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THEORY OF THIN-LAYER CHROMATOGRAPHY

EFFECT OF THE STRUCTURE OF THE ADSORPTION LAYER ON SPREADING OF SPOTS

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SUMMARY

Two types of inhomogeneity of the structure of the adsorbent layer have been shown to exist, chaotic and regular (sedimentation), depending on the method of preparation of the layer. The effect of these types of structure on the dynamics of the spreading of chromatographic spots was investigated both theoretically and experimentally.

Values of different terms of chromatographic spreading (HETP) were calculated, taking into account the properties of the solvent and the geometrical parameters of the plate. The contributions of individual terms to the overall HETP were estimated.

A comparison of calculated with experimental values for different variations of thin-layer chromatography showed good agreement between them.

INTRODUCTION

If we consider thin-layer chromatography (TLC) to be a two-dimensional non-ideal and non-equilibrium process occurring in a homogeneous adsorption layer, then, as has already been shown[1], it can be described by the following differential equation:

$$\frac{\partial C}{\partial t} + uR_F \cdot \frac{\partial C}{\partial x} = R_F \left[D_{tx} \cdot \frac{\partial^2 C}{\partial x^2} + D_{ty} \cdot \frac{\partial^2 C}{\partial y^2} + (1-R_F)\, u^2 \tau \cdot \frac{\partial^2 C}{\partial x^2}\right] \quad (1)$$

where C is the average concentration of the substance at a point (x,y) of the adsorption layer, u is the flow-rate, x is the direction of eluent migration, y is the transverse direction, R_F is the relative mobility of the substance under equilibrium conditions (relative mobility of the centre of the chromatographic spot), $D_{tx,y}$ is the effective diffusion coefficient in the mobile phase, τ is a kinetic parameter characterizing the lag in the establishment between the mobile and the stationary phase and t is time.

The solution of this equation under initial and boundary conditions corresponding to the point spotting is given by

$$C(x,y,t) = C_m \exp\left\{-\frac{1}{2}\left[\frac{(x - R_F ut)^2}{\sigma_x^2} - \frac{y^2}{\sigma_y^2}\right]\right\} \quad (2)$$

where

$$\sigma_x^2 = 2\,[D_{lx} + (1-R_F)\,u^2\tau]\,R_F t \tag{3}$$

$$\sigma_y^2 = 2\,D_{ly}\,R_F t \tag{4}$$

The eluent flow-rate, u, in a thin layer is a function of time or of the distance of migration along the plate. The method of continuous chromatography in which the flow-rate is essentially constant is an exception. In a general case, the migration of a liquid in a granular layer obeys the Darcy law[2], which, in combination with the Kozeny–Carman equation[3], permits the equations for the solvent flow-rate, u, as a function of geometrical parameters of the layer and the properties of the liquid to be obtained. For ascending TLC, this equation becomes

$$u = \frac{V_1^2}{15\,(1-V_1)^2} \cdot \frac{d_p}{\eta} \left(\frac{\lambda \cos\theta}{R_s} - \frac{d_p \varrho g}{12}\right) \tag{5}$$

where V_1 is the volume of the intergranular space, d_p is the mean grain diameter, η, ϱ, λ and θ are the viscosity, density, surface tension and contact angle, respectively, g is the acceleration due to gravity and R_s is the distance of migration. Eqn. 5 takes into account the effect of two forces upon the liquid: capillary rise and hydrostatic pressure. As $u = \mathrm{d}R_s/\mathrm{d}t$, it follows that by solving the ordinary differential eqn. 5 we obtain

$$\exp\left(\frac{\beta R_s}{\alpha}\right)\left(1 - \frac{\beta R_s}{\alpha}\right) = \exp\left(-\frac{\beta^2 t}{\alpha}\right) \tag{6}$$

where

$$\alpha = V_1^2 d_p \lambda \cos\theta / 15(1-V_1)^2\eta \quad \text{and} \quad \beta = V_1^2 d_p^2 \varrho g / 180(1-V_1)^2\eta.$$

In order to obtain the explicit dependence $R_s(t)$ and then $u(t)$, we can expand the exponent on the left-hand side of eqn. 6 into a series for $(\beta R_s)/\alpha$ and use only two terms of the expansion. Finally, we have

$$u = \left(\frac{V_1^2 \lambda \cos\theta d_p}{15(1-V_1)^2\eta}\right)^{\frac{1}{2}} t^{-\frac{1}{2}} - \frac{V_1^2 d_p^2 \varrho^2 g^2}{180(1-V_1)^2 \eta\lambda \cos\theta} \tag{7}$$

Eqn. 7 expresses a well known parabolic law of the dependence of the eluent flow-rate on time formulated for paper chromatography[4–7]. This equation shows that the greatest change in the flow-rate occurs in the course of a short initial period: $\mathrm{d}u/\mathrm{d}t\ t^{-3/2}$, or along a small initial length of the layer. As the eluent migrates further, its flow-rate decreases smoothly with time.

Fig. 1 shows the experimental dependence of the flow-rate of the solvent front on time for dichloroethane and chloroform in ascending chromatography on a plate with silica gel KSK ($d_p = 11\ \mu$m).

To the right of the vertical dashed line in Fig. 1 the rate changes only very slightly with time. Hence, if the mixture under investigation is applied to the plate to the right of the dashed line (*i.e.*, at a distance of 3 cm from the line of initial contact with the solvent), the rate of the chromatographic process may be considered, to a good approximation, to be constant and equal to the mean rate over the time interval

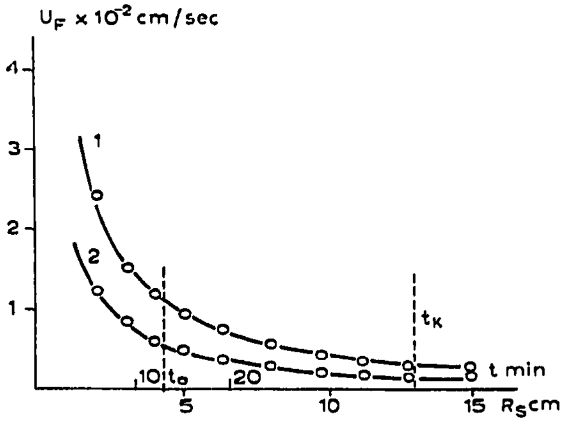

Fig. 1. Flow-rate of the solvent front *versus* time for dichloroethane and chloroform in ascending chromatography on a plate with silica gel KSK (d_p = 11 μm).

considered, $\bar{u}(t_0 + t)$ = constant·(t). Thus, in eqns. 1–4, in a general case, the rate can be interpreted simply as the mean rate, $\bar{u}$, over the interval $t_0 + t$. However, if we take into consideration that the rate is not constant with time (eqn. 7), it can be shown[8] that the solution of the differential eqn. 1 satisfies eqns. 2–4 when u is replaced with $\int_{t_0}^{t} u\mathrm{d}t$.

Eqns. 2–4 were derived for a homogeneous chromatographic layer. Nevertheless, it should be remembered that the adsorption layer is virtually inhomogeneous, *i.e.*, the grains are always distributed by size in the volume of the layer (specifically, throughout the thickness). This distribution may be either regular (*e.g.*, as a result of sedimentation of particles of different size) or chaotic. Giddings and others investigated the effect of the inhomogeneity of the absorbent packing on chromatographic spreading in column chromatography[8–10]. Similar investigations have not previously been carried out for TLC.

THEORETICAL

In this work, the theory of TLC is developed for the case of an inhomogeneous absorption layer with regular or chaotic inter-layer grain distribution by size. This inhomogeneity is responsible for the inhomogeneity of hydrodynamic flow in the plane perpendicular to its direction, which in turn leads to a corresponding deformation of the chromatographic zone. In general, eqn. 1 takes account of the local structural inhomogeneity of the layer, as it is assumed that the effective diffusion coefficients (D_{ex} and D_{ey}) that determine mechanical dispersion (granulation effects) and molecular diffusion depend both on the size of the adsorbent grains and on the irregularity and asymmetry of their arrangement. In addition to this local micro-inhomogeneity of the layer, it is advisable to consider its macro-inhomogeneity, *i.e.*, the inhomogeneity of its packing throughout its whole thickness. In this case, the change in the fraction of the free space throughout the thickness leads to the appearance of an overall gradient of the eluent flow-rate that produces additional spreading of spots in the direction of migration (the x axis). In this case, owing to diffusion mixing, the inter-layer concentration gradient of the substance should reduce the size of the regions with the greatest deformation and thus should decrease the effective size of the spot.

We will consider this phenomenon independently of the factors of spreading taken into account in eqn. 1. The differential equation for this case becomes

$$\frac{\partial C}{\partial t} + R_F u(z,t) \cdot \frac{\partial C}{\partial x} = R_F D_\perp \cdot \frac{\partial^2 C}{\partial z^2} \qquad (8)$$

where $D_\perp$ is the effective coefficient of transverse mixing (the z coordinate) accounting for both the molecular and the eddy inter-layer diffusion. Eqn. 8 may be derived from eqn. 1 if it is assumed that the chromatographic process is ideal and equilibrium is attained but that its rate is not constant with respect to either time (the plate length) or thickness $u = u(z,t)$. This separate consideration of the effect of inter-layer inhomogeneity in packing on the spreading of spots is based on the assumption of the additivity law for different sources of spreading[1].

Assuming that at the start the substance is distributed throughout the thickness homogeneously as an infinitely narrow zone, we can write the following initial and boundary conditions for eqn. 8:

$$C(x,z,t)\mid_{t=0} = q\delta(x)/h_0 \qquad (9)$$

where q is the total amount of the substance in a spot, h_0 is the layer thickness, $\delta(x)$ is the delta-function and

$$C(x,z,t)\mid_{x\to\pm\infty} \to 0 \qquad (10)$$

Further, as a gradient exists in the z direction, it should be taken into account that the concentration flows on the interface with air and with the glass support (when $z = 0$ and $z = h_0$) are absent, *i.e.*

(11)

As we wish to find only the contribution of the inter-layer rate gradient to the dispersion of spot spreading, it is not necessary to determine the type of the function $C(x,z,t)$; it is sufficient to calculate its dispersion. According to determination of dispersion

$$\sigma^2 = \tilde{m}_2 - \tilde{m}_1^2 \qquad (12)$$

where $\tilde{m}_1$ and $\tilde{m}_2$ are the first and the second normalized moments of the distribution function $C(x,z,t)$ determined in a general case according to the equations

$$m_i = \frac{1}{h_0}\int_0^{h_0}\int_{-\infty}^{+\infty} C(x,z,t)x^i \mathrm{d}x\mathrm{d}z,\ i = 0, 1, 2, \ldots \qquad (13)$$

and

$$\tilde{m}_i = m_i/m_0 \qquad (14)$$

As the variables z and t are independent, the flow-rate of the mobile phase is given by

$$u(z,t) = \omega(z)\tau(t) \tag{15}$$

where $\omega(z)$ is the function describing the transverse rate gradient and $\tau(t)$ determines the time dependence (*e.g.*, for ascending TLC it is determined by eqn. 7).

The solution of eqn. 3 under the conditions of eqns. 9–11, taking into account eqn. 15, by consecutive application of Laplace transformation and expansion for its own functions of the arbitrary operator $R_F D_\perp \cdot \partial^2/\partial z^2$ permits us to find the moments and dispersion determined from eqns. 12–14. A detailed scheme of the solution is shown in the Appendix and we report here only the final result for dispersion, σ_x^2:

$$\begin{aligned}\sigma_x^2 = {} & \frac{R_F^2 \omega_0^2}{2} \left\{ \int_0^t \tau(t'') \int_0^{t''} \tau(t')\mathrm{d}t'\mathrm{d}t'' - \frac{1}{2}\left[\int_0^t \tau(t')\mathrm{d}t' \right]^2 \right\} + \\ & + R_F^2 \sum_{n=1}^{\infty} \omega_n^2 \int_0^t \tau(t'') \exp\left[-R_F D_\perp \left(\frac{\pi n}{h_0}\right) t''\right] \int_0^{t''} \tau(t') \exp\left[R_F D_\perp \left(\frac{\pi n}{h_0}\right)^2 t'\right] \mathrm{d}t'\mathrm{d}t''\end{aligned} \tag{16}$$

where ω_n are the coefficients of expansion into the Fourier series determined by the equation

$$\omega_n = \frac{2}{h_0} \int_0^{h_0} \omega(z) \cos\left(\frac{\pi n z}{h_0}\right) \mathrm{d}z, \quad n = 0, 1, 2, \ldots \tag{17}$$

If we assume that the flow-rate of the mobile phase is constant and equal to the mean flow-rate during the time interval considered ($t_0 + t$),

$$\bar{u}_t = \frac{2\,(V_1^2 \lambda \cos\theta\, d_p)^{\frac{1}{2}}}{[15\,(1 - V_1)^2 \eta]^{\frac{1}{2}}\,(t_k^{\frac{1}{2}} + t_0^{\frac{1}{2}})} \tag{18}$$

and express its inter-layer change (associated with the change in d_p) as a stepwise gradient (Fig. 2),

$$\bar{u}_t(t) = \begin{cases} u_0, & 0 \leqslant z \leqslant h_v \\ u_0 + \Delta u, & h_v \leqslant z \leqslant h_0 \end{cases} \tag{19}$$

where Δu is the value of the rate jump and h_v is its coordinate, then the expression for dispersion (eqn. 16) is simplified and becomes

$$\sigma_x^2 = \frac{2\, R_F v^2 (1 - v)^2 h_0^2 (\Delta u)^2}{3\, D_\perp} \cdot t \tag{20}$$

where $v = h_v/h_0$. It is convenient to use this equation for practical calculations (in the Appendix we give other particular types of eqn. 16).

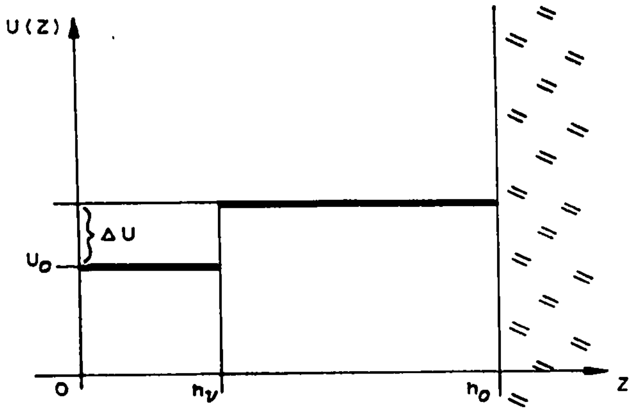

Fig. 2. A stepwise model of the rate profile in a column.

If we multiply the numerator and denominator on the right-hand side of eqn. 20 by $R_F t$, we reveal the existence of opposite effects that influence the longitudinal spreading of spots:

$$\sigma_x^2 = \frac{2}{3} h_0^2 \nu^2 (1 - \nu)^2 \cdot \frac{R_F^2 \Delta u^2 t^2}{D_\perp R_F t} \tag{21}$$

The numerator on the right-hand side of eqn. 21 characterizes the divergence of the concentration points in different inter-layer sections and hence the increase in the total projection of the spot on the direction of development (the x axis). This increase is proportional to the distance of migration of the spot along the plate and to the layer thickness, $\sigma_x \approx R_F \bar{u}_h h_0 t$. The denominator describes the equalization of inter-layer concentration caused by "thawing" of the front edge of the spot in the lower section and of the back edge in the upper section.

It is interesting to compare with eqn. 21 the Giddings expression for HETP related to spreading in the column owing to the rate profile[11]:

$$H = \left(\frac{\varkappa - 1}{\varkappa + 1}\right)^2 \frac{r_c^2 u}{24 \gamma D_m} \tag{22}$$

where κ is the ratio of the rates on the periphery and in the centre of the column [$\kappa = (u + \Delta u)/u$], r_c is the radius of the column and D_m is the coefficient of molecular diffusion.

Rewriting eqn. 22 for a column of length L:

$$\sigma_{x\perp}^2 = LH \tag{23}$$

where $L = \bar{u}_t R_F t$, we obtain

$$\sigma_{x\perp}^2 = \frac{(\Delta u)^2 R_F^2 t}{96 \gamma D_m} r_c^2 \tag{24}$$

If we assume that $D_m = D_{\perp}$, this expression corresponds to the expression for σ_x^2 in TLC (eqn. 21) to within the coefficient in the denominator:

$$\sigma_{x\perp}^2 = \frac{2(\Delta u)^2 R_F t}{3 D_{\perp}} [\nu^2 (1-\nu)^2] h_0^2 \tag{25}$$

(The expression in square brackets is equal to 0.047 when $\nu = 0.66$).

It is clear that the dispersion of chromatographic spreading owing to the rate profile is higher in TLC than in column chromatography. This is presumably associated with a uniform flow of the substance throughout the whole thickness of the plate, in contrast to column chromatography in which the flow in the peripheral parts of the column is much faster than in the central zone.

EXPERIMENTAL AND RESULTS

A study of inter-layer distribution of particles in the layers prepared by different methods was carried out by cutting from the layer successive sections (starting from the upper edge) and by measuring the mean size of the particles in each section. For this purpose we used a microtome to cut layers 5 ± 1 μm thick with a cutting interval of 10 μm.

Fig. 3 shows the results of measurements for three fractions of silica gel KSK with large pores differing both in the mean grain diameter and in the fraction width. When the plates were prepared by pouring a dilute suspension of the adsorbent (Fig. 3a), the inter-layer particle distribution became ordered (by size) owing to sedimentation. When the layer was prepared from a concentrated suspension, *e.g.*, by using an applicator or by immersing the glass plate into such a suspension prepared from a solvent of low volatility, the mean grain sizes in different sections were virtually identical but in individual sections the size of the particles varied greatly. Thus, the two different methods of preparation of the layer create different inhomogeneities in its structure.

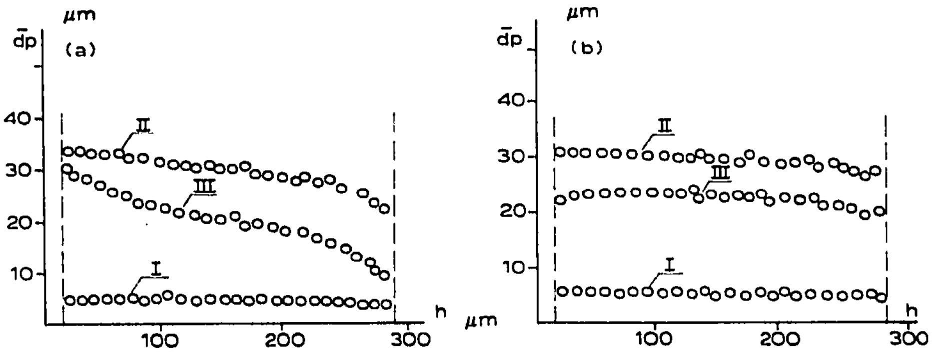

Fig. 3. Size distribution of adsorbent particles *versus* their distance (h) from the glass support for a plate prepared (a) by pouring with the use of a dilute suspension of adsorbent, (b) by immersion in a concentrated suspension based on a volatile solvent. I, $d_p = 4.0$ μm, $\Delta d_p = 4.5$–3.5 μm; II, $d_p = 30.0$ μm, $\Delta d_p = 3.50$–28.0 μm; III, $d_p = 20.0$ μm, mixture of I and II in a 1:3 weight ratio.

When the layer is poured and the inter-layer of particles is sedimented, a transverse gradient of the eluent flow-rate appears, and this contributes to additional spreading of spots in the direction of development. When the layer is prepared by immersion, the inhomogeneity of the structure is localized over the range of only one or a few grain diameters. In this case, the main contribution to the spreading of spots is due to diffusion in the mobile phase, in particular to the eddy diffusion component. As the effective coefficient of the coupling eddy diffusion is proportional to the mean grain diameter and to the flow-rate, $D_{lx} = (1/2\lambda d_p + D_m/\omega d_p^2 u)^{-1}$, where λ and ω are structural parameters[5], it follows that in micro-TLC (optimum d_p and u) for which D_{lx} is small, the spreading of spots will be much lower for the immersion method of layer preparation than for the pouring method.

Fig. 4 shows the spreading of spots (when the same amounts of the substance are used) as a function of the layer thickness for layers prepared from dilute and concentrated suspensions of silica gel KSK. As the experimental dependence shows that $\sigma_x^2 \approx h_0^2$, it satisfies eqn. 20. In this case, the slopes of the straight lines for different fractions of silica gel are different and this is also consistent with eqn. 20. For fractions II and III, the slopes of the straight lines for the immersion method are lower than for the pouring method. Hence, the inhomogeneity of the layer structure in the inter-layer distribution of the absorbent particles affects the spreading of spots (particularly when the layer thickness increases) to a greater extent than does the local inhomogeneity. It can be concluded that in order to obtain highly effective chromatographic plates they should be prepared from concentrated suspensions of the adsorbent. It is characteristic that in the pouring method the region of the highest structural inhomogeneity is located near the layer surface. The removal of this region from the layer should decrease the spreading of spots. This suggestion was confirmed experimentally. Fig. 5 shows as an example the dependence of the spreading of DNP-alanine spots on the height of the removed upper part of the layer (Δh). The dependence of spreading on the layer thickness without removal of the upper part is shown by the broken line. It can be seen from Fig. 5 that the removal of the surface part of

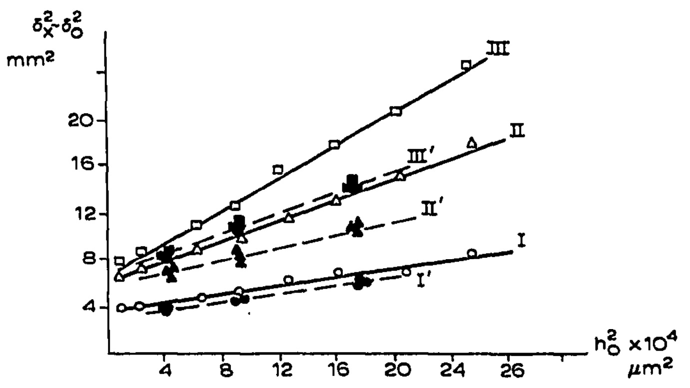

Fig. 4. Spreading of the Dns-glycine spot *versus* the thickness of the layer prepared from dilute (solid line) and concentrated (broken line) suspensions of silica gel KSK.

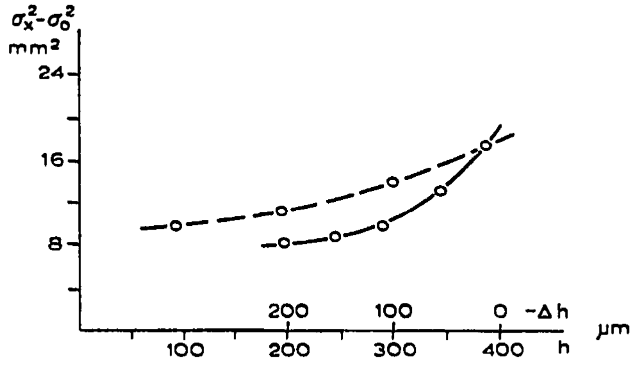

Fig. 5. Spreading of the DNP-alanine spot *versus* the thickness of the layer section.

the layer ($\Delta h \approx 100\ \mu$m) led to a considerable decrease in spreading, but when further sections were cut off the homogeneity of structure was not improved and the dependence of spreading on the layer thickness became less pronounced.

Experiments were carried out to confirm the above assumptions concerning the mechanism of longitudinal spreading of spots due to the inter-layer rate gradient. For this purpose, thin-layer plates were prepared with different grain size distributions in the layer section. This was attained by mixing homogeneous adsorbent fractions of different grain sizes. Table I shows the values of h_v and v calculated for a plate with sedimentational distribution of the adsorbent grains*. The plates obtained were spotted with Dns-amino acids. The concentration distribution of a substance in the spots was determined with an MFTKh-1 microabsorbtiometer–fluorimeter[12,13] as the upper parts of the layer were successively cut off. The results (Figs. 6 and 7) show that as the parts of the layer were cut off, the dispersion of the spot decreased and its centre shifted along the axis of development.

TABLE I

CHARACTERISTICS OF ADSORPTION LAYERS ON THIN-LAYER PLATES

No.	*Initial fractions*		*$\bar{d}_p$ (mixture) (μm)*	*h_0 (μm)*	*h_v (μm)*	*v*
	$\bar{d}_p$ (μm)	*Weight (g)*				
1	30 5	0.06 0.24	10	290	240	0.8
2	25 3	0.09 0.21	10	290	175	0.6
3	14 3	0.19 0.11	10	300	90	0.3

By extrapolating the values of the standard deviations for the concentration

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[*] Calculations were made with the assumption that precipitation is consecutive, *i.e.*, large adsorbent particles are precipitated faster than small particles.

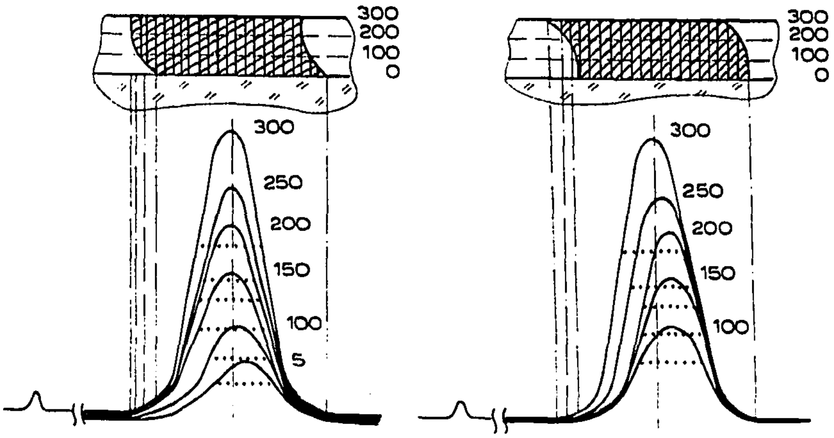

Fig. 6. Overlapping plots of interlayer concentration distribution of a fluorescent substance for three gradient models.

profile in the spot to a layer of zero thickness, it is possible to distinguish the spot spreading related only to the rate gradient and to determine its contribution to the total spot spreading. This contribution for layers of types 1, 2 and 3 is 30, 50 and 20%, respectively, *i.e.*, it is very high.

We have also investigated the effect of R_F on spot spreading caused by the

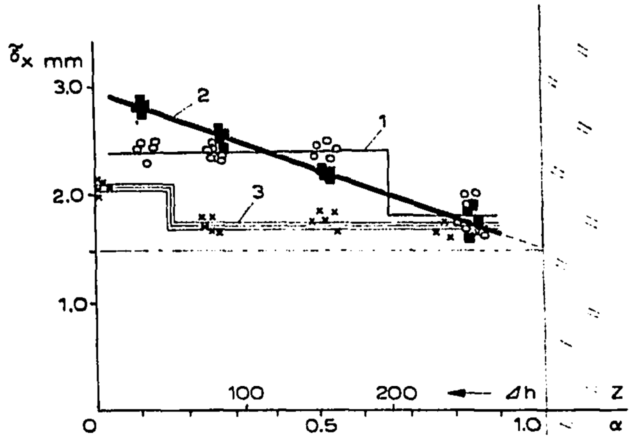

Fig. 7. Change in the value of the projection of the spot on the direction of the inter-layer development for three gradient models.

rate gradient (Fig. 8). It is clear that the dependence $\sigma_x^2(R_F)$ is linear, as follows from eqn. 20.

We can compare the experimental with the calculated results for $\sigma_{\perp}^2(H)$, taking into account the properties of the solvent and the geometrical parameters of the plate: length, L; mean grain diameter, $\bar{d}_p$; difference in the grain diameter near the support, d_{p0}, and near the layer surface, d_{ph0} ($\Delta d_p = d_{p0} - d_{ph0}$). Combining eqns. 21, 24 and

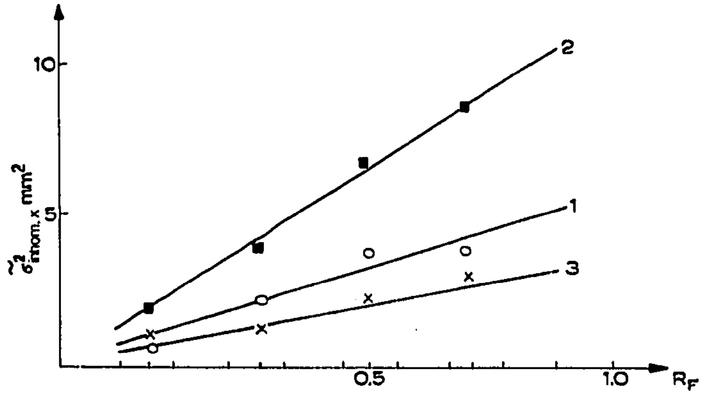

Fig. 8. Reduced spot dispersion versus R_F value.

7 and neglecting the hydrostatic pressure of the liquid, we obtain the expression for $H(R_s) = \mathrm{d}\sigma^2/R_F\mathrm{d}R_s$*:

$$H_{\perp}(R_s) = \frac{2(\Delta u)^2}{3D_{\perp}}[\nu^2(1-\nu)^2]h_0^2 \cdot \frac{R_s}{\bar{d}_p\xi} \tag{25}$$

where

$$\xi = [V_1^2/15(1-V_1)^2](\lambda\cos\theta/\eta) \tag{26}$$

The expression for Δu can be written as

$$\Delta u = \frac{(d_{p0}^2 - d_{ph0}^2)}{\bar{d}_p} \cdot \frac{\xi}{R_s} \tag{27}$$

assuming $\bar{d}_p = (d_{p0} + d_{ph0})/2$ for a linear inter-layer distribution of particles by size. Substituting it into eqn. 27 and then into eqn. 26, we obtain the expression for $H_{\perp}(R_s)$ in a form suitable for calculations:

$$H_{\perp}(R_s) = \frac{8\Delta d_p^2\xi}{45\bar{d}_pD_{\perp}R_s}[\nu^2(1-\nu)^2]h_0^2 \tag{28}$$

The choice of the value of $D_{\perp}$ in eqn. 20 is of fundamental importance. As has been shown above, the coefficient of the inter-layer diffusion, $D_{\perp}$, is related to molecular and eddy diffusion: $D_{\perp} = D_{m\perp} + D_{\text{eddy}\perp}$. The coefficient $D_{m\perp}$ is determined by the coefficient of molecular diffusion, D_m, multiplied by the tortuosity factor γ: $D_{m\perp} = \gamma D_m$. The coefficient of inter-layer eddy diffusion can be obtained as follows. It has been shown[14] that transverse transfer of the substance by a distance of one grain diameter, $\Delta x_{\perp} = d_p$, occurs during the time t_D needed for the solvent to

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* Integrating both parts of this equality from R_{s0} to R_s and dividing them by the plate length ($R_s - R_{s0}$), it is possible to find the mean value of HETP for the plate, $H_{\perp}$, at various elution rates. This procedure is justified for practical calculations.

migrate at a distance of 20 grain diameters, $t_D = 20\, d_p/u$. Hence, the coefficient of quasi-diffusion, $D_{\text{eddy}\,\perp}$, according to the Einstein equation is given by

$$D_{\text{eddy}\,\perp} = \frac{\Delta\, x^2_\perp}{2\, t_D} = 0.025\, d_p u \tag{29}$$

Hence, the total coefficient of transverse diffusion, D, is the sum of coefficients related to the true diffusion transfer of the substance, $D_{m\,\perp} = \gamma D_m$, and to the inter-layer quasi-diffusional mass transfer, $D_{\text{eddy}\,\perp} = 0.025\, d_p u$:

$$D_\perp = \gamma\, D_m + 0.025\, d_p u = \gamma\, D_m + 0.025\, \xi\, d_p^2/R_s \tag{30}$$

Substituting eqn. 30 into eqn. 28, integrating both parts of the equality from R_{s0} to R_s and dividing the result by $\Delta R_s = R_s - R_{s0}$, we obtain the equation for the mean value of HETP, $\overline{H}_\perp$, related to the inter-layer effect in TLC:

$$\overline{H}_\perp = \frac{8\,(\Delta d_p)^2\, \xi}{3\, \bar{d}_p \Delta R_s \gamma D_m}\, [\nu^2\,(1-\nu)^2]\, h_0^2 \ln \left[\frac{R_s + (0.025\, d_p^2\, \xi/\gamma\, D_m)}{R_{s0} + (0.025\, d_p^2 \xi/\gamma\, D_m)}\right] \tag{31}$$

Eqn. 31 can be compared with the equation for HETP obtained for the case in which transverse mixing of the substance in the chromatographic layer is induced only by molecular diffusion, $D_\perp = D_{m\,\perp} = \gamma D_m$:

$$\overline{H}_\perp = \frac{8\,(\Delta d_p)^2\, \xi}{3\, \bar{d}_p \Delta R_s \gamma D_m}\, [\nu^2\,(1-\nu)^2]\, h_0^2 \ln\,(R_s/R_{s0}) \tag{32}$$

It is clear that the introduction of "eddy diffusion" decreases the HETP owing to the presence of a logarithmic term. This decrease is the higher the greater is the ratio d_p/D_m.

We calculate below the HETP related to the inter-layer effect for several TLC conditions: $d_p = 10^{-4}$, $3 \cdot 10^{-4}$, 10^{-3} and $3 \cdot 10^{-3}$ cm, $R_s = 6$ cm, $R_{s0} = 1$ cm for substances with $D_m = 10^{-5}$, 10^{-6} and 10^{-7} cm²/sec when a typical solvent for TLC is used; $\lambda = 30$ dyne/cm $= 30$ g/sec², $\eta = 5 \cdot 10^{-3}$ poise $= 5 \cdot 10^{-3}$ g/cm·sec, $\cos\theta = 1$. In this case, $V_1 = 0.4$, $\Delta d_p = 0.5\, d_p$ and $0.1\, d_p$. Table II shows the results obtained.

As can be seen from Table II, the HETP passes through a maximum and, when

TABLE II

CALCULATION OF $H_\perp$ (cm) ACCORDING TO EQN. 31

d_p (cm)	D_m (cm²/sec)					
	10^{-5}		10^{-6}		10^{-7}	
	$\Delta d_p = 0.5\, \bar{d}_p$	$\Delta d_p = 0.1\, \bar{d}_p$	$\Delta d_p = 0.5\, \bar{d}_p$	$\Delta d_p = 0.1\, \bar{d}_p$	$\Delta d_p = 0.5\, \bar{d}_p$	$\Delta d_p = 0.1\, \bar{d}_p$
$3 \cdot 10^{-3}$	$1.3 \cdot 10^{-1}$	$5.2 \cdot 10^{-1}$	$1.2 \cdot 10^{-1}$	$4.8 \cdot 10^{-3}$	$1.3 \cdot 10^{-1}$	$5.1 \cdot 10^{-3}$
10^{-3}	$8.4 \cdot 10^{-2}$	$3.7 \cdot 10^{-3}$	$4.3 \cdot 10^{-1}$	$1.7 \cdot 10^{-2}$	$4 \cdot 10^{-1}$	$1.6 \cdot 10^{-2}$
$7 \cdot 10^{-4}$	$6.7 \cdot 10^{-2}$	$2.7 \cdot 10^{-3}$	$3.2 \cdot 10^{-1}$	$1.3 \cdot 10^{-2}$	$5.5 \cdot 10^{-1}$	$2.2 \cdot 10^{-2}$
$3 \cdot 10^{-4}$	$2.9 \cdot 10^{-2}$	$1.5 \cdot 10^{-3}$	$2.5 \cdot 10^{-1}$	$1 \cdot 10^{-2}$	1.3	$5.2 \cdot 10^{-2}$
10^{-4}	$1.1 \cdot 10^{-2}$	$4.4 \cdot 10^{-4}$	$9.5 \cdot 10^{-2}$	$3.8 \cdot 10^{-3}$	$8.4 \cdot 10^{-1}$	$3.4 \cdot 10^{-2}$

D_m increases, the position of the maximum shifts to higher values of d_p. The table shows clearly that in order to decrease H for the TLC of low-molecular-weight compounds ($D_m = 10^{-5}$ cm²/sec), one should use plates covered with a fine-grain absorbent ($d_p = 10^{-4}$ cm), whereas for TLC of high-molecular-weight compounds ($D_m = 10^{-7}$ cm²/sec) the absorbent should be coarse-grained ($d_p \geqslant 3 \cdot 10^{-3}$ cm).

It is of interest to compare the value of $\overline{H}_{\perp}$ with other terms of HETP for TLC that are related to molecular diffusion ($\overline{H}_M$), coupling eddy diffusion ($\overline{H}_{CED}$) and internal mass transfer ($\overline{H}_{MT}$):

$$\overline{H} = \overline{H}_M + \overline{H}_{CED} + \overline{H}_{\perp} + \overline{H}_{MT} \tag{33}$$

A term related to adsorption kinetics and external mass transfer has been omitted from eqn. 33. As Giddings[15] points out, this term is a small fraction of $\overline{H}_M$ under the usual conditions of TLC ($R_s = 0.1$–0.9 when absorbents with energetically homogeneous surfaces are used).

According to Giddings[15] and in agreement with our previous work[1], we will select the expressions for the terms of HETP in eqn. 33 for TLC on dry plates when the solvent movement is due to capillary forces. Thus, when the spreading is induced only by molecular diffusion, $H_M(R_s)$, the HETP will be described by the expression

$$H_M(R_s) = \frac{2\,\gamma D R_s}{\xi\, d_p} \tag{34}$$

and the mean value of $\overline{H}_M$ for a chromatographic layer over the range from R_{s0} to R_s ($\Delta R_s = R_s - R_{s0}$) is given by

$$\overline{H}_M = \frac{\gamma\,(R_s^2 - R_{s0}^2)\,D_m}{\xi\,\Delta R_s\, d_p} \tag{35}$$

The corresponding values of HETP for coupling eddy diffusion (in the range of one grain) are given by

$$H_{CED}(R_s) = (1/2\lambda' d_p + D_m R_s/\omega d_p^3 \xi)^{-1} \tag{36}$$

$$\overline{H}_{CED} = \frac{\omega \xi d_p^3}{\Delta R_s D_m} \ln \left[\frac{R_s + (\omega \xi d_p^2/2\lambda' D_m)}{R_{s_0} + (\omega \xi d_p^2/2\lambda' D_m)}\right] \tag{37}$$

where ω and λ' are the structural factors. For coupling eddy diffusion in the range of one absorbent grain, ω is equal to λ' and is 0.5 (ref. 5). For intradiffusion mass transfer, we can use the equation obtained by Hawkes[16] for an absorbing spherical particle:

$$H_{MT} = q(1 - R_F)R_F d_p^3 \xi/(L\gamma_p D_m) \tag{38}$$

$$\overline{H}_{MT} = q(1 - R_F)R_F \xi \ln (R_s/R_{s0}) d_p^3/(\gamma_p D_m \Delta R_s) \tag{39}$$

where q is the structural factor:

$$q = [1 + K'(1 - \beta)]/30 \tag{40}$$

where β is the part of the solvent outside the adsorbent pores:

$$\beta = 1/[1 + (1 - V_1)\,0.8/V_1]$$

K' is the ratio of the mass of the adsorbed substance to that of the unadsorbed substance:

$$K' = [(1 - R_F)/R_F][V_1/(1 - V_1)\,0.8] - 1$$

and γ_p is the lag factor (according to our measurements, $\gamma_p = 0.05$).

Using eqns. 35, 37 and 39 and the above values of the terms in these equations, we calculated the values of $\bar{H}_M$, $\bar{H}_{CED}$ and $\bar{H}_{MT}$. The results are plotted in Fig. 9, which also shows the dependence of single terms of the chromatographic spread and of the total HETP on the grain diameter in the TLC of substances with diffusion coef-

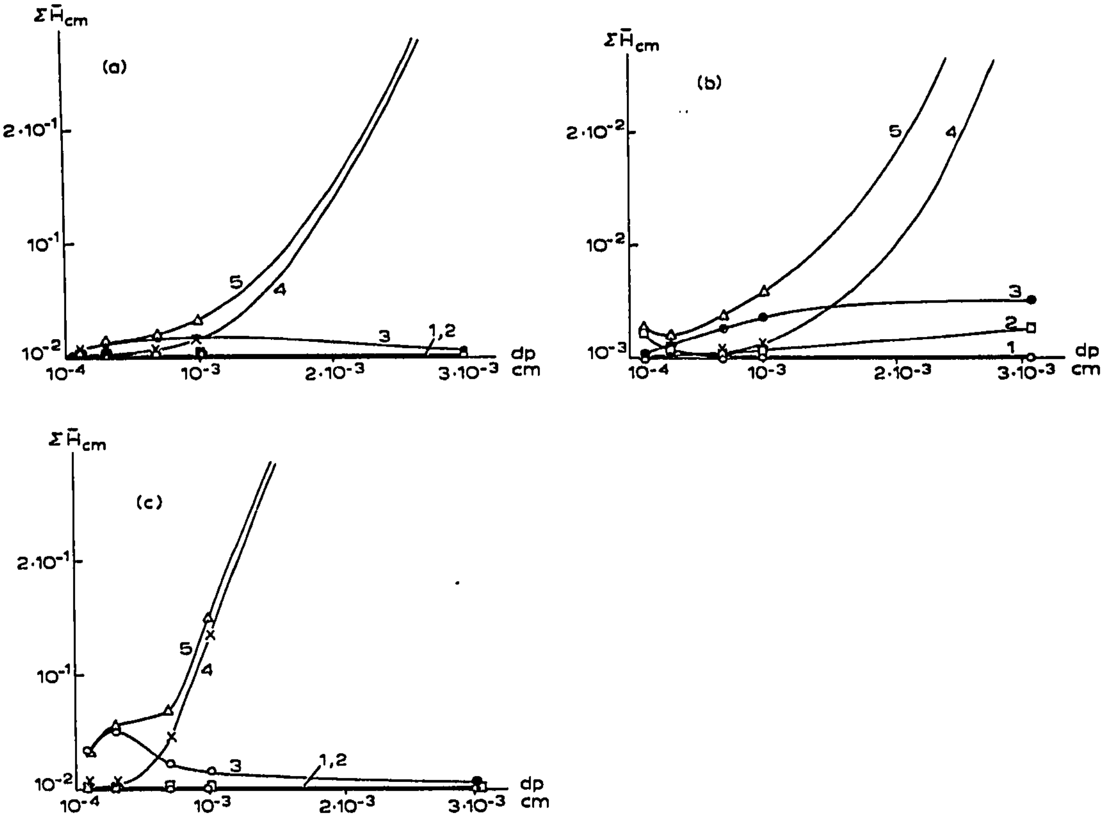

Fig. 9. Calculated curves for HETP related to molecular diffusion, $\bar{H}_M$ (curve 1), coupling eddy diffusion, $\bar{H}_{CED}$ (curve 2), inter-layer effect, $\bar{H}_{\perp}$ (curve 3), internal mass transfer, $\bar{H}_{MT}$ (curve 4) and total HETP, $\Sigma\bar{H}$ (curve 5), for different variants of TLC on dry plates [$u = u(R_s)$]. $R_{s0} = 1$ cm; $R_s = 6$ cm; $\gamma = 0.6$; $\gamma_p = 0.05$; $\omega = \lambda' = 0.5$; $V_1 = 0.4$; $\lambda = 30$ dyn/cm; $\eta = 5 \cdot 10^{-3}$ poise; cos $\theta = 1$; $R_F = 0.3$; $\Delta d_p = 0.1$. (a) $D_m = 10^{-5}$ cm²/sec; (b) $D_m = 10^{-6}$ cm²/sec; (c) $D_m = 10^{-7}$ cm²/sec.

ficients, D_m, of 10^{-5}, 10^{-6} and 10^{-7} cm^2/sec. The peculiarity of the curves in Fig. 9 is that the HETP decreases sharply in passing from $d_p = 3 \cdot 10^{-3}$–10^{-3} cm, which is associated with a decrease in the mass transfer term H_{MT} in this range. The range of minimum HETP values (at $d_p = 1 \cdot 10^{-4}$–$7 \cdot 10^{-4}$ cm) depends on the diffusion coefficient of the compounds undergoing chromatography and is $2 \cdot 10^{-3}$, $4 \cdot 10^{-3}$ and $2 \cdot 10^{-2}$ cm for $D_m = 10^{-5}$, 10^{-6} and 10^{-7} cm^2/sec, respectively. It should be noted that a very flat minimum in the HETP value is observed for $d_p = 3 \cdot 10^{-3}$ cm in the TLC of substances with $D_m = 10^{-5}$ cm^2/sec. For substances with $D_m = 10^{-6}$–10^{-7} cm^2/sec, this minimum lies at $d_p < 10^{-4}$ cm. These relationships are valid for the case of a relatively homogeneous layer ($\Delta d_p = 0.1\ d_p$) and, consequently, for weak inter-layer spreading. When the inhomogeneity of the layer increases, *e.g.*, up to $\Delta d_p = 0.5\ d_p$ (the corresponding values of $\bar{H}_{\perp}$ are shown in Fig. 9 with a dashed line), these relationships cannot be observed as the HETP value related to the inter-layer effect passes through a maximum that shifts to high d_p values when the diffusion coefficient, D_m, increases (see also Table II).

Hence, it can be concluded that the most effective method for increasing the efficiency of TLC, in particular for low-molecular-weight compounds ($D_m = 10^{-5}$ cm^2/sec), is the preparation of plates with well (regularly) packed adsorbent layers. When such plates are used, the size of the adsorbent grains should be $d_p = 1 \cdot 10^{-4}$–$7 \cdot 10^{-4}$ cm. The decrease in the grain size in this range leads to an increase in the efficiency of TLC (for $D_m = 10^{-5}$ cm^2/sec, the maximum efficiency corresponds to $d_p = 3 \cdot 10^{-4}$ cm). However, the rate of development drops simultaneously. As follows from eqn. 5, this rate is proportional to the grain diameter, *i.e.*, for routine analysis with the aid of TLC an adsorbent with $d_p = 7 \cdot 10^{-4}$ cm is appropriate and for highly efficient TLC an adsorbent with $d_p = 1 \cdot 10^{-4}$–$3 \cdot 10^{-4}$ cm (depending on D_m for the substances undergoing chromatography).

A further possible means of increasing the effectiveness of TLC is to decrease the elution rate; this is particularly advisable for the TLC of high-molecular-weight compounds (with $D_m = 10^{-7}$ cm^2/sec). The decrease in the elution rate reduces the terms related to mass transfer and eddy diffusion (including the inter-layer effect) and increases the term determined by molecular diffusion. The latter effect is not significant in the TLC of high-molecular-weight compounds, whereas the two former factors determine completely the HETP. A decrease in the elution rate with constant d_p can be achieved by using TLC. In this case, the flow-rate of the solvent is not determined by capillary forces but is governed by the hydrostatic pressure of the liquid in the channels of the layer, which is easily controlled by changing the slope of the plate.

As Fig. 9 shows, our calculated data are in fairly good agreement with experimental results for different variants of TLC.

These results show that, depending on the conditions of chromatography (d_p, D_m) used, the importance of the terms of spreading in the total HETP varies. Consequently, we cannot agree with Snyder's opinion[17] that eddy diffusion is the main contribution to HETP in TLC. If the eddy diffusion is understood to be a process that occurs within the range of one grain, H_{CED}, Snyder's statement is not valid, as is shown in Table III. But if we include the inter-layer effect in the eddy diffusion, the spreading associated with the rate profile occurs in TLC only when badly prepared plates are used (made of an inhomogeneous absorbent with sedimentational layer packing).

It is characteristic that Snyder[18] estimates the HETP in TLC to be 10^{-2} cm (100 μm), whereas the HETP in micro-TLC[6] is $5 \cdot 10^{-4}$–10^{-3} cm (5–10 μm) when well made plates are used ($\Delta d_p/d_p \rightarrow 0$). This ensures an effectiveness of 2–3 ETP/sec in the separation of DHS-amino acids, which is comparable with the effectiveness of high-speed column chromatography[18]. (Incidentally, Snyder[18] estimates the effectiveness of TLC as 0.05 ETP/sec.)

APPENDIX

The initial differential equation and the corresponding initial and boundary conditions are shown in eqns. 8, 9 and 11. Applying to these equations a Laplace transformation for the variable x according to the equation

$$y(s,z,t) = \int_{-\infty}^{+\infty} C(x,z,t)\, e^{-sx} dx \tag{A1}$$

we obtain

$$\frac{\partial y}{\partial t} + R_F u(z,t) s y = R_F D_\perp \cdot \frac{\partial^2 y}{\partial z^2} \tag{A2}$$

$$y \mid_{t=0} = q/h_0. \tag{A3}$$

$$(\partial y/\partial z) \mid_{z=0,h_0} = 0. \tag{A4}$$

Comparing eqns. A1 and 14, it can be seen that

$$m_0 = \left(\int_0^{h_0} y \mid_{s=0} dz\right)/h_0 \tag{A5}$$

$$m_1 = \left(-\int_0^{h_0} (\partial y/\partial s) \mid_{s=0} dz\right)/h_0 \tag{A6}$$

$$m_2 = \left(\int_0^{h_0} (\partial^2 y/\partial s^2) \mid_{s=0} dz\right)/h_0 \tag{A7}$$

Thus, finding the functions $y \mid_{s=0} = y_0$, $(\partial y/\partial s) \mid_{s=0} = y_1$, $(\partial^2 y/\partial s^2) \mid_{s=0} = y_2$, we can determine the necessary moments and dispersion from eqn. 13.

Assuming in eqn. A2 that $s = 0$, we obtain

$$\frac{\partial y_0}{\partial t} = R_F D_\perp \cdot \frac{\partial^2 y_0}{\partial z^2} \tag{A8}$$

Under conditions for y_0 analogous to eqns. A3 and A4, eqn. A8 is solved by a standard method and is written as

$$y_0 = q/h_0 \tag{A9}$$

We will differentiate eqns. A2–A4 with respect to s and then, assuming $s = 0$ and taking into account eqn. A9, we obtain

$$\frac{\partial y_1}{\partial t} + \frac{R_F q}{h_0} \cdot u(z,t) = R_F D_\perp \cdot \frac{\partial^2 y_1}{\partial z^2} \tag{A10}$$

$$y_1 \mid_{t=0} = 0 \tag{A11}$$

$$(\partial y_1/\partial z) \mid_{z=0,h_0} = 0 \tag{A12}$$

Under the conditions of eqns. A11 and A12, the solution of eqn. A10 is given by

$$y_1(z,t) = \sum_{n=0}^{\infty} A_n(t) \cos\left(\frac{\pi n z}{h_0}\right) \tag{A13}$$

where cos $(\pi n z/h_0)$ are the normalized fundamental functions of the operator $R_F D \cdot (\partial^2/\partial z^2)$. It should be remembered that, according to eqn. 16, $u(z,t)$ is equal to $\tau(t)\omega(z)$. The function $\omega(z)$ can be expanded into a Fourier series:

$$\omega(z) = \frac{\omega_0}{2} + \sum_{n=1}^{\infty} \omega_n \cos\left(\frac{\pi n z}{h_0}\right) \tag{A14}$$

where ω_n is determined from eqn. 18.

Substitution of eqns. A13 and A14 into eqn. A10 gives

$$\frac{\mathrm{d}}{\mathrm{d}t}[A_n(t)] + R_F D_{\perp} \left(\frac{n\pi}{h_0}\right)^2 A_n(t) + \frac{R_F q}{h_0} \omega_n \tau(t) \tag{A15}$$

It follows from eqn. A11 that

$$A_n \mid_{t=0} = 0 \tag{A16}$$

In order to solve eqn. A15 under the conditions of eqn. A16, a Laplace transformation for t is introduced:

$$\tilde{A}_n(s) = \int_0^{\infty} A_n(t) \mathrm{e}^{-st} \mathrm{d}t \tag{A17}$$

Applying eqn. A17 to eqn. A15, we obtain

$$\tilde{A}_n(s) = -\frac{R_F q}{h_0} \cdot \omega_n \cdot \frac{\theta(s)}{s + R_F D_{\perp}(n\pi/h_0)^2} \tag{A18}$$

where

$$\theta(s) = \int_0^{\infty} \tau(t)\, \mathrm{e}^{-st}\, \mathrm{d}t \tag{A19}$$

It then follows that the original term $A_n(t)$ is given by

$$A_n(t) = -\frac{R_F q \omega_n}{h_0} \cdot \exp\left\{-R_F D_{\perp}\left(\frac{n\pi}{h_0}\right)^2 t\right\} \int_0^t \tau(t') \exp\left\{R_F D_{\perp}\left(\frac{n\pi}{h_0}\right)^2 t'\right\} \mathrm{d}t' \tag{A20}$$

Substitution of eqn. A20 into eqn. A13 gives

$$y_1(z,t) = -\frac{R_F q\, \omega_0}{2h_0} \int_0^t \tau(t')\, \mathrm{d}t' - \int \frac{R_F q}{h_0} \sum_{n=1}^{\infty} \left[\omega_n \exp\left\{-R_F D_{\perp}\left(\frac{n\pi}{h_0}\right)^2 t\right\} \cdot \int_0^t \tau(t') \exp\left\{R_F D_{\perp}\left(\frac{n\pi}{h_0}\right)^2 t'\right\} \mathrm{d}t'\right] \cos\left(\frac{n\pi z}{h_0}\right) \tag{A21}$$

After differentiating eqns. A2–A4 twice with respect to s and assuming $s = 0$, we obtain

$$\frac{\partial y_2}{\partial t} + 2\, R_F u(z,t) y_1 = R_F D_\perp \cdot \frac{\partial^2 y_2}{\partial z^2} \tag{A22}$$

$$y_2 \mid_{t=0} = 0 \tag{A23}$$

$$(\partial y_2/\partial z) \mid_{z=0,\, h_0} = 0 \tag{A24}$$

It is possible to find m_2 without solving eqn. A22 in order to find y_2.

In fact, multiplying both sides of eqn. A22 by $1/h_2$, integrating over z from 0 to h_0 and taking into account eqn. A24, we obtain

$$m_2 = -\frac{2\, R_F}{h_0} \int_0^t \int_0^{h_0} u(z,t') y_1(z,t') \mathrm{d}z \mathrm{d}t' \tag{A25}$$

Substituting eqn. A21 into eqns. A6 and A25 and carrying out calculations according to eqn. 13, we finally obtain

$$\sigma^2 = \frac{R_F^2 \omega_0^2}{2} \left\{ \int_0^t \tau(t'') \int_0^{t''} \tau(t') \mathrm{d}t' \mathrm{d}t'' - \frac{1}{2} \left[\int_0^t \tau(t') \mathrm{d}t' \right]^2 \right\} +$$
$$+ R_F^2 \sum_{n=1}^{\infty} \omega_n^2 \int_0^t \tau(t'') \mathrm{e}^{-R_F D_\perp \left(\frac{n\pi}{h_0}\right)^2 t''} \int_0^{t''} \tau(t') \mathrm{e}^{R_F D_\perp \left(\frac{n\pi}{h_0}\right)^2 t'} \mathrm{d}t' \mathrm{d}t'' \tag{A26}$$

We give below some particular types of eqn. A26.

For a model expressed by eqns. 19 and 20, direct substitution of these equations into eqn. A26 gives

$$\sigma^2 = \frac{2\, R_F^2 h_0^2 \nu^2 (1-\nu)^2}{3\, D_\perp} \cdot (\Delta u)^2 t \tag{A27}$$

One can use the equality

$$\sum_{n=1}^{\infty} \frac{\sin^2(n\nu\pi)}{n^4} = \frac{\pi^4}{6} \cdot \nu^2 (1-\nu)^2 \tag{A28}$$

For a parabolic inter-layer rate gradient,

$$\bar{u}_t(z) = a_0 + a_1 z + a_2 z^2 \tag{A29}$$

we find

$$\sigma^2 = \frac{R_F h^2}{60\, D_\perp} [\bar{u}_t(h_0) - \bar{u}_t(0)]\, t \tag{A30}$$

For solving eqn. A30, the following ratios were also used:

$$\sum_{n=1}^{\infty} \frac{1}{n^6} = \frac{\pi^6}{945} \text{ and } \sum_{n=1}^{\infty} \frac{1}{(2n-1)^6} = \frac{\pi^6}{960} \tag{A31}$$

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