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Spatially Resolved Anomalous Kinetics of a Catalytic Reaction: Enzymatic Glucose Oxidation in Capillary Spaces

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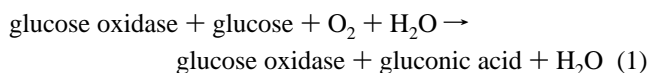
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A reaction involving the catalytic oxidation of glucose by the enzyme glucose oxidase is employed as a first spatially resolved experimental realization of a nonclassical A + C → C + Products chemical reaction. This heterogeneous kinetics experiment models the operation of optical glucose sensors. One-dimensional nonclassical reaction kinetics, manifested in anomalous rate laws, are observed. The time evolution of the density gradient of O_2 in the zone near the catalytic trap was monitored. The temporal dependence of the growth of this depletion zone M, predicted to scale as $t^{1/2}$, was measured to be $M \sim t^{0.50\pm0.05}$. This time dependence of the growth in M is directly proportional to the anomalous, time-dependent, reaction rate coefficient.

1. Introduction

Many chemical reaction processes are heterogeneous; that is, they take place at interfaces of different phases or in solid, viscous, or porous media, where particles, molecules, excitons, etc. are not freely stirred or able to diffuse in three dimensions. Examples include industrial surface catalysis, enzymatic reactions, and atmospheric reactions. We study here an effectively one-dimensional heterogeneous reaction of importance to biomedical science and technology, particularly to glucose sensors. Under such conditions it is usually difficult to impose mechanical stirring or to obtain complete convective mixing. Thus, a reaction occurring under heterogeneous conditions is often limited by the diffusion of the reactants. Also, in low dimensions, the diffusive self-stirring is not an effective stirring mechanism and this causes the reaction process to exhibit anomalous, dimension-dependent rates or rate coefficients.

Theory[1−17] and simulations[2,3,7,18−24] have established that the kinetics of *all* diffusion-limited elementary reactions are highly affected by the spatial dimension in which they occur. Below some critical dimension, these reaction processes exhibit nonclassical reaction kinetics manifested in a time-*dependent* rate coefficient $k(t) = kt^{-h}$. This is a result of the formation of a nonrandom spatial distribution of the reactants. That is, in contrast to the Smoluchowski model,[25−30] there is a time-dependent growth of depletion zones. Therefore, time-resolved studies of such elementary reactions are called for.

The A + C → C trapping reaction has been examined in quasi-1-D systems by *physical* exciton annihilation experiments in porous membranes and Vycor glass[27,28] and by the *physical* photobleaching experiments in capillaries and also in quasi-2-D sample geometries.[22] The trapping reactions studied in these experiments were shown to obey the theoretical predictions of low-dimensional nonclassical reaction kinetics. However, no spatial resolution of the reaction progress was obtained. Here we employ a two-phase enzymatic, catalytic *chemical* reaction, which is more complex but, nevertheless, analogous to the simple trapping reaction. Specifically, we used oxygen in water (phase 1) as the reactant A and glucose oxidase embedded in an acrylamide polymer matrix as the catalytic trap C (phase 2) in an effort to observe this behavior experimentally via the chemical reaction:

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glucose oxidase + glucose + O_2 + H_2O →
$$\text{glucose oxidase + gluconic acid} + H_2O \quad (1)$$

The experiments were run under conditions of *excess* glucose and water. Thus, effectively, the reaction can be written as the pseudo-monomolecular reaction

$$O_2 + \text{glucose oxidase} \rightarrow \text{glucose oxidase + products} \quad (2)$$
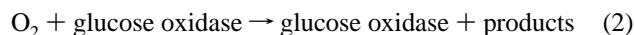
Measuring the instantaneous, O_2 sensitive fluorescence, of tris-(1,10-phenanthroline)ruthenium(II) chloride, a molecular complex known to respond linearly to O_2 concentrations at 0.24 mM (air saturation) and below,[31] the depletion of oxygen was monitored as a function of time.

Classically, the A + C → C reaction obeys the pseudo-first-order rate law

$$-d\rho_A/dt = k\rho_A\rho_C = k'\rho_A \quad (3)$$

where ρ is the concentration and $k' = k\rho_C$ is a constant, since C is not consumed by the reaction process. This leads to the integrated rate law

$$\ln\left(\frac{\rho_A}{\rho_{A_0}}\right) = -k't \quad (4)$$

which is equivalent to

$$\rho_A = \rho_{A_0}e^{-k't} \quad (5)$$

In the nonclassical kinetics formalism[4] for the trapping reaction, the rate constant k' is replaced in the differential form of the rate law by k_1t^{-h}. Here h is the heterogeneity coefficient and is equal to $1 - (d_s/2)$, where d_s is the spectral dimension[30] and is given by the relation

$$P \sim t^{-d_s/2} \quad (6)$$

where P is the probability that a random walker will return to its origin after a certain time t. In one dimension $d_s = 1$ and $h = \frac{1}{2}$; thus a nonclassical rate coefficient of

$$k(t) = k' = k_1t^{-1/2} \quad (7)$$

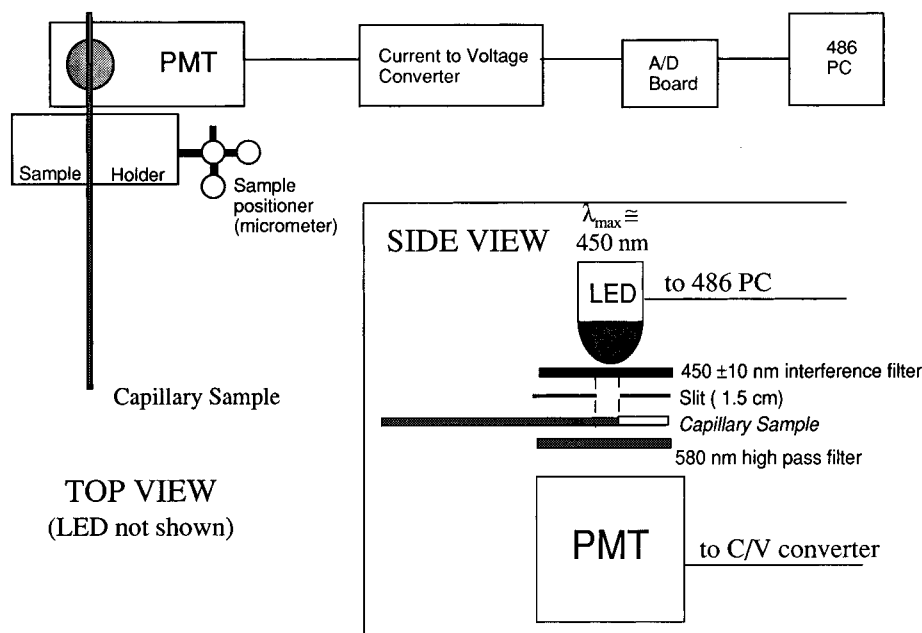is predicted for the differential rate law. Substitution of (7)

Figure 1. Experimental setup.

into (4) leads to a dependence of

$$\ln\left(\frac{\rho_A}{\rho_{A_0}}\right) \sim t^{1/2}, \quad t \to \infty \tag{8}$$

in one dimension. We denote the left-hand side quantity in (8) as Y. Thus,

$$Y \equiv \ln\left(\frac{\rho_A}{\rho_{A_0}}\right) = -k_1 t^{1/2}, \quad t \to \infty \tag{9}$$

where k_1 is a positive constant. It is this dependence that we tested experimentally using the chemical reaction of the enzyme catalyzed oxidation of glucose. Note that the nonclassical behavior described above is an asymptotic result. Related to this anomalous rate law is a depletion zone that grows in time[16,17,21] as $t^{1/2}$. Also, note that this is a heterogeneous (two-phase), reaction-diffusion system with a single, immobile catalyst (trap) in contrast to a homogeneous (single-phase) reaction-diffusion system with a distribution of traps. Although the enzyme catalyst is an imperfect trap, the asymptotic kinetics should be the same as a perfect trap.

2. Experimental Section

2.1. Trap Preparation. The polyacrylamide matrix employed in our experiments was the same as that used in the fabrication of submicron optical fiber oxygen sensors.[31] A 0.1 M phosphate buffer solution of pH 6.5 was prepared with 27% acrylamide and 3% *N,N*-methylenebis(acrylamide) as cross-linker to which was added 40 μL of a 225 units/mg solution of glucose oxidase. The polymerization was catalyzed with 1 μL triethylamine and 20 μL of 10% ammonium persulfate. The polymer was washed three times with 2 mL of tris(hydroxy-methyl)aminomethane hydrochloric acid buffer and then soaked in 5 mL of 20 mM pH 6.5 phosphate buffer/135 mM NaCl solution for at least an hour. The acrylamide was obtained from Gibco, the glucose oxidase was purchased from Sigma, and all other chemicals were obtained from Aldrich Chemical Co.

2.2. Sample Preparation. A 16 mM glucose, 0.4 mM tris-(1,10-phenanthroline)ruthenium(II) chloride solution (phase 1) was prepared in a phosphate buffer of pH 7.6 (approximately 5

mM concentration). In blank experiments, the glucose was omitted from the sample solution. All solutions were air saturated. The prepared solution was introduced into a 10 cm long glass capillary tube with a diameter of 0.53 mm. One end of the tube was then sealed with Krytox Teflon grease. The trap was introduced at the other end of the capillary tube by pressing the unsealed end of the capillary into a piece of the previously prepared polyacrylamide (phase 2), which had been soaking for about 10 min in pH 7.6 phosphate buffer. After placement of the trap, this end of the tube was also sealed with Krytox grease. The capillary tubes were purchased from Sutter Instrument Co., and the Krytox grease was obtained from DuPont Chemical Co.

2.3. Experimental Setup. A schematic of the experimental setup is pictured in Figure 1. We used a blue LED, with λ_{max} = 455 nm, as an excitation source. The capillary sample was positioned behind a 1.5 cm × 2 mm slit such that the volume of solution at a distance of $\leq$1.5 cm from the acrylamide trap is exposed to the excitation source. A 580 nm high-pass filter was positioned between the capillary sample and a photomultiplier tube (PMT). The signal from the PMT was passed through a current-to-voltage converter and sent to an IBM PC-486. Data acquisition was initiated within 5 min of sample construction. The fluorescence of the Ru(II) complex in water was monitored in arbitrary intensity units over a 12 h period. The fluorescence quenching of the Ru(II) dye is linearly proportional to the density of O_2 in the density range of $\rho_{O_2} \leq$ 0.24 mM, the density of air-saturated aqueous solution.[31] Data were acquired at 20 s time intervals. The fluorescence was taken in 500 ms time bins and averaged over 1 s for each data point.

3. Results and Discussion

For the A + C → C + Products reaction (as well as for the A + A → 0 reaction), it is useful to discuss the concept of a depletion zone,[16,17,21] which can be thought of in terms of a plot of the distance from a point to *every* A particle in the system. In the case of the trapping problem, if we consider that the trap is located at this point, we can monitor how the annihilation of A particles at the trap causes the depletion zone to grow in time. Specifically, we can monitor the reactant density profile in the vicinity of the trap and observe how it

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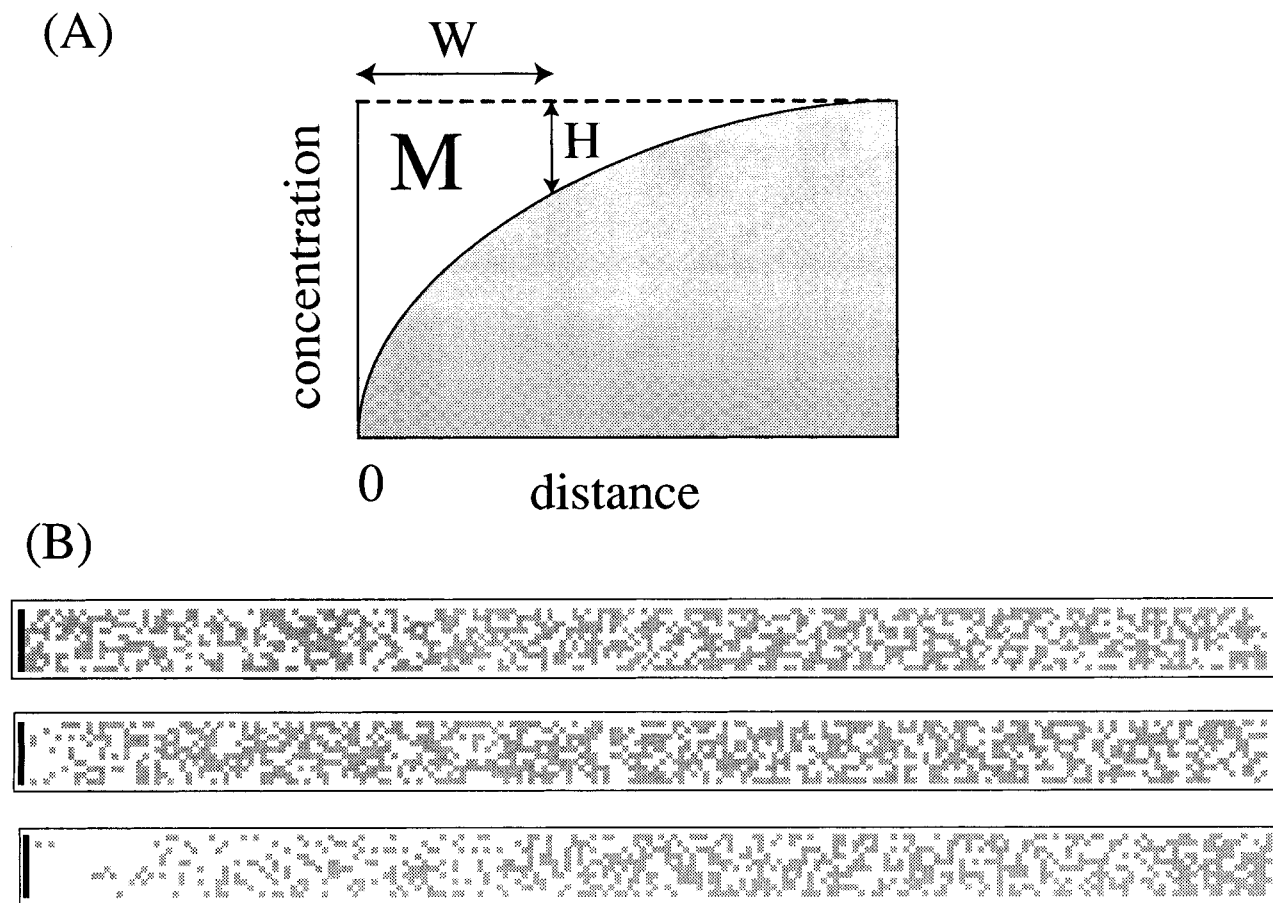
Figure 2. (A) Schematic of the depletion mass, M (e.g. $\rho(x,0) - \rho(x,t)$ as a function of distance from the trap. (B) Three snapshots, at $t = 0$, $t = 256$, and $t = 16\,384$ Monte Carlo time steps (top to bottom), which show the reactant depletion zone formed in a Monte Carlo computer simulation of a "trapping" reaction in a 2-D tube. "A particles" (gray pixels) are landed randomly on a 10×200 site square lattice. The "trap" (black pixels) is located at the 0 position of the x-axis of the lattice and spans its entire width (y-axis). Diffusion of the A particles is modeled as a random walk of the particles on the lattice. Boundary conditions are reflective, multiple site occupancy is allowed (except in the trap), and the initial reactant density is 0.75 particle/site. "Reaction" is simulated by removing from the system the particles that walk onto a trap site. The asymptotic time dependent rate coefficient recovered from the simulation is $k'(t) \sim t^{-1/2}$, in agreement with theory.

changes with time. A convenient way to do this is to measure the depletion mass, M, which is the area of the depletion zone in the particle density profile with respect to the vertical axis. Figure 2A is a schematic representation of the depletion mass M adjacent to a trap at some $t > 0$. Figure 2B shows snapshots of particles on a lattice in the presence of a trap at $t = 0$, 256, and 16 384 Monte Carlo time steps (see ref 22 for description of the simulation method). The formation of the depletion zone near the trap is clearly visible. It has been shown via Monte Carlo simulations[21] that the rate of the growth of M in time, $\partial M/\partial t$, scales as $t^{-1/2}$ asymptotically and that this scaling of $\partial M/\partial t$ with time is directly proportional to the scaling of the asymptotic trapping rate. We chose to measure the ρ_{O_2} depletion; thus samples were prepared with an excess of glucose to ensure that ρ_{O_2} was the limiting reagent. In the analysis of the experimental data, therefore, oxygen was considered to be the reactant A and we monitor the rate at which it is consumed by reaction with glucose at the acrylamide trap containing glucose oxidase. To observe the density profile in the vicinity of the trap (i.e., the depletion mass, M, in Figure 2) for the one-dimensional A + C → C system, the slit is positioned directly over the depletion mass, that is, directly over the volume where the reactant density is decreasing. The density of O_2 in the volume of solution adjacent to the acrylamide trap was monitored by measuring the instantaneous fluorescence intensity of the entire sample solution a distance $\leq$1.5 cm away from the trap. To calculate the slit width necessary to accommodate

a 12 h experiment, Einstein's diffusion equation, $x = (2Dt)^{1/2}$, was applied for O_2. Using $D = 2.6 \times 10^{-5}$ cm^2/s and a time of 12 h, a length of 1.5 cm is calculated. As long as the slit is this wide, we are able to monitor the entire depletion mass created by the trapping reaction. We are assuming an infinite source of reactant, which is essentially true in our 10 cm long capillary tube. We defined a quantity Z to be analogous to Y (see (9)), with respect to the fluorescence intensity. Thus, we obtain the experimental measure of the integrated rate law

$$Z \equiv \ln(I/I_0) \qquad (10)$$

where I_0 is the total (integrated) fluorescence, at $t = 0$, from the solution volume $\leq$1.5 cm away from the trap. (10) is proportional to Y since the fluorescence intensity (I) increases linearly as ρ_{O_2} decreases linearly. In addition, the minus sign in (9) disappears when Z is substituted for Y because the Ru(II) dye is *quenched* by O_2. On the basis of the theoretical predictions and our experimental observable, we expect an increase in fluorescence such that Z scales with the square root of time asymptotically. Figure 3 is a a plot of Z vs t on a log–log plot. The data were run through a computer program that averaged the points in such a way as to give equal weight to all points on a logarithmic scale. Data obtained from experiments with glucose were averaged over several experiments, and the average of the data obtained from experiments without glucose (blank run) was subtracted.
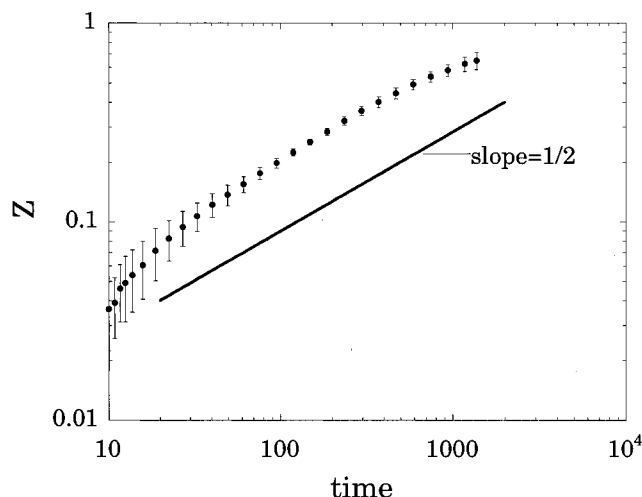
Figure 3. Integrated rate law measure Z vs time t on a log−log scale. The solid line has a slope of $1/2$. A linear least-squares fit of the data to a power law, $Z \sim t^x$, gives $x = 0.50 \pm 0.05$.

The behavior observed was an increase in the fluorescence, $I/I_0 \sim t^{0.50}$ (the value of the exponent is from the linear least-squares fit of the data to a power law). One may note in Figure 3 that the data appear to be falling away from the solid line, which has a slope of $1/2$, at early times. This is to be expected, since the $t^{1/2}$ behavior is theoretically an infinite time result. In addition, the fact that the power law is approaching $t^{1/2}$ from above is the norm and also is consistent with predictions for imperfect trapping rates. Simulations of the trapping reaction with different reaction probabilities (that is, the probability that a given collision between a reactant particle and the trap will result in a reaction) have been done by Schoonover.[21,23] For probabilities less than 1, his data fit powers of t that are greater than $1/2$ and less than 1 *at early time*, but as $t \to \infty$ the $t^{1/2}$ dependence is eventually obtained. This is in agreement with our observations of powers greater than $1/2$ at early times, which gradually approach the value $1/2$ asymptotically.

4. Summary

Using a spatially resolved technique, we tested quantitatively the spatial distribution of a reactant depletion mass and also observed the approach of the reaction rate, at an imperfect trap, from $R \sim t^x$, $1/2 < x < 1$, to the asymptotic $R \sim t^{-1/2}$ predicted for one-dimensional spaces. This was achieved using the catalytic oxidation of glucose to gluconic acid and water by the enzyme glucose oxidase, under spatially confined reactant conditions in capillary tubes. This catalytic enzyme−substrate reaction is a *chemical* analogue of a physical trapping reaction. It is also of importance to the design of glucose sensors for diabetics[31] and other biomedical applications.

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