Journal of Chromatography, 151 (1978) 267–289

CHROM. 10,084

THEORETICAL STUDY OF THE GRADIENT ELUTION PROFILES OBTAINED WITH SYRINGE-TYPE PUMPS IN LIQUID CHROMATOGRAPHY

MICHEL MARTIN and GEORGES GUIOCHON

École Polytechnique, Laboratoire de Chimie Analytique Physique, Route de Saclay, 91128 Palaiseau Cedex (France)

(First received February 24th, 1977; revised manuscript received January 4th, 1978)

SUMMARY

Because of the different viscosities of the two solvents used, a gradient run in liquid chromatography results in either a large change in the flow-rate (constant-pressure pumps) or a large change in the inlet pressure (constant flow-rate pumps). Because of the compressibility of liquids and the large volume of their reservoirs compared with the usual values of the flow-rate, the pressure change observed with a syringe pump makes the actual concentration profile of the eluent very different from the set profile, most often a linear one, and the flow-rate during the gradient different from the constant set value.

The deviation from linearity and also the importance of the transitory change in total flow-rate are smaller when the second solvent, with the highest eluting strength, is less viscous than the first solvent. These deviations are also reduced if check valves are used on the solvent line and if the second solvent is pressurized to the column inlet pressure before the beginning of the gradient run. The correct use of pressure controllers or of feedback control of the flow-rate could in practice make negligible the consequences of these effects.

A theory is suggested for the prediction of the pressure and flow-rate profiles and calculation of the concentration profile as a function of time when the variation of the solvent mixture viscosity with its composition is known.

INTRODUCTION

Gradient elution is a useful technique for the liquid chromatographic analysis of mixtures of components with a wide range of retention characteristics, such as a wide range of polarity on a silica column or a wide range of molecular weight in reversed-phase chromatography. In this technique, the composition of the eluent is gradually varied from a solvent of low eluting strength to a solvent of high eluting strength. Among the several devices used for this aim[1], one can use an assembly of two syringe-type pumps with continuously variable speeds and mix the two solvent streams. The optimal gradient profile, *i.e.*, the concentration of one solvent in the eluent *versus* time, depends on the sample being analyzed. Its determination is not within the scope of this work, which deals only with the difficulties encountered in the preparation of a given gradient profile.

The flow behaviour of single syringe-type pumps in liquid chromatography has been studied previously[2]. In such a pump, the eluent contained in a cylindrical reservoir is displaced towards the column using a piston which is moved at a constant speed[1]. It was shown theoretically[2] that, because of the compressibility of the solvent, a relatively long time (typically 5–15 min) is necessary before a steady flow-rate can be reached. Although the compressibility of liquids is small (about 10^{-4} per atmosphere for most solvents), this effect is greatly amplified by the large volume of the reservoir (200–500 ml) and the relatively slow piston velocity. The transitory time depends on the characteristics of the liquid (compressibility and viscosity) and of the column (length and particle and column diameters) and on the volume of the reservoir, but it depends very little on the rate of piston movement or piston flow, *i.e.*, the value of the steady eluent flow-rate we wish to achieve, as the effect of this flow on the necessary steady-state pressure is nearly completely nullified by its effect on the speed at which the eluent is compressed in the pump[2]. During this transitory time, as all chromatographic characteristics (retention time, theoretical plate number, apparent capacity factor and resolution) depend on the flow velocity, chromatographic work during the transitory period must be avoided.

Experimental results on the compressibility effect are worse than those predicted by theory[3] because real systems are second- or higher-order systems and not first-order systems as assumed in our earlier work[2]. In spite of some discussion about the actual magnitude of the effect[4,5], there is a basic agreement on its existence and order of magnitude.

As recently pointed out[4], however, it is possible to reduce the transitory period by using momentarily a high piston flow until the column inlet pressure reaches a value corresponding to the steady-state flow-rate. This is useful mainly for routine analysis as in practice it is required that the pressure be known from previous measurements. Further, the high pumping speed has to be significantly greater than the working speed, which means that the pump motor will need a high power. An interesting safety device is a valve placed at the pump outlet which stays closed while the pump pressure has not yet reached a pre-set value, which prevents the analyst from operating the pump during the transitory period. The situation is more complex in gradient elution analysis because, owing to the change in the composition of the mobile phase and hence in the viscosity of the eluent, the column inlet pressure varies continuously, again resulting in compressibility effects which modify each of the individual pump flow-rates and hence the composition of the mixture. The aim of this paper is to investigate the theory of flow behaviour and the gradient profile obtained with a gradient elution device using two syringe-type pumps.

ANALYSIS OF FLOW BEHAVIOUR

Description of system

A schematic diagram of the gradient elution device using two syringe-type pumps is shown in Fig. 1. The solvent of low eluting strength (A) and that of high eluting strength (B) are pumped by pumps a and b, respectively. Each solvent flows through a flow-through pressure transducer, possibly through a ball-check valve (one-

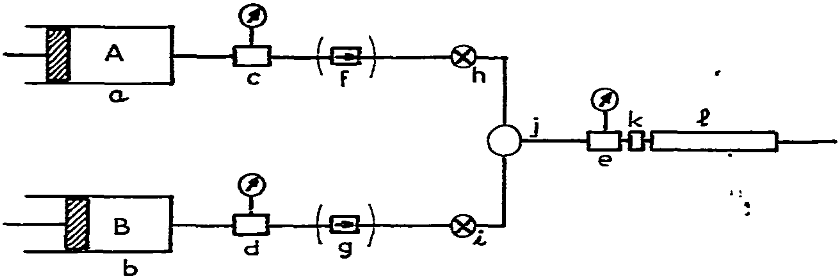

Fig. 1. Schematic diagram of the gradient elution assembly with two syringe-type pumps. a, b = syringe-type pumps; c. d, e = flow-through pressure transducers; f, g = ball-check valves; h, i = shut-off valves; j = mixing chamber; k = injection port; l = column.

way valve) and through a shut-off valve before entering the mixing chamber, then the mixture flows through a flow-through pressure transducer, the injection port, the column and the detector.

Pressure transducers c and d measure the pressure in reservoirs a and b, respectively, while the transducer e measures the column inlet pressure. Ball-check valves f and g can sometimes be introduced in the solvent lines in order to prevent reversal of flow into the reservoirs. Shut-off valves h and i are used when necessary in order to isolate one solvent line from the remainder of the assembly (isocratic operation, filling of syringe reservoir, etc.).

In the following discussion, we assume that the volumes of the valves, pressure transducers, mixing chamber and tubing between the reservoirs and the column are very small in comparison with the volumes of the pump reservoirs, so that we can neglect the former. When the solvent-line volumes are not negligible, they have to be added to the corresponding reservoir volumes. Further, we assume that there is no pressure drop between the reservoirs and the column when all valves are opened so that, in the case of Fig. 1, the readings of transducers c, d and e are identical.

Gradient operation

Before starting a gradient, solvent A is generally allowed to flow alone through the column for a period and the piston in the reservoir a moves at a constant speed so that the volume of A displaced by this piston per unit time, which we shall term the "piston flow-rate", is Q_0. We assume that this isocratic run is carried out under such conditions[2] that the steady-state pressure, $P_{1_{\mathrm{lim}}}$, is reached before it starts.

When the gradient program is run, the two piston speeds are varied continuously in such a way that the sum of the two piston flow-rates is kept constant and equal to Q_0. The ratio of these two flow-rates varies according to the required gradient profile. In the following discussion we assume that the required gradient profile is linear during time T, which is the most general and simplest profile used. More complex profiles could be studied with the same theory.

According to our assumption, the piston flow-rates Q_A and Q_B, corresponding to reservoirs a and b, respectively, are given by:

$$Q_A(t) = Q_0\left(1 - \frac{t}{T}\right) \qquad (1)$$

$$Q_B(t) = Q_0 \frac{t}{T} \tag{2}$$

the origin of time ($t = 0$) being taken as the beginning of the gradient run.

The actual volumes, V_A and V_B, available to the solvents in reservoirs a and b, respectively, at time t, are calculated from

$$\int_{V_{A,0}}^{V_A(t)} \mathrm{d}V_A = -\int_0^t Q_A(t)\,\mathrm{d}t \tag{3}$$

$$\int_{V_{B,0}}^{V_B(t)} \mathrm{d}V_B = -\int_0^t Q_B(t)\,\mathrm{d}t \tag{4}$$

where $V_{A,0}$ and $V_{B,0}$ are the volumes of reservoirs a and b, respectively, at the beginning of the gradient run. Hence, using eqns. 1 and 2, we obtain

$$V_A(t) = V_{A,0} - Q_0\, t\left(1 - \frac{t}{2T}\right) \tag{5}$$

$$V_B(t) = V_{B,0} - Q_0 \frac{t^2}{2T} \tag{6}$$

The gradient run ends at time T. After it is over, piston A is stationary and piston B is moving with a piston flow-rate Q_0. Hence the volumes of the reservoirs at the instant $t > T$ are

$$V_A(t) = V_A(T) = V_{A,0} - \frac{Q_0\, T}{2} \tag{7}$$

$$V_B(t) = V_B(T) - Q_0(t - T) = V_{B,0} + \frac{Q_0\, T}{2} - Q_0\, t \tag{8}$$

Calculation scheme and assumptions

The volumes given by eqns. 5–8 are the physical volumes inside the pumps available to the solvents. The elasticity of the pump metal is neglected[2]. These volumes can be filled with different masses of solvent, depending on the pressure, because of the compressibility of the solvent.

The basic principle of the calculation scheme is that the actual flow-rate of the eluent entering the column is related to the pressure drop, P, through the column by the Darcy law. While this law is a limiting expression at low velocities, we assume that, during the gradient run, the Reynolds number remains sufficiently small for this law to be valid[6]. This assumption seems valid in chromatography. We shall also assume that P is large and that we can neglect the pressure drops in positions other than in the column.

Flow-rate equations. The variation, $\mathrm{d}V$, of the volume V occupied by the liquid in a revervoir due to a pressure variation $\mathrm{d}P$ is given by the definition of the solvent compressibility coefficient:

$$\mathrm{d}V = -\chi V\,\mathrm{d}P \tag{9}$$

Hence, when the reservoir is filled with solvent A, the actual flow-rate, Q_1, of solvent A at the reservoir outlet is[2]

$$Q_1(t) = Q_A(t) - \chi_A\, V_A(t) \frac{\mathrm{d}P}{\mathrm{d}t} \tag{10}$$

where χ_A is the compressibility coefficient of solvent A and $Q_A(t)$ is the piston flow-rate, which is also the flow-rate set on the pump by the operator.

Similarly, for reservoir b, filled with solvent B of compressibility coefficient χ_B, the actual outlet flow-rate, Q_2, is

$$Q_2(t) = Q_B(t) - \chi_B V_B(t) \frac{dP}{dt} \tag{11}$$

where $Q_B(t)$ is the piston flow-rate.

The compressibility coefficients are pressure dependent: the higher the pressure, the smaller is the compressibility[4]. This variation, however, is not very important in the pressure range usually considered in chromatography[6] and can be neglected by taking average compressibility coefficients, which often are the only data available.

Eqns. 10 and 11 are valid when the reservoirs are filled with pure solvents and the valves on the corresponding solvent lines are opened, *i.e.*, if Q_1 and Q_2 are positive. There are conditions, however, when flow reversal may occur. In this instance, the ball-check valve g would close, Q_2 becomes zero and, from eqn. 11, the variation of pressure, P_B, in reservoir b is such that

$$\chi_B V_B(t) \frac{dP_B}{dt} = Q_B(t) \tag{12}$$

One-way valves may be used to prevent one solvent from flowing into the other solvent reservoir, but it does not seem that this method is widely used. As discussed later, it can prove useful only if the pressure is equal in both pumps at the beginning of the gradient (*cf.*, Results). The use of flow resistance (short tubes filled with glass beads) cannot prevent flow reversal and has further adverse effects owing to the higher pressure in the pump reservoirs, because of the pressure drop in these columns.

If there is no one-way valve on the two solvent lines, flow reversal becomes possible. For example, if solvent B is allowed to flow into reservoir a, this reservoir will contain solvents A and B; we shall assume that these two solvents are not mixed in reservoir a or that the compressibility of a mixture is linear (*i.e.*, $\chi = \chi_A Z_A + \chi_B Z_B$), an assumption which is necessary for our calculations and which is realistic. Let P_{tr} and $V_{A,tr}$ be the pressure in the system upstream of the column and the volume of reservoir a at the time t_{tr} when solvent B begins to enter reservoir a, respectively. At any time $t > t_{tr}$, the volume $V_{A,A}$ of solvent A in the reservoir a, from eqn. 9, is

$$V_{A,A} = V_{A,tr} \exp\left[-\chi_A (P - P_{tr})\right] \tag{13}$$

as the mass of A in the reservoir a is now constant. The volume $V_{A,B}$ of solvent B in this reservoir is

$$V_{A,B} = V_A(t) - V_{A,A} = V_A(t) - V_{A,tr} \exp\left[-\chi_A (P - P_{tr})\right] \tag{14}$$

where $V_A(t)$ is given by eqn. 5.

Hence, if we assume that in reservoir a the two solvents are compressed separately and additively, the flow-rate Q_1 is

$$Q_1(t) = Q_A(t) - (\chi_A V_{A,A} + \chi_B V_{A,B}) \frac{dP}{dt} \tag{15}$$

Q_1 is negative if solvent B enters reservoir a. Eqn. 15 becomes eqn. 10 if no flow reversal occurs.

To express the Darcy law, we shall write that the column inlet flow-rate, $Q(t)$, is the sum of the two outlet flow-rates, Q_1 and Q_2; hence we assume that the excess volume of mixing is zero, that is, the solution is ideal:

$$Q(t) = Q_1(t) + Q_2(t) \tag{16}$$

In some instances of solvent mixtures very far from an ideal solution, the volume of the mixture can differ by several percent from the volume of the unmixed solvents. We consider that this correction is negligible.

Darcy law equation. The Darcy law is given in a differential form by

$$Q = -\frac{kS}{\eta} \cdot \frac{dP}{dx} \tag{17}$$

where Q is the local flow-rate, k the local permeability, S the average section of the column which is occupied by the liquid, η the viscosity of the liquid and dP/dx the local pressure gradient. This law is easily integrated for a homogeneous column of constant permeability and an incompressible liquid of constant viscosity. Then, the pressure gradient $-dP/dx$ is constant and equal to P/L, where L is the column length. The pressure dependences of the liquid volume in the column, the viscosity and the permeability have been studied earlier[6] and it has been shown that it can be neglected for pressure drops less than about 200 atm.

The situation is different in gradient elution as the composition of the liquid is continuously changing along the column. Hence the viscosity depends on the position along the column (column abscissa, x) and the pressure profile along the column is not linear. In general, it is not possible to measure or calculate the composition of the solvent along all the column because, during its migration in the column, the composition of a given volume of liquid is modified by adsorption of the solvent of highest elution strength. To overcome this difficulty, we shall assume that the composition of the solvent is constant along the column at any time and equal to the composition of the eluent entering the column. This assumption amounts to neglecting the column volume, or assuming that the residence time of an unretained compound is small compared with the gradient time, T, which is most often the case. If it were not, this assumption limits the validity of the calculations, but does not change the general trend of the flow behaviour.

Variation of viscosity with composition. Finally, it is necessary to know the viscosity of the eluent entering the column. When this eluent is a mixture of solvents A and B, its viscosity, η, is expressed following a law that is generally valid for ideal mixtures[7]:

$$\ln \eta = x_A \ln \eta_A + x_B \ln \eta_B \tag{18}$$

where η_A and η_B are the viscosities of solvents A and B, respectively, and x_A and x_B are their molar fractions. In some instances, especially for non-ideal mixtures, the variation of the viscosity is very complex and eqn. 18 fails to describe it.

The molar fractions are related to the volume fractions, Z_A and Z_B, as a function of the molar volumes, v_A and v_B, of the pure solvents. Hence:

$$x_A = \frac{\dfrac{Z_A}{v_A}}{\dfrac{Z_A}{v_A} + \dfrac{Z_B}{v_B}} = \frac{\dfrac{Q_A}{v_A}}{\dfrac{Q_A}{v_A} + \dfrac{Q_B}{v_B}} \quad \text{or} \quad x_A = \frac{Q_A}{Q_A + \dfrac{v_A}{v_B} Q_B} \tag{19}$$

The molar volumes are functions of the actual pressure, and so

$$\frac{v_A}{v_B} = \frac{v_{A,0}}{v_{B,0}} \exp\left[-(\chi_A - \chi_B) P\right] \tag{20}$$

where $v_{A,0}$ and $v_{B,0}$ are the molar volumes of solvents A and B at atmospheric pressure, respectively. These molar volumes can be derived from literature data on molecular weights and densities at the experimental temperature.

As mentioned above, the viscosity of the solvents depends on the pressure. We shall neglect their variations in the pressure range studied and take the mean values satisfying the Darcy law under the isocratic steady-state conditions at the beginning and end of the gradient profile (steady flows of pure A and B), when pressures $P_{1_{\mathrm{lim}}}$ and $P_{2_{\mathrm{lim}}}$ are obtained for a flow-rate Q_0 of pure solvents A and B, respectively:

$$\eta_1 = \frac{kS\, P_{1_{\mathrm{lim}}}}{Q_0\, L} \tag{21}$$

$$\eta_2 = \frac{kS\, P_{2_{\mathrm{lim}}}}{Q_0\, L} \tag{22}$$

Of course, the pressures $P_{1_{\mathrm{lim}}}$ and $P_{2_{\mathrm{lim}}}$ reflect the influence of the viscosity dependence on pressure[6], and hence:

$$P_{1_{\mathrm{lim}}} = \frac{1}{\theta_1} \left[\exp\left(\frac{\eta_{1,0}\, L\, \theta_1\, Q_0}{kS}\right) - 1\right] \tag{23}$$

$$P_{2_{\mathrm{lim}}} = \frac{1}{\theta_2} \left[\exp\left(\frac{\eta_{2,0}\, L\, \theta_2\, Q_0}{kS}\right) - 1\right] \tag{24}$$

where $\eta_{1,0}$ and $\eta_{2,0}$ are the viscosities of solvents A and B, respectively, at atmospheric pressure and θ_1 and θ_2 are the relative coefficients of pressure variation of the viscosity of these solvents.

Using eqns. 18–24, it is possible to calculate the actual viscosity of the eluent entering the column as a function of its composition and so, with the above assumption (constant composition of solvent in the column), it is possible to write the basic equation of the calculation scheme. There is no analytical solution to this problem, so a numerical solution must be found. In the following discussion, this has been made using a step-to-step method, the time increment being one thousandth of the gradient time T.

RESULTS

Different calculations have been made using *n*-pentane, *n*-heptane, diethyl ether and ethanol, which are miscible in all proportions, as solvents. The viscosity, compressibility and molar volume data of these solvents are summarized in Table I and are taken from previous references[6]. We know that some of these data, especially the compressibility data, are questionable; in particular the compressibility of *n*-pentane is probably too high[4]. Nevertheless, this does not change the general shape of the curves or the qualitative results. Much can be learned about the possible effect of the exact value of compressibility by comparing the results obtained with *n*-pentane and *n*-heptane. For the sake of simplicity of calculations, in all instances the volumes $V_{A,0}$ and $V_{B,0}$ are taken to be 500 cm^3 and the piston flow-rate Q_0 to be 1 cm^3/min. These volumes correspond to the largest volume of the syringe pumps presently available. It is, of course, not possible that, after reaching a steady-state pressure $P_{1_{\text{lim}}}$ in an isocratic run with solvent A and compressing solvent B before starting the gradient run, the volume of the two reservoirs is still 500 cm^3. However, these volumes are chosen in order to illustrate the behaviour of the pump.

TABLE I

DATA USED IN THE CALCULATIONS

Solvent	*Viscosity at atmospheric pressure (cP)*	*Pressure coefficient of viscosity*	*Compressibility (bar^{-1})*	*Molar volume under atmospheric pressure (cm^3)*	*Steady-state pressure* (bar)*
n-Pentane	0.23	$1.1 \cdot 10^{-3}$	$3.14 \cdot 10^{-4}$	115	51.9
n-Heptane	0.37 (0.35)**	$1.1 \cdot 10^{-3}$ $(1.09 \cdot 10^{-3})$**	$1.42 \cdot 10^{-4}$	146	84.9 (80.0)**
Diethyl ether	0.23 (0.212)*	$1.1 \cdot 10^{-3}$ $(1.11 \cdot 10^{-3})$**	$1.87 \cdot 10^{-4}$	103.7	51.9 (47.7)**
Ethanol	1.02	$0.585 \cdot 10^{-3}$	$1.10 \cdot 10^{-4}$	58.2	239.0

* Column: length 50 cm, I.D. 2.2 mm, particle diameter 10 μm.
** Values in parentheses are data used for calculation of Fig. 3.

The column is assumed to give the same permeability and pressure behaviour as a column of length 50 cm and I.D. 2.2 mm, packed with 10-μm particles. Under these conditions, the steady-state pressures corresponding to the different solvents are as indicated in Table I.

The calculations are carried out for three cases: firstly, with no ball-check valve (f or g) on the solvent lines and with a pressure $P_{1_{\text{lim}}}$ in both reservoirs when the gradient starts; secondly, with the two ball-check valves (f and g) and a pressure $P_{1_{\text{lim}}}$ in both reservoirs when the gradient starts; and thirdly, with the two ball-check valves and with pressures equal to $P_{1_{lim}}$ in reservoir a and zero in reservoir b when the gradient starts. This last case is certainly uncorrect practice.

Gradient operation with no ball-check valves on solvent lines

The shut-off valve i is used to compress solvent B in reservoir b up to the steady-state pressure of solvent A, $P_{1_{lim}}$, then it is kept open. Two cases must be considered, depending on whether $P_{2_{lim}}$ is greater or smaller than $P_{1_{lim}}$.

First case: $P_{2_{lim}} < P_{1_{lim}}$. As the coefficients which measure the pressure effect on viscosity are similar for all solvents (*cf.*, Table I), such a condition ($P_{2_{lim}} < P_{1_{lim}}$) means that the viscosity of solvent B at atmospheric pressure is lower than the viscosity of solvent A. From eqn. 18, the column inlet pressure decreases continuously when the volume fraction of solvent B increases.

Such a case is illustrated in Fig. 2 for a 30-min linear gradient of *n*-heptane (solvent A) and diethyl ether (solvent B). The variations with time of the inlet pressure (curve a) and flow-rate (curve b) are given.

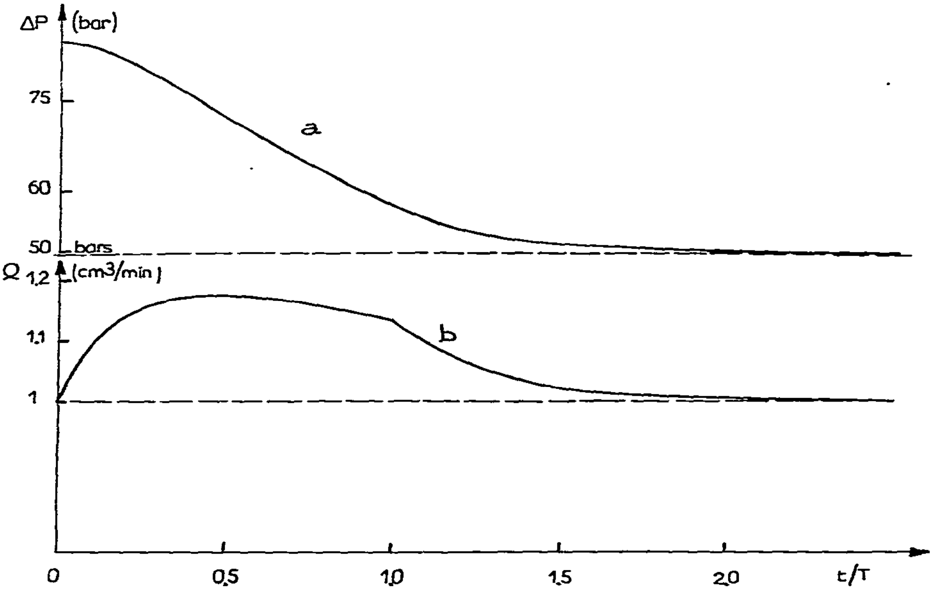

Fig. 2. Pressure (a) and flow-rate (b) *versus* time profiles for a gradient of *n*-heptane (solvent A) and diethyl ether (solvent B). $T = 30$ min.

As the pressure decreases, the solvents A and B are decompressed and the eluent flow entering the column is greater than the total piston flow. This flow Q reaches a maximum for $t/T \approx 0.5$. When the gradient time is finished the flow-rate and the inlet pressure have not yet reached the steady-state values Q_0 and $P_{2_{lim}}$, so that after the gradient run operation is believed to be ended and although the piston in reservoir a is not moving, some solvent A continues to flow through the column; the eluent is not pure diethyl ether before about a further 30 min. When the gradient time T is decreased to 10 min, this phenomenon is amplified, as can be seen in Fig. 3. The

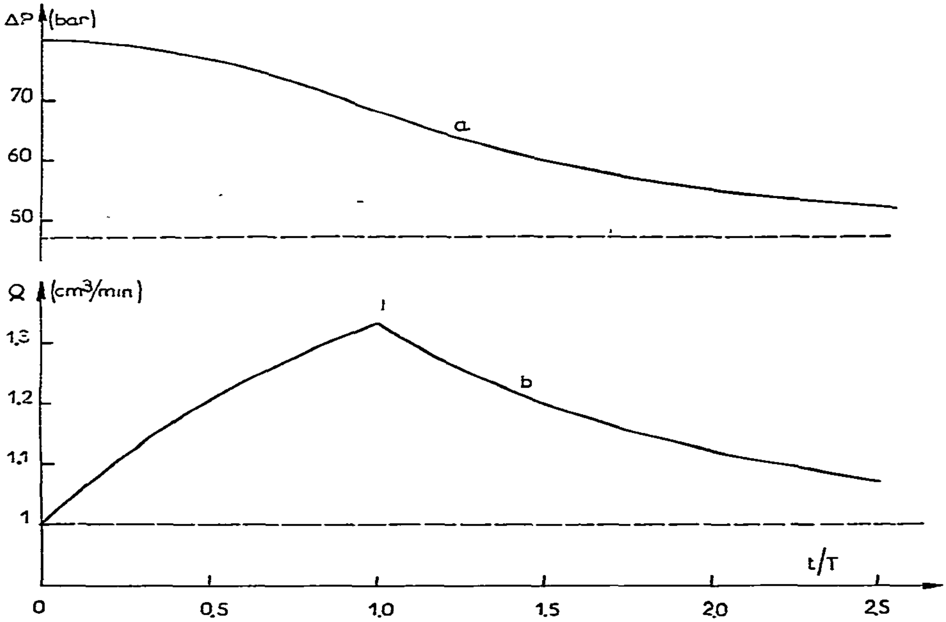

Fig. 3. Gradient of *n*-heptane (A) and diethyl ether (B). $T = 10$ min. Variations of pressure (a) and flow-rate (b) *versus* time.

maximum flow-rate is now reached at the end of the gradient time. At this point, the flow-rate excess is greater than 30%. Even after a time $t = 2\,T$, the flow-rate excess is larger than 10%. The time necessary to reach the steady state is very long, a flow deviation of less than 1% being reached only after $t = 4.5\,T$. Provided that the inlet pressure is decreasing, even long after the end of the gradient time, solvent A is flowing through the column, due to its decompression. Fig. 4 shows the variation of the volume fraction, Z_B, of solvent B with time. The broken line represents the ideal gradient, which would be obtained if the solvents were not compressible. It should be

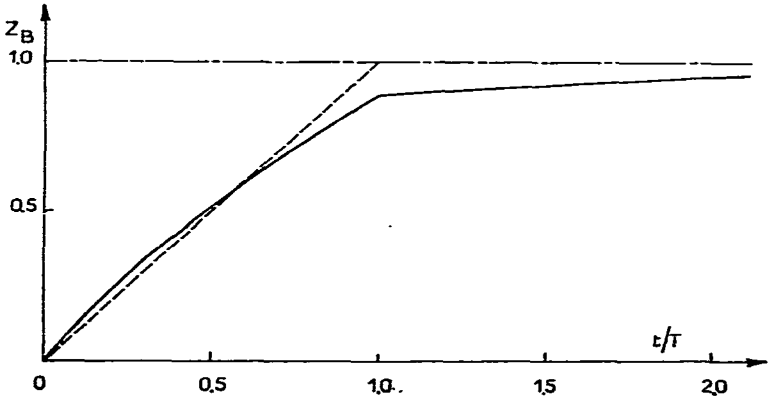

Fig. 4. Variation of volume fraction Z of solvent B *versus* time, with same gradient conditions as for Fig. 3.

noted in this instance that the effect on chromatographic data of the excess of A at the end of the gradient run is not very large, as the solvent A has, by definition of gradient operation, a much lower elution strength. More significant might be the deviation from linearity in the first part of the run.

Second case: $P_{2_{\mathrm{lim}}} > P_{1_{\mathrm{lim}}}$. In this case the inlet pressure increases steadily during the programme and part of the piston flow-rate must serve to compress the solvent in both reservoirs. Hence the flow-rate Q through the column is smaller than Q_0. At the beginning of the gradient run, the piston flow in reservoir b is small, but Q_2 is always positive as it is because of the introduction of the more viscous solvent B in the eluent that the inlet pressure increases, and the system is autoregulated.

At some time during the gradient run, however, the piston flow in reservoir a, which decreases with increasing time, cannot compensate for the volume reduction of the mass of A in that reservoir due to the compression of solvent A. Then solvent B enters the reservoir a and the eluent becomes pure solvent B, even though pump A is apparently delivering a positive flow-rate of A: the gradient is finished too early.

Fig. 5 shows the variation of pressure and flow-rate with time for a gradient of diethyl ether (A) and *n*-heptane (B), a gradient reversed from that described in Figs. 2–4. A minimum flow-rate is reached when solvent B begins to enter the reservoir of solvent A.

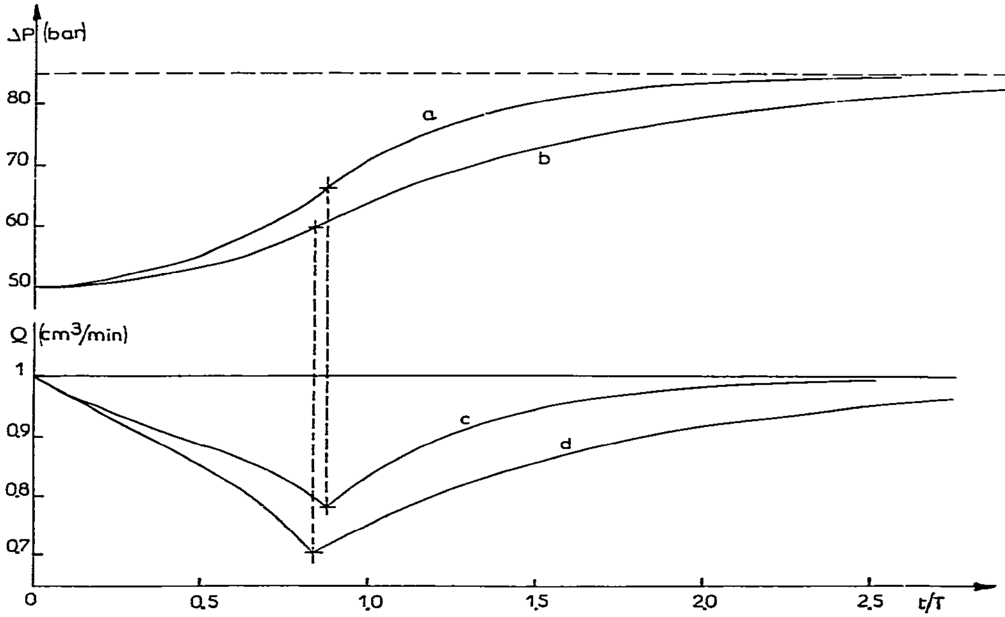

Fig. 5. Variation of pressure (a and b) and flow-rate (c and d), with gradient of diethyl ether (A) and *n*-heptane (B). Gradient times: 15 min (b and d) and 30 min (a and c).

As shown in Fig. 6, the shapes of the flow-rate *versus* time curves are different for *n*-heptane (A) and ethanol (B) gradients, a case in which the difference in viscosity is very large. Fig. 6 shows profiles for gradient times ranging from 1 min to 10 h. The extreme cases are not representative of actual gradient operation but they have been

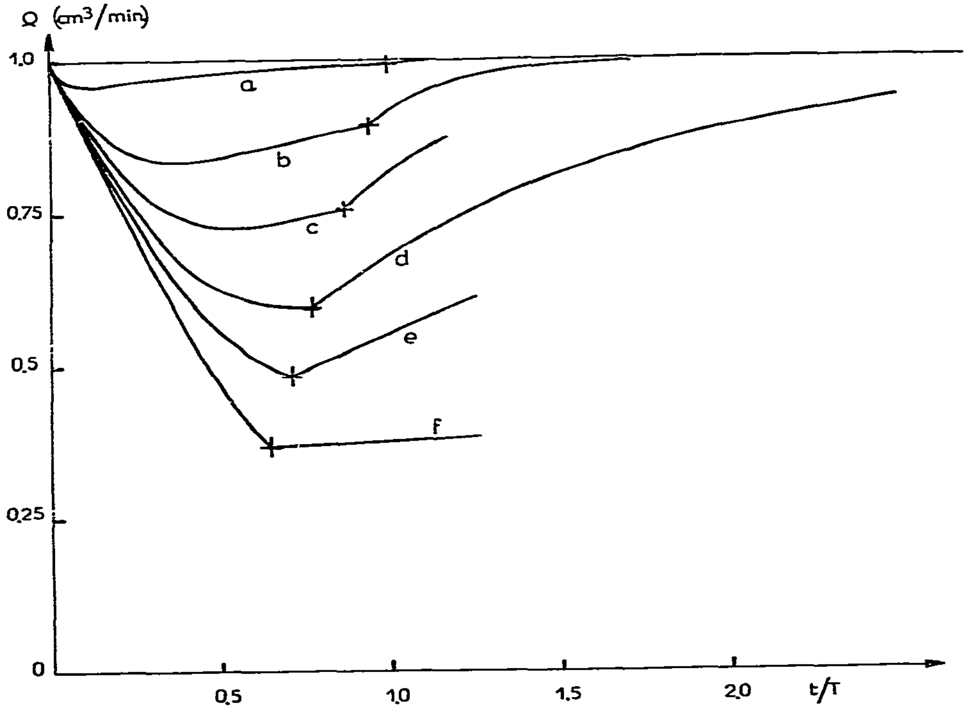

Fig. 6. Variation of flow-rate with time for gradients of *n*-heptane (A) and ethanol (B). Gradient times: a = 10 h; b = 2 h; c = 1 h; d = 30 min; e = 15 min; f = 1 min.

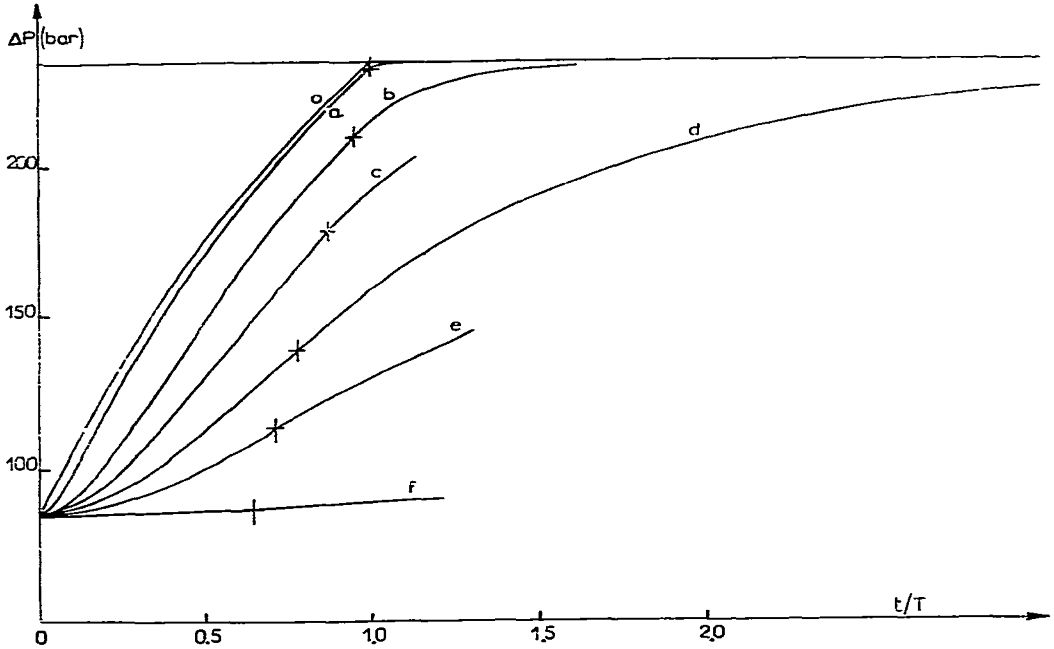

Fig. 7. Variation of pressure with time, with same gradients as for Fig. 6. The line labelled 0 corresponds to an ideal gradient.

included for the following reasons. The first one illustrates the trend of variation of the flow-rate profile with very rapid variations in concentration, although in this instance the critical assumption of constant composition of the eluent in the column is not fulfilled. It shows the difficulties associated with too rapid re-setting of the original conditions with an ethanol–*n*-heptane gradient in reversed-phase chromatography. The slow gradients show that when the variation of pressure with time is small, the flow behaviour approaches the ideal case. Nevertheless, even in this case, the deviation can reach several percent. It can be seen in Fig. 6 that the smaller the gradient time *T*, the smaller is the ratio *t*/*T* for which the solvent B begins to flow into reservoir a.

The transient decrease in flow-rate can be very important, more than 50% for a gradient time of 15 min, which is not exceptional (*cf.*, Fig. 6). Comparison of curve c in Fig. 5 and curve d in Fig. 6, corresponding to the same gradient time, shows that a greater decrease in flow-rate is obtained with the *n*-heptane–ethanol gradient. This effect is due to the larger difference in viscosity between the two solvents in the latter case and hence to the larger variation of pressure during the gradient.

In Fig. 7 are plotted the corresponding variations of the inlet pressure. The smaller the gradient time *T*, the greater is the ratio *t*/*T* at which a given fraction of the steady-state pressure is reached. The curve marked 0 represents the pressure–time profile for an ideal gradient (no compressibility). This curve is determined entirely by the variations in the viscosity with the volume fraction of solvent B. Fig. 8 shows the variations in the volume fraction of ethanol (solvent B) with time in the *n*-heptane–ethanol gradient. In all instances the volumic fraction Z_B is smaller than that corresponding to the set gradient (straight line) at the beginning of the gradient run, up to $Z_B \approx 0.45$, in the case in point. Then it becomes increasingly larger and, as mentioned

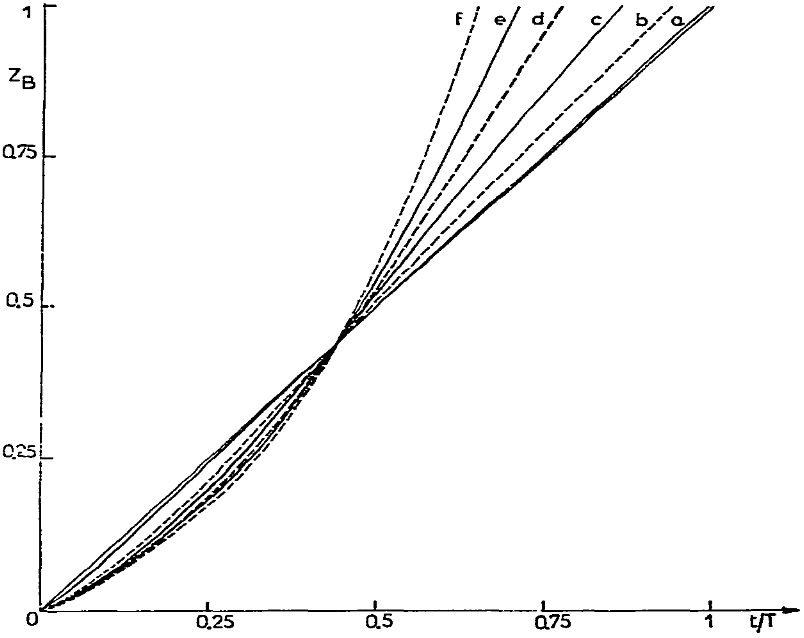

Fig. 8. Time profile of the volume fraction of solvent B, with same gradient conditions as for Fig. 6.

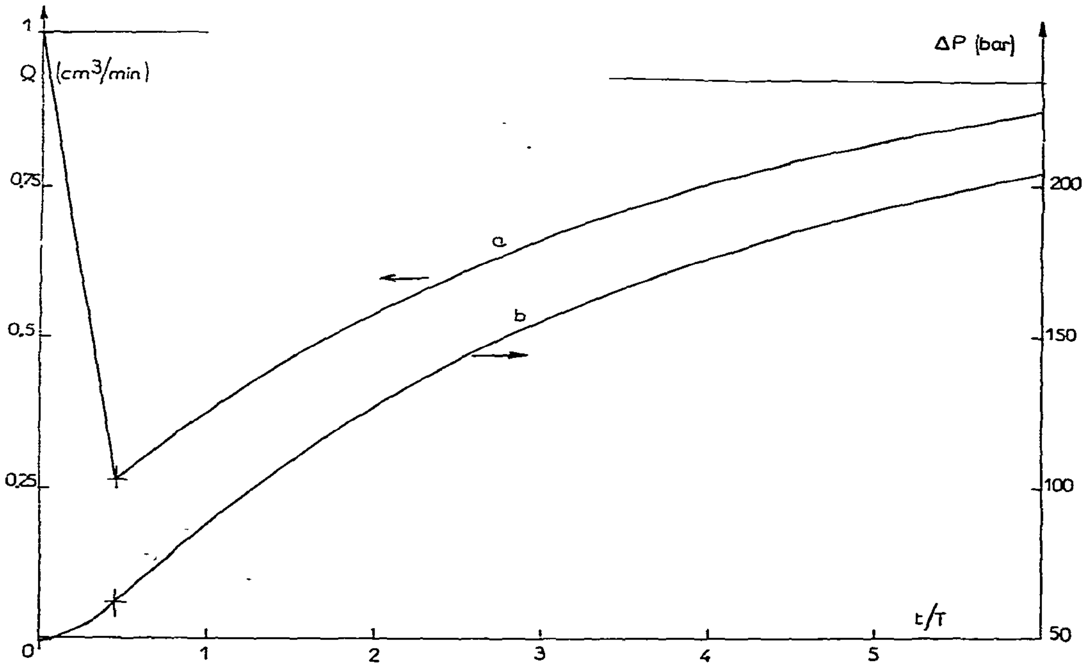

Fig. 9. Profiles of flow-rate (a) and pressure (b) for a gradient of *n*-pentane (A) and ethanol (B). T = 15 min.

above, pure solvent B flows through the column when there is flow reversal in the line to reservoir a. The faster the gradient, the earlier is the time when this happens.

These effects are more important with a very compressible solvent and a higher steady-state pressure, as shown in Figs. 9 and 10 for an *n*-pentane (A)–ethanol (B) gradient with $I = 15$ min. Fig. 9 shows the flow-rate and pressure profiles while Fig. 10 shows the corresponding volume fraction profile. In this instance pure solvent B appears before half of the gradient time has elapsed and at this moment the flow-rate

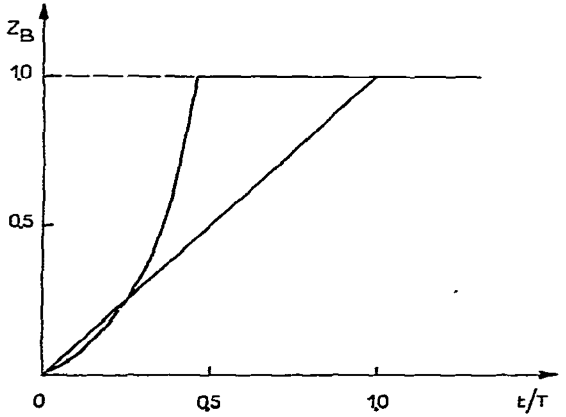

Fig. 10. Time profile of the volume fraction of solvent B, with same gradient conditions as in Fig. 9.

is about 25% of the set "constant" flow-rate. Admittedly, the deviation predicted is larger than that which would be expected in practice because we used a slightly too large value for the compressibility of *n*-pentane[5].

To minimize these effects, it is advisable to select solvents with similar viscosities, which, of course, is not easy. On all of these curves, it can be seen that the point of inflexion of the pressure profile corresponds to the extreme (maximum or minimum) of the flow profile.

Finally, a word of caution: if flow reversal occurs, the reservoir of the solvent of low elution strength should be purged carefully before starting the next analysis, otherwise during the following analysis a mixture of solvents A and B, rich in the strongly eluting solvent, will flow through the column for a few minutes, giving unexpected and irreproducible results.

Gradient elution with ball-check valves on the solvent lines and identical high starting pressures in both reservoirs

Ball-check valves serve to prevent the flow reversal of one solvent into the other solvent reservoir. Such a flow reversal happens during the gradient of a viscous solvent in a less viscous solvent when the piston flow-rate in reservoir a becomes too small to compensate for the decrease in the volume of solvent A due to the increase of the inlet pressure. In this instance, the ball-check valve f is closed and solvent B can flow only through the column. The flow-rate of B increases faster towards the steady-state value than in the previous case while the pressure in reservoir a lags behind the column inlet

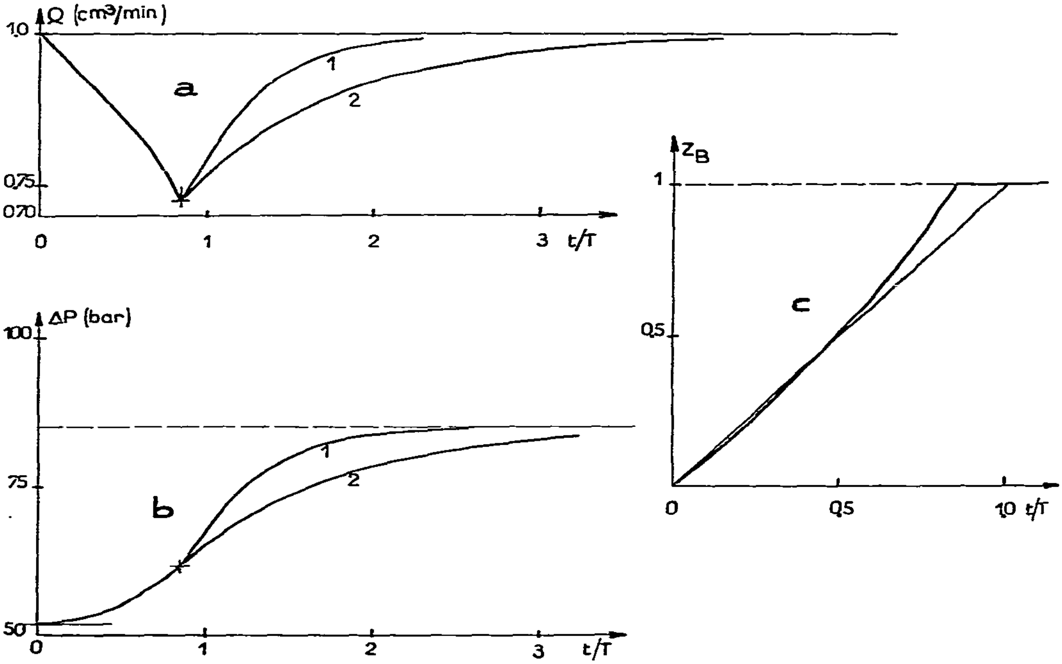

Fig. 11. Comparison of the gradient profiles obtained (1) with and (2) without a ball-check valve. Gradient of *n*-heptane (B) in diethyl ether (A). $T = 15$ min. (a) Flow-rate profile; (b) pressure profile; (c) profile of the volume fraction of *n*-heptane (solvent B).

pressure. In Fig. 11 the flow profiles with and without a ball-check valve during a diethyl ether–*n*-heptane gradient with $T = 15$ min are compared. Obviously, the steady-state flow-rate and pressure are reached faster with a ball-check valve. The volume fraction profiles, however, are identical in both instances, as the ball-check valve closes just when pure B starts to flow through the column.

The same comparison is shown for the *n*-heptane–ethanol gradient, with different gradient times, in Fig. 12 (flow-rate profile) and Fig. 13 (pressure profile). Figs. 14 and 15 are plotted for gradients of *n*-pentane and ethanol.

It should be noted that the pressure profiles obtained with ball-check valves exhibit a point of inflexion at $t = T$, whereas without the valves the point of inflexion corresponds to the minimum of the flow-rate.

Gradient elution with ball-check valves on solvent lines and a zero initial pressure in the reservoir b

The results are as easy to calculate as in the previous instances but more difficult to understand as they are surprising at first sight and seem to be contrary to common-sense predictions: the actual mass flow-rate of solvent through the column is determined by the algebraic sum of the piston volume flow-rate and the volume flow-rate which result from compressibility of the solvent.

If a pure solvent of compressibility χ is pumped from a syringe pump through a set valve, under equilibrium conditions the flow-rate through the valve is equal to the piston flow, Q_0. If we now start to move the valve so that its permeability decreases in such a way that the inlet pressure increases linearly ($P = P_0 + at$), the flow-rate will decrease abruptly to $Q_0 - a\chi V$, where V is the volume of the reservoir (*cf.*, eqn. 10). Although the pressure profile is continuous, if there is a sudden change in its time derivative there is a discontinuity in the flow profile.

In the case in point, the column inlet pressure is $P_{1_{\lim}}$ at the beginning of the gradient run, which is also the pressure in the reservoir a, but the pressure in reservoir B is zero and the ball-check valve g is closed. As the set gradient profile requires a decrease in the flow-rate of A and an increase in the flow-rate of B, the two piston flows will change accordingly, with surprising results that are completely different from what would be expected.

The actual flow of solvent A through the column decreases, and there is no flow of B provided that the pressure P_B is smaller than P and valve g is closed. Thus, the column inlet pressure, P, decreases and the actual flow-rate, Q_1, of solvent A through the column is greater than the piston flow Q_A, because of the additional contribution $V\chi \dfrac{dP}{dt}$ due to the decompression of A. Meanwhile, solvent B is compressed by its piston at an increasing rate. As P decreases while P_B increases, eventually they become equal and the check valve g opens. Then solvent B starts suddenly to flow at a finite rate into the mixing chamber, causing the pressure to increase. This effect is shown in Fig. 16, which gives the pressure profile for gradients of *n*-heptane (A) and ethanol (B).

When valve g opens and solvent B starts to flow through the column, as the pressure begins to increase, the flow-rate of solvent A decreases suddenly, because now part of the piston flow Q_A is used to compress A in its reservoir. There is a discontinuity in the flow-rate of A. Obviously, there is an interaction between this decrease and the

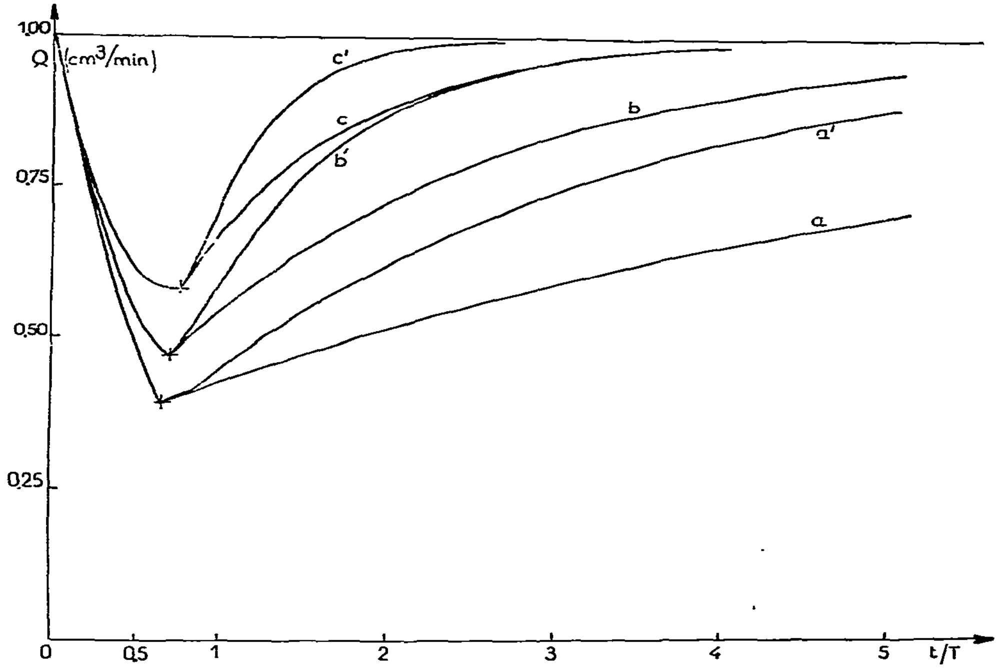

Fig. 12. Flow-rate profile without (a, b, c) and with (a', b', c') ball-check valves. Gradient of *n*-heptane (A) and ethanol (B). a, a', $T = 5$ min; b, b', $T = 15$ min; c, c', $T = 30$ min.

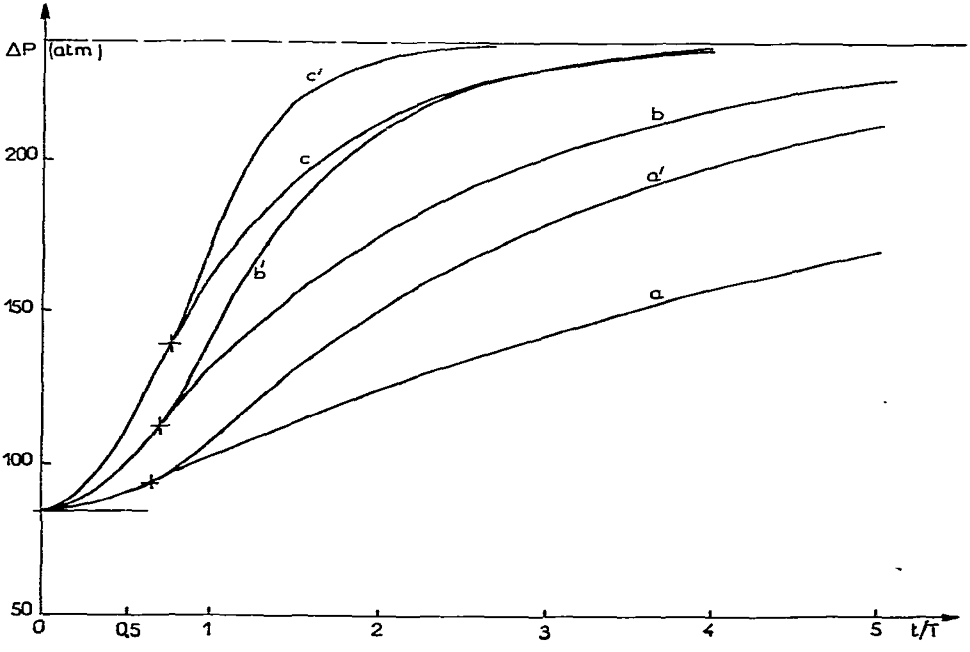

Fig. 13. Pressure profiles, with same gradient conditions as for Fig. 12.

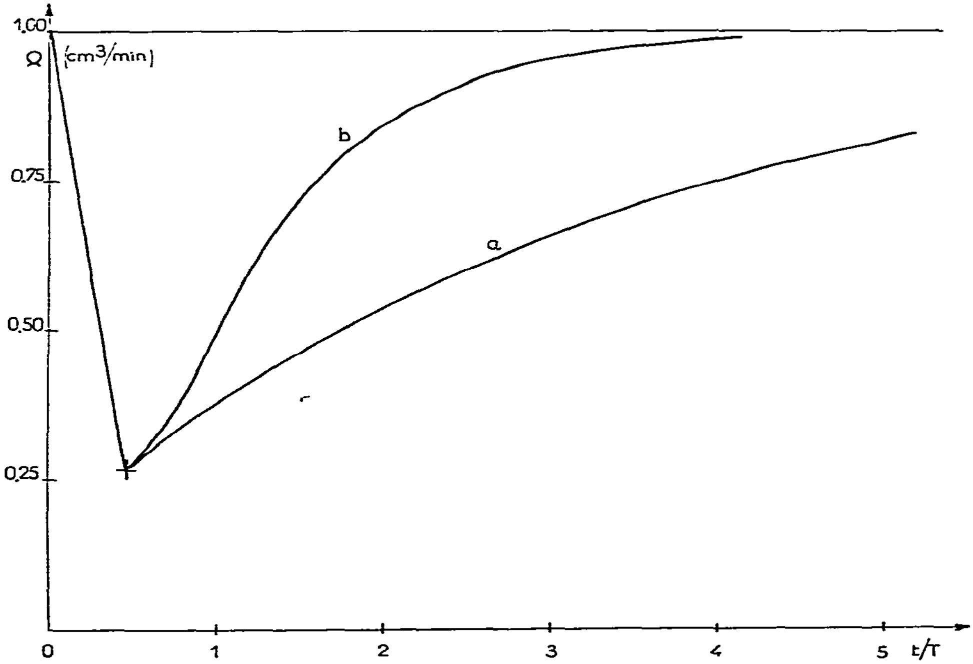

Fig. 14. Flow-rate profile (a) without and (b) with ball-check valves. Gradient of *n*-pentane (A) and ethanol (B). $T = 15$ min.

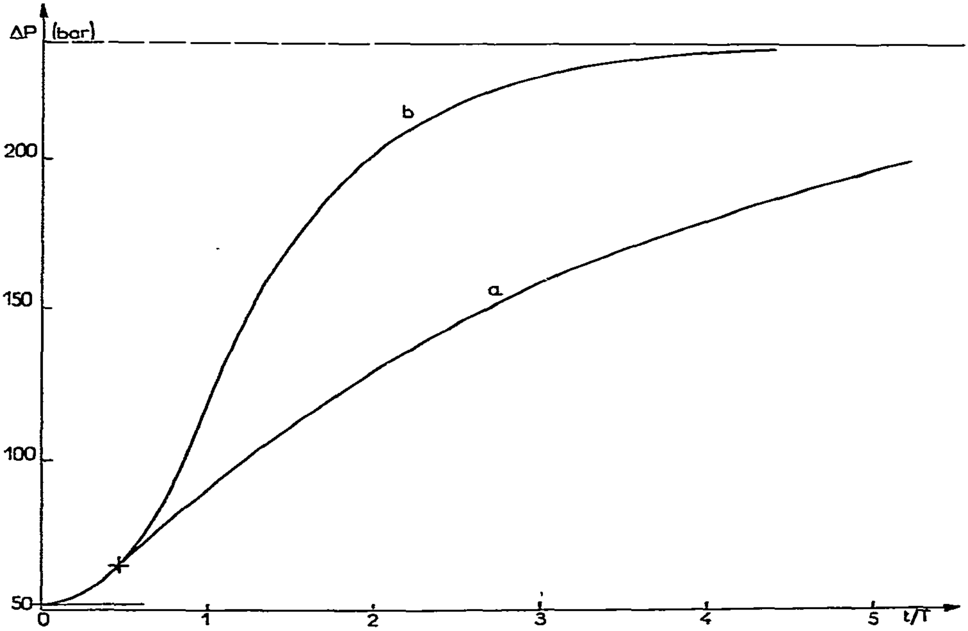

Fig. 15. Pressure profile with same gradient conditions as for Fig. 14.

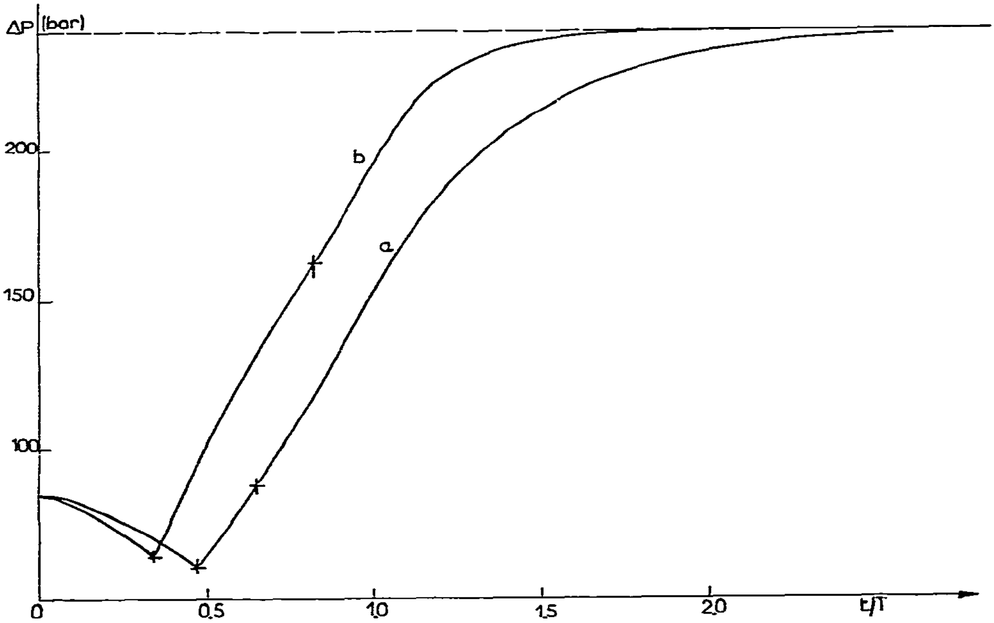

Fig. 16. Pressure profiles for an *n*-heptane (A)–ethanol (B) gradient with ball-check valves on solvent line and zero initial pressure in the reservoir b. Curve a, $T = 15$ min; curve b, $T = 30$ min.

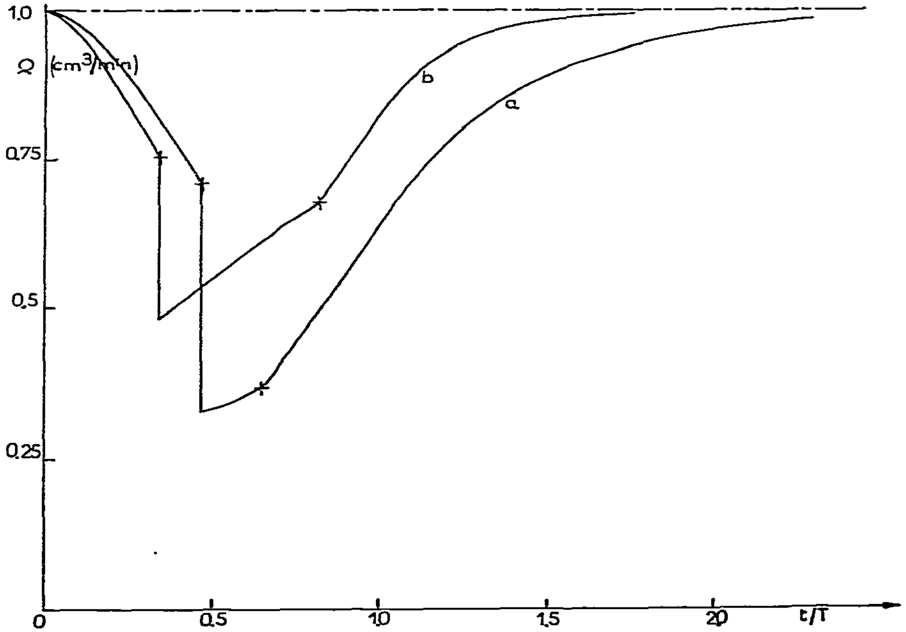

Fig. 17. Flow-rate profiles, with same gradient conditions as for Fig. 16.

sudden jump in the flow-rate of B, so that there is no discontinuity in the inlet pressure. However, as there is a difference between the viscosities of the two solvents, there is also a considerable change in the total flow-rate. The flow profile for gradients of *n*-heptane (A) and ethanol (B) is shown in Fig. 17. Admittedly, these flow discontinuities are not instantaneous, because there are sources of inertia in the system which are not accounted for in the equations used here, but they are small and the flow changes take place in a very short time. After that sudden event, the situation is similar to that described in the previous section; the flow-rate of B increases while the flow-rate of A decreases until eventually the ball-check valve f closes and the eluent is pure solvent B.

Obviously, the volume fraction profile is very different from the smooth straight line set on the programmer, as shown in Fig. 18. During the first part, pure solvent A flows through the column and when the valve opens solvent B immediately starts to flow at a finite flow-rate, and hence there is a step gradient from pure A to a mixture of A and B, the composition of which depends on the gradient time. It can be seen that the smaller the gradient time, the smaller is the fraction t/T of gradient time during which both solvents are flowing through the column. If the viscosity of solvent B is large and the gradient is run fast enough, it can even happen that when the check valve g opens, ball-check valve f closes, because the pressure begins to increase so steeply that the piston flow in reservoir a, which is decreasing, cannot compensate for the compressibility effect on solvent A. Hence, although a linear gradient is programmed, actually a step gradient from 0 to 100% of solvent B takes place.

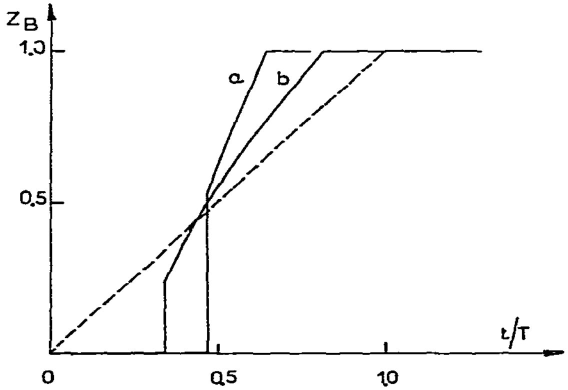

Fig. 18. Profile of the volume fraction of solvent B in the eluent, with same gradient conditions as for Fig. 16.

DISCUSSION

The conclusions which can be drawn are more qualitative than quantitative: we have considered only linear gradients of a few solvents for which we have used data taken from the literature (*cf.*, Table I). The following assumptions restrict the validity of our quantitative results: (1) no excess mixing volume; (2) constant compressibility; (3) the viscosity of the mixture is given by eqn. 17; (4) this viscosity is independent of the pressure; (5) Darcy's law is valid; (6) a pressure drop takes place only in the column; (7) the inert peak residence time is short compared with the gradient time; and

(8) the pressure effects on the column properties are negligible. Some of these assumptions are really valid, *i.e.*, either they are realistic (assumptions 5, 6 and 8) or they amount to neglect effects which would result only in very minor changes of pressure and flow-rate profiles and eluent composition (assumptions 1, 2 and 4).

The excess volume of mixing is usually a few percent or less when we deal with 10–50% changes in flow-rate. Deviation from ideal laws of mixing have a much more pronounced effect on the viscosity of a mixture which might be different from that given by eqn. 17. The compressibility of liquids decreases with increasing pressure[4] but the effect can be accounted for satisfactorily by using a value of the compressibility averaged over the pressure range studied. In fact, differences between the effects predicted by us for *n*-pentane[2] and those measured by Achener *et al.*[4] results from our use of a compressibility value taken from the literature which appears to be too large. The correction for pressure variation of the compressibility is small in the most usual pressure range of 0–200 atm. Similarly, the variation in viscosity is small and can be accounted for by using an average value, as we did. Darcy's law is valid in the range of low velocities normally used in liquid chromatography[6] and the effects of pressure on the column hold-up volume or on the residence time of an inert substance have been shown to be negligible[6]. Only in poorly designed equipment or in equipment working with a very permeable column can the pressure drop in the ball-check valves, mixing chamber, sampling port and connection tubing be important compared with that in the column. Operating a modern liquid chromatographic apparatus with an empty tube in place of the column shows that a negligible pressure (less than 1 atm) is needed in order to achieve a flow-rate of 1 cm^3/min.

The exponential-like dependence of the logarithm of the elution time of a compound on the volume fraction of the strongly eluting solvent[8] gives a much faster variation than the exponential dependence of the retention time on the column temperature in gas chromatography. A calculation similar to that developed by Habgood and Harris[9] shows that the performance in gradient elution, *i.e.*, the resolution between two closely eluted compounds, is not drastically reduced only if the gradient is slow enough[10]. Hence, in all practical applications the residence time of an inert substance has to be small compared with the gradient time (at least 3–5 times smaller).

Finally, we consider that eqn. 17, giving the viscosity of a mixture, is the least acceptable of the approximations made here. In fact as shown by Abbott *et al.*[11] there are better equations which could be used in place of eqn. 18 if needed. This is especially true for non-ideal mixtures, which is mostly the case in gradient elution. It should be pointed out, however, that although a marked change in the relationship between viscosity and concentration may result in a viscosity profile of the eluent quantitatively different from the one assumed here, the shape of the flow-rate and pressure profiles will not change, as long as the viscosity profile remains constant, which is the case for mixtures of most non-associated liquids. Mixtures of water and either methanol or acetonitrile, on the other hand, exhibit a maximum viscosity for some intermediate water concentration[11]; in these cases the pressure and flow-rate profiles will accordingly be more complex.

Consequently, the results described in Figs. 2–18 certainly give a good qualitative description of what happens in practice. They illustrate the great difficulty in achieving a linear gradient when using syringe-type pumps, because of the effect of liquid compressibility.

The effects are different when the viscosity of the mixture increases during the gradient and when it decreases. The latter situation, which tends to prevail in reversed-phase chromatography, is certainly more favourable, the ideal case being that with pH or similar gradients. When the viscosity of the eluent decreases during the gradient, the effect on the composition profile of the eluent is smaller and of less importance as (*cf.*, Fig. 4) the deviation from linearity is moderate and the existence of a small concentration of the "weak" solvent in the "strong" one at the end of the run has only a minor effect on the retention of the last components.

Certainly, for theoretical calculations and applications to measurements in physical chemistry, for which gradient chromatography is not particularly convenient, the deviation from linearity is very significant and will result in important systematic errors. The consequences, however, are of lesser importance in analytical applications. In this instance a linear gradient is selected merely because it is the easiest to achieve, but reproducibility is most important; it is easier to achieve in the case when the viscosity of the eluent decreases during the gradient run than in the opposite case, when the viscosity of the eluent increases during the run; this is the typical case in chromatography with a polar stationary phase. Even so, an acceptable gradient profile and gradient reproducibility may be achieved if the second solvent reservoir is pressurized; otherwise, it may be impossible to achieve a normal gradient operation (*cf.*, Figs. 16–18). We should point out that careful purging of the "strong" solvent volume which has entered the "weak" solvent reservoir is necessary before starting a new analysis.

At any rate, we feel that a good check of the reproducibility of a gradient system requires measurement of the composition of the eluent and its time variation at the column inlet, *i.e.* using a high pressure detector or a small dead-volume valve in place of the column. The column changes the concentration profile, damping its fluctuations but making it steeper, as the column retains the strong solvent and delays its exit, thus giving the outlet profile little resemblance to the input one[10]. As theoretical prediction of the optimum gradient profile at the column inlet is not possible, the task of experimentors and manufacturers is difficult. Reproducibility is however a critical requirement.

The lack of reproducibility of the gradient composition and of the flow-rate profile of the stream generated by two syringe pumps derives essentially from the variation in the viscosity of the mixture as a result of its changing composition. Two kinds of device can be used to compensate for the effect of varying pressure: first a pressure controller, which would maintain, during the whole run, the pressure at the exit of the two pumps at a value larger than the one which is necessary to sustain the desired flow-rate through the column of the most viscous mixture used. This can be achieved in different ways, for example by using two constant pressure valves, one at the end of each pump, or only one such valve at the exit of the mixing chamber. In this last case the total volume available to the mixture, from the point where the two solvents begin to mix to the column inlet, should remain small in comparison to column volume. In both cases the volume available to the liquid inside the valve should not change appreciably when its flow resistance varies. The valve described recently by Abbot *et al.*[11] and Achener[12] seems to fit these requirements. Another solution would be to use a feed-back system similar to those employed with alternating pumps[1]: the flow rate at the outlet of each pump is measured, the result compared to the theoretical value resulting from the program and the error signal used to modify the piston speed.

While the feed-back system works the same way, independently of the pump model used, the pressure controllers would retain the basic advantage of syringe pumps: indeed when the pressure inside the cylinder is kept constant, the flow-rate delivered by the pump is as constant as the geometrical or piston flow-rate can be.

This work is purely theoretical. Its conclusions are in excellent agreement with those of the experimental work by Parrish[13] who fully demonstrated the effect of viscosity and compressibility of the solvents on the shape of the gradient delivered by two syringe pumps. There is also a good qualitative agreement with the findings by Abbott *et al.*[11]. These authors conclusively demonstrated the advantage of using a pressure controller, as shown above. Furthermore they show that with syringe pumps used without such a controller to deliver a water–acetonitrile gradient, the retention times of the first compounds are longer and those of the last eluted compounds shorter than when the controller is used. The viscosity of water–acetonitrile mixtures increases with acetonitrile concentration, passes through a maximum and then decreases. In the first part of the run the actual solvent flow-rate is accordingly lower than the set flow-rate, while it is larger towards the end. The effect on the first compounds is furthermore decreased by a compensation which results from the fact that when viscosity increases, the flow-rate decreases but at the same time the concentration of the stronger solvent also increases. Precise calculations of this effect are made difficult by the fact that the actual gradient profile varies along the column due to the frontal analysis effect[10]. Further study of the associated phenomena is in progress.

REFERENCES

1 M. Martin and G. Guiochon, in J. F. K. Huber (Editor), *Instrumentation for High-Performance Liquid Chromatography*, Elsevier, Amsterdam, Oxford, New York, 1978, in press.
2 M. Martin, G. Blu, C. Eon and G. Guiochon, *J. Chromatogr.*, 112 (1975) 399.
3 M. Martin and G. Guiochon, unpublished results.
4 P. Achener, S. R. Abbott and R. L. Stevenson, *J. Chromatogr.*, 130 (1977) 29.
5 M. Martin and G. Guiochon, *J. Chromatogr.*, 130 (1977) 428.
6 M. Martin, G. Blu and G. Guiochon, *J. Chromatogr. Sci.*, 11 (1973) 641.
7 W. R. Gambill, *Chem. Eng.*, 66 (1959) 151.
8 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
9 H. B. Habgood and W. E. Harris, *Programmed Temperature Chromatography*, Wiley, New York, 1966, pp. 122–132.
10 H. Colin and G. Guiochon, unpublished results.
11 S. R. Abbott, J. R. Berg, P. Achener and R. L. Stevenson, *J. Chromatogr.*, 126 (1976) 421.
12 P. Achener, private communication, patent pending.
13 Anon., *Caracteristics of Gradient Elution Chromatography*, Du Pont, Wilmington, Del., 1976.