Journal of Chromatography A, 1344 (2014) 66–75

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Separations by gradient elution: Why are steep gradient profiles distorted and what is their impact on resolution in reversed-phase liquid chromatography

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ARTICLE INFO

Article history:
Received 25 February 2014
Received in revised form 2 April 2014
Accepted 3 April 2014
Available online 18 April 2014

Keywords:
Gradient chromatography
Distorted gradients
Excess adsorption isotherms
Methanol
Acetonitrile
Symmetry-C_{18}

ABSTRACT

The formation of a concentration shock layer of either methanol or acetonitrile used as the modifier in steep RPLC aqueous/organic gradients was predictable from their excess adsorption isotherm and was directly observed from their elution profiles recorded in UV absorption. A discontinuity of the concentration profile of the organic modifier arises and grows along the column. Its effects on the peak shapes and the resolution levels achieved in steep gradient chromatography were measured in the recorded chromatograms of a sample mixture containing 14 different analytes uniformly distributed across the gradient retention window. The results showed severe peak shape deformation for some compounds and a significant degradation of the baseline resolution of weakly (when using acetonitrile) and moderately (when using methanol) retained analytes. Solutions to limit this loss of resolution may lie in changing the initial gradient conditions or in adjusting the curvature of the gradient profile at the column inlet.

1. Introduction

Gradient elution methods are widely applied in both analytical [1,2] and preparative [3,4] chromatography. They are applied in most retention modes, including ion-exchange, normal-phase, and RPLC chromatography. They permit the resolution of highly complex sample mixtures in a much shorter time than could be expected in isocratic elution. Additionally, peak widths and overloaded band profiles are thinner, allowing better resolution and sample purification. To implement gradient elution, the eluent concentration of a strong solvent of the sample compounds is progressively increased at the column inlet. Most often, for the sake of simplicity, the HPLC instrument software programs a linear increase of the modified concentration. However, nonlinear programs may be used to properly modulate retention patterns and improve the quality of the separation [5].

The main theories of gradient elution chromatography [2,6,7] are based on the assumption that the strong eluent has no affinity for the solid adsorbent. As a result, the whole inlet gradient profile of this solvent conserves its initial shape and propagates at the same chromatographic velocity along the column, as an unretained plug. Additionally, these theories often assume that the retention behavior follows the linear solvent strength model (LSSM) [6]. The elution times expected in gradient elution with a quadratic solvent strength model (QSSM) were recently derived analytically [8,9] but their expression was not found convenient by analysts and is rarely used. To cope with that problem, Neue and Kuss [10] proposed an “integrable” expression for a non-linear retention behavior, which somehow differs from the true parabolic retention behavior. Finally, if the modifier is adsorbed, peak retention times [11–13], peak variances [13], and the band compression factor [14] can be predicted if the uptake of the strong eluent onto the stationary phase were described by a strictly linear adsorption isotherm.

Unfortunately, the excess adsorption isotherms of the classical organic modifiers used to elute columns packed with RPLC-C_{18} adsorbents is not constant over the whole range of mobile phase composition (i.e., from pure water to pure organic modifier). Past and recent measurements of excess adsorption isotherms of these modifiers onto old and modern HPLC stationary phases showed that they are non-linear [5,15–21]. Therefore, the theory of non-linear chromatography [3,22,23] predicts a progressive deformation of these modifier profiles during their migration along the column. The shorter the gradient time and the smaller the eluent flow rate, the most likely the formation of a concentration shock layer. This

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http://dx.doi.org/10.1016/j.chroma.2014.04.010

shock layer grows along the column depending on the intensity of the second derivative of the excess adsorption isotherm with respect to the bulk concentration at the beginning of the gradient [24]. However, no experimental result has yet validated this result nor characterized the impact of this non-linear adsorption phenomenon on the elution profile of the modifier, on the shape of eluted peaks, and on the resolution achieved with steep gradients.

A first goal of this work is to predict the modifier concentration profiles (either methanol or acetonitrile) under linear gradient conditions from (1) their excess adsorption isotherm (measured with the minor disturbance method) onto a brand new column; and (2) their elution band profiles calculated with the equilibrium dispersive (ED) model of chromatography. These profiles will be compared to the chromatograms recorded in UV at wavelengths below 200 nm for methanol and acetonitrile eluted in water. A second goal of this work is to investigate the impact of the distorted band profiles of methanol and acetonitrile on the performance of steep gradients. Finally, practical solutions will be proposed to limit the loss of peak capacity when applying very steep gradients in analytical throughput attempts.

2. Theory

2.1. Excess adsorption isotherm for a binary mixture of eluent

All the definitions needed for the elution volume V_R of perturbation peaks on an equilibrium plateau, the thermodynamic void volume V_M of the column, and the excess adsorption isotherm data, n_A^e, were previously given in Ref. [24]. The unique experimental value of n_A^e is accessible by minor disturbance experiments on an equilibrium plateau [17,18,20,21,25]. It is given by

$$n_A^e = \frac{1}{\nu_A^*}\int_0^{x_A}(V_R - V_M)dx_A \tag{1}$$

where ν_A^* is the molar volume of the pure organic eluent and x_A is its bulk volume fraction.

The total amount of modifier adsorbed, n_A^a, depends on the location of the Gibbs's dividing surface that separates the bulk phase of composition x_A and the adsorbed phase. Therefore, it is an arbitrary quantity that has no direct physical sense. It simply obeys the principle of mass conservation in the adsorption system. The volume assumed to be occupied by the bulk phase of uniform composition x_A is called V_0. If $V_0 = V_M$, $n_A^a = n_A^e$ and the volume of adsorbed phase is zero [20]. In general, f is defined as the fraction of the thermodynamic void volume occupied by the adsorbed phase in which the composition is uniform. By definition [24]:

$$n_A^a = x_A\frac{fV_M}{\nu_A^*} + n_A^e \tag{2}$$

The number of mole, n_A^m of organic solvent in the bulk phase is then [24]:

$$n_A^m = x_A\frac{(1-f)V_M}{\nu_A^*} \tag{3}$$

2.2. Early shock formation

The theory of shock formation in the framework of the ideal model was developed in [3,22–24]. It was shown that a concentration shock of height zero for the organic modifier takes place in the column at time $t_{s,0}$ and axial position $z_{s,0}$ [24]. The values of $t_{s,0}$ and $z_{s,0}$ are provided below for a linear gradient starting at $x_A = 0$ and ending at $x_A = 1$ during a gradient time t_g and at a flow rate F_ν:

$$t_{s,0} = -\frac{\dfrac{V_M}{\nu_A^*} + \left[\dfrac{dn_A^e}{dx_A}\right]_{x_A=0}}{\left[\dfrac{d^2n_A^e}{dx_A^2}\right]_{x_A=0}}t_g \tag{4}$$

$$z_{s,0} = -\frac{LF_\nu}{\nu_A^*\left[\dfrac{d^2n_A^e}{dx_A^2}\right]_{x_A=0}}t_g \tag{5}$$

The shock is formed and grows along the column if $z_{s,0} < L$, or when

$$t_g < -\frac{\nu_A^*\left[\dfrac{d^2n_A^e}{dx_A^2}\right]_0}{F_\nu} \tag{6}$$

The smaller the gradient time, the more likely a concentration shock of organic modifier will form and develop further along the column.

Actually, the formation of a true shock is physically impossible because chromatography can never be implemented under the conditions postulated by the ideal model. The column efficiency is finite and mass transfer cannot be infinitely fast as required by this model. So, during the band migration, two phenomena affect the modifier band profile. Because its isotherm is langmuirian, the modifier concentration velocities increase with increasing concentration and the higher concentrations move farther ahead in the column but the higher concentrations enter the column after the lower ones and a fast moving concentration cannot pass a slower one: there can be only one finite concentration in any given point of the column. So the high concentrations tend to pile up at the peak front and cause an even higher raising profile that would become a shock if it were not for mass transfer resistance. Because the column efficiency is limited, the building up of the shock is limited by the finite rate of diffusion. As shown by Rhee et al. [3,26–29], a dynamic equilibrium between these two phenomena takes place, a shock layer is formed and elutes at the time predicted for the shock itself. The front boundary of the elution band becomes extremely steep; its slope is related to the column efficiency [3,28]. The rear boundary is diffuse. Experimental results have confirmed the validity of these conclusions [3,30–33].

2.3. Numerical calculations

Calculations of methanol and acetonitrile band profiles along the column during gradient elution were performed using the equilibrium-dispersive (ED) model of chromatography [3]. This model assumes instantaneous equilibrium between the mobile and the stationary phases and a finite column efficiency characterized by an apparent axial dispersion coefficient, D_a. This coefficient accounts for the band broadening contributions due to axial dispersive phenomena (molecular and eddy diffusion), to the finite kinetics of mass transfer between the two phases in the column, and to the extra-column effects (dwell and post-column volumes). The apparent axial dispersion coefficient is related to the apparent column efficiency through:

$$D_a = \frac{uL}{2N} \tag{7}$$

where L is the column length, N is the number of theoretical plates or apparent efficiency of the column measured under linear conditions, i.e., with samples small enough that the column efficiency is independent of the sample size, and u is the apparent linear velocity of the mobile phase associated with the position of the Gibbs' dividing surface:

$$u = \frac{F_\nu}{(1-f)\epsilon_t\pi r_c^2} \tag{8}$$