A MODEL OF RANDOM FIBER NETWORKS THAT DESCRIBES THE SIEVING OF PARTICLES DURING GEL ELECTROPHORESIS

GARY COBBS, *Department of Biology, University of Louisville, Louisville, Kentucky 40292*

ABSTRACT A model is presented to describe the sieving of particles during gel electrophoresis by considering the movement of a spherical particle through a random network of straight, rigid fibers. The movement of the particle through the network is approximated by a discrete model of the network composed of parallel planes containing fibers through which the particle must pass. Unlike previous models this model does not assume that the rate of movement is proportional to the proportion of cross-sectional area available to the particle. The results provide a new justification for approximately linear Ferguson plots and suggest that for large particles, Ferguson plots may become nonlinear.

INTRODUCTION

Ogston (1958) presents an analysis of the distribution of spaces available to a spherical object randomly placed within a three-dimensional network of fibers. His model assumed infinitely thin fibers, all with the same length. The essential result reported by Ogston (1958) is a formula for the probability a spherical space of radius R, randomly inserted into the above network of fibers will include no part of any fibers. This probability is given by

$$P(\text{no contact}) = \exp[-2\Pi\nu LR^2 - (4/3)\Pi\nu R^3] \quad (1)$$

where ν denotes the concentration of fiber centers per unit volume and fiber length is $2L$. If the fibers are much longer than the radius of the sphere, then Eq. 1 is approximately equal to

$$P(\text{no contact}) = \exp(-2\Pi\nu LR^2) \quad (2)$$

because the second term in Eq. 1 becomes negligible in comparison to the first term. Ogston points out that under Eq. 2 the probability for given R depends only on the total length of fibers per unit volume and not on ν or L separately. Ogston (1958) also points out that the above theory can easily be extended to fibers of finite thickness. If the fibers are cylinders of radius r, then $(R + r)$ is simply substituted in place of R in Eq. 1 and 2.

Eq. 2 has been used extensively by Morris (1966), Rodbard and Chrambach (1970, 1971), Rodbard (1976), Morris and Morris (1971), and Fawcett and Morris (1966) to describe the sieving effect on molecules during gel electrophoresis and gel filtration. During electrophoresis, molecules are forced by an electric field to penetrate some type of gel matrix. The matrix may be any of several kinds: starch, polyacrylamide, agar, agarose, and others. All of these gels are thought to be composed of long fibers. In all cases it is observed that the gel retards the movement of molecules in the electric field and that higher concentration gels retard more

than lower concentration gels. This sieving effect has been studied extensively for several kinds of gels and with numerous macromolecules (Ferguson, 1964; Hedrick and Smith, 1968; Rodbard and Chrambach, 1971). In all studies it has been found that the log of electrophoretic mobility is very closely a linearly decreasing function of gel concentration. A plot of the log of electrophoretic mobility (EM) against gel concentration is frequently referred to as a Ferguson plot because of its use by Ferguson (1964). The slope of this empirically measured linear relationship depends on the size of the molecule under study and larger molecules have larger negative slopes.

A theoretical explanation of these observations was proposed by Rodbard and Chrambach (1970) who used the results of Ogston (1958). They employed the principle of Delesse to show that Eq. 2 also represents the area fraction of spaces available to a molecular of radius R in a random cross section of the fiber network. He then assumes that EM is proportional to the cross-sectional area available to a molecule. No justification for this assumption is given. This leads to the expression

$$U = U_0 \exp(-2\Pi \nu L R^2) \tag{3}$$

where U denotes EM and U_0 is EM in the absence of fibers. Because the exponent in Eq. 3 is proportional to total fiber length per unit volume and gel concentration is also proportional to total fiber length Eq. 3 suggests

$$U = U_0 \exp(-K_R T) \tag{4}$$

as the relation between U and gel concentration. In Eq. 4 T denotes gel concentration and K_R is a constant for any choice of molecule and gel condition. The constant, K_R is the slope of the Ferguson plot and has been called the retardation coefficient (Rodbard and Chrambach, 1970). It is well known that K_R depends on the size of the molecule and Rodbard and Chrambach (1970) have used Eq. 3 to justify

$$K_R = c(R + r)^2 \tag{5}$$

as a proposed relation between K_R and molecular radius, R. Here c is a constant for any gel system, and r is fiber radius.

Here a model is presented using methods similar to those of Ogston (1958) and Rodbard and Chambach (1970) and which may be a more accurate description of the sieving effect of gel electrophoresis. This model does not make the unsupported assumption that EM is proportional to the cross-sectional area in a gel, though it does have its own set of a priori assumptions. Also, its derivation considers more directly the movement of molecules through a gel.

DISCRETE MODEL OF A GEL

The sieving effect during gel electrophoresis is generally conceived as a continuous process in a homogeneous gel; however, it is convenient to treat it as a series of separate and independent sieving stages. The network or gel is assumed to be composed of randomly distributed, rigid, straight, infinitely thin fibers not necessarily all of the same length. A spherical particle will be forced to penetrate the gel by a force field with the same direction and magnitude at all points within the gel. Also, the medium within the gel, excluding the gel fibers, has resistance to

movement of a particle such that its terminal velocity is U_0. Consider a line parallel to the force field projecting through the gel with points equally spaced by distance W. At each point on the line is a plane perpendicular in all directions to the line. Now consider all fibers whose centers lie within $W/2$ of each plane and their projections onto the plane. The projections are perpendicular to the plane so that if a fiber of length $2L$ has inclination α with the plane the length of the projection on the plane is $2L\cos\alpha$. If the fibers in the three-dimensional network have mean length $2\bar{L}$ then the mean length of the projections can be shown to be $(2/\Pi)\bar{L}$.

The discrete model of the gel consists of the projections of the fibers onto the parallel planes. The passage of a particle through the gel is assumed to be equivalent to passage through the parallel planes in a direction perpendicular to the planes. Collisions of the particle with fibers in one plane are considered to be independent of what may happen in all other planes. Also, it will be assumed that the particle has its maximum velocity, U_0, when it is halfway between any two planes. This is equivalent to assuming that whatever effect collision with fibers in a plane have on the particle's velocity, the particle will accelerate to U_0 before it travels a distance $W/2$ from the plane.

The net velocity of the particle in the direction of the force field and in the region $W/2$ on either side of a plane, U_n, is a random variable that depends on the nature of the collisions with the fibers in the plane. If by chance there is no contact with any fiber during passage through a plane, then $U_n = U_0$, otherwise, $U_n < U_0$. Because passages through all planes are independent, the mean velocity in one plane, $U = E(U_n)$, is also the mean velocity the particle will have when passing through any number of adjacent planes. Thus, to determine the relation between U and gel characteristics such as fiber concentration and fiber length, we need consider only events occurring during passage through a single plane. This representation of a gel by successive "plates" is similar to a model of Ackers (1964) who used plates containing pores of constant radii.

PASSAGE THROUGH A SINGLE PLANE

A spherical particle of radius R is to pass through a plane containing projections of the fibers. The sphere is located randomly on the plane with respect to the fiber locations. Consider the projection of the sphere onto the plane. Again, the projection is perpendicular to the plane and is equivalent to a randomly placed circle on the plane. Denote by p_k the probability that this circle includes any part of exactly k different fibers, where $k = 0, 1, 2, \ldots$. Explicit formulae for p_k in terms of gel characteristics will be provided in the next section. A complete model for the effect of collisions with fibers on mobility will not be given here but will be described by a set of parameters. Specifically, for a given passage through the plane, let t_i denote the time elapsed during travel of the particle from $W/2$ on one side to $W/2$ on the other side of the plane when initial contact is made with exactly i different fibers. The term "initial contact" here denotes fibers included in the projection of the particle onto the plane. The quantities, t_i, are random variables because the time required for passage depends on the exact geometry of the initial contacts as well as on the number of fibers involved. The quantity t_0 has a similar definition except that it is a constant equal to W/U_0. Now define

$$h_i = E_{t_i}\left(\frac{t_0}{t_i}\right) \qquad i = 0, 1, 2, \ldots$$

where E_{t_i} denotes the operation of expectation with respect to t_i. The quantity h_i has the interpretation that $h_i U_0$ is the average velocity of a particle that has initial contact with exactly i fibers. The h_i values are assumed to be constant with respect to R, W and ν; however, they may depend on L. Thus, for any gel system we assume that $\bar{L}$ is constant with respect to R, W and ν. The above formula gives $h_0 = 1$ and it is reasonable to suppose $h_{i+1} < h_i$ for all $i > 0$.

The average velocity of the particle through the region around the plane can now be written as

$$U = U_0 \sum_{k=0}^{\infty} p_k h_k. \tag{6}$$

In this model the quantities, h_k, are considered underlying parameters describing the effects of collisions with fibers on mobility. Formulae for the p_k values are now needed.

DISTRIBUTION OF NUMBER OF CONTACTS

Consider a random point on the plane which we shall consider the origin. The mean number of fibers whose centers lie in the annulus defined by the distances X, $X + \mathrm{d}X$ from the origin is

$$(\mathrm{d}n)_X = 2\Pi X \nu W \mathrm{d}X \tag{7}$$

where ν and W are the same as before and νW is the density of fiber centers on the plane. Following Ogston (1958), contacts will be considered to be of two kinds, tangential and end, and may be counted separately. A tangential contact occurs when a fiber with some portion of its length included in the circle of radius R is also tangent to a circle of radius less than R about the origin. Any fiber with some portion of its length within the circle that does not make tangential contact is said to make end contact. The total contacts are the sum of tangential and end contacts.

Tangential Contacts

At each direction from the origin the fraction of fibers making angles in the interval $(\psi, \psi + \mathrm{d}\psi)$ with the line joining the fiber center to the origin is

$$(\alpha)_\psi = (2/\Pi)\mathrm{d}\psi. \tag{8}$$

Multiplying Eqs. 7 and 8 gives the average number of fibers with centers in the annulus given by distances X, $X + \mathrm{d}X$ and with angle in $(\psi, \psi + \mathrm{d}\psi)$ to be

$$(\alpha)_\psi (\mathrm{d}n)_X = 4X\nu W \mathrm{d}X \mathrm{d}\psi. \tag{9}$$

Now, for a given fiber, consider the ends to be extended as straight lines so that tangential contact is made, either by the fiber itself or its extension, with a circle of radius D. Then, for a given value of ψ we have

$$D = X \sin \psi$$

$$\mathrm{d}D = (\sin \psi)\mathrm{d}X$$

and thus

$$X\mathrm{d}X = \frac{D\mathrm{d}D}{(\sin\psi)^2}. \tag{10}$$

Substituting Eq. 10 in Eq. 9 gives the average number of fibers with angles in $(\psi, \psi + \mathrm{d}\psi)$ and centers in the annulus $X, X + \mathrm{d}X$ which would make tangential contact with a circle of radius D, assuming fibers infinitely long to be

$$(\alpha)_\psi(\mathrm{d}n)_X = \frac{4\nu W\,\mathrm{d}\psi\, D\,\mathrm{d}D}{(\sin\psi)^2}. \tag{11}$$

Let $n_{t,\ell,D}$ be the average number of fibers with projected length 2ℓ that make tangential contact with a circle with radius in (O,D) and let ν_ℓ denote the concentration of fibers with projected length 2ℓ. Using Eq. 11 we write

$$\frac{\mathrm{d}n_{t,\ell,D}}{\mathrm{d}D} = 4\upsilon_\ell WD\int_{\cot^{-1}(\ell/D)}^{\pi/2}\frac{\mathrm{d}\psi}{(\sin\psi)^2} = 4\upsilon_\ell W\ell. \tag{12}$$

Here the limits of the integration take into account the finite length of the fibers. To account for fibers of all lengths, Eq. 12 is integrated with respect to ℓ to give

$$\mathrm{d}n_{t,D} = 4\nu W\bar{\ell}\,dD \tag{13}$$

where $\mathrm{d}n_{t,D}$ denotes the average number of tangential contacts with circles whose radii are in D, $D + dD$ and $\bar{\ell}$ is the mean length of the fiber projections. Since $\bar{\ell} = (2/\Pi)\bar{L}$ Eq. 14 may also be written as

$$\mathrm{d}n_{t,D} = (8/\Pi)\nu W\bar{L}\mathrm{d}D. \tag{14}$$

Thus for a circle of radius R the average number of tangential contacts, n_t, can be found by integrating Eq. 14 to be

$$n_t = \int_0^R \mathrm{d}n_{t,D}dD = (8/\Pi)\nu W\bar{L}R. \tag{15}$$

Now since all fibers are distributed in space randomly, the number of tangential contacts within a circle of radius R should follow a Poisson probability function with expectation n_t. Letting k_t denote the number of tangential contacts gives the probability function

$$\text{Prob}\,(k_t = m) = \frac{n_t^m\exp(-n_t)}{m!},\ m = 0, 1, 2\ldots \tag{16}$$

where n_t is given by Eq. 15.

End Contacts

The integration in Eq. 12 excluded all fibers making end contact and they need to be included when counting total contacts. The average number of end contacts, n_e, may be counted by using the fact that in the annulus composed of circles with radii in $(D,\ D + \mathrm{d}D)$ the concentration of fiber ends is twice the concentration of fiber centers. Only those fibers with

ends in the annulus that enter from outside the circle of radius $D + dD$ are counted as making end contact, as any fiber that enters from inside will be counted as a tangential contact, or will be counted as an end contact in an annulus with smaller radii. Therefore

$$dn_e = 2\Pi \upsilon W D dD$$

and

$$n_e = \int_0^R dn_e = \Pi \upsilon W R^2 \tag{17}$$

is the average number of end contacts for a circle of radius R. Like tangential contacts, end contacts will follow the Poisson probability function but with mean n_e given by Eq. 17.

Total Contacts

The random variable $k = k_t + k_e$ follows the Poisson probability function with mean $n_t + n_e$ since k_t and k_e are independent Poisson-distributed random variables with means n_t and n_e, respectively. Using Eqs. 15 and 17 gives the desired formula for the distribution of the number of contacts, p_k, as

$$p_k = \frac{\lambda^k e^{-\lambda}}{k!} \tag{18a}$$

where

$$\lambda = (8/\Pi)\upsilon W \bar{L} R + \Pi \upsilon W R^2. \tag{18b}$$

MOBILITY OF PARTICLE IN GEL

Substitution of Eq. 18 into Eq. 6 yields

$$U = U_0 e^{-\lambda}\left(1 + h_1\lambda + h_2\frac{\lambda^2}{2!} + h_3\frac{\lambda^3}{3!} + \ldots\right) \tag{19}$$

which gives EM as a function of: (a) the particle properties U_0 and R, (b) the gel properties υ and $\bar{L}$ and (c) the constants $\{h_i\}$. Eq. 19 is analogous to Eq. 3 but suggests a more complicated relation between U, R, and fiber concentration and fiber length. If $\bar{L} \gg R$, as is thought to be the case in most kinds of electrophoresis experiments, then the second term of Eq. 18b is negligible in comparison to the first and

$$\lambda = (8/\Pi)\upsilon W \bar{L} R \tag{20}$$

should be an accurate approximation of λ. Eq. 19 and 20 can be used in the same way that Eq. 3 was used to obtain Eq. 4 to suggest a relation between EM and gel concentration. First an algebraic rearrangement of Eq. 19 is useful. Since in Eq. 19 we know $h_i \geq 0$ for all i and $\lambda \geq 0$, we may rewrite Eq. 19 as

$$U = U_0 \exp(a_1\lambda + a_2\lambda^2 + a_3\lambda^3 \ldots) \tag{21}$$

where

$$a_1 = h_1, a_2 = h_2 - h_1^2$$

$$a_3 = (h_3 - h_1^3) + 3h_1(h_2 - h_1^2)$$

$$a_4 = (h_4 - h_1^4) - 4(h_3 - h_1^3) + (9h_1 - 3h_1^2)(h_2 - h_1^2) - 3(h_2 - h_1^2)^2.$$

Expressions for a_k for $k > 4$ in terms of $\{h_i\}$ can be obtained but will not be reported here. Now Eqs. 20 and 21 suggest

$$U = U_0 \exp(K_1 T + K_2 T^2 + K_3 T^3 + \ldots) \tag{22}$$

as a formula appropriate for describing the relation between U and T. Here U_0 and K_i for $i = 1, 2, \ldots$ are constants to be estimated for any particular molecule and gel system. Also, the K_i values have the interpretation

$$K_i = c_i(R + r)^i \qquad i = 1, 2, 3 \ldots \tag{23}$$

where $\{c_i\}$ are constants for any particular molecule and gel system and r is the fiber radius.

DISCUSSION

The analysis presented here suggests that the Ferguson relationship, Eq. 4, may be only an approximation of Eq. 22. This would be true if K_i for $i > 1$ were all small. There is convincing experimental evidence that Ferguson plots are very closely linear, though slight deviations from linearity have not been rigorously tested for. There are two ways that Eq. 19 or Eq. 21 can produce linear Ferguson plots. The first way is if $h_i = 0$ for all $i \geq 1$. This is analagous to Rodbard and Chrambach's (1970) assumption that EM is proportional to cross-sectional area. The second way Eq. 19 or Eq. 21 can predict linear Ferguson plots is if the sequence $\{h_i\}$ satisfies the relation $h_i - h_1^i = 0$ for all $i \geq 2$. This latter assumption seems more reasonable as an explanation for approximately linear Ferguson plots. Most likely this assumption is only approximately correct. There are no theoretical arguments supporting its validity. If it is only approximately correct, then one would expect that Ferguson plots involving high concentration gels may show some nonlinearity. Also, Eq. 23 suggests that any such nonlinearity should be more extreme for larger particles.

Another phenomenon that may contribute to nonlinear Ferguson plots is deviation from the assumption that the terminal velocity, U_0, is achieved when the particle is halfway between planes. In a real three dimensional gel, this assumption corresponds to assuming that the particle has sufficient time between collisions to accelerate to U_0. If the fiber concentration is sufficiently high or the particle is sufficiently large or both, then this assumption may be inaccurate. This phenomenon could be incorporated into the present model by allowing the particle velocity at the point midway between the planes to be a random variable that depends on the number of planes penetrated since the last contact and on the acceleration properties of the particle. Such an extension would require substantial justification and will not be developed here since it is not presently known whether it is necessary or not when modeling gel electrophoresis.

Clearly, a detailed statistical analysis of Ferguson plot data will be necessary to determine

if the present results Eqs. 22 and 23 are an improvement over the established model Eqs. 4 and 5. This work is in progress.

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