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Mass transfer coefficients determination from linear gradient elution experiments

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ABSTRACT

A procedure to estimate mass transfer coefficients in linear gradient elution chromatography is presented and validated by comparison with experimental data. Mass transfer coefficients are traditionally estimated experimentally through the van Deemter plot, which represents the HETP as a function of the fluid velocity. Up to now, the HETP was obtained under isocratic elution conditions. Unfortunately, isocratic elution experiments are often not suitable for large biomolecules which suffer from severe mass transfer hindrances. Yamamoto et al. were the first to propose a semi-empirical equation to relate HETPs measured from linear gradient elution experiments to those obtained under isocratic conditions [7]. Based on his pioneering work, the approach presented in this work aims at providing an experimental procedure supported by simple equations to estimate reliable mass transfer parameters from linear gradient elution chromatographic experiments. From the resolution of the *transport model*, we derived a rigorous analytical expression for the HETP in linear gradient elution chromatography.

1. Introduction

The dispersive processes in chromatographic columns are traditionally characterized by measuring the HETP at different flow rates. The equation relating the broadness of the chromatograms to the linear mobile phase velocity is the well-known van Deemter equation:

$$HETP = \breve{A} + \frac{\breve{B}}{u} + \breve{C}u \tag{1}$$

where $\breve{A}$, $\breve{B}$ and $\breve{C}$ are related to the Eddy-diffusion, the axial dispersion and to the overall mass transfer resistance between the mobile and stationary phases, respectively [1]. This equation is only valid for HETPs obtained with linear isotherms and under isocratic conditions. Unfortunately isocratic elution chromatography is often not convenient for large macromolecules which suffer from severe steric hindrance and whose elution profiles tend to flatten out, thus affecting the detectability and the precision of the measurements [2]. Instead, gradient elution is a very convenient method, well adapted to bio-macromolecules resulting in sharper peaks. Extensive work was developed by Yamamoto et al. to estimate equilibrium isotherm parameters [3,4] and mass transfer coefficients [5–7] from linear gradient elution (LGE) experiments.

To relate the HETP in LGE to the one obtained under isocratic conditions, Yamamoto introduced the so-called *compression factor* defined by:

$$C_f = \frac{\sigma_{LGE}}{\sigma_{iso}(c_M^R)} \tag{2}$$

where σ_{LGE} and σ_{iso} are the standard deviations of the chromatograms measured under LGE and isocratic conditions, respectively. To be comparable to the LGE experiment, the isocratic elution is performed at $c_M^R = c_M(L, t_R)$, which is the modifier concentration measured at the end of the column ($z = L$) when the solute of interest is eluting (at time t_R) while applying a linear gradient. Knowing the HETP in LGE and the compression factor, the HETP in isocratic elution is obtained from the following equation:

$$HETP_{iso} = HETP_{LGE}\left(\frac{t_R}{t_R^{iso}}\right)^2 \frac{1}{C_f^2} \tag{3}$$

where t_R is the solute retention time from LGE while t_R^{iso} is the corresponding retention time from isocratic elution. For the compression factor Yamamoto et al. derived a semi-empirical expression [5,8]:

$$C_f = \begin{cases} \sqrt{\Lambda} & \text{for } \Lambda < 0.25 \\ \dfrac{3.22\Lambda}{1+3.13\Lambda} & \text{for } 0.25 < \Lambda < 12 \\ 1 & \text{for } \Lambda > 12 \end{cases}, \quad \text{where } \Lambda = \frac{1}{2}\frac{1+K_R}{1+K_\infty}\frac{1+\beta}{\beta} \tag{4}$$

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The parameters used in the definition of Λ are defined in the next section.

In this work, we propose an analytical expression of the HETP in LGE based on the analytical resolution of the *transport model*. This classical model for chromatography has been solved analytically in the Laplace space for LGE and an expression of the HETP was derived by calculating the moments of the thus obtained solution.

2. Theory

2.1. Transport model

At low solute concentration, the equilibrium concentration of solute retained in the stationary phase (q^*) is linearly related to the solute concentration in solution (c) by a linear isotherm defined as follows:

$$q^* = Hc \tag{5}$$

where H is known as the Henry coefficient. It is related to the retention factor by $K = \nu H$, where $\nu = 1 - \varepsilon/\varepsilon$ is the phase ratio defined with respect to the bed porosity ε. In the frame of the *stoichiometric displacement* model (*SD*) [9,10] the retention factor is related to the modifier concentration (often a salt as NaCl in ion exchange chromatography) by a power function:

$$K = K_\infty + \alpha c_M^{-\beta} \tag{6}$$

where $K_\infty = \varepsilon_p \nu$ is the retention factor at infinite modifier concentration, and α and β are the two parameters used to relate the retention factor to the modifier concentration. $c_m(z,t)$ (written c_M for short) is the modifier concentration at time t and position z along the column.

The intra-particle porosity, ε_p, represents the volume fraction of the porous particles accessible to the solute. For large macromolecules, ε_p is defined in terms of accessible porosity: $\varepsilon_{p,i}$. Therefore, $\varepsilon_{p,i}$ is ranging from ε_p obtained for small non-excluded tracers which can access all the pores without restrictions, to 0 for completely excluded molecules. In terms of retention factor at infinite modifier concentration, it comes $K_{\infty,i} = \varepsilon_{p,i}\nu$. The total, and total accessible porosities are defined by $\varepsilon_t = \varepsilon(1 + K_\infty)$ and $\varepsilon_{t,i} = \varepsilon(1 + K_{\infty,i})$, respectively. The porosity distributions of the resin under consideration are given in our previous article [11].

Among the different chromatographic models available in the literature [2,12], the *transport model* [13,14] offers a good approximation of the dissipative mechanisms by lumping together all the mass transfer resistances in a single overall parameter k_m. In the frame of the *transport model*, the chromatographic column is modeled as an ideal plug flow (negligible axial dispersion). This assumption is valid when the mass transfer resistance is dominated by the diffusion of the macromolecules in the porous particles. The mass balance equation for a given solute (or modifier) is written as follows:

$$u\frac{\partial c}{\partial z} + \varepsilon\frac{\partial c}{\partial t} + (1-\varepsilon)\frac{\partial q}{\partial t} = 0 \tag{7}$$

where u is the linear velocity. In the frame of the *linear driving force* approximation (LDF), the equation describing the kinetic transport in the pores is described as follows:

$$\frac{\partial q}{\partial t} = k_m(q^* - q) \tag{8}$$

where q^* is defined by the equilibrium isotherm given in Eq. (5). The boundary and initial conditions are $c(z,0) = q(z,0) = 0$ for $0 \le z \le L$ and $c(0,t) = m_0\delta(t)$ where m_0 is the initial amount injected and $\delta(t)$ is the Dirac function.

As shown in our previous article [11], under the assumption that the modifier is not excluded and not interacting with the ligands and that the transport into to pore is infinitely fast (large k_m), for linear gradients, the concentration of modifier at the position z along the column as a function of time and gradient slope is described by the following equation:

$$c_M(z,t) = c_M^0 + g\left(t - \frac{\varepsilon_t z}{u}\right), \quad t \ge \frac{\varepsilon_t z}{u},\ 0 \le z \le L \tag{9}$$

where c_M^0 in mM is the initial modifier concentration, and g the gradient slope in mM/min. The modifier retention time is given by $t_{0,M} = \varepsilon_t L/u = t_0(1 + K_\infty)$, where $t_0 = \varepsilon L/u$.

The analytical resolution of Eqs. (7) and (8) under isocratic condition has been early proposed by Thomas [15], who smartly introduced the coordinates transformation $x \to z\varepsilon_t/u$ and $y \to (t - z\varepsilon_t/u)/\nu$.

From Eqs. (6) and (9), it is clear that K is a function of y exclusively. In addition, k_m is known to be a function of the retention factor K, and therefore we assume that k_m is also a function of y exclusively and does not depend on x. Introducing Eq. (5) in (8) and applying the above-mentioned variable change leads to the following system of equations:

$$\frac{\partial c}{\partial x} + \frac{\partial q}{\partial y} = 0 \tag{10}$$

$$\frac{\partial q}{\partial y} = k_m(y)[K(y)c - \nu q] \tag{11}$$

The initial and boundary conditions in the new coordinate system become $c(x,0) = q(x,0) = 0$ for $0 \le x \le L\varepsilon_t/u$ and $c(0,y) = m_0\delta(y)/\nu$, for $0 \le y$.

The differentiation of Eq. (11) with respect to the coordinate x, and the substitution of the variable c with Eq. (10), leads to the following equation:

$$\frac{\partial^2 q}{\partial x \partial y} = -k_m(y)\left[K(y)\frac{\partial q}{\partial y} + \nu\frac{\partial q}{\partial x}\right] \tag{12}$$

Eq. (12) has an analytical solution in the Laplace space after taking the Laplace transform with respect to the x coordinate [16]. This solution has been proposed by Hao et al. for impulse injection for $y \ge 0$ [17]:

$$\hat{q}(s,y) = \frac{m_0 k_m(0)K(0)}{\nu(s + k_m(0)K(0))}\exp\left(-\int_0^y \frac{\nu k_m(w)s}{s + k_m(w)k(w)}\,dw\right) \tag{13}$$

And for the solute concentration in the mobile phase:

$$\hat{c}(s,y) = \frac{m_0 k_m(0)K(0)}{(s + k_m(0)K(0))}\frac{k_m(y)}{(s + k_m(y)K(y))} \times \exp\left(-\int_0^y \frac{\nu k_m(w)s}{s + k_m(w)K(w)}\,dw\right) \tag{14}$$

2.2. HETP

Under isocratic elution conditions, the inverse Laplace transform can be obtained analytically and the corresponding expression of $c(z,t)$ corresponds to the very well-known Gaussian profile derived originally by van Deemter et al. [1]. For LGE, the inverse Laplace transform cannot be derived analytically, however we can use moment analysis to derive the first and second order moments of the chromatogram which are sufficient to compute the HETP. The first and the second order central moments of the mobile phase concentration $c(x,y)$ in the (x,y) coordinate system with respect to the x variable are obtained from the analytical solution in the Laplace space according to following equation [17]:

$$\mu_i = (-1)^i \lim_{s\to 0}\frac{\partial^i \ln(\hat{c}(s,y))}{\partial s^i}, \quad i = 1, 2 \tag{15}$$