Journal of Chromatography A, 1340 (2014) 50–58

Contents lists available at ScienceDirect

Journal of Chromatography A

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

The distortion of gradient profiles in reversed-phase liquid chromatography

Fabrice Gritti, Georges Guiochon*

Department of Chemistry, University of Tennessee, Knoxville, 37996–1600, USA

ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form 2 March 2014
Accepted 3 March 2014
Available online 18 March 2014

Keywords:
Gradient chromatography
Distorted gradients
Excess adsorption isotherms
Gibbs' dividing surface
Acetonitrile
RPLC-C_{18}

ABSTRACT

Severe distortions of the axial concentration profiles of modifiers in steep RPLC gradients were recently observed. These distortions are directly explained by the results of measurements of the excess adsorption isotherms of the strongest mobile phase component, the concentration of which is made to increase linearly with time at the column inlet. A front shock or a discontinuity of the organic modifier concentration may arise and grow along the column. The position where it forms is determined by the reciprocal of the second derivative of the excess adsorption isotherm with respect to the concentration of the strongest mobile phase component. It forms when two characteristic lines intersect for the first time. Gradient profiles are continuous and diffuse as long as characteristic lines do not intersect but diverge from each other. However, acetonitrile–water gradients are systematically distorted and deviate significantly from assumed ideal, linear, non-retained gradients. This challenges the validity of classical theories of gradient chromatography regarding the prediction of retention times, peak widths, and band compression factors when steep gradients are applied.

1. Introduction

Gradient elution chromatography is a widely applied separation technique [1]. It allows analysts to resolve successfully complex sample mixtures (particularly those characterized by a wide range of physico-chemical properties, *e.g.*, mass, polarity, *etc.*) in a short time. In gradient elution, the concentration of the strongest mobile phase component is made to increase with time at the column inlet. In most applications, gradients are linear, the concentration of the strongest eluent increasing linearly with the elapsed time. The classical theory of gradient chromatography [2–4] predicts the retention times and peak widths assuming that the strong eluent (the organic modifier in RPLC) does not adsorb onto the stationary phase. Consequently, the whole gradient profile moves along the column at the constant chromatographic velocity and remains linear during its migration. The conventional theory of gradient chromatography was extended to particular cases in which the equilibrium isotherm of the strong mobile component is strictly linear over the whole concentration range encountered during the gradient. The retention times [5–7], the peak widths [7], and the band compression factors [8] were then corrected for the uptake of the strong eluent onto the stationary phase. This more general theory is valid only when the isotherm remains strictly linear or when the amplitude of the gradient is small. However, adsorption isotherms of adsorbed organic modifiers onto RPLC-C_{18} stationary phases are not linear over the whole range of mobile phase composition, from pure water to pure organic modifier. Past and recent measurements of excess adsorption isotherms of strong eluents with respect to the bulk concentration clearly showed that they are non-linear [9–15]. The rate of uptake of the organic modifier is maximum in the water-rich eluent. It decreases continuously with increasing content of organic solvent. Therefore, the theory of nonlinear chromatography [16–18] predicts that the gradient profile deforms progressively and deviates from a linear behavior during its migration along the column. Then, the classical theories of gradient elution become incorrect and do not account accurately for the experimental gradient times nor for peak resolutions. The main goal of this work was to predict the concentration profile along a column of the strongest mobile phase component as a function of the elapsed gradient time. The gradient is assumed to be linear at the column inlet. The calculations are based on the results of measurements of the excess adsorption isotherm of the organic modifier and apply rigorously the concept of a Gibbs' dividing surface separating the adsorbed from the bulk phase [19]. A gradient performed with a symmetry-C_{18} stationary phase and a mixture of

* Corresponding author. Tel.: +1 865 974 0733; fax: +1 865 974 2667.
E-mail addresses: guiochon@utk.edu, guiochon@ion.chem.utk.edu (G. Guiochon).

http://dx.doi.org/10.1016/j.chroma.2014.03.004
0021-9673/

acetonitrile and water were used. The excess adsorption isotherm of acetonitrile from water was acquired by the minor disturbance method [10]. The equilibrium-dispersive model of chromatography was used [16] to calculate the band profiles. This work relates the distortion of the gradient profile, the emergence of a concentration discontinuity (shock layer), and the steepness of the gradient. Finally, it assesses the deviation of actual gradient profiles from the ideal, linear, and non-retained gradients assumed to be true in the conventional theory of gradient chromatography.

2. Theory

2.1. Definitions

In this work, the mobile phase is a binary mixture of water and an organic modifier (methanol, ethanol, iso-propanol, acetonitrile, or tetrahydrofuran). This mixture is assumed to be ideal, so the partial molar volumes are equal to the molar volumes of the pure organic solvents, ν_A^*. The eluent is assumed to be incompressible. The volume fraction of the organic solvent in the bulk eluent is x_A while y_A is the volume fraction of the organic eluent inside the accessible volume in the chromatographic column at equilibrium. Knox and Kaliszan showed that the elution volume, V_R, of a pulse of organic modifier in a column equilibrated with the binary eluent of volume fraction x_A in the organic solvent is given by [10]:

$$V_R = V_M \frac{dy_A}{dx_A} \tag{1}$$

where V_M is the thermodynamic void volume, defined as the sum of the volumes of each solvent component. Integration of Eq. (1) between the volume fractions $x_A = 0$ and $x_A = 1$ provides the volume V_M [10]:

$$\int_0^1 V_R dx_A = V_M \tag{2}$$

The excess number of moles n_A^e (n_A^e can be either positive or negative) of the organic solvent is defined as the equilibrium number of moles of the organic solvent present in the column volume V_M after subtracting the number of its moles present in the same volume if the adsorbent does not adsorb any solvent component ($y_A = x_A$). Accordingly,

$$n_A^e = \frac{V_M y_A}{\nu_A^*} - \frac{V_M x_A}{\nu_A^*} \tag{3}$$

where V_M is the void volume defined as the sum of the individual volumes of each solvent component. It is important to note that n_A^e is unique and is accessible by minor disturbance experiments on a plateau [12–14,20,15]:

$$n_A^e = \frac{1}{\nu_A^*} \int_0^{x_A} (V_R - V_M)\, dx_A \tag{4}$$

In contrast, the total amount of organic solvent 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. The volume of the bulk phase is V_0. If $V_0 = V_M$, $n_A^a = n_A^e$ and the volume of adsorbed phase is zero [14]. In practice, let define f as the fraction of the thermodynamic void volume V_M occupied by the adsorbed phase. By definition:

$$n_A^a = x_A \frac{f V_M}{\nu_A^*} + n_A^e \tag{5}$$

The volume of the bulk phase is then

$$V_0 = (1 - f) V_M \tag{6}$$

and the number of mole, n_A^m of organic solvent in the bulk phase is:

$$n_A^m = x_A \frac{(1 - f) V_M}{\nu_A^*} \tag{7}$$

2.2. The mass balance

The Gibbs's dividing surface or the volume fraction f need to be defined. The differential mass balance equation under ideal chromatography (when the apparent axial dispersion coefficient is assumed to be equal to zero) is written [16]:

$$\frac{\partial n_A^a}{\partial t} + \frac{\partial n_A^m}{\partial t} + u_0 \frac{\partial n_A^m}{\partial z} = 0 \tag{8}$$

where u_0 is the chromatographic linear velocity defined by:

$$u_0 = F_v \frac{L}{(1 - f) V_M} \tag{9}$$

where L is the column length and F_v is the applied flow rate. Eq. (8) can be rewritten as:

$$\left[1 + \frac{dn_A^a}{dn_A^m}\right] \frac{\partial x_A}{\partial t} + u_0 \frac{\partial x_A}{\partial z} = 0 \tag{10}$$

According to Eqs. (5) and (7),

$$\frac{dn_A^a}{dn_A^m} = \frac{f}{1 - f} + \frac{\nu_A^*}{(1 - f) V_M} \frac{dn_A^e}{dx_A} \tag{11}$$

So, by combining Eqs. (10) and (11), we obtain

$$\left[1 + \frac{\nu_A^*}{V_M} \frac{dn_A^e}{dx_A}\right] \frac{\partial x_A}{\partial t} + u_0 (1 - f) \frac{\partial x_A}{\partial z} = 0 \tag{12}$$

This equation provides the characteristics lines of the problem that describe the propagation of finite concentrations along the column.

2.3. Characteristic lines

Along a characteristic line, the volume fraction x_A of the organic solvent is constant. The reciprocal of its propagation velocity along the column is given by Eq. (12):

$$\left[\frac{dt}{dz}\right]_{x_A} = \frac{1 + (\nu_A^*/V_M)[(dn_A^e/dx_A)]_{x_A}}{u_0 (1 - f)} = \frac{V_M + \nu_A^* [(dn_A^e/dx_A)]_{x_A}}{L F_v} \tag{13}$$

If the gradient is assumed to be linear at the column inlet

$$x_A(z = 0, t) = x_{A,i} \quad t < 0 \tag{14}$$

$$x_A(z = 0, t) = x_{A,i} + (x_{A,f} - x_{A,i}) \frac{t}{t_g} \quad 0 < t < t_g \tag{15}$$

$$x_A(z = 0, t) = x_{A,f} \quad t_g < t \tag{16}$$

where $x_{A,i}$ and $x_{A,f}$ are the initial and final volume fractions of the organic solvent, respectively, and t_g is the gradient time. Integration of Eq. (13) between $t(z = 0, x_A)$ and time $t(z, x_A)$ leads to:

$$t(z, x_A) - \frac{x_A - x_{A,i}}{x_{A,f} - x_{A,i}} t_g = \frac{V_M + \nu_A^* [dn_A^e/dx_A]_{x_A}}{L F_v} z \tag{17}$$

Eq. (17) is the characteristic line for the volume fraction of organic solvent x_A. It is clearly determined from the results of the minor disturbance experiments (V_M and n_A^e), the linear gradient conditions ($x_{A,i}$, $x_{A,f}$, and t_g), the column length (L), and the applied flow rate (F_v).