

Note

Stop-flow method of sample injection and unsteady-state flow of the mobile phase through high-performance liquid chromatographic columns

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Stop-flow injection is widely used for the introduction of samples into high-performance liquid chromatographic (HPLC) columns¹. This mode of injection involves the following: the flow of the mobile phase through the column is stopped, a suitable volume of the sample is injected on to the open top of the column, the column is re-sealed and the flow of the mobile phase is re-started. The important advantage is the simple construction of the injectors and their reliable functioning at pressures up to 500 atm. On the other hand, the irreproducibility of the separation process resulting from the unsteady-state flow of the mobile phase at the beginning of the analysis is the main disadvantage of the stop-flow injection¹⁻³.

THEORETICAL

Let us examine the origin of the unsteady-state flow of the mobile phase in an idealized HPLC apparatus, the duration of this unsteady-state flow and its possible influence on the column efficiency. The idealized apparatus includes a supply unit for the mobile phase, which is delivered at a constant pressure p_1 , a valve and an HPLC column. During the sample injection, the pressure on the top and bottom of the column is atmospheric (p_0). After re-sealing the column and opening the valve, the atmospheric pressure p_0 on the the top of the column will increase sharply to a new pressure p_1 . It is evident that, owing to the compressibility of the mobile phase, the linear flow velocity on the top of the column will be higher after the pressure increase $p_0 \rightarrow p_1$ than the linear flow-rate corresponding to the steady-state flow of the mobile phase. This increase in the linear flow velocity is generally assumed to influence the separation efficiency of HPLC columns.

The flow of the mobile phase through a chromatographic column packed with a porous medium is described by Darcy's law⁴:

$$u = (-K/\mu) \text{ grad } p \quad (1)$$

The linear flow velocity (u) is therefore directly proportional to the pressure gradient on the column ($\text{grad } p$) and inversely proportional to the viscosity (μ) of the mobile phase. The permeability (K) characterizing the hydrodynamic resistance of a porous

medium has been approximated by Halász and Walkling⁵ for HPLC columns packed with porous microparticles of diameter d_p by the empirical relationship

$$K \approx d_p^2/1000 \quad (2)$$

Let us suppose that the porous medium is incompressible and that the compressibility (β) of the mobile phase, which is defined by the equation

$$\beta = (-1/V) \left(\frac{dV}{dp} \right) \quad (3)$$

is constant. Assuming that in a linear approximation⁴ the liquid density depends on the pressure p according to the equation

$$\rho = \rho_0 + \beta \rho_0 (p - p_0) \quad (4)$$

the isothermal flow of the mobile phase through a porous medium can be expressed by the second-order differential equation

$$\frac{\partial^2 p}{\partial x^2} = \left(\frac{\mu \varepsilon \beta}{K} \right) \left(\frac{\partial p}{\partial t} \right) + \alpha \left(\frac{\partial^2 p}{\partial t^2} \right) \quad (5)$$

where ε is the column porosity, x is the distance from the end of the column and t is time. The value of ε for columns packed with ordered silica gel microparticles is usually 0.7–0.8 (ref. 6). According to Bennion and Goss⁷, the term $\alpha(\partial^2 p/\partial t^2)$, which is related to inertial forces, can be neglected in eqn. 5 when $\mu \varepsilon \beta / K > 3 \cdot 10^{-7}$; this condition is fulfilled here.

The simplified form of eqn. 5, which is formally identical with that used to describe one-dimensional unsteady-state heat conduction^{4,8}, can be solved for the set of boundary conditions 6a–6c by the method of variable separations^{8,9}. Using this method, Shchelkachev⁹ obtained a relationship (eqn. 7) that reflects the pressure changes with time for an arbitrary cross-section of a column of porous particles.

$$p = p_0 \quad \text{for } x = 0 \quad \text{and } t \langle 0, \infty \rangle \quad (6a)$$

$$p = p_1 \quad \text{for } x = L \quad \text{and } t \langle 0, \infty \rangle \quad (6b)$$

$$p = p_0 \quad \text{for } x \langle 0, L \rangle \quad \text{and } t = 0 \quad (6c)$$

$$\frac{p - p_0}{p_1 - p_0} = \frac{x}{L} - \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{(-1)^{n-1}}{n} \cdot \exp \left(- \frac{n^2 \pi^2 K t}{L^2 \mu \varepsilon \beta} \right) \sin \left(n \pi \cdot \frac{x}{L} \right) \quad (7)$$

where L is column length.

RESULTS AND DISCUSSION

From eqn. 1, it follows that the attainment of the steady-state pressure in the column is accompanied by the simultaneous attainment of the steady-state linear flow velocity of the mobile phase. This effect is very important from the practical point of view, as the changes in the linear flow velocity of the mobile phase could influence the course of the separation process.

Eqn. 8 expresses Darcy's law for unsteady-state flow. Substituting $(\partial p/\partial x)_t$, derived from eqn. 7 in eqn. 8, one obtains a relationship (eqn. 9) that describes the time dependence of the linear flow velocity (u_u) under conditions of unsteady-state flow:

$$u_u = \left(-\frac{K}{\mu}\right) \left(\frac{\partial p}{\partial x}\right)_t \quad (8)$$

$$u_u = u_s \left[1 - 2 \sum_{n=1}^{\infty} (-1)^{n-1} \exp\left(-\frac{n^2 \pi^2 K t}{L^2 \mu \varepsilon \beta}\right) \cos\left(\frac{n \pi x}{L}\right)\right] \quad (9)$$

where u_s is the linear flow velocity under conditions of steady-state flow of the mobile phase.

In Fig. 1, the term u_u/u_s is plotted for several cross-sections of the column as a function of time, which was expressed by the dimensionless parameter $Kt/L^2 \mu \varepsilon \beta$. This plot shows that 99% of the steady-state linear flow velocity can be attained at the top of the column ($x = L$) when the condition given by the following equation is fulfilled:

$$Kt/L^2 \mu \varepsilon \beta = 1 \quad (10)$$

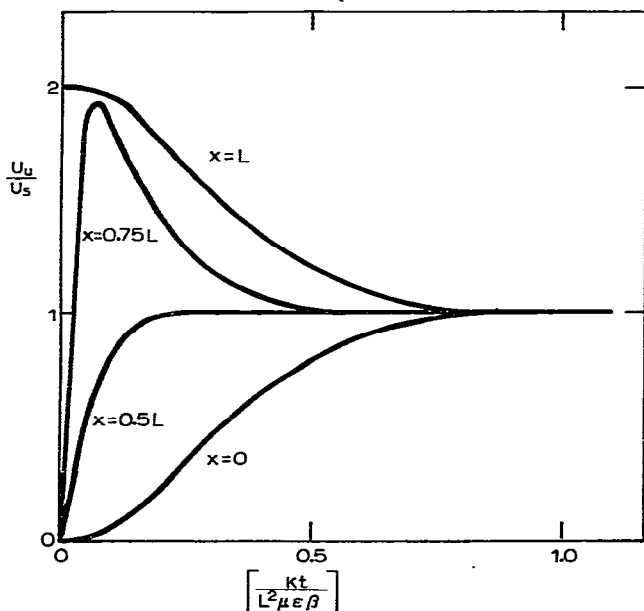


Fig. 1. Time dependence of u_u/u_s for different cross-sections of HPLC columns.

The duration of the unsteady-state flow, t_u , will then be expressed approximately by the relationship

$$t_u \approx L^2 \mu \varepsilon \beta / K \quad (11)$$

The maximum increase in the linear flow velocity attained for a short time interval at the top of a HPLC column will therefore be twice as great as the linear flow velocity u_s under conditions of steady-state flow (Fig. 1).

Let us now estimate the duration of the unsteady-state flow of a mobile phase for an usual HPLC column 20 cm in length packed with porous silica gel micro-particles ($d_p = 10 \mu\text{m}$); the porosity ε is assumed to be 0.75, the permeability $K = 1 \cdot 10^{-13} \text{ m}^2$, the viscosity (μ) of the liquid used as the mobile phase is 0.5 cP and the compressibility $\beta = 1 \cdot 10^{-9} \text{ Pa}^{-1}$. Under these conditions, the duration of the unsteady-state flow, t_u , calculated from eqn. 11 corresponds to about $4 \cdot 10^{-2} \text{ sec}$; this means that a small increase in the linear flow-rate on the top of the column has only a negligible influence on the separation process. A decrease in the separation efficiency of an HPLC column will therefore be observable only at very high linear flow velocities of the mobile phase, *i.e.*, when the duration of the unsteady-state flow is comparable with the retention times, on long HPLC columns with a relatively high value of the constant C in the Van Deemter equation, or using viscous mobile phases of high compressibility. These conclusions hold for HPLC systems in which, as emphasized above, the pressure on the top of the column changes sharply from p_0 to p_1 . However, when the pressure within the mobile-phase supply unit at the beginning of the analysis is p_0 , the duration of the unsteady-state flow would increase depending upon the volumes and hydrodynamic resistances of the pump, valve, connection tubing and other parts of the apparatus^{2,3,10,11}. Consequently, the use of a "hard" mobile-phase supply unit operating under a constant pressure p_1 is desirable for the sample injection on to HPLC columns by the stop-flow method.

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