Published in final edited form as:

Sens Actuators B Chem. 2012 November ; 174: 373–379. doi:10.1016/j.snb.2012.08.046.

Silica-dispersed glucose oxidase for glucose sensing: in vitro testing in serum and blood and the effect of condensation pH

James M. Harris, Gabriel P. Lopez, and William M. Reichert*

Department of Biomedical Engineering Duke University Durham, NC 27708-0281

Abstract

The objectives of this study were to examine the feasibility of using glucose oxidase (GOx) dispersed in a silica matrix for glucose monitoring in whole blood, and then to assess whether the flexibility of silica sol-gel chemistry could be exploited to enhance glucose sensor performance and stability. Silica-dispersed GOx was deployed on platinized platinum (Pt) wire to form a Clark-type amperometric glucose sensor. Sensors were calibrated using buffered glucose standard solutions, and then tested against glucose spiked human serum and whole blood. All serum and whole blood measurements met the minimum FDA requirement of falling within the “A+B region” of a Clark Error Grid. To our knowledge this is the first report of using silica-dispersed GOx to measure glucose in whole blood. The effect of condensation pH on sensor performance was assessed by dispersing GOx in silica condensed at pH 3, 7 and 12, and then testing the sensor response against glucose calibration standards. The pH 12 silica sensors had statistically faster response time, and higher sensor sensitivity compared to pH 7, pH 3 silica and glutaraldehyde crosslinked sensors. Membranes of the pH 12 silica had statistically higher glucose diffusion coefficient than did the pH 7 and 3 sensors. GOx dispersed in pH 12 silica also had the longest half life. We hypothesize that the gel-like pH 12 silica gels provided reduced barriers to glucose diffusion, and the more aqueous microenvironment provided greater stability for the enzyme.

Keywords

Glucose sensing; silica; condensation pH; glucose oxidase; glutaraldehyde; blood; serum

INTRODUCTION

Glucose oxidase (GOx) mediated amperometric sensing has been the gold standard for monitoring blood glucose since Leland Clark first designed a H₂O₂ based enzyme electrode to measure the conversion of glucose to gluconic acid. A number of FDA approved glucose monitors continue to utilize modifications of the Clark electrode design, including the Abbott Freestyle Navigator®, DexCom STS®, Medtronic iPro2 CGMS®, and Medtronic Guardian RT®. All of these devices employ GOx immobilized at the surface of the working electrode and covered by a glucose restriction membrane, typically polyurethane.

The most common mode of GOx immobilization is curing with homo-bifunctional glutaraldehyde that covalently crosslinks the adsorbed [1] or chemically tethered enzyme

© 2012 Elsevier B.V. All rights reserved.

*corresponding author (reichert@duke.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

[2–4]. However, glutaraldehyde can also affect catalytic or structural amino acids populating the active site of the enzyme that can compromise enzyme catalytic power and lifetime [5]. Finding an alternative to glutaraldehyde crosslinking of GOx that does not chemically react with the enzyme and that provides greater steric support is attractive. For acute applications such as glucose monitoring of blood droplets, the negative effects of glutaraldehyde crosslinking on enzyme function is minimal; however for longer-term applications these effects could become significant.

Silica serves as a well-established dispersing material for proteins because it is non-toxic, chemically and biologically inert, subject to negligible hydration swelling, hydrophilic, and inexpensive to synthesize [6, 7]. Researchers have established silica sol gel encapsulation as an effective means of stabilizing enzymes [8–12]. The improved enzyme stability in silica arises from matrix-protein interactions that constrain the protein conformational state, such as surface hydration [13], hydrophobic interactions [14], electrostatic forces [15, 16], surface curvature [17, 18], and macromolecular crowding [14]. Constraining the enzyme's conformational changes also reduces enzyme susceptibility to proteolytic degradation [19, 20], an important factor *in vivo*. A number of researchers have reported the use of silica-dispersed GOx for glucose sensing [8, 21–26], only three of which tested their sensor in human serum, and none of which have tested their sensor in either whole blood or intact tissue.

Sol gel synthesis of silica is a two-step chemistry: hydrolysis of the silicon alkoxide precursor, and condensation of the silanol intermediates. Control of the buffer pH during the condensation reaction dramatically changes the properties in silica [27]: acidic conditions promote cluster-cluster growth resulting in branched surface fractals and a dense and brittle solid; basic conditions promote non-fractal particulate sol formation and a less dense and soft gel; neutral pH values produce silica of intermediate density and hardness.

In the current study, a simple three wire Clark-type glucose sensor was produced by deploying silica-dispersed GOx on a platinized Pt working electrode. The sensors were calibrated using standard solutions of buffered glucose, and tested against glucose spiked human serum and whole blood. All serum and whole blood measurements met the minimum FDA requirement of falling within the “A+B region” of a Clark Error Grid [28]. The effect of condensation pH on sensor performance was assessed by encapsulating GOx in silica at pH 3, 7 and 12, and then testing the sensor response against glucose calibration standards. These results were correlated with measurements of glucose diffusion and GOx half life in silica produced at the different condensation pH values.

To our knowledge, this is the first report of using silica-dispersed GOx based sensors to measure glucose in whole blood. Our research utilized a simple Clark-type electrode to allow for proper parity to be drawn against other works but the novelty our work hinges optimization of the silica gel using varying condensation pHs. The literature lacks studies on this subject and our report would be the first to claim a higher pH produces a favorable microenvironment for the GOx and favorable mass transport conditions that benefit the sensor performance. It is also the first report that examines whether varying the silica sol-gel chemistry could be exploited to improve glucose sensor performance and stability.

MATERIALS AND METHODS

Reagents

GOx was purchased from Sigma Aldrich (Lot: 040M1349) and EMD Bioscience/Calbiochem (Lot: D00099683). Lead (II) acetate trihydrate (Lot: 03697PJV), chloroplatinic acid. Sulfuric Acid was purchased from JT Baker. D-Glucose was purchased from Sigma

Aldrich (Lot: 040M6185). Phosphate buffered saline was purchased from EMD Bioscience (Lot: 22706212). Milli-Q water was obtained from university facilities. Tetramethyl orthosilicate was purchased from Alfa Aesar (Lot: USLF001196). The pH meter calibration standards were purchased from VWR International (pH 4.0 Lot: 2011506, pH 7.0 Lot: 1008163, pH 10.0 Lot: 2011584). The storage solution for the Ag/AgCl was purchased from VWR (Lot: OP1). THF was purchased from Sigma Aldrich (Lot: 16996TMV).

Electrodes

Platinum wire, silver wire and the potentiostat were purchased from Pine Research Instrumentation (Wave-Now). The 0.5mm diameter Pt wire for the working electrodes was purchased from Sigma Aldrich. The Ag/AgCl reference electrode and Pt counter electrodes were purchased from CH Instruments. The working electrode was “platinized” by exposing segments of 0.5mm ID Pt wire to 0.072 M (3.5%) chloroplatinic acid and 1.3×10^{-4} M (0.005%) lead acetate at a current density of 30 mA/cm² for 10 minutes in a potentiostat [29].

Enzyme-silica matrix

GOx was applied to the working electrode either by encapsulation in a silica matrix or through glutaraldehyde crosslinking as described below. A 2wt% solution of polyurethane (equal parts Tecoflex and Hydrothane in 50:50 THF and ethanol) was applied to the working electrode and allowed to dry at room temperature. Prepared working electrodes were used 24 hours following fabrication.

Using the technique of Gupta et al. [30], a silica sol gel was prepared by chemical vapor deposition of TMOS into buffered saline (either pH 12, 7 or 3). 4mg of the resulting silica gel was mixed with 100 μ L of GOx creating a paste with 40mg/mL GOx. 10 μ L of the GOx paste was applied to 1–2cm length of the working electrode creating a GOx coating with 400 μ g load of enzyme. Following the protocol of Zodak [31] or glutaraldehyde crosslinking of GOx, 15 μ L of 27mg/mL GOx in PBS buffer was mixed with 1.7 μ L of 8% glutaraldehyde (v/v) in a micro centrifuge tube and allowed to cure for 1 hr. 10 μ L of the cross-linked GOx was added to a 1–2cm length of the working electrode creating a GOx coating with 400 μ g load of enzyme. In both cases, the applied layer of GOx was allowed to cure for 1h before the final layer of polyurethane was added.

Flow cell configuration

Figure 1A shows the overall sensing configuration. The three wire electrodes were placed in a 1.5cm $\times$ 5.5cm $\times$ 1.2cm laminar flow cell perpendicular to the direction of fluid flow and held in place by a 1.5cm thick silicon rubber gasket sandwiched between two polycarbonate plates. The gasket defined a flow cell volume of 10mL. Approximately 60 cm of a 0.32cm ID silicone rubber tubing was connected to the flow cell inlet and outlet and to a Master flex® pump. The total volume of the flow loop and flow cell was 15mL. Test solutions were introduced into the flow loop using a 3-way stopcock and circulated thorough the flow cell at 1.8mL/min. Per Abdel-Humid et al. [32], the electrodes were connected to the potentiostat at a voltage difference of 0.536V between the working and counter electrodes was established. This voltage difference was used because of the noted benefits of operating at lower voltages in minimizing the effect electro-active interfering species. The currents between the working and counter electrode detected by the potentiostat during glucose measurements were on the order of 0–200 μ A.

Sensor calibration and response time

The sensor current was calibrated using a series of glucose in PBS standard solutions (0mM, 2.3mM, 6.6mM, 15mM, and 25mM) before each measurement series. Upon the introduction of each glucose standard the sensor current increased rapidly and then leveled off at a new baseline (Figure 2). The sensor sensitivity was the slope of the baseline sensor current versus glucose concentration plot. The sensor response time was the duration between the introduction of the glucose standard and the establishment of 99% of the next baseline current. The limit of detection (LOD) was estimated using the method of Cunningham [33]

$$\text{LOD} = (k\epsilon_r) \times [\text{Sensitivity of sensor}]^{-1} \quad \text{Equation 1}$$

where k is the number of standard deviations of separation that constitutes 'different' from noise, ϵ_r is the standard deviation from the amperometric signals ($n=5$) for 2.3 mM glucose samples.

Serum and whole blood measurements

Human whole blood was acquired according to Duke University IRB protocol 2257-08-1R17ER. 50mL of blood was collected in sealed tubes from healthy volunteers and refrigerated, then and used within 24 hrs. Heparin was utilized as an anticoagulant for the whole blood. Human serum was obtained by spinning 50mL of whole blood at 1500g for 10 minutes in a 50mL conical tube. Serum or whole blood was introduced into the flow system by an injection loop (12.7 cm length 3.175 cm ID silicone tube) and the samples were spiked by injections of glucose solutions into the flow loop. The glucose concentration of each sample (whole blood and serum) was determined using the pH 7.0 silica glucose sensor in a simple three electrode setup. The blood and serum samples were only exposed to air prior to use and were transferred to an oxygen limited flow cell. To avoid biofouling, the silica sensor was replaced after a single use. The glucose concentration of each serum and whole blood test solution was determined independently using an OneTouch® Ultra hand held glucose meter.

Glucose Diffusion Determination

The diffusion of glucose across silica membranes was determined using a two chamber configuration. Silica membranes were prepared on Transwell® membrane plates overnight a condensation pH 3, 7, and 12 based similar to the approach described in the enzyme-silica matrix section.

The polyurethane layer was added to silica membranes outer layer. The bottom chamber of the transwell was filled with 10mM PBS buffer at pH 7.0 and the upper chamber above the silica membrane was filled with 25mM (450mg/dL) glucose in 10 mM PBS buffer at pH 7.0. At set time intervals the concentration of glucose in the bottom chamber was determined using an OneTouch® Ultra glucose meter. The diffusion coefficient was determined using Quasi Steady State analysis of Fick's Law [34]. The diffusion coefficient of the polyurethane blend was also determined ($4.2 \pm 1.4 \times 10^{-6} \text{ cm}^2/\text{s}$) and subtracted from the diffusion coefficients calculated from the silica membranes using Equation 2, where D_{PU+Si} is the combined diffusion coefficient of polyurethane and silica, D_{PU} is the diffusion coefficient of polyurethane, and D_{Si} is the diffusion coefficient of silica. T-tests were used to determine statistical significance seen in the sensors response.

$$[D_{PU+Si}]^{-1} = [D_{PU}]^{-1} + [D_{Si}]^{-1} \quad \text{Equation 2}$$

Scanning Electron Microscopy

Scanning electron microscopy was used to image bare and silica coated working electrodes. The electrodes were dried in argon prior to three minute sputtering with gold using a Denton Desk IV sputter. The surfaces were imaged using FEI XL30 SEM.

GOx Half Life Determination

Sensors with silica-dispersed GOx at pH 3, 7 or 12 or with glutaraldehyde cross-linked GOx were prepared and assembled in the flow cell as described above. 14mM (250mg/dL) glucose in PBS solution (pH 7) was circulated over the sensor at 0.80mL/min at room temperature 24h. Sensor half life was determined by fitting the data to a first and second order logarithmic decay equations, and extrapolating to the time where the sensor current decayed to 50% of the original value.

RESULTS

Sensor response in serum and whole blood

Silica-dispersed GOx sensors for serum and whole blood studies were fabricated at pH 7. Figure 1B shows an SEM image of the silica layer on top of a working electrode. The silica formed conformal films of uniform thickness that surrounded the electrode surface. While high vacuum conditions caused cracking in the silica for SEM imaging, the cracks revealed the underlying working electrode surface consisting of Pt black nodules.

The pH 7 silica sensors were configured into the flow system and were calibrated using a series of glucose in PBS standards. Figure 2 shows a typical sensor response to calibration standards: rapid decline in sensor current to the initial baseline upon introduction into PBS (0mgM glucose), followed by increments in sensor response to equilibrium current plateaus following the introduction of 100mg/dL (5.5mM) and 200mg/dL (11.0mM) glucose in PBS.

Following calibration, the pH 7 sensors were tested in separate trials against glucose increments in PBS, human serum and human whole blood all within a physiological range (0–450mg/dL, 0–25mM). The results of these measurements were displayed using a Clark Error Grid (Figure 3), where the y-axis is the glucose concentration measured by the silica sensor and the x-axis is the corresponding reference values measured using a hand held OneTouch® glucose meter. The color-coding for these data points are black for PBS, green for serum, and red for whole blood measurements. Using the Clark Error Grid analysis, 89% of PBS measurements, 100% of serum measurements and 75% of whole blood measurements fell within the “A” region of the Clark Error Grid, and 100% of all measurements fell within the “A+B” region. In order to minimize dilution effects in the serum and whole blood measurements, glucose was introduced into the flow loop using a concentrated stock solution of 200 mM glucose in PBS.

Effect of varying condensation pH

Silica condensed at basic, neutral and acidic pHs have very different physical characteristics, suggesting that these physical changes could be used to favorably affect the performance of glucose sensors. This possibility was tested by preparing silica-dispersed GOx at condensation pHs of 3, 7 and 12 and characterizing the sensor response to glucose in PBS, glucose diffusion through silica membranes, and the half life of the GOx.

Figure 4 compares the equilibrium sensor currents over a range of physiologically relevant glucose in PBS solutions of 0–25mM (0–450 mg/dL) for sensors made at three condensation pHs (3, 7, and 12), and for glutaraldehyde crosslinked sensors. The linear best fits to the calibration data were:

$$\begin{aligned} \text{pH } 12 \quad \mu\text{Amp} &= 8.7[\text{Glucose}] + 20.9 \quad (r^2=0.943) \\ \text{pH } 7 \quad \mu\text{Amp} &= 6.4[\text{Glucose}] + 7.5 \quad (r^2=0.937) \\ \text{pH } 3 \quad \mu\text{Amp} &= 3.0[\text{Glucose}] + 17.7 \quad (r^2=0.874) \\ \text{GA} \quad \mu\text{Amp} &= 3.4[\text{Glucose}] + 5.8 \quad (r^2=0.974) \end{aligned}$$

All fits had correlation coefficients of greater than 0.85.

Table 2 summarizes the sensitivities measured for each GOx encapsulation condition. Statistical analysis using t-tests showed that the sensitivities of the pH 12 and pH 7 silica sensors were significantly higher than the glutaraldehyde crosslinked sensors and pH 3 silica sensors ($p < 0.05$); whereas the glutaraldehyde crosslinked and condensation pH 3 silica were not significantly different.

Table 2 also contains the 99% response time of the sensors measured for a 25mM glucose in PBS solution. The response times measured for pH 12, 7 and 3 sensors were significantly different from each other ($p < 0.05$) and from the glutaraldehyde crosslinked GOx. The sensor response times followed the same trend as the sensor sensitivities – i.e. the most rapidly responding sensor also displayed the highest sensitivity – all of which had significantly faster response times than the glutaraldehyde crosslinked sensors.

To test whether the observed trends in sensor sensitivity and response time were correlated with changes in the properties of the silica matrix, we determined the diffusion coefficients for glucose through silica membranes condensed at pH 3, 7 and 12. The diffusion coefficients measured for the pH 12 silica membranes were significantly higher than the other values ($p < 0.05$), while the values for pH 3 and 7 silica membranes were not significantly different. This hydrophilic polyurethane blend exhibited a diffusion coefficient of $4.2 \pm 1.4 \times 10^{-6} \text{ cm}^2/\text{s}$. By comparison the diffusivity of glucose in buffer at room temperature is $6.6 \times 10^{-6} \text{ cm}^2/\text{s}$.

The time it took the current of silica-dispersed GOx sensors placed in 13.8mM glucose in PBS to decay to 50% of the original value (i.e. enzyme half life) was also measured. While only determined for experimental trial, the pH 12 silica sensor displayed the longest half life (Table 2). This could translate to a longer in vivo functionality due to improved stability of the sensing enzyme but this must be determined with further study.

DISCUSSION

The dispersion of enzymes in silica serves as an excellent alternative to glutaraldehyde crosslinking because it is non-toxic, chemically and biologically inert, subject to negligible swelling, hydrophilic, and inexpensive to synthesize [6, 7]. Studies have shown that encapsulation of proteins in porous silica can result in dramatic stabilization of the protein's structure. In addition, the hydrophilic nature of silica allows for a degree of intrinsic biocompatibility. In spite of these advantages, few studies have implemented silica-dispersed GOx for measuring glucose in biological samples (Table 1).

Gupta et al. recently reported a one-step approach to disperse biological macromolecules in silica that avoids solvent byproducts and acid/base catalysts that could compromise the structure and function of the biological components. This technique was used in the current study to disperse GOx in a silica matrix that formed conformal coatings on the surface of working electrode of an amperometric Clark type glucose sensor, followed by application of a polyurethane over layer. The silica-dispersed GOx accurately detected glucose concentrations in glucose-spiked samples of PBS, human serum, and human whole blood. We were concerned about possible dilution of samples with the addition of glucose leading

to inaccurate glucose predictions. To overcome this possible flaw, we added only highly concentrated glucose stock solution (200 mM) to minimize the change in total volume of the sample being tested.

The condensation pH was varied to disperse GOx in silica matrices of different physical properties: acidic pH 3 produced a brittle and dense glass, basic pH 12 produced a soft and less dense gel, and neutral pH 7 produced intermediately flexible silica. Consistent with these physical changes, glucose diffused the fastest in pH 12 silica membranes, followed by the pH 7 and then pH 3 membranes. Testing of these sensors in solutions of glucose in PBS showed the pH 12 silica sensors to also have the highest sensitivity, the fastest response time, and the longest half life, which were followed in order by the pH 7, pH 3, and glutaraldehyde sensors.

These results were consistent with our hypothesis that the gel-like pH 12 silica gel provided reduced barriers to glucose diffusion, and the more aqueous microenvironment therefore providing greater stability for the enzyme. Although pH 12 silica sensors exhibited uniformly superior sensing characteristics, because it is a soft gel presents some concerns regarding its physical robustness.

Table 3 compares the sensing characteristics of the pH 12 silica sensor described here to previously reported glucose sensors that utilized silica (Table 1) and other comparable matrix-based GOx encapsulation techniques. Salimi et al. developed a carbon nanotube silica hybrid glucose sensor that exhibited lower linear range, similar response time, and a lower sensitivity than our pH 12 silica sensor. Overall, our sensitivity and response time was comparable to the other systems, but our limit of detection was notably higher mostly due to variance in the data and possibly the use of the simple 3 electrode sensing format.

Dung et al. [42] described a TiO₂-Single Wall Carbon Nanotube sensor that had a linear range of detection significantly less than the pH 12 silica sensor we produced, a similar response time to our sensor, and a lower sensitivity than our pH 12 silica sensor. The addition of carbon nanotubes are known to improve glucose sensor sensitivity and response time; therefore it is encouraging that our sensitivity was comparable. Li et al. developed a glucose sensor with silica gel layered on Prussian blue. This sensor had a lower sensitivity and linear range than our pH 12 silica sensor. Xue-Cai et al. developed a chitosan-silica gel layered on Prussian blue and this sensor also exhibited a lower sensitivity and a comparable linear range while maintaining a similar response time as our pH 12 silica sensor. Additionally, Zuo et al. reported a Silica Polyvinyl Alcohol Prussian Blue Modified sensor had a linear range of detection from 50 μ M to 2.5 mM, a response time of 8 seconds, and sensitivity of 3.41 μ A mM⁻¹. Our sensor outperformed Zuo's sensor in terms of linear range and sensitivity while displaying a similar response time. Finally, Trivedi [47] developed an organic polymer matrix (polyethylenimine and poly(carbamoylsuphonate) glucose sensor. The sensitivity, time response, and linear range underperformed compared to our glucose sensor. The limit of detection obtained from our sensor has room for improvement but for most sensor characteristics our sensor is on par with other matrix-based glucose sensors. In addition, our simple silica-based glucose sensor system provides superior flexibility to these other sensors with a mere adjustment of the condensation pH.

While this study characterized the feasibility of using silica-dispersed GOx, in order to develop a true glucose biosensor based on this technology it is absolutely necessary to more fully characterize the biosensor in terms of sensitivity, specificity, detection limit, response time, linear range, operational and storage stability, reproducibility in order to define and enhance the glucose sensor performance and stability. Next steps in the further development of our sensor include fully characterizing the performance of the pH 12 silica

sensor. An extension of this feasibility study will incorporate the following critical elements: performing operational and storage stability of the sensor, improving the detection limit of the sensor (mM to μ M), perform an interference study of our sensor, determine the minimum amount of blood required for a measurement. In addition, we will focus on optimization of the pH 12 silica matrix to improve mechanical stability to allow for testing in real samples, and testing the functionality of the optimized pH 12 silica biosensor in human blood and serum.

CONCLUSIONS

Clark-type glucose sensors with silica-dispersed GOx were tested against buffered glucose calibration standards as well as glucose spiked human serum and whole blood. All serum and whole blood measurements met the minimum FDA requirement of falling within the “A +B region” of a Clark Error Grid. Silica condensation pH significantly affected sensor performance. The pH 12 silica sensors had faster response times, higher sensor sensitivity, and a longer half life than the glutaraldehyde crosslinked sensor and the pH 7 and 3 silica sensors. Membranes of the pH 12 silica also had higher diffusion coefficients than the pH 7 and 3 silica membranes. Compared to glucose sensors with similar matrix encapsulation techniques, the pH 12 silica sensors exhibited similar values of sensor sensitivity, linear range, and response time; however, had a notably higher limit of detection of glucose. To our knowledge this is the first report of using silica-dispersed GOx based sensors to measure glucose in whole blood, and also the first report that examines whether varying the silica sol-gel chemistry could be exploited to improve glucose sensor performance and stability.

Acknowledgments

We gratefully acknowledge Dr. Plamen B. Atanasov of the University of New Mexico for aid in preparing the platinum black electrodes, Dr. Mark Schoenfisch of the University of North Carolina, Chapel Hill for aid in constructing the glucose sensors, and the thoughtful comments of reviewers. This work was funded by NIH grant DK44972 (WMR) and NSF grant CBET1050176 (GPL).

CITATIONS

1. Fernandez-Lafuente R, Rosell CM, Rodriguez V, Guisan JM. Strategies for enzyme stabilization by intramolecular crosslinking with bifunctional reagents. *Enzyme and Microbial Technology*. 1995; 17:517–523.
2. Zhou QZK, Dong Chen X. Immobilization of β -galactosidase on graphite surface by glutaraldehyde. *Journal of Food Engineering*. 2001; 48:69–74.
3. Magnan E, Catarino I, Paolucci-Jeanjean D, Preziosi-Belloy L, Belleville MP. Immobilization of lipase on a ceramic membrane: activity and stability. *Journal of Membrane Science*. 2004; 241:161–166.
4. Seyhan TS, Alptekin O. Immobilization and kinetics of catalase onto magnesium silicate. *Process Biochem*. 2004; 39:2149–2155.
5. House JL, Anderson EM, Ward WK. Immobilization Techniques to Avoid Enzyme Loss from Oxidase-Based Biosensors: A One-Year Study. *J Diabetes Sci Technol*. 2007; 1:18–27. [PubMed: 19888375]
6. Nassif N, Coiffier A, Coradin T, Roux C, Livage J, Bouvet O. Viability of Bacteria in Hybrid Aqueous Silica Gels. *Journal of Sol-Gel Science and Technology*. 2003; 26:1141–1144.
7. Chiriac PN, Nita I, Nistor L, Sol M. Gel Method Performed for Biomedical Products Implementation. *Mini Reviews in Medicinal Chemistry*. 2010; 10:990–1013. [PubMed: 20690901]
8. Kros A, Gerritsen M, Sprakel VSI, Sommerdijk N, Jansen JA, Nolte RJM. Silica-based hybrid materials as biocompatible coatings for glucose sensors. *Sensors and Actuators B-Chemical*. 2001; 81:68–75.

9. Lu HY, Yang J, Rusling JF, Hu NF. Vapor-surface sol-gel deposition of titania alternated with protein adsorption for assembly of electroactive, enzyme-active films. *Electroanalysis*. 2006; 18:379–390.
10. Yu JH, Ju HX. Pure organic phase phenol biosensor based on tyrosinase entrapped in a vapor deposited titania sol-gel membrane. *Electroanalysis*. 2004; 16:1305–1310.
11. Zhang T, Tian BZ, Kong JL, Yang PY, Liu BH. A sensitive mediator-free tyrosinase biosensor based on an inorganic-organic hybrid titania sol-gel matrix. *Analytica Chimica Acta*. 2003; 489:199–206.
12. Rabinovich L, Lev O. Sol-gel derived composite ceramic carbon biosensors. 2001
13. Brennan JD, Benjamin D, DiBattista E, Gulcev MD. Using sugar and amino acid additives to stabilize enzymes within sol-gel derived silica. *Chemistry of Materials*. 2003; 15:737–745.
14. Mena B, Mena F, Aiolfi-Guimaraes C, Sharts O. Silica-based nanoporous sol-gel glasses: from bioencapsulation to protein folding studies. *International Journal of Nanotechnology*. 2010; 7:1–45.
15. Heller J, Heller A. Loss of activity or gain in stability of oxidases upon their immobilization in hydrated silica: Significance of the electrostatic interactions of surface arginine residues at the entrances of the reaction channels. *Journal of the American Chemical Society*. 1998; 120:4586–4590.
16. Nguyen DT, Smit M, Dunn B, Zink JJ. Stabilization of creatine kinase encapsulated in silicate sol-gel materials and unusual temperature effects on its activity. *Chemistry of Materials*. 2002; 14:4300–4306.
17. Kandimalla VB, Tripathi VS, Ju HX. Immobilization of biomolecules in sol-gels: Biological and analytical applications. *Critical Reviews in Analytical Chemistry*. 2006; 36:73–106.
18. Rottman C, Grader G, De Hazan Y, Melchior S, Avnir D. Surfactant-induced modification of dopants reactivity in sol-gel matrixes. *Journal of the American Chemical Society*. 1999; 121:8533–8543.
19. Danowski TS, Sunder JH. Jet Injection of Insulin During Self-Monitoring of Blood Glucose. *Diabetes Care*. 1978; 1:27–33. [PubMed: 400127]
20. Nakanishi, N.; Yamamoto, H.; Tsuchiya, K.; Uetsuji, Y.; Nakamachi, E. Development of wearable medical device for bio-MEMS - art. no. 60390P. In: Nicolau, DV., editor. *BioMEMS and NanoTechnology II*. Bellingham: Spie-Int Soc Optical Engineering; 2006. p. 390-P
21. Zink JJ, Yamanaka SA, Ellerby LM, Valentine JS, Nishida F, Dunn B. Biomolecular Materials Based on Sol-Gel Encapsulated Proteins. *Journal of Sol-Gel Science and Technology*. 1994; 2:791–795.
22. Pandey PC, Upadhyay S, Pathak HC. A new glucose sensor based on encapsulated glucose oxidase within organically modified sol-gel glass. *Sensors and Actuators B-Chemical*. 1999; 60:83–89.
23. Blin JL, Gerardin C, Carteret C, Rodehuser L, Selve C, Stebe MJ. Direct One Step immobilization of Glucose Oxidase in Well Ordered Mesostructured Silica Using a Nonionic Fluorinated Surfactant. *Chemical Materials*. 2005; 17:1479–1486.
24. Li J, Chia LS, Goh NK, Tan SN, Ge H. Mediated amperometric glucose sensor modified by the sol-gel method. *Sensors and Actuators B-Chemical*. 1997; 40:135–141.
25. Musholt PB, Schipper C, Thome N, Ramljak S, Schmidt M, Forst T, et al. Dynamic electrochemistry corrects for hematocrit interference on blood glucose determinations with patient self-measurement devices. *J Diabetes Sci Technol*. 2011; 5:1167–1175. [PubMed: 22027312]
26. Jia W-Z, Wang K, Zhu Z-J, Song H-T, Xia X-H. One-Step Immobilization of Glucose Oxidase in a Silica Matrix on a Pt Electrode by an Electrochemically Induced Sol-Gel Process. *Langmuir*. 2007; 23:11896–1900. [PubMed: 17929847]
27. Oliver N, Toumazou C, Cass A, Johnston D. Glucose sensors: a review of current and emerging technology. *Diabetic Medicine*. 2009; 26:197–210. [PubMed: 19317813]
28. Krouwer JS, Cembrowski GS. A Review of Standards and Statistics Used to Describe Blood Glucose Monitor Performance. *J Diabetes Sci Technol*. 2010; 4:75–83. [PubMed: 20167170]
29. Feltham AM, Spiro M. Platinized platinum electrodes. *Chemical Reviews*. 1971; 71:177–193.

30. Gupta G, Rathod SB, Staggs KW, Ista LK, Abbou Oucherif K, Atanasov PB, et al. CVD for the Facile Synthesis of Hybrid Nanobiomaterials Integrating Functional Supramolecular Assemblies. *Langmuir*. 2009; 25:13322–13327. [PubMed: 19883092]
31. Zoldák G, Zubrik A, Musatov A, Stupák M, Sedlák E. Irreversible Thermal Denaturation of Glucose Oxidase from *Aspergillus niger* Is the Transition to the Denatured State with Residual Structure. *Journal of Biological Chemistry*. 2004; 279:47601–47609. [PubMed: 15342626]
32. Abdel-Humid I, Atanasov P, Wilkins E. Needle-type glucose biosensor with an electrochemically codeposited enzyme in a platinum black matrix. *Electroanalysis*. 1995; 7:738–741.
33. Cunningham, A. *Introduction To Bioanalytical Sensors*. New York: John Wiley & Sons, Inc.; 1998. *Biosensors and Bioanalytical Challenges*; p. 40
34. Harvey DR, Cooper LV, Fancourt RF, Levene M, Schoberg T. The use of dextrostix and dextrostix reflectance meters in the diagnosis of neonatal hypoglycemia. *J Perinat Med*. 1976; 4:106–110. [PubMed: 966130]
35. Priya M, Mohan Anjana R, Pradeepa R, Jayashri R, Deepa M, Bhansali A, et al. Comparison of capillary whole blood versus venous plasma glucose estimations in screening for diabetes mellitus in epidemiological studies in developing countries. *Diabetes Technol Ther*. 2011; 13:586–591. [PubMed: 21406012]
36. Badjic JD, Kostic NM. Effects of encapsulation in sol-gel silica glass on esterase activity, conformational stability, and unfolding of bovine carbonic anhydrase II. *Chemistry of Materials*. 1999; 11:3671–3679.
37. Gill I, Ballesteros A. Encapsulation of Biologicals within Silicate, Siloxane, and Hybrid Sol-Gel Polymers: An Efficient and Generic Approach. *Journal of the American Chemical Society*. 1998; 120:8587–8598.
38. Livage J, et al. Encapsulation of biomolecules in silica gels. *Journal of Physics: Condensed Matter*. 2001; 13:R673.
39. Ronda L, Pioselli B, Bruno S, Micalella C, Bettati S, Mozzarelli A. Biocatalysis in a confined environment - Lessons from enzymes immobilized in wet, nanoporous silica gels. *Chimica Oggi-Chemistry Today*. 2007; 25:10–15.
40. Kortessuo P, Ahola M, Karlsson S, Kangasniemi I, Yli-Urpo A, Kiesvaara J. Silica xerogel as an implantable carrier for controlled drug delivery—evaluation of drug distribution and tissue effects after implantation. *Biomaterials*. 2000; 21:193–198. [PubMed: 10632401]
41. Salimi A, Compton RG, Hallaj R. Glucose biosensor prepared by glucose oxidase encapsulated sol-gel and carbon-nanotube-modified basal plane pyrolytic graphite electrode. *Analytical Biochemistry*. 2004; 333:49–56. [PubMed: 15351279]
42. Dung NQ, Patil D, Duong TT, Jung H, Kim D, Yoon SG. An amperometric glucose biosensor based on a GOx-entrapped TiO₂-SWCNT. *Sensors and Actuators B-Chemical*. 2012; 166–167:103–109.
43. Im JS, Yun J, Kim JG, Bae T-S, Lee Y-S. The effects of carbon nanotube addition and oxyfluorination on the glucose-sensing capabilities of glucose oxidase-coated carbon fiber electrodes. *Applied Surface Science*. 2012; 258:2219–2225.
44. Li T, Yao Z, Ding L. Development of an amperometric biosensor based on glucose oxidase immobilized through silica sol-gel film onto Prussian Blue modified electrode. *Sensors and Actuators B: Chemical*. 2004; 101:155–160.
45. Xue-Cai T, Yuan-Xin T, Pei-Xiang C, Xiao-Yong Z. Glucose biosensor based on glucose oxidase immobilized in sol-gel chitosan/silica hybrid composite film on Prussian blue modified glass carbon electrode. *Analytical and Bioanalytical Chemistry*. 2005; 381:500–507. [PubMed: 15657706]
46. Zuo SH, Zhao HL, Zhang LF, Yuan HH, Lan MB, Lawrence GA, et al. An Amperometric Biosensor Based on Glucose Oxidase Immobilized in a Sol-Gel Polyvinyl Alcohol/Silica Hybrid Composite Film on a Prussian Blue Modified Electrode. *Advanced Science Letters*. 2010; 3:476–481.
47. Trivedi UB, Lakshminarayana D, Kothari IL, Patel NG, Kapse HN, Patel PB, et al. Amperometric Glucose Biosensor Based on Immobilization of Glucose Oxidase in Polyethylenimine and Poly(carbamoylsuphonate) Polymer Matrix. *Sensors and Transducers*. 2010; 119:129–141.

Figure 1.

A. Flow cell design used in measuring glucose concentration and electrode positioning within the flow cell. 1B. Schematic of the working electrode containing platinum black nanoparticles and silica segments layer on top of these nanoparticles. The inset is a 1000x SEM image of the silica-dispersed GOx overlayer.

Figure 2. Measurement of sensor response time at 99% of pH 12 silica sensor signal response to addition of glucose in PBS standard solutions.

Figure 3.

Clark Error Grid analysis of silica glucose sensor with condensation pH 7.0. Black points for glucose in 10 mM PBS pH 7.0 (N=9, A:89%, B:11%, C:0%, D:0%, E:0%), green points for human serum (N=13, A:100%, B:0%, C:0%, D:0%, E:0%), red points for whole blood (N=8, A:75%, B:25%, C:0%, D:0%, E:0%). Data points plotted may be obstructed from view due to superposition of other data points. The number of sensors used in the whole blood testing was higher than in serum and PBS+glucose testing.

Figure 4. Sensor response of silica glucose sensors made at pH 12, 7.0, 3.0 and glutaraldehyde cross-linked film all containing GOx. Blue diamonds (pH 12), red squares (pH 7.0), green triangles (pH 3.0), purple cross (glutaraldehyde-cross-linked). The sample size $n=15$ for each pH silica condition and the confidence level is 95%.

Table 1

Silica-entrapped GOx for glucose sensing in biological samples.

	Blood Serum	Whole Blood	Intact Tissue
<u>GOx entrapped w/in silica matrix</u>			
Salimi et al. (2004) [27]	Yes	No	No
Xue-Cai et al. (2005) [28]	Yes	No	No
<u>GOx entrapped between silica films</u>			
Li et al. (2004) [29]	Yes	No	No

Table 2

Characterization of sensors made at condensation pH 3, 7 and 12.

GOx encapsulation matrix	Sensor sensitivity to glucose in PBS ($\mu\text{A}/\text{mM}$ Glucose)	Sensor response time in 25mM glucose (sec)	Glucose diffusivity in silica ($10^{-6}\text{cm}^2/\text{s}$)	Half life of dispersed GOx (hours)
3.0	3.0 ± 0.8 (n=15)	209.3 ± 75 (n=15)	0.9 ± 0.2 (n=9)	58
7.0	6.5 ± 1.1 (n=15)	80.9 ± 30 (n=15)	1.1 ± 0.3 (n=9)	37
12.0	8.6 ± 1.3 (n=15)	10.3 ± 5.9 (n=15)	1.7 ± 0.3 (n=9)	120
Glutaraldehyde	3.4 ± 0.8 (n=15)	648.7 ± 90.1 (n=15)	---	27

Table 3

Comparison of the pH 12 silica sensor to sensors with similar matrix encapsulation configurations.

Sensor matrix configuration	Sensitivity ($\mu\text{A}/\text{mM}$ glucose)	Response time in 25mM glucose (sec)	Detection limit	Linear Range	Ref.
pH 12.0 Silica	8.6	10.3	1.1 mM	0mM – 25mM	This paper
pH 7.0 Silica	6.5	80.9	0.2mM	0mM – 25mM	This paper
pH 3.0 Silica	3.0	209.3	0.2mM	0mM – 25mM	This paper
Glutaraldehyde	3.4	648.7	0.5mM	0mM – 25mM	This paper
Carbon Nanotube and Silica	0.2	5	50 μM	0.2mM – 20mM	[27]
Silica and Prussian Blue	1.1	---	20 μM	0mM – 4.70mM	[29]
Chitosan, Silica, and Prussian Blue	0.4	10	8 μM	50 μM – 26mM	[28]
TiO ₂ -Single Wall Carbon Nanotube sensor	5.3	9	10 μM	10 μM – 1.40mM	[42]
Silica, Polyvinyl Alcohol, and Prussian Blue	3.4	8	50 μM	50 μM – 2.5mM	[44]
Polyethylenimine and Poly(carbamoylsulphonate)	0.7	15	0.35 μM	3.5mM – 5.0mM	[45]