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Enzymatic Glucose Sensor Compensation for Variations in Ambient Oxygen Concentration

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Abstract

Due to the increasing prevalence of diabetes, research toward painless glucose sensing continues. Oxygen sensitive phosphors with glucose oxidase (GOx) can be used to determine glucose levels indirectly by monitoring oxygen consumption. This is an attractive combination because of its speed and specificity. Packaging these molecules together in “smart materials” for implantation will enable non-invasive glucose monitoring. As glucose levels increase, oxygen levels decrease; consequently, the luminescence intensity and lifetime of the phosphor increase. Although the response of the sensor is dependent on glucose concentration, the ambient oxygen concentration also plays a key role. This could lead to inaccurate glucose readings and increase the risk of hyper- or hypoglycemia. To mitigate this risk, the dependence of hydrogel glucose sensor response on oxygen levels was investigated and compensation methods explored. Sensors were calibrated at different oxygen concentrations using a single generic logistic equation, such that trends in oxygen-dependence were determined as varying parameters in the equation. Each parameter was found to be a function of oxygen concentration, such that the correct glucose calibration equation can be calculated if the oxygen level is known. Accuracy of compensation will be determined by developing an overall calibration, using both glucose and oxygen sensors in parallel, correcting for oxygen fluctuations in real time by intentionally varying oxygen, and calculating the error in actual and predicted glucose levels. While this method was developed for compensation of enzymatic glucose sensors, in principle it can also be implemented with other kinds of sensors utilizing oxidases.

Keywords

enzymatic glucose sensors; oxygen compensation; luminescence lifetime

1. Introduction

In recent years, there has been a growing demand to measure glucose concentrations in many biotechnology industries. The increasing number of people with diabetes, in

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particular, has led to an increased need for *in vivo* sensors to monitor blood glucose levels in order to prevent long-term health complications.¹⁻²

Most proposed glucose sensors are electrochemical in nature, and utilize glucose oxidase (GOx) as a means of signal transduction because of its specificity, stability, and high turnover rate.³ Although glucose cannot be measured directly, this enzyme can produce a measurable signal using a variety of means as can be seen in the reaction equations:

where E_{ox} is the oxidized form of GOx, G is glucose, E_{red} is the reduced form of GOx, GDL is glucono- δ -lactone, O_2 is oxygen, H_2O_2 is hydrogen peroxide, and the k_n terms refer to the binding or reaction rates. It is also important to note that GDL will eventually hydrolyze to gluconic acid. Glucose levels can then be determined indirectly by monitoring consumption of oxygen, the production of hydrogen peroxide, or the change in pH.²

Commercially available glucose sensors typically rely on the amperometric determination of hydrogen peroxide (H_2O_2). However, the electrodes employed are susceptible to interference from a variety of chemicals which can lead to inaccurate analyte measurements.⁴⁻⁶ As an alternative, optical sensors have been gaining interest in recent years due to their measurement flexibility, fast response time, reversibility, and nanoscale resolution.⁷⁻⁸ In addition, luminescent detection of oxygen has been shown to be an effective method of glucose detection where the luminophores utilized are able to provide high selectivity and sensitivity compared to amperometric methods.⁹⁻¹³

A challenge to implementing both types of sensors is the dependence of the response on ambient oxygen concentrations.^{9-10, 14} Because GOx uses both glucose and oxygen as substrates, the response will be dependent on the concentration of both analytes.¹⁴ This could be especially problematic for *in vivo* applications where oxygen concentrations cannot be controlled. An example of the effect varying oxygen and glucose levels can have on a sensor response can be seen in Figure 1. The results were obtained through modeling of a sensor response using parameters and settings listed below. Initial conditions at time = 0 were 0 mM glucose and 250 μ M oxygen for each transient response. (A) The glucose concentration is much larger than oxygen concentration, leading to quick depletion of oxygen inside of the sensor. (B) Oxygen is larger than glucose so the response is determined by the rate of reaction of the enzyme. (C) Oxygen is much larger than glucose. Again the response is determined by the reaction rate but the time to reach steady-state is slower and the magnitude of the response is reduced. (D) When the sensor is exposed to zero oxygen and glucose, the response is dependent on the diffusion of oxygen out of the matrix. Although this is much slower than the enzymatic reaction, the response will eventually reach the same steady-state magnitude as that in (A). (E) and (F) also show a zero glucose response but with oxygen concentrations slightly lower and higher than the initial concentration, respectively. Despite this obvious dependence of enzymatic sensor response

on ambient oxygen levels, relatively little work has been performed in attempt to find a solution for this problem.

Zhang *et al.* were the first to investigate the oxygen-dependence problem using amperometric sensors.¹⁴ They found that by reducing the diffusion of glucose into the sensor, oxygen-dependence was also reduced. However, sensitivity is sacrificed in this process and low oxygen levels could conceivably still lead to an oxygen-dependent response. Other methods to reduce the oxygen-dependent response of amperometric sensors include supplying oxygen internally¹⁵ and circumventing the use of oxygen by wiring the enzyme directly to an electrode using a mediator.¹⁶ However, these approaches are not completely oxygen-dependent.^{6, 9}

Rather than reduce oxygen-dependence of the sensor response, another approach is to incorporate a second sensor to monitor ambient oxygen concentrations.^{6, 9, 17} This method is usually utilized for sensors that monitor consumption of oxygen. This method was first demonstrated using luminescence by Li *et al.* who used a glucose sensor and a reference oxygen sensor in a fiber optic probe.⁹ The oxygen sensor was identical to the glucose sensor, with the exception that GOx was excluded from the sensing matrix. By measuring the response of both sensors simultaneously, glucose levels were related to the difference in the oxygen concentration at each sensor.^{9, 17} They also found that sensors with lower glucose sensitivity were less dependent on ambient oxygen concentrations. Pasic *et al.* used a similar approach where the difference in oxygen concentration from the glucose sensor to a reference oxygen sensor was used to determine glucose concentrations.⁶ The amount of oxygen-dependence was then determined by analyzing the results using Michaelis-Menten kinetics similar to previous methods.⁴ In all cases, this approach was only demonstrated for sensors with a linear response and will probably not be applicable with non-linear responses.

Another approach was proposed by Wolfbeis *et al.* where the *difference* in oxygen concentration at the glucose sensor and at the reference oxygen sensor plays a key role.¹⁰ With this approach, however, several assumptions must be made: the oxygen and glucose sensor have similar oxygen responses, the difference in oxygen concentration between each sensor is linear with glucose levels, and this difference must also be proportional to ambient oxygen concentrations. Using both linear and non-linear Stern-Volmer relationships and these assumptions, equations were derived that could calculate glucose concentrations with variable ambient oxygen concentrations. Although these equations were straightforward, the authors did not show any *in vitro* testing results; thus, the validity of this method has not been demonstrated.

These methods are limited to sensors with a specific kind of response because of the assumptions made in the compensation algorithm. We propose the development of a new oxygen compensation technique that can be applied to any glucose sensor with the aid of a reference oxygen sensor. This technique will remove many assumptions, but will require more thorough calibration of the glucose sensor so that trends in the oxygen-dependent glucose response can be found. In principle, accurate glucose measurements can be made at any ambient oxygen concentration.

2. Materials and Methods

2.1 Modeling with Finite Element Analysis

Initially, static and transient modeling of enzymatic optical glucose sensor response was performed using COMSOL Multiphysics, similar to previous reports.¹³ To begin, a 1-D model was used to simulate a hydrogel slab with immobilized GOx. To simulate the GOx reaction, the following simplified reaction equation was utilized:¹⁸

$$\frac{[GOx]}{R} = \frac{1}{k_3} + \frac{k_2 + k_3}{k_1 k_3 [G]} + \frac{1}{k_4 [O_2]} \quad (3)$$

where $[GOx]$, $[G]$, and $[O_2]$ are the concentration of GOx, glucose, and oxygen respectively. The reaction rate constants are represented by k_1 , k_2 , k_3 , and k_4 and R is the reaction rate. The values used for these and other parameters used in the model can be found in Table 1, unless otherwise stated.

The steady-state ambient glucose and oxygen concentrations used as constant-concentration boundary conditions were changed to allow observation of the oxygen-dependent response of the glucose sensors. The apparent volume-averaged optical signal was calculated by first averaging the steady-state oxygen concentration inside of the hydrogel and determining the luminescent lifetime response of the sensor using a Stern-Volmer relationship:

$$\frac{\tau_0}{\tau} = 1 + K_{sv} [O_2] \quad (4)$$

where τ_0 is the lifetime in the absence of the quencher, τ is the lifetime at each oxygen concentration, $[O_2]$, and K_{sv} is the Stern-Volmer constant. The values for τ_0 and K_{sv} were previously obtained experimentally (Table 1). The glucose response at each oxygen concentration was then analyzed for trends in the response using MATLAB.

2.2 Sensor Formulation

Following modeling of sensor response, enzymatic glucose sensors were fabricated using a procedure similar to one previously reported.¹⁹ Unless otherwise stated all chemicals were from Sigma-Aldrich and were used as received. A custom tetra-methacrylated porphyrin (TMAP) was made by functionalizing palladium(II) meso-Tetra(4-carboxyphenyl) porphine (Frontier Scientific) with 2-aminoethylmethacrylate in order to easily immobilize it in the sensor matrix (see Acknowledgements). A 5 mM solution of TMAP was made by dissolving it in dimethyl sulfoxide. A precursor solution for the sensor matrix was made by mixing 2.28 g acrylamide (Amresco), 3.87 ml of 2-hydroxyethylmethacrylate (HEMA, Polysciences), 192 μ L tetraethylene glycol dimethacrylate (TEG-DMA, Polysciences), 3.75 mL of ethylene glycol, and 2 mL of de-ionized (DI) water. 6.6 mg of glucose oxidase (EC 1.1.3.4, BBI Enzymes) and 5.2 mg of catalase (EC 11.1.1.6) were dissolved in 100 μ L and 75 μ L of DI water, respectively. To initiate polymerization, 1.8 mg of 2,2-dimethoxy-2-phenylacetophenone was dissolved in 775 μ L of the precursor solution. 50 μ L of TMAP solution was then added followed by addition of the enzyme solutions. This solution was

then placed between two glass slides using a 750 micron Teflon spacer. The solution was then polymerized by exposing to UV light for 3 minutes on each side. Following polymerization, the gel was placed in 20 mL of PBS with 20 mg of N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and 46 mg of N-hydroxysulfosuccinimide sodium salt (NHS, Toronto Research Chemicals) for >24 hours to covalently cross-link the enzymes to the matrix. After 24 hours, the gel was rinsed and placed in PBS until tested.

2.3 *In vitro* Testing

Testing of the glucose sensor response was performed using a test system automated by custom LabVIEW software (National Instruments), similar to one previously reported.¹³ Glucose concentrations were controlled by mixing a glucose/PBS solution and a PBS solution using positive-displacement pumps. The 10 mM phosphate-buffered saline (PBS) solution was made by dissolving 8 g sodium chloride, 0.2 g potassium phosphate monobasic, 1.15 g sodium phosphate dibasic, and 0.2 g potassium chloride for each liter of DI water. Nitrogen (Acetylene Oxygen Company) and compressed air were also used to control the oxygen concentration in the solutions using mass flow controllers (MKS Instruments).

To test a sensor, a 2.5 mm biopsy punch was used to remove a sample from the hydrogel created. The sensor was then immobilized in a custom reaction chamber inside of an incubator held at 37°C. Three sensors were tested at three different oxygen concentrations (132.75, 199.125, and 265.5 µM). Incremental glucose solutions at a set oxygen concentration were then pumped into the reaction chamber and the lifetime response recorded using a custom time-domain lifetime measurement system.²¹

3. Results and Discussion

3.1 Modeling Results

To investigate the oxygen-dependent response of enzymatic glucose sensors, the model previously described was used to predict the response of sensors exposed to four different oxygen concentrations (100, 150, 200, and 250 µM) and a range of glucose concentrations (0-25 mM). The steady-state response at each glucose and oxygen concentration can be seen in Figure 2. As has been observed in our previous theoretical and experimental work with microspheres using the same sensing chemistry, as oxygen levels increase, the range of the sensor decreases while the sensitivity increases.¹² The modeled responses were then fit using:

$$\tau \frac{A}{(1 + Be^{-C[\text{glucose}]})} + D(1 - Ee^{-F[\text{glucose}]}) \quad (5)$$

where τ is the lifetime, $[\text{glucose}]$ is the glucose concentration, and A - F are the fit parameters. For all fits, R^2 was greater than 0.99. The parameters A - F are plotted in Figure 3 to illustrate how they vary as a function of oxygen concentration. It is clear that these values increase or decrease monotonically with oxygen concentration. Therefore, it was anticipated

that measurement of oxygen would enable direct calculation of the corresponding parameter value, resulting in an accurate equation for relating lifetime and glucose concentration. In order to provide a means of compensation, trends in each parameter as a function of oxygen concentration were fit using simple linear regression. Using the parameter values obtained from these linear fittings, R^2 values for Equation 5 were re-calculated and again found to be greater than 0.99 for each oxygen-dependent response. This indicates for a given oxygen concentration, a variable, oxygen-dependent calibration curve can be used to accurately predict glucose concentrations.

Using these equations, the benefits of oxygen compensation were examined by comparing to the error in the glucose prediction for a lifetime at a given oxygen and glucose concentration using a *static* calibration curve (*i.e.* one that does not change with oxygen concentration). For this analysis, the calibration curve obtained for 200 μM oxygen was used. The percent error was also determined when compensation is applied and can be seen in Table 2.

In several cases, glucose levels could not be predicted when there is not any oxygen compensation because the lifetime fell outside of the realistic range of values. This is due to the asymptotic nature of the fits or the prediction of a negative glucose value. However, when compensation is employed, there is a considerable improvement in the accuracy of the predicted glucose concentration; in some cases more than two orders of magnitude. In fact, only at extremely low glucose levels (physiologically irrelevant) is the error predicted to exceed 27% when compensation is applied. In contrast, the error from a static calibration model increases as the oxygen goes further from the level used for calibration and renders the calibration clearly unusable.

3.2 *In vitro* Experimental Results

Glucose responses of three different hydrogel sensors were measured at different ambient oxygen concentrations. The lifetime results recorded for each glucose concentration can be seen in Figure 4. As can be seen, the responses follow a similar sigmoidal same shape as the modeled results. Again, as oxygen levels increased, the response was shifted towards lower glucose levels. The *in vitro* data also showed that the sensor quickly consumed all of the available oxygen at higher glucose levels independent of the ambient oxygen concentration. This is in contrast to the *in silico* data seen in Figure 2, which shows similar trends but the model predicted that the response would settle on different final (maximum) lifetime values than what was observed experimentally. Equation 5 was again used to fit the glucose responses; however, a reliable relationship between the fit parameters and the oxygen concentration was not found (data not shown). This can be attributed to the variability in the response from sensor to sensor and the *lack* of variability at higher glucose levels especially at the two lower oxygen concentrations (compared to Figure 2, these saturate at very similar lifetimes). Future work will require more thorough testing of glucose sensors so that trends can found in the response curves.

4. Conclusions

Oxygen-dependency of enzymatic, glucose sensors was investigated *in silico* using finite element analysis software and *in vitro* using a custom glucose response measurement

system. Fittings of the modeled data showed a relationship between oxygen concentration and the calibration curve parameters. With this information, unique calibration curves can be obtained leading to improved accuracy of glucose predictions. Although *in vitro* results do not currently demonstrate a similar relationship, we believe this method will be useful for future studies using glucose and other types of sensors.

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Figure 1.

These modeling results show the transient response of an enzymatic glucose sensor for different oxygen and glucose levels. See text for more information.

Figure 2.

These modeled results show the steady-state response of an enzymatic glucose sensor for different levels of oxygen and glucose.

Figure 3. Oxygen-dependent parameters found from fitting the modeled steady-state data. Lines indicate the linear trend of each parameter which are used for oxygen compensation.

Figure 4.

In vitro results can be seen above for three different ambient oxygen concentrations.

Table 1

Values and description of the parameters used to model enzymatic, glucose sensor response.

Parameter	Value	Description
h	750 μm	Height of hydrogel
m	1 μm	Mesh size of model
$[GOx]$	20 mM	Concentration of GOx in the sensor matrix
D_g	2.5e-10 m^2/s	Diffusivity of glucose in the hydrogel ¹⁹
D_o	8e-10 m^2/s	Diffusivity of oxygen in the hydrogel ²⁰
k_1	2100 $\text{L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$	Binding rate of glucose to GOx ¹⁸
k_2	90 s^{-1}	Dissociation rate of glucose from GOx ¹⁸
k_3	784 s^{-1}	Rate of glucose oxidation ¹⁸
k_4	1.2e6 $\text{L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$	Rate of H_2O_2 release ¹⁸
τ_0	470 μs	Un-quenched lifetime
K_{sv}	0.12e6 μM^{-1}	Stern-Volmer constant

Table 2

Calculated percent errors in glucose prediction for can be seen below. The shaded rows represent error calculated for a static calibration curve (at 200 μM oxygen) while non-shaded rows represent the error when compensation is performed by varying the calibration curve as needed. a) Lifetimes were above the maximum possible value for the calibration fit (at 200 μM oxygen) preventing a glucose prediction. b) Lifetime was below the minimum expected lifetime leading to a negative glucose prediction.

Oxygen Concentration (μM)	Cal. Type	Glucose Concentration (mM)							
		0.1	0.5	1	2	5	10		
100	Static	-324.0	-494.8	-677.7	X ^a	X ^a	X ^a		
	Comp.	39.0	4.6	-9.2	-26.8	4.4	13.0		
150	Static	-131.0	-64.6	-94.8	-137.9	-185.6	X ^a		
	Comp.	40.0	-11.2	21.9	7.8	15.5	25.3		
250	Static	X ^b	45.4	24.1	51.0	50.2	54.7		
	Comp.	-102.0	-6.2	-16.4	-20.2	-26.0	-133		