# THEORY OF STOP-FLOW SAMPLE INJECTION

## EFFECT OF NON-STEADY-STATE FLOW ON RETENTION TIME AND COLUMN EFFICIENCY IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND FAST GEL PERMEATION CHROMATOGRAPHY

M. KUBÍN and S. VOZKA

*Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague (Czecho-slovakia)*

(Received May 20th, 1977)

## SUMMARY

A partial differential equation has been derived that describes the non-steady-state pressure profile along a chromatographic column for the case of a liquid mobile phase of finite compressibility. This equation was solved subject to boundary conditions that pertain to the flow disturbance at the early stages of elution in the stop-flow sample injection method in liquid chromatography. The effect of stop-flow sample introduction on the separation efficiency is generally small both in high-performance liquid chromatography (HPLC) and in fast gel permeation chromatography (GPC). The shift in retention time (or volume) due to this mode of sample introduction increases with the (steady-state) pressure drop across the column and with the compressibility factor of the mobile phase, and decreases with the extent of sample retention, but in HPLC this effect is significant only when very fast analyses on columns of low permeability are attempted. When stop-flow sample injection in combination with a positive displacement pump is used in fast GPC, some caution has to be exercised.

## INTRODUCTION

In the modern high-performance liquid chromatography (HPLC) and fast gel permeation chromatography (GPC), where efficient columns packed with microparticulate sorbents are used, the method of sample introduction can markedly affect the separation efficiency. It has been shown theoretically[1] and verified experimentally[2] that best results are obtained if the sample is injected centrally onto the top of the column packing[2] or even several millimetres below[3]. Owing to difficulties connected with the choice of a septum material that can withstand high pressures of the often aggressive solvents, the stop-flow injection technique is more and more preferred. However, this mode of sample introduction brings about problems of its own, particularly when used with a positive-displacement pump[4]; it has been shown[5,6] that to obtain a good reproducibility of retention data under these conditions, it is im-

perative to insert a valve between the pump and the injection port so that the large reservoir is kept permanently under a pressure that corresponds to the steady-state pressure drop across the column at the solvent flow-rate employed.

There exists another feature of stop-flow injection not recognized so far: as soon as the depressurized column is suddenly connected to the pressure reservoir, a very steep pressure gradient is initially built up in the top region of the column. This decays more or less slowly (depending on the column permeability and on the compressibility of the mobile phase) to a steady-state, virtually linear dependence of the pressure along the column axis. As the solvent linear velocity is proportional to the negative local pressure gradient, the sample zones move initially with a velocity much higher than in the steady state; the retention times therefore tend to be lower than under comparable conditions without the stop-flow injection, and also the separation efficiency could be impaired as the sample travels through at least part of the column at a high speed.

It is very difficult to estimate the magnitude of these effects without a mathematical analysis of the transitory state; in this article the relevant partial differential equation is derived and solved, and results of numerical calculations performed for conditions (flow-rate, pressure drop, solvent compressibility, *etc.*) encountered in modern HPLC and fast GPC are presented.

## THEORETICAL

Let us denote by $x$ the spatial coordinate along the column axis and assume for simplicity that both the viscosity, $\eta$, and the compressibility, $\chi$, of the mobile phase are independent of pressure. From the Darcy Law written in the differential form

$$\frac{\partial P}{\partial x} = -\frac{\eta u}{k_o} \tag{1}$$

where $P$ is the pressure, $u$ is the linear velocity of the mobile phase and $k_0$ is the column permeability, and from the definition of the compressibility factor,

$$\chi = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right) \tag{2}$$

a partial differential equation can be derived that governs the dependence of pressure in a non-steady-state flow of a compressible liquid through a chromatographic column:

$$(k_o/\eta\chi)\frac{\partial^2 P(x,t)}{\partial x^2} + (k_o/\eta)\left[\frac{\partial P(x,t)}{\partial x}\right]^2 = \frac{\partial P(x,t)}{\partial t} \tag{3}$$

where $t$ is time.

If we introduce the dimensionless variables

$$\xi = x/L, \; p = P/P_0, \text{ and } \tau = tk_0/(\eta\,\chi\,L^2) \tag{4}$$

where $L$ is the column length and $P_0$ the outlet pressure, eqn. 3 assumes the form

$$\frac{\partial^2 p}{\partial \xi^2} + \beta\left(\frac{\partial p}{\partial \xi}\right)^2 = \frac{\partial p}{\partial \tau} \tag{5}$$

where the dimensionless parameter $\beta$ is given by

$$\beta = P_0 \chi \tag{6}$$

In solving eqn. 5 we will use mostly the boundary conditions

$$p = 1, \tau = 0, 0 \leqslant \xi \leqslant 1 \tag{7a}$$

$$p = p^* \equiv P_1/P_0, \tau > 0, \xi = 0 \tag{7b}$$

$$p = 1, \tau > 0, \xi = 1 \tag{7c}$$

($P_1$ is the inlet pressure assumed to be constant) that are relevant in the case of stop-flow injection with a positive-displacement pump and a valve before the injection port (it is assumed that the volume of the reservoir is large so that the pressure does not change appreciably when the valve is opened at $t = 0$); other boundary conditions are mentioned briefly in the Discussion.

Eqn. 5 is non-linear and not amenable to an analytical solution. At the expense of a certain approximation it is possible to linearize it by a perturbation method. To do this, we first derive the pressure drop along the column in the steady state, i.e., we find the function $p_s(\xi)$ that represents a solution of eqn. 5 with the right-hand side zero:

$$\frac{d^2 p_s}{d\xi^2} + \beta \left( \frac{dp_s}{d\xi} \right)^2 = 0 \tag{8}$$

with $p_s(0) = p^*$ and $p_s(1) = 1$.

The integration of eqn. 8 is straightforward, if $(dp_s/d\xi)$ is introduced as a new dependent variable, and leads to

$$p_s = p^* + \beta^{-1} \ln \left\{ 1 - \xi \left[ 1 - e^{-\beta(p^*-1)} \right] \right\} \tag{9}$$

However, $\beta$ is very small (typically of most liquids $\beta \simeq 10^{-4}$), and therefore eqn. 9 can be replaced by the straight-line dependence

$$p_s = p^* - \xi(p^* - 1) \tag{10}$$

with excellent accuracy. (The validity of eqn. 10 can be verified by expanding the exponential and logarithmic functions in eqn. 9 and retaining only the first terms.)

Let us now express the quantity $p(\xi, \tau)$ as a sum of two terms,

$$p(\xi, \tau) = p_s(\xi) + \Delta p(\xi, \tau) \tag{11}$$

where $p_s(\xi)$ is given by eqn. 10 and the quantity $\Delta p(\xi, \tau)$ can be looked upon as a perturbation. Hence, the partial differential equation, eqn. 5, can be written as

$$\frac{\partial^2 p_s}{\partial \xi^2} + \beta \left( \frac{\partial p_s}{\partial \xi} \right)^2 + \frac{\partial^2 \Delta p}{\partial \xi^2} + 2\beta \frac{\partial p_s}{\partial \xi} \frac{\partial \Delta p}{\partial \xi} + \beta \left( \frac{\partial \Delta p}{\partial \xi} \right)^2 = \frac{\partial \Delta p}{\partial \tau} \tag{12}$$

The sum of the first two terms is zero according to eqn. 8; if we now assume that the last term on the left can be neglected (an assumption justified below), we have to solve, instead of the original non-linear boundary value problem, its linear counterpart

$$\frac{\partial^2 \Delta p}{\partial \xi^2} - 2\beta (p^* - 1) \frac{\partial \Delta p}{\partial \xi} = \frac{\partial \Delta p}{\partial \tau} \tag{13}$$

for the new quantity $\Delta p (\xi, \tau)$; in view of eqns. 11 and 7, the boundary conditions are

$$\tau = 0, \; \Delta p = \xi (p^* - 1) - (p^* - 1) \qquad 0 \leqslant \xi \leqslant 1 \tag{14a}$$

$$\tau > 0, \; \Delta p = 0 \qquad \xi = 0 \tag{14b}$$

$$\tau > 0, \; \Delta p = 0 \qquad \xi = 1 \tag{14c}$$

where $p^*$ is again defined as $p^* = P_i/P_0$.

This boundary value problem can be solved by standard methods[7]; the solution reads

$$\Delta p = 2\pi (p^* - 1) \left\{ e^{\alpha \xi} \left[ 2\alpha S_1 (\xi, \tau) - S_2 (\xi, \tau) \right] - 2\alpha e^{\alpha(\xi - 1)} S_3 (\xi, \tau) \right\} \tag{15}$$

where

$$\alpha = \beta (p^* - 1) = P_0 \chi (p^* - 1) \tag{16}$$

$$S_1 (\xi, \tau) = \sum_{k=1}^{\infty} \frac{k \sin(k\pi\xi) \exp[-(\alpha^2 + k^2\pi^2) \tau]}{(\alpha^2 + k^2\pi^2)^2} \tag{17a}$$

$$S_2 (\xi, \tau) = \sum_{k=1}^{\infty} \frac{k \sin(k\pi\xi) \exp[-(\alpha^2 + k^2\pi^2) \tau]}{\alpha^2 + k^2\pi^2} \tag{17b}$$

$$S_3 (\xi, \tau) = \sum_{k=1}^{\infty} \frac{(-1)^k k \sin(k\pi\xi) \exp[-(\alpha^2 + k^2\pi^2) \tau]}{(\alpha^2 + k^2\pi^2)^2} \tag{17c}$$

The final solution for the dimensionless pressure $p (\xi, \tau)$ is then obtained from eqns. 10 and 11 as

$$p (\xi, \tau) = p^* - \xi (p^* - 1) + \Delta p (\xi, \tau) \tag{18}$$

with $\Delta p (\xi, \tau)$ given by eqn. 15. (It is obvious that eqn. 15 satisfies the boundary conditions 14b and 14c as the three sums $S_1$, $S_2$ and $S_3$ are all zero, both for $\xi = 0$ and $\xi = 1$. In proving that eqn. 15 satisfies also the condition 14a, the sums[8]

$$\sum_{k=1}^{\infty} \frac{k\sin(kx)}{k^2a^2 + b^2} = \frac{\pi}{2a^2} \frac{\sinh[(\pi - x)b/a]}{\sinh(\pi b/a)}$$

and

$$\sum_{k=1}^{\infty} \frac{(-1)^{k-1} k \sin(kx)}{k^2 a^2 + b^2} = \frac{\pi}{2a^2} \frac{\sinh(xb/a)}{\sinh(\pi b/a)}$$

and their derivatives with respect to $b$ are useful.)

In the following, eqn. 18 will serve as a basis in showing how to calculate the effect of the stop-flow injection on the retention time, $t_R$, and on the column efficiency (expressed by the plate height $H$) for a solute characterized by a certain value of the capacity factor $k'$.

It follows from eqns. 1 and 4 that the solvent linear velocity, $u$ (cm/sec), is given by

$$u = -\varkappa \frac{\partial p}{\partial \xi} \tag{19}$$

where

$$\varkappa = k_0 P_0 / L \eta \tag{20}$$

We can calculate the derivative in eqn. 19 by means of eqns. 15 and 18 and we have:

$$u(\xi, \tau) = \varkappa(p^* - 1) \left\{ 1 + 2\pi e^{a\xi} \left[ a S_2(\xi, \tau) + \pi C_2(\xi, \tau) - 2a^2 S_1(\xi, \tau) - \right. \right.$$

$$\left. \left. - 2a\pi C_1(\xi, \tau) \right] + 4a\pi e^{-a(1-\xi)} \left[ a S_3(\xi, \tau) + \pi C_3(\xi, \tau) \right] \right\} \tag{21}$$

where

$$C_1(\xi, \tau) = \sum_{k=1}^{\infty} \frac{k^2 \cos(k\pi\xi) \exp[-(a^2 + k^2\pi^2)\tau]}{(a^2 + k^2\pi^2)^2} \tag{22a}$$

$$C_2(\xi, \tau) = \sum_{k=1}^{\infty} \frac{k^2 \cos(k\pi\xi) \exp[-(a^2 + k^2\pi^2)\tau]}{a^2 + k^2\pi^2} \tag{22b}$$

$$C_3(\xi, \tau) = \sum_{k=1}^{\infty} \frac{(-1)^k k^2 \cos(k\pi\xi) \exp[-(a^2 + k^2\pi^2)\tau]}{(a^2 + k^2\pi^2)^2} \tag{22c}$$

We can assume that also under non-steady-state conditions the velocity of the solute zone, $v$, remains proportional to the linear velocity of the solvent, i.e.,

$$v = dx/dt = R u(x,t) = u(x,t)/(1 + k')$$

where $R$ is a constant.

In the dimensionless variables,

$$\frac{d\xi}{d\tau} = \frac{R\eta\chi}{k_o}\frac{L}{} u(\xi, \tau)$$

For actual calculations it is more convenient to rewrite this differential equation in the form

$$\frac{d\tau}{d\xi} = \frac{k_o}{\eta\chi L}\frac{1}{Ru(\xi, \tau)} = f(\xi, \tau) \tag{23}$$

Owing to the complicated form of eqn. 21 defining $u(\xi, \tau)$, this ordinary differential equation must be solved numerically; its solution $\tau = \tau(\xi)$ gives the (dimensionless) time necessary for the solute to reach the position in the column characterized by the dimensionless coordinate $\xi$, under the non-steady-state conditions of solvent velocity encountered in the stop-flow method. (In the following, we shall designate the quantities that refer to the non-steady state by a bar.)

The retention time is given by $\bar{\tau}_R = \tau(1)$ or, in view of eqn. 4,

$$\bar{t}_R = (\eta\chi L^2/k_0)\tau(1) \tag{24}$$

Under steady-state conditions we have from the definition of the plate height $H$

$$\sigma_t^2 = (1/L)H t_R^2 \tag{25}$$

where $\sigma_t$ is the standard deviation of the peak (in time units); this must be replaced (see Giddings[9]) under non-uniform conditions in the column by

$$\sigma_t^2 = \int_0^L \frac{H(u_{loc})}{R^2 u_{loc}^2} dx \tag{26}$$

where $u_{loc}$ is the (local) velocity of the solvent at a point $x$ in the column, and the dependence of $H$ on $u_{loc}$ must be described by an appropriate form of the Van Deemter equation. The resulting plate height, $\bar{H}$, observed under the non-steady-state conditions, is therefore given by

$$\bar{H} = L\sigma_t^2/\bar{t}_R^2 = (L/\bar{t}_R^2)\int_0^L \frac{H(u_{loc})}{R^2 u_{loc}^2} dx$$

or, in dimensionless variables,

$$\bar{H} = \frac{k_o^2}{L^2\eta^2\chi^2}\frac{1}{R^2\tau^2(1)}\int_0^1 \frac{H(u_{loc})}{u_{loc}^2} d\xi \tag{27}$$

and the local solvent velocity, $u_{loc}$, is calculated as

$$u_{loc} = u[\xi, \tau(\xi)] \tag{28}$$

from eqn. 21 and from the solution $\tau(\xi)$ of the differential eqn. 23.

## RESULTS AND DISCUSSION

The approximations involved in the perturbation method of solving the basic partial differential equation have been justified by comparing the pressure profiles along the column, calculated for different values of the dimensionless time, $\tau$, by means of the approximate eqn. 18, with profiles obtained by solving numerically the original, non-linear boundary value problem as described by eqns. 5–7. The numerical solution was obtained for $\beta = 1.42 \cdot 10^{-4}$ (a value corresponding to the compressibility of $n$-heptane) by the method of Douglas and Jones[10]. The results are plotted in Figs. 1a and 1b for two values of the inlet pressure characterized by $p^* = 10$ and $p^* = 300$; the full lines represent the numerical solution of the original differential eqn. 5, and the points were calculated from the approximate solution, eqn. 18. The respective algorithms were programmed in BASIC and implemented on the desk minicomputer Wang 2200 B. The results of the perturbation method clearly agree very closely with the true shape of the pressure gradient along the column, even for very low values of $\tau$.

In order to demonstrate the effect of non-steady-state solvent flow due to the stop-flow injection method in HPLC and GPC, retention times $\bar{t}_R$ and plate heights
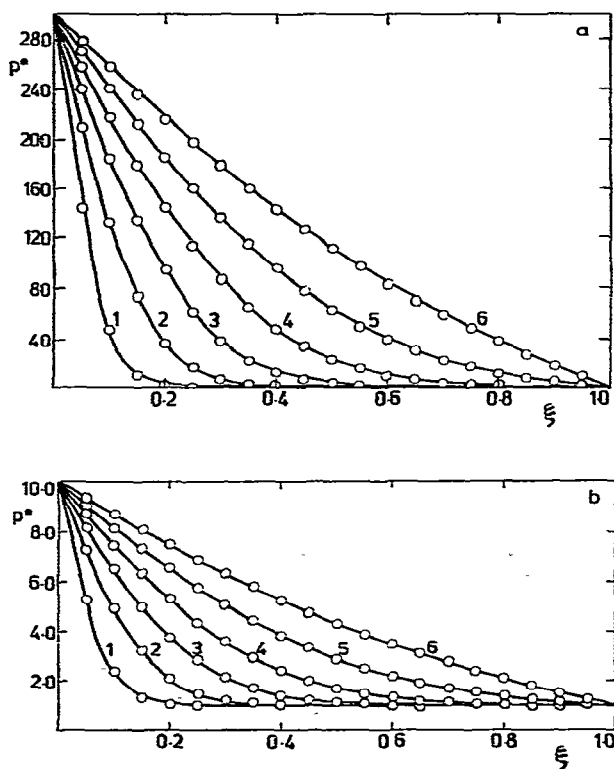


Fig. 1. Pressure profiles along a chromatographic column at the early stages of elution with stop-flow injection technique for different values of the dimensionless time $\tau$. (a) $p^* = P_i/P_0 = 300$; (b) $p^* = P_i/P_0 = 10$. Full lines are calculated from a numerical solution of the exact boundary value problem, eqns. 5 and 7. Points are calculated on the basis of the approximate solution, eqn. 18. 1, $\tau = 2.5 \cdot 10^{-3}$; 2, $\tau = 8.28 \cdot 10^{-3}$; 3, $\tau = 1.93 \cdot 10^{-2}$; 4, $\tau = 3.98 \cdot 10^{-2}$; 5, $\tau = 7.94 \cdot 10^{-2}$; 6, $\tau = 0.16$.

$\bar{H}$ were calculated for typical column parameters, typical solvent–solute pairs and under conditions of solvent flow-rate that can be encountered in high-speed analyses, and compared with the corresponding steady-state values $t_R$ and $H$ as observed under comparable conditions with "uninterrupted-flow" injection (e.g. with septum injectors or injection valves).

Unless explicitly stated otherwise, the following values were used in the calculations: column length $L = 30$ cm for HPLC and $L = 120$ cm for fast GPC; inner column diameter $d_c = 0.3$ cm for HPLC and $d_c = 0.4$ cm for GPC; interparticle porosity in the column $\varepsilon_0 = 0.4$, intraparticle porosity $\varepsilon_i = 0.8$.

The steady-state inlet pressure, $P_i$, was calculated from

$$P_i/P_0 = p^* = \eta\, L\, Q/60\, k_0\, S_{ef} \tag{29}$$

where $Q$ (ml/min) is the solvent flow-rate, $S_{ef}$ the effective cross-sectional area of the column

$$S_{ef} = (\pi/4)\, d_c^2\left[\varepsilon_o + (1 - \varepsilon_o)\varepsilon_i\right] \tag{30}$$

and the specific permeability, $k_0$, was taken[11] simply as

$$k_o = d_p^2/1000 \tag{31}$$

with $d_p$ (cm) the particle diameter of the packing.

The other steady-state values were calculated from the relations

$$u = \varkappa\,(p^* - 1) = k_0\, P_0\,(p^* - 1)/L\,\eta \tag{32}$$

$$t_R = L/R\,u \tag{33}$$

and the dependence of the plate height $H$ on the linear solvent velocity $u$ was described by the empirical Kennedy–Knox[12] equation

$$H = 2D_m/u + A(u/D_m)^{0.33}\, d_p^{1.33} + Cud_p^2/D_m \tag{34}$$

with the dimensionless constants $A = 1$ and $C = 0.03$ (see ref. 13); the diffusion coefficient $D_m$ of the solute (benzene) was estimated from the Wilke–Chang equation[14].

As a first step in the calculation of the non-steady-state quantities $\bar{t}_R$ and $\bar{H}$, the dependence $\tau = \tau(\xi)$ was obtained by solving numerically the ordinary differential eqn. 23, with $u\,(\xi, \tau)$ given by eqn. 21, by means of the Runge–Kutta method. (A fine integration step $\Delta\xi = 0.005$ was necessary at the top of the column, i.e., for $0 \leqslant \xi \leqslant 0.2$, where the most dramatic changes of $u$ with both $\xi$ and $\tau$ took place; $\Delta\xi = 0.05$ was sufficient for the remaining part of the column). The final value thus obtained, $\tau = \tau\,(\xi = 1)$, yielded the retention time (eqn. 24).

These values of $\xi_i$ and $\tau\,(\xi_i)$ were then used, together with the dependence $H(u)$ given by eqn. 34, to evaluate by the Simpson rule the integral defining $\bar{H}$ in eqn. 27.

In accordance with the parameters of chromatographic instruments now commercially available, the maximum pressure drop across the column was restricted to some 70 MPa (ca. 700 atm). Results of these calculations for some solvents com-

## TABLE I

EFFECT OF FLOW DISTURBANCE IN STOP-FLOW INJECTION ON RETENTION TIME AND PLATE HEIGHT AS REFLECTED IN THE RATIO OF NON-STEADY-STATE (BARRED) AND STEADY-STATE VALUES

Unretained peak ($R = 1$) in $n$-pentane ($\eta = 0.22$ mPa sec, $\chi = 3.14 \cdot 10^{-3}$ MPa$^{-1}$); column $L = 30$ cm, $d_c = 0.3$ cm; $\Delta P$ is the stationary pressure drop, $Q$ is the solvent flow-rate.

| $Q$ (ml/min) | $\Delta P$ (MPa) | $H \cdot 10^3$ (cm) | $\bar{H}/H$ | $\bar{t}_R/t_R$ |
|---|---|---|---|---|
| Particle diameter $d_p = 20\,\mu$m | | | | |
| 2 | 0.88 | 6.53 | 1.005 | 0.997 |
| 4 | 1.77 | 9.13 | 1.005 | 0.996 |
| 8 | 3.54 | 13.35 | 1.005 | 0.994 |
| 12 | 5.31 | 17.06 | 1.006 | 0.992 |
| Particle diameter $d_p = 10\,\mu$m | | | | |
| 2 | 3.54 | 2.64 | 1.004 | 0.994 |
| 4 | 7.07 | 3.41 | 1.008 | 0.991 |
| 8 | 14.15 | 4.68 | 1.013 | 0.984 |
| 12 | 21.22 | 5.77 | 1.020 | 0.977 |
| Particle diameter $d_p = 5\,\mu$m | | | | |
| 2 | 14.15 | 1.13 | 1.012 | 0.984 |
| 4 | 28.29 | 1.33 | 1.023 | 0.969 |
| 8 | 56.59 | 1.71 | 1.047 | 0.941 |

## TABLE II

EFFECT OF STOP-FLOW INJECTION ON RETENTION TIME AND PLATE HEIGHT IN HPLC WITH HEXANE AS MOBILE PHASE

Unretained peak in $n$-hexane ($\eta = 0.296$ mPa sec, $\chi = 1.62 \cdot 10^{-3}$ MPa$^{-1}$); column $L = 30$ cm, $d_c = 0.3$ cm.

| $Q$ (ml/min) | $\Delta P$ (MPa) | $H \cdot 10^3$ (cm) | $\bar{H}/H$ | $\bar{t}_R/t_R$ |
|---|---|---|---|---|
| Particle diameter $d_p = 20\,\mu$m | | | | |
| 3 | 1.78 | 9.63 | 1.005 | 0.997 |
| 6 | 3.57 | 14.21 | 1.005 | 0.996 |
| 10 | 5.95 | 19.52 | 1.005 | 0.995 |
| Particle diameter $d_p = 10\,\mu$m | | | | |
| 2 | 4.76 | 3.03 | 1.004 | 0.995 |
| 4 | 9.52 | 4.06 | 1.006 | 0.993 |
| 8 | 19.03 | 5.74 | 1.010 | 0.988 |
| 12 | 28.55 | 7.21 | 1.014 | 0.983 |
| Particle diameter $d_p = 5\,\mu$m | | | | |
| 2 | 19.03 | 1.22 | 1.009 | 0.988 |
| 4 | 38.07 | 1.52 | 1.017 | 0.978 |
| | | 2.04 | 1.034 | 0.958 |
| 8 | 76.17 | 4.65* | 1.035* | 0.958* |
| | | 2.04** | 0.998** | 1.005** |

\* Calculated with $A = 2$, $C = 0.1$ in eqn. 34.
\*\* Calculated for the case of boundary conditions, eqn. 35, that correspond to zero initial pressure in the column and in the small-volume reciprocating pump.

monly employed in HPLC are summarized in Tables I–V. As the data in the tables refer to the unretained peak, where the most pronounced effects are to be expected, the influence of the stop-flow injection in HPLC can be said to be small. This is

TABLE III

EFFECT OF STOP-FLOW INJECTION ON RETENTION TIME AND PLATE HEIGHT IN HPLC WITH CARBON TETRACHLORIDE AS MOBILE PHASE

Unretained peak in CCl₄ ($\eta = 0.845$ mPa sec; $\chi = 1.07 \cdot 10^{-3}$ MPa$^{-1}$); column $L = 30$ cm, $d_c = 0.3$ cm.

| $Q$ (ml/min) | $\Delta P$ (MPa) | $H \cdot 10^5$ (cm) | $\bar{H}/H$ | $\bar{t}_R/t_R$ |
|---|---|---|---|---|
| Particle diameter $d_p = 20\ \mu$m | | | | |
| 2 | 3.40 | 10.63 | 1.004 | 0.996 |
| 4 | 6.79 | 15.79 | 1.004 | 0.996 |
| 10 | 16.98 | 28.87 | 1.007 | 0.992 |
| Particle diameter $d_p = 10\ \mu$m | | | | |
| 2 | 13.58 | 3.85 | 1.006 | 0.993 |
| 4 | 27.17 | 5.39 | 1 010 | 0 989 |
| 10 | 67.92 | 9.13 | 1.022 | 0.975 |
| Particle diameter $d_p = 5\ \mu$m | | | | |
| 1.5 | 40.75 | 1.32 | 1.012 | 0.984 |
| 2 | 54.34 | 1.46 | 1.016 | 0.979 |
| | | 3.11* | 1.017* | |
| 2.5 | 67.92 | 1.58 | 1.02 | 0.975 |

* Calculated with $A = 2$, $C = 0.1$ in eqn. 34.

TABLE IV

EFFECT OF STOP-FLOW INJECTION ON RETENTION TIME AND PLATE HEIGHT IN HPLC WITH DIETHYL ETHER AS MOBILE PHASE

Unretained peak in diethyl ether ($\eta = 0.212$ mPa sec; $\chi = 1.87 \cdot 10^{-3}$ MPa$^{-1}$); column $L = 30$ cm, $d_c = 0.3$ cm.

| $Q$ (ml/min) | $\Delta P$ (MPa) | $H \cdot 10^3$ (cm) | $\bar{H}/H$ | $\bar{t}_R/t_R$ |
|---|---|---|---|---|
| Particle diameter $d_p = 20\ \mu$m | | | | |
| 2 | 0.85 | 6.39 | 1.004 | 0.997 |
| 4 | 1.70 | 8.90 | 1.004 | 0.997 |
| 8 | 3.41 | 13.00 | 1.004 | 0.996 |
| 12 | 5.11 | 16.54 | 1.004 | 0.995 |
| Particle diameter $d_p = 10\ \mu$m | | | | |
| 2 | 3.41 | 2.60 | 1.004 | 0.996 |
| 4 | 6.82 | 3.34 | 1.005 | 0.994 |
| 8 | 13.63 | 4.57 | 1.009 | 0.990 |
| 12 | 20.45 | 5.62 | 1.012 | 0.986 |
| Particle diameter $d_p = 5\ \mu$m | | | | |
| 2 | 13.63 | 1.12 | 1.008 | 0.990 |
| 4 | 27.27 | 1.31 | 1.014 | 0.982 |
| 8 | 54.53 | 1.68 | 1.028 | 0.965 |
| | | 3.70* | 1.029* | |

* Calculated with $A = 2$, $C = 0.1$ in eqn. 34.

TABLE V

EFFECT OF STOP-FLOW INJECTION ON RETENTION TIME AND PLATE HEIGHT IN HPLC WITH WATER AS MOBILE PHASE

Unretained peak in water ($\eta = 0.8$ mPa sec; $\chi = 4.6 \cdot 10^{-4}$ MPa$^{-1}$); column $L = 30$ cm, $d_c = 0.3$ cm.

| $Q$ (ml/min) | $\Delta P$ (MPa) | $H \cdot 10^3$ (cm) | $\bar{H}/H$ | $\bar{t}_R/t_R$ |
|---|---|---|---|---|
| Particle diameter $d_p = 10 \,\mu$m | | | | |
| 4 | 25.72 | 7.28 | 1.005 | 0.994 |
| 8 | 51.44 | 11.22 | 1.009 | 0.990 |
| 10 | 64.31 | 13.04 | 1.010 | 0.989 |
| Particle diameter $d_p = 5 \,\mu$m | | | | |
| 1 | 25.72 | 1.38 | 1.005 | 0.994 |
| 1.5 | 38.58 | 1.60 | 1.008 | 0.990 |
| 2 | 51.44 | 1.81 | 1.008 | 0.990 |
| 2.5 | 64.30 | 1.99 | 1.009 | 0.989 |

certainly true with regard to the column efficiency as expressed by the plate height, where the maximum observed effect for n-pentane at a rather high flow-rate of 8 ml/min (Table I) is probably still within the limits of experimental error. For less compressible solvents (water, carbon tetrachloride), the effect on the retention time is also small in all instances considered. However, for solvents with relatively higher values of $\chi$ (diethyl ether, Table IV, and particularly n-pentane, Table I), the effect on retention time amounts to several per cent under the conditions of high inlet pressure, and thus can no longer be neglected.

The very small influence of the parameters $A$ and $C$ in eqn. 34 on the calculated values of $\bar{H}$ (see Tables II–IV) is in accord with the general observations made by Giddings[9] in connection with the non-uniform properties of columns in gas chromatography.

As shown by Kraak et al.[15], it is possible to create liquid chromatographic columns with an exceptionally high plate number by coupling several shorter columns in series. Table VI shows the influence of stop-flow injection on the retention data for such a long column ($L = 120$ cm) and n-pentane. The non-steady-state velocity profile characteristic of the early stages of elution, when the stop-flow injection is used, can shift the retention time of an inert peak significantly downwards in such long columns, but the magnitude of the effect decreases rapidly for retained solutes, as seen from the bottom rows of Table VI.

So far the discussion has been concerned with stop-flow injection used in connection with a positive-displacement pump and a back-valve; accordingly, the partial differential equation, eqn. 5, was solved with boundary conditions 7. However, the stop-flow injection is often used with reciprocating pumps where no valve is considered necessary. Assuming for simplicity that the volume of the cylinder in the reciprocating pump is so small that we can neglect volume changes due to non-zero compressibility of the liquid occupying this space, the boundary conditions for eqn. 5, relevant to this situation, are

$$p(\xi, 0) = 1, \, p(1, \tau) = 1, \, (\delta p / \delta \xi)_{\xi = 0} = -\gamma \tag{35}$$

TABLE VI

EFFECT OF STOP-FLOW INJECTION ON RETENTION TIME AND PLATE HEIGHT IN HPLC WITH PENTANE AS MOBILE PHASE ON A VERY LONG COLUMN

Unretained peak in $n$-pentane ($\eta = 0.22$ mPa sec; $\chi = 3.14 \cdot 10^{-3}$ MPa$^{-1}$); column $L = 120$ cm, $d_c = 0.3$ cm.

| $Q$ (ml/min) | $\Delta P$ (MPa) | $H \cdot 10^3$ (cm) | $\bar{H}/H$ | $\bar{t}_R/t_R$ |
|---|---|---|---|---|
| Particle diameter $d_p = 20 \mu$m | | | | |
| 2 | 3.54 | 6.78 | 1.005 | 0.994 |
| 4 | 7.07 | 9.33 | 1.007 | 0.991 |
| 8 | 14.15 | 13.52 | 1.014 | 0.984 |
| 12 | 21.22 | 17.21 | 1.020 | 0.977 |
| Particle diameter $d_p = 10 \mu$m | | | | |
| 2 | 14.15 | 2.66 | 1.013 | 0.984 |
| 4 | 28.29 | 3.42 | 1.024 | 0.970 |
| 8 | 56.59 | 4.69 | 1.049 | 0.941 |
| Particle diameter $d_p = 5 \mu$m | | | | |
| 1 | 28.29 | 1.11 | 1.020 | 0.970 |
| 1.5 | 42.44 | 1.10 | 1.031 | 0.955 |
| | | | 1.043 | 0.941 |
| 2 | 56.59 | 1.13 | 1.022 * | 0.969 * |
| | | | 1.010 ** | 0.987 ** |
| | | | 1.055 | 0.928 |
| 2.5 | 70.74 | 1.18 | 1.028 * | 0.962 * |
| | | | 1.012 ** | 0.984 ** |

\* Calculated for a solute with $R = 0.5$.
\*\* Calculated for a solute with $R = 0.2$.

where $\gamma = Q_0 \eta L/(k_0 S_{er} P_0)$, with $Q_0$ (ml/sec) being the preset volume output of the pump.

This modified problem can be solved analogously. In this case, the pressure builds up gradually at the top of the column and therefore no dramatic changes in the linear velocity of the solvent can be expected. The results of the calculations show that the sign of the effect is opposite to that in the previous case but that its magnitude is much lower. One of the results is included in Table II for comparison.

In the case of fast GPC, calculations have been made for tetrahydrofuran (THF, a typical GPC solvent) and polystyrene fractions of different molecular weight. A linear calibration was assumed to be valid within the range of molecular weight ($M$) between $10^2$ and $10^7$; the corresponding extreme values of retention volume were calculated from the dimensions of the column ($L = 120$ cm, $d_c = 0.4$ cm) and from $\varepsilon_0 = 0.4$ and $\varepsilon_i = 0.8$. This yielded $V_R$ (ml) $= 16.166 - 1.448 \log_{10} M$ as the equation of the calibration line. As no value for the compressibility factor for THF was found in the literature, it was estimated from the critical data according to Reid and Sherwood[16] as $\chi \simeq 1.2 \cdot 10^{-3}$ MPa$^{-1}$. The Knox equation, eqn. 34, was rather arbitrarily assumed to be valid also for macromolecular solutes with $D_m = K M^{-\alpha}$.

The calculations were made only with the boundary conditions described by eqns. 7. It was again found that the values of the plate height $H$, influenced by the variation in local solvent velocity due to stop-flow injection, departed only slightly

from the corresponding steady-state values $H$ (the deviation never exceeded 5%). The calculated values of the ratio $\bar{t}_R/t_R$ that characterizes the influence of stop-flow injection on the retention time in GPC are summarized in Table VII. Although at first sight the deviations from unity of the data in Table VII are not large, in the GPC mode they can profoundly influence the calculated values of $M$. This effect has been visualized by calculating the relative error in $M$ brought about by the corresponding shift in calibration; these values are also given in Table VII and are seen to be quite large for fast GPC analyses.

TABLE VII

EFFECT OF STOP-FLOW INJECTION IN FAST GPC ON RETENTION TIMES OF POLYMER FRACTIONS OF DIFFERENT MOLECULAR WEIGHT ($M$) AND THE RESULTING SHIFT IN CALIBRATION LINE EXPRESSED BY RELATIVE ERROR IN $M$
Column length $L = 120\ cm$, $d_c = 0.4\ cm$; tetrahydrofuran ($\eta = 0.51$ mPa sec; $\chi = 1.2 \cdot 10^{-3}$ MPa$^{-1}$); $Q$ is the volume flow-rate.

| $d_p$ ($\mu m$) | $Q$ ($ml/min$) | $\Delta P$ ($MPa$) | $M \cdot 10^{-3}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 | | 50 | | 500 | | 5000 | |
| | | | $\bar{t}_R/t_R$ | % Error | $\bar{t}_R/t_R$ | % Error | $\bar{t}_R/t_R$ | % Error | $\bar{t}_R/t_R$ | % Error |
| 5 | 0.2 | 7.4 | 0.994 | 11 | 0.994 | 9 | 0.993 | 9 | 0.992 | 7 |
| | 0.5 | 18.4 | 0.989 | 21 | 0.988 | 20 | 0.986 | 19 | 0.984 | 18 |
| | 1 | 36.9 | 0.981 | 39 | 0.978 | 39 | 0.974 | 39 | 0.969 | 38 |
| | 2 | 73.8 | 0.963 | 89 | 0.958 | 88 | 0.950 | 87 | 0.940 | 85 |
| 10 | 0.2 | 1.8 | 0.997 | 5 | 0.997 | 5 | 0.996 | 5 | 0.996 | 4 |
| | 1 | 9.2 | 0.994 | 11 | 0.993 | 11 | 0.992 | 11 | 0.991 | 10 |
| | 4 | 36.9 | 0.981 | 39 | 0.978 | 39 | 0.974 | 39 | 0.969 | 38 |
| | 8 | 73.8 | 0.963 | 89 | 0.958 | 88 | 0.950 | 87 | 0.940 | 85 |
| 20 | 0.2 | 0.5 | 0.998 | 4 | 0.998 | 3 | 0.998 | 3 | 0.997 | 3 |
| | 1 | 2.3 | 0.997 | 6 | 0.996 | 6 | 0.996 | 5 | 0.996 | 4 |
| | 4 | 9.2 | 0.994 | 11 | 0.993 | 11 | 0.992 | 11 | 0.991 | 10 |
| | 8 | 18.4 | 0.989 | 21 | 0.988 | 20 | 0.986 | 20 | 0.983 | 19 |

Accordingly, in fast GPC one should not establish a calibration line using stop-flow injection and then switch to some "uninterrupted-flow" injection mode (or vice versa). Moreover, it is known that in GPC the pressure drop (at a constant flow-rate) across a column set that has been in use for some time may begin to rise gradually; when stop-flow injection is employed, the resulting shift in the calibration curve can markedly affect the accuracy and reproducibility of the results.

CONCLUSION

In the stop-flow injection method as practised with a positive-displacement pump and a valve, the steep changes in the velocity profile in the initial stages of elution impair the separation efficiency only to a negligible extent. Their effect on the retention time (or volume) is significant only when very fast analyses on columns of low permeability are attempted. The relative downward shift in retention time due to stop-flow injection increases with the (steady-state) pressure drop across the col-

umn and with the compressibility factor of the mobile phase, and decreases with the extent of sample retention. As a consequence of the latter dependence, stop-flow injection must be used with some caution in fast GPC, where it is necessary to calibrate the columns under exactly the same conditions as those employed in the subsequent analyses and their permeability must be checked frequently.

## REFERENCES

1 J. H. Knox, G. R. Laird and P. A. Raven, *J. Chromatogr.*, 122 (1976) 129.
2 T. J. N. Webber and E. H. McKerrell, *J. Chromatogr.*, 122 (1976) 243.
3 B. Coq, C. Gonnet and J.-L. Rocca, *J. Chromatogr.*, 106 (1975) 249.
4 M. Martin, G. Blu, C. Eon and G. Guiochon, *J. Chromatogr.*, 112 (1975) 399.
5 P. Achener, S. R. Abbott and R. L. Stevenson, *J. Chromatogr.*, 130 (1977) 29.
6 M. Martin, G. Guiochon, G. Blu and C. Eon, *J. Chromatogr.*, 130 (1977) 458.
7 H. Jeffreys and B. Jeffreys, *Methods of Mathematical Physics*, Cambridge University Press, London, 3rd ed., 1962, p. 529.
8 E. R. Hansen, *A Table of Series and Products*, Prentice-Hall, Englewood Cliffs, N.J., 1975, p. 222.
9 J. C. Giddings, *Anal. Chem.*, 35 (1963) 353.
10 J. Douglas, Jr. and B. F. Jones, *J. Ind. Appl. Math.*, 11 (1963) 195.
11 R. Endele, I. Haiász and K. Unger, *J. Chromatogr.*, 99 (1974) 377.
12 G. J. Kennedy and J. H. Knox, *J. Chromatogr. Sci.*, 10 (1972) 549.
13 M. Martin, G. Blu, C. Eon and G. Guiochon, *J. Chromatogr. Sci.*, 12 (1974) 438.
14 C. R. Wilke and P. Chang, *Amer. Inst. Chem. Eng.*, 1 (1955) 264.
15 J. C. Kraak, H. Poppe and F. Smedes, *J. Chromatogr.*, 122 (1976) 147.
16 R. C. Reid and T. K. Sherwood, *The Properties of Gases and Liquids*, McGraw-Hill, New York, 2nd ed., 1966, p. 89.