Flexible thick-film glucose biosensor: Influence of mechanical bending on the performance

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ABSTRACT

The influence of the bending-induced mechanical stress of flexible Nafion®/GOx/carbon screen-printed electrodes (SPEs) upon the performance of such glucose biosensors has been examined. Surprisingly, such flexible enzyme/polymer-SPEs operate well following a severe bending-induced mechanical stress (including a 180° pinch), and actually display a substantial sensitivity enhancement following their mechanical bending. The bending-induced sensitivity enhancement is observed only for the amperometric detection of the glucose substrate but not for measurements of hydrogen peroxide, catechol or ferrocyanide at coated or bare SPEs. These (and additional) data indicate that the bending effect is associated primarily with changes in the biocatalytic activity. Such sensitivity enhancement is more pronounced at elevated glucose levels, reflecting the bending-induced changes in the biocatalytic reaction. Factors affecting the bending-induced changes in the performance are examined. While our data clearly indicate that flexible enzyme/polymer-SPEs can tolerate a severe mechanical stress and hold promise as wearable glucose biosensors, delivering the sample to the active sensor surface remains the major challenge for such continuous health monitoring.

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1. Introduction

On-body wearable sensors are of considerable recent interest owing to their great promise for monitoring the wearer's health and its surrounding environment [1,2]. Such sensing devices can thus be used for a wide range of healthcare, military or sport applications. While early efforts in this direction have been devoted for continuous physical monitoring of vital signs such as ECG or heart rate, wearable chemical sensors and biosensors are currently receiving a growing interest in connection to personalized health monitoring. In addition, wearable chemical sensors may be used for identifying related threats in the surrounding environment.

One promising route that our group is exploring for addressing the challenge of wearable chemical sensors involves the use of flexible thick-film (screen-printed) transducers. Since the mid-1980s, screen-printing electrode (SPE) technology has been widely employed for fabricating single-use disposable glucose biosensor strips [3,4]. Various amperometric and potentiometric thick-film sensors have since been developed for a wide range of biomedical or environmental applications [5,6]. The screen-printing technol-ogy relies on printing patterns of conductor and insulators onto the surface of planar substrates. Different conducting and insulating ink materials can be used for this task in connection to rigid (ceramic) or flexible (plastic) substrates. The latter, including Kapton, Mylar or polyethylene naphthalate, are particularly suitable for use as wearable on-body sensors. The development of wearable flexible screen-printed on-body sensors requires proper attention to the influence of their mechanical stress, induced during the bending (associated with daily activities of the user) upon the electrochemical behavior, and sensor performance, in general. We have recently examined the effect of electrode bending upon the electrical properties and voltammetric behavior of bare flexible screen-printed electrodes [7].

This article is devoted to examining and understanding the influence of mechanical stress upon the amperometric biosensing at enzyme/polymer-coated SPEs. Since wearable flexible SPE biosensors hold a considerable promise for the management of diabetes, we used a glucose oxidase (GOx)/Nafion-coated SPE for illustrating the impact of mechanical bending and stress. A polyanionic perfluorinated Nafion coating was used in view of the attractive permselective and biocompatibility properties of this polymer and its widespread use in amperometric biosensors [8]. Our findings indicate that extreme bending of flexible GOx/Nafion-coated SPE biosensors (including a 180° pinch) does not cause a sensor fail-ure but actually leads to a substantial sensitivity enhancement
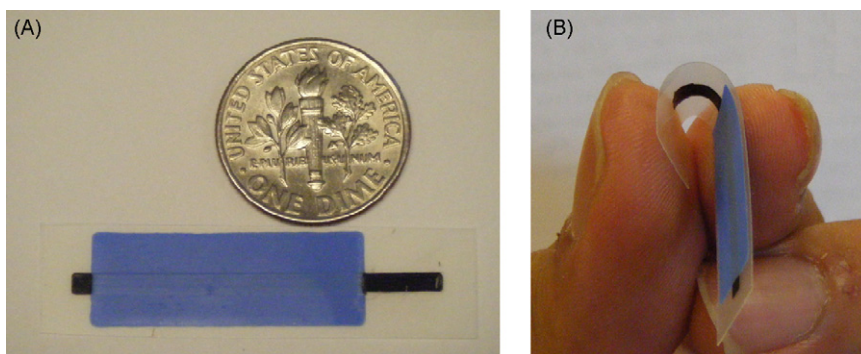

Fig. 1. Images of a bare carbon SPE (black) with an insulating layer (blue) on a flexible Mylar substrate (A), and of the SPE during a 180° inward bend of the working-electrode active portion (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

and to an improved sensor performance. The mechanical-bending enhancement effect has been attributed to changes in the biocatalytic activity. The new data have broad implications beyond the sensing of glucose, and is highly relevant to a wide range of biomedical and security sensing applications involving flexible wearable enzyme/polymer-coated SPEs.

2. Materials and methods

2.1. Chemicals and materials

D(+)-Glucose, potassium chloride (KCl), hydrogen peroxide, potassium ferrocyanide, catechol and the Nafion® (5 wt.%) solution were obtained from Sigma–Aldrich (St. Louis, MO). Glucose oxidase (GOx, EC 1.1.3.4) from *Aspergillus niger* was also purchased from Sigma–Aldrich (St. Louis, MO) and was properly stored in refrigerator before use. Deionized water (>18 MΩ cm), from a NANOpure Diamond system (Thermo Scientific Barnstead, Waltham, MA) was used to prepare all solutions. Electrochemical measurements were carried out in a 0.1 M phosphate buffer (pH 7.0). Glucose stock solutions were equilibrated for at least 24 h prior to use.

2.2. Preparation of sensing electrodes

A semi-automatic screen-printer (Model TF 100, MPM, Franklin, MA) was used to print ~75 μm thick carbon electrodes. Carbon-based ink (E3449, Ercon, Wareham, MA) was squeegeed through a patterned stencil onto a Mylar polyester film (125 μm, DuPont, Wilmington, DE). An insulator ink (E6165, Ercon, Wareham, MA) printing was followed to define a geometric working-electrode area as 10.5 mm^2 (see Fig. 1A). Subsequent to the printing processes, the patterned electrode was cured for 20 min at 120 °C. A 2 μL of a 10 U/mL GOx solution in 0.1 M phosphate buffer (pH 7.0) was cast onto the carbon electrode surface. The electrode was dried at room temperature for 20 min at 23 °C. Subsequently, a 2 μL droplet of 1% Nafion® (diluted in 95% alcohol) was cast onto the enzyme-coated electrode surface and was allowed to dry. The electrode was stored in the refrigerator when not in use.

2.3. Apparatus and measurements

The effect of bending on the resistance of the bare carbon SPE was measured using a digital multimeter Elenco LCM-1950 (Elenco Electronics, Wheeling, IL). The resistance of the electrode was measured in its flat position with probing two points 6 mm apart. To understand the bending effect upon the electrochemical/sensing behavior of electrode, the bending was only applied on the electroactive part of working electrode (i.e., the electrode section not covered by the insulating layer) of freshly prepared

electrodes. All bending were inward (as illustrated in Fig. 1B), for a selected angle and time, and were conducted by the same individual. Following its bending, the working electrode was placed in the cell (described below), containing the reference and counter wire electrodes.

All electrochemical measurements were performed at room temperature using a 620A Electrochemical Analyzers (CH Instruments, Austin, TX). To facilitate the electrochemical measurements, the planar printed working electrode was placed horizontally on one custom-designed plastic plate, integrating external platinum wire (0.25 mm diameter) counter and Ag/AgCl wire reference electrodes through a non-conducting epoxy 'ring', forming the miniaturized electrochemical cell. A 40 μL sample droplet was pipetted onto the electrode strip covering completely the working electrode as well as the counter and reference electrodes. Either cyclic voltammetry (CV) or chronoamperometry (with current sampled after 60 s) was used to evaluate the effect of bending on the performance of the SPE. Images for the sensing electrode strip were acquired by using a scanning electron microscope (XL30, Phillips, Andover, MA).

3. Results and discussion

The bending of flexible sensors and biosensors, associated with the daily activity of the wearer, requires a detailed evaluation of the influence of their mechanical stress upon the sensor performance. Here, we examined the influence of mechanical bending upon the sensing performance of flexible Nafion/GOx/carbon SPE glucose biosensors and evaluated various factors associated with such mechanical stress. The biosensors were exposed to different curvatures of inward bending of the electroactive working-electrode area of the SPE, including a maximal 180° pinch (e.g., Fig. 1B), and changes in their electrochemical and electrical properties, and of their overall sensing performance, due to such mechanical stress were evaluated.

In order to characterize the electrical robustness of the flexible carbon SPE as well as the influence of bending upon its electrical resistance, an inward bending (illustrated in Fig. 1B) was applied to the bare carbon working electrode resulting in a compressive strain. The SPE was bent to 3 and 1 mm curvature radii, corresponding to 90° and 180° bending angles, respectively. The unbent SPE exhibited the lowest resistance of 175 Ω, which increased to 220 and 225 Ω following a 5 min bending of 45° and 90°, respectively. Similar resistance changes (increases) to around 230 Ω were observed following 135° and 180° bending for 5 min. Subsequently, we evaluated the influence of the bending time upon the electrical resistance of the SPEs. Prolonging the bending time had a negligible effect upon the resistance, with resistances around 225 Ω measured following 10 and 20 min bending.
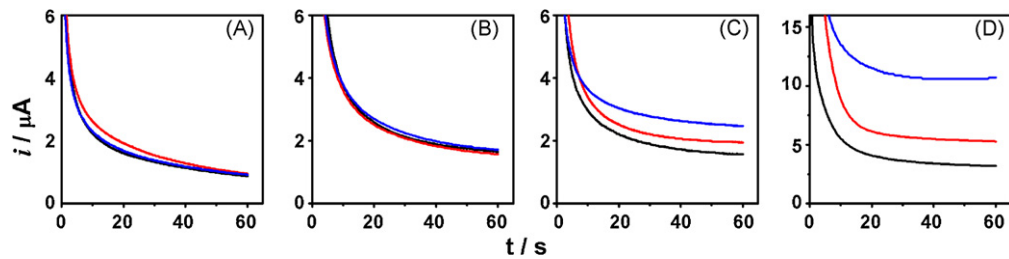
Fig. 2. Chronoamperometric curves recorded at the flexible Nafion/GOx/carbon SPEs before (black) and after 90° (red) and 180° (blue) bending. Test solutions are: (A) 0.1 M phosphate buffer (pH 7), (B) 0.6 mM hydrogen peroxide, (C) 4 mM glucose, and (D) 40 mM glucose. Bending time, 5 min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The influence of the bending-induced mechanical stress upon the electrochemical response of the SPE is illustrated in Fig. 2. These data show chronoamperograms recorded at the flexible Nafion/GOx/carbon SPE for the background buffer solution (A), for 0.6 mM hydrogen peroxide (B) and for 4 (C) and 40 (D) mM glucose solutions, in connection to different extents of bending. The chronoamperometric background and hydrogen peroxide signals exhibit minimal change before and after the (90° and 180°) bending. In contrast, the bending of the SPE has a profound effect upon the glucose response. Such bending-induced signal enhancement is more profound following the 180° bending and at elevated glucose

levels. For example, while the response to 4 mM glucose (measured 60 s after the potential step) increases from 1.57 µA (before bending) to 2.48 µA (following 180° bending), a 235% increase in the 40 mM glucose response (from 3.19 to 10.7 µA) is observed following a similar bending.

In order to further investigate the influence of bending upon the behavior of the Nafion/GOx/carbon SPE, we examined the effect of
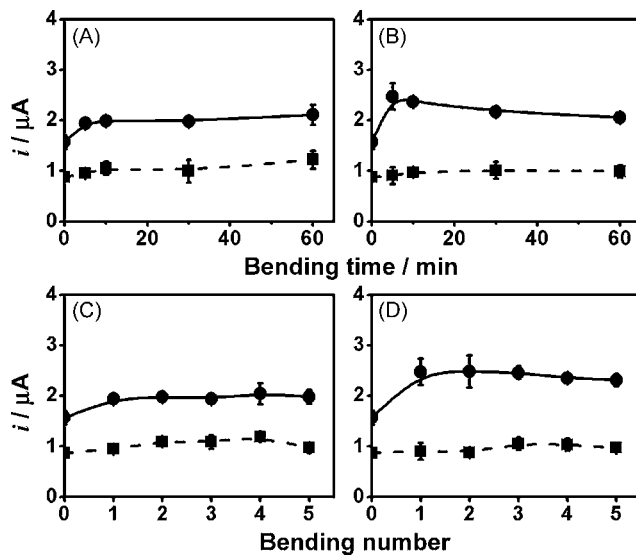

Fig. 3. Effects of bending time (A and B) and bending number (C and D) on the current response to 0.1 M (pH 7) phosphate buffer (■) and 4 mM glucose (●) using 90° (A and C) and 180° (B and D) bending. Bending time (C and D), 5 min. Other conditions, as in Fig. 2 (with sampling after 60 s).
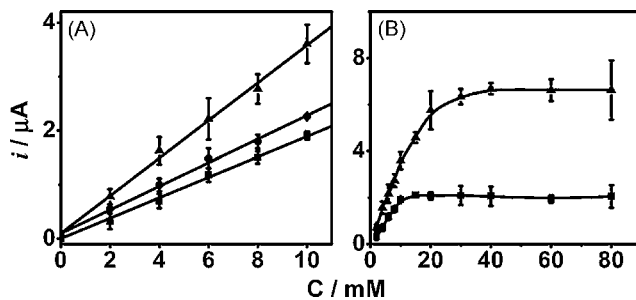

Fig. 4. Calibration curves corresponding to the detections of 2–10 mM (A) and 2–80 mM (B) glucose using the flexible Nafion/GOx/carbon SPE obtained before (■), after a 90° (●), and after a 180° (▲) bending. Bending time, 5 min. Other conditions, as in Fig. 2 (with current sampling after 60 s).
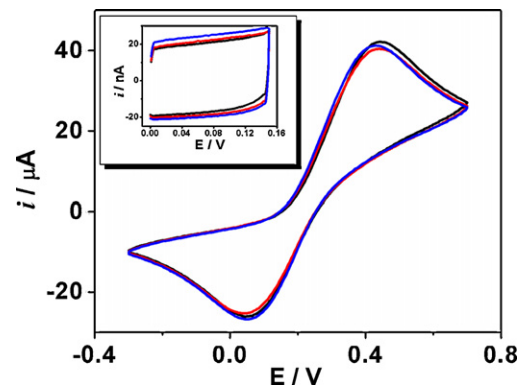

Fig. 5. Cyclic voltammograms for 5 mM ferrocyanide recorded using the bare carbon SPE before (black) and after 90° (red) and 180° (blue) 5 min bending. Inset: Corresponding CVs of the bare carbon SPE recorded in a 0.1 M phosphate buffer (pH 7) blank solution. Scan rate, 0.1 V/s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
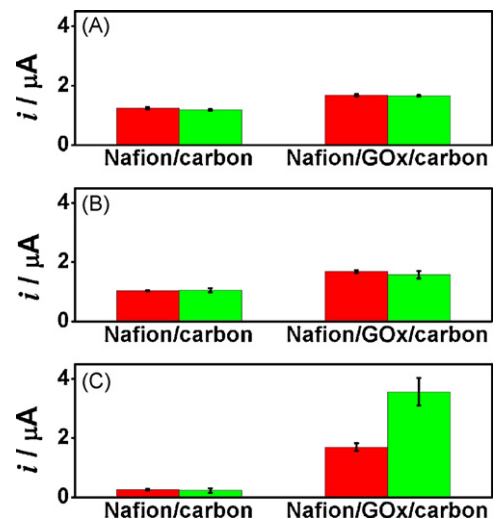

Fig. 6. Bar diagrams featuring the response of Nafion/carbon and Nafion/GOx/carbon SPEs before (red) and after a 180° (green) 5 min bending to (A) 0.2 mM catechol, (B) 0.6 mM hydrogen peroxide, and (C) 4 mM glucose. Other conditions, as in Fig. 2 (with sampling after 60 s). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
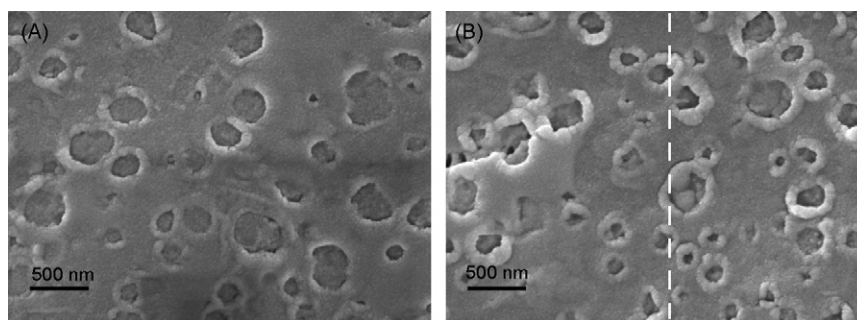
Fig. 7. SEM images of Nafion/GOx/carbon SPEs before (A) and after a 180° (B) 5 min bending. The dashed line in image (B) indicates the place where bending took place.

the bending time and of the number of repeated bending. Fig. 3A and B illustrates the effect of the bending time upon the background (■) and glucose (●) signals in connection to 90° and 180° bending, respectively. A marked (42%) increase in the glucose response is observed following a 5 min 90° bending. Longer bending periods have a negligible effect on the response. Using the 180° bending, the current increases greatly (125%) following the initial 5 min stress, and decreases slightly using longer bending periods. Note that the background currents are nearly independent of the bending time for both 90° and 180° bending (■). The number of repeated bending steps also have a profound effect upon the chronoamperometric glucose response and a negligible effect upon the corresponding background signal (Fig. 3C and D). The biosensor clearly 'survives' such repeated bending, with the glucose current actually increasing by 125% following a single 180° bending and decreases slightly upon additional bending steps (D). A smaller (42%) increase of the glucose signal is observed following a single 90° bending, with no further current changes upon repetitive bending (C).

The influence of the bending-induced mechanical stress upon the concentration-dependence (calibration data) for glucose is illustrated in Fig. 4. For both the 2–10 mM (A) and 2–80 mM (B) glucose ranges, the sensitivity is enhanced following the bending. For example, while a 2.18-fold increase in the signal is observed over the lower concentration range following the 180° bending, an even larger improvement (>3.24-fold) in the response is obtained using a similar bending for elevated (>30 mM) glucose concentrations. Note also that the leveling off of the glucose signals starts at 10 mM and 25 mM for calibration plots obtained before and after bending, respectively, with $K_{m,app}$ values of 5.6 and 9.4 mM (B).

The data of Figs. 2–4 clearly imply that the bending of flexible SPE biosensors results in a higher sensitivity. Such enhancement could be the result of changes in the electrochemical reactivity of the product or of the effective electrode area, of changes in the mass-transport of the substrate towards the enzyme or of changes in the kinetics of the enzymatic reaction. A series of experiments, described below, was conducted for gaining better understanding of the bending effect and to identify the cause of the observed changes.

Cyclic voltammograms (CV) for the blank and ferrocyanide solutions (Fig. 5) were used to shed useful insights into the influence of the mechanical stress upon the electrochemical behavior. Nearly identical ferrocyanide redox signals, with no apparent change in the peak currents or potentials, are observed before and after the 90° and 180° bending (black vs. red and blue). These data indicate that the mechanical stress has a negligible affect upon the electrochemical reactivity. Similarly, the bending of the SPE has a minimal effect upon background CVs recorded in the phosphate buffer over the 0.0–0.15 V range (Fig. 5, inset). Such nearly similar charging background current contributions (and ferrocyanide redox signals) indicate that the effective surface area of the work-

ing electrode is hardly affected by the mechanical stress. Overall, the minimal changes in the electrochemical behavior and reactivity cannot account for the substantially increased glucose signal observed throughout this study following the sensor bending.

While our early data (e.g., Figs. 2–4) illustrate the bending-induced signal enhancement for the glucose substrate of GOx, we examined the influence of the bending of different SPEs upon the response of catechol and hydrogen peroxide (Fig. 6). We expected that such use of electroactive species of different sizes may reveal possible changes in mass-transport through the Nafion and Nafion/GOx layers. Fig. 6 clearly indicates that the bending of the Nafion/GOx/carbon electrode has no affect upon the response to catechol (A) or hydrogen peroxide (B), but has a profound effect upon the response of the glucose substrate (C). Similarly, no changes in the catechol or peroxide signals are observed at the Nafion/carbon electrode (without the enzyme layer, where, as expected, a negligible glucose signal is observed). Overall, the data of Fig. 6 indicate that the enhanced glucose response is not associated with changes in the permeability of the Nafion coating. The SEM images of Fig. 7 indicate no major changes in the morphology of the Nafion coating following the bending of the SPE (compare A and B), supporting the observation of Fig. 6 that the permeability of the polymeric coating is hardly affected by the bending-induced mechanical stress.

The data of Figs. 5 and 6 clearly indicate the bending-induced improved glucose biosensing is not associated with changes in the electrochemical reactivity of the peroxide product or in the permeability of the polymer film. These observations, along with the calibration profiles of Fig. 4, clearly indicate that the larger glucose response reflects primarily changes in the biocatalytic reaction associated with changes in the morphology of the enzyme layer or surface orientation of the enzyme during the mechanical stress. In view of the complexity of the surface layer it is difficult to identify the exact reason for these changes. Changes in the accessibility of the substrate to GOx, in the substrate–enzyme interactions, or transport of the peroxide product, may all account for the observed behavior.

4. Conclusions

In summary, we have examined the influence of mechanical bending upon the sensing performance of the flexible Nafion/GOx/carbon SPEs. Bending the SPEs over wide range of curvatures does not cause any adverse effect on the current response, but actually leads to an enhanced sensitivity. Factors affecting the bending-induced changes in the biosensor performance have been elucidated to indicate that the observed sensitivity improvements reflect primarily changes in the biocatalytic reaction associated with changes in the morphology of the enzyme layer. The data

also indicate promise for bending-induced enhanced selectivity, as the bending effect appears to be associated primarily with the biocatalytic activity (and not redox activity of solution species). The results illustrate the potential of flexible enzyme/polymer-SPEs as wearable glucose biosensors for monitoring of diabetes patients and for a wide range of health monitoring applications. However, the key challenge in developing such wearable glucose sensors, and for wearable flexible biosensors, in general, remains the sample delivery to the active surface of the sensor [2]. Such sample delivery will be addressed in our future studies. Our finding have broader implications onto the operation of flexible enzyme electrode strips and suggest that a simple and short bending period may be used for activating such SPE biosensor strips. Such possibility should be examined for other enzyme/polymer-SPE systems.

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