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Analysis of the separability of plate height into overload and intrinsic contributions using the kinetic model of non-linear chromatography

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ABSTRACT

In developing optimization strategies for preparative-scale chromatography it is very convenient, if not entirely valid, to the represent overall peak broadening in terms of the sum of two distinct, independent contributions to the plate height: that portion due to band broadening under linear chromatographic conditions and that due to the effect of mass overload. The kinetic model of non-linear elution chromatography is used to demonstrate that this separation of terms is a reasonable approximation under a wide range of chromatographic conditions and to define the limits of this approximation.

INTRODUCTION

In their seminal work on the optimization of sample throughput in preparative chromatography, Knox and Pyper [1] analyzed the effect of sample overload on peak width in terms of two distinctly different and assumed independent contributions to the plate height. For the present purposes, the first factor will be termed the intrinsic (H_{int}) contribution. This corresponds to the height equivalent to a theoretical plate for a column operating under perfectly linear isotherm conditions. The second contribution, that due to isotherm broadening, H_{iso}, results from overloading the isotherm by injection of a negligibly small volume of solution containing an excessively large amount of solute. Knox and Pyper [1] wrote an equation equivalent to

$$H = H_{int} + H_{iso} \quad (1)$$

Their justification for the decomposition of the total plate height into these two types of terms was based on the prior theoretical work of Haarhoff and Van der Linde [2] and the experimental work of De Jong *et al.* [3]. The peak-shape equation developed by Haarhoff and Van der Linde [2] was based on the assumption that the mobile and

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stationary phase solutes are in perfect equilibrium and that broadening under linear conditions, *i.e.*, at very low sample load, is due only to axial diffusion and eddy dispersion processes. Additionally, a parabolic isotherm was assumed to make the mathematics tractable, *i.e.* the relationship between the mobile (C) and stationary phase (q) solute concentrations was taken as

$$q = a_1 + a_2C + a_3C^2 \tag{2}$$

Under conditions where the volume injected *per se* does not broaden the peak, the concentration–time relationship derived by Haarhoff and Van der Linde [2] can be expressed as

$$\frac{C}{C_0} = \frac{\sqrt{P}(1+k')}{\sqrt{\pi}YC_0k'} \cdot \frac{\exp - \left[\dfrac{\left(\dfrac{\bar{t}}{1+k'} - 1\right)^2}{4P}\right]}{\left[\dfrac{1}{\exp\left(\dfrac{k'YC_0}{2P(1+k')^2}\right) - 1}\right] + 0.5\left[1 + \mathrm{erf}\left(\dfrac{\dfrac{\bar{t}}{1+k'} - 1}{2\sqrt{P}}\right)\right]} \tag{3}$$

where $P = D_{ax}t_0/L^2$ (dimensionless dispersion coefficient); $\bar{t} = t/t_0$ (dimensionless time); $k' = k'_{C=0}$ (thermodynamic k'); $Y = -1/k'\,(\mathrm{d}^2q/\mathrm{d}C^2)_{C=0}$; C_0 = (moles of solute injected)/(dead volume); D_{ax} is the axial dispersion coefficient, t_0 is the column dead time and L is the column length. In the limit of a very small number of moles of solute, *i.e.*, under linear isotherm conditions, the above equation takes on the much simpler Gaussian form:

$$\frac{C}{C_0} = \frac{1}{2\sqrt{P\pi}(1+k')} \cdot \exp - \left[\frac{\left(\dfrac{\bar{t}}{1+k'} - 1\right)^2}{4P}\right] \tag{4}$$

with the dimensionless plate height given by

$$H_{\mathrm{int,disp}} = 2P \tag{5}$$

P is directly related to D_{ax}, which is formally equivalent to the spreading by pure axial molecular diffusion, which in turn is formally inversely dependent on flow-rate. Therefore, eqn. 5 is only an approximate representation of the overall intrinsic plate-height behavior under overload conditions. However, physically D_{ax} is a dispersion coefficient which is coupled to the linear velocity, u [*i.e.*, $D_{ax} = f(u)$]. Thus,

$$P = \frac{D_{ax}t_0}{L^2} = \frac{f(u)}{uL}$$

Therefore, P has a complicated dependence on the linear velocity, and eqn. 5 is a

reasonable approximation of the band broadening under linear chromatographic conditions.

Recently, the solution to a very different model of non-linear elution chromatography was presented. Based on the work of Thomas [4], Heister and Vermuelen [5] and Arnold *et al.* [6,7], a solution was obtained to the non-linear boundary value problem in which the rate of transfer of solute between the phases is taken as the sole band-broadening process under linear conditions [8]. That is, interphase equilibrium is not assumed, rather the solute concentrations in the mobile and stationary phases were assumed to be related by

$$\frac{\partial q}{\partial t} = k_a(S_0 - q)C - k_d q \tag{6}$$

where S_0 is the concentration of binding sites (M; same units as q). The rate constants k_a ($\mathrm{lmol^{-1}s^{-1}}$) and k_d ($\mathrm{s^{-1}}$) are "lumped" rate parameters corresponding to the net rate constants of solute adsorption and solute desorption.

In order to make the problem mathematically tractable, dispersion was assumed to be negligible ($D_{ax}=0$). One advantage of this particular theoretical approach is that a Langmuir isotherm is retained. This physically more realistic isotherm, in comparison with eqn. 2, allows this model to be used for higher degrees of column overload than the equilibrium model using a parabolic isotherm [9]. The solution for an impulse injection of sample is [8]

$$\frac{C}{C_0} = \left(\frac{1 - \exp(-\gamma K C_0)}{\gamma K C_0}\right)\left\{\frac{[\gamma\sqrt{k'/y}\, I_1(2\gamma\sqrt{k'y}) + \delta(y)]\exp[-\gamma(y + k')]}{1 - T(\gamma k', \gamma y)[1 - \exp(-\gamma K C_0)]}\right\} \tag{7}$$

where $y \equiv t/t_0 - 1$; $\gamma \equiv k_d t_0$ (dimensionless rate parameter); $k' \equiv (k_a/k_d)S_0\varepsilon$ (thermodynamic k'); $K \equiv k_a/k_d$; $C_0 \equiv$ (moles of solute injected)/(column dead volume).

In the above set of definitions, $S_0\varepsilon$ is the maximum adsorption capacity of the column. In eqn. 7, I_1 is a first-order modified Bessel function of the first kind, and the T-function is a Bessel function integral:

$$T(u,v) = e^{-v}\int_0^u e^{-t} I_0(\sqrt{vt})\,dt \tag{8}$$

in which I_0 is the zeroth-order Bessel function of the first kind. The T-function acts as a "switching" function which produces the skew in the peak profile when the column is overloaded.

In the limit of a very small number of moles of sample, *i.e.*, linear isotherm conditions, eqn. 7 takes on a much simpler form:

$$\frac{C}{C_0} = [\gamma\sqrt{k'/y}\, I_1(2\gamma\sqrt{k'y}) + \delta(y)]\exp(-\gamma y - \gamma k') \tag{9}$$

This result is mathematically identical with the Giddings–Eyring first-order stochastic model of chromatography [10]. The dimensionless plate height corresponding to this equation is

$$H_{\text{int}} = \frac{2k'}{(1 + k')^2\gamma} = 2\frac{k'}{(1 + k')^2} \cdot \frac{u}{k_d L} \tag{10}$$

Eqn. 10 is the usual result for a plate height resulting from resistance to interphase equilibrium originating in the stationary phase under linear chromatographic ($KC_o = 0$) conditions.

The separability of the total plate height into intrinsic and isothermal contributions is commonly used in theoretical treatments of preparative chromatography [11–13]. However, the justification for this separability thus far is based solely on the Haarhoff–Van der Linde model [2]. As the conditions for the validity of the Haarhoff–Van der Linde [2] and the kinetic models are in complete opposition, *i.e.*, one is an axial dispersion–equilibrium model and the other is based solely on slow kinetics, we felt that it would be of considerable importance to determine whether and under what conditions the non-linear kinetic equation (eqn. 7) would lead to the same separation of plate height contribution shown in eqn. 1.

COMPUTATIONS

The dimensionless plate heights were obtained from

$$H = m'_2/m_1^2 \tag{11}$$

where m_1 and m'_2 are the first normalized and second normalized centralized statistical moments, respectively. The moments were computed for concentration–time profiles generated using eqn. 7 such that 75 evenly spaced points were taken on each side of the maxima. Integrations were performed using Simpson's 1/3 rule.

For the reversed-phase chromatographic simulations, k' was varied between 1 and 10, γ was varied from 100 to 1000 and KC_o was varied from 0 to 0.1. For high-performance affinity chromatography (HPAC), k' was 25, γ was varied from 4 to 40 and KC_o was varied from 0 to 2.0.

RESULTS AND DISCUSSION

At the outset of this work is was not at all clear to us that the separation of plate height as shown in eqn. 1 would be possible. Indeed, we were surprised that eqn. 1 had been given much credence at all [11–13]. In essence, our concern was as follows: the extent of peak broadening due to overload must depend on the *local* solute concentration averaged over the entire column. Obviously the local concentration under linear chromatographic conditions depends on both axial dispersion and broadening due to slow interphase transfer. Consequently, the intrinsic and isotherm broadening should be strongly coupled effects for overloaded columns, such that the contribution from isotherm broadening effects would diminish as the intrinsic broadening increased.

Despite this argument, we find that eqn. 1 is a surprisingly good approximation both for high-performance reversed-phase and affinity chromatographic conditions. The expected coupling between the intrinsic and isotherm broadening was observed, but was sufficiently small that it would generally be easily overwhelmed by experimental uncertainties in real chromatographic data.

Reversed-phase chromatography

The range of the model parameters (k', KC_0 and γ) used in the computations here was based on the values observed in experimental studies of small uncharged solutes in preparative reversed-phase chromatography [9]. In that study, 3-phenylpropanol was studied from linear chromatographic conditions ($KC_0 = 0.0$) to "moderate" column overload conditions ($KC_0 = 0.1$) [9].

In the kinetic model of non-linear chromatography, axial dispersion is assumed to be negligible and band broadening under linear chromatographic conditions is described solely by γ, the dimensionless rate parameter. The magnitude of this rate parameter results from the combined effects of slow solute desorption ad slow interphase mass transfer. The effects of these two mechanisms are combined in the "lumped" desorption rate constant. In reversed-phase chromatography, the kinetics of solute desorption at the surface are fairly fast. Therefore, the "lumped" desorption rate constant reflects the effect of mass transfer, which is independent of k' and, if resistance to mass transfer resides in the stationary phase, it will be essentially independant of flow-rate. Therefore, k_d can be considered to be constant, and so the dimensionless rate parameter, γ, is directly related to the dead time of the column, t_0, or indirectly to the linear velocity. Thus, instead of the traditional H *versus* linear velocity plot, H *versus* $1/\gamma$ will used.

Fig. 1 shows a series of plots of H *vs.* $1/\gamma$ for the range of overload conditions previously studied [9] at k' values of 1, 3 and 10. At all values of k' the plots are qualitatively similar. For the case of zero overload ($KC_0 = 0$), which corresponds to H_{int} in eqn. 1, the plot is exactly linear in accord with eqn. 10, and passes through the origin at $1/\gamma = 0$. As the column becomes slightly overloaded ($KC_0 = 0.02$), the plot of H *vs.* $1/\gamma$ appears to translate upwards. Close inspection of the plots for $KC_0 = 0.02$ reveals a slight curvature at low $1/\gamma$ (*i.e.*, low linear velocity). However, for higher degrees of overload this curvature is more gradual and less distinct. Experimentally it would be difficult to discern any curvature or change in slope given the expected random error in the measurement of H.

Thus all of the plots are roughly parallel and show an increase in the intercept at $1/\gamma = 0$ as the degree of overload increases. This is the qualitative behavior predicted by eqn. 1. The overload effect acts as a flow-rate-independent contribution to the overall H in these pseudo-Van Deemter plots.

In order to obtain a clearer view of the overload term, the computed H_{int} based on eqn. 9 was subtracted from the total H values shown in Fig. 1:

$$H_{iso} = H - H_{int} \quad \text{(eqn. 9)} \tag{12}$$

The results are summarized in Fig. 2. If H_{iso} were truly independent of the intrinsic broadening effects, then these plots should have a slope of zero. It is evident that H_{iso} does depend on the linear velocity ($1/\gamma$) and thus the separation of terms shown in eqn. 1 can not be perfect. As the intrinsic band broadening in the column increases

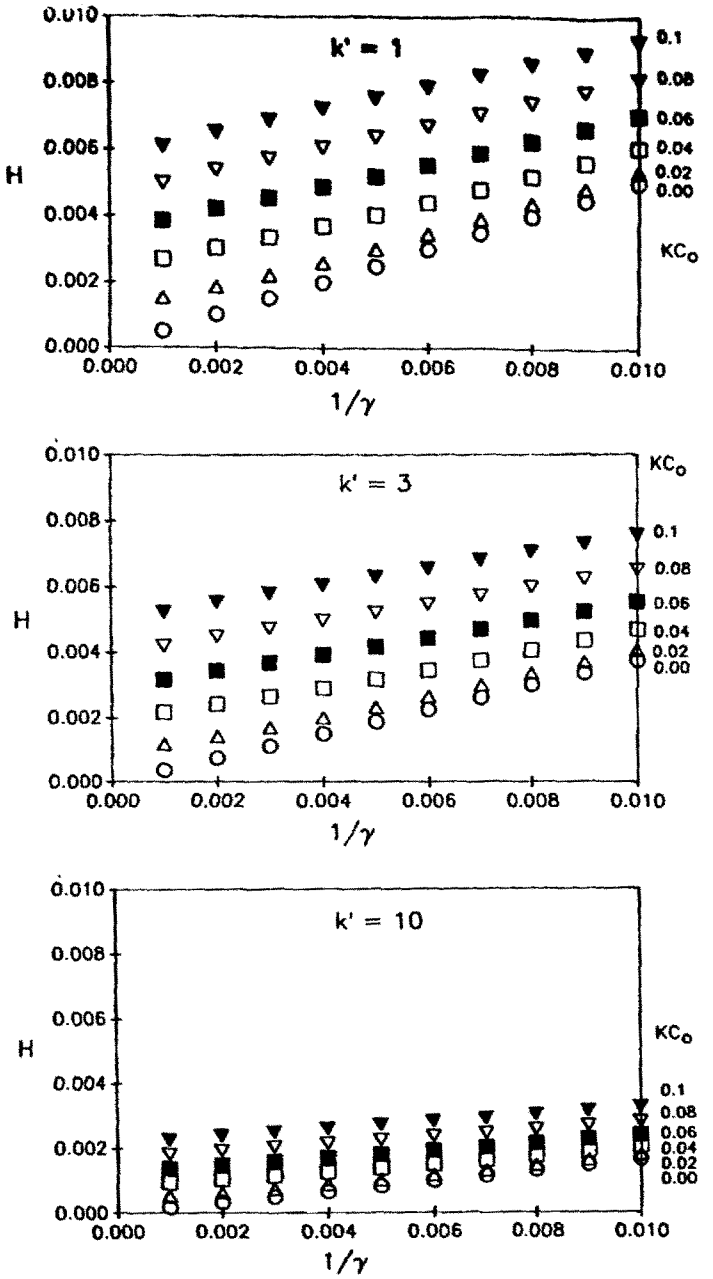

Fig. 1. H *vs.* $1/\gamma$ dependence under preparative reversed-phase conditions at $k' = 1$, 3 and 10, for γ in the range 100–1000. The degree of column overload, KC_o, associated with each curve is indicated on the plots.

with increasing flow-rate, the average solute concentration in the sample band does decrease. Hence, as H_{iso} is concentration dependent, it will diminish. This is the behavior that we initially expected. However, as can be seen in comparing Figs. 1 and

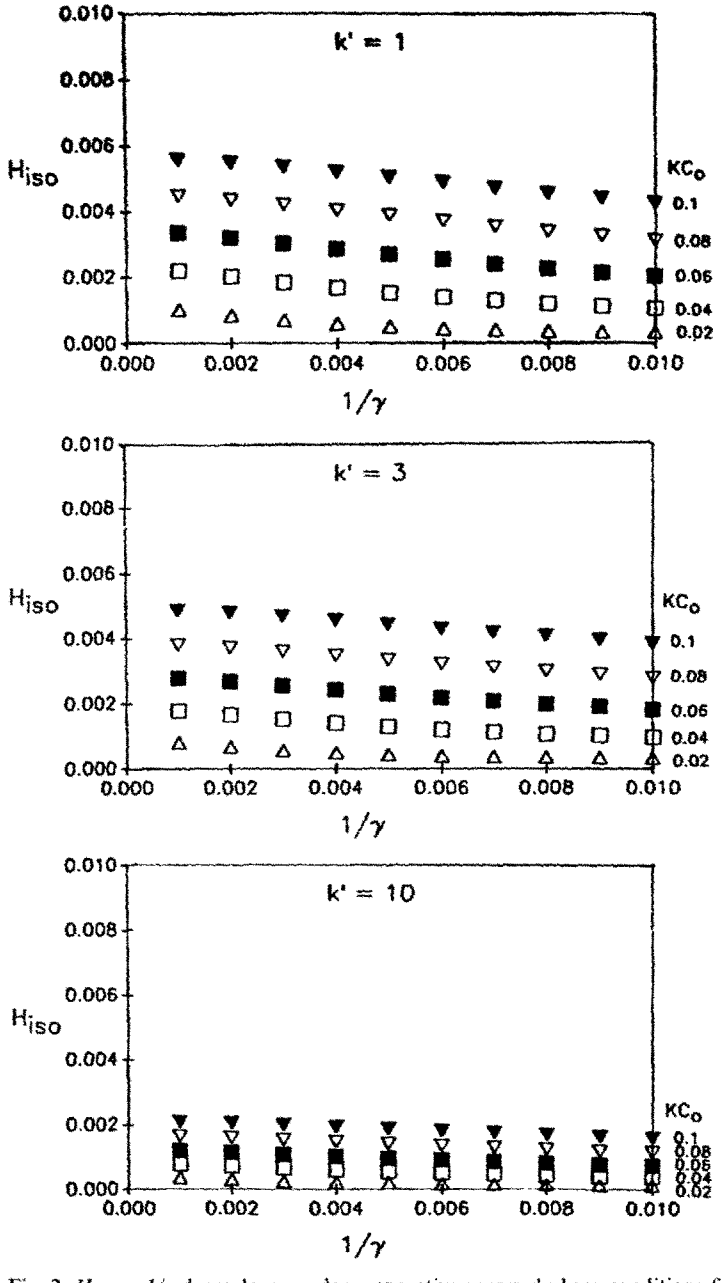

Fig. 2. H_{iso} *vs.* $1/\gamma$ dependence under preparative reversed-phase conditions for data given in Fig. 1. H_{iso} was calculated using eqn. 12.

2, the decrease in H_{iso} which results from this coupling is small enough relative to the change in the intrinsic plate height that eqn. 1 will appear to be obeyed in experimental studies.

It is also evident in Fig. 2 that for a given degree of column overload (KC_o), H_{iso}

decreases with increasing k'. This trend is observed at constant KC_o because m_1^2 increases more rapidly than m_2' with increasing k'. The net result of these two opposing processes is a decrease in H_{iso} with increasing k'. However, in any experimental study in which the number of moles injected is held constant, KC_o would not be constant but rather would increase linearly with increasing k'. The effect of this more realistic situation is given in Fig. 3, where it is shown that for a constant solute concentration given by a constant value of KC_o/k', H_{iso} increases with increasing k'. This is intuitively what one would expect.

Affinity chromatography

In high-performance affinity chromatography (HPAC), the low density of binding sites can result in overloading of the column under even analytical conditions and the strong binding constants between the immobilized ligand and the solute result in column efficiencies far below those associated with reversed-phase HPLC. Thus HPAC provides a distinctly different test of the separability of the plate height under non-linear chromatographic conditions from the preparative reversed-phase HPLC case discussed above.

To test the validity of the separation of plate height shown in eqn. 1 under HPAC conditions, H *vs.* u plots were calculated using conditions previously observed for the retention of *p*-nitrophenyl-α-D-mannopyranoside on a silica-bound concanavalin A affinity column [8]. One very approximate assumption that has been made is that the affinity medium is homogeneous, *i.e.*, all sites have the same dissociation rate constant, k_d, and thus the observed k_d is independent of the amount injected. Under these conditions $1/\gamma$ is proportional to linear velocity, u, for a constant k'. Fig. 4 shows the variation of (A) the total plate height and (B) H_{iso} with $1/\gamma$ for $k' = 25$. The plots in Fig. 4 display similar behavior to that observed in Figs. 1 and 2 despite the large differences in the parameter ranges between the two cases. Again there is evidence of significant coupling between the intrinsic band broadening and that caused by the overloading of the isotherm. However, the effect of the coupling is such that H_{iso} would in all likelihood appear constant in an experimental study of preparative HPAC.

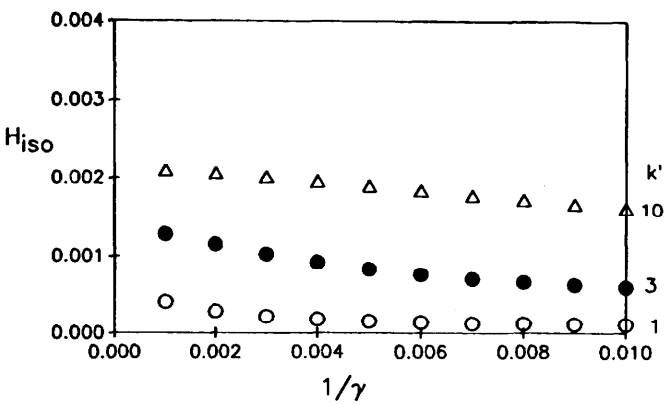

Fig. 3. Effect of k' on the H_{iso} *vs.* $1/\gamma$ dependence for a constant level of column overload under preparative reversed-phase conditions. KC_o/k' is constant at 0.01 for the three plots.

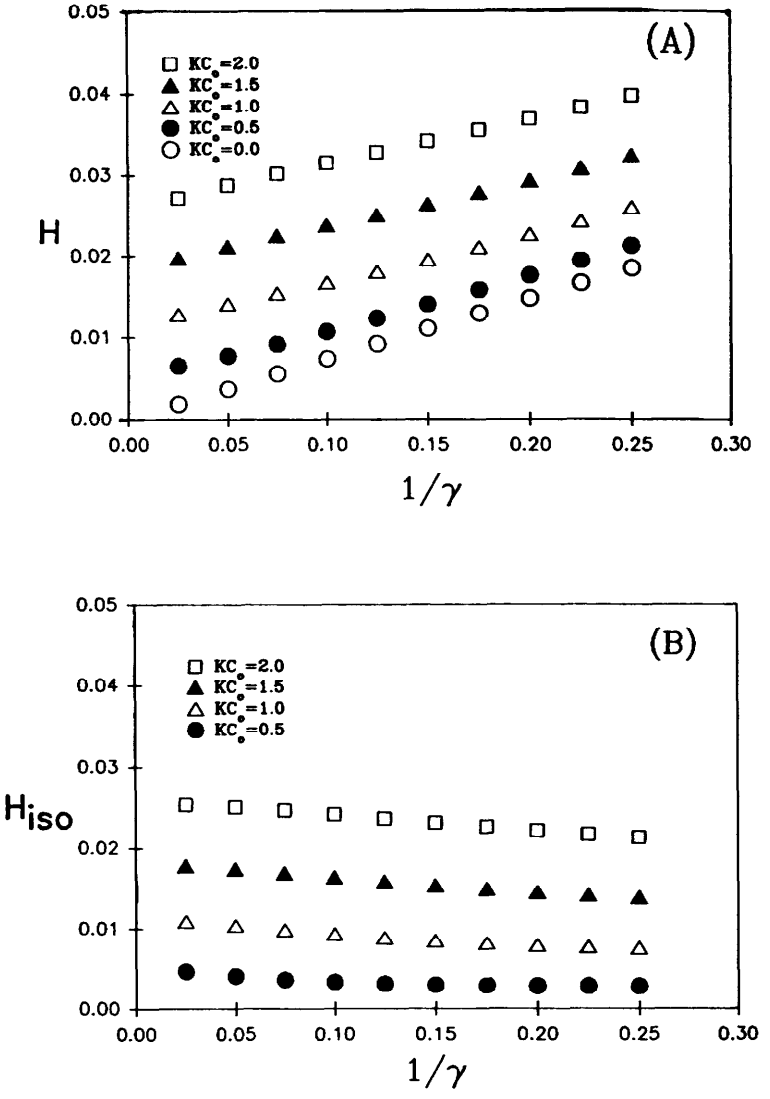

Fig. 4. Plate-height dependence under high-performance affinity chromatographic conditions. $k' = 25$; $\gamma = 4$–40; KC_o = (○) 0.0; (●) 0.5; (△) 1.0; (▲) 1.5 and (□) 2.0. (A) Total plate height H *vs.* $1/\gamma$; (B) H_{iso} *vs.* $1/\gamma$.

CONCLUSIONS

The kinetic model of non-linear chromatography has been used to test the validity of the separation of the total plate height observed in preparative chromatography into intrinsic and isotherm contributions. *H versus u* plots were generated using physico-chemical parameters typical of both high-performance reversed-phase and affinity chromatography. These plots indicate that regardless of the mode of

chromatography, the intrinsic and isotherm contributions to the plate height are coupled, such that increases in the intrinsic band broadening on the column will reduce the band broadening due to the isotherm overload. However, it was found that the degree of this coupling is sufficiently small that in most instances the two contributions could be considered independent.

Under conditions of extreme overload the peak width is dominated by the isotherm broadening effect and thus eqn. 1 will appear to be observed as H_{iso} will be much larger than H_{int}.

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