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MOVEMENT AND BAND SPREADING OF UNSORBED SOLUTES IN LIQUID CHROMATOGRAPHY

CSABA HORVATH and HUNG-JYE LIN

Chemical Engineering Group, Department of Engineering and Applied Science, Yale University, New Haven, Conn. 06520 (U.S.A.)

SUMMARY

The different mobile phase velocities which can be measured in liquid chromatography have been described in order to facilitate an unambiguous specification of the velocity frame and the mobile phase hold-up volumes required to relate experimental data to the theory. The interplay of convective and diffusional mixing, which is responsible for band spreading in the mobile phase, has been treated by using the interstitial stagnant fluid model. Plate height equations have been derived for un-sorbed tracers, which are totally excluded from the column material, as well as for un-sorbed solutes, which permeate the stagnant mobile phase inside the particles. Band spreading was measured over a wide range of the reduced velocity with a single column packed with glass beads. The results show that the equation derived from the interstitial stagnant fluid model describes the velocity dependence of plate height more accurately than those found in the literature. It is suggested that the packing structure of a column be characterized by three dimensionless parameters.

INTRODUCTION

Recent advances in liquid chromatography (LC) with precision instruments allow us to obtain chromatographic data of greater accuracy than before. The interpretation of data, however, requires a knowledge of the thermodynamic, kinetic, and transport phenomena underlying the chromatographic process in order to exploit the full potential of the technique for more efficient separation of complex mixtures as well as for physico-chemical measurements. Whereas the theory of gas chromatography, which has mainly been established in the past twenty years, can greatly facilitate our understanding of LC, there are certain differences between the two techniques. In LC, the viscosity of the mobile phase is higher, the diffusivity of the solutes is lower, and the sample components can be partially or completely excluded from the interior of the porous column material.

In this study, we first examine the different types of fluid velocities which can be measured in chromatographic experiments. We feel that this subject requires clarification because a correct interpretation of band spreading and solute retention is impossible without an unambiguous specification of the fluid velocity or the pertinent

mobile phase hold-up volume¹. In addition, the measurement of the flow velocity with different tracers can be very useful to evaluate important column parameters. The main part of the present study deals with the band spreading of an unadsorbed solute in packed columns which is caused by convective and molecular diffusion. A great deal of study has been conducted both by chemical engineers^{2,3} and chromatographers^{4,5} to shed light on factors affecting axial dispersion. The classical eddy diffusion theory^{6,7} has been refuted by Giddings^{8,9}, whose coupling theory is the result of a thorough analysis of the interplay of flow and diffusion in packed beds. Yet, the simple coupling theory does not agree well with the experimental data, whereas the more elaborate version of the coupling theory escapes experimental verification according to the extensive study of mobile phase band spreading by Knox⁵. In dealing with the factors determining axial dispersion of retarded peaks, Huber and Hulsman¹⁰ and Huber^{11,12} also introduced a term to account for the interplay of convective and diffusional mixing in the mobile phase. His expression, however, has not been related to any model or verified experimentally.

Consequently, there is a need for a relatively simple theory which involves measurable parameters only and can be used to characterize columns with respect to band spreading in the mobile phase. In our approach, we modified the classical eddy diffusion concept, assuming that there is a stagnant fluid space in the interstices of the column packing. The plate height expression derived from this model is not unlike the simple coupling term introduced by Giddings or the term of Huber, but it shows better agreement with experimental data. Our model has also been extended to express band spreading due to the stagnant mobile phase inside the porous packing material.

FLOW VELOCITIES AND HOLD-UP VOLUMES OF UNSORBED SOLUTES IN LIQUID-SOLID CHROMATOGRAPHY

Superficial or empty tube velocity

Superficial or empty tube velocity, u_s , is used most commonly in the chemical engineering literature. It can be evaluated without tracer experiments and is not subject to interpretation as the hold-up volume is arbitrarily but unambiguously defined as the empty tube volume. The superficial velocity is given by

$$u_s = \frac{1}{O} \frac{dV}{dt} \quad (1)$$

where O is the cross-section of the empty column and dV/dt is the volumetric flow-rate. At constant flow, $dV/dt = F$, and $u_s = F/O$. As shown below, u_s is related to the other flow velocities by important column parameters. Therefore, it is highly desirable that in chromatographic experiments aimed toward physico-chemical studies the volumetric flow-rate is accurately measured and reported together with the column inner diameter.

Interstitial velocity

Interstitial velocity, u_e , is probably the most significant linear flow velocity in LC. It is defined by

$$u_e = \frac{u_s}{\epsilon_e} \quad (2a)$$

where ε_e is the interparticulate porosity, which is given by

$$\varepsilon_e = \frac{V_e}{V} \quad (2b)$$

where V_e and V are the interstitial fluid volume in the column and the empty column volume, respectively. The interstitial velocity can be measured from the hold-up time of an unsorbed tracer, t_e , which cannot penetrate the particles in the packing, *i.e.*, is completely excluded. In exclusion chromatography, u_e is routinely measured with a solute of sufficiently high molecular weight in order to evaluate the interstitial volume, which equals the hold-up volume of the excluded tracer. The following relationships are of interest

$$u_e = \frac{L}{t_e} \quad (2c)$$

where L is the length of the column, and at constant flow

$$V_e = Ft_e \quad (2d)$$

Thus, the interparticulate porosity is readily obtained by one of the following expressions

$$\varepsilon_e = \frac{u_s}{u_e} = \frac{V_e}{V} = \frac{Ft_e}{V} \quad (2e)$$

Chromatographic velocity

Chromatographic velocity, u , is measured by the hold-up time of an unsorbed solute that can explore not only the interstitial but also the total intraparticulate fluid space in the column. It is given by

$$u = \frac{u_s}{\varepsilon_T} \quad (3a)$$

where ε_T is the total porosity defined by

$$\varepsilon_T = \frac{V_e + V_i}{V} = \varepsilon_e + \varepsilon_i (1 - \varepsilon_e) \quad (3b)$$

V_i is the intraparticulate pore volume in the column packing, which is filled with the eluent, and ε_i is the corresponding intraparticulate porosity of the packing material given by $V_i/(V - V_e)$. The chromatographic velocity can be measured with a tracer which has the same molecular dimensions as the solvent. The corresponding hold-up time, t_0 , and hold-up volume, V_0 , is expressed by

$$t_0 = \frac{L}{u} \quad (3c)$$

and

$$V_0 = V_e + V_i = Ft_0 \quad (3d)$$

The relationship between the chromatographic and interstitial velocities is given by

$$u_e = u \left[1 + \frac{\varepsilon_i(1 - \varepsilon_e)}{\varepsilon_e} \right] \quad (3e)$$

In chromatographic practice, u is measured most commonly and the "unretained" solute is usually a small molecule which explores the intraparticle fluid space. With pellicular packings the ratio $\varepsilon_i(1 - \varepsilon_e)/\varepsilon_e$ is usually small so that the chromatographic velocity is about the same as the interstitial velocity.

Velocity of the unsorbed solute

Velocity of the unsorbed solute, u_M , is the velocity of a given solute when it is eluted without being sorbed by the stationary phase, *i.e.*, at very high eluent strength. As the molecular dimensions of the solute can be between those of the solvent and a totally excluded solute, it does not necessarily explore the total intraparticle fluid space. If a φ fraction of this space is accessible to the solute, we obtain that

$$u_M = \frac{u_s}{\varepsilon_e + \varphi\varepsilon_i(1 - \varepsilon_e)} = \frac{u_e\varepsilon_e}{\varepsilon_e + \varphi\varepsilon_i(1 - \varepsilon_e)} = u \frac{\varepsilon_e + \varepsilon_i(1 - \varepsilon_e)}{\varepsilon_e + \varphi\varepsilon_i(1 - \varepsilon_e)} \quad (4a)$$

and the intraparticle fluid volume explored by the solute is φV_i . The value of φ depends on the size of the solute molecule and the pore size distribution of the porous column material. Usually φ decreases with increasing molecular weight of the solute and becomes zero for completely excluded solutes.

The hold-up time of the unsorbed solute, t_M , and the corresponding hold-up volume, V_M , are given by

$$t_M = \frac{L}{u_M} \quad (4b)$$

and

$$V_M = V_e + \varphi V_i \quad (4c)$$

It is seen that the hold-up volume of an intermediate-size solute under elution conditions when it is not sorbed is between the hold-up volume of the excluded tracer and the eluent. With a sufficiently large and a sufficiently small solute, the unsorbed solute velocities become u_e and u , respectively.

Fig. 1 illustrates the hold-up times and volumes which characterize the movement of unsorbed solutes in LC columns. The limiting values V_e and V_i have been of great interest in exclusion chromatography. Only very recently Scott¹³ demonstrated that siliceous column materials commonly used in adsorption chromatography have also exclusion properties and that the mobile phase hold-up volume of solutes of relatively small molecular weight lies between the two limits at sufficiently high eluent strength where no sorption occurs. Rigid porous materials are widely used in high-pressure LC. It is expected that their pore structure and the interparticle porosity of the column are unchanged under a wide range of elution conditions. Then the fluid velocities listed above are proportional to each other and subject to a precise definition. In order to extract physico-chemical data from chromatographic measure-

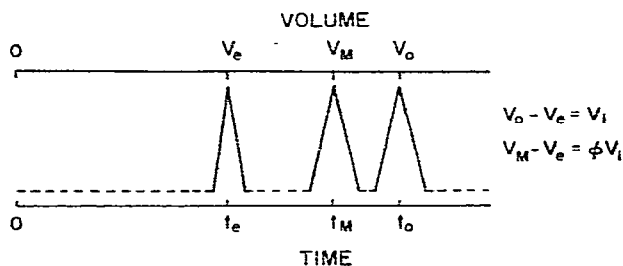


Fig. 1. Illustration of the three different mobile phase hold-up times and the corresponding hold-up volumes which can be measured with unsorbed tracers of different sizes in chromatographic experiments.

ment the appropriate flow velocity or hold-up volume have to be employed and clearly specified.

In liquid-liquid chromatography, the situation is more complicated because a tracer which is not totally excluded can explore the intraparticulate volume occupied by the liquid stationary phase. Under such conditions, the above definitions of the velocity of the unsorbed solute and the chromatographic velocity do not apply and more detailed approach such as given by Huber¹¹ is needed to relate the flow velocity and hold-up volume obtained from chromatographic measurements to the theory.

On the other hand, the situation is greatly simplified for columns packed with glass beads which have often been used to study mobile phase band spreading. In this case, the column material is impervious to the solute so that $\varepsilon_T = \varepsilon_e$. Then

$$u_e = u = u_M = \frac{u_s}{\varepsilon_e} \quad (5a)$$

$$t_e = t_o = t_M \quad (5b)$$

$$V_e = V_o = V_M = V\varepsilon_e \quad (5c)$$

Reduced velocity

Reduced velocity, v , has frequently been used in chromatographic science. In order to avoid ambiguities, it is most appropriate to define v with the interstitial velocity, so that the reduced velocity is given by

$$v = \frac{u_e d_p}{D_e} \quad (6)$$

where d_p is the effective particle diameter of the column material and D_e is the molecular diffusivity of the solute in the eluent.

It is noted that all velocities defined above represent the mean value of the corresponding local velocities in the packed column.

INTERSTITIAL STAGNANT FLUID MODEL

This model postulates that when a liquid flows through a packed bed at low Reynolds numbers an appreciable fraction of the interstitial fluid is essentially stag-

nant with respect to the actual stream in the center region of the interparticulate channels, as shown in Fig. 2. Whereas in reality the fluid velocity in the channels changes continuously from its maximum value to zero at the surface, the assumption of a fluid space which is stagnant with respect to the free fluid stream has certain physical justification. First of all, the hydrodynamic boundary layer at the surface of the particles can be relatively thick under conditions employed in LC^{14,15}. In addition, as seen in Fig. 2, around the points of contact of the particles, the passages available for flow are very narrow and vanish at the points of contact. It is obvious then that the flow in these cusp regions will be considerably smaller than the mean flow through the voids. Hence, a significant fraction of the fluid will be, in fact, essentially stagnant as compared to the mean flow of the fluid streaming through the voids.

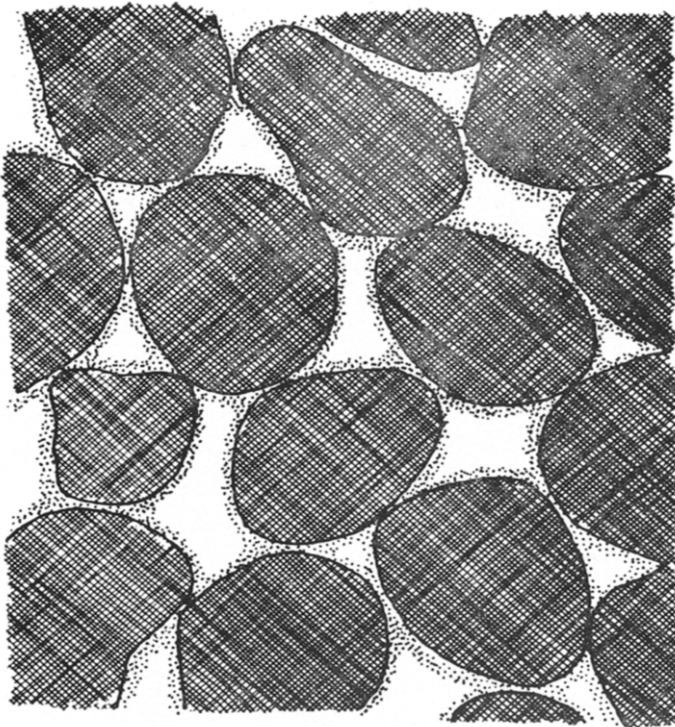


Fig. 2. Illustration of a cross-sectional area in a packed column. The fluid stream in the middle of the interstitial channels is normal to the cross-sectional plane. The particles are surrounded by quasi-stagnant fluid.

Our goal is to express the axial dispersion of an unadsorbed solute in the mobile phase by using the interstitial stagnant fluid model. As shown schematically in Fig. 3, the fluid space in the column is considered to consist of three domains: the free streaming fluid space, the stagnant interstitial fluid space, and the intraparticulate fluid space, which is assumed to be also stagnant in LC.

In order to evaluate the relative magnitude of the stagnant interstitial fluid volume, we employ the theory of Pfeffer¹⁶, who described steady-state mass transfer

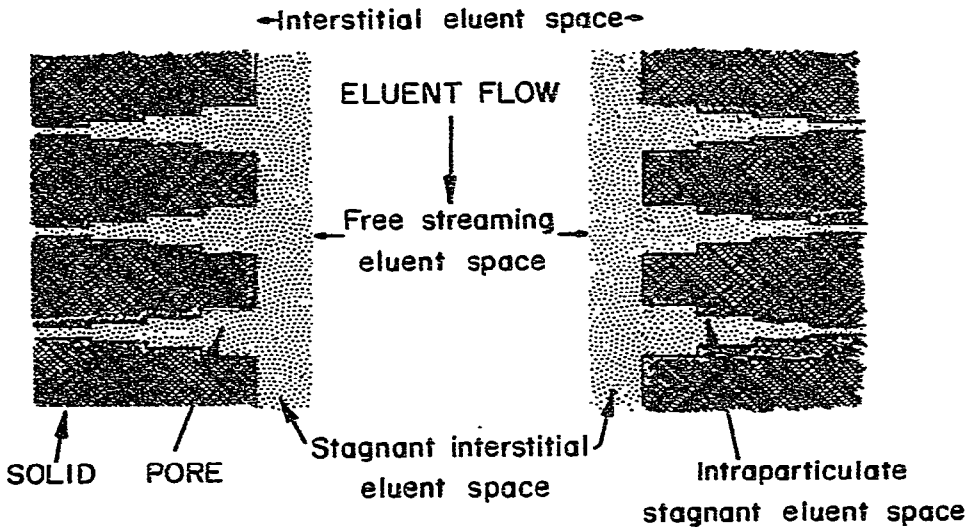


Fig. 3. Schematic illustration of the fluid spaces in a column packed with porous particles.

in packed beds at low Reynolds numbers by using the "free surface" model¹⁷. The mass transfer coefficient, k_e , derived by Pfeffer is given by

$$k_e = \frac{\Omega D_e v^{1/3}}{d_p} \quad (7)$$

The value of Ω is a function of the interparticulate porosity only and changes from 3.6–3.2 when ε_e varies from 0.36–0.42. The data calculated from eqn. 7 showed very close agreement with experimental data in the domain of $Re < 70$ and $\nu < 450,000$, which is of most interest in LC.

We assume that the stagnant liquid is a thin film surrounding spherical particles and the film thickness, δ , can be evaluated from eqn. 7 by the following relationship

$$\delta = \frac{D_e}{k_e} = \frac{d_p}{\Omega v^{1/3}} \quad (8)$$

When the stagnant fluid occupies the fraction η of the interstitial space the corresponding streaming fluid space can be evaluated from δ as follows

$$1 - \eta = \frac{1}{1 + \omega v^{-1/3}} \quad (9a)$$

where ω is related to Ω by

$$\omega = \frac{6(1 - \varepsilon_e)}{\varepsilon_e \Omega} \quad (9b)$$

and is a function of the porosity only. The theoretical value of ω varies from 2.9–2.6 in the range of ε_e from 0.36–0.42. It is noted that eqn. 9a is applicable also when η becomes large, *i.e.*, the thin film assumption does not hold at low values of ν .

The expression of ω in eqn. 9b has been derived for steady-state conditions from the "free surface" model, which is based on certain idealizations. Therefore, the ω values stated probably represent the lower limit for this parameter. It is expected that the departure from steady state also manifests itself in an increased value for ω . In fact, ω has to be a measure of the quality of the packing with respect to band spreading in the mobile phase and the value of ω is expected to decrease with increasing interstitial porosity.

BAND SPREADING OF A COMPLETELY EXCLUDED NONSORBED SOLUTE

In columns packed with fluid impervious particles such as glass beads, any unsorbed solute moves with the interstitial velocity, u_e . In columns packed with porous particles, solutes that are completely excluded from the interior of the particles also move with u_e .

The band spreading which independently occurs due to longitudinal molecular diffusion in the mobile phase⁹ is expressed by the plate height contribution, H_L , given by

$$H_L = \frac{2\gamma D_e}{u_e} \quad (10a)$$

where γ is the obstruction factor. It is more convenient to use the reduced value of H_L which is given by

$$h_L = \frac{H_L}{d_p} = \frac{2\gamma}{v} \quad (10b)$$

The band spreading caused by non-uniform flow and anastomosis in packed beds has been originally related⁶ to an eddy diffusion coefficient, E , which has the dimension of diffusivity and is given by

$$E = \lambda d_p u_e \quad (11)$$

where λ is a measure of the flow inequality in the bed. The eddy diffusion process independently contributes to band spreading with respect to the longitudinal diffusion discussed above. Giddings¹⁸ suggested and experimental data with unsorbed solutes showed⁵ that the plate height contribution derived from E is not independent of the flow velocity as had been thought originally⁶.

Our interstitial stagnant fluid model postulates that flow occurs only in the free stream space. Consequently, eddy diffusion can only take place in this space and the solute is subject to eddy diffusion only during the time t^* that it spends in the free eluent stream. This time is readily evaluated from the relationship

$$t^* = \frac{L(1 - \eta)}{u_e} \quad (12a)$$

as $u_e/(1 - \eta)$ is the free stream velocity.

We assume that the expression for E in eqn. 11 is correct and can evaluate the peak variance, σ_E^2 , caused by eddy diffusion as

$$\sigma_E^2 = 2Et^* \quad (12b)$$

Substituting E from eqn. 11 we obtain that the plate height contribution from eddy diffusion, H_E , is given by

$$H_E = \frac{\sigma_E^2}{L} = 2\lambda d_p(1 - \eta) \quad (13a)$$

Substituting the expression for $1 - \eta$ from eqn. 9a into eqn. 13a we obtain that

$$H_E = \frac{2\lambda d_p}{1 + \frac{\omega}{\nu^{1/3}}} \quad (13b)$$

and the corresponding reduced plate height increment, h_E , is given by

$$h_E = \frac{H_E}{d_p} = \frac{2\lambda}{1 + \frac{\omega}{\nu^{1/3}}} = \frac{1}{\frac{1}{2\lambda} + \frac{\omega}{2\lambda\nu^{1/3}}} \quad (13c)$$

By combining eqns. 10b and 13c we obtain for the reduced plate height of a nonsorbed solute, which cannot permeate the interior of the packing material, the following expression

$$h = \frac{2\gamma}{\nu} + \frac{2\lambda}{1 + \omega\nu^{-1/3}} \quad (14)$$

Eqn. 14 has also been derived from the interstitial stagnant fluid model by solving the pertinent mass balance equation. When $\nu^3 \ll \omega$, i.e., $\nu \ll \omega^3$, eqn. 14 can be simplified to

$$h = \frac{2\gamma}{\nu} + \frac{2\lambda}{\omega} \nu^{1/3} \quad (15a)$$

On the other hand, at sufficiently high reduced velocities the effect of longitudinal diffusion vanishes and h_E approaches a constant value of 2λ so that

$$h = 2\lambda \quad (15b)$$

From eqn. 14 the optimum value of the reduced velocity, ν_{opt} , where the h vs. ν plot goes through a minimum, h_{min} , has been evaluated analytically. The values of ν_{opt} and h_{min} are solely determined by γ/λ and ω . As the mathematical expression is rather complex, the relation is graphically illustrated in Figs. 4 and 5. The value of γ was fixed at 0.6, which has been frequently found experimentally¹⁹.

BAND SPREADING IN A BED PACKED WITH PARTICLES PERMEABLE BY THE NON-SORBED SOLUTE

As shown schematically for a single porous particle in Fig. 6, the solute molecules diffuse from the free stream across the diffusional boundary layer first to the surface, then into the stagnant fluid inside the particle. The solute concentration in the free stream space, C_e , is assumed to be uniform. In our model, we use the mass

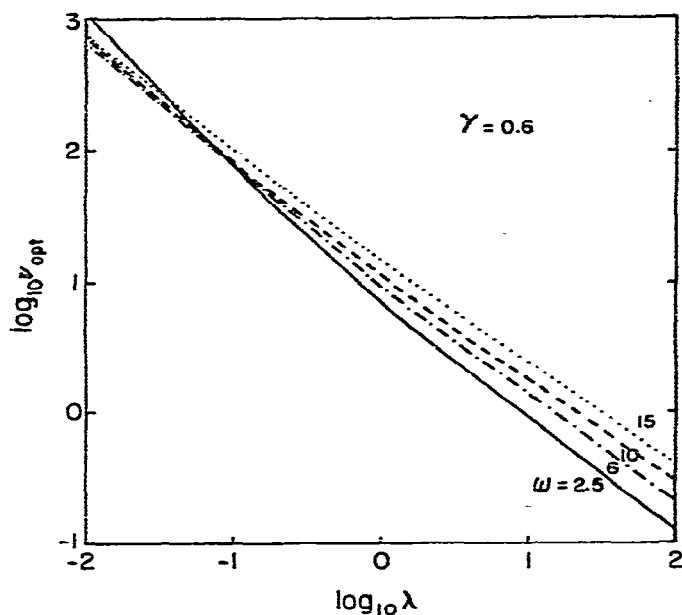


Fig. 4. Plot of the optimum reduced velocity for unsorted solutes which do not permeate the packing material, as a function of λ with ω as the parameter. The value of the obstruction factor is arbitrarily chosen as $\gamma = 0.6$.

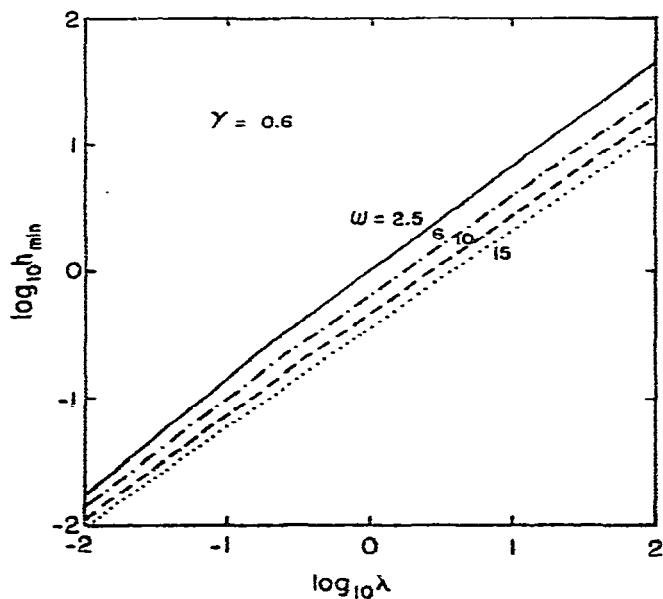


Fig. 5. Plot of the minimum reduced plate height for unsorted solutes which do not permeate the packing material, as a function of λ with ω as the parameter for $\gamma = 0.6$.

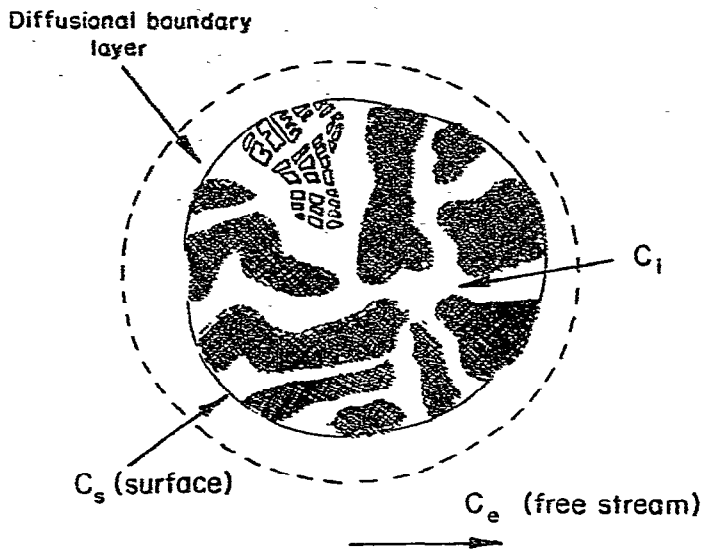


Fig. 6. Illustration of the instantaneous solute concentrations inside and outside a porous particle.

transfer coefficient, k_e , given in eqn. 7 to calculate the solute concentration at the surface, C_s , for any value of C_e in the Laplace domain. The transient penetration of the solute into and out of the accessible interparticulate stagnant space is characterized by the transfer function, $H(s)$, which is given by

$$H(s) = \frac{\bar{C}_i}{\bar{C}_s} \quad (16a)$$

where s is the parameter of the Laplace transform, \bar{C}_i is the Laplace transform of the average instantaneous solute concentration inside the particle, C_i , and \bar{C}_s is the Laplace transform of C_s .

In order to express the overall band spreading in terms of the plate height, we first evaluated \bar{C}_i from the pertinent mass balance equation and derived the relationship between C_i and C_e by using the method described by Villiermaux²⁰. Thereafter, the diffusion equation has been solved with the axial dispersion coefficient, \mathcal{D} , by using Laplace transform to evaluate the overall band spreading due to mobile phase effects both in the interstitial and intraparticulate space. The axial dispersion coefficient which expresses band spreading due to longitudinal molecular diffusion and eddy diffusion was obtained from eqns. 10a and 13b as

$$\mathcal{D} = \gamma D_e + \frac{\lambda d_p u_e}{1 + \omega \nu^{-1/3}} \quad (16b)$$

After going through the elaborate mathematical procedures, we obtained the following relationship for the reduced plate height of a nonsorbed solute

$$h = \frac{2\gamma}{\nu} + \frac{2\lambda}{1 + \omega \nu^{-1/3}} + \frac{\omega \varphi \varepsilon_t \varepsilon_e k_0}{18(1 - \varepsilon_e)(1 + k_0)^2} \nu^{2/3} + \frac{k_0}{30\tau(1 + k_0)^2} \nu \quad (17)$$

where k_0 is defined as $\varphi V_i/V_e$, i.e., the ratio of the intraparticulate void space accessible to the solute and the interstitial space in the column. τ is the tortuosity factor which relates the effective solute diffusivity inside the particles, D_i , to D_e by $D_i = \tau D_e$. Eqn. 17 yields for the plate height proper the following expression

$$H = \frac{A}{u_e} + \frac{B}{1 + Cu_e^{-1/3}} + Du_e^{2/3} + Eu_e \quad (18a)$$

with the parameters

$$A = 2\gamma D_e \quad (18b)$$

$$B = 2\lambda d_p \quad (18c)$$

$$C = \frac{\omega D_e^{1/3}}{d_p^{1/3}} \quad (18d)$$

$$D = \frac{\varphi \varepsilon_i \varepsilon_e \omega}{18(1 - \varepsilon_e)} \frac{k_0}{(1 + k_0)^2} \frac{d_p^{5/3}}{D_e^{2/3}} \quad (18e)$$

$$E = \frac{1}{30} \frac{k_0}{(1 + k_0)^2} \frac{d_p^2}{\tau D_e} \quad (18f)$$

At low reduced velocities eqn. 18a can be simplified to give

$$H = \frac{A}{u_e} + B'u_e^{1/3} + C'u_e^{2/3} + D'u_e \quad (19a)$$

where

$$B' = \frac{2\lambda d_p^{4/3}}{\omega D_e^{1/3}} \quad (19b)$$

$$C' = D \text{ and } D' = E \quad (19c)$$

At sufficiently high reduced velocities, when $v \gg \omega^3$, the second term of eqn. 18a becomes constant, so that we have

$$H = \frac{A}{u_e} + B + C'u_e^{2/3} + D'u_e \quad (20a)$$

It is noted that the mobile phase velocity in eqns. 18a, 19a, and 20a is expressed by the interstitial velocity. As frequently the chromatographic velocity is measured experimentally, both the inter- and intraparticulate porosity have to be evaluated independently in order to calculate u_e from eqn. 3e.

EXPERIMENTAL

The chromatograph used in the measurement of band spreading was assembled from the following moduli: Altex Model 100 reciprocating pump, Rheodyne Model 7010 sample injector with a 20- μ l loop, Perkin-Elmer Model LC-55 detector, and

Perkin-Elmer Model FGA-1 recorder. The variable-wavelength detector was set to 240 nm. The dead volume between the sampling valve and the column inlet as well as between the column outlet and the flow cell was minimized by using the shortest possible lengths of 0.25 mm I.D. connector tubing. The column was prepared from a 98.3 cm \times $\frac{1}{4}$ ft. O.D. \times 0.467 cm I.D. stainless-steel tubing which was packed dry with Code 0202 glass beads (Corning Glass Works) having an average particle diameter of 48.5 μ m. The inner wall of the stainless-steel tube was not polished. Whatman low dead volume fittings were used at both ends of the column, which was straight and kept in vertical position.

Experiments were carried out with three solvent-solute systems at 25°. In one series of experiments *n*-hexane containing 0.3% (v/v) methanol was used as the mobile phase and a 1% (v/v) acetone solution in *n*-hexane was injected. In another series, the same eluent was used but a 1% (v/v) benzene solution in *n*-hexane was injected. The third set of experiments was carried out with a 1% (w/v) benzoic acid solution in ethylene glycol as the sample. The flow-rate was varied between 0.02 and 9.99 ml/min. The sensitivity of the detector-recorder system was adjusted so that the peak height was at least half of the full scale length of the chart paper.

The peaks obtained with hexane as the mobile phase were symmetrical, but those obtained with ethylene glycol as the eluent showed slight fronting. Plate heights were calculated from half-height widths and baseline intercepts²¹ and average values were taken. In each run, the flow-rate was measured by using a buret and stopwatch. The linear flow velocity, u_e , was calculated from the elution time of the peak center as measured from the chromatogram, and the column length.

The porosity of the bed was evaluated by using eqn. 2e and the average value was found to be 0.38. The reduced velocities were obtained by using the following values for solute diffusivity calculated by the Wilke-Chang equation²²: acetone in hexane, $D_e = 4.6 \times 10^{-5}$ cm²/sec, and benzene in hexane, $D_e = 5.8 \times 10^{-5}$ cm²/sec. For benzoic acid in ethylene glycol, $D_e = 4.3 \times 10^{-7}$ cm²/sec was taken from the literature²³.

RESULTS AND DISCUSSION

In the chromatographic literature, several simple equations have been proposed to express the plate height of a non-permeating and unsorbed solute as a function of the flow velocity. These equations, together with eqn. 14, are listed in Table I. The simple coupling equation of Giddings is shown because his more complex coupling theory does not yield an expression which can be verified experimentally.

It is seen that the essential difference between eqn. 14 and those put forward by Giddings and by Huber is the exponent of the flow velocity. The equation of Done and Knox²⁴ is identical with eqn. 14 at sufficiently low values of the reduced velocity, as has been shown in eqn. 15a. None of the equations accounts for the effect of turbulence which may occur at high Reynolds numbers in packed beds⁵, but in usual chromatographic practice, the Reynolds number is sufficiently low so that turbulence does not play a role.

In order to test the dependence of the reduced plate height on the reduced velocity experimentally, a wide range of ν has to be covered. In addition, we have to consider the following points. Measurements have to be carried out with the same

TABLE I

EQUATIONS DESCRIBING THE PLATE HEIGHT OF AN UNSORBED AND NONPERMEATING SOLUTE AS A FUNCTION OF THE FLOW VELOCITY

For the sake of comparison the equations are presented in dimensionless form and the same symbols are used for the three column parameters.

Giddings ¹⁰	$h = \frac{2\gamma}{\nu} + \frac{2\lambda}{1 + \omega\nu^{-1}}$
Huber ¹¹	$h = \frac{2\gamma}{\nu} + \frac{2\lambda}{1 + \omega\nu^{-1/2}}$
Done and Knox ²⁴	$h = \frac{2\gamma}{\nu} + \frac{2\lambda}{\omega \nu^{1/3}}$
Eqn. 14	$h = \frac{2\gamma}{\nu} + \frac{2\lambda}{1 + \omega\nu^{-1/3}}$

column because columns which have particles of different size, shape or size distribution or different tube to particle diameter or aspect ratios are expected to have different λ and ω values. In fact, the large body of literature data available²⁵ is not adequate for testing the equations because the experiments have been performed with different columns and plate height values have been obtained with each in a relatively narrow reduced velocity range only. It is also important that the experimental conditions are such that the effect of turbulence is negligible even at very high reduced velocities. In addition, great care has to be taken to avoid entrance effects as well as extra column band spreading.

In accordance with these considerations, we selected a relatively long column packed with intermediate size fluid impervious particles so that high values of u_e could be obtained at the available inlet pressure and the extra column dead space was negligibly small. By varying both the eluent and the solutes, we covered a wide range of u_e at the available flow-rate range and the maximum Reynolds number was less than 1 with hexane at $\nu = 250$ and less than 5×10^{-3} with ethylene glycol, even at a reduced velocity of 10,000.

The results are illustrated in Fig. 7, which shows the experimental data and the curves obtained by fitting the data to the various equations given in Table I, except the equation of Knox, which has not been claimed to be valid at high reduced velocities. The curve fitting was performed by the least squares method on a PDP-11 minicomputer. We assumed $\gamma = 0.6$ in each case and the parameters calculated with the different equations are listed in Table II. The average absolute error was 28% for the simple coupling equation of Giddings, 12% for the equation of Huber, and 9% for eqn. 14. It is seen that with this set of data both the Huber equation and eqn. 14 agree reasonably well with the experimental results. Nevertheless, the fit of the data to the latter equation is better than that to Huber's equation as the average error is a poor measure of the fit.

There is other support for the superiority of eqn. 14. This equation is derived from a theoretical model which showed excellent agreement with data measured for steady state mass transfer in packed beds. In addition, at low reduced velocities eqn.

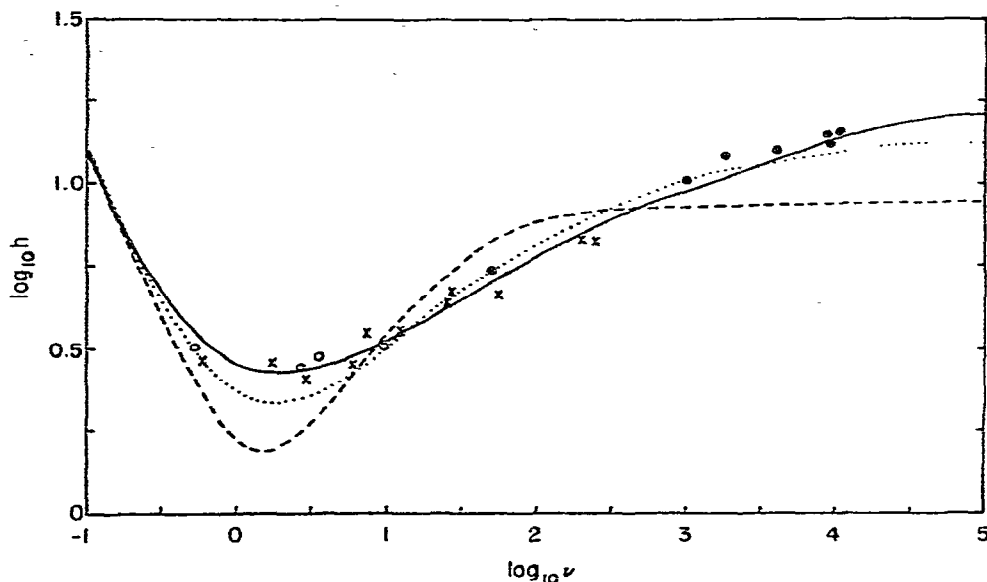


Fig. 7. Plots of the reduced plate height against the reduced velocity. The data points were obtained with a single glass bead column by using acetone in *n*-hexane (\times), benzene in *n*-hexane (\circ), and benzoic acid in ethylene glycol (\oplus). The curves represent the equations given in Table I with the parameters which gave the best fit to the experimental data which are listed in Table II. ---, Giddings' equation; \cdots , Huber's equation; —, eqn. 14.

TABLE II

VALUES OF THE PARAMETERS OBTAINED FOR THE EQUATIONS GIVEN IN TABLE I WHICH REPRESENT THE VARIOUS CURVES IN FIG. 7

Equation	γ	λ	ω
Giddings	0.6	4.35	16.1
Huber	0.6	6.85	11.1
Eqn. 14	0.6	10.47	11.2

14 reduces to the equation given by Knox on the basis of extensive investigations of band spreading in glass bead columns. Consequently, the empirical equation of Done and Knox is a limiting form of eqn. 14.

In order to express the interplay of flow and diffusion in the anfractuous interstitial space of packed columns, we arrived at eqn. 14 by applying the mixing concept of eddy diffusion to the movement of the solute in the free streaming fluid only. Whereas the coupling theory of Giddings considers the relaxation of convective axial mixing by the transverse diffusion of the solute between fixed stream channels, our model postulates the attenuation of the classical eddy diffusion by a velocity-dependent quasi-stagnant fluid fraction in the interstices. Although the calculation of this fluid fraction proper entails several approximations, eqn. 14 appears to represent the band spreading of a nonpermeating solute in packed beds adequately.

It has been mentioned previously that λ is a measure of the velocity inequality of the streaming fluid flowing through the column and ω is related to the stagnant

fraction of the interstitial fluid in the bed. Together with the obstruction factor γ they represent the parameters of the column packing in view of eqn. 14. The value of γ , however, does not vary significantly from column to column and is assumed to be 0.6–0.7 (ref. 4). Consequently the quality of a given column packing can be quantified by the overall values of λ and ω . Eqn. 14 allows us to evaluate for any given column the two parameters from experimental data obtained with a totally excluded tracer, provided there are sufficient data points relating h to v at least over the domain from $v = v_{\text{opt}}$ to $v = 10^3 v_{\text{opt}}$.

The effect of ω on the dependence of h on v has been calculated from eqn. 14 for a nonpermeating solute and is illustrated in Fig. 8. It can be seen that at a fixed λ the minimum of the h vs. v curve shifts to higher values of v with increasing values of ω , in agreement with the results shown in Figs. 4 and 5. Generally, when λ is the same, a higher ω appears to represent a "better" column packing because it yields a given reduced plate height at a higher reduced velocity with a totally excluded tracer. The value of ω is dependent on the interstitial porosity but the relationship is likely to be more complicated than suggested by eqn. 9b. When v is not much higher than the minimum, the column packing can be characterized by λ/ω according to eqn. 15a. On the other hand, λ is a packing characteristic which represents the limiting value of h for a nonpermeating solute at high reduced velocities in the absence of turbulence. Both λ and ω are determined not only by the materials used to make a column but

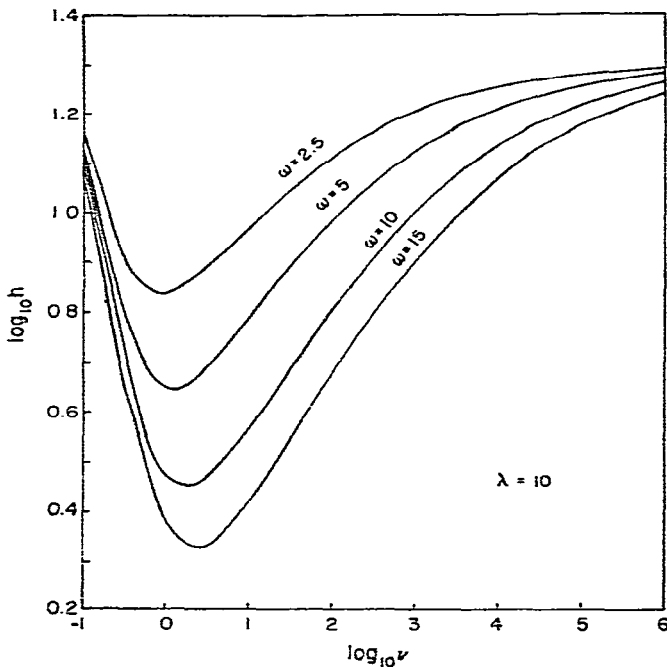


Fig. 8. Graph illustrating the effect of ω on band spreading of a totally excluded unsorted solute. The reduced plate height is plotted against the reduced velocity according to eqn. 14 with the fixed parameters $\lambda = 10$, $\gamma = 0.6$, and $\epsilon_s = 0.4$, and with the ω values shown on the graph.

also by the packing procedure itself. Thus, for a given type of column the comparison of the corresponding λ and ω values can shed light on the efficacy of the various packing procedures employed.

The experimentally obtained column parameters are overall values, *i.e.*, the averages of numerous local λ and ω values, because no column packing can be considered completely uniform. Thus, the overall λ and ω values account for, among others, the five velocity inequalities discussed by Giddings⁹. With columns of low aspect ratio, entrance and exit effects could be related to the corresponding local λ and ω values but these phenomena still require further study. On the other hand, the so-called wall effect, *i.e.*, perturbances in the packing at the wall region, has been extensively investigated²⁶. It is intriguing to view the wall effect as the result of significant differences between the average local λ and ω values in the wall and the center regions of the packing. The disturbances caused by the wall in the packing can result in an annulus of relatively high interstitial porosity. According to our model, the local ω values in this annulus, which may extend over many particle diameters, could be significantly lower than in the center region. Consequently, the local band spreading of a nonpermeating solute would be much greater in this annulus even if the local average interstitial velocity would be close to that in the center part of the packing. Of course the locally greater "persistence-of-velocity-span"⁹ in the wall region would also result in an increase in the average local value of λ with respect to that in the center region.

Whereas the study of the detailed structure of a packed bed by measuring local values of the column parameters is of great interest, in our view a separation of the packing and the tube wall as two different entities with respect to axial dispersion is not justifiable to a chromatographer who is interested in the overall effect. As soon as the solute molecules explore the wall region, the tube wall becomes a part of the packing as far as band spreading is concerned and it is a quodlibetical proposition that dispersion due to wall effect should not be considered "true" dispersion since it is not a function solely of the packed bed²⁷. Furthermore, the wall effect is only one of several factors which can give rise to local λ and γ values significantly different from the overall parameters obtained with the whole column.

When the unsorbed solute can diffuse into the intraparticulate void space of the packing, the plate height equation becomes more complicated than that for the excluded solute, because two additional terms have to be added, as shown in eqn. 17. One of these terms accounts for the mass transfer resistance in the diffusional boundary layer surrounding the particles, the other for the mass transfer resistance in the intraparticulate stagnant fluid. In essence, this stagnant fluid acts as an "apparent" stationary phase that is associated with a hold-up volume, ϕV_i , as shown in Fig. 1. This hold-up volume results in an "apparent" capacity factor, k_0 , when the interstitial volume of the column is used as the reference volume for the mobile phase.

Once the axial dispersion coefficient is evaluated by eqn. 16b, a straightforward and rigorous mathematical procedure clearly yields eqns. 17 and/or 18a. These equations can be used to assess the significance of the effect of the intraparticulate stagnant mobile phase on the band spreading of a permeating unsorbed solute. In Fig. 9 this effect is exemplified by h vs. ν plots calculated from eqns. 14 and 17. In both cases, the column parameters except ε_i are the same, so that the relatively higher h values shown by the upper curve are solely caused by the last two terms of eqn. 17. It is noted that

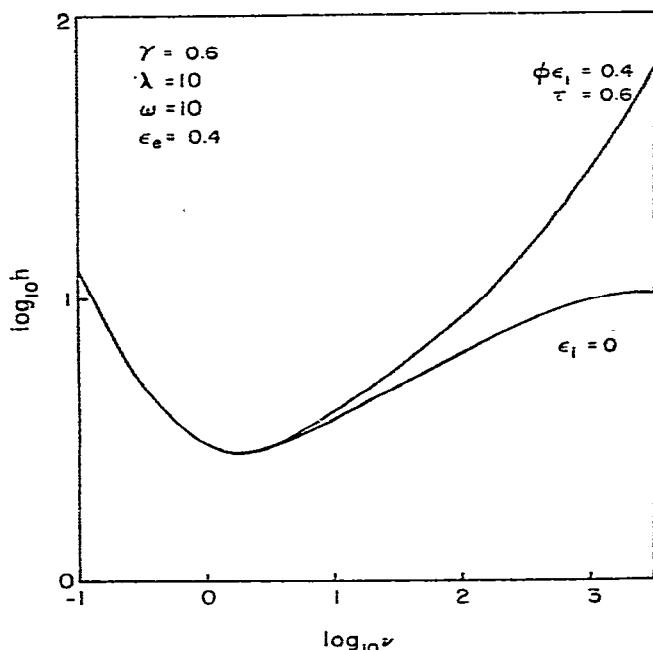


Fig. 9. Graph illustrating the effect of the mass transfer resistance arising from solute diffusion into the intraparticulate space on the reduced plate height as a function of the reduced velocity. The lower curve refers to a completely excluded solute ($\epsilon_1 = 0$), the upper curve to a solute which explores the intraparticulate void ($\phi \epsilon_1 = 0.4$). The other column parameters in both cases are as shown on the graph.

with porous particles, when τ is greater than 0.3 and ω is about 10, the ratio of D/E is of the order of unity. Then the last term in eqn. 17, which is linear in velocity, would dominate band spreading only at reduced velocities as high as 10^6 . Consequently, under practical conditions the plate height does not depend linearly on the flow velocity, except with microreticular resinous stationary phases when τ can be very small, as long as ω is sufficiently large.

We should note that ω appears in the denominator of the second term and in the numerator of the third term in eqns. 17 and 18a. Accordingly, the stagnant fluid in the interstices not only attenuates convective band spreading but can also augment zone dispersion at relatively high flow velocities when the solute diffuses into the particles. In the latter case, which reflects the usual chromatographic situation, the stagnant fluid represents a mass transfer resistance and the relationship between ω and the quality of the packing with respect to column efficiency becomes obscured. More precisely the effect of ω on band spreading depends on the properties of the solute, the flow velocity and other features of the column packing as well. An interesting conclusion from this is that for various types of chromatographic systems in the practical flow velocity range different overall ω values, *i.e.*, different packing structure in the column could yield optimum efficiency, whereas in columns packed with fluid impervious particles axial dispersion invariably decreases with increasing ω .

Eqn. 19a allows us to make a rough estimate of the flow dependence of the

plate height at low reduced velocities and with columns of practical interest. With pellicular packings the ratio $\varphi\varepsilon_i/\varepsilon_e$ is relatively small and the band spreading of an unsorbed solute is dominated by the second term in eqn. 19a. As a result, the plate height is expected to depend largely upon u_e^{\dagger} under practical conditions. On the other hand, with totally porous particles the third term of eqn. 19a may dominate and then the plate height would depend roughly on $u_e^{2/3}$. According to Snyder²⁸, in the two cases experimental data indicate a flow dependence of the plate height with approximately $u_e^{0.4}$ and $u_e^{0.6}$, respectively. In view of the foregoing discussion and eqn. 18a these exponents can readily be explained. The increase in the apparent exponent from the expected value of 0.33–0.4 with pellicular packings can be caused by the influence of the last two terms in eqn. 19a which have a stronger velocity dependence than the second term. With totally porous particles the observed exponent is smaller than the expected value of 0.67 and this is indicative of the influence of the second term in eqn. 18a, which has a weaker velocity dependence than the third and fourth terms.

In the present study, we examined the effect of the mobile phase on the band spreading of unsorbed solutes in LC and derived plate height equations for solutes which can or cannot permeate the packing material. Our approach is also applicable to describe band spreading of sorbed solutes, the treatment of which is the subject of a subsequent investigation.

NOTE ADDED IN PROOF

Professor J. Villiermaux brought to our attention that mass exchange between the streaming and stagnant interstitial fluid also has to be included in our model. This generates an additional term in eqn. 14 which is correctly given by

$$h = \frac{2\gamma}{v} + \frac{2\lambda}{1 + \omega v^{-1/3}} + \frac{2\omega/\Omega^2}{1 + \omega v^{-1/3}}$$

Nevertheless, the form of eqn. 14 remains unchanged when λ is replaced by the term $\lambda + (\omega/\Omega^2)$. We expect that under practical conditions $\lambda \gg \omega/\Omega^2$.

Professor Villiermaux also pointed out that the effective interparticulate diffusivity is calculated by $D_i = \varepsilon_i D_e / \theta$ with θ as the tortuosity factor²⁹. Accordingly, $\tau = \varepsilon_i / \theta$ in this paper.

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