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## Solution of Boundary Spreading Equations for Electrophoresis and the Velocity Ultracentrifuge

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By direct solution of the boundary spreading equations for ideal electrophoresis or velocity ultracentrifuge experiments, respectively, expressions are obtained relating the experimentally measured refractive index gradient curves to the mobility or sedimentation constant distributions in the sample. Discussion of the distribution functions and derivation of the boundary spreading equations are included, together with the conditions which must be satisfied for the spreading to be ideal. It is shown that the correct distribution of mobilities or sedimentation constants may be obtained by an appropriate extrapolation method regardless of whether all molecules have the same diffusion constant.

The heterogeneity of proteins or of other high molecular weight substances with respect to sedimentation constant or electrophoretic mobility has been examined, in a semi-quantitative way, by comparing the spreading of experimental concentration gradient curves with that expected from diffusion alone.<sup>1-4</sup> In experiments where spreading by diffusion was negligible, interpretation of the observed spreading was simplified, and for this case heterogeneity has been measured by ratios of curve areas to curve heights,<sup>5,6</sup> by the second moment of the mobility distribution curve,<sup>7</sup> and by the actual distribution of sedimentation constants.<sup>8-10</sup> By assuming a Gaussian distribution of mobilities Alberty<sup>11</sup> solved the electrophoretic boundary spreading equation of Sharp, *et al.*,<sup>7</sup> to obtain both the mobility distribution and diffusion coefficient, while Brown and Cann<sup>12</sup> developed a general solution for any mobility distribution in terms of Hermite polynomials and higher moments of concentration gradient curves. Both of these solutions for mobility distributions assumed the solute to be homogeneous with respect to diffusion coefficient.

Recent work in this Laboratory has shown that the distribution of mobilities<sup>13</sup> or sedimentation constants<sup>14,15</sup> in a protein sample may be obtained by extrapolation of an "apparent" distribution to infinite time. In this way the spreading due to

diffusion, which depends on the square root of the time, becomes negligible compared to spreading by the electrical or centrifugal field, which varies with the first power of the time. The following development provides additional theoretical support for this extrapolation procedure and also shows it to be valid when the sample contains a distribution of diffusion coefficients. At the same time it points out limitations in the current procedures and provides correction terms and some alternative methods of calculation.

Because of the increased difficulty of handling equations in which the diffusion coefficient, sedimentation coefficient or electrophoretic mobility are allowed to vary with solute concentration, the effect of these variations will be left for further research. Consequently the following results apply rigorously only to sedimentation or electrophoresis experiments which satisfy the criteria of ideal spreading as defined below. In non-ideal experiments it may be possible to obtain the correct sedimentation constant or mobility distribution curves by extrapolation to infinite dilution of solute while the composition of the solvent, including any buffer salts, is held constant.

### Definition of the Distribution Functions

It has been customary<sup>7,11</sup> to assume that all molecules of the solute possess the same diffusion constant,  $D$ , and then to represent the distribution of mobilities by a function  $g(U)$  where

$$f_U = g(U)dU \quad (1)$$

is that fraction of the sample having mobility  $U$  or having mobilities between  $U$  and  $U + dU$  and

$$\int_{-\infty}^{\infty} g(U) dU = 1 \quad (2)$$

Analogous relations define the sedimentation constant distribution function,  $q(S)$ , except [we consider only positive values of the sedimentation constant,  $S$ ,<sup>16</sup> so that

$$\int_0^{\infty} q(S) dS = 1 \quad (3)$$

(1) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940, p. 287.

(2) A. Tiselius, *Nova Acta Reg. Soc. Sci. Upsala*, [IV] **7**, No. 4 (1930).

(3) A. M. Pappenheimer, H. P. Lundgren and J. W. Williams, *J. Exp. Med.*, **71**, 247 (1940).

(4) M. A. Lauffer, *J. Biol. Chem.*, **143**, 99 (1942).

(5) A. Tiselius and F. L. Horsfall, *Arkiv Kemi, Mineral, Geol.*, **13A**, No. 18 (1939).

(6) N. Gralen, Dissertation, Upsala, 1944.

(7) D. G. Sharp, M. H. Hebb, A. R. Taylor and J. W. Beard, *J. Biol. Chem.*, **142**, 217 (1942).

(8) R. Signer and H. Gross, *Helv. Chim. Acta*, **17**, 726 (1934).

(9) W. Bridgman, *THIS JOURNAL*, **64**, 2349 (1942).

(10) I. Jullander, *Arkiv. Kemi, Mineral, Geol.*, **21A**, No. 8 (1945).

(11) R. A. Alberty, *THIS JOURNAL*, **70**, 1675 (1948).

(12) R. A. Brown and J. R. Cann, *J. Phys. Colloid Chem.*, **54**, 364 (1950).

(13) R. L. Baldwin, P. M. Laughton and R. A. Alberty, *ibid.*, **55**, 111 (1951).

(14) R. L. Baldwin, J. W. Williams, *THIS JOURNAL*, **72**, 4325 (1950).

(15) J. W. Williams, R. L. Baldwin, W. M. Saunders and P. G. Squire, *ibid.*, **74**, 1542 (1952).

(16) Here  $S$  is the variable of integration, not necessarily expressed in Svedberg units. The symbol  $s$  is reserved to denote a particular sedimentation constant corresponding to a given position in the cell at a given time (equation (33)).

to avoid the problem of boundaries sedimenting in opposite directions.

In general, molecules having different mobilities or sedimentation constants will have different diffusion constants, and there may also be a range of diffusion constants among molecules having the same mobility or sedimentation constant. Consequently, we define a mobility-diffusion constant distribution function,  $g(U, D)$ , by

$$f_{U,D} = g(U, D) dD dU \quad (4)$$

and

$$\int_{-\infty}^{\infty} \int_0^{\infty} g(U, D) dD dU = 1 \quad (5)$$

where  $f_{U,D}$  is that fraction of the solute having mobilities between  $U$  and  $U + dU$  and diffusion constants between  $D$  and  $D + dD$ . An analogous distribution function,  $q(S, D)$  is defined for sedimentation velocity by considering only positive values of  $S$ . Visualization of these functions may be aided by Fig. 1, which shows the two surfaces for a hypothetical protein.<sup>17</sup>

It is seen that the fraction of material, irrespective of diffusion constant, with mobilities between  $U$  and  $U + dU$  is  $G(U) dU$  where

$$G(U) = \int_0^{\infty} g(U, D) dD \quad (6)$$

and the fraction of material with sedimentation constants between  $S$  and  $S + dS$  is  $Q(S) dS$  where

$$Q(S) = \int_0^{\infty} q(S, D) dD \quad (7)$$

Thus  $G(U)$  and  $Q(S)$  are equivalent to  $g(U)$  and  $q(S)$  except that they represent distributions for samples in which  $D$  is variable.

The differential equation relating the concentration gradient curve to the distribution functions will now be derived in a form which may be applied subsequently to either electrophoresis or sedimentation velocity. This makes possible a more straightforward and rigorous treatment than may be made by attempting to derive a spreading equation for sedimentation from the corresponding equation for electrophoresis.<sup>7,11</sup> Furthermore, it will then be possible to delineate the meaning of "fraction" as used in defining the distribution functions. While equations in the next paragraph are derived for spreading in the velocity ultracentrifuge, the development for electrophoretic spreading is analogous. It should be remembered that the distribution of sedimentation constants or mobilities may not be continuous on a microscopic scale.

(17) The function  $g(U, D)$  is always positive and approaches zero as  $D \rightarrow 0$ ,  $D \rightarrow \infty$ , or  $U \rightarrow \pm \infty$ . In addition to restrictions of this sort  $q(S, D)$  must also be zero in the region where  $S$  and  $D$  are large. The approximate boundary of this forbidden region may be obtained by expressing both  $S$  and  $D$  in terms of the frictional ratio,  $(f/f_0) \geq 1$ , and eliminating the molecular weight,  $M$ , instead of  $(f/f_0)$ , between the two equations to give

$$SD^2 = \frac{R^2 T^2}{162 \pi^2 \bar{v}_2 \eta^3} (1 - \bar{v}_2 \rho) (f/f_0)^{-3}$$

Here  $R$  is the gas constant,  $T$  is the absolute temperature,  $\eta$  and  $\rho$  represent the viscosity and density, respectively, of the solution, and the (approximately constant) partial specific volume of the solute molecules is denoted by  $\bar{v}_2$ . It will be noted that straight lines of constant molecular weight radiate from the origin into the  $S, D$  plane.

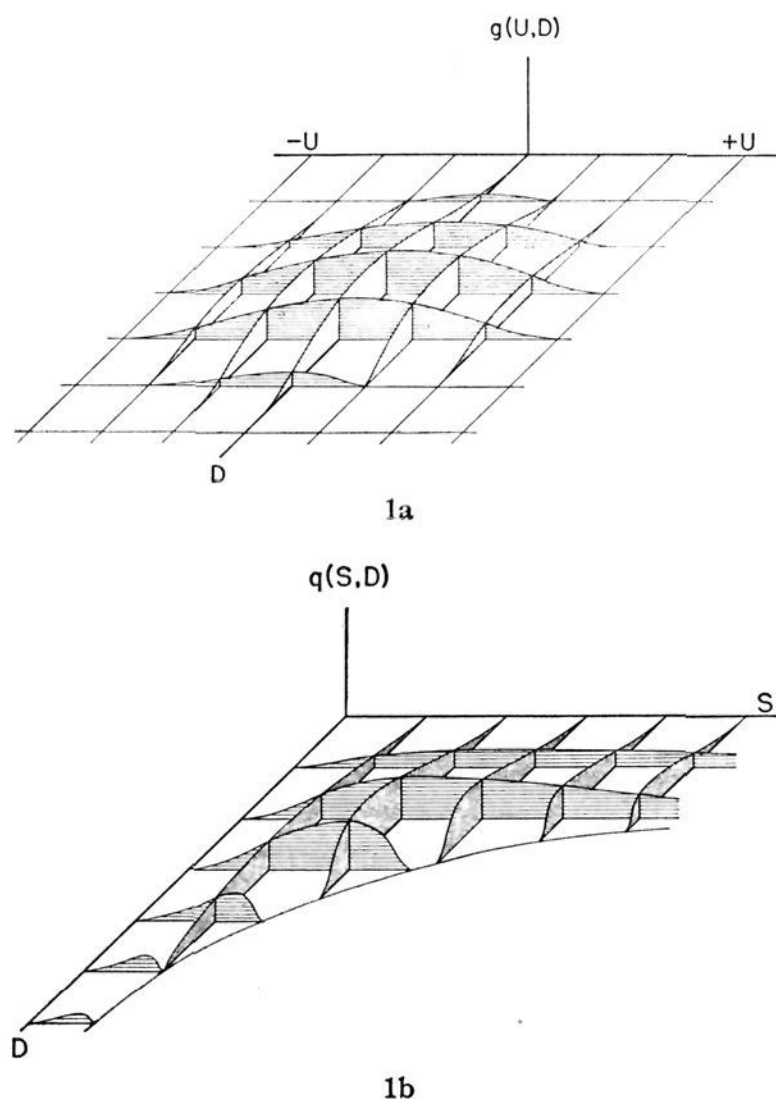


Fig. 1.—Surfaces representing (a) the mobility-diffusion constant distribution function,  $g(U, D)$ , and (b) the sedimentation constant-diffusion constant distribution function,  $q(S, D)$ , for a hypothetical protein.

Denote the total solute concentration in the cell at level  $x$  by  $C$  and the concentration at  $x$  of those molecules of solute with sedimentation constant  $S$  by  $c_s$ . At the start of the experiment  $C = c_s = 0$  on one side of the boundary or meniscus, while on the other  $C = C_0$  and  $c_s = (c_0)_s = f_s C_0$ . Expressing all concentrations as weight of solute per unit volume of solution and defining  $f_s$  as weight fraction of the total solute having sedimentation constant  $S$ , we write the concentration gradient expression

$$\frac{\partial C}{\partial x} = \sum_S \frac{\partial c_s}{\partial x} = C_0 \sum_S f_s \frac{\partial (c/c_0)_s}{\partial x} \quad (8)$$

where  $(c/c_0)_s$  at level  $x$  is the concentration relative to the concentration of that species in the original solution, and we have temporarily assumed that all species have the same diffusion constant. If the distribution of sedimentation constants is continuous,  $f_s$  may be replaced by  $q_w(S) dS$ , the weight fraction of molecules having sedimentation constants between  $S$  and  $S + dS$ , so

$$\frac{\partial C}{\partial x} = C_0 \int_0^{\infty} q_w(S) \frac{\partial (c/c_0)_s}{\partial x} dS \quad (9)$$

Even if the distribution of sedimentation constants is not continuous, diffusion smooths the  $\partial C/\partial x$  curve until it and all of its derivatives are continuous within the error of measurement. Consequently, we will obtain from experiment

smooth distribution curves which closely approximate any step-like or discontinuous distributions.

Since refractive index gradients,  $\partial n/\partial x$ , are ordinarily measured instead of concentration gradients, the distribution function  $q_n(S)$ , based on the specific refractive increments,  $k_S = \partial n/\partial c_S$ , is defined by

$$\frac{\partial n}{\partial x} = (n_s - n_0) \int_0^\infty q_n(S) \frac{\partial(c/c_0)_S}{\partial x} dS \quad (10)$$

where  $(n_s - n_0)$  is the refractive index difference between the starting solution and solvent. Providing only that each  $k_S$  is independent of the concentration,  $c_S$ , of every species, it may be shown from equations (8), (9) and (10) that  $q_n(S)$  is a well defined distribution function and related to  $q_w(S)$  by

$$q_n(S) = k_S \frac{\sum_S (c_0)_S}{\sum_S k_S (c_0)_S} q_w(S) \quad (11)$$

If  $k_S$  is the same for every species it is seen that  $q_n(S) = q_w(S)$  and the sedimentation constant distribution function on a weight basis is obtained directly from refractive index measurements.

Analogous relations hold for  $g_n(U)$ ,  $q_n(S, D)$  and  $g_n(U, D)$ , and when  $D$  is not constant we have, for example

$$\frac{\partial n}{\partial x} = (n_s - n_0) \int_{-\infty}^\infty \int_0^\infty g_n(U, D) \frac{\partial(c/c_0)_{U, D}}{\partial x} dD dU \quad (12)$$

instead of equation (10).

### Boundary Spreading during Electrophoresis

Experimental precautions which must be observed to ensure that electrophoretic spreading is ideal, *i.e.*, due only to the distribution of mobilities and to diffusion, have been investigated in detail.<sup>11,13,18,19</sup> The equivalent mathematical requirements are that the electric field strength,  $E$ , and the mobility,  $U$ , and diffusion coefficient,  $D$ , of each species remain constant throughout the cell during the experiment.

Subject to these restrictions, the flow equation

$$J_{U, D} = -D \frac{\partial c_{U, D}}{\partial x} + EUc_{U, D} \quad (13)$$

for the weight,  $J_{U, D}$ , of solute with mobility  $U$  and diffusion constant  $D$  crossing a unit area per second may be substituted into the equation of continuity

$$\frac{\partial c_{U, D}}{\partial t} = - \frac{\partial J_{U, D}}{\partial x} \quad (14)$$

for a cell of uniform cross section to yield the differential equation for ideal electrophoretic boundary spreading. Its solution for the relative concentration is readily obtained by assuming that  $(c/c_0)_{U, D}$  is a function only of

$$\lambda = (x - UEt_E)/\sqrt{t_D}^{20}$$

Thus

$$\frac{\partial(c/c_0)_{U, D}}{\partial x} = \frac{1}{2\sqrt{\pi D t_D}} e^{-\frac{(x-UEt_E)^2}{4Dt_D}} \quad (15)$$

(18) R. A. Albery, E. A. Anderson and J. W. Williams, *J. Phys. Colloid Chem.*, **52**, 217 (1948).

(19) E. A. Anderson and R. A. Albery, *ibid.*, **52**, 1345 (1948).

(20) This is analogous to solution of the differential equation for diffusion, alone, by the substitution  $c = c(x/\sqrt{t})$ .

providing the starting boundary is infinitely sharp and located at height  $x = 0$  at time  $t_D = 0$ . The time of electrophoresis,  $t_E$ , is distinguished from the time of diffusion,  $t_D$ , because the electric field is usually applied after some diffusion has occurred.<sup>11</sup> Assuming for the present that all molecules have the same diffusion constant, we may rewrite equation (10) using mobilities instead of sedimentation constants and substitute the relative concentration gradient from equation (15) to obtain the customary electrophoretic boundary spreading equation.<sup>7,11</sup>

$$\frac{\partial n}{\partial x} = \frac{(n_s - n_0)}{2\sqrt{\pi D t_D}} \int_{-\infty}^\infty g_n(U) e^{-\frac{(x-UEt_E)^2}{4Dt_D}} dU \quad (16)$$

Integration of this equation is carried out by observing that at long times the exponential becomes a sharply peaked function of  $U$  about  $EUt_E$ , so any variation of  $g_n(U)$  during the integration is adequately given by its Taylor expansion

$$g_n(U) = \sum_{j=0,1,2,\dots} \frac{(U-u)^j}{j!} \left[ \frac{d^j g_n(U)}{dU^j} \right]_{U=u} \quad (17)$$

where

$$u = x/(Et_E) \quad (18)$$

is the mobility corresponding to a particular value of  $x$  at time  $t_E$ . Using  $(U-u)$  as the variable of integration, we obtain the final result

$$g_n^*(u) = \frac{Et_E(\partial n/\partial x)_u}{(n_s - n_0)} = \sum_{k=0,1,2,\dots} \frac{1}{k!} \left( \frac{Dt_D}{E^2 t_E^2} \right)^k \frac{d^{2k} g_n(u)}{du^{2k}} \quad (19)$$

Thus the current procedure of plotting  $g_n^*(u)$  versus  $1/t$  to obtain  $g_n(u)$  from the intercept<sup>13</sup> is seen to be completely rigorous, though straight lines will be obtained only if  $t_D = t_E$  and if the times are sufficiently long so that terms beyond the second (*i.e.*,  $k \geq 2$ ) in the series are negligible. If  $t_E \neq t_D$  a plot of  $g_n^*(u)$  versus  $t_D/t_E^2$  is indicated.

By measuring derivatives of the  $\partial n/\partial x$  versus  $x$  curve it should be possible to determine the  $g_n(u)$  curve from a single photograph providing  $D$  is known, since equation (19) may be inverted to express  $g_n(u)$  as follows.<sup>21</sup>

$$g_n(u) = \frac{Et_E}{(n_s - n_0)} \left[ (\partial n/\partial x)_u - Dt_D \frac{\partial^2(\partial n/\partial x)_u}{\partial x^2} + \frac{(Dt_D)^2}{2!} \frac{\partial^4(\partial n/\partial x)_u}{\partial x^4} - \dots \right] \quad (20)$$

The convergence of this series should be good since at long times  $\partial^{2k}(\partial n/\partial x)_u/\partial x^{2k}$  is nearly proportional to  $1/t_E^{2k+1}$ .

When the solute molecules possess a range of diffusion constants the mobility distribution curve may still be determined by plotting  $G_n^*(u)$  versus  $t_D/t_E^2$ , but the limiting slope is more complicated. For this case we substitute equation (15) in equation (12) to obtain the spreading equation

(21) A method of solving the spreading equation for  $g(u)$  was suggested, but not developed, by Sharp, *et al.*, footnote 3 of ref. (7). Expanding  $\partial n/\partial x$  in their equation as a Taylor series, distinguishing between  $t_E$  and  $t_D$ , and integrating across the saddle point yields the expression

$$g_n(u) = \frac{Et_E}{(n_s - n_0)} \sum_{k=0,1,2,\dots} \frac{(-Dt_D)^k}{k!} \frac{\partial^{2k}(\partial n/\partial x)_u}{\partial x^{2k}}$$

which is seen to be identical with equation (20) above.

$$\frac{\partial n}{\partial x} = \frac{(n_s - n_0)}{2\sqrt{\pi l_D}} \int_{-\infty}^{\infty} \int_0^{\infty} \frac{g_n(U, D)}{\sqrt{D}} e^{-\frac{(x-U E t_E)^2}{4 D t_D}} dD dU \quad (21)$$

Inverting the order of integration and solving by the same procedure used for equation (16) gives

$$G_n^*(u) = \frac{E t_E}{(n_s - n_0)} \left( \frac{\partial n}{\partial x} \right)_u = \sum_{k=0,1,2,\dots} \frac{1}{k!} \left( \frac{l_D}{E^2 t_E^2} \right)^k \int_0^{\infty} D^k \frac{\partial^{2k} g_n(u, D)}{\partial u^{2k}} dD \quad (22)$$

In terms of the different average diffusion constants,  $\overline{D}_u^k$ , of all species having mobility  $u$ , where

$$\overline{D}_u^k = \frac{\int_0^{\infty} D^k g_n(u, D) dD}{\int_0^{\infty} g_n(u, D) dD} \quad (23)$$

the final expression for this case becomes

$$G_n^*(u) = \sum_{k=0,1,2,\dots} \frac{1}{k!} \left( \frac{l_D}{E^2 t_E^2} \right)^k \frac{\partial^{2k} [\overline{D}_u^k G_n(u)]}{\partial u^{2k}} \quad (24)$$

which reduces to equation (19) if  $D$  is the same for all solute molecules.

**Boundary Spreading in the Velocity Ultracentrifuge**

In this case the centrifugal field is proportional to the distance,  $x$ , from the axis of rotation, so the flow equation becomes

$$J_{S,D} = -D \frac{\partial c_{S,D}}{\partial x} + S \omega^2 x c_{S,D} \quad (25)$$

where  $\omega$  is the angular velocity of the rotor and  $J_{S,D}$  is the mass of material with sedimentation constant  $S$  and diffusion constant  $D$  crossing a unit area per second. The cross-sectional area,  $A$ , of the cell is also proportional to  $x$ , so instead of equation (14) the more complicated continuity equation

$$\frac{\partial c_{S,D}}{\partial t} = -\frac{1}{A} \frac{\partial (A J_{S,D})}{\partial x} \quad (26)$$

is required. The time,  $t$ , of sedimentation is considered equal to the time of diffusion with  $t = 0$  when the two processes begin. In practice this may occur slightly before the rotor reaches its operating speed, so a starting time correction should be determined by a suitable extrapolation of the experimental data.<sup>10,15,22</sup>

Combination of equations (25) and (26) leads to a differential equation<sup>23</sup> for the ultracentrifuge which was solved by Faxen<sup>24,25</sup> when  $S$  and  $D$  are both independent of  $x$  and  $t$ . For the case of relatively rapid sedimentation in which the sedimentation and diffusion processes are not disturbed by the meniscus, Faxen's expression for the relative concentration during the early stages of sedimentation (*i.e.*,  $S \omega^2 t \ll 1$ ) may be written<sup>26</sup>

(22) P. G. Squire, M.S. Thesis, University of Wisconsin, 1951.  
 (23) O. Lamm, *Arkiv Mat., Astron., Fysik.*, **21B**, No. 2 (1929).  
 (24) H. Faxen, *ibid.*, **21B**, No. 3 (1929).  
 (25) Ref. 1, p. 22.  
 (26) Faxen computed only the first term, unity, of the series in the second brackets. The above expression was obtained by repeating his development and retaining more terms.

$$\left( \frac{c}{c_0} \right)_{S,D} = \frac{r}{\sqrt{\pi}} \left\{ \frac{\sqrt{\pi}}{2} [1 - \phi(\xi)] + \frac{e^{-\xi^2}}{4w} \left[ 1 - \frac{\xi}{4w} + \frac{(1 + 2\xi^2)}{16w^2} - \frac{(9\xi + 10\xi^3)}{128w^3} + \frac{9 + 36\xi^2 + 28\xi^4}{512w^4} - \dots \right] \right\} \quad (27)$$

providing the boundary is still far from the bottom of the cell. Here

$$r = e^{-2S \omega^2 t} \quad (28)$$

$$\phi(\xi) = (2/\sqrt{\pi}) \int_0^{\xi} e^{-t^2} dt \quad (29)$$

$$w = x \sqrt{\omega^2 S / [2D(e^{2S \omega^2 t} - 1)]} \quad (30)$$

$$\xi = (x_0 e^{S \omega^2 t} - x) \sqrt{\omega^2 S / [2D(e^{2S \omega^2 t} - 1)]} \quad (31)$$

and  $x_0$  is the value of  $x$  at the meniscus. His corresponding expression for long times<sup>27</sup> ( $S \omega^2 t \gg 1$ ) does not converge rapidly enough to be useful in the usual experiment, so we must use equation (27) even though the apparent sedimentation constant distribution function,  $q^*(s)$ , must eventually be extrapolated to infinite time. Differentiation of equation (27) with respect to  $x$  yields the required concentration gradient expression

$$\frac{\partial (c/c_0)_{S,D}}{\partial x} = \frac{r}{\sqrt{\pi}} e^{-\xi^2} \left( -\frac{\partial \xi}{\partial x} \right) \left[ 1 + \frac{\xi}{2w} - \frac{(3 + 2\xi^2)}{16w^2} + \frac{(3\xi + 2\xi^3)}{32w^3} - \frac{(15 + 36\xi^2 + 20\xi^4)}{512w^4} + \dots \right] \quad (32)$$

If all molecules have the same diffusion constant, we expand  $q_n(S)$  as a Taylor series about some sedimentation constant,  $s$ , which is related to a given value of  $x$  at time  $t$  by the expression

$$x = x_0 e^{s \omega^2 t} \quad (33)$$

and use  $v = (S - s) \omega^2 t$  as the variable of integration so equation (10) becomes

$$\frac{\omega^2 t}{(n_s - n_0)} \frac{\partial n}{\partial x} = \int_{-\infty}^{\infty} \frac{\partial (c/c_0)_S}{\partial x} \sum_{j=0,1,2,\dots} \frac{v^j}{j! (\omega^2 t)^j} \frac{d^j q_n(s)}{ds^j} dv \quad (34)$$

Expressing  $r$ ,  $w$  and  $\xi$  in equation (32) as power series in  $v$  allows the integration to be carried out yielding the final expression for this case

$$q_n^*(s) = \frac{x \omega^2 t}{(n_s - n_0) e^{-2s \omega^2 t}} \left( \frac{\partial n}{\partial x} \right)_s = \left[ 1 + \frac{5}{6} \sigma_2 Z^2 + O(Z^4) + \dots \right] q_n(s) - [\sigma_3 + O(Z^2) + \dots] \frac{Z^2}{(\omega^2 t)} \frac{dq_n(s)}{ds} + [1 + 3\sigma_4 Z^2 + O(Z^4) + \dots] \frac{Z^2 \sigma_1}{4(\omega^2 t)^2} \frac{d^2 q_n(s)}{ds^2} - [\sigma_5 + O(Z^2) + \dots] \frac{Z^4 \sigma_1}{4(\omega^2 t)^3} \frac{d^3 q_n(s)}{ds^3} + [1 + O(Z^2) + \dots] \frac{Z^4 \sigma_1^2}{32(\omega^2 t)^4} \frac{d^4 q_n(s)}{ds^4} - \dots \quad (35)$$

where

$$Z^2 = 4Dt/x^2 \quad (36)$$

and

$$\begin{aligned} \sigma_1 &= 1 + (s \omega^2 t) + (2/3)(s \omega^2 t)^2 + 1/3(s \omega^2 t)^3 + \dots \\ &= (e^{2s \omega^2 t} - 1)/(2s \omega^2 t) \\ \sigma_2 &= 1 + (3/5)(s \omega^2 t) + (7/25)(s \omega^2 t)^2 + \dots \\ \sigma_3 &= 1 + (5/6)(s \omega^2 t) + (1/2)(s \omega^2 t)^2 + \dots \\ \sigma_4 &= 1 + (1/3)(s \omega^2 t) + (7/90)(s \omega^2 t)^2 + \dots \\ \sigma_5 &= 1 + (2/3)(s \omega^2 t) + \dots \end{aligned} \quad (37)$$

During the usual experiments  $s \omega^2 t \leq 0.2$  and  $Z^2 \leq 0.002$ , so terms of order  $Z^2$ , denoted by  $O(Z^2)$ , or

(27) Equation (3) of ref. (24).

higher are negligible compared to unity and may be dropped from the series in brackets to give

$$q_n^*(s) = \left[ q_n(s) - \frac{4D\sigma_2}{\omega^2 x^2} \frac{dq_n(s)}{ds} \right] + \frac{D}{\omega^4} \left( \frac{\sigma_1}{x^2 t} \right) \left[ \frac{d^2 q_n(s)}{ds^2} - \frac{4D\sigma_3}{\omega^2 x^2} \frac{d^3 q_n(s)}{ds^3} \right] + \frac{1}{2} \frac{D^2}{\omega^8} \left( \frac{\sigma_1}{x^2 t} \right)^2 \frac{d^4 q_n(s)}{ds^4} + \dots \quad (38)$$

The terms  $\frac{4D\sigma_3}{\omega^2 x^2} \frac{dq_n(s)}{ds}$  and  $\frac{4D\sigma_6}{\omega^2 x^2} \frac{d^3 q_n(s)}{ds^3}$  are seldom more than a few per cent. of the maximum values of  $q_n(s)$  and  $d^2 q_n(s)/ds^2$ , respectively, so they may also be neglected in most experiments.

It is not evident from equation (35) that plots of  $q_n^*(s)$  versus  $\sigma_1/(x^2 t)$  will extrapolate linearly to  $q_n(s)$  at very long times, *i.e.*, when  $s\omega^2 t \gg 1$ . However, these plots should be quite straight over an intermediate range of times in which  $t$  is so large that terms containing the fourth and higher derivatives of  $q_n(s)$  are negligible but in which  $s\omega^2 t$  is still small. The values of  $q_n(s)$  may therefore be obtained by extrapolating these straight regions, which sometimes include most of the experimental points, to  $\sigma_1/(x^2 t) = 0$ .

When  $D$  is not the same for all solute molecules, we start with the sedimentation spreading equation analogous to equation (12) and arrive at an expression identical to equation (35) except that  $Q_n^*(s)$  is substituted for  $q_n^*(s)$  and the products  $D^j \frac{d^k q_n(s)}{ds^k}$ ,

where  $j = 0, 1, 2, \dots$ , and  $k = 0, 1, 2, \dots$ , are replaced by  $\frac{\partial^k [D_s^j Q_n(s)]}{\partial s^k}$  in which

$$D_s^j = \int_0^\infty \frac{D^j q_n(s, D) dD}{q_n(s, D) dD} \quad (39)$$

Since solutes which have a distribution of sedimentation constants are almost certain to have a distribution of diffusion constants, no attempt has been made to invert equation (35) or equation (38) to express  $q_n(s)$  in terms of  $\partial n/\partial x$  and its derivatives as was done for electrophoretic spreading in equation (20). To compute  $Q_n(s)$  in this way would require a knowledge of average diffusion constants, equation (39), as a function of the sedimentation constant.

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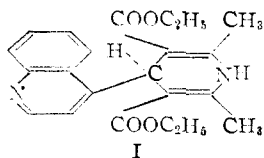
[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

## Absorption Spectra and Structure of Some 4-Arylpyridines Derived from the Hantzsch Pyridine Synthesis

BY ARTHUR P. PHILLIPS AND PHOEBE LEE GRAHAM

Comparison of the ultraviolet absorption spectra of certain 4-aryl-2,6-lutidines, both with and without 3,5-dicarbethoxy groups on the pyridine ring, reveals valuable information concerning the configuration of these compounds. When the 4-aryl is phenyl the presence of the carbethoxy groups appears to block internuclear resonance, or conjugation, relating to coplanarity between the two rings. Conjugation, and thus presumably coplanarity, is readily apparent in 4-phenyl-2,6-lutidine in which the carbethoxys are absent. When the 4-aryl is quinoline, results suggest no significant resonance interaction between the quinoline and pyridine rings in the presence of the two carbethoxys. Absorption spectra of the quinoline derivatives also indicate little or no conjugation between rings even in the absence of carbethoxy groups.

In an earlier study<sup>1</sup> it was hypothesized that in certain products derived from the Hantzsch pyridine synthesis, exemplified by I, the aryl and dihydropyridine rings should be non-coaxial and non-coplanar. The former suggestion derives from the



concept of the tetrahedral structure of carbon, the latter is related to the currently accepted views of the structure of substituted biphenyls. This paper presents some work in support of the latter idea.

(1) A. P. Phillips, *THIS JOURNAL*, **71**, 4003 (1949).

It has long been known<sup>2-6</sup> that biphenyls suitably substituted in the ortho positions are resolvable into optical antipodes. These results led to the concept of a coaxial arrangement of the two phenyls which were also capable of coplanarity in non-hindered biphenyl systems. Optical activity in this series was ascribed to molecular asymmetry depending upon restriction of rotation about the central bond, by bulky ortho groups which made coplanarity difficult or impossible through steric hindrance.

Although numerous substituted biphenyls were resolved by Adams<sup>6</sup> and co-workers and the earlier

- (2) G. H. Christie and J. Kenner, *J. Chem. Soc.*, **121**, 614 (1922).
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