

Note

Liquid chromatography plate height equations

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High-performance liquid chromatography (HPLC) has been used extensively to measure equilibrium binding constants¹ and, recently, to determine the actual rate constants of the binding processes involved in biochemical interactions^{2,3}. In many of these studies the data are analyzed using the chromatographic plate height expressions presented by Horváth and Lin^{4,5}. These expressions are strictly applicable only when strong biological interactions are not present, as in the study of mass transfer contributions to peak spreading^{6,7}, or when the interactions are very weak and can be described adequately by a linear equilibrium isotherm. However, many of the experiments aimed at measuring binding constants and sorption rates have been performed under conditions where the equilibrium relationships are highly non-linear. This problem will be addressed in a later paper⁸. The present discussion is confined to the chromatographic theory for systems displaying linear equilibria.

Expressions for plate heights can be obtained by solving the partial differential equations that govern the movement of the sorbing species through the column. Briefly, the set of equations describing the conservation of mass in the packed bed, the equilibrium between solute and sorbate, and the nonequilibrium effects of axial dispersion, diffusion in the axial direction, and mass transfer into the particles are solved in the Laplace domain for the column response to a pulse input. It is possible to derive from this transformed solution expressions for the statistical moments of the exiting peak. The first absolute moment μ_1 is related to the peak retention time, and therefore to the strength of binding (*i.e.* the equilibrium constant). The second central moment μ'_2 is related to peak spreading, caused by departures from equilibrium. When the exiting peak is Gaussian, μ'_2 equals σ^2 , the variance. In general, however, the peak is not Gaussian, and μ'_2 must be measured by numerical integration or Fourier analysis of the experimental data.

$$\mu_1 \equiv \frac{\int_0^{\infty} c(L,t)tdt}{\int_0^{\infty} c(L,t)dt} \quad (1)$$

$$\mu'_2 \equiv \frac{\int_0^{\infty} c(L,t) (t - \mu_1)^2 dt}{\int_0^{\infty} c(L,t) dt} \quad (2)$$

Both the first and second moments are combined in an expression which defines the chromatographic plate height, H .

$$H = \frac{\mu'_2 L}{\mu_1^2} = f(\text{geometry, solute, flow ...}) \quad (3)$$

H is a function of the column and packing geometry, operating conditions, the properties of the solute, and the interactions of the solute with the mobile and stationary phases. For a given separation in which the system, packing and solute, is already defined, H is a function of the mobile phase velocity (u). One widely-used function is the Van Deemter equation (eqn. 4)

$$H = A + \frac{B}{u} + Cu \quad (4)$$

The first two terms include the effects of molecular diffusion in the axial direction and axial dispersion, while the third term accounts for the contributions from fluid film mass transfer, particle diffusion, and slow sorption kinetics.

Giddings⁹ argued that this plate height expression is too simplistic because it does not consider flow and diffusive coupling in the void spaces between the particles. According to Giddings, "flow and radial diffusion work simultaneously to exchange molecules between flow paths of unequal velocity. A molecule in a fast streampath may end up in a slow one at the next particle diameter, or it can diffuse laterally into a new flow channel or velocity extreme". Instead of having a constant axial dispersion contribution to H (the A term in eqn. 4), Giddings argued that it should have some flow-rate dependence, and he proposed a term with the form $a/(1 + bu^{-1})$. This coupling term, however, did not agree well with experiments¹⁰, and other forms have been proposed¹¹.

In order to include coupling between flow and diffusion, Horváth and Lin chose a simple model in which each particle is surrounded by a stagnant film of thickness δ . Axial dispersion occurs only in the free-streaming fluid outside this stagnant film, whose thickness depends inversely on the fluid velocity. Thus the residence time of the free-streaming fluid and, therefore, the axial dispersion plate height contribution depend on the fluid velocity. To obtain an expression for δ , Horváth and Lin turned to the "free-surface" cell model of Pfeffer and Happel¹² for mass and heat transfer to a bed of spherical particles. These authors found that, for high Peclet numbers, the Sherwood number is proportional to the Peclet number to the one-third power.

$$Sh = \Omega Pe^{1/3} \quad (5)$$

(Ω is a function of ε , the void fraction, and Pe is identical to the reduced velocity v used in refs. 4 and 5). From the definition of Sh , the fluid film mass transfer coefficient k_f is

$$k_f = \frac{ShD}{d_p} = \frac{\Omega D}{d_p} Pe^{1/3} \quad (6)$$

δ was obtained by equating the stagnant layer thickness to D/k_f , the Nernst diffusion layer thickness

$$\delta = \frac{d_p}{\Omega Pe^{1/3}} \quad (7)$$

Using this, Horváth and Lin derived their axial dispersion plate height equation, which has the form $a/(1 + bu^{1/3})$.

There are a number of problems with this plate height equation. In the first place, eqn. 5 for the Sherwood number is valid for large Peclet numbers. At Pe less than about 50, the Sherwood number approaches a constant which depends on ε (ref. 12). Thus at low Pe , or low reduced velocities, the stagnant boundary layer thickness approaches a constant value, and so should the plate height. The expression derived by Horváth and Lin is strictly applicable for $Pe > 50$, although it is plotted in Fig. 6 of ref. 4 for $Pe = 10^{-1}$ to 10^5 . In the region $Pe \approx 1$, where the coupling effect is most likely to be observed, the Horváth and Lin equation cannot be used. The fluid film plate height expression proposed in ref. 4 is also invalid for small Pe , since it, too, was derived using eqn. 5. At higher Pe , the predicted velocity dependence is so small that in most cases it would be well within experimental error.

The effects of axial dispersion will be most important when the effects of slow sorption kinetics and the mass transfer contributions to peak spreading are small (small θ , Pe). The various plate height contributions, calculated using Horváth's equations, are plotted in Fig. 1 for a solute that diffuses into the particles but does not adsorb. Only for $Pe < 200$ are the deviations from linearity in the axial dispersion and fluid film mass transfer contributions noticeable. At these smaller Peclet numbers, the validity of the equation proposed by Horváth and Lin is most questionable. When sorption occurs, the mass transfer contributions increase, and axial dispersion is even less important. Finally, if there is a slow desorption step, the relative importance of axial dispersion is reduced even further, as illustrated in Fig. 2 for a weakly adsorbing solute.

The basis for the calculation of the film thickness, δ , is also questionable. The film mass transfer coefficient k_f , used by Horváth and Lin to determine δ , was derived from the concentration boundary layer profile obtained by solving the coupled momentum and mass conservation equations for flow around a sphere¹². The correct film thickness would be the concentration boundary layer thickness, which will be less than the Nernst diffusion layer thickness used by Horváth and Lin. This difference will be more pronounced at higher Reynolds numbers.

The remaining terms of Horváth and Lin's general plate height equation (eqn. 14 in ref. 5) account for the contributions from fluid film mass transfer, diffusion into the particle, and slow desorption. It can be shown that these are identical to the plate

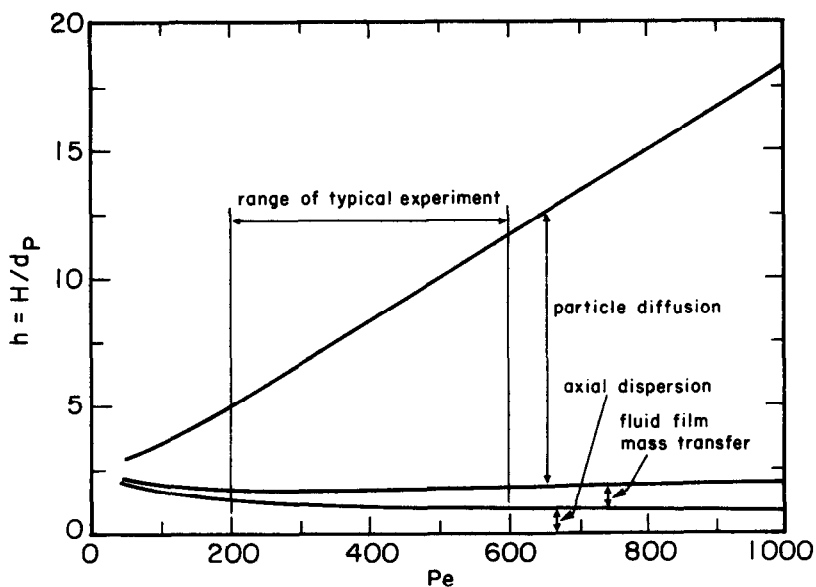


Fig. 1. Reduced plate heights calculated using eqn. 14, ref. 5, for an unadsorbed solute. $\lambda = 10$, $\omega = 2.5$, $\theta = 2$, $k_0 = 0.75$, $k' = 0$.

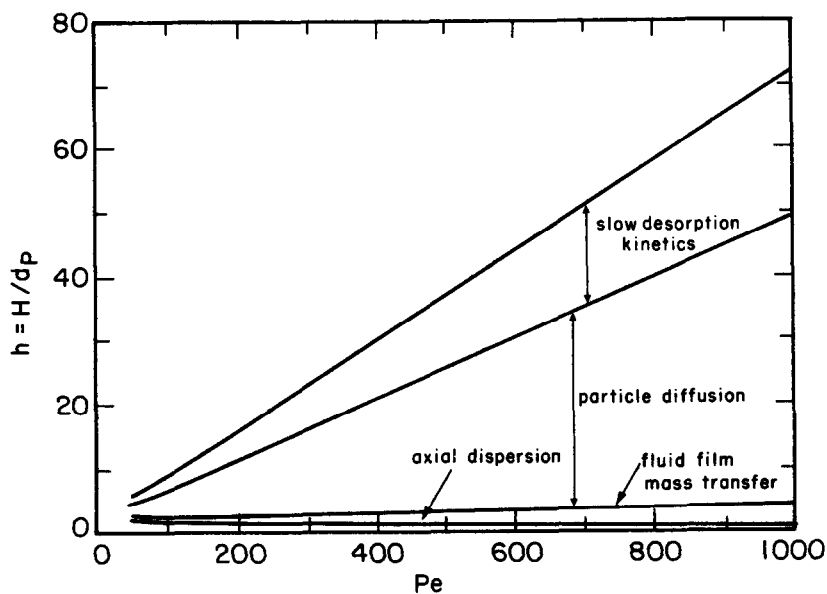


Fig. 2. Reduced plate heights calculated using eqn. 14, ref. 5, for a weakly adsorbing solute. $\lambda = 10$, $\omega = 2.5$, $\theta = 2$, $k_0 = 0.75$, $k' = 1.0$, $d_p = 0.005$ cm, $D = 1 \cdot 10^{-6}$ cm²/sec, $k_d = 0.5$ sec⁻¹.

heights assembled from the expression for the statistical moments derived by Kucera¹³ and Furusawa *et al.*¹⁴ for short pulses. From the model of Furusawa *et al.*:

$$\mu_1 = \frac{L}{u_0} \{ \varepsilon + (1 - \varepsilon) \beta + (1 - \varepsilon) \rho_p K \} \tag{8a}$$

$$\begin{aligned} \mu'_2 = \frac{2L}{u_0} \left\{ \frac{E_z}{u_0^2} \left[\varepsilon + (1 - \varepsilon) \beta \left(1 + \frac{\rho_p K}{\beta} \right) \right]^2 + \right. \\ \left. (1 - \varepsilon) \left[\frac{\rho_p K}{k_d} + \frac{d_p \beta^2}{60} \left(1 + \frac{\rho_p K}{\beta} \right)^2 \left(\frac{1}{D_i} + \frac{10}{k_t d_p} \right) \right] \right\} \end{aligned} \tag{8b}$$

and, therefore, with eqn. 3, the plate height is given by

$$H = \frac{2d_p}{Pe_p} + \frac{2(1 - \varepsilon) \left[(\beta + \rho_p K)^2 \frac{d_p^2}{60} \left(\frac{1}{D_i} + \frac{10}{k_t d_p} \right) + \frac{\rho_p K}{k_d} \right] u_0}{[\varepsilon + (1 - \varepsilon) \beta + (1 - \varepsilon) \rho_p K]^2} \tag{9}$$

where $Pe_p = u_0 d_p / E_z$. The second term on the right hand side includes the effects mentioned above. These contributions are identical to the corresponding terms in Horváth and Lin's equation, using eqn. 6 for the fluid film mass transfer coefficient. The nomenclature of the two derivations is compared in Table I.

TABLE I
COMPARISON OF EQN. 14, REF. 5 WITH EQN. 10

<i>Ref. 5</i>	<i>This paper</i>
κ	1/15
k_0	$\frac{\beta(1 - \varepsilon)}{\varepsilon}$
k'	$\frac{K\rho_p(1 - \varepsilon)}{\varepsilon + (1 - \varepsilon)\beta} = \frac{K_L Q_{max} \rho_p (1 - \varepsilon)}{\varepsilon + (1 - \varepsilon)\beta} = \frac{K'}{\alpha}$
ν	$Pe = \frac{u_0 d_p}{\varepsilon D}$
θ	$\frac{\beta D}{D_i}$
$\frac{\Omega D}{d_p} \nu^{1/3}$	k_t
K	K_L
φ	$\frac{\rho_p Q_{max} (1 - \varepsilon)}{\varepsilon + (1 - \varepsilon)\beta}$
u_c	$u = u_0 / \varepsilon$

The first term in eqn. 9 includes axial mixing and diffusion and would encompass any coupling phenomena. In many cases, explicitly including coupling adds unnecessary complexity to the overall plate height expression. Coupling is observed at small Pe (≈ 1), *i.e.* when the flow-rate or particles are extremely small, or when the diffusivities are large. The expression proposed by Horváth and Lin for axial dispersion is not valid in this range, and its validity for higher Pe is arguable, based on the preceding discussions.

We believe that a reasonable approach to the problem of estimating axial dispersion plate height contributions is to use directly correlations for dispersion coefficients in packed beds. The axial dispersion coefficient is often given in the form of a packing Peclet number, Pe_p , similar to the flow Peclet number Pe . In their study of axial dispersion in packed beds, Miller and King¹⁵ found that the packing Peclet number is roughly constant at low Reynolds number for a wide range of particles sizes. Pulse studies have confirmed this result for chromatographic packings at $Pe > 50$ (refs. 7 and 16). An axial dispersion coefficient in this form corresponds to a constant plate height contribution ($A = 2d_p/Pe_p$, $B = 0$ in eqn. 4). This is, of course, valid when coupling is unimportant.

If it becomes necessary to treat systems with small reduced velocities, one can turn to more complete expressions for Pe_p . Eidsath *et al.*¹⁷ recently published a thorough investigation of axial dispersion phenomena over a very wide range of Pe . Their results are similar to those of Miller and King at $Pe \approx 10$, although they do find a weak dependence on Pe ($Pe_p \propto Pe^{-0.2}$). At very low Pe , axial dispersion approaches the molecular diffusion limit, and Pe_p becomes directly proportional to Pe . One could fit a number of functional forms to the intermediate region and still be within experimental error.

A SIMPLIFIED PLATE HEIGHT EQUATION

As stated previously, Horváth and Lin have used eqn. 6, valid for $Pe > 50$, to write k_f in terms of the fluid velocity. For this and other possible relations, k_f depends rather weakly on the fluid velocity. Over the range of fluid velocity in a typical experiment, a factor of about 3 to 10, k_f can often be assumed constant. If Pe_p and k_f are constants, then eqn. 10 is of the Van Deemter form (with $B = 0$), where the C term takes into account the mass transfer and slow desorption. Eqn. 9 can then be rewritten in a simpler form:

$$H = \frac{2d_p}{Pe_p} + \frac{2 [K'/k_d + (\alpha - \varepsilon + K')^2/K_{OL}a_p] u_0}{(\alpha + K')^2} \quad (10)$$

where

$$\begin{aligned} K' &= (1 - \varepsilon) \rho_p K = (1 - \varepsilon) \rho_p Q_{\max} K_L \\ &= \frac{\text{maximum bound solute}}{\text{column volume}} \cdot K_L \end{aligned}$$

$$\alpha = \varepsilon + (1 - \varepsilon) \beta = \text{fraction of column volume available to the solute}$$

$$K_{OL}a_p = \left[\frac{d_p^2}{60(1-\varepsilon)} \left(\frac{1}{D_i} + \frac{10}{k_f d_p} \right) \right]^{-1}$$

$$= \left(\begin{array}{c} \text{overall mass} \\ \text{transfer coefficient} \end{array} \right) \cdot \left(\begin{array}{c} \text{particle} \\ \text{surface area} \end{array} \right)$$

The simple form of this plate height equation makes it convenient for analyzing experimental results. Since the particle mass transfer and slow kinetics contributions have the same fluid velocity dependence, mass transfer effects must be measured in separate control experiments in which all binding is suppressed. The column fraction available to the solute (α), is obtained directly from the first moment, or retention time (eqn. 8) in the control experiment. K' is then obtained from the retention time of the sorbed species. Finally, k_d , the desorption rate constant, can be found from the slope of the H vs. u_0 plot for the sorbed material. It is important to stress that this analysis is strictly valid only when the sorption rate equation is linear (*i.e.* when one is operating in the linear region of the isotherm). If the axial dispersion coefficient is desired, the intercept must be corrected for the slight velocity dependence of k_f (ref. 7).

SYMBOLS

a_p	surface area of particles per unit bed volume (cm^{-1})
c	bulk liquid solute concentration (g cm^{-3})
d_p	particle diameter (cm)
h	reduced plate height ($= H/d_p$)
k_d	desorption rate constant (sec^{-1})
k_f	fluid film mass transfer coefficient (cm sec^{-1})
u_0	liquid superficial velocity (cm sec^{-1})
u	liquid linear velocity (u_0/ε) (cm sec^{-1})
D	solute diffusivity in bulk liquid ($\text{cm}^2 \text{sec}^{-1}$)
D_i	effective particle diffusion coefficient ($\text{cm}^2 \text{sec}^{-1}$)
E_z	axial dispersion coefficient ($\text{cm}^2 \text{sec}^{-1}$)
H	plate height (cm)
K	linear equilibrium constant ($= Q_{\max}K_L$) ($\text{cm}^3 \text{g}^{-1}$)
K_L	binding constant ($\text{cm}^3 \text{g}^{-1}$)
K_{OL}	overall mass transfer coefficient (cm sec^{-1})
K'	$K_L Q_{\max} \rho_p (1 - \varepsilon)$
L	bed length (cm)
Q_{\max}	maximum loading capacity (g solute/g particle)
Pe	Peclet number ($= u_0 d_p / D\varepsilon$)
Pe_p	packing Peclet number ($= u_0 d_p / E_z$)
Sh	Sherwood number ($= k_f d_p / D$)
α	column liquid fraction
β	particle porosity
δ	stagnant boundary layer thickness (cm)
ε	column void fraction

μ_1	first absolute moment of peak (sec)
μ_2	second central moment of peak (sec ²)
v	reduced velocity (= Pe)
ρ_p	particle density (g cm ⁻³ particle)
σ^2	variance of Gaussian peak (sec ²)
θ	ratio of bulk diffusivity to diffusivity in pore liquid
Ω	constant in eqn. 5

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