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Realization and potential advantages of gradient separations performed under steady state temperature regime

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ABSTRACT

This paper describes a new method designed to perform gradient separations while keeping constant the distribution of temperature throughout the column during the three phases of a gradient run (the ramp up of the strong eluent concentration, the column wash, and the re-equilibration steps) in high pressure liquid chromatography (vHPLC). The method is based on the solution of the integrated heat balance equation along the column knowing the variations of the eluent specific heat, its thermal expansion coefficient, and its viscosity with the concentration of the strong eluent. This new method is called gradient under constant wall heat (cWH). Its value is demonstrated by the stability of the eluent temperature at the column outlet during a series of consecutive gradient runs. The potential advantages of cWH gradients over classical gradients *execution mode* at constant flow rate (cF) are illustrated in the example of two such gradients run in the same time. They *include* (1) a diminution by a factor 8 of the largest temperature variation during the sequence of injections (0.4 °C *versus* 3.2 °C); (2) a reduction of the baseline noise by a factor two (0.05 mAU *versus* 0.10 mAU); and (3) the achievement of an excellent injection-to-injection repeatability of the vHPLC system used to measure the first (average RSDs, 0.04%) and the second central (average RSDs $\simeq$ 0.75%) moments of the peaks of an eight-compounds mix, baseline separated, from the first to the last injection.

1. Introduction

The advent of very high-pressure liquid chromatography (vHPLC) methods in the mid 2000s [1–6] allowed a considerable decrease of analysis times in liquid chromatography. Gradient analyses made with short, narrow-bore columns packed with sub-2 μm particles can routinely be made in less than a minute when performed with 1 kbar inlet pressures. Excellent peak capacity can be generated [7] since the minimum plate height of modern columns is as low as 3.5 μm [8].

The exceptional performance of new sub-2 μm particles also shed light on the limits of the new generation of vHPLC systems that can operate columns at inlet pressures of 1.2 kbar. First, the peaks eluted in vHPLC are so *narrow* that the contribution of the instrument to the overall band broadening cannot be neglected any more [8]. The diameter of connecting tubes, the volumes of the sample and of the detector cell must be chosen carefully [9–11]. Original injection protocols were proposed to focus the injected sample zone at the column inlet [12,13]. Also, the combination of high linear velocities and high pressure drops generates a significant amount of heat inside the column. This heat originates from the friction forces between the eluent streamlines in the hydrodynamic boundary layer between the packed particles [14]. This heat is dissipated by axial convection (at the column end), by radial diffusion and radiation (through the column wall), and is absorbed by the eluent as it decompresses along the column. The heat balance in HPLC columns was reported elsewhere [15]. Briefly, one third of the *frictional heat* in liquids is absorbed by the eluent, another third is lost to the external environment, and the last third serves to increase the eluent temperature as it moves along the column. Under steady-state temperature regime, the axial and radial temperature gradients are fully developed along and across the column, respectively [16]. The first gradient affects the retention time of the analyte [17] while the second can significantly deteriorate the column efficiency [4,18].

Gradient elution is usually a three steps process: first, the volume fraction of the strong eluent ramps up, then the column is washed, and the column is finally re-equilibrated with the mobile phase at its initial composition. Under classical constant flow rate (cF) gradients, the pressure drop constantly changes from the beginning to the end of the gradient, during all these three steps. So does the *frictional heat* generated in the column and the temperature gradients. In fast chromatography, the re-equilibration time

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between two successive injections is short compared to the time required to return to the steady state temperature regime. Then, the initial temperature and pressure will necessarily differ to some extent. This limits the repeatability of the retention times and peak variances for a series of successive injections. In a previous study [19], gradients were designed to be performed under constant *frictional heat*. The experimental results showed that the temperature of the column outlet did not remain completely stable at the end of the gradient because the variation of the energy absorbed by the decompressing eluent was neglected. A more sophisticated theoretical approach needed to be investigated.

The goal of this work was to perform gradient separations under a steady-state thermal regime that lasts for the whole three phases of a gradient run (ramp up, wash, and re-equilibration steps) in order to minimize the variations of the initial pressure and temperature between successive injections for short re-equilibration times. A new gradient program (or flow rate and mobile phase composition timetable) is proposed, which require knowledge of (1) the variations of the specific heat ($c_{p,m}$), of the thermal expansion coefficient (α) of the eluent and its viscosity (η) as a function of the content of strong eluent (acetonitrile in water) and (2) the solution of the integrated heat conservation equation during a gradient run under a pseudo steady state thermal regime along the column length. The validity of this new gradient designed for vHPLC analyses will be tested by recording the temperature at the column outlet. Its potential advantages over classical gradients run under constant flow rate are discussed in terms of the *baseline noise amplitude* and *of the* repeatability of the first and second central moments *of* a series of peaks compared to those recorded in the *constant flow mode*.

2. Theory

2.1. Conservation of heat in gradient chromatography

The general differential energy balance of a column was derived by Slattery [20] and applied to chromatographic columns by Horvath [14]. The derivation and the main different assumptions of this heat balance equation were recalled in a previous report [19]. Its final expression is [14,19,21,22,16,15]:

$$[\epsilon_e c_{p,m} + (1-\epsilon_e)c_{p,s}]\frac{\partial T}{\partial t} + \epsilon_e \alpha T \frac{\partial P}{\partial t} = -c_{p,m} u_S \frac{\partial T}{\partial z} + \lambda_{eff,z}\frac{\partial^2 T}{\partial z^2} + \lambda_{eff,r}\left(\frac{1}{r}\frac{\partial T}{\partial r} + \frac{\partial^2 T}{\partial r^2}\right) - u_S\frac{\partial P}{\partial z} - \alpha T u_S \frac{\partial P}{\partial z} \quad (1)$$

where ϵ_e is the external porosity of the bed, $c_{p,m}$ is the specific heat capacity (J/m^3/K) of the mobile phase, $c_{p,s}$ is the effective specific heat capacity (J/m^3/K) of the stationary phase, T is the local temperature (K) that depends on the time t and on the cylindrical coordinates r and z, α is the isobaric thermal expansion coefficient (K^{-1}) of the eluent, P is the local pressure (J/m^3), u_S is the local superficial linear velocity (m/s), $\lambda_{eff,z}$ is the effective heat conductivity (W/m/K) of the packed bed along the column, and $\lambda_{eff,r}$ is the effective heat conductivity (W/m/K) of the packed bed across the column diameter.

It is important to note that in Eq. (1), the isobaric thermal expansion coefficient α of the eluent is defined as:

$$\alpha = \frac{1}{\rho}\frac{\partial \rho}{\partial T} < 0 \quad (2)$$

So, for liquids such as acetonitrile–water mixtures, α is negative. Also, the heat power per volume unit, Φ'', created by the dissipation of the viscous force along the column is positive. It is expressed with the convention that the sign of the pressure increment along the column is opposite to the sign of the distance increment [19]. Therefore,

$$\Phi'' = -u_S\frac{\partial P}{\partial z} > 0 \quad (3)$$

The two terms in the LHS of Eq. (1) are related to the temporal variation of the local temperature and pressure at any position in the column under unsteady conditions, when the temperature and the pressure profiles are not fully developed throughout the column. The first term in the RHS of this equation accounts for the axial convection of heat due to the flow of mobile phase along the column. The second and third terms represent the axial and radial diffusion of heat, along and across the column, respectively. The fourth term expresses the heat power produced by friction of the mobile phase layers migrating in the inter-particle volume, and the last term accounts for heat absorbed by the eluent during its decompression along the column.

Under isocratic conditions, with a constant flow and fixed thermal boundary conditions at the column inlet and at the external surface wall, a steady state regime is eventually reached and the LHS of Eq. (1) reduces to zero because the term $(1+\alpha T)u_S(\partial P)/(\partial z)$ (creation and absorption of heat) is constant. In contrast, in either *constant flow (cF)* or *constant pressure (cP)* gradient elution, the local pressure gradients (in cF mode), the superficial linear velocity u_S (in cP mode), the thermal expansion coefficient α (in both modes), the effective heat conductivities (in both modes), the specific heat capacities (in both modes), and, therefore, the temperature and pressure profiles $T(z, r, t)$ and $P(z, r, t)$ vary continuously with time. The heat generated in and evacuated out of the column by convection, the heat loss due to *dissipation* through the column wall, the heat power due to friction forces, and the heat absorbed by the eluent decompression change continuously during the gradient run for either type of gradient.

Significant variations of the temperature and pressure profiles along and across the column are expected to take place between successive injections. They are difficult to control if the time between two successive runs is not sufficiently long and irregularly chosen, and does not permit the full re-equilibration of the temperature distribution throughout the column. Fluctuations of the initial column conditions inevitably affect the injection-to-injection repeatability of retention times and peak widths of all analytes when the time elapsed between two consecutive injections is shorter than the column equilibration time. This is particularly critical in fast vHPLC gradient elution. A practical solution to this reproducibility problem is presented later.

2.2. Reference volume-based gradient $\varphi(z, t)$

In this work, a same reference linear volume-based gradient of slope β_v was considered. The origin for the time variable t is when the gradient starts in the pump mixer, a time that is assumed to coincide exactly with the sample injection. The total volume of eluent delivered by the pump at time t is given by:

$$V(t) = \int_0^t F_v(t')dt' \quad (4)$$

where t' is the time dummy variable.

The column length is L and z is the axial coordinate. The total volume of the linear gradient is V_G. *It includes a final isocratic washing step during which a wash volume of one column hold-up volume, V_0, is injected at the column inlet. The gradient stops exactly when the end of this plug reaches the column outlet.* This plug is assumed to be non-retained, so it is not distorted upon its migration along the column length. The initial and final mobile phase compositions are