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Enhancing the longevity of microparticle-based glucose sensors towards one month continuous operation

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Abstract

Luminescent microspheres encapsulating glucose oxidase have recently been reported as potential implantable sensors, but the operational lifetime of these systems has been limited by enzyme degradation. We report here that the longevity of these enzymatic microparticle-based sensors has been extended by the coimmobilization of glucose oxidase (GOx) and catalase (CAT) into the sensor matrix. A mathematical model was used to compare the response and longevity of the sensors with and without catalase. To experimentally test the longevity, sensors were continuously operated under normoglycemic dermal substrate concentrations and physiological conditions (5.5 mM glucose and 140 μ M O₂, 37°C and pH 7.4). The sensors incorporating CAT were experimentally shown to be ~5 times more stable than those without CAT; nevertheless, the response of sensors with CAT still changed by approximately 20%, when operated continuously for seven days. The experimentally-determined trends obtained for the variation in sensor response due to enzyme deactivation were in close agreement with modeling predictions, which also revealed a significant apparent loss in enzyme activity upon immobilization. It was further predicted via modeling that by incorporating 0.1 mM each of active GOx and CAT, the sensors will exhibit less than 2% variation in response over one month of continuous operation.

Keywords

Glucose; biosensors; luminescence; microparticles; finite element modeling; layer-by-layer self assembly

1. Introduction

Diabetes mellitus affects millions of people worldwide, leading to a huge economic and social burden on society. To reduce the risk of complications, Type I diabetics are counseled to frequently monitor blood glucose levels and take an appropriate dosage of insulin to restore normoglycemia. Recently, continuous glucose monitoring systems (CGMS) based on highly selective enzyme electrodes have been made available commercially; these sensors are inserted through the skin, much like an infusion set for an insulin pump (Klonoff 2005). However, these systems still suffer from poor stability/longevity (up to 7 days), potential infection of the percutaneous leads, slow response, susceptibility to electromagnetic interference, and unpredictable drift in the signal (Pickup et al. 2005a; Pickup et al. 2005b). Non-invasive optical glucose analysis methods have been investigated as attractive alternatives (Amerov et al. 2005; Stuart et al. 2005; Wan et al. 2005), but lack selectivity and exhibit poor sensitivity, making them unreliable for accurate glucose predictions.

To overcome the problems associated with abovementioned sensing techniques, an ideal approach would be to use optical “readout” to monitor a selective interaction between a specific receptor and glucose. We have recently reported one possibility for this in the form of enzymatic luminescent microspheres (Brown and McShane 2006; Brown et al. 2006; McShane et al. 2006; Stein et al. 2007; Stein et al. 2008). These systems employ a phosphorescent indicator to transduce oxygen, which is consumed in proportion to the glucose level in the reaction with glucose oxidase (GOx), to luminescence intensity or lifetime. They exhibit very high sensitivity and selectivity, tunable response range, and a response time of less than a minute *in vitro*. While this approach is very promising, there is concern about the operational lifetime due to the use of sensitive proteins; therefore, the longevity of these microsphere-based sensors must be investigated to determine the viability of this sensing approach for long-term *in vivo* glucose monitoring.

We investigated one means of extending the longevity of our GOx-based enzymatic microparticle sensors by drawing on success of electrochemical sensors (Baker and Gough 1993; Gough and Bremer 2000; Tse and Gough 1987), for which it has been reported that operating lifetime can be substantially improved via incorporation of a second enzyme, catalase (CAT). Hydrogen peroxide, which deactivates GOx via hydrolytic cleavage of peptide bonds, is a substrate for CAT; therefore, the incorporation of CAT minimizes the exposure of GOx to peroxide, enhancing stability (Tse and Gough 1987). Accordingly, the goal of this study was to assess the stability of microsphere-based glucose sensors with and without CAT. For this, a mathematical model was created to estimate the effect of immobilized enzyme (GOx and CAT) concentrations on the stability of sensor responses, and prototype sensors were then prepared and the models were validated through experimentation. The findings of this study point the way forward in the development of stable microparticle-based glucose sensors for *in vivo* application.

2. Experimental

2.1 Chemicals

Sodium alginate (low viscosity, 250 centipoise, MW 12–80 kDa), (3-glycidyloxypropyl)trimethoxysilane (GPTS), and ammonium hydroxide were obtained from Sigma and used for the synthesis of mesoporous alginate-silica (“algilica”) microspheres. Pt(II) octaethylporphine (PtOEP, Frontier Scientific), tetrahydrofuran (THF, Fluka), glucose oxidase (GOx, EC 232-601-0, 198 units/mg of solid, Sigma), catalase (CAT, EC 232-577-1, 2950 units/mg of solid, Sigma), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, Fluka), *N*-hydroxysulfosuccinimide sodium salt (NHSS, Toronto Research Chemicals Inc.), and sodium acetate (Sigma) were used to prepare PtOEP/GOx-doped algilica particles. Poly-(allylamine hydrochloride) (PAH, MW 70 kDa, Aldrich), poly-(sodium 4-styrenesulfonate) (PSS, MW 70 kDa, Aldrich), and sodium chloride (Sigma) were used during the deposition of multilayer thin films. Additionally, rhodamine B isothiocyanate (RITC, Aldrich) was conjugated to PAH (PAH-RITC), which was also used in LbL NF deposition. β-D-glucose (MP Biomedicals, Inc.), oxygen and nitrogen gases (PraxAir), and phosphate-buffered saline (PBS) (Sigma) were used during dynamic testing. All necessary pH adjustments were performed using titrations of 1.0 M HCl and 1.0 M NaOH (Fluka). All chemicals listed above were reagent grade and used as received. Ultrapure water with a resistivity of greater than 18 MΩ-cm was used to prepare all aqueous solutions. All experiments were conducted at 37°C and the oxygen concentration in the glucose and buffer reservoirs was maintained at 140 μM to mimic skin environment (Stucker et al. 2002).

2.2 Sensor preparation and characterization

Alginate microspheres were synthesized using a protocol detailed elsewhere (Stein et al. 2007; Stein et al. 2008). Briefly, alginate and GPTS were mixed in water, and ammonium hydroxide was added to the mixture to initiate microsphere formation. Dried microspheres were sent for surface area and pore size analysis (Delta Lab, North Huntingdon, PA). A particle size analyzer (ElZone 540, Micromeritics®) was used to measure the concentration and size distribution of the microspheres. The indicator dye, PtOEP, was loaded into the microspheres via insolubility-induced precipitation (Stein et al. 2007; Stein et al. 2008). Subsequently, two batches of PtOEP-loaded microspheres ($\sim 10^8$ microspheres/batch) were incubated in a 250 μ L acetate buffer solution (pH 4) containing enzyme. The first batch was incubated in a 0.2 mM GOx solution and the second batch in a GOx and CAT solution in which the concentration of each enzyme was 0.1 mM. A UV-Vis spectrophotometer (Lambda 45, Perkin Elmer) was used for absorbance measurements to determine enzyme uptake into microspheres (Ozyilmaz and Tukul 2007).

After four hours of incubation with enzyme, particles were rinsed with water and incubated in 50 mg/mL of EDC and NHS solution for two hours for covalent coupling of protein amines to alginate carboxylate moieties. After rinsing, the particles were coated with [PAH-RITC/PSS]₂-[PAH/PSS]₂₃-PAH NFs using the LbL technique (Stein et al. 2007). The NFs act as a transport barrier, allowing for the tailoring of the response range and sensitivity (Stein et al. 2008). The reference dye, RITC, was conjugated to PAH using a standard amine labeling protocol. The incorporation of RITC into the precursor layers provides reference fluorescence that is not sensitive to the ambient oxygen concentration, therefore enabling the sensing scheme to be insensitive to nonspecific signal modulations.

The sensor response was experimentally determined using a flow-through system controlled via a custom-designed software suite (LabVIEW, National Instruments), which has been described elsewhere (Stein et al. 2007). A fixed dissolved oxygen concentration was achieved by aerating the solution with a gas mixture of nitrogen and oxygen via glass diffusers. Precise control over dissolved oxygen concentration was attained via mass flow controllers connected to oxygen and nitrogen supplies. Briefly, $\sim 10^7$ microsensors were immobilized using double-sided pressure-sensitive adhesive attached to a glass slide, which was mounted inside a custom designed plastic reaction chamber. A bifurcated optical fiber bundle was coupled into the bottom of the reaction chamber to excite the sensors and collect the emitted fluorescence. A green LED with emission at ~ 518 nm was used as an excitation light source. Fluorescence signals were monitored using a charge-coupled device array detector (USB 2000, OceanOptics). Emission was recorded at 580 and 645 nm, corresponding to the emission maxima for RITC and PtOEP, respectively. The reaction chamber was placed inside an incubator at 37°C and all solutions were prepared in PBS to maintain physiological conditions.

2.3 Theory

The redox reaction of glucose and oxygen catalyzed by GOx may be expressed as

where G and O_2 are the GOx co-substrates, glucose and oxygen, respectively, E_{ox} and E_{red} are the oxidized and reduced forms of the enzymes, respectively, $E_{red}P_1$ and $E_{ox}'P_2$ are the enzyme substrate complexes; and k_1 , k_2 , k_3 , and k_4 are the rate constants of the respective reaction steps (Gibson et al. 1964).

The catalytic consumption of H_2O_2 via CAT can be expressed as

where I is the intermediate form of the enzyme, which is converted back to the native form of CAT by reacting with a molecule of peroxide; k_5 and k_6 are the rate constants of the reaction steps (Lardinois et al. 1996). If we combine reaction schemes (1) and (2), it can be observed that glucose and oxygen are the consumed substrates. Figure 1 depicts the model for the glucose sensing scheme. When sensors are exposed to bulk glucose and oxygen, these substrates diffuse inside the sensor matrix and trigger reaction (1) and, subsequently, reaction (2). In a few seconds, steady state is attained within the microspheres, in which the substrate spatial and average concentrations depend on the delicate balance between reaction and diffusion rates. The reaction-diffusion model has been used to describe such phenomena (Pao 1993). As bulk oxygen is assumed to be fixed, the reaction and diffusion rates primarily depend on bulk glucose concentrations. With increased glucose level, we expect to observe higher reaction rates, resulting in depleted oxygen levels inside microspheres. As the transduction mechanism is based on quenching of fluorescence by oxygen, an increased fluorescence will be observed for increased glucose levels. However, it is important to notice that the above scheme assumes constant concentrations of enzymes, which is not realistic. Enzymes, like any other protein molecules, undergo degradation via different mechanisms, which has been described in supplementary text S1.

The reaction schemes (1) and (2) can be combined together, resulting in a system of nine coupled PDEs that describe the behavior of the system in time and space. For purposes of this study, it was assumed that the boundary conditions for the sensor are constant and uniform throughout the surface of the sphere and therefore the solution only depends on the radial coordinate (r). The PDE used to model such system can be written as

$$\frac{\partial C_i}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} \left(-D_i r^2 \frac{\partial C_i}{\partial r} \right) = R_i \quad (3)$$

where i is the subscript denoting one of the nine reactive species, G , O_2 , E_{ox} , $E_{red}P_1$, E_{red} , $E_{ox}P_2$, H_2O_2 , I , and CAT . D_i and R_i represent the diffusivity and reaction rates of the respective species involved in the reaction scheme. To convert the predicted O_2 level to luminescence, the Stern-Volmer equation

$$\frac{F_0}{F} = 1 + K_{sv} \cdot [O_2] \quad (4)$$

was applied. In this equation, F_0 is the fluorescence intensity in the absence of O_2 , F is the fluorescence intensity in the presence of O_2 , $[O_2]$ is the oxygen concentration, and K_{sv} is the Stern-Volmer constant. For PtOEP immobilized in the algilica matrix, a previously-determined value for K_{sv} was used ($14,200 \text{ M}^{-1}$ (Stein et al. 2007)).

2.4 Simulations

The model described herein was designed to predict the enzymatic loss that occurs within microspheres when exposed to certain glucose and O_2 concentrations. To do this, it was assumed that at $t=0^-$ substrate concentration inside the sensors (algilica+film) was equal to the

bulk. The bulk glucose and O_2 concentration were set to 5.5mM (100 mg/dL) and 140 μ M, respectively, which are close to the physiological substrate level in normoglycemia (Stucker et al. 2002). At $t=0$, the reaction was “turned on,” and the diffusion-reaction process was modeled to predict the loss of GOx and CAT with time. The system of equation was solved for $t = 30$ days, and enzyme concentrations were extracted for intermediate time points $t = 0, 4, 7, 15$, and 30 days. The enzyme distributions at different time points for different kinds of sensors were plotted together to estimate the enzyme degradation that can be expected to occur after implantation.

The response of these sensors depends on the reaction-diffusion kinetics, which is expected to vary with time because of enzyme degradation. Therefore, the system of equations was also used to predict the corresponding drift in the response profile. For this, the bulk glucose concentration was varied from 0 to 33 mM (600mg/dL) in increments of 1.5 mM (20mg/dL). This provides 23 data points that were used in curve fitting using nonlinear least squares analysis. In these predictions, the pseudo-steady-state solution—the steady state solution at a given moment, considering no enzyme deactivation—was attained within two seconds; therefore, the solution at $t=20$ sec after each glucose step was taken as the steady-state sensor response. Finally, the response profile was constructed by plotting the percentage increase in luminescence versus glucose concentration, where luminescence intensity at zero glucose concentration was taken as the baseline. For these simulations, the initial condition for enzyme distribution was obtained from the longevity of simulations, where the enzyme distribution at each intermediate time point was predicted. In these simulations, glucose concentration was represented in SI units (molar); however, clinical units (mg/dL) for glucose measurements were used to show results (1 mM = 18.0 mg/dL).

The sensor model was solved numerically using diffusion mode in COMSOL 3.5a (COMSOL, Inc, Burlington, MA), which estimates the approximate solution using the finite element method. The solution of the model was obtained using parameters and assumptions that have been stated in the supplementary text S1. The output solution was plotted using COMSOL or was exported as a matrix file and imported into MATLAB (Mathworks, Natick, MA) workspace for further analysis.

2.5 Sensitivity and range calculations

The procedure used to estimate sensitivity and range from the experimental data have been described in detail elsewhere (Stein et al. 2007; Stein et al. 2008). Briefly, the data obtained from dynamic testing experiments were analyzed using a MATLAB script file, where the last 20 data points at each concentration step were taken to calculate the mean and standard deviation of the steady-state response. The percent increase from the baseline (0 mM glucose) was calculated at each concentration step. Finally, the response was plotted by taking the global average (average of three steady-state % increase in fluorescence) for each concentration. Equation (5) was fitted to the response curve, where I_{max} and k were estimated using the method of nonlinear least squares analysis (Levenberg-Marquardt algorithm). Sensitivity—defined as the slope of the linear response—was estimated by performing a simple linear regression on the first few data points such that $R^2 \geq 0.95$. Range was defined as the glucose concentration at which the response deviates from linearity by 10%.

$$I = I_{max} (1 - e^{-kc}) \quad (5)$$

3. Results and discussion

3.1 Sensor characterization

The mean diameter of the particles as measured with the Coulter counter was $\sim 12 \mu\text{m}$. For the microparticles loaded with GOx only, the enzyme concentration was found to be $\sim 0.2 \text{ mM}$ using UV-Vis absorption measurements. In the case of microparticles loaded with both GOx and CAT, the individual enzyme concentration was determined to be $\sim 0.1 \text{ mM}$ of each. Nevertheless, it is known that a significant loss in enzyme activity can occur after covalent immobilization of enzyme and, therefore, the concentration of the immobilized active enzyme will be less than the experimentally determined concentration, also referred as total enzyme concentration. The enzyme distribution, as determined via confocal microscopy, was found to be homogeneous, matching previous observations (Stein et al. 2007).

3.2 Estimated enzyme deactivation

The system of nine PDEs was used to model the enzyme degradation occurring inside microsenors during exposure to physiological levels of glucose and oxygen. Figure 2A shows the predicted active GOx concentration and its radial distribution at different time points, assuming sensors initially loaded with 0.2 mM GOx. It is obvious that a higher GOx degradation rate was estimated in the near center region compared to the near surface region. Peroxide-mediated deactivation was estimated to be slower in the near surface region, because the surface boundary condition assumed zero peroxide concentration. Moreover, it has been shown that the oxidized form of GOx is not subjected to peroxide-mediated deactivation, while all other forms are strongly affected (Kleppe 1966). The higher O_2 concentration in the near surface region results in an increased percentage of GOx to be present in the oxidized state, consequently slowing down the net deactivation rate of GOx. Almost 70% of the total GOx is lost within 4 days of continuous operation; this increases to more than 95% loss after 15 days.

In comparison, Figure 2B shows the active GOx concentration for sensors loaded with 0.1 mM GOx and 0.1 mM CAT. It is obvious that the degradation of GOx is expected to be much more uniform when CAT has been co-immobilized within the same matrix. The turnover rate of CAT is very high, which results in an immediate consumption of peroxide upon production; the effect on GOx, then, is a slower and more homogenous degradation by peroxide. Furthermore, only $\sim 2\%$ of the total GOx is lost within 4 days of continuous operation, with an increase to $\sim 11\%$ loss after 15 days. This theoretical analysis suggests a major impact on GOx stability with the inclusion of catalase.

Figure 3 shows the volume average concentration of active GOx or CAT present at any given time in both types of sensors. For sensors having no CAT, it is apparent that the average active GOx degradation follows an exponential decay profile. However, in the presence of CAT, the average active GOx concentration follows a more linear trend. The decay profile of CAT was predicted to be linear as well. The linear decay trend is due to the fact that peroxide mediated deactivation of both enzymes is minimized in the presence of CAT. Under these conditions, nearly all deactivation occurs via spontaneous denaturation, a process exhibiting first-order decay with infinitesimally small rate constants (k_{sGOx} and k_{sCAT}). Although first-order deactivation is expected to result in an exponential decay trend, one month is a very short time to observe the entire decay profile in this case and, therefore, the deactivation appears to be linear for the initial first month. As the spontaneous deactivation rate of CAT is almost twice of GOx, a faster deactivation of CAT was estimated for sensors containing both GOx and CAT. In addition, CAT is even more susceptible to the peroxide-mediated deactivation than GOx ($k_9 > k_8$) and the oxidized form of GOx is not deactivated by peroxide, whereas all forms of CAT are equally susceptible to peroxide.

3.3 Estimated calibration shift

As noted in the discussion of Figure 2, the loss of active enzyme is not always uniform throughout the microsphere. A changing enzyme distribution can influence the overall reaction-diffusion kinetics of the system, altering the sensor response. Therefore, when predicting the sensor response profile over different bulk glucose levels at different time points, the calculated enzyme distribution at that particular time was used for the response prediction. Figure 4A shows the predicted response of sensors loaded with 0.2 mM of active GOx. Although approximately 90% loss in active GOx was estimated in first 7 days, it seems that the response profile of the sensor was not proportionally affected. A decrease in sensitivity of only ~9% was predicted, along with no significant variation in response range. This latter observation leads to the interpretation that if the GOx concentration is higher than 0.02 mM, the sensing scheme will be primarily diffusion-limited. Therefore, in this situation, the effect of decreased enzyme concentration on the response was not significant. However, when the average GOx concentration in the microsphere falls below 0.02 mM, the predicted response was severely affected. At such low GOx concentration, a linear relationship between GOx decay and sensitivity loss can be noticed. This suggests that the sensing scheme at lower GOx concentrations (<0.02 mM) becomes reaction-limited. As long as the GOx concentration is such that the sensing scheme is not reaction limited, we will not expect a significant change in the sensor response.

Figure 4B shows the predicted response of sensors loaded with 0.1 mM GOx and 0.1 mM CAT. Comparing the initial response of these two types of sensors (without and with catalase, Figures 4A and 4B), it can be noticed that the sensors with CAT are expected to exhibit lower sensitivity than sensors without CAT. This observation can be attributed to the fact that the overall consumption rate of oxygen is lower for sensors with CAT; from equations (1) and (2) we can observe that one O₂ molecules is produced for every two O₂ molecules consumed. It can also be noticed that for sensors containing both GOx and CAT, less than 10% variation in the response range and sensitivity is expected even when continuously exposed to 100 mg/dL glucose for one month. As approximately 85% active GOx ([GOx] > 0.02 mM) is present even after a month, the sensing scheme is predicted to remain diffusion-limited and, therefore, a stable response can be expected.

3.4 Measured calibration shift

To validate the modeling results that predict an enhancement in stability with the inclusion of catalase, experiments were performed to compare the response of sensors with and without catalase over time. Figure 5 contains the measured response profiles for both sensor types. The initial response was measured for each sensor type immobilized inside the reaction chamber, after which the responses were measured after 4 and 7 days of continuous operation. The response of the fresh sensors was recorded under physiological conditions by exposing them to step changes in glucose concentration. Subsequently, the sensors were incubated in 100 mg/dL glucose for 4 days, after which the response was tested using the same procedure. After the second response measurement the sensors were again incubated in 100 mg/dL glucose for an additional 3 days, and finally the response profile was measured for the third time.

Figure 5A shows the response of sensors (without CAT) that was measured initially, after 4 days, and after 7 days. Approximately 40% loss in sensitivity was observed for the measurements performed after 4 days. For comparison, the model predicted less than 5% loss in activity for sensors loaded with 0.2 mM active GOx. Approximately 65% loss in sensitivity can be observed after seven days. The percent loss in sensitivity was almost uniform throughout the testing period, which suggests that the sensing scheme for these sensors was reaction-limited right from the very beginning. Therefore, perhaps more than 90% loss in the enzyme activity occurred because of immobilization. The discrepancies between theoretical and

experimental data are likely due to the fact that the enzyme immobilization via covalent attachment results in a significant loss of enzyme activity. Therefore, the concentration of the active enzyme immobilized in the microspheres is expected to be less than the value determined with spectrophotometric measurements. Since only total protein concentration can be measured via absorption measurements, and activity assays are confounded by transport resistance, accurate direct measurements of active enzyme were not possible.

Figure 5B shows the measured response of sensors that were loaded with both GOx and CAT. Using spectrophotometric absorption measurements, the concentration of GOx and CAT was determined to be 0.1 mM each. It can be noticed that the sensitivity values obtained from initial experiments were significantly lower than the measured sensitivities for sensors without CAT, as predicted from modeling. For these concentrations, modeling predicted almost no change in sensor response over 7 days of continuous operation. However, we observed ~8% and ~11% losses in sensitivity after 4 and 7 days, respectively, in experimental measurements. This again is likely due to the loss in active enzyme that occurred in the immobilization process. Therefore, the initial active enzyme concentration could be less than the one-tenth of the total immobilized enzyme concentration predicted using absorption measurements. Regardless of inaccurate values for active enzyme, a significant improvement in the stability of sensor response was observed with the coimmobilization of CAT with GOx. For a 7-day period, the sensitivity loss was reduced from 65% (no CAT) to 11% (with CAT), which can be considered as a substantial improvement in the stability of sensors. The observed loss in sensitivity is attributed to the loss in active GOx resulting from spontaneous and peroxide-mediated deactivation. In addition, the other reaction byproduct, gluconolactone, will induce a drop in the local pH, which has been reported to affect the spontaneous stability of GOx (Wilson and Turner 1992). The enzyme is most stable at around pH 5; below pH 2 and above pH 8, catalytic activity is dramatically decreased. When sensors are exposed to high substrate concentrations, high levels of peroxide and gluconolactone will be produced, which will result in faster enzyme deactivation. Nevertheless, a good correspondence was observed between the general response trends predicted via modeling and the trends that were experimentally determined.

3.5 Variation of k with time

Evaluation of the change in response over time was performed to more clearly understand the magnitude of calibration shifts with time. Equation 5 was used as a model equation to fit the response of sensors using nonlinear least squares analysis. The shape of the response profile and range primarily depend on k . Although sensitivity is directly related to R_{max} , it is difficult to draw conclusions based on R_{max} variation in luminescent sensors, as it is strongly affected by photobleaching. In contrast, k will only be affected by the enzyme concentration and distribution. Therefore, models were solved to predict the variation in k with time for sensors consisting of different GOx and CAT loadings. Figure 6 contains plots of k versus time for such sensors, wherein a decrease in k is understood to be an increase in the response range of the sensors due to the decreased overall reaction rate from less enzyme available for catalysis. A close correspondence between k and its decay trend can be observed between the experimental data and models for sensors loaded with 0.02 mM GOx (without CAT). This matches the previous discussion, and further supports the inference that almost 90% of the total enzyme lost activity during the immobilization process. These curves further illustrate that a noticeable change in k would not be expected for sensors loaded with 0.1 mM GOx and CAT. However, a linear decay in k is expected if the loaded concentration for each enzyme was 0.01 mM or less. The decay rate of k that we observed experimentally was in between the one predicted for 0.01 mM and 0.005 mM (GOx and CAT), suggesting the loss in active enzyme is perhaps 90–95% in this case. While these results highlight a major inefficiency in immobilization of enzymes, the experimental k decay rates reveal a reduction in the decay rate of k by five times due to the incorporation of CAT.

3.5 Conclusion

Considering the rate of change of k as a parameter defining the response stability of a sensor, the sensors obtained by the incorporation of 0.1 mM CAT can be considered as 5X more stable than sensors having no CAT, despite undergoing an estimated 90% loss in enzyme activity upon immobilization. Increasing the concentration of both active GOx and CAT to 10X is expected to result in a very stable response: only ~2% decrease in k value over a one month period, which translates to less than 5% drift in sensitivity and range. Increased enzyme loading can be attained by using microparticles with higher porosity than the particles employed here, which had a rather low porosity of 0.005. Mesoporous microparticles with porosity exceeding 0.5 can be synthesized (Katiyar et al. 2006), and we have already observed major improvements when mesoporous silica substrates were used for immobilization. Therefore, it is reasonable to believe that microsphere-based sensors exhibiting less than 10% deviation in sensitivity and range over a 3-month period can be fabricated. Such an approach could overcome one of the primary obstacles for deployment of enzymatic systems in minimally-invasive continuous glucose monitoring.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Schematic of microsphere sensors with dimensions used in the model.

Figure 2. Theoretical estimation of the radial distribution of GOx inside microsphere sensors at different time points during constant operation. (A) predicted active GOx concentration for microspheres loaded with GOx only. (B) predicted active GOx concentration for microspheres loaded with both GOx and CAT.

Figure 3.
Predicted volume average of the active enzyme concentration during constant operation for sensors loaded with GOx only and for sensors loaded with both GOx and CAT.

Figure 4. Theoretically predicted response of two types of sensors if they are continuously exposed to 5.5 mM glucose and 140 μM O_2 at 37 $^\circ\text{C}$. (A) response of sensors that are loaded with 0.2 mM GOx and (B) response of sensors that are loaded with 0.1 mM GOx and 0.1 mM CAT.

Figure 5. Experimentally determined response of both types of sensors that were continuously exposed to 5.5 mM glucose and 140 μ M O_2 at 37 $^{\circ}$ C. (A) response of sensors loaded with 0.2 mM GOx and (B) response of sensors loaded with 0.1 mM GOx and 0.1 mM CAT

Figure 6.
Theoretically predicted and experimentally determined variation in the curve fit parameter, k , with respect to time.