Band broadening along gradient reversed phase columns: A potential gain in resolution factor

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ABSTRACT

The advantages of using columns packed with stationary phases having a composition gradient so that retention factors increase toward the outlet and eluting them with an isocratic mobile phase may provide increased peak resolution in liquid chromatography. This approach is discussed from a theoretical viewpoint. The retention factor is assumed to increase linearly along the column. The peak width can be predicted under three different conditions: (1) the possible band compression is neglected (the Giddings model); (2) band compression is taken into account (the Poppe model); and (3) both band compression and extra-column effects are considered in the calculations. The impact of a stationary phase gradient on the resolution of a pair of compound difficult to separate (constant selectivity $\alpha = 1.05$) is illustrated for a 3 mm × 100 mm silica monolithic column of the second generation (porosity $\epsilon_t = 0.85$, plate height $H = 6\,\mu m$) operated with a new generation of liquid chromatograph (pre- and post-column volume variances of 10 and 5 μL^2, respectively). The results show that the resolution factor R_S becomes optimum for a specific positive value of the retention gradient along the column. This optimum depends on the retention factor at the column inlet.

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1. Introduction

A wide variety of mobile phase gradients are commonly used in liquid chromatography, including gradients of eluent composition, pH, and mobile phase velocity [1,2]. Thermal gradients were also applied along the column [3]. Efforts were devoted towards the preparation of optimized stationary phase gradients when the nature and the composition of the eluent are imposed by the separation problem [4] or when, for some economic reasons, alternative eluents are necessary [5]. The advantages of using a stationary phase gradient are the reduction of the mobile phase costs since it is simpler to prepare isocratic than gradient mobile phases, of the analysis time and the consumption of chemicals since it is not necessary to re-equilibrate the column between successive runs, and of instrument maintenance since the pressure along the instrument is stable.

Gradient stationary phase columns are characterized by a variation of their physico-chemical properties (chemical nature, material, temperature due to frictional heating [6–11], their

bonding density [12,13], their packing density [14], etc.) along their length and diameter. For instance, superhydrophobic monolith porous polymer layers with a gradient of hydrophobicity were prepared and used for 2-D thin layer chromatography of peptides [12]. A series of two plates (silica gel and coated cellulose) was used to separate water-soluble vitamins [13]. Even though the practical relevance of gradient stationary phase is unquestionable, the advantages of columns packed with these phases in terms of peak width and peak capacity has yet to be assessed from a theoretical viewpoint. It still remains unknown whether columns packed with gradient stationary phases can provide larger peak capacities and better resolution than classical columns packed with a uniform stationary phase in gradient elution chromatography.

The widths of bands eluted along gradient stationary phase gas chromatographic (GC) columns were determined by Giddings in 1963 [15,16]. His results were applied to account for the effects of gas compression in GC, in which the linear velocity of the carrier gas depends on the position or local pressure along the column, and for the determination of the apparent height equivalent to a theoretical plate (HETP) of a series of columns. This theory holds as long as the gradient applied can be considered as negligible along the bandwidth or when the phenomenon of band compression described by Poppe [17] in liquid chromatography can be neglected. In Giddings model, the order in which the series of columns are connected

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has no impact on the apparent HETP. However, the orientation of the gradient of the stationary phase properties with respect to the direction of the flow stream should have an impact on the apparent HETP. For instance, assuming that the surface bonding density and the retention factors increase from the inlet to the outlet of the column, the front part of the band profile moves slightly faster than the rear part, which is more retained. This is the essence of the band compression reported by Poppe [17].

The goal of this work is the determination of the bandwidth and the apparent HETP of gradient reversed phase columns due to a continuous gradient of the stationary phase properties. For the sake of simplicity, the gradients of physico-chemical properties are such that the retention factors are assumed to vary linearly along the column. This is approximately true for endcapped RPLC-C_{18} stationary phases for which the gradient of the surface density in octadecyl bonded ligands is linear between 0 and 2 μmol/m^2 [18]. In a first calculation, the phenomenon of band compression is neglected and Gidding model is used. In a second one, the impact of band compression on the apparent HETP is predicted from a direct extension of the general theory of peak broadening in solvent-programmed elution to stationary gradient stationary phase. In a third one, the impact of the extra-column band broadening contribution when a gradient stationary phase is used on the peak width observed is investigated quantitatively. Finally, the advantage of using a stationary phase gradient over classical solvent-programmed elution is discussed in terms of band compression factor and resolution factor for the same difference of retention factor between the column inlet and the column outlet.

2. Theory

In this work, the retention factor k' is assumed to vary linearly along the chromatographic column. Therefore, the local retention factor of the sample at the axial position z is given by:

$$k'(z) = k'_0 + az \tag{1}$$

where k'_0 is the retention factor at the column inlet and a is a constant accounting for the retention factor gradient (dk'/dz). Note that a can be either positive (if retention increases from the column inlet to its outlet) or negative (if retention decreases from the column inlet to its outlet). The parameter a takes values from $(-k'_0/L)$ to $+\infty$. When $a = 0$, the column is uniform.

2.1. Elution time with gradient stationary phases

The chromatographic linear velocity is called u_0. By definition:

$$u_0 = \frac{F_v}{\epsilon_t \pi r_c^2} \tag{2}$$

where F_v is the flow rate, ϵ_t is the total porosity of the column, and r_c is the column inner radius. u_0 is assumed to be constant along the whole length of the column (the liquid mobile phase can be considered as incompressible). The elution time t_R of the sample zone reaching the axial position μ_1 is given by the classical gradient equation [1,2]:

$$\int_0^{t_R} dt = \int_0^{\mu_1} \frac{dz}{u(z)} \tag{3}$$

where $u(z)$ is the linear migration velocity of the sample at the axial position z. So,

$$\int_0^{t_R} dt = \int_0^{\mu_1} (1 + k'_0 + az) \frac{dz}{u_0} \tag{4}$$

The result of this integration gives the gradient time at position μ_1:

$$t_R = \frac{\mu_1}{u_0} \left(1 + k'_0 + a\frac{\mu_1}{2} \right) \tag{5}$$

In conclusion, the gradient elution time at the column outlet ($\mu_1 = L$) is the elution time along a virtual and uniform column for which the unique retention factor is equal to the one observed at half the length of the actual gradient stationary phase column.

2.2. Bandwidth with gradient stationary phases

The prediction of the bandwidth for gradient stationary phases is carried out under three different conditions. By increasing order of system complexity:

- Band compression for $a > 0$ or band enlargement for $a < 0$ are neglected. The calculations are based on the simple Giddings model for gradient stationary phase columns [15,16].
- Band compression or enlargement are taken into account according to the general model of Poppe [17].
- The contribution of the extra-column volume on the observed bandwidth is added in the prediction.

2.2.1. Absence of band compression or band enlargement

In this section, the apparent plate height $\langle H \rangle_z$ along the stationary phase gradient is given by the Giddings model [15] for gradient stationary phase columns. The theory of Giddings on the time and spatial band variance along non-uniform columns is general, irrespective of whether this heterogeneity is due to the mobile phase or to the stationary phase properties. What matters in this problem is to account properly for the variation of the migration linear velocity along the column, no matter what is causing it. In this model, band compression is omitted. $\langle H \rangle_z$ is derived from the equation given in reference [15]:

$$\langle H \rangle_z = L \frac{\int_0^L \frac{H(u_0, z)}{u(z)^2} dz}{\left[\int_0^L \frac{1}{u(z)} dz \right]^2} \tag{6}$$

Let assume that the chromatographic linear velocity u_0 is constant along the column and that the local plate height $H(z)$ is independent of the local retention factor $k'(z)$. Then $H(u_0, z) = H = $ Cte, where Cte represents one constant value for H. The local linear velocity $u(z)$ of the peak is:

$$u(z) = \frac{u_0}{1 + k'_0 + az} \tag{7}$$

Therefore, introducing Eq. (7) into Eq. (6) gives:

$$\langle H \rangle_z = HL \frac{\left[\frac{1}{3a} \left(1 + k'_0 + az \right)^3 \right]_0^L}{\left(\left[\frac{1}{2a} \left(1 + k'_0 + az \right)^2 \right]_0^L \right)^2} \tag{8}$$

After some basic algebra, the final result is:

$$\langle H \rangle_z = H \frac{1 + p(L) + \frac{p(L)^2}{3}}{\left(1 + \frac{p(L)}{2} \right)^2} \tag{9}$$

where $p(L)$ is given by:

$$p(L) = \frac{aL}{1 + k'_0} \tag{10}$$

The most remarkable result is that the ratio of the two polynomials in $p(L)$ in the right-hand-side of Eq. (9) following H is strictly larger than unity, irrespective of the sign of $p(L)$, which depends on the sign of the constant a (negative or positive gradient of the retention