MONTE CARLO SIMULATION OF PARTICLE ADSORPTION RATES AT HIGH CELL CONCENTRATION

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ABSTRACT A practical method of simulating Brownian diffusion of small particles and their adsorptions by randomly placed cells is used to estimate the adsorption process rate constant. The ratio of the rate constant to its classical value, $4\pi RD$ for dilute perfectly adsorbing spheres, is found to be determined by cellular excluded volume. This ratio varies from 1 for dilute solutions of spheres to ~40 for spheres in the maximum possible concentration. A function that usefully estimates the rate constant for all possible values of cell concentration, cell radius, and particle diffusion constant is given for random fields of identical spherical cells. The method is also applied to primative cubic, body centered, and face centered lattices of spheres. At any given excluded volume and concentration the face and body centered lattices have about the same adsorption rate constant whereas the primitive cubic lattice has a smaller one which is, in turn, greater than that for randomly placed spheres. The results will be useful in determining diffusion limited reaction rates under high excluded volume conditions. These include adsorption by red blood cells at normal concentration, the adsorption of molecules by beads in a column, and adsorption of bacteriophage at very high bacterial concentrations.

INTRODUCTION

If there are n freely diffusing particles in a suspension of C cells/ml and if the particles adhere to the cells on first collision then the equation of mass action $dn/dt = -kCn$ describes the rate of decrease of free particles in solution. This relation fails to account for cell size; thus 10 bacterial cells/m^3 should adsorb particles at the same rate as ten bowling balls in the same volume. If cells are attributed nonzero volume then the collision rate can be expected to increase faster than C for large C. For example, for spherical cells in a closest packed lattice or "cannon-ball stack," the maximum possible diffusion distance is only a small fraction of the distance between cell centers. This gives the appearance of a concentration much higher than the number of cells per unit volume.

An experiment conducted at this Institute by Margret Bayer required 2×10^9 ANS labeled bacteria/ml to detect differential fluorescence emission following adsorption of bacteriophage ϵ_{15}. To interpret the time course of fluorescence emission we had to find the upper limit of cell concentration for which the classical rate constant, $4\pi RD$ (4) (R = cell radius, D = particle diffusion constant) is applicable. In doing so we also obtained an upward adjustment of the rate constant by a factor depending only on cellular excluded volume per unit volume. This empirical result: $k = 4\pi RD\{p\exp[\alpha_1(R^3C)^{\beta_1}] + (1-p)\exp[\alpha_2(R^3C)^{\beta_2}]\}$ with $p = 0.963$, $\alpha_1 = 1.405 \times 10^5$, $\beta_1 = 6.13$, $\alpha_2 = 8.91$, $\beta_2 = 0.254$, R = radius of spherical cells at concentration C, and D = diffusion constant of the particle, is a good fit to the Monte

Carlo simulation data presented here. Although derived from simulation of various concentrations of spherical cells about the size of log phase *Escherechia coli* the result is shown to be applicable to spheres of any size and concentration. The formula may be applied over the entire range of excluded volume per unit volume, namely: $0 \leq (4/3)\pi R^3 C \leq \pi/(3\sqrt{2}) \simeq 0.7405$.

Other possible applications of this result are: (*a*) Adsorption experiments with red blood cells at in vivo concentrations. The blood cells occupy ~50% of the total blood volume. (*b*) Adsorption of molecules by beads in a column. Here the bead concentration is very small but excluded volume is near its limiting value. (*c*) Adsorption by cells in natural environments such as in soil or on plant stems and leaves. Here cell concentrations may approach space limiting values. (*d*) Enzyme-protein interaction within cells. To the extent that enzymes might be considered perfectly adsorbing spheres this analysis can set upper bounds on diffusion limited reaction rates.

BROWNIAN MOTION

To avoid time-consuming simulation of zig-zag Brownian paths and their dependence on time step size we make use of the distribution of the first passage time from a point to a sphere of radius r. A conceptual balloon is expanded from the position of the diffusing particle until it becomes tangent to the nearest cell in a field of randomly placed, nonoverlapping, cells. Subsequently the particle is moved to a point selected at random uniformly over the balloon's surface. A deviate from the appropriate first passage distribution (see below) is determined to account for the elapsed time. This process is repeated and the deviates accumulated until the particle is within 0.1% of a cell radius from some cell. The resulting average cell acquisition time (over many trials) was found to be insensitive to variation in this capture distance as long as it was not > 0.5% of a cell radius.

Thus, in this procedure the motion of the particle to the balloon surface of radius r, which it must in any case acquire, is accounted for by one computational step instead of many. The amount of computer time saved for this operation, compared to following the particle in Brownian motion, is considerable. Fig. 1 illustrates the process in two dimensions.

DESCRIPTION OF THE EMPIRICAL RESULTS

Fig. 2 shows the results of fitting a curve of the form $k(C) = 4\pi RD\{p \exp[(C/C_1)^{r_1}] + (1 - p)\exp[(C/C_2)^{r_2}]\}$ to the simulated data. 5,000 trials were completed at each of 18 cell concentrations. This number is adequate to detect a 5% change in the rate constant k. The solution of the rate equation, $1 - (n/n_0) = 1 - e^{-kCt}$, gives the fraction of particles adsorbed and the distribution of adsorption times. However, at very high cell concentrations the simulated adsorption time distribution deviates significantly from the simple exponential. By defining the estimated rate constant $\hat{k} = 1/C\overline{T}$, with $\overline{T}$ being the mean of 5,000 observed adsorption times, a first order stochastic process is identified that has the same mean as the simulated one. This $\hat{k}$ is the maximum likelihood estimate of k in the exponential distribution which fits all but the very highest cell concentrations.

The cell radius, R, was calculated to give Schlesinger's spherical equivalent (1) to log phase *E. coli*, ($R = 8.3 \times 10^{-5}$ cm), and D was taken as the diffusion constant for bacteriophage ϕX174 in water at 37°C (2), ($D = 1.78 \times 10^{-7}$ cm^2/s).

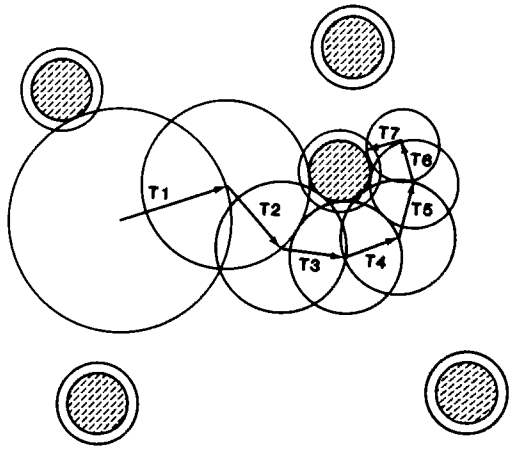

FIGURE 1 Two-dimensional illustration of a sequence of ballooning steps leading from a random starting position to adsorption of the diffusing particle. The first balloon is expanded from the starting point until it becomes tangent to the nearest cell's inner boundary. Next, a random point on this balloon is selected and a deviate, T_1, obtained from the first passage time distribution to such a balloon. This process is repeated, leading to T_2, T_3, ..., T_7 at which the process is terminated because the seventh random point lies within the outer and inner boundaries of the tangent cell. $T_1 + T_2 + \ldots + T_7$ is the traversal time for this particular trial. The width of the annulus is greatly exaggerated in the figure, R(outer)/R(inner) = 1.001.

GENERALIZATION

The rate constant determined from simulating one cell concentration, C_0, one cell radius, R_0, and one particle diffusion constant, D_0, yields the rate constant for every pair (C, R) on the curve $R = R_0 (C_0/C)^{1/3}$ for any D. To see this let $R = fR_0$, and f be a linear magnification factor. A cube containing n cells having side s_0 goes over, with this enlargement, to one with n bigger cells in a cube of side $s = fs_0$. Hence if $C_0 = n/s_0^3$, $C = n/s^3 = n/(fs_0)^3$ or $C = C_0/f^3$. Thus $(C_0/C)^{1/3} = f = (R/R_0)$ ties together the new cell radius and new cell concentration and by eliminating f, describes a curve, $R^3C = R_0^3C_0$, of constant total cell volume per milliliter.

The sequence of balloons created for the given simulation would be geometrically similar to those needed for any (C, R) pair lying on the above curve. All members of the class are merely magnifications, by f, of the original. Spheres in closest packed stacks form the limiting excluded volume curve for which $(4/3)\pi R^3C = 0.74$, (3).

Deviates from the first passage distribution, $F(t)$, are obtained by setting a uniform (0, 1) random deviate, U, equal to $F(t)$, and solving for t. Thus, if T_0 is such a deviate appearing in the given simulation then $U = F(T_0)$ where (see Appendix) $F(t) = 1 + 2\sum_{n=1}^{\infty}(-1)^n \exp[-(n\pi/r_0)^2 D_0 t]$, $t > 0$, $F(0) = 0$, and r_0 is the balloon radius corresponding to T_0. If T_0 satisfies this equation then T, the corresponding deviate under magnified dimensions, will too if $D_0T_0/r_0^2 = DT/r^2$. Here, D is the new diffusion constant and $r = fr_0$. Hence: $T = (D_0/D)(r/r_0)^2T_0 = D_0f^2T_0/D$. Thus, if $\overline{T}_0$ is the average cell acquisition time in the given simulation then $\overline{T} = (D_0/D)f^2\overline{T}_0$ is the average that would be obtained when dimensions are magnified by f and the diffusion constant is changed to D. This result yields the rate constant, $k = 1/C\overline{T}$, that would be obtained under magnification by f, namely: $k = 1/[(C_0/f^3)(D_0f^2\overline{T}_0/D)] = fDk_0/D_0$, or $(kD_0)/(k_0D) = f$, and $k = k_0RD/R_0D_0$. This

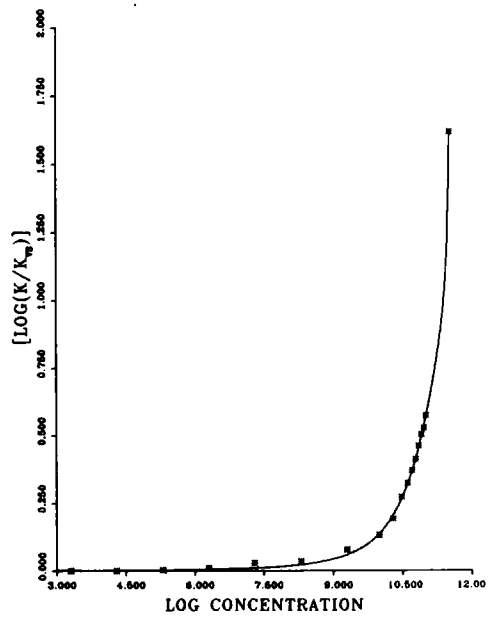

FIGURE 2 Relation between $\log(k/k_{vs})$ and $\log(C)$ where $k_{vs} = 4\pi RD$. $R = 8.3 \times 10^{-5}$ cm to approximate log phase *E. coli*, and $D = 1.78 \times 10^{-7}$ cm^2/s for bacteriophage ϕX174 in water at 37°C. Both logs are base ten. Closest packed spheres were used to obtain the maximum point for which k/k_{vs} is ~40.

establishes our ability to find the rate constant k for any pair (C, R) on the curve $R^3C = R_0^3C_0$ and any D given its value, k_0, for the triple (C_0, R_0, D_0).

Next, we suppose that we are given a function $g(C)$ that gives the values of k for cells of radius R_0, at concentration C and particles with diffusion constant D_0. This function combined with the above result will yield the values of k for all triples (C, R, D).

The value of k at (C, R, D) may be obtained from that at (C', R_0, D_0), where $R^3C = R_0^3C'$, i.e., $C' = C(R/R_0)^3$. The value of k at this point is $g[C(R/R_0)^3]$ by hypothesis; this value is k_0 in the above formulation. Hence: $k(C, R, D) = (RD/R_0D_0)g[C(R/R_0)^3]$. Using the $k(C)$ from above as $g(C)$ we obtain the result given in the Introduction. In our simulation, the cells are fixed during any one trial and the diffusing particles considered points. Putting $R = R_{\text{cell}} + R_{\text{particle}}$ will account for the latter. Setting $D = D_{\text{particle}} + D_{\text{cell}}$ should account for cell motion (5). Other factors such as the cell's surface being only partly composed of receptive sites may reduce the effective rate constant but may also be taken into account (6).

ARBITRARY DISTRIBUTION OF CELL SIZES AND SHAPES; EXCLUDED VOLUME

R^3C in the last formula is proportional to the volume per milliliter excluded by cells to particle diffusion. We show here that the rate constant is proportional to a function of the average excluded volume per milliliter, the particle diffusion constant, and the cube root of the average cell volume. The function may itself depend on the specific shapes and their distribution. The analysis is parallel to that of the previous section.

Let a given simulation determine the rate constant k_0 for the triple (C_0, V_0, D_0) where V_0 is

the average volume of the cells in the distribution. The cells may vary in both size and shape. Cells would be selected according to the given distribution and placed at random nonoverlapping loci in random orientations so as to achieve C_0 cells per milliliter.

Magnifying the linear dimensions of this simulation by a factor f gives a new concentration $C = C_0/f^3$ as before, and a new average cell volume $V = f^3V_0$. The curve of constant excluded volume per milliliter, $VC = V_0C_0$ gives the set of pairs (C, V) for which k is determined by the given simulation. As before, $k = fDk_0/D_0$ since the two sets of spherical balloons are geometrically similar. Also, $k = Dk_0(V/V_0)^{1/3}/D_0 = Dk_0(C_0/C)^{1/3}/D_0$, so we may determine k for any pair (C, V) on the curve $VC = V_0C_0$ and any D given the value of k_0 corresponding to (C_0, V_0, D_0). Let $h(C)$ give the values of k for various concentrations of the given distribution of cell sizes and shapes, i.e., at average cell volume V_0 and diffusion constant D_0. k at (C, V, D) may be obtained from that at (C', V_0, D_0) where $VC = V_0C'$.

k at this point is $h[VC/V_0]$. Hence, $k(C, V, D) = (D/D_0)\,(V/V_0)^{1/3}h[VC/V_0]$.

A future paper will report work done on various distributions of cell sizes and shapes. In particular, work is in progress for cylindrical bodies with spherical end caps intended to simulate log phase *E. coli*.

APPLICATION TO ADSORPTION BY LATTICES OF SPHERES

Face centered, body centered, and primitive cubic lattices of spheres were simulated for excluded volumes ranging from zero to each lattice's maximum packing fraction. In each case a diffusing particle is placed at random within the open area between the spheres and allowed to diffuse until it makes contact with one of them. The rate constant was calculated from $k = 1/C\overline{T}$ where C is the concentration of spheres and $\overline{T}$ is the average time to first contact. Fig. 3

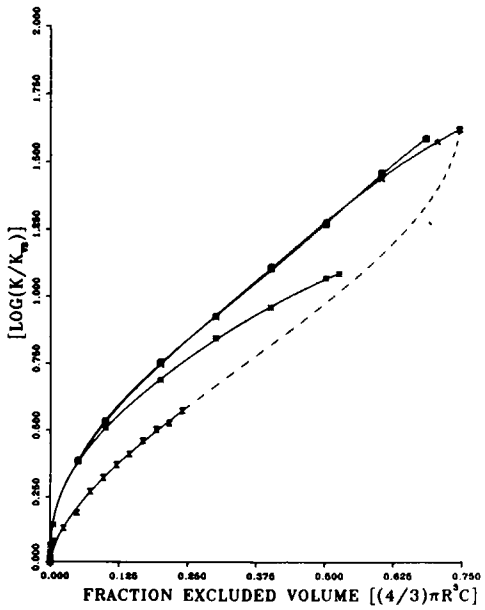

FIGURE 3 $\log(k/k_m)$ vs. excluded volume for three lattice structures: (★) face centered cubic lattice; (□) body centered cubic lattice; (*) primitive cubic lattice; and (×) random placement of spheres

TABLE I
AVERAGE DISTANCE BETWEEN A RANDOM POINT AND THE NEAREST SPHERE FOR EXCLUDED VOLUME OF 0.125

	Average distance*
Random	0.782
Primitive cubic	0.672
Body centered cubic	0.619
Face centered cubic	0.623

*Radii of spheres is taken as 1.

shows $\log(k/k_{vs})$ as functions of excluded volume for the three lattices and also for randomly placed spheres, $k_{vs} = 4\pi RD$. The face and body centered lattices cannot be distinguished by these data but each of them yields faster adsorption than the primitive cubic lattice, which in turn adsorbs faster than random spheres. At any particular excluded volume each of the structures has the same concentration of spheres and the same surface area exposed to the diffusing particle; however, they vary in the average distance available within the open areas. Table I shows the mean distance between a random point in the open area and the nearest sphere for each of the structures. An excluded volume of 0.125 was used for each table entry. These distances correspond to the average adsorption time in the expected way.

The dashed line extension of $\log(k/k_{vs})$ for random spheres indicates merely that random spheres may be packed into concentrations all the way up to closest packing. This end point can be considered the limit of random packing and so is connected by the dashed line. No data were collected for any of the excluded volumes in the dashed region.

APPENDIX

Distribution of First Passage Time to a Sphere of Radius R[1]

Consider a small sphere of radius $a << R$ containing a unit amount of concentrated diffusing material at the center of the sphere of radius R at time zero. The outer sphere is considered to be an absorbing boundary. At time zero the small ball of material starts to diffuse and is eventually entirely absorbed by the outer boundary. The integral from zero to time t of the total flux at the outer boundary is the fraction of the material that has reached the outer boundary by time t. With $a << R$ this integral also approximates the probability that a randomly selected particle of the material has been absorbed by time t. The limiting value of this distribution as the small sphere radius, a, vanishes is the desired result. Letting $U(r, t)$ be the radially symmetric concentration of material we have the boundary conditions:

$$U(r, 0) = U_0 \quad ,0 \leq r \leq a$$
$$= 0 \quad , r \geq a \text{ , and}$$
$$U(R, t) = 0$$

Substituting $V = rU$ into the radially symmetric diffusion equation, $U_{rr} + (2/r)U_r = (1/D)U_t$, gives $V_{rr} = (1/D)V_t$. Separating variables yields solutions of the form $[\alpha \sin(\omega r) + \beta$

[1]The solution to this problem may appear in the early literature on Brownian motion, but I was unable to find it.

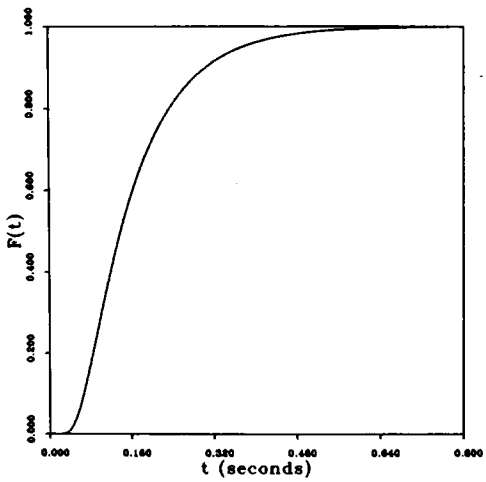

FIGURE 4 Distribution of first passage time to a sphere of radius 1 of a particle with diffusion coefficient 1. $F(t) = 1 + 2\,\Sigma_{n=1}^{\infty}(-1)^n e^{-(n\pi/r)^2 Dt}$.

$\cos(\omega r)]e^{-\omega^2 Dt}$ for any α, β, and ω. The cosine terms in $V(r, t) = \Sigma_{n=1}^{\infty}[\alpha_n \sin(\omega_n r) + \beta_n \cos(\omega_n r)]e^{-\omega_n^2 Dt}$ are eliminated and the boundary condition at $r = R$ met by extending the initial conditions on V as an odd function with period $2R$. The result is: $V(r, 0) = \Sigma_{n=1}^{\infty} \alpha_n \sin(2\pi n r/2R)$ for $-R \le r \le R$, where $\alpha_n = (2U_0/n\pi)[a\cos(n\pi a/R) - (R/n\pi)\sin(n\pi a/R)]$. Thus: $U(r,t) = (1/r)\,\Sigma_{n=1}^{\infty}(2U_0/n\pi)[a\cos(n\pi a/R) - (R/n\pi)\sin(n\pi a/R)] \times \sin(n\pi r/R)\exp[-(n\pi/R)^2 Dt]$. The flux at the outer surface is: $-4\pi R^2 D(\partial U/\partial r)_{r=R} = f_R(t)$ and we require $4\pi a^3 U_0/3 = 1$, i.e., unit amount of initial material. Finally, $F_R(t) = \int_0^t f_R(x)dx$, giving $F_R(t) = (6/\pi^2)(R/a)^3\,\Sigma_{n=1}^{\infty}\{(-1)^n/n^2)[(a/R)\cos(n\pi a/R) - (1/n\pi)\sin(n\pi a/R)](1-\exp[-(n\pi/R)^2 Dt]\}$. We use the facts that: $F_R(\infty) = 1$, that $\lim_{a\to 0}[(1/n)\sin(n\pi a/R) - (a/R)\cos(n\pi a/R)]/(a/R)^3 = (n\pi)^2/3$, and that $F_R(0) = 0$ for every $a > 0$, to obtain $\lim_{a\to 0} F_R(t)$ and hence $F(t)$ given in the Generalization section. The mean and variance of $F(t)$ are $(r_0^2/6D_0)$ and $(r_0^4/90D_0^2)$, respectively. A plot of $F(t)$ for $r_0 = D_0 = 1$ is shown in Fig. 4.

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