Concentration Scaling of Protein Deposition Kinetics

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Measurement of the phase velocities of guided modes in optical waveguides yields information about the optical parameters of the waveguide and the medium surrounding it. Application of this technique to a planar waveguide upon which proteins are allowed to adsorb from solution has yielded the first experimental data accurate enough to be able to test analytical expressions for the kinetics of random sequential deposition in two dimensions. The equation of Schaaf and Talbot for intermediate coverages describes the measurements with good accuracy. The rate of deposition should scale linearly with bulk concentration, but this was found to be the case only at low values ($\gtrsim 20$ $\mu g/cm^3$), above which a marked deviation occurs.

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Strong and growing interest is being shown in random sequential adsorption (RSA). In the simplest model, rigid objects are placed sequentially at random onto a surface, after which they remain immobile [1]. Two-dimensional RSA appears to offer a good description of a great variety of natural processes, such as the deposition of proteins, living cells, latex and pigment particles, etc., onto solid surfaces. It has very recently been shown that the effects of diffusion and hydrodynamic interactions, neglected in simple RSA, fortuitously cancel each other out [2]. RSA is therefore an even better and more general model for adsorption processes than might have been thought.

The one-dimensional problem ("random parking") was first solved by Rényi [3], as a step towards tackling the problem in higher dimensions. Unfortunately, complete solutions even in two dimensions have remained analytically intractable. However, the coverage at the jamming limit (54.7% for disks) has been determined [4], as has the asymptotic rate of approach to the jamming limit [5], and, recently, an analytical expression in powers of the coverage θ, exact up to θ^3, for low to intermediate values of θ [6]. These results have been tested by numerical simulations, but no experimental work of sufficient accuracy to test these results has been reported.

In this Letter the kinetics of irreversible adsorption of a protein, transferrin, onto a planar waveguide with a smooth, well characterized surface are described. The number of adsorbed molecules is calculated from the changes in the phase velocities of the guided modes in the waveguide. The present resolution of the method is about 75 molecules/μm^2, for a molecule of the size of transferrin. Transferrin was chosen as a well characterized [7], approximately spherical, highly soluble protein.

The experimental arrangement is described in detail elsewhere [8–10]. The substrate is a planar optical waveguide made from $Ti_xSi_{1-x}O_2$, $x \approx 0.3$, of refractive index $n_F \approx 1.8$ and thickness $d_F \approx 180$ nm, supported on glass, and incorporating a diffraction grating (period $\Lambda = 2400$ mm^{-1}) in the upper surface (type 2400, ASI AG, Zürich, Switzerland). Before use, the waveguides were cleaned in hot concentrated permonosulfuric acid (H_2SO_5) for half an hour, rinsed in distilled water, and finally aged overnight in the same buffer solution used for dissolving the protein. Helium-neon laser light (wavelength $\lambda = 632.8$ nm) impinging with angle α onto the grating is coupled into the waveguide when the following relation is satisfied [8,11]:

$$N = n_{\text{air}} \sin\alpha + l\lambda/\Lambda , \tag{1}$$

where N is the effective refractive index of the waveguide and l the diffraction order. α was varied by mounting the waveguide on a goniometer with which the intensity of light coupled into the waveguide was recorded while α was varied using photodiodes positioned at each end (instrument IOS-1, ASI AG, Zürich). Zeroth order transverse electric (TE) and transverse magnetic (TM) modes were recorded. The waveguide forms one wall of a small flowthrough cuvette (radius $R = 0.025$ cm) positioned above the grating region and sealed to it with an O ring. An exponentially decaying evanescent wave penetrates the medium beyond the confines of the waveguide proper, and the optical parameters of the surrounding medium therefore influence N [11].

Iron-free transferrin molecules dissolved with concentration c in aqueous buffer solution (0.01M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid–NaOH pH 7.4) flowing through the cuvette with velocity V are adsorbed at random on the waveguide surface, to form a uniform, isotropic layer of thickness d_A and refractive index n_A. If c_A is the concentration of protein in this layer, and n_B ($= 1.331\,660$ at 25.2 °C) the refractive index of the aqueous buffer solution in which the protein is dissolved, then $n_A = n_B + c_A dn/dc$, where dn/dc is the refractive index increment of bulk solutions containing proteins, and which has an almost universal value of 0.182 cm^3/g [12]. The adsorbed mass per unit area, defined as $M = c_A d_A$, is then given by [12]

$$M = \frac{d_A(n_A - n_B)}{dn/dc} . \tag{2}$$

The relevant mode equations for the measured TE and

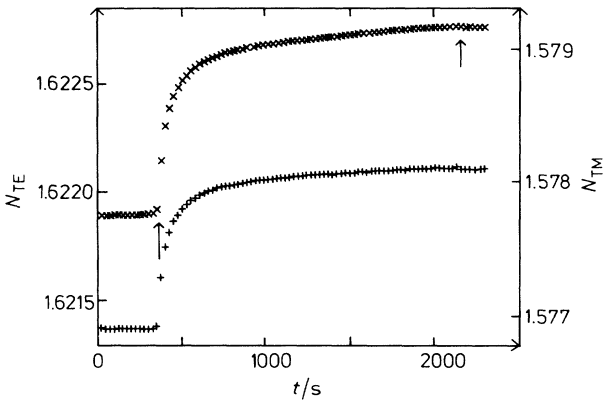

FIG. 1. Plot of measured N_{TE} (×) and N_{TM} (+) against time. The arrow in the lower left marks the beginning of protein flow (at a concentration of 400 $\mu g/cm^{-3}$), and the arrow in the upper right the end of protein flow and the start of flow of pure buffer solution.

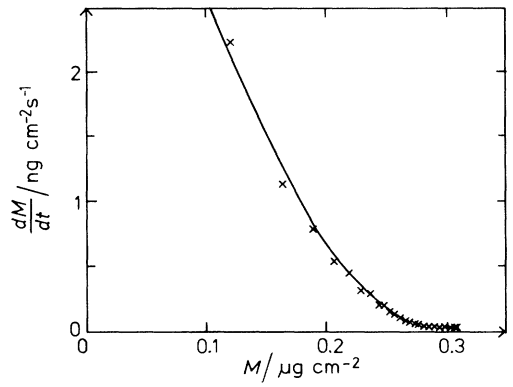

FIG. 2. The data from Fig. 1 (early to intermediate coverage) converted to $M(t)$ using the mode equations [8,9] and (2). The solid line is a least-squares fit of (3).

TM modes can be derived [7] from the Fresnel equations and solved simultaneously to yield n_A and d_A, and hence M [8,9]. The other optical parameters of the waveguide were measured separately in prior experiments.

Figure 1 shows the measured N_{TE} and N_{TM} data for a typical deposition experiment. The rate of adsorption always slowed down after a time and appeared to asymptotically come to a halt. This observation allows one to exclude multilayer formation. At the end of a run, the protein solution was replaced with pure buffer, whereupon a negligible change in N occurred, showing that adsorption to the $Ti_xSi_{1-x}O_2$ waveguide surface is indeed irreversible. Some experiments were also carried out in which the cuvette was flushed at short (10 min) intervals with pure buffer solution; in this case too, negligible amounts of protein were removed.

If v is the number of adsorbed particles (disks of area a) per unit area at time t, then $dv/dt = K\phi$, where ϕ is the average fraction of the surface available to the center of a new particle. According to Schaaf and Talbot [6], dv/dt may be expressed as a series in powers of the number of adsorbed particles. Defining $\theta = va$, then up to θ^3 the expression is [6]

$$dv/dt = K[1 - 4\theta + (6\sqrt{3}/\pi)\theta^2 + (40/\sqrt{3}\pi - 176/3\pi^2)\theta^3] . \quad (3)$$

The connection between θ and the measured quantity M is given by

$$\theta = Ma/m , \quad (4)$$

where m is the mass ($=1.33\times10^{-13}$ μg) of a single molecule. a can be estimated from the radius of gyration $R_g = 3.025$ nm [6], as $\pi R_g^2 = 29$ nm^2. The time scale is embodied in the parameter K, which gives the rate of deposition onto a perfectly empty surface, i.e., the rate of mass transport to the surface. This occurs chiefly by convective diffusion, for which a suitable expression may be obtained from the work of Lévêque [13]:

$$K = (9/2)^{2/3}[1/\Gamma(1/3)]D^{2/3}(V/Rx)^{1/3}c , \quad (5)$$

where D is the diffusion coefficient of the protein, estimated to be 8×10^{-7} cm^2/s. The laser beam impinged at a point $x = 3.5$ mm distant from the inlet tube to the cuvette. Hence K/c is calculated as 1.09×10^{-4} cm/s.

Figure 2 shows dM/dt vs M calculated from the data of Fig. 1 using the mode equations [8,9] to determine n_A and d_A, and then (2) to calculate M, together with (3) fitted by the Levenberg-Marquardt least-squares method with a/m and K as adjustable parameters. The fitted parameters are plotted against bulk protein concentration c in Fig. 3. Whereas according to the basic RSA model a/m should be invariant with c, it decreases from the predicted value as c increases, to reach a plateau at about 70% of the predicted value at concentrations of 200 μg/cm^3 and above. On the other hand, the experimentally determined K agrees with the prediction of (5) only for bulk concentrations up to 20 μg/cm^3, after which it seems to undergo an abrupt transition and thereafter increases with a slope about an order of magnitude less than at low concentrations.

The approach to the asymptotic limit was also investigated. It should have the form [5]

$$M_\infty - M = \xi t^{-1/2} \quad (6)$$

where ξ is a constant. M_∞ is the jamming limit coverage, which should equal $0.547m/a = 0.251$ μg/cm^2. Equation (6) was least-squares fitted to the later part ($\theta > 0.4$) of the experimental data. Figure 4 shows M calculated as before, plotted to show the asymptotic approach to the jamming limit. The predicted $t^{-1/2}$ dependence indeed seems to be followed, but the fitted parameters vary with c (see Fig. 5). In particular, the jamming limit increases sharply with c. M_∞ is inversely proportional to a/m and

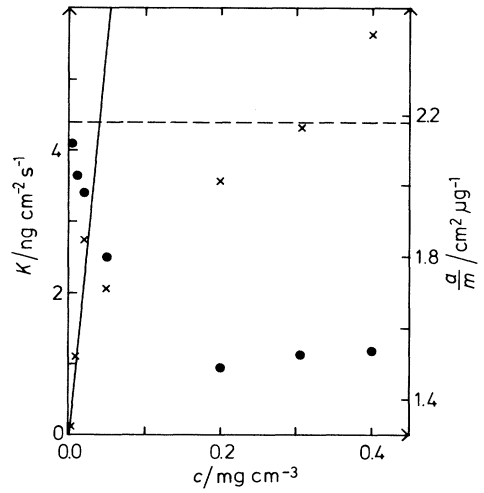

FIG. 3. Parameters from the least-squares fitting of (3) to the experimental data (early to intermediate coverage) from runs at different bulk protein concentrations. ×, K; ●, a/m. The solid line shows the predicted variation of K with c according to (5). The dashed line shows the value of a/m predicted from data given in Ref. [7].

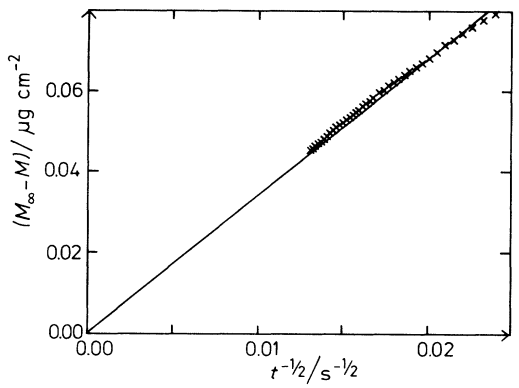

FIG. 4. Data from Fig. 1 (late coverage) converted to $M(t)$. The solid line is a least-squares fit of (6).

there is good agreement between the two sets of fits, by (3) and by (6), using respectively the early to intermediate and late portions of the experimental data.

At the highest bulk concentration for which measurements were carried out (400 μg/cm^3), the mean center-to-center distance of protein molecules in the solution is 70 nm, about 12 molecular diameters, and the volume fraction of protein in the aqueous solution is 3×10^{-4}. Therefore deviation from RSA behavior due to correlations between particle motion imposed by crowding of the molecules seems to be ruled out.

The variations of a/m, and hence M_∞, are more likely to be due to postadsorption relaxation processes. In general, these could include intramolecular rearrangements which change the symmetry of the molecule (e.g., in order to form more bonds with the surface), but since transferrin is actually somewhat oblate [7], the molecule may simply first land on the surface in an orientation which minimizes the area it occupies, and then slowly relax to maximize this area [14]. The rate of relaxation will be determined by the internal clock of the molecule and is therefore independent of the rate of impingement of protein molecules onto the surface, which depends on the bulk protein concentration. It may, however, be supposed that the relaxation of a molecule is blocked by the presence of a sufficiently close neighbor. The higher the concentration, the greater the probability that a molecule lands close enough to one which landed previously to prevent its own relaxation, and soon enough after the landing of the first to prevent its relaxation.

From the bulk concentration (30 μg/cm^{-3}) at the observed changeover from the predicted a/m (ideal RSA) to the diminished a/m, one may obtain a rough estimate of the time scale of the molecular relaxation: Suppose that a single molecule landing within a center-to-center distance of $3R_g$ from one which landed earlier is sufficient to block its relaxation. The area available to the incoming molecule for landing is $8\pi R_g^2$, and the rate ρ at which molecules land in this area is therefore $8\pi R_g^2 K/m$, i.e., 5×10^{-3} molecule/s for $c=30$ μg/cm^{-3}. The relaxation time for an individual molecule should be of order $1/\rho$ and therefore appears to be rather long—of the order of 200 s.

Another point which needs to be taken into account in models of RSA in two dimensions is the finite thickness of the particles. All the calculations and computer simulations of 2D RSA have assumed that the particles are infinitely thin. It is conceivable that molecules arriving

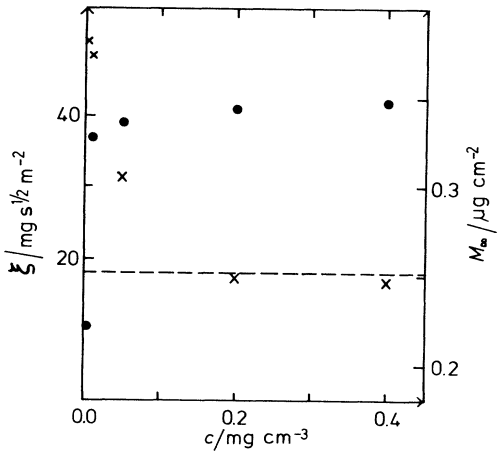

FIG. 5. Parameters from the least-squares fitting of (6) to the experimental data (late coverage) from runs at different bulk protein concentrations. ×, ξ; ●, M_∞. The dashed line shows the value of M_∞ calculated from the data in Ref. [7] and the jamming limit from Ref. [4].

near a surface which is no longer sparsely occupied may be steered between those already deposited, enhancing the probability of landing in an orientation which minimizes the area of a molecule in contact with the surface.

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