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Enzyme Deposition by Polydimethylsiloxane Stamping for Biosensor Fabrication

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Abstract

High-performance biosensors were fabricated by efficiently transferring enzyme onto Pt electrode surfaces using a polydimethylsiloxane (PDMS) stamp. Polypyrrole and Nafion were coated first on the electrode surface to act as permselective films for exclusion of both anionic and cationic electrooxidizable interfering compounds. A chitosan film then was electrochemically deposited to serve as an adhesive layer for enzyme immobilization. Glucose oxidase (GOx) was selected as a model enzyme for construction of a glucose biosensor, and a mixture of GOx and bovine serum albumin was stamped onto the chitosan-coated surface and subsequently crosslinked using glutaraldehyde vapor. For the optimized fabrication process, the biosensor exhibited excellent performance characteristics including a linear range up to 2 mM with sensitivity of $29.4 \pm 1.3 \mu\text{A mM}^{-1} \text{cm}^{-2}$ and detection limit of $4.3 \pm 1.7 \mu\text{M}$ ($S/N = 3$) as well as a rapid response time of ~ 2 s. In comparison to those previously described, this glucose biosensor exhibits an excellent combination of high sensitivity, low detection limit, rapid response time, and good selectivity. Thus, these results support the use of PDMS stamping as an effective enzyme deposition method for electroenzymatic biosensor fabrication, which may prove especially useful for the deposition of enzyme at selected sites on microelectrode array microprobes of the kind used for neuroscience research *in vivo*.

Keywords

Enzyme deposition; Electroenzymatic biosensor; Glucose biosensor; Microcontact printing; Polydimethylsiloxane stamping

1. Introduction

Electroenzymatic biosensors utilize an enzyme as the biological recognition element, which is coupled to an electrode that serves as a transducer to convert the recognition event into a measurable signal. Typically, the means by which the enzyme is deposited and immobilized on the electrode surface constitute the critical steps in biosensor fabrication, as the immobilized enzyme concentration and activity directly impact sensor performance. Many approaches have been taken in an effort to deposit enzyme in an active state on the electrode surface including drop coating, layer-by-layer assembly, adsorption, and electrodeposition.

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[1] Commercially available glucose sensors for home blood glucose monitoring commonly are fabricated by screen-printing,^[2] which typically has a resolution of 50–150 μm .^[3] However, we ultimately are searching for a simple technique enabling the simultaneous transfer of enzyme at high concentration to multiple preselected sites on our microelectrode array microprobes with micron-scale resolution for neurochemical sensing applications *in vivo*.^[4]

Tseng *et al.* recently demonstrated the use of electrodeposited chitosan to direct the adsorption of glutamate oxidase (GlutOx) on selected microelectrodes so as to fabricate glutamate microbiosensors.^[5] Chitosan may be deposited selectively on microelectrodes at negative potential, as the high local pH in the vicinity of the electrodes results in the deprotonation of amine groups on chitosan resulting in its precipitation.^[6] These amine groups on the electrodeposited chitosan attract typically negatively charged proteins for adsorption and are available for subsequent crosslinking. However, simple electrodeposition of GlutOx on the chitosan film resulted in a less active, presumably lower concentration deposit than that obtained by manual deposition, which was reflected in a lower performance glutamate sensor.

In recent years, polydimethylsiloxane (PDMS) stamps have been employed to transfer protein in a micron to submicron-resolution pattern to various substrates in a process referred to as microcontact printing.^[7] The mechanical properties of the PDMS stamps provide sufficient mechanical stability for printing features as small as 500 nm.^[7b] Importantly, many proteins retain their biological activity after printing, and the transfer of proteins can occur in a few seconds.^[7c] In addition, the stamp often does not pick up protein already printed. Thus, the PDMS stamping process has been regarded as a unidirectional process for protein transfer. After protein deposition, the PDMS stamps can be washed and reused. Therefore, we hypothesized that the PDMS stamping process may be an excellent, high-resolution method for transferring concentrated enzyme onto arrays of Pt microelectrodes on micromachined silicon microprobes. Such microprobes fabricated by us were used by our collaborators to monitor neurotransmitter release in the brains of freely moving laboratory rats.^[4, 8]

In this study, the transfer of glucose oxidase (GOx) to modified Pt electrode surfaces was employed as a model system to demonstrate, for the first time, the utility of the PDMS stamping method for creation of high-performance (*e.g.*, high sensitivity, low detection limit, excellent selectivity and fast response time), electroenzymatic biosensors. In these biosensors, the immobilized GOx catalyzes the oxidation of glucose to D-glucono-1,5-lactone with concomitant production of hydrogen peroxide. The underlying electrode, held at positive potential, electrooxidizes the hydrogen peroxide giving rise to a measurable Faradaic current. Sensor selectivity against common interferents is achieved by modifying the electrode surface with permselective polymers such as Nafion and polypyrrole. Thicker polypyrrole films exclude both cations and anions and Nafion rejects anionic interferents.^[1b, 9] Glucose biosensors obviously are useful for a variety of applications both *in vitro* and *in vivo*. Based on recent comprehensive reviews, home use blood glucose biosensors have been the most successful biosensor products to date by a wide margin, and amperometric glucose biosensors based on immobilized GOx or glucose dehydrogenase are the most

common. The typical test range of these commercial glucose biosensors is ~0.6 mM–33.3 mM and the reported assay time varies from 5 to 20 s.^[2, 10] However, our interest is in sensors useful for neuroscience research *in vivo*, thus the emphasis on response time, sensitivity (so that sensors may be miniaturized) and rejection of dopamine and ascorbic acid, which are electrooxidizable interferents common to brain extracellular fluid.

2. Experimental Section

2.1. Reagents

Glucose oxidase (from *Aspergillus niger*, CAS NO. 9001-37-0), pyrrole (Py), Nafion® (5%), glutaraldehyde solution (25%), bovine serum albumin (BSA) lyophilized powder, hydrogen peroxide solution (30%), chitosan (From crab shells, minimum 85% deacetylated), D-(+)-glucose, L-ascorbic acid, dopamine hydrochloride, potassium hexacyanoferrate (II) trihydrate, and potassium hexacyanoferrate (III) were purchased from Sigma-Aldrich (St. Louis, MO). Isopropyl alcohol and 1 M sulfuric acid solutions were obtained from Fisher Scientific (Pittsburgh, PA). Ag/AgCl glass-bodied reference electrodes with 3 M NaCl electrolyte, 0.5-mm-diameter Pt wire auxiliary electrodes and disk Pt electrodes (1.6 mm dia.) were purchased from BASi (West Lafayette, IN). Sodium phosphate buffer (PBS, pH 7.4) was composed of 50 mM sodium phosphate (dibasic) and 100 mM sodium chloride. Microcloth (PSA, 2–7/8") for electrode polishing was purchased from Buehler (Lake Bluff, Illinois). Ultrapure water was generated using a Millipore Milli-Q Water System and was used for preparation of all solutions.

2.2. Instrumentation

Electrochemical experiments for sensor development, evaluation and calibration were performed using a Versatile Multichannel Potentiostat (model VMP3) equipped with the 'p' low current option and N' Stat box driven by EC-LAB software (Bio-Logic USA, LLC, Knoxville, TN) in a three electrode configuration consisting of the sensing electrode, a Pt wire auxiliary electrode, and a Ag/AgCl glass-bodied reference electrode. A Nova Nano 230 was used for environmental SEM images. An Infinite® M1000 PRO (Tecan) was used for fluorescence assays.

2.3. Fabrication of polydimethylsiloxane stamps

Polydimethylsiloxane (PDMS) stamps were fabricated using the Sylgard® 184 silicone elastomer kit from Dow Corning. The curing agent and monomer were mixed at a 1:10 ratio in a Petri dish to give a ~2-mm-thick polymer film. Subsequently, the mixture was carefully degassed under vacuum and cured at 60 °C for 4 hrs. A cylindrical feature of ~1.6 mm diameter and ~2 mm height was punched from the film and a rectangular PDMS support (~5 mm square) was cut as well. Next, the cylindrical PDMS piece was glued at the center of the rectangular piece using an uncured mixture of curing agent and base monomer. The assembled PDMS stamp was ready after curing at 60 °C for 4 hours. The PDMS stamps were washed with ethanol/water (v/v = 1:2) and reused.^[7g]

2.4. Sensor preparation

The sensor was prepared layer-by-layer to achieve the final configuration illustrated in Figure 1. The Pt disk electrode (1.6 mm dia.) was polished using a microcloth with a 0.05- μm -particle suspension. After rinsing with ultrapure water, it was sonicated in isopropyl alcohol followed by electrochemical cleaning with 0.5 M sulfuric acid and ultrapure water, respectively. Next, a polypyrrole (PPy) film was electrodeposited (200 mM Py in stirred PBS, 0.85 V *vs.* Ag/AgCl, ~5 min) onto the Pt surface, followed by Nafion dip-coating twice with a 5% Nafion® solution and baking in an oven at 180 °C for 3 min.^[1b, 9, 11]

The pH of the chitosan solution (0.04% m/v) was adjusted to pH 3 using hydrochloric acid (HCl) to dissolve the chitosan flakes. After filtering with a 0.2 μm syringe filter, the pH was adjusted to 5 using sodium hydroxide (NaOH) solution (0.5 M). A constant potential of -0.7 V *vs.* Ag/AgCl was applied at the PPy/Nafion-coated Pt electrode surface for 2 min while immersed in the chitosan solution to electrodeposit a chitosan film.^[5a, 6b] This chitosan-coating process was repeated twice more.

A droplet of GOx/BSA solution, mixed in a 1:1 mass ratio (BSA: 10 mg/mL; GOx 10 mg/mL) in PBS, was placed on the cleaned PDMS stamp surface and left at room temperature for ~10 min (inking time). The excess protein solution was carefully wicked from the stamp with a Kimwipe, and the stamp was dried under a stream of argon for ~30 s. The stamp then was placed horizontally in contact with the chitosan-coated electrode surface for 10–15 s. Subsequently, the disk electrode surface was exposed to the vapor from a 12.5% glutaraldehyde (GAH) solution at room temperature for 10 s to 10 min. If the sensor was treated with multiple layers of stamped protein, each layer was treated with GAH vapor prior to stamping of the next layer. Chitosan was deposited only before the first enzyme layer transfer. Finally, the sensors were washed with ultrapure water and kept at 4 °C under dry conditions when not in use.

2.5. Electrochemical measurements

Constant potential amperometric measurements were conducted in PBS buffer at 0.7 V *vs.* Ag/AgCl and at ambient laboratory temperature. More than 30 min of equilibration time in PBS buffer was required to achieve a stable current before adding analytes. Faradaic impedance measurements were performed in the presence of $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ (1:1)-mixture as a redox probe in PBS, using an AC voltage amplitude of 5 mV. A bias potential of 0.2 V *vs.* Ag/AgCl also was used over a frequency range from 0.1 to 1×10^5 Hz.^[1a, 12]

2.6. Quantification of immobilized glucose oxidase

To estimate the thickness of the enzyme layer and enzyme concentration on the electrode surface, a fluorescence assay was implemented using an Infinite® M1000 PRO (Tecan) microplate reader. The two fluorescent FAD moieties per GOx protein enable measurement of GOx concentration as FAD fluorescence using a method developed by Gooding *et al.*^[1e, 13] A calibration curve was created from recordings of the fluorescence intensity of various concentrations of FAD in an aqueous solution of 8 M urea and 0.05 M KCl. The FAD solutions were stored in the dark until use. The emission intensity at 525 nm scaled linearly with the FAD concentration between 19.2 nM and 1229.7 nM at an excitation

wavelength of 375 nm. Enzyme-coated electrodes were soaked in 0.7 mL of 8 M urea solution overnight to ensure that FAD was leached completely from the electrode surface. The emission intensity at 525 nm was then measured and correlated to enzyme concentration using the calibration curve described above.

3. Results and Discussion

3.1. Biosensor surface morphology

The surface morphology of each layer of the glucose biosensor was examined using environmental SEM (Figure 2). The dip-coated, Nafion film was smooth without noticeable structure at the magnification used (Figure 2a). The lines shown in Figure 2a reflected scratches on the underlying platinum disk electrode. Previously reported SEM images of electrodeposited chitosan [1a] showed a sponge-like structure, however the chitosan surface shown in Figure 2b appears to consist of a somewhat non-uniform assembly of small particles. This difference likely is due to the fact that in this work, the chitosan solution was filtered through a 0.2- μ m-pore membrane immediately before use. In contrast, SEM images of a chitosan film, electrochemically deposited two days after filtration, showed large chitosan aggregates (data not shown).

The first GOx-BSA film stamped on the chitosan layer was quite uniform (Figure 2c) despite some inconsistency in the underlying chitosan layer, however the second stamped layer of GOx-BSA appeared to be incomplete (Figure 2d). This difference in stamping efficiency between the two layers may be indicative of the importance of the chitosan film in providing an adhesive layer for the GOx-BSA deposit.^[5–6]

3.2. Electrochemical impedance spectroscopy (EIS)

The fabrication process for the glucose biosensor was monitored by EIS and demonstrated that each layering step resulted in an expected change in impedance.^[1a, 12, 14] Figure 3 shows the impedance features as Nyquist plots ($-\text{Im}(Z)$ vs. $\text{Re}(Z)$) during the electrode modification process. The Nyquist plot consists of two regimes; a semicircular part at high frequency reflecting electron transfer resistance (R_{ct}) and a linear part at low frequency corresponding to diffusion limitations. The spectrum for the PPy-modified electrode consisted of an almost straight line (Figure 3a) without a noticeable semicircular regime due to the fact that the thin PPy layer on the electrode acted primarily as a diffusional resistance. However as more insulating layers were added, the diameter of the semicircular regime increased as expected. Figure 3b is the spectrum after Nafion and chitosan films were added to the Pt/PPy electrode, and Figures 3c–e show significant differences in resistance after the addition of each of three protein layers by PDMS stamping.

3.3. Effect of interferents

The glucose biosensor selectivity was tested with ascorbic acid and dopamine, common electrooxidizable interferents in brain extracellular fluid. Physiologically relevant concentrations of 5 and 10 μ M dopamine and 250 and 500 μ M ascorbic acid were used, and a negligible biosensor response was observed at the constant operating potential of 0.7 V (vs. Ag/AgCl) (Figure 4), although this representative biosensor exhibited the expected

response to glucose and hydrogen peroxide. This result shows that polypyrrole and Nafion block access of these key electroactive interferents, which suggests that the biosensor may be useful for neuroscience research *in vivo*. In order to achieve the selectivity shown, the applied potential for chitosan deposition atop the permselective polymer coatings was set at -0.7 V, which is sufficient to create a high local pH for chitosan precipitation on the electrode surface without disrupting the underlying PPy and Nafion films.^[5a, 6b]

3.4. GOx deposition by PDMS stamping

After a droplet of GOx and BSA mixture (1:1 mass ratio) was placed onto the PDMS stamp surface for ~ 10 min, the excess solution was removed and the stamp surface was dried. GOx and BSA on the PDMS stamp surface were transferred to the modified electrode surface by stamping for 10–15 seconds. A smooth electrode surface likely facilitated the enzyme transfer due to uniform surface contact, and filtration of the chitosan solution before electro-deposition likely helped to generate the smooth chitosan deposit (see Figure 1).

The stamped enzyme layers were stabilized by exposure to saturated glutaraldehyde vapor for cross-linking. A short exposure time (*i.e.*, <30 s) resulted in an unstable protein layer that was washed away easily, while a long exposure time (*i.e.*, >5 min) resulted in unacceptable loss in enzyme activity. In addition, one layer of stamped enzyme was found to give unsatisfactory sensor performance, while three layers of enzyme commonly resulted in a long biosensor response time (*i.e.*, >3 s). Finally, the exposure time to glutaraldehyde was set at 45 s for each stamped enzyme layer and two layers of enzyme were stamped to obtain a rapid response time (~ 2 s) while still providing good sensitivity and a low detection limit (see below).

3.5. Biosensor performance

Figure 5 shows current recordings of a typical biosensor in real time in response to successive step changes in glucose concentration at 0.7 V vs. Ag/AgCl. The biosensor reached 95% of the steady-state current within 2 s in response to changes in glucose concentration, indicating excellent electrocatalytic behavior of the biosensor.

The apparent Michaelis-Menten constant (K_m^{app}), estimated from the non-linear plot of current vs. glucose concentration, was 1.85 ± 0.08 mM (Figure 5 and Figure 6). The low K_m^{app} , which is much lower than the reported range for the free enzyme (*i.e.*, $K_m = 33$ mM–110 mM),^[15] likely was due to oxygen-limited enzyme kinetics at glucose concentrations in the millimolar range.^[16] Such oxygen limitations at high glucose concentrations, due to relatively low oxygen solubility and mass transfer resistances, causes a reduction in $V_{\text{max}}^{\text{app}}$, which resulted in the lower K_m^{app} reported here. Further insight into the kinetics was had through a determination of the apparent k_{cat} , which is interpreted as the maximum number of substrate molecules converted to product per enzyme active site per second. The constant, k_{cat} , generally is calculated from the quotient of the maximum observed reaction rate and the enzyme concentration, $V_{\text{max}}/[E]_0$. In this case, the maximum reaction rate corresponds to the maximum biosensor current observed. For our glucose biosensor, $V_{\text{max}}^{\text{app}}$ was estimated at ~ 0.541 nmol s $^{-1}$ cm $^{-2}$ by noting that two electrons are generated for each molecule of H₂O₂ oxidized and one molecule of H₂O₂ is produced upon

enzyme catalyzed oxidation of a molecule of glucose. The GOx concentration and thickness of GOx layer immobilized by PDMS stamping were estimated by FAD extraction followed by fluorescence assays. The surface concentration of the enzyme active sites was estimated at $\sim 2.26 \text{ nmol cm}^{-2}$ after two GOx transfers by stamping, which gave the best biosensor performance. Here, the GOx surface concentration estimated from a FAD measurement is based on the assumption that all the FAD-containing, immobilized enzyme is active. With this assumption, $k_{\text{cat}}^{\text{app}}$ was estimated at $\sim 0.24 \text{ s}^{-1}$, which is relatively low.^[15b] However, this k_{cat} value is an apparent quantity that likely is influenced by mass transport in the electrode coatings and subsequent oxygen limitation at high glucose concentration (see above), and by the fact that most H_2O_2 diffuses into the bulk solution.^[1e] Based on the amount of GOx obtained from FAD experiments and the diameter of the Pt disk electrode, the thickness of the enzyme and BSA layer was estimated to be $7.0 \text{ }\mu\text{m}$. The thickness of one enzyme layer is estimated to be $\sim 3.5 \text{ }\mu\text{m}$, which corresponds to ~ 435 enzyme molecule layers.

A typical calibration curve for the glucose biosensor is presented as Figure 6. Glucose biosensors fabricated on the same day exhibited a repeatable high sensitivity of $29.4 \pm 1.3 \text{ }\mu\text{A mM}^{-1} \text{ cm}^{-2}$ ($n = 3$) and detection limit of $4.3 \pm 1.7 \text{ }\mu\text{M}$ ($n = 3$) at a signal-to-noise ratio of 3. The sensor displayed a linear detection range of up to 2 mM ($R^2 = 0.998$) and a fast response time ($\sim 2 \text{ s}$). A larger linear range could be achieved by adding a glucose mass transfer resistance in the form of an additional polymer layer, for example, but this would come at the expense of a longer response time.^[16] The performance of our electroenzymatic biosensor fabricated by PDMS stamping compares favorably with recently reported glucose biosensors based on immobilized GOx,^[17] the best of which tend to rely on more exotic materials including nanoparticles, nanotubes and graphene. For example, Feng *et al.* reported the glucose sensor fabrication by immobilizing GOx into nanostructured graphene-conducting polyaniline nanocomposite.^[18] The biosensor showed some characteristics similar to those reported here (*i.e.*, a sensitivity of $22.1 \text{ }\mu\text{A mM}^{-1} \text{ cm}^{-2}$ and detection limit of $2.769 \text{ }\mu\text{M}$). In further comparison, a sensor utilizing Pt nanoparticles showed a good sensitivity of $17.40 \text{ }\mu\text{A mM}^{-1} \text{ cm}^{-2}$ but a significantly higher limit of detection of $18 \text{ }\mu\text{M}$ and slower response time of $15\text{--}20 \text{ s}$.^[19] The use of maghemite nanoparticles in carbon paste gave rise to a sensor with higher sensitivity, $45.85 \text{ }\mu\text{A mM}^{-1} \text{ cm}^{-2}$, and a lower detection limit of $0.9 \text{ }\mu\text{M}$, but no response time was given.^[20] In another report where magnetic nanoparticles were used, a high sensitivity ($62.45 \text{ }\mu\text{A mM}^{-1} \text{ cm}^{-2}$) and low detection limit ($0.23 \text{ }\mu\text{M}$) also were reported but the response time was $\sim 5 \text{ s}$.^[21] Shi and Ma described an amperometric glucose biosensor based on GluOx immobilized in a composite film of silver “nanoprisms” in chitosan. They also reported a relatively high sensitivity of $67.17 \text{ }\mu\text{A mM}^{-1} \text{ cm}^{-2}$ and a more typical detection limit of $1 \text{ }\mu\text{M}$, but the sensor showed “serious” interference from ascorbic acid.^[1c] Recently, a glucose biosensor constructed of GOx immobilized on chitosan nanoparticles on gold was described that exhibits a response time similar to our biosensor of $\sim 2 \text{ s}$, yet provides a higher sensitivity of $156.27 \text{ }\mu\text{A mM}^{-1} \text{ cm}^{-2}$ and a lower detection limit of $1.1 \text{ }\mu\text{M}$.^[22] However, no selectivity data was given, which is an important consideration for sensors to be used *in vivo* or with biological samples. Another recent review describes the impressive performance characteristics of a number of glucose biosensors based on nanostructured metal oxides including some

amperometric electroenzymatic biosensors with several-fold higher sensitivity than our biosensor, yet none exhibit a response time of 2 s or less.^[23] Thus, the impressive combination of performance characteristics exhibited by our relatively simple glucose biosensor created with a PDMS stamp appears to be unusual in the recent literature.

4. Conclusions

In summary, PDMS stamping has proved, for the first time, to be an excellent enzyme deposition method for the preparation of an amperometric glucose biosensor. GOx was successfully transferred onto the electrode surface with its activity retained. The resulting sensor exhibited a superior combination of high sensitivity ($\sim 29 \mu\text{A mM}^{-1} \text{cm}^{-2}$), low detection limit ($\sim 4 \mu\text{M}$), fast response time ($\sim 2 \text{ s}$) and good selectivity relative to recently reported glucose biosensors. This PDMS stamping method for enzyme transfer therefore may provide a means for the high throughput deposition of different enzymes on different selected sites on the same microelectrode array to give especially useful, multifunctional microprobes for neuroscience research *in vivo*.

Acknowledgments

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Fig. 1. Schematic diagram of the glucose sensor configuration (not to scale). Ascorbic acid and dopamine were rejected primarily by Nafion and PPy, respectively.

Fig. 2. Scanning electron microscopic (SEM) images of (a) Pt/PPy/Nafion, (b) Pt/PPy/Nafion/Chitosan, (c) Pt/PPy/Nafion/Chitosan/GOx-BSA (Inset: 50 $\times$ light microscope image of stamped GOx-BSA on a Pt surface showing the edge of the stamped area) and (d) Pt/PPy/Nafion/Chitosan/GOx-BSA/GOx-BSA.

Fig. 3. Electrochemical impedance spectra for the modified Pt electrode at sequential steps in preparation of the glucose biosensor in 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1 molar ratio) in PBS solution. (a) Pt/PPy; (b) Pt/PPy/Nafion/chitosan; (c)–(e) Pt/PPy/Nafion/chitosan with successive layers of GOx/BSA protein ((c) one layer; (d) two layers; (e) three layers.)

Fig. 4.

Current responses of the glucose biosensor to interferents, glucose, and H₂O₂. The biosensor response at a constant potential of 0.7 V (vs. Ag/AgCl) was monitored in stirred PBS solution upon sequential injections to give 5 and 10 μ M of dopamine (DA), followed by 250 and 500 μ M of ascorbic acid (AA), 0.8 and 1.6 mM of glucose (Glu), and 20 μ M and 40 μ M of hydrogen peroxide (H₂O₂).

Fig. 5.

Current response of the biosensor to glucose. The biosensor response in stirred solution was recorded for sequential injections of glucose to give concentrations of 0, 80, 160, 240, 440, 640, 840, 1240, 1640, 2040, 2840, and 3640 μM , at a constant potential of 0.7 V (vs. Ag/AgCl) in PBS buffer (pH 7.4).

Fig. 6. A calibration curve for glucose biosensor. The inset plot shows the lower analyte concentration range. Error bars: standard error of the mean.