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Plate-height equation for non-linear chromatography

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ABSTRACT

A height equivalent to a theoretical plate (HETP) equation for non-linear chromatography was derived analytically based on an equilibrium–non-equilibrium theory. The band-broadening factors including non-linear behaviour of the adsorption isotherm and kinetics of adsorption–desorption and mass transfer were all considered. The contributions of sample concentration, inhibitor concentration and sample size to plate height were explicitly expressed. Some significant advantages of the proposed plate-height equation were demonstrated by the agreement between the experimental data from a concanavalin A–sugar system and the calculated results. In a limiting case, this plate-height equation can reduce to the HETP expression for linear chromatography. It is easier to use this HETP equation in scaling-up a non-linear chromatographic process and in determining the thermodynamic and kinetic constants characterizing non-linear chromatography.

INTRODUCTION

The theory of non-linear chromatography has been well developed during the last few years. Excellent reviews of theoretical analyses and modelling methods of non-linear chromatography include the works of Lin *et al.* [1], Lee *et al.* [2], Cowan *et al.* [3] and Liapis [4]. The concept of non-linear chromatography takes into account the non-linear isotherm of equilibrium between the solute in the mobile phase and the solute on the inner surface of the adsorbent. Experimentally, samples with a very large volume and/or mass have to be considered in preparative chromatography, which can rarely be regarded as a linear process as interactions between solute molecules are unavoidable. Preparative chromatography is therefore sometimes called non-linear chromatography [1]. In non-linear chromatography, peak shapes and band spreading are affected by the solute's competition for adsorbent sites, sample size and various mass transfer resistances. As a result, peak shape and band spreading are complex functions of the equilibrium isotherm, mass transfer parameters, packing properties and operating conditions [2]. Unfortunately, no theory of non-linear chromatography that encompasses all the usual chromatographic broadening factors is available. For the past few decades, the height equivalent to a theoretical plate (HETP) has been used to characterize the chromatographic efficiency and band broadening of the elution peak [5]. Typical HETP equations for linear chromatography explicitly contain terms contributed by eddy diffusion, longitudinal molecular diffusion, film mass transfer, pore diffusion and surface adsorption resistances [6]. However, the plate-height equations generally found in the literature are limited to linear chromatography. In this work, a novel, closed-form plate-height equation for non-linear chromatography was developed based on an equilibrium–non-equilibrium theory. The focos was placed on the effects of isotherm non-linearity.

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THEORY

Equilibrium–non-equilibrium theory

In this work the plate-height equation for non-linear chromatography is based on equilibrium–

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non-equilibrium theory. In this theory, the capacity factor is regarded as a thermodynamic invariant and can be predicted by the local equilibrium model, whereas the plate-height is obtained from both the isotherm non-linearity and the departure from equilibrium. To obtain a plate-height expression we used the same assumption as made by Knox and Pyper [7] and Golshan-Shirazi and Guiochon [8] that the column HETP is the sum of two independent contributions:

$$H = H_{\text{ther}} + H_{\text{kin}} \tag{1}$$

The thermodynamic contribution to the plate-height, H_{ther}, results from the non-linear behaviour of the equilibrium isotherm. On the other hand, the contribution H_{kin} is attributed to the non-equilibrium due to a finite rate of adsorption–desorption and mass transfer. Eqn. 1 is not exactly correct as the convolution theorem used to sum the contributions for plate-height is only valid for a linear system. It is nevertheless a reasonable approximation which is often used by investigators working in overload chromatography [7–10]. Lucy and Carr [11] recently demonstrated that eqn. 1 is valid under a wide range of chromatographic conditions. The derivation of each contribution in eqn. 1 is given as the follows.

Solution of local equilibrium model and capacity factor

The local equilibrium model (theory), also called the ideal model [8], has been thoroughly studied for non-linear chromatography in the measurement of association and inhibition constants [12]. Golshan-Shirazi and Guiochon [8,13] used this model for the simulation of band profiles in chromatography in the case of a Langmuir isotherm. In a local equilibrium model, the mass transfer resistances due to axial dispersion, film mass transfer and pore diffusion are assumed to be negligible and the adsorption–desorption interaction is assumed to be very rapid. As a result, the concentrations of the solute in the pore and in the bulk fluid are all the same and denoted by C. If equilibrium prevails throughout the column, the concentration of the solute in the mobile phase, C, and the concentration of the solute on the inner surface of the solid, q, is related by an equilibrium isotherm. The governing equations for a single solute pulse and Langmuir equilibrium are

$$u_0 \cdot \frac{\partial C}{\partial z} + \varepsilon_t \cdot \frac{\partial C}{\partial t} + (1-\varepsilon)\rho_p \cdot \frac{\partial q}{\partial t} = 0 \tag{2}$$

$$q = \frac{q_s K_L C}{1 + K_L C} \tag{3}$$

The solutions for this local equilibrium model with a rectangular input which has concentration C_0 and width t_0 are obtainable by applying the method of characteristics. The concentrations of the solute at the outlet of a column of length L are given by [12,14]

$$C = \begin{cases} \dfrac{1}{K_L}\left[\sqrt{\dfrac{(1-\varepsilon)\rho_p q_s K_L L}{u_0(t-t_0) - \varepsilon_t L}} - 1\right], & t_f \leqslant t \leqslant t_e \\ 0, \quad \text{otherwise} \end{cases} \tag{4}$$

where t_f and t_e are the retention times of the shock front and the tailing edge, respectively, and given by

$$t_f = t_0 + \frac{L}{u_0}\left[\varepsilon_t + \left(\sqrt{(1-\varepsilon)\rho_p q_s K_L} - \sqrt{t_0 u_0 K_L C_0 / L}\right)^2\right] \tag{5}$$

and

$$t_e = t_0 + \frac{L}{u_0}\left[\varepsilon_t + (1-\varepsilon)\rho_p q_s K_L\right] \tag{6}$$

If

$$t_0 > (1-\varepsilon)\rho_p q_s K_L \left(\frac{L}{u_0}\right) \frac{K_L C_0}{(1 + K_L C_0)^2}$$

then the concentrations are given by

$$C = \begin{cases} C_0, & t_f \leqslant t \leqslant t_x \\ \dfrac{1}{K_L}\left[\sqrt{\dfrac{(1-\varepsilon)\rho_p q_s K_L L}{u_0(t-t_0) - \varepsilon_t L}} - 1\right], & t_x \leqslant t \leqslant t_e \\ 0, \quad \text{otherwise} \end{cases} \tag{7}$$

where

$$t_x = t_0 + \frac{L}{u_0}\left[\varepsilon_t + \frac{(1-\varepsilon)\rho_p q_s K_L}{(1 + K_L C_0)^2}\right] \tag{8}$$

t_e is defined as in eqn. 6, but t_f is given by

$$t_f = \frac{L}{u_0}\left[\varepsilon_t + \frac{(1-\varepsilon)\rho_p q_s K_L}{(1 + K_L C_0)}\right] \tag{9}$$

Substituting the concentration profiles, eqns. 4–9, into the definition of the first moment,

$$\mu_1 = \int_0^\infty Ct\mathrm{d}t / \int_0^\infty C\mathrm{d}t \qquad (10)$$

an expression for the first moment can be obtained. The capacity factor, k', is thus given by $k' = (\mu_1 - t_m)/t_m$, where t_m is the retention time of the unretained solute. After taking the integration for μ_1 (*i.e.*, eqn. 10) and assuming that the injection volume of the sample is relatively small compared with the retention volume, *i.e.*, $(u_0 t_0/L\varepsilon_t)/k'_0 \ll 1$, we obtain the capacity factor as

$$k' = k'_0 \Phi(a) \qquad (11)$$

where $\Phi(a)$ is a universal function presented in Table I and a is defined as

$$a = \sqrt{\left(\frac{t_0 u_0}{L}\right)\frac{C_0}{(1-\varepsilon)\rho_p q_s}}$$

The value of a reflects the amount of relative feed concentration and sample size. The parameter a can be defined as

$$a = \sqrt{\left(\frac{t_0 u_0}{L\varepsilon_t}\right)\left(\frac{K_L C_0}{k'_0}\right)}$$

a combination of three non-dimensional factors, k'_0, $(t_0 u_0/L\varepsilon_t)$ and $K_L C_0$, where the last two represent the non-dimensional quantities of sample size and sample concentration, respectively. The quantity k'_0 represents the capacity factor in the case of zero concentration, *i.e.*, the capacity factor when the chromatographic process is linear and no interaction occurs between solutes. As shown in eqn. 11, the capacity factor in non-linear chromatography is no longer a constant as it is in linear chromatography. The Langmuir isotherm derives from the linear isotherm by a universal function Φ of a, due to the overload effect of sample concentration. This universal function $\Phi(a)$ is obtained directly by integration of the first moment using the concentration profiles, eqns. 4–9. As listed in Table I, the universal function is simply $1 - 4a/3 + a^2/2$ for $a \leqslant 1$ and $1/(6a^2)$ for $a > 1$.

TABLE I

UNIVERSAL FUNCTIONS OF LOADING FACTOR IN NON-LINEAR CHROMATOGRAPHY

Universal function			Application
$\Phi(a) =$	$1 - \frac{4}{3}a + \frac{a^2}{2}$	$a \leqslant 1$[a]	Capacity factor: $k' = k'_0\Phi(a)$
	$\frac{1}{6a^2}$	$a \geqslant 1$	
$\Psi(a) =$	$\frac{\frac{2}{9}a^2 - \frac{4}{15}a^3 + \frac{1}{12}a^4}{\left(1 - \frac{4}{3}a + \frac{1}{2}a^2\right)^2}$	$a \leqslant 1$	Thermodynamic contribution to HETP: $H_{ther} = \left(\frac{k'}{1+k'}\right)^2 L\Psi(a)$
	$\frac{36}{15}a^2 - 1$	$a \geqslant 1$	
$\theta(a) =$	$\frac{a^2}{2a - a^2}$	$a \leqslant 1$	Average equilibrium concentration: $K_L C^* = \theta(a)$
	a^2	$a \geqslant 1$	

[a] The universal function $\Phi(a)$ in ref. 25 was derived based on the solution with a printed error presented in ref. 12, it should be corrected to that in this table.

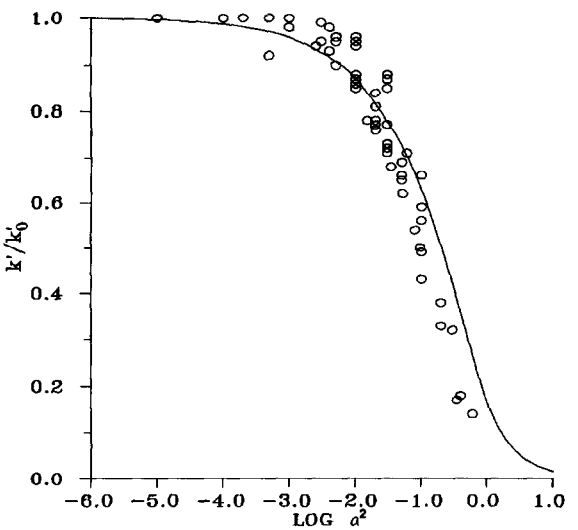

Fig. 1. Comparison of capacity factors calculated by eqn. 11 (solid line) and obtained from Craig model simulation given by Eble *et al.* [15] (data points).

To confirm the validity of eqn. 11, we compared the calculated results using eqn. 11 with the data from Craig simulation of overload separations. The "band" values of the capacity factor k' from Craig simulation are read from Table I in the paper by Eble *et al.* [15]. Fig. 1 shows that the data from Craig simulations cluster about the curve predicted by eqn. 11. As the Craig simulations for these data are carried out for a broad range of conditions, we believe that eqn. 11 is valid not only for local equilibrium but also for the cases with a finite rate of adsorption–desorption and mass transfer. Fig. 1 also shows that the onset of the concentration overload effect seems to occur at $a \approx 0.08$ (10% deviation from linear theory).

The capacity factor can be regarded as a thermodynamic property only when the elution time is independent of various mass transfers, *i.e.*, flow velocity. Wade *et al.* [16] have shown, in the case of infinitesimal sample volume, that the slow kinetics of mass transfer and adsorption–desorption tend to delay the decrease in elution time with increasing solute concentration. However, the kinetic dependence of the capacity factor is a trivial problem since the effect of mass transfers on capacity factor is relatively small, as Wade *et al.* [16] concluded. With the support of Fig. 1, we may conclude that the capacity factor can be regarded as a thermodynamic invariant. Eqn. 11 shows that k'/k'_0 also can be represented as a function of non-dimensional sample concentration $K_L C_0$ and relative sample volume, denoted by $(t_0 u_0/L\varepsilon_t)/k'_0$. In contrast to the effect of slow kinetics, an increase in relative sample volume promotes a decrease in capacity factor (and elution time) beginning at lower solute concentration, as shown in Fig. 2.

Thermodynamic contribution to the plate height

Golshan-Shirazi and Guiochon [8] used the concentration profiles (eqns. 4, 5 and 6) to obtain the column height equivalent to a theoretical plate. They called the "thermodynamic" contribution the plate height, H_{ther}, which results from the non-linear behaviour of the equilibrium isotherm. Accordingly, they derived two equations for the plate number of an ideal model band, *i.e.*, eqns. 38 and 40 in their work. Actually, the non-linear isotherm effect is coupled with that of concentration overload through the loading factor, denoted a^2 in this paper. The pure volume overload due to a larger injection interval is negligible. Other similar plate-height equations for the contribution of non-linear isotherms are summarized in Table II.

Using the concentration profiles given by eqns. 4–9 and the following definition of plate height [11]:

$$H = \frac{\mu'_2}{\mu_1^2} \cdot L \tag{12}$$

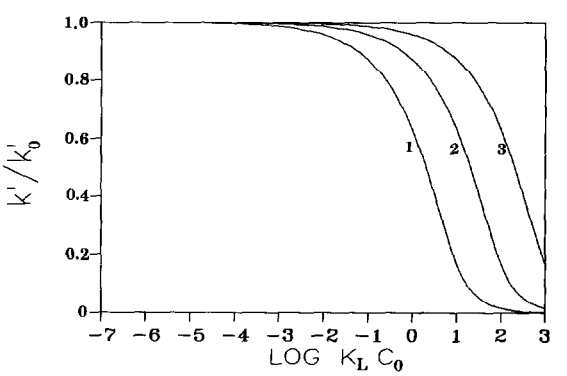

Fig. 2. Effects of sample size on the dependence of capacity factor on concentration, $\left(\frac{t_0 u_0}{L\varepsilon_t}\right)\Big/k'_0$ = (1) 0.1, (2) 0.01 and (3) 0.001.

TABLE II

EXPRESSIONS FOR THE THERMODYNAMIC CONTRIBUTION TO PLATE HEIGHT

Source	H_{ther}
Golshan-Shirazi and Guiochon, eqn. 38 [8]	$L\left[5.54\frac{(2-a)^4}{(4a-a^2)^2}\right]^{-1}\left[1+\frac{1}{(1-a)^2}\left(\frac{a^2}{K_L C_0}+\frac{1}{k'_0}\right)\right]^{-2}$
Golshan-Shirazi and Guiochon, eqn. 40 [8]	$L\left[\frac{16}{(2a-a^2)^2}\right]^{-1}\left[\frac{1}{k'_0}+a^2/(K_L C_0)+(1-a)^2\right]^{-2}$
This work	$L\left[\frac{k'_0\Phi(a)}{1+k'_0\Phi(a)}\right]^2\Psi(a)$
Knox and Pyper [7]	$\frac{L}{4}\left(\frac{k'_0}{1+k'_0}\right)^2 a^2$
Snyder *et al.* [17]	$\frac{3}{8}L\left(\frac{k'_0}{1+k'_0}\right)^2 a^2$
Jenke [18]	$\frac{c}{k'}La^2$ (c is an experimental constant)

in which μ'_2 is the second central moment, we obtained the thermodynamic contribution to the plate height as

$$H_{ther} = L\left[\frac{k'_0\Phi(a)}{1+k'_0\Phi(a)}\right]^2\Psi(a) \qquad (13)$$

where $\Psi(a)$ and $\Phi(a)$, resulting from the integrations for the first moment and the second central moment with the application of the concentration profiles given by eqns. 4–9, are universal functions of the loading factor in non-linear chromatography as shown in Table I. A calculation not shown here revealed that eqn. 13 gives values in the middle of those predicted by eqns. 38 and 40 of Golshan-Shirazi and Guiochon [8] with the parameter values for typical affinity chromatography.

The plate height defined by eqn. 12 takes advantage of the fact that the plate height can be calculated from the experimental chromatogram or from the profile predicted by the mass balance model. In addition, the assumption of a Gaussian chromatographic peak is not needed in applying this definition. With the aid of computer calculations and data acquisition, it becomes easier to characterize a peak by its statistical moments, which can be measured from the chromatogram.

The other expressions in Table II are either empirical or experimental. It should be noted that the notation a^2 used in this paper is exactly the same as that of L_f in Golshan-Shirazi and Guiochon's work [8]. The loading factor a^2 also is equal to w_x/w_s, the ratio of the mass of solute injected to the saturation capacity of the column [7,17,18]. The larger is a^2, the greater is the concentration overload effect. We may conclude that the plate height resulting from the non-linear behaviour of the equilibrium isotherm is the contribution of concentration overload through a loading factor.

Plate height due to non-equilibrium

To obtain H_{kin} in eqn. 1, we should reconsider the mass balance model as represented by eqns. 2 and 3. When a Langmuir kinetics relationship is used to account for a finite rate of adsorption–desorption kinetics, eqn. 3 should be replaced by

$$\frac{\partial q}{\partial t} = k_a^*(q_s - q)C - k_d^* q \qquad (14)$$

while eqn. 2 remains unchanged. If adsorption–desorption processes are slow compared with all diffusion processes, the rate parameters k_a^* and k_d^* in eqn. 14 are the true adsorption and desorption rate constants, respectively. Experimentally, Chase [19] and Arnold and Blanch [20] used the model eqns. 2

and 14 successfully to interpret the results of affinity chromatography for protein purification.

Whenever the mass transfer contribution is comparable to the adsorption–desorption contribution, the rate constants k_a^* and k_d^* should be regarded as the effective overall rate constants that combine the effects of mass transfer and chemical kinetic limitations of the binding interaction. For this reason, the model including eqns. 2 and 14 is called the lumped rate constants model. Based on the "linear driving force" concept of Hiester and Vermeulen [21], as noted by Arnold and Blanch [20], combination of the mass transfer rate constant k_{mass} and the true desorption rate constant k_d yields the following effective desorption rate constant:

$$\frac{1}{k_d^*} = \frac{1}{k_d} + \frac{(1-\varepsilon)\rho_p q_s K_L}{2k_{mass}}\left(\frac{2 + K_L C_0}{1 + K_L C_0}\right) \quad (15)$$

where

$$k_{mass} = \frac{6(1-\varepsilon)}{d_p}\left(\frac{1}{k_f} + \frac{1}{10}\cdot\frac{d_p}{D_i}\right)^{-1} \quad (16)$$

Wade *et al.* [16] also obtained a similar relationship between true and effective rate constants. However, in the following derivation of plate height, k_d^* is regarded as independent of concentration, which is believed to be realistic in ion-exchange and affinity chromatography.

The lumped rate constants model (eqns. 2 and 14) has often been used for the simulation of chromatography operated in the frontal mode and in the zonal mode. Although the lumped rate constants model with different kinds of boundary conditions has been solved and reported by Thomas [22], Hiester and Vermeulen [21], Goldstein [23], Aris and Amundson [14], Chase [19], Arnold and Blanch [20] and Wade *et al.* [16], the calculations for the moments can only be done numerically and an explicit form of the plate-height expression is not possible.

In this work, an extension of the non-equilibrium theory developed originally by Giddings [24] was made to obtain the plate height due to the non-equilibrium by starting from the lumped rate constants model (eqns. 2 and 14). According to the non-equilibrium theory [24], the solute in a chromatographic zone is slightly out of equilibrium owing to the moving concentration gradient and the inability of equilibration to keep up with it. Plate height can be related to the extent of departure from equilibrium, as the departure is found to be directly responsible for zone (band) spreading. By following the approach developed by Giddings, the plate height resulting from non-equilibrium for non-linear chromatography with a Langmuir isotherm was derived as eqn. A10 in the Appendix. It is a function of equilibrium concentration in the mobile phase, C^*. A detailed derivation is given in the Appendix. An average equilibrium concentration was then introduced to express C^* by taking the average value of solute concentrations between the elution times for the shock front and the tailing edge when the chromatographic process is in equilibrium, *i.e.*,

$$C^* = \int_{t_f}^{t_e} C\,dt \Big/ \int_{t_f}^{t_e} dt \quad (17)$$

where the concentration profile C is given by eqns. 4 and 7. Eqn. 17 results in the third universal function $\theta(a)$ as presented in Table I. Accordingly, the kinetic contribution to the plate height is given by

$$H_{kin} = \frac{2u_0}{\varepsilon_t}\cdot\frac{1}{k_d^*}\cdot\frac{k_0'[1+\theta(a)]}{\{k_0' + [1+\theta(a)]^2\}^2} \quad (18)$$

Plate-height equation for non-linear chromatography

Finally, substituting eqns. 13 and 18 into eqn. 1 we obtained the following plate-height equation for non-linear chromatography:

$$H = L\left[\frac{k_0'\Phi(a)}{1 + k_0'\Phi(a)}\right]^2 \Psi(a) + \frac{2u_0}{\varepsilon_t}\cdot\frac{1}{k_d^*}\cdot\frac{k_0'[1+\theta(a)]}{\{k_0' + [1+\theta(a)]^2\}^2} \quad (19)$$

Clearly, when the feed concentration C_0 approaches zero, both $\theta(a)$ and $\Psi(a)$ vanish, and eqn. 19 becomes the plate height due to slow kinetics in linear chromatography. It is noted that eqn. 19 is so far the only explicit expression of plate height for non-linear chromatography. Further, it seems useful in optimizing and scaling up non-linear chromatographic processes.

In eqn. 19, we used an effective rate constant k_d^* to lump the effects of slow adsorption–desorption and all mass transfers but not axial dispersion, and assumed that k_d^* is independent of sample concentration. This assumption is realistic when the

adsorption–desorption contribution to the peak spreading outweighs the mass transfer contribution, as in ion-exchange and affinity chromatography. We may let N_0 denote the plate number determined under linear conditions, and rewrite eqn. 19 as

$$\frac{1}{N} = \frac{1}{N_{\text{ther}}} + \frac{1}{N_0}\left(\frac{H_{\text{kin}}}{H_0}\right) \tag{20}$$

where

$$\frac{H_{\text{kin}}}{H_0} = \frac{[1 + k'_0]^2[1 + \theta(a)]}{\{k'_0 + [1 + \theta(a)]^2\}^2} \tag{21}$$

In eqn. 20, $H_0 = L/N_0$ and $H_{\text{ther}} = L/N_{\text{ther}}$. The plate number predicted by eqns. 20 and 21 is comparable to that obtained from the lumped rate constants model and calculated by Craig simulation. A general agreement was found from the plot of N/N_0 values calculated by eqns. 20 and 21 *versus* the simulated N/N_0 by the Craig model as shown in Fig. 3. The Craig simulation data are read from the "band" values of N/N_0 in Table I in the paper by Eble *et al.* [15].

Obviously, eqn. 20 is slightly different from that given in the literature by a factor H_{kin}/H_0. The contribution of this factor is significant provided that the plate height due to kinetic non-equilibrium is much greater than that due to isotherm non-linearity. This case occurs when the rate of adsorption and desorption kinetics are slow. In all instances, the plots of N *versus* the loading factor a^2

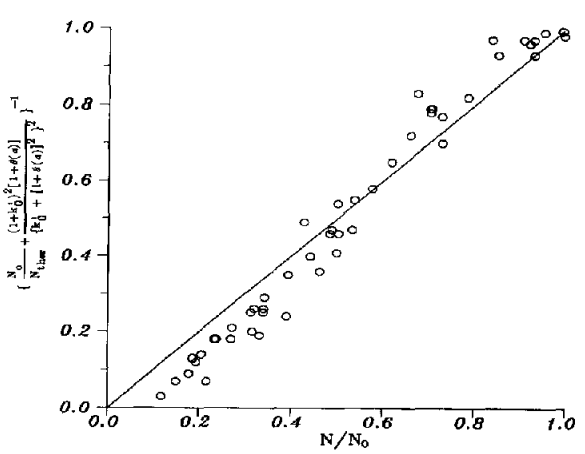

Fig. 3. Comparison of N/N_0 simulated from Craig model, as in Table I in the paper by Eble *et al.* [15] (abscissa) and the values calculated by eqns. 20 and 21 (ordinate).

according to eqns. 20 and 21 will reveal that the plate number decreases with increasing loading factor in a manner very similar to that previously published by Colin [10] and Golshan-Shirazi and Guiochon [8].

For multi-component systems, the afore-mentioned plate-height equations and the universal functions presented in Table I are still useful with minor modification. For example, consider a competing free (soluble) inhibition system in an affinity process [25,26]. A solute inhibitor (I), which competes with an immobilized ligand for the desired macromolecular compound, is present in the sample solution pre-equilibrated buffer. Assuming a constant concentration of I throughout the column during elution stage, all the above-mentioned equations are valid when the limiting capacity factor for the desired solute k'_0 is replaced by $k'_0/(1 + K_I C_I)$, where C_I and K_I are the concentration and the binding constant, respectively, of inhibitor I. According to ref. 25, the limiting capacity factor in the definition of a should also be changed to $k'_0/(1 + K_I C_I)$.

EXAMPLE

The use of this plate-height equation is demonstrated by the following example. Consider the adsorption of sugar, *p*-nitrophenyl-α-D-mannopyranoside (pNp)-mannoside, on to the inner surface of 50-μm concanavalin A (Con A)–silica packed in a column (5.0 cm × 0.3 cm I.D., 38 mg/g Con A, pore diameter 42 nm, $\varepsilon = 0.5$, $\varepsilon_p = 0.6$). The chromatographic system is operated at a flow-rate of 1 ml/min with an injection volume of 25 μl ($t_0 = 1.5$ s, $u_0 = 0.236$ cm/s). Variation of the experimental capacity factor and total plate height with injected concentration has been reported by Muller and Carr [27]. Using the equations derived in the previous section by assuming the capacity factor k' to be independent of mass transfer, the best fitting of the experimental and calculated capacity factors yielded $k'_0 = 13$ and $K_L = 1.6 \cdot 10^4$ lmol^{-1}. It was found that this fitted value of the binding constant K_L is identical with that reported by Muller and Carr [28]. The calculated plate height is obtained using eqn. 19 with a constant effective desorption constant $k_d^* = 0.1$ s^{-1}. The assumption employed here that the kinetics of adsorption–desorption are rate limiting is realistic in affinity chromatography, especially when

TABLE III

EXPERIMENTAL AND THEORETICAL VALUES OF THE CAPACITY FACTOR AND PLATE HEIGHT FOR THE EXAMPLE AS DESCRIBED IN THE TEXT[a]

C_0 (mM)	Experimental		k' calculated using eqn. 11 ($k'_0 = 13, K_L = 1.6 \cdot 10^4$ lmol^{-1})	Equilibrium–non-equilibrium theory			Lumped rate constants model: HETP (μm)
	k'	HETP (μm)		H_{ther}	H_{kin}	HETP (μm)	
0.1	11.4	4050	11.26	119	4063	4182	3980
0.2	10.6	4280	10.58	253	4127	4380	4110
0.4	9.7	5400	9.67	551	4219	4770	4350
0.6	9.2	4710	8.99	882	4292	5174	4560
0.8	8.4	5630	8.45	1243	4354	5597	4850
1.0	8.0	6090	7.99	1633	4409	6042	5180

[a] Theoretical values were generated from the best-fit parameters.

the immobilized ligand is a macromolecule such as Con A in this example. As suggested by Hethcote and DeLisi [29] and Arnold and Blanch [20], the mass transfer contribution can be reduced by immobilizing the macromolecular species and allowing the small molecules to diffuse to the binding site. The results are summarized in Table III for comparison. Obviously, the calculated HETP agrees with the experimental data. Also, this rate constant k_d^* is close to the value of k_d reported by Muller and Carr [28] (0.3 s^{-1}), but unfortunately they incorrectly took the HETP equation of linear chromatography from a paper by Horváth and Lin [6] for this nonlinear system. Table III indicates that the equilibrium–non-equilibrium theory is comparable to the lumped rate constants model in obtaining HETP to take account of band spreading. However, the lumped rate constants model predicted lower HETP values than the experimental values. The advantage of the proposed HETP equation based on the equilibrium–non-equilibrium theory, however, is very clear: it is simpler and easier to use.

Plots of the experimental and calculated HETP *versus* concentration are shown in Fig. 4. The calculated HETP is generated by eqn. 19 with the parameter values from the best fitting. It is clear that the HETP starting from a limiting value in the linear region at zero concentration increases with sample concentration. This is because at higher sample concentration, the fraction of unbound solutes increases and tends to be eluted rapidly. Further, the solute concentrations are scattered over the entire column and serious mixing is induced by concentration gradients, which increase the plate height. Plots of HETP *versus* linear velocity u_0 with various sample concentrations are shown in Fig. 5. A higher plate height accounts for much of the inefficiency of the chromatographic column when a higher concentration is applied. It is clear that the effect of concentration cannot be ignored even when the concentration is not really high. The larger is K_L, the much pronounced is the contribution of concentration overload. However, at very high concentrations, the concentration effect on the plate height may be traded off by the high flow velocity. The reason may be that a large fraction of the solute does not adsorb immediately, and elutes together at the

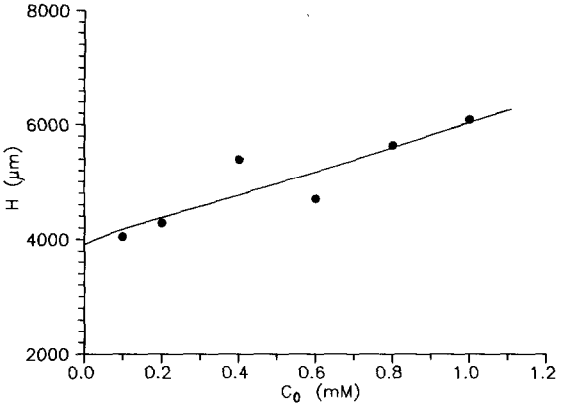

Fig. 4. Effects of sample concentration on plate height with $k'_0 = 13$, $K_L = 1.6 \cdot 10^6$ lmol^{-1}, $L = 5$ cm, $\varepsilon_t = 0.8$, $u_0 = 0.236$ cm/s, $t_0 = 1.5$ s and $k_d^* = 0.1$ s^{-1}. Calculated values were generated by eqn. 19.

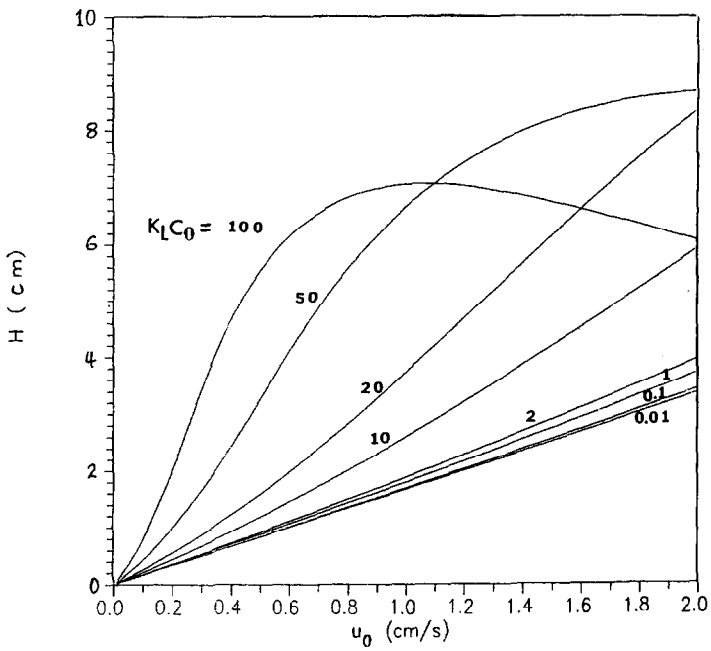

Fig. 5. Plate height as a function of flow velocity in non-linear chromatography. Parameter values are $L = 5$ cm, $\varepsilon_t = 0.8$, $t_0 = 1.5$ s, $k'_0 = 13$ and $k_d^* = 0.1$ s^{-1}.

dead volume under the condition of heavy concentration overload. Regardless the variation of plate height at high flow velocities, it is suggested that the effective desorption rate constant can be measured from the slope of these plots in the region of low velocity.

As shown in this example, one of the greatest advantages of the equations obtained in this work is that they provide a easier way for the determination of the thermodynamic constant K_L and rate constants. Experimentally, by varying the amount of sample mass or sample concentration, one can easily obtain measurements of the site density (k'_0/K_L) and equilibrium binding constant by using eqn. 11. Further, eqn. 19 provides a theory for the estimation of the effective desorption rate constant.

CONCLUSIONS

In non-linear chromatography, the sample concentration tends to sharpen the front of the sample pulse and to prolong the tailing. If the time interval of sample injection is relatively small compared with the total retention time, the effect of sample concentration on the capacity factor can be estimated using the local equilibrium model. The contribution of concentration to plate height due to the isotherm non-linearity is also obtainable from the same model. In the limiting case of zero sample concentration, the plate height due to a non-linear isotherm vanishes. This thermodynamic plate height contributed by isotherm non-linearity is given by eqn. 13.

A plate-height equation for non-linear chromatography (under isocratic condition) has been derived based on the equilibrium–non-equilibrium theory. With this theory, the retention time is assumed to be identical with that of the local equilibrium model and the capacity factor is calculated from this retention time. Both the departure from equilibrium contributed by slow adsorption–desorption and mass transfer and the self-sharpening and tailing effect of concentrations due to non-linear isotherms are responsible for band broadening (zone spreading). The overall plate-height equation, eqn. 19, accounts for this band broadening. For a multi-component inhibition system, the con-

tribution of competing inhibitor concentration to plate height is via the limiting capacity factor of the desired solute k'_0 and the loading factor a.

An example of non-linear chromatography has been used to demonstrate the application of the plate-height equation derived in this work. A comparison of calculated HETP and simulation results from the Craig model and the lumped rate constants model showed general agreement. Finally, it should be noted that the plate-height equation derived in this work can be easily used in scaling-up chromatography of biomolecules and in determining the thermodynamic and kinetic constants characterizing non-linear chromatography.

SYMBOLS

C Concentration of solute in bulk fluid phase, mol l^{-1}
C_0 Sample concentration, mol l^{-1}
C_I Concentration of inhibitor, mol l^{-1}
C_m Total concentration of solute in mobile phase, $= \varepsilon_t C$
C_s Total concentration of solute in stationary phase, $= (1 - \varepsilon)\rho_p q$
$\bar{C}$ $= C_m + C_s$
d_p Diameter of adsorbent particle, cm
D^* Apparent diffusion coefficient, $cm^2\ s^{-1}$
D_i Pore diffusivity of solute, $cm^2\ s^{-1}$
H HETP, plate height, cm
k' Capacity factor
k'_0 Capacity factor at zero sample concentration, $= (1 - \varepsilon)\rho_p q_s K_L/\varepsilon_t$
k_a Adsorption rate constant, $l mol^{-1}\ s^{-1}$
k_d Desorption rate constant, s^{-1}
k_d^* Effective desorption rate constant, s^{-1}
k_f Fluid film mass transfer coefficient of solute, cm s^{-1}
K_I Inhibition constant, $l mol^{-1}$
K_L Equilibrium binding constant, $= k_a/k_d$, $l mol^{-1}$
L Length of the column, cm
N Plate number, $= L/H$
q Sorbate concentration, mmol (g particle)$^{-1}$
q_s Maximum number of available binding sites, mmol (g particle)$^{-1}$
t Time, s
t_m Retention time of the unretained solute, $= \varepsilon_t L/u_0$, s
t_0 Time interval of sample injection, s
u Chromatographic velocity, $= u_0/\varepsilon_t$, cm s^{-1}
u^* Velocity of solute in local equilibrium, cm s^{-1}
u_0 Fluid superficial velocity, cm s^{-1}
z Linear coordinate along the packed bed, cm

Greek letters

ε Void fraction of packed bed, equal to the volume outside the particles divided by the empty column volume
ε_t Total void fraction in column
ρ_p Particle density, the density of packed stationary phase in column, g (cm^3 particle)$^{-1}$
μ_1 First moment, s
μ'_2 Second central moment, $= \int_0^\infty C(t - \mu_1)^2 dt / \int_0^\infty C dt$, s^2
$\in$ Parameter of departure from equilibrium

Superscript

* Value estimated at equilibrium

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APPENDIX

Define C_m and C_s as the total concentrations of the solute in the mobile phase and the stationary phase, respectively. We can rewrite eqns. 2 and 14 as

$$\frac{\partial C_m}{\partial t} + u \cdot \frac{\partial C_m}{\partial z} \equiv S_m = k_d^* \left\{ C_s - \left[\frac{(1 - \varepsilon)\rho_p q_s - C_s}{\varepsilon_t} \right] K_L C_m \right\} \quad \text{(A1)}$$

where $u = u_0/\varepsilon_t$ and $K_L = k_a^*/k_d^*$.

When the net rate of mass transfer between phases through adsorption and desorption approaches zero, we have

$$0 = k_d^* \left\{ C_s^* - \left[\frac{(1 - \varepsilon)\rho_p q_s - C_s^*}{\varepsilon_t} \right] K_L C_m^* \right\} \quad \text{(A2)}$$

Obviously, the isotherm is Langmuiran. If we substitute $C_m^*(1 + \in_m)$ for C_m and $C_s^*(1 + \in_s)$ for C_s, we then have

$$S_m = k_d^* C_s^* \epsilon_s - \frac{k_d^* K_L}{\varepsilon_t} [(1-\varepsilon) q_s C_m^* + C_m^{*2} - C_s^* C_m^*] \epsilon_m \quad \text{(A3)}$$

where the ϵ terms represent the departures from equilibrium. In eqn. A3, the higher order term $\epsilon_s \epsilon_m$ has been ignored and ϵ_s relates to ϵ_m by $\epsilon_s = -\epsilon_m \cdot C_m^*/C_s^*$. With eqns. A2 and A3, we have

$$S_m = -k_d^* \left\{ C_m^* + \frac{K_L}{\varepsilon_t} [(1-\varepsilon)\rho_p q_s C_m^* + C_m^{*2} - C_s^* C_m^*] \right\} \epsilon_m \quad \text{(A4)}$$

When the near-equilibrium approximation applied, S_m can also given by

$$S_m \approx \frac{\partial C_m^*}{\partial t} + u \cdot \frac{\partial C_m^*}{\partial z} = (u - u^*) \frac{\partial C_m^*}{\partial z} \quad \text{(A5)}$$

where we have used the relationship of mass balance in local equilibrium:

$$\frac{\partial C_m^*}{\partial t} + u^* \cdot \frac{\partial C_m^*}{\partial z} = 0 \quad \text{(A6)}$$

In eqn. A6, u^* is given by $u^* = u/[1 + k_0'(1 + K_L C_m^*/\varepsilon_t)^{-2}]$. It is noted that $k_0' = (1-\varepsilon)\rho_p q_s K_L/\varepsilon_t$.

Following the preceding formulation of plate height by Giddings [24], we define a mass flux related to u, $J = C_m u$. The zone spreading originates in the ΔJ term and $\Delta J = J - J^* = C_m^* \epsilon_m u$. Let $\bar{C}$ denote the sum of C_m and C_s. Applying the equilibrium relationship of C_m^* and C_s^*, we have

$$\frac{\partial \bar{C}}{\partial z} = \left[1 + \frac{k_0'}{(1 + K_L C_m^*/\varepsilon_t)^2} \right] \frac{\partial C_m^*}{\partial z} = \frac{u}{u^*} \cdot \frac{\partial C_m^*}{\partial z} \quad \text{(A7)}$$

For the total concentration $\bar{C}$, we may make a mass balance with an additional dispersion term:

$$\frac{\partial \bar{C}}{\partial t} + u \cdot \frac{\partial C_m}{\partial z} \approx \frac{\partial \bar{C}}{\partial t} + u^* \cdot \frac{\partial \bar{C}}{\partial z} \equiv D^* \cdot \frac{\partial^2 \bar{C}}{\partial z^2} \quad \text{(A8)}$$

where D^* is the apparent diffusion coefficient and is given by $D^* = -\Delta J/(\partial \bar{C}/\partial z)$. By definition, the plate height is related to the apparent diffusion coefficient D^* by

$$H = \frac{2D^*}{u^*} = \frac{-2C_m^* \epsilon_m u}{u^*(\partial \bar{C}/\partial z)} \quad \text{(A9)}$$

Solving for ϵ_m by equating eqns. A4 and A5 and substituting it into eqn. A9, we finally obtain the plate height H as a function of equilibrium concentration and lumped rate constant:

$$H = \frac{2(u - u^*)}{k_d^* [1 + k_0' + (K_L C_m^*/\varepsilon_t) - (K_L C_s^*/\varepsilon_t)]} \quad \text{(A10)}$$

It is noted that $C_m^* = \varepsilon_t C^*$ and $C_s^* = (1-\varepsilon)\rho_p q_s K_L C^*/(1 + K_L C^*)$.

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