM A CYLINDER FULL OF LIQUID WITH A CONSTANT PISTON FLOW

From eqn. 3, in almost all cases $t \approx \frac{\chi V_0 \Delta P}{Q_0}$.

Volume under atmospheric pressure (cm ³)	Piston flow (cm ³ /min)	Liquid	Time (min)
500	0.2	Water	115
	0.2	n-Pentane	787
	0.2	n-Heptane	350
	1	Water	23
	1	n-Pentane	157
	1	n-Heptane	70
	5	Water	4.6
	5	n-Pentane	31
	5	n-Heptane	14
100	0.2	Water	23
	0.2	n-Pentane	157
	1	Water	4.6
	1	n-Pentane	31
	5	Water	0.9
	5	n-Pentane	6.3

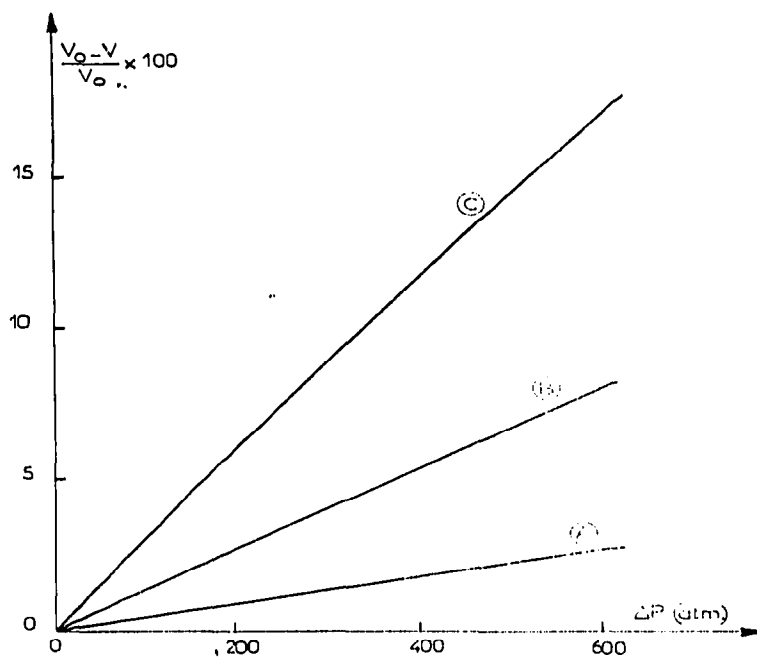


Fig. 2. Relative variation of the volume of a liquid under pressure. Liquids as in Fig. 1.

viscosity varies with time during the transitory period. As this variation is limited (θ is typically 10^{-3} atm $^{-1}$), it is appropriate and sufficiently accurate to assume that the viscosity is constant and equal to its average value, $\bar{\eta}$, under the steady-state conditions⁴. Then,

$$Q_0 = \frac{kS}{\bar{\eta}L} \cdot \Delta P_\infty \quad (6)$$

where ΔP_∞ is the column pressure drop at infinite time. By integration of the Darcy equation along the column⁴, it can be shown that

$$\Delta P_\infty = \frac{1}{\theta} \left(e^{\frac{\eta_0 L Q_0 \theta}{kS}} - 1 \right) \quad (7)$$

Combination of eqns. 6 and 7 gives $\bar{\eta}$. If Q is the volume flow-rate of carrier liquid through the column at time t and the pressure is ΔP , then, from our assumption that the viscosity is constant,

$$\frac{Q}{Q_0} = \frac{\Delta P}{\Delta P_\infty} \quad (8)$$

At time t , the liquid flow-rate at the outlet of the pump is the piston flow-rate, Q_0 , less the decrease in volume of the liquid in the cylinder per unit time, $\chi V \cdot dP/dt$. This is equal to the flow-rate through the column given by the Darcy law. We thus obtain the differential equation

$$Q_0 - \chi V \cdot \frac{d\Delta P}{dt} = \frac{kS}{\bar{\eta}L} \cdot \Delta P \quad (9)$$

where ΔP , the pressure drop at time t , and V (given by eqn. 2) are functions of time. Eqn. 9 can then be rewritten as

$$\frac{\chi d\Delta P}{Q_0 - \frac{kS}{\bar{\eta}L} \cdot \Delta P} = \frac{dt}{V_0 - Q_0 t} \quad (10)$$

Integration of eqn. 10 is straightforward, the boundary conditions being $t = 0$, $\Delta P = 0$ and t , ΔP . Taking into account eqn. 6, we obtain

$$t = \frac{V_0}{Q_0} \left[1 - \left(1 - \frac{\Delta P}{\Delta P_\infty} \right)^{\chi \Delta P_\infty} \right] \quad (11)$$

ΔP_∞ is given by eqn. 7.

These equations show that the transitory state is determined by the pump (V_0 , Q_0), the column (L , S , k) and the liquid used (χ , η_0 , θ). The influence of these parameters is illustrated in Figs. 3–6. All numerical calculations were made with a computer. The pressure under steady-state conditions depends on the viscosity of the liquid, the flow-rate desired and the flow resistance. The period necessary for the steady-state to be approached is controlled in addition by the compressibility of the liquid and the

ratio V_0/Q_0 . Under given experimental conditions (χ and ΔP_∞ constant), the time during which the pump can deliver mobile phase to the column is also proportional to V_0/Q_0 , so that a constant fraction of the analyst's time will be spent waiting for equilibrium to be achieved, whether he uses small or large volume pumps. In this case, large pumps will probably be preferred, in order to reduce the number of re-fill and pressurization steps necessary in a working day. Fig. 3, however, shows that over 20 min are necessary under favourable conditions for 98% of the steady-state pressure to be attained.

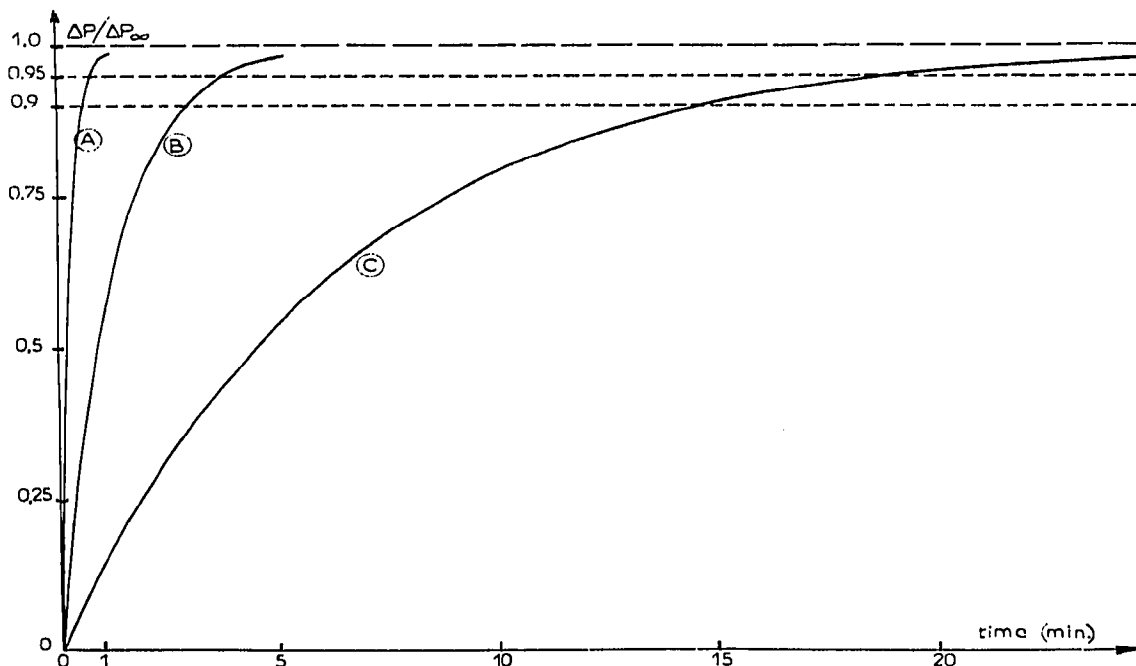


Fig. 3. Variation of inlet pressure with time at the start of an LC analysis: effect of pump volume. Liquid: *n*-heptane. $Q_0 = 1 \text{ cm}^3/\text{min}$. Steady-state pressure: 92 atm. Column: $L = 50 \text{ cm}$; I.D. = 2.2 mm; particle size = $10 \mu\text{m}$. Pump volume (cm^3): A, 20; B, 100; C, 500.

The effect of the carrier liquid is more complex because both its viscosity and compressibility are important factors. A lower viscosity means a lower inlet pressure, which is achieved more rapidly at constant compressibility. Equilibrium is therefore reached more rapidly when using liquids with low viscosity and compressibility (Fig. 4).

The influence of the column resistance on the liquid flow is illustrated in Fig. 5. For a given flow-rate, the resistance depends on the column length and diameter and on the particle size. The results show that it is much easier to use columns at low or moderate pressures, as is now possible with modern column technology^{5,6}; short columns packed with small particles permit the separation of mixtures that require several thousand plates in about 10 min with moderate inlet pressures (5–20 atm), if they are operated near the optimum flow velocity.

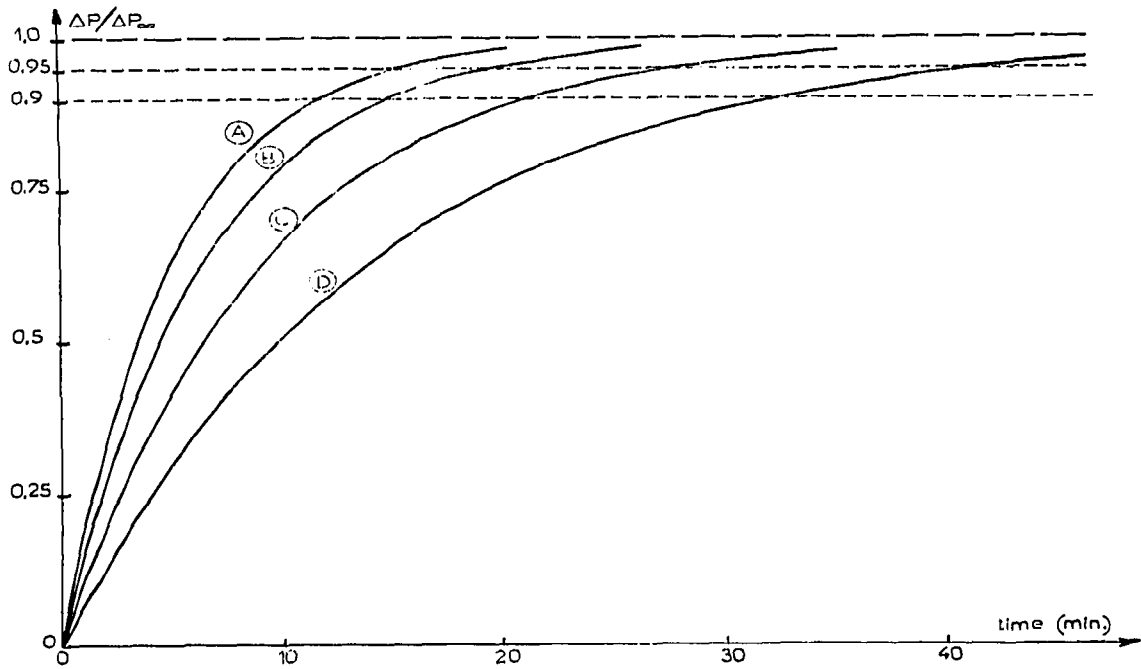


Fig. 4. Variation of inlet pressure with time at the start of an LC analysis: effect of the carrier liquid. Pump: $Q_0 = 1 \text{ cm}^3/\text{min}$; $V_0 = 500 \text{ cm}^3$. Column as in Fig. 3. A: Water, $\Delta P_{\infty} = 220 \text{ atm}$. B: *n*-Heptane, $\Delta P_{\infty} = 92 \text{ atm}$. C: *n*-Pentane, $\Delta P_{\infty} = 59 \text{ atm}$. D: Ethanol, $\Delta P_{\infty} = 247 \text{ atm}$.

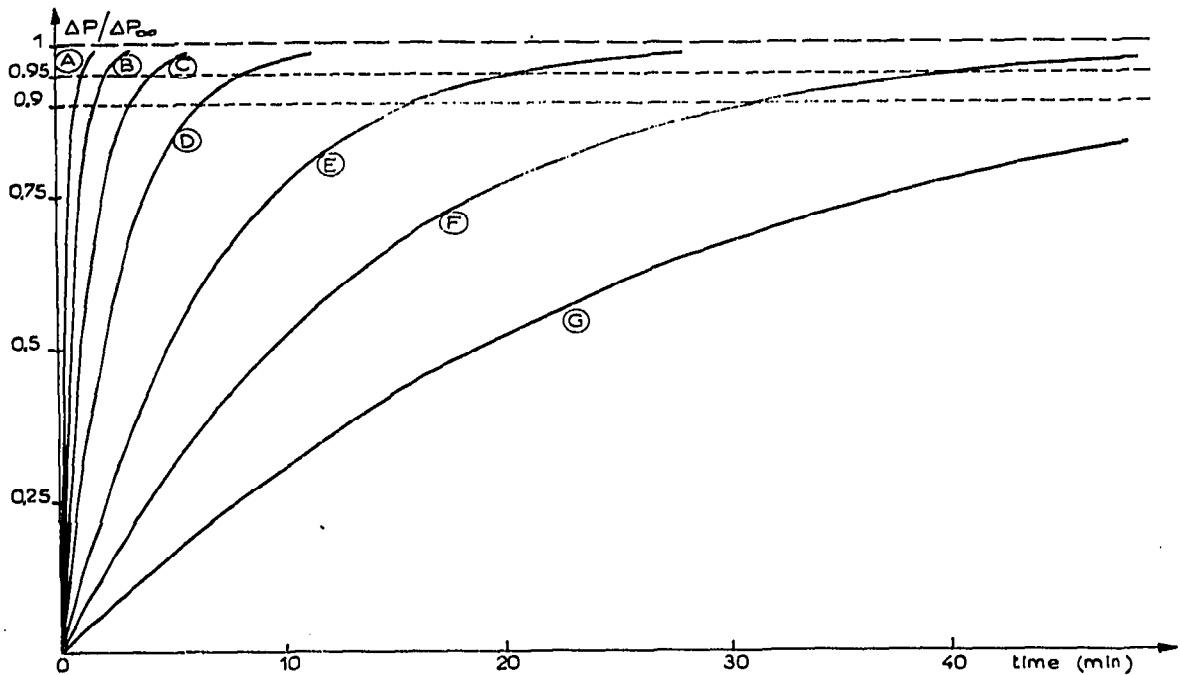


Fig. 5. Variation of inlet pressure with time at the start of an LC analysis: effect of column permeability. Liquid: *n*-heptane. Pump: $Q_0 = 1 \text{ cm}^3/\text{min}$; $V_0 = 500 \text{ cm}^3$. Steady-state pressure (atm): A, 5; B, 10; C, 20; D, 40; E, 100; F, 200; G, 400.

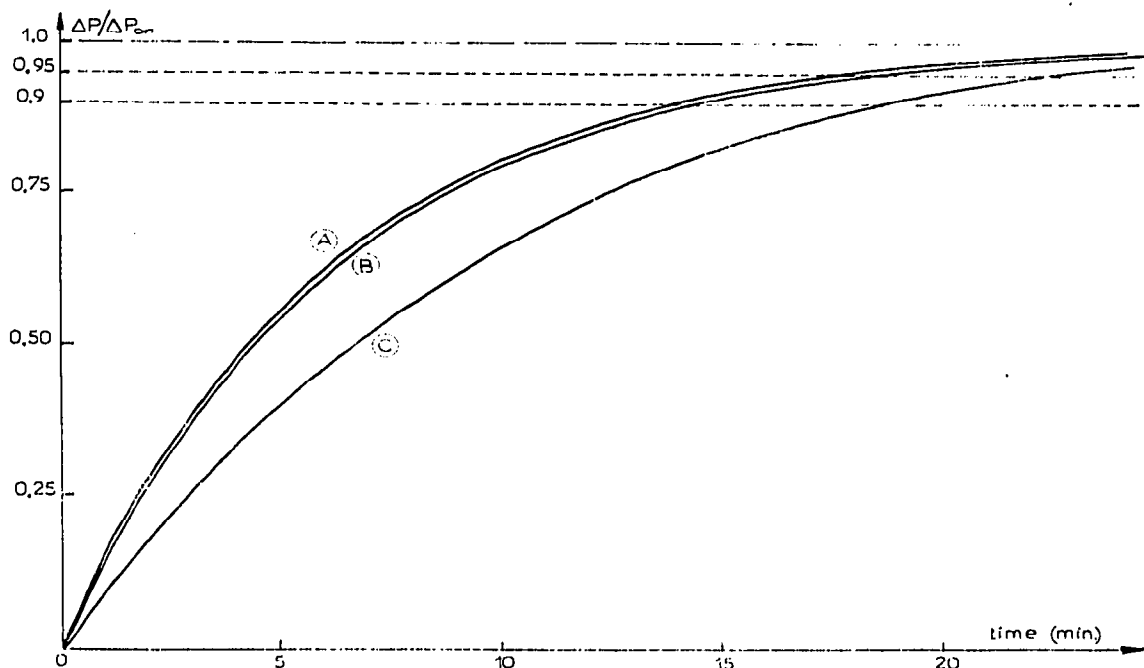


Fig. 6. Variation of inlet pressure with time at the start of an LC analysis: effect of flow-rate. Liquid: *n*-heptane. Pump: $V_0 = 500 \text{ cm}^3$. Column: $L = 50 \text{ cm}$; I.D. = 2.2 mm; particle size = $10 \mu\text{m}$. A: $Q_0 = 0.1 \text{ cm}^3/\text{min}$, $\Delta P_\infty = 8.8 \text{ atm}$. B: $Q_0 = 1 \text{ cm}^3/\text{min}$, $\Delta P_\infty = 92 \text{ atm}$. C: $Q_0 = 10 \text{ cm}^3/\text{min}$, $\Delta P_\infty = 1480 \text{ atm}$.

Fig. 6 illustrates the effect of the flow-rate selected for a given system. Obviously, the steady-state pressure increases with the flow-rate (cf., eqns. 5 and 6). The effect is small provided that the physical characteristics of the liquid (η , χ) do not change significantly with the average pressure.

All of the curves in Figs. 3-6 are similar to exponentials. Identification of eqn. 11 with an exponential of time constant τ_0 [$\Delta P/\Delta P_\infty = 1 - \exp(-t/\tau_0)$] gives

$$\frac{1}{\tau_0} = \frac{1}{\chi \Delta P_\infty} \left(\frac{Q_0}{V_0} + \frac{Q_0^2}{V_0^2} \cdot t + \dots \right) \quad (12)$$

When the pump volume is large compared with the flow-rate, the liquid volume in the pump can be assumed to be constant ($Q_0 t/V_0 \ll 1$), and the variation of pressure is exponential with a time constant given by

$$\tau_0 = \frac{V_0 \chi \Delta P_\infty}{Q_0} = V_0 \chi \bar{\eta} \cdot \frac{L}{kS} \quad (13)$$

which is proportional to the pump volume, the product $\chi \bar{\eta}$ and to the resistance of the column to the liquid flow. The time necessary for 95% of the steady-state pressure to be attained is then $3 \tau_0$.

This time could be reduced by pressurizing the liquid in the pump cylinder before opening the valve between the pump and the column and starting the analysis.

It is difficult to estimate ΔP_∞ when beginning work with a new column, but it is much easier when the column has been tested and its resistance (L/kS) measured. If the initial pressure is ΔP_i , then the time-pressure dependence becomes

$$t = \frac{V_0}{Q_0} \left[1 - \left(\frac{1 - \frac{\Delta P}{\Delta P_\infty}}{1 - \frac{\Delta P_i}{\Delta P_\infty}} \right)^{\frac{\Delta P_\infty}{\Delta P_i}} \right] \quad (14)$$

The variation of ΔP with time for different values of $\Delta P_i/\Delta P_\infty$ is shown in Fig. 7, which shows that a steady state can be achieved much faster by this procedure

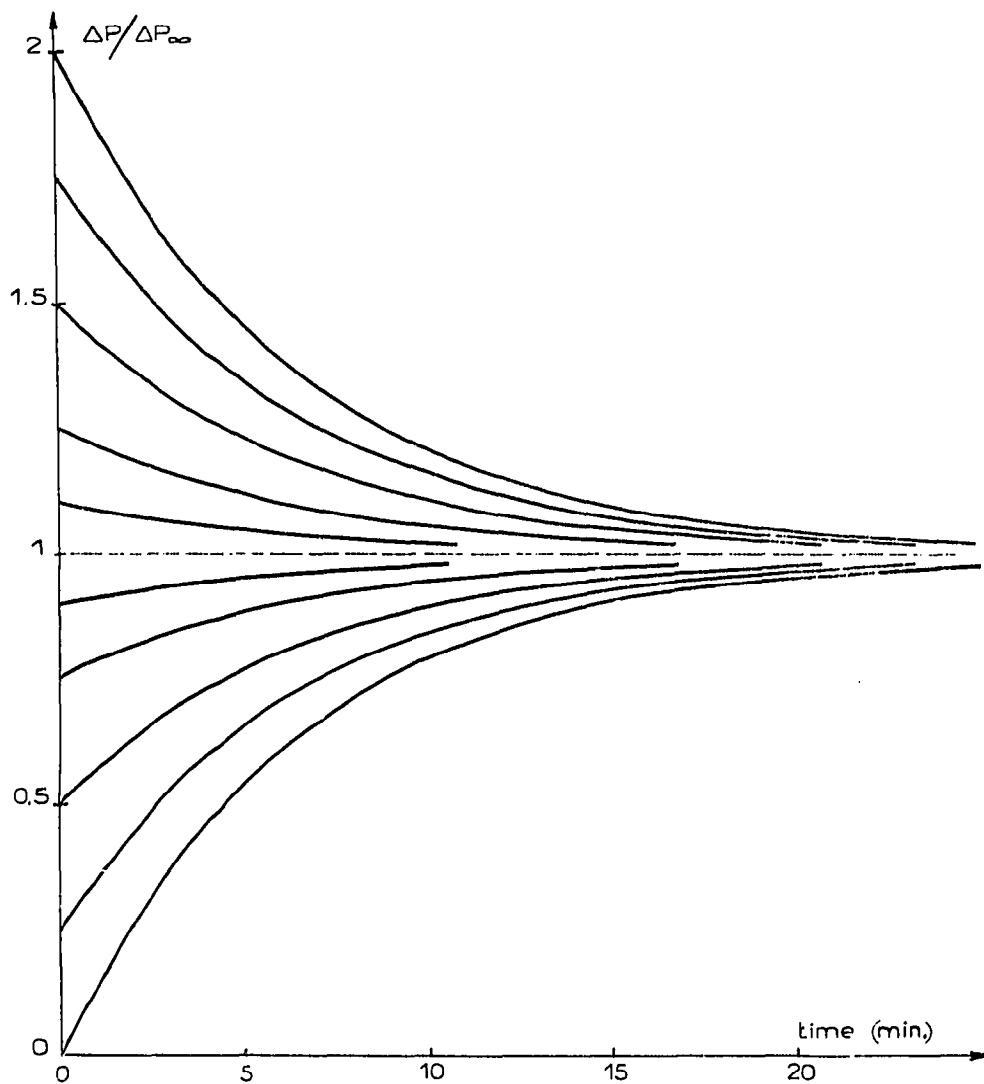


Fig. 7. Variation of inlet pressure with time at the start of an LC analysis: effect of the initial pressure in the pump. Conditions as in Fig. 4 for *n*-heptane.

if no large error is made in the evaluation of ΔP_{∞} . It would be wrong, for example, to begin an analysis at the maximum pressure available. Note that the data in Fig. 7 were obtained by neglecting the variation of liquid volume with pressure, which is only approximate, as shown in Fig. 2; the error, however, is not large in practice.

INFLUENCE OF THE TRANSITORY STATE ON THE REPRODUCIBILITY OF CHROMATOGRAPHIC DATA

In this section, it is assumed that the initial pressure is zero. If the original pressure is intermediate between 0 and ΔP_{∞} , the effect will be smaller, as the transitory state is shorter and the relative pressure variation smaller.

Effect on the retention time

As the flow-rate is lower than the steady-state value, the retention time will be larger than V_R/Q_0 , where V_R is the retention volume. The ratio $t_R/t_{R\infty}$ is larger for an inert peak or for compounds with a low retention than for compounds that are strongly retained on the column: the larger the column capacity ratio, k' , the larger is the average flow-rate during elution (*cf.*, Fig. 3).

Fig. 8 shows the variation of retention time observed as a function of the injection time for compounds with different retentions. Under the conditions selected, which are typical, injections should be made at least 20 min after starting the pump if the true retention time is to be obtained.

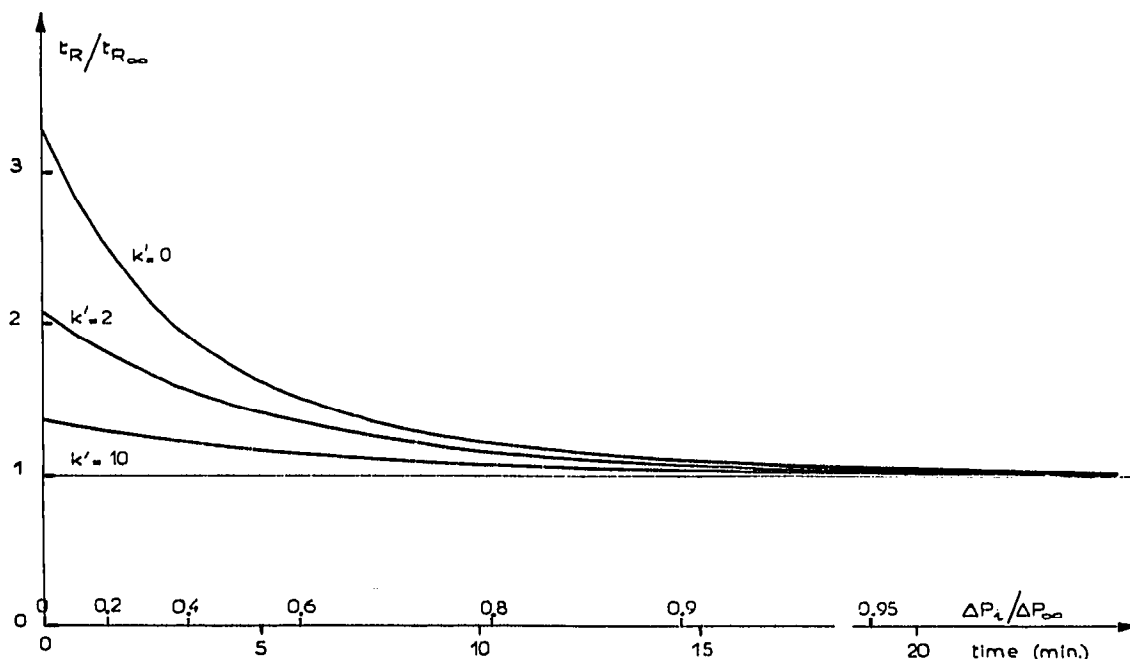


Fig. 8. Effect of the delay between starting the pump and sample injection on the retention time observed. The value of the column capacity factor are the true ones, as measured under steady-state conditions. Conditions as in Fig. 4 for *n*-heptane.

The effect is much greater on an inert peak than on those of retained compounds, which has an important influence on the reproducibility of relative retention data.

It must be noted that it is not equivalent either to inject at pressure ΔP_i some time after starting the pump and allowing the liquid to flow through the column or to inject under stop-flow conditions, the carrier liquid in the pump being pressurized at ΔP_i . In the latter case, the volume of liquid in the pump is greater and so the transitory state and the retention time are longer.

Effect on the apparent column capacity factor (k')

The conventional definition

$$k' = \frac{t_R - t_0}{t_0}$$

results in a meaningful value of the column capacity factor only if the residence times of the unknown compound and the inert compound in the mobile phase are the same, which is not the case here as the speed of the liquid increases with increasing time.

The error made in the determination of k' can be very large, as shown in Fig. 9, as the mobile phase moves much more slowly during the elution of the inert peak than during the elution of the retained peaks. It will therefore be difficult to make reproducible measurements of retention data, and this problem is discussed below.

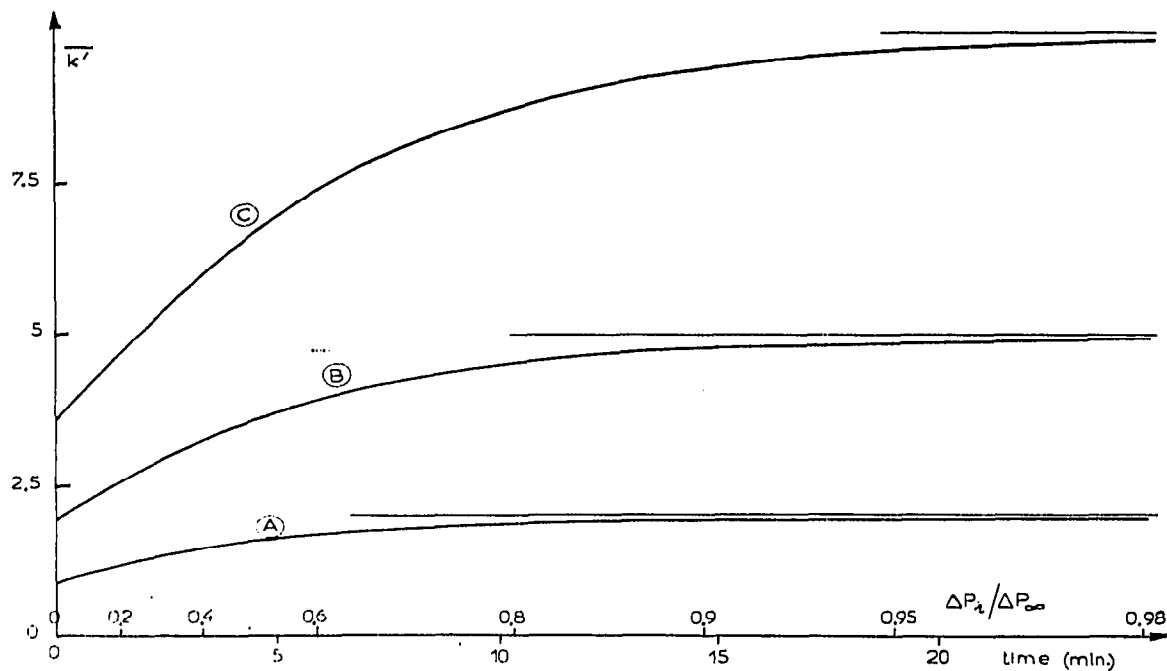


Fig. 9. Effect of the delay between starting the pump and sample injection on the measured column capacity factor, \bar{k}' . Conditions as in Fig. 8. True value of k' : A, 2; B, 5; C, 10.

Effect on the apparent relative retention (α)

As the flow-rate changes during the period the compounds spend in the column, the relative retention will also vary with the injection time. The nearer the two retention times, however, the smaller is the effect, because any variation of flow-rate during the period that the two compounds are both inside the column has no effect on their relative retention time. Only the difference between the average flow-rates during this first period and during the period the second compound alone is inside the column has an effect, and this is prone to decrease steadily with $\alpha - 1$.

As an example, Fig. 10 shows the variation of apparent relative retention with injection time for two compounds with a true relative retention of 1.2. The maximum error is 3%. This error increases rapidly, however, with increasing $\alpha - 1$. The results are virtually the same whether the column capacity factor for the first compound is 2, 5 or 10.

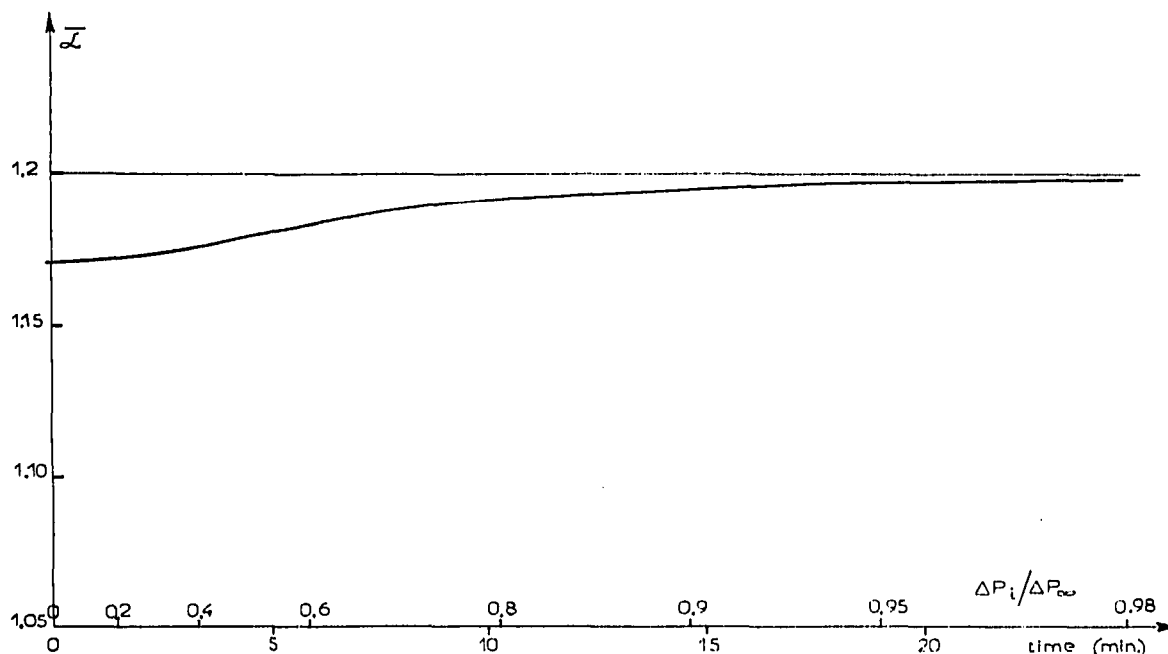


Fig. 10. Effect of the delay between starting the pump and sample injection on the apparent relative retention, $\bar{\alpha}$. Conditions as in Fig. 8. True relative retention: 1.2.

Effect on column performance

Similarly, the column efficiency calculated by applying in a straightforward manner the definition $N = 16(t_R/\omega)^2$, where N is the number of theoretical plates, is an average value that is different from that measured under steady-state conditions, as the observed peak width integrates the effects of all sources of peak broadening during peak migration. As some of these sources do not depend linearly on flow-rate, the overall result is complicated. Depending on the k' value and whether the flow-rate under steady-state conditions is much greater than the optimum value or is of the same

order, the peak width, in time units, at the outlet of the column is lower or higher than the true peak width. However, in all instances, the increase in retention time is greater than that of time variance, and so the apparent column plate number is greater than the true value (Fig. 11). The effect can be very important, the plate number appearing to be several times greater than that under steady-state conditions.

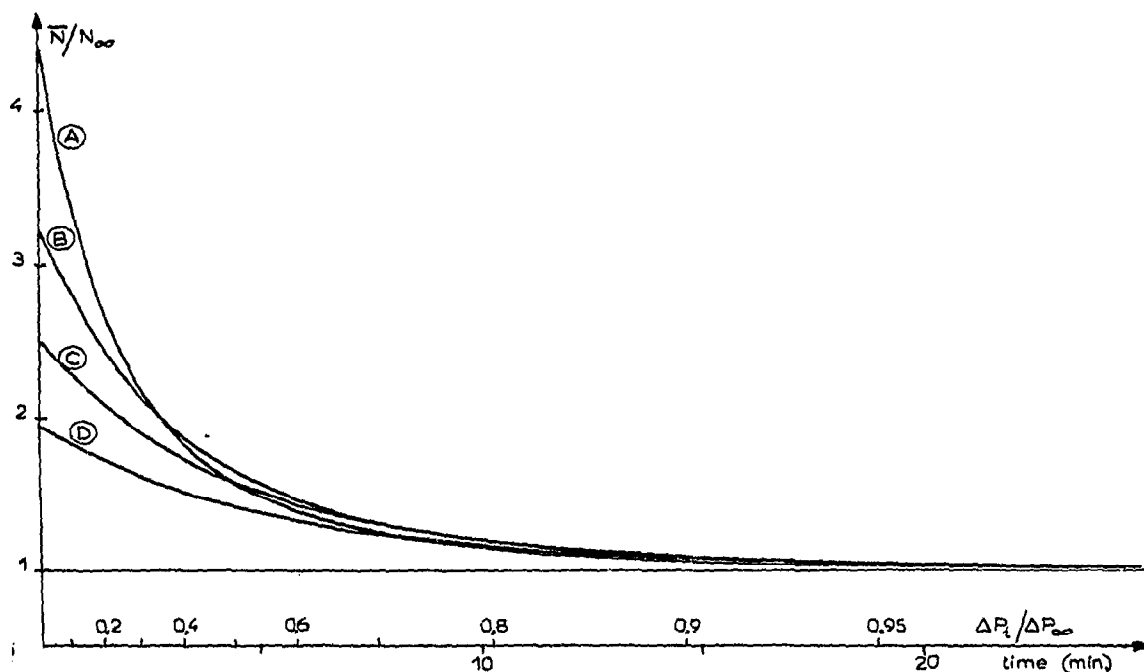


Fig. 11. Effect of the delay between starting the pump and sample injection on the apparent efficiency of the peak of a pure compound, \bar{N} . Conditions as in Fig. 8; $N = 9700$; v_r (reduced velocity in steady-state conditions) = 15 ($v_{opt} = 2.15$). k' : A, 0; B, 2; C, 5; D, 10.

The resolution between two peaks is also changed. Depending on the relative increase in the retention time difference and in the peak width, the resolution is greater or smaller than the steady-state value. These two possibilities are illustrated by Figs. 12 and 13. In both instances, however, the change in resolution is less than 15%.

PRACTICAL CONSEQUENCES ON THE OPERATION OF SYRINGE-TYPE PUMPS

From the previous section, the different parameters measured during the transitory state, t_R , k' , α , N and R , can be seen to be meaningless. The retention data obtained under such conditions, and also the HETP curves, are incorrect and unreliable, and many data in the literature should unfortunately be discarded. *Only retention volumes actually measured* (and not calculated by multiplying retention times by Q_0) are correct. Hence this type of pump should really be used with a volume-based and not a time-based recorder.

It is not even possible to compare chromatograms obtained with the same

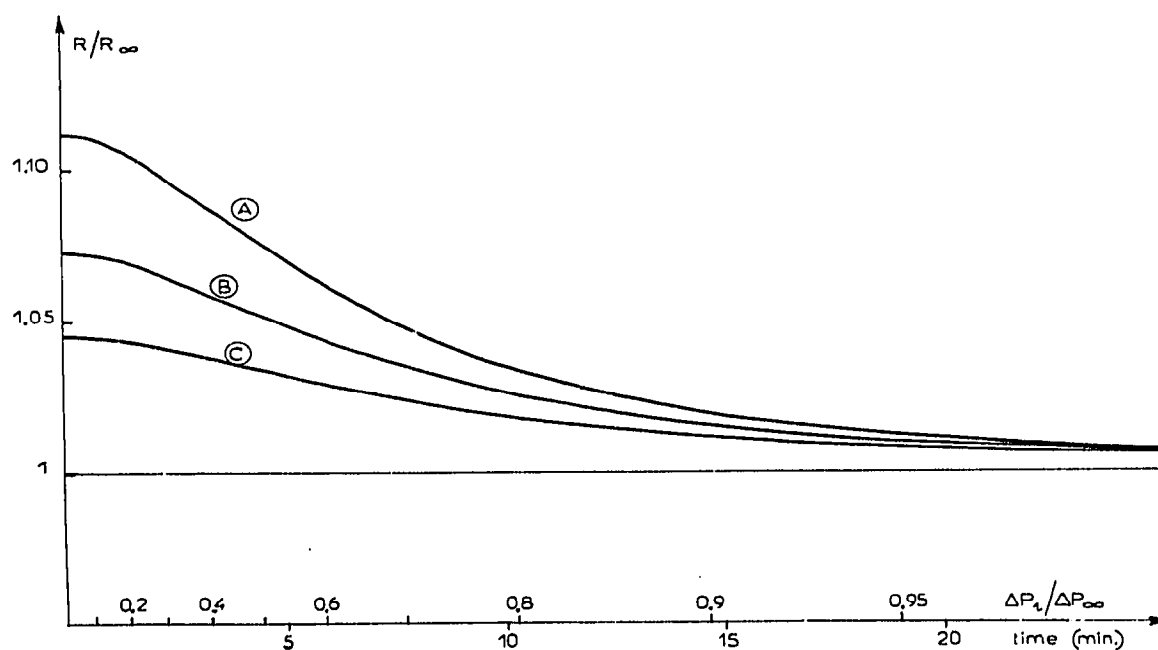


Fig. 12. Effect of the delay between starting the pump and sample injection on the resolution between two peaks. Conditions as for Figs. 8 and 11. True relative retention: 1.1. k' : A, 2; B, 5; C, 10.

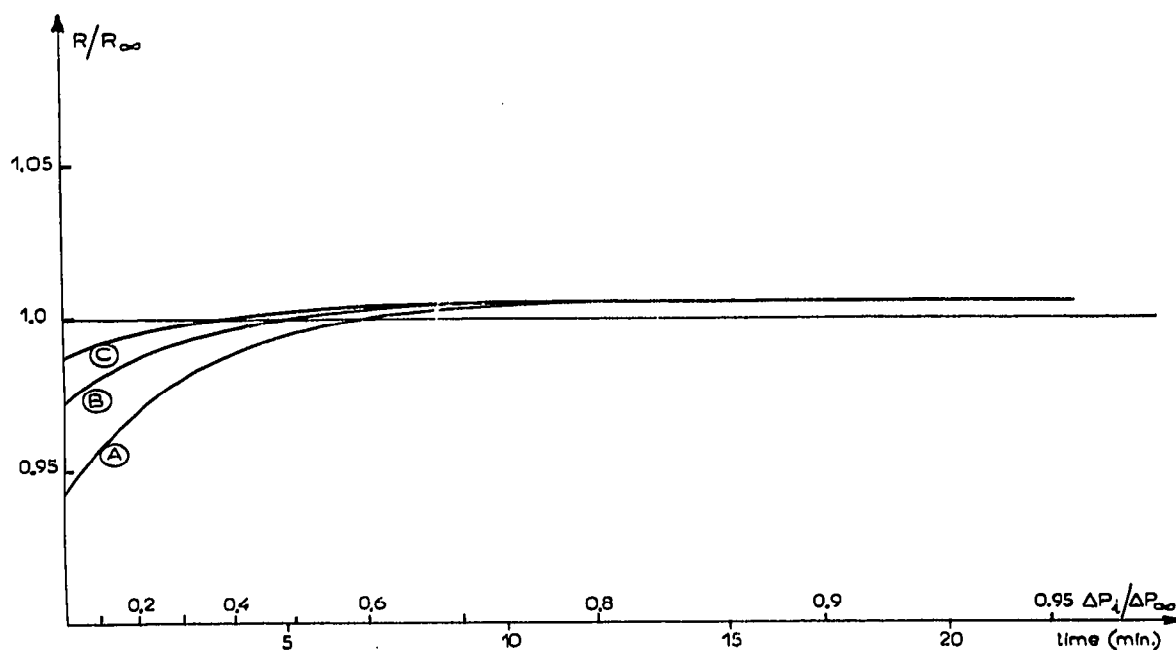


Fig. 13. As Fig. 12, except: flow-rate, 3 cm³/min; column length, 15 cm; particle size, 5 μ m; $N_z = 9550$; $\nu_{\infty} = 2.35$; $\Delta P_{\infty} = 32$ atm.

column and the same apparatus in order to make identifications on the basis of measurements of retention time, or even relative retention times, unless samples are injected under exactly the same conditions (same volume V_0 at the starting time; same injection delay).

Quantitative measurements are correct only if they are performed under steady-state flow conditions. Otherwise, the peak area, which is inversely proportional to the flow-rate during peak elution³, is not reproducible at constant sample size and not proportional to the sample mass.

The resolution is changed during the transitory state and the plate number is meaningless. The determination of an HETP curve can be a lengthy process, as it is necessary to wait long enough for the flow-rate to reach its steady-state value before measurements are made at any different flow-rate. Obviously, optimization is possible only under steady-state conditions.

In practice, it will be necessary to work under steady-state conditions. This needs time (10–30 min) and reduces the volume of liquid available. These drawbacks can be limited by pressurizing the liquid in the pump after filling it and before resuming pumping, but the pressure reached in that step should be very close to the steady-state pressure, which is possible only when the column has already been used. Even so, it is better to wait at least for a few minutes before beginning analytical work. This means that injection has to be on-line, without changing the liquid flow-rate.

If stop-flow injection is necessary or if for any other reason analytical work has to be carried out in the transitory state (remembering that measurements of physico-chemical parameters are meaningless), reproducible results can be obtained only if (a) V_0 is the same (the pump should be re-filled each time a new analysis is carried out), (b) the initial pressure is the same, and (c) the delay between starting the pump and injecting the sample is the same. There is some change in resolution, but reasonably reproducible quantitative results can still be obtained.

It is therefore not surprising that the syringe-type pumps have evolved into very sophisticated and expensive devices. It is true that the application of electronics and micro-processors can do much to improve equipment design, but they cannot change the laws of physics. The use of syringe-type pumps is often more difficult and less satisfactory than the use of other types of pumps. Also, we have discussed here the problem of their use under isocratic conditions. One can imagine how much more complicated it is to combine two positive-displacement pumps and to use gradient elution chromatography. Results in this field will be reported soon⁷.

REFERENCES

- 1 H. M. McNair and C. H. Chandler, *J. Chromatogr.*, 12 (1974) 425.
- 2 L. Berry and B. L. Karger, *Anal. Chem.*, 45 (1973) 819A.
- 3 B. L. Karger, M. Martin and G. Guiochon, *Anal. Chem.*, 46 (1974) 1640.
- 4 M. Martin, G. Blu and G. Guiochon, *J. Chromatogr. Sci.*, 11 (1973) 641.
- 5 M. Martin, C. Eon and G. Guiochon, *J. Chromatogr.*, 99 (1974) 357.
- 6 M. Martin, C. Eon and G. Guiochon, *J. Chromatogr.*, 110 (1975) 213.
- 7 M. Martin and G. Guiochon, to be published.