analytical chemistry

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Rational Design and One-Step Formation of Multifunctional Gel Transducer for Simple Fabrication of Integrated Electrochemical Biosensors

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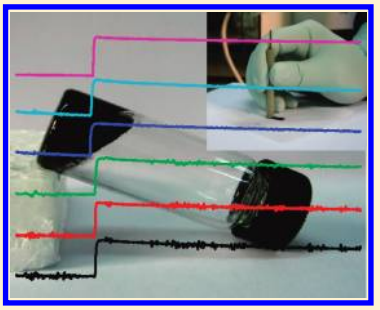
Supporting Information

ABSTRACT: This study demonstrates a new strategy to simplify the biosensor fabrication and thus minimize the biosensor-to-biosensor deviation through rational design and one-step formation of a multifunctional gel electronic transducer integrating all elements necessitated for efficiently transducing the biorecognition events to signal readout, by using glucose dehydrogenase (GDH) based electrochemical biosensor as an example. To meet the requirements for preparing integrated biosensors and retaining electronic and ionic conductivities for electronically transducing process, ionic liquids (ILs) with enzyme cofactor (i.e., oxidized form of nicotinamide adenine dinucleotide) as the anion were synthesized and used to form a bucky gel with single-walled carbon nanotubes, in which methylene green electrocatalyst was stably encapsulated for the oxidation of nicotinamide adenine dinucleotide. With such kind of rationally designed and one-step-formed multifunctional gel as the electronic transducer, the GDH-based electrochemical biosensors were simply fabricated by polishing the electrodes onto the gel followed by enzyme immobilization. This capability greatly simplifies the biosensor fabrication, prolongs the stability of the biosensors, and, more remarkably, minimizes the biosensor-to-biosensor deviation. The relative standard deviations obtained both with one electrode for the repeated measurements of glucose and with the different electrodes prepared with the same method for the concurrent measurements of glucose with the same concentration were 3.30% (n = 7) and 4.70% (n = 6), respectively. These excellent properties of the multifunctional gel-based biosensors substantially enable them to well-satisfy the pressing need of rapid measurements, for example, environmental monitoring, food analysis, and clinical diagnoses.



With the development of society's economy and improvement of the level of people's standard of living, it is very imperative to develop rapid and reliable analytical methods for environmental monitoring, food analysis, clinical diagnoses, and so forth.[1] Among the methods demonstrated so far to meet such requirements, electrochemical biosensors remain particularly attractive, because the integration of specific recognition of biorecognition elements (e.g., enzymes, proteins, and aptamers) toward the targets with the technical simplicity and high sensitivity of electrochemical methods substantially endows the electrochemical biosensors with excellent properties, including good sensitivity, easy adaptability for in situ/on-spot analysis, and relatively cheap instrumentation.[2]

Generally, electrochemical biosensors are mainly based on efficient transduction of biorecognition events into physically readable electronic signal, and as a consequence, the biosensors have often been constructed by coimmobilizing electronic transducers and biorecognition units onto solid substrate to accomplish the requirements for development of integrated biosensing devices.[2a,b,3] Due to the difficulties in directly wiring the biorecognition units (i.e., enzymes and proteins) onto substrate to efficiently transduce the biorecognition events into electronic signal in a straightforward manner, some kinds of electron transfer mediators or electrocatalysts are inevitably involved in the electronically transducing process to facilitate the electronic communication between the biorecognition units and electrode in an indirect approach.[2a,b,3b,4] For instance, in the oxidase-based electrochemical biosensors, electron transfer mediators are frequently employed to shuttle the electron transfer between enzymes and electrode.[2a,b,3b,5] More complicated than this, the uses of dehydrogenases as the biorecognition units basically requires the concurrent uses of cofactor (e.g., oxidized form of nicotinamide adenine dinucleotide, NAD) and of electrocatalysts for the catalytic oxidation of the reduced form of the cofactor (e.g., reduced form of nicotinamide adenine dinucleotide, NADH) to efficiently transduce the biorecognition events into the physically readable current signal in the dehydrogenase-based biosensors.[2a,b] These requirements, along with those for the electronic and ionic conductivities and for the biocompatibility toward the biorecognition units, substantially complicated the components and their surface assembling of the electronic transducers. In fact, the fabrication of dehydrogenase-based electrochemical biosensors

Received: April 12, 2011
Accepted: June 4, 2011
Published: June 06, 2011

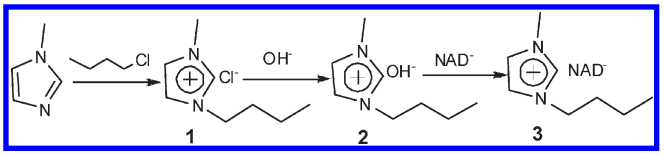
often included several step-by-step procedures for surface immobilization of electrocatalysts for NADH oxidation and NAD cofactor for dehydrogenases, which were technically complicated and time-consuming.[2a,b,3b] More importantly, the involvement of multiple steps for biosensor fabrication inevitably makes it difficult to minimize the biosensor-to-biosensor and person-to-person as well as lab-to-lab deviations, even though the surface enzyme immobilization can be carefully controlled. These deviations eventually make it difficult to apply the electrochemical biosensors to satisfy the pressing need of rapid and in situ/on-spot measurements, although the excellent analytical properties of this kind of biosensor substantially enable them potentially to meet the requirements mentioned above.

This study demonstrates a new strategy to simplify the biosensor fabrication and thus minimize the biosensor-to-biosensor deviation through rational design and one-step formation of a multifunctional gel electronic transducer integrating all elements necessitated for efficiently transducing the biorecognition events to signal readout, by using glucose dehydrogenase-based biosensor as an example. Considering the requirements of both electronic and ionic conductivities for the electrochemical and biochemical reactions in the signal transducing processes, a gel formed by single-walled carbon nanotubes (SWNTs) and ionic liquids (ILs) with enzyme cofactor as the anion is chosen as the bulk in terms of the good electronic and ionic conductivities of SWNTs and ILs, respectively.[6] Moreover, both the rich surface chemistry of SWNTs and excellent physiochemical properties of ILs substantially facilitate the encapsulation of other kinds of elements necessitated for signal transducing within the gel to eventually form a multifunctional gel transducer for electrochemical biosensing.[7] With the multifunctional gel as the electronic transducer, the electrochemical biosensors can thus be simply and reproducibly fabricated by grinding the electrodes onto the gel, followed by enzyme surface immobilization. Compared to the existing methods for preparing the integrative electrochemical biosensors, the strategy demonstrated in this study is facile and reproducible. This capability is envisaged to further enable the electrochemical biosensors to meet well the requirements for practical measurements such as environmental monitoring, food analysis, clinical diagnoses, and so forth.

EXPERIMENTAL SECTION

Chemicals and Materials. SWNTs were purchased from Shenzhen Nanoport Co. Ltd. and were purified by refluxing the as-received SWNTs in 2.6 M nitric acid for 5 h, followed by centrifugation, resuspension, filtration, and air-drying to evaporate the solvent. Glucose dehydrogenase (EC 1.1.1.47, from *Pseudomonas* sp.), bovine serum albumin (BSA), and D-(+)-glucose were all obtained from Sigma. Methylene green (MG) and glycine were purchased from Chemical Reagent Co. Ltd. (Beijing, China). 1-Methylimidazole, chlorobutane, 1-bromotetradecane, 3-bromopropyltrimethylammonium bromide, 1,10-dibromodecane, and 717 ion-exchange resins were obtained from the Aladin Reagent Co. (Shanghai, China). The oxidized form of nicotinamide adenine dinucleotide (NAD) was purchased from Aldrich. Other chemicals were of at least analytical grade and used as received.

Synthesis of NAD-Based Ionic Liquids. Synthesis of NAD-based ILs was preformed according to the following reaction scheme, with 1-butyl-3-methylimidazolium as a typical example. Other kinds of NAD-based ILs were synthesized according to the same scheme only by changing the corresponding cations.



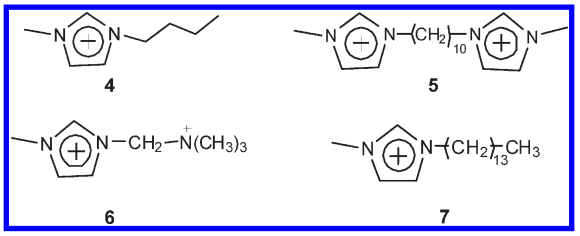
To synthesize NAD-based IL with 1-butyl-3-methylimidazolium ($Bmim^+$) as the cation (i.e., $Bmim^+NAD^-$, **3**), $Bmim^+Cl^-$ (**1**) was first synthesized according to a previous report.[8] Briefly, in a typical experiment, a solution of 1-methylimidazole (33 mL, 0.31 M) in methylene chloride (50 mL) was added to a three-neck flask, and then 1-chlorobutane (20 mL, 0.25 M) was mixed into the solution at room temperature. The resulting mixture was refluxed at 50 °C under nitrogen atmosphere for 24 h. After being cooled to room temperature, the solution forms two phases. The bottom phase was first separated from the mixture and then precipitated by adding 50 mL of ethyl acetate. The solvent was filtered off and the residue solvent in the precipitate was further removed by vacuum drying at 50 °C overnight to give a white product, **1**: ^{1}H NMR (400 Hz, D_2O) δ 0.88 (t, 3H), 1.28 (m, 2H), 1.81 (m, 2H), 3.85 (s, 3H), 4.16 (t, 2H), 7.39 (s, 1H), 7.44 (s, 1H), 8.68 (s, 1H).

In order to synthesize **3**, **2** was first obtained through anion exchange with **1** using 717 ion-exchange resin. After that, to an aqueous solution of NAD (1 g, 1.5 mM), an equivalent aqueous solution of **2** was added drop by drop under vigorously stirring and the resulting mixture was kept stirring at room temperature for 48 h. Then, the solvent was evaporated at 50 °C. To this reaction mixture, methanol and acetonitrile (volume ratio, 9:1) were added, and the resulting mixture was first stirred vigorously and then filtered to remove excess NAD. The filtrate was evaporated to remove solvents and dried in vacuo for 24 h at 80 °C to obtain **3**: ^{1}H NMR (400 Hz, D_2O) δ 0.85 (t, 3H), 1.26 (m, 2H), 1.77 (m, 2H), 3.83 (s, 3H), 4.12 (t, 2H), 4.22−4.71 (m, 15H), 5.91 (d, 1H), 5.99 (d, 1H), 7.26 (s, 1H), 7.31(s, 1H), 8.06 (s, 1H), 8.11 (m, 1H), 8.34 (s, 1H), 8.55 (s, 1H), 8.73 (s, 1H), 8.76 (s, 1H), 9.09 (d, 1H), 9.25 (s, 1H). Anal. Calcd for **3** (i.e., $Bmim^+NAD^-$): C, 41.51; H, 5.53; N, 15.03. Found: C, 41.90; H, 5.54; N, 15.10. The IR and UV−vis results for **3** are given in the Supporting Information (Figure S1).

Synthesis of 1-Butyl-3-methylimidazolium Glysine ($Bmim^+Gly^-$). $Bmim^+Gly^-$ was synthesized according to the method reported previously with minor modification.[9] Typically, to the aqueous solution of glycine (1 g, 13.3 mM) was added an equivalent aqueous solution of **2** drop by drop under vigorously stirring at room temperature. The resulting mixture was kept stirring at room temperature for 12 h in an ice−water bath. Then, water solvent was evaporated at 45 °C. To this reaction mixture, methanol and acetonitrile (volume ratio, 9:1) were added, and the resulting mixture was stirred vigorously. After that, the mixture was filtered to remove excess amino acid and the filtrate was evaporated to remove solvents. The product was dried in vacuo for 2 days at 80 °C: ^{1}H NMR (D_2O): δ 0.8 (t, 3H), 1.22 (m, 2H), 1.78 (m, 2H), 3.10 (s, 3H), 3.77 (s, 3H), 4.08 (t, 2H), 7.31 (s, 1H), 7.36 (s, 1H).

Preparation of Multifunctional Gel. To prepare the multifunctional gel, a nanocomposite of SWNTs and MG was first prepared according to our previous report.[2d] Typically, SWNTs (40 mg) were mixed into ethanol solution (4 mL) containing 10 mg/mL MG. After being stirred overnight, the mixture was

Scheme 1. Chemical Structures of Cations of NAD-Based Ionic Liquids



filtered and washed with ethanol to remove the excess MG. After evaporating the solvent, the SWNT/MG nanocomposite was obtained. A 10 mg portion of SWNT/MG nanocomposite was mixed with 0.2 mL of $Bmim^+Gly^-$ and 2 mg of $Bmim^+NAD^-$, and the resulting mixture was ground for about 30 min to form a black gel. Alternatively, the multifunctional gel could also be prepared simply by grinding 10 mg of SWNTs with 0.2 mL of $Bmim^+Gly^-$ containing 2 mg of $Bmim^+NAD^-$ and 2 mg of MG. For comparison, a composite was also prepared by simply grinding the mixture consisting of 10 mg of SWNTs, 2 mg of natural NAD, 2 mg of MG, and 0.2 mL of $Bmim^+Gly^-$ containing 20% water.

Apparatus and Electrochemical Measurements. Electrochemical measurements were performed with a computer-controlled electrochemical analyzer (BAS 100B/W, BAS) with a conventional three-electrode cell. For glucose biosensing, the multifunctional gel-based biosensor was used as working electrode, platinum spiral wire as counter electrode, and Ag/AgCl (KCl-saturated) electrode as reference electrode. A 0.10 M phosphate buffer (pH 7.0) was used as the supporting electrolyte. To prepare the GDH/MG/SWNT-modified GC electrode, MG/SWNT composite was prepared by mixing 2 mg of SWNTs and 5 mg of MG into 5 mL of distilled water. After being sonicated for 2 h, the mixture was isolated by centrifugation (5000 rpm) for 1 min and rinsed with distilled water and dried at 75 °C for 5 h to obtain the MG/SWNT adduct. The MG−SWNT adduct was dispersed into distilled water to give a suspension (1 mg mL^{-1}), and 5 μL of the as-formed suspension was dip-coated onto GC electrode to give the MG/SWNT-modified GC electrode. For immobilizing GDH onto the MG/SWNT- modified electrode, 1 μL of GDH (10 mg/mL) solution was mixed with 1 μL of BSA aqueous solution (1%) and 1 μL of gluteraldehyde (1%), and the resulting mixture was totally coated onto the MG/SWNT-modified electrode. After being rinsed with distilled water, the GDH/MG/SWNT-modified electrode was dried at ambient temperature. The reproducibility for the repeated measurements of glucose with one biosensor was conducted in a continuous-flow system with the biosensor as the online detector, as reported in our earlier works.[2d] The reproducibility of the biosensor fabrication was evaluated through concurrent measurements of the same concentration of glucose with the different multifunctional gel-based biosensors prepared with the same procedures with a multichannel electrochemical analyzer (CHI 1030, Chenhua, Shanghai, China).

^{1}H NMR was measured on a Bruker Avance 400 in D_2O. Elements analysis was measured on a Flash EA1112. UV−vis spectrometry was performed on TU-1900 spectrophotometer (Beijing, China). For UV−vis spectrometric measurements, $Bmim^+NAD^-$ and natural NAD were both dissolved into Milli-Q water. CD and IR spectrometry were performed on a J-815 spectrophotometer (JASCO) and Tensor-27 FTIR spectrometer (Bruker), respectively.

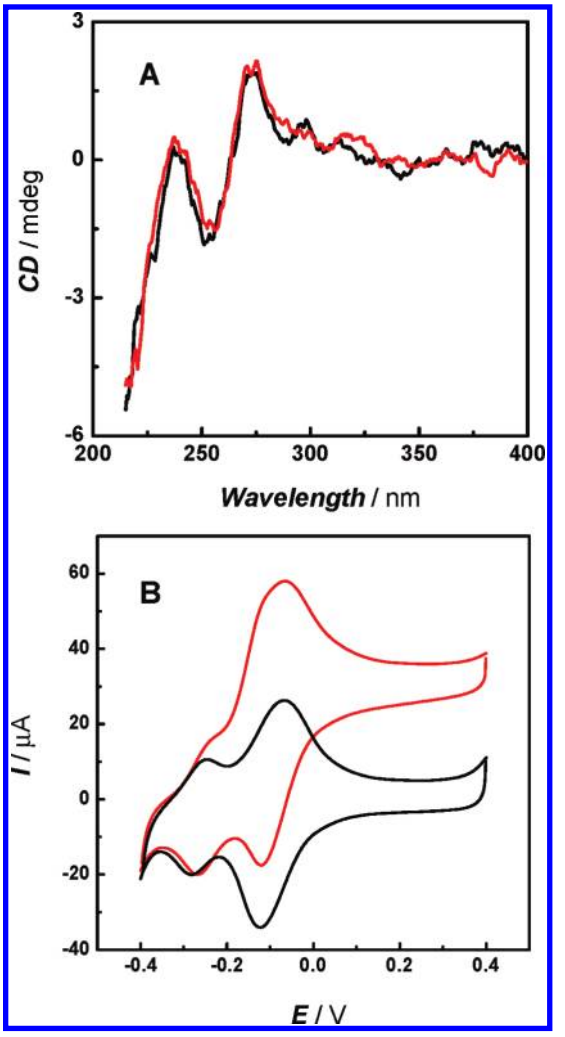


Figure 1. (a) CD spectra of aqueous solutions of 250 μM $Bmim^+NAD^-$ (red curve) and natural NAD (black curve). (b) CVs obtained at the GDH/MG/SWNT-modified GC electrode in 0.10 M phosphate buffer (pH 7.0) containing 40 mM glucose in the absence (black curve) and presence (red curve) of 10 mM $Bmim^+NAD^-$. Scan rate, 1 mV/s.

RESULTS AND DISSCUSSION

Design and Synthesis of NAD-Based Ionic Liquids. As mentioned above, to efficiently transduce the biorecognition events into the physically readable signal readout in the GDH-based electrochemical biosensors, the electronically transducing system generally requires the concurrent uses of NAD as the cofactor and of electrocatalyst to accelerate the oxidation of NADH and converse NADH into NAD. Due to the poor solubility of natural NAD cofactor in most kinds of ILs and the complicated procedures to prepare NAD/SWNT nanocomposite through the interaction between both components,[10] we synthesized IL with $Bmim^+$ as the cation (**4**, Scheme 1) and NAD as the counteranion since the oxidized form of nicotinamide adenine dinucleotide bears one negative charge. To prepare the gel transducer, one kind of redox organic dye, MG, was employed as the electrocatalyst for NADH oxidation due to its excellent electrocatalytic activity and capability of being well-encapsulated into the gel by taking advantage of the interaction with SWNTs and dissolution into the ILs.[2d] Unfortunately, the synthetic

$Bmim^+NAD^-$ was solid state at room temperature (mp 95 °C) and was not able to form a bucky gel with SWNTs. It was reported that the melting points of ILs were closely associated with the kinds of cations.[11] We thus synthesized other three kinds of NAD-based ILs with different kinds of cations, of which two were composed of the cations with two positive charges (**5** and **6**, Scheme 1), with the same methods for $Bmim^+NAD^-$, since the ILs based on this kind of the cations may have the lower melting points, as compared with those based on the cations with one positive charge, as reported previously.[11b] However, the synthetic NAD-based ILs with these two kinds of cations were also solid at room temperature. We then synthesized another kind of IL composed of a cation with a high asymmetric property (**7**, Scheme 1) to prepare the NAD-based IL since, according to the previous report,[11c] the melting points of ILs decrease with increasing the asymmetry of cation. Although the synthetic IL was liquid at room temperature, it was very viscous and difficult to use to form a gel with SWNTs. Consequently, we decided to use another kind of IL with glycine as the counteranion (i.e., $Bmim^+Gly^-$) to assist $Bmim^+NAD^-$ to form a bucky gel with SWNTs. $Bmim^+Gly^-$ was chosen as the second kind of IL, since this kind of IL was present as liquid at room temperature and, more remarkably, was predicted to exhibit a better biocompatibility toward biomacromolecules, as compared with those based on the halogen anions.[12]

Prior to the formation of the multifunctional gel with the synthetic ILs, the conformation and bioactivity of the NAD counteranion in $Bmim^+NAD^-$ were investigated. As displayed in Figure 1A, the circular dichroism (CD) spectrum of $Bmim^+NAD^-$ shows a negative signal and positive signal at 255 and 275 nm, respectively (red curve). These signals were also observed for the natural NAD (black curve), suggesting that NAD maintains its natural conformation when it serves as the counteranion of IL. Moreover, the NAD counteranion well retains its bioactivity as the cofactor for GDH, as was evident from cyclic voltammograms (CVs) for bioelectrocatalytic oxidation of glucose at the GDH/MG/SWNT-modified glassy carbon (GC) electrode with the presence of $Bmim^+NAD^-$ in 0.10 M phosphate buffer (pH 7.0) containing glucose (Figure 1 B). With the absence of $Bmim^+NAD^-$ in the buffer containing glucose, the GDH/MG/SWNT-modified GC electrode exhibits two couples of redox waves at ca. −0.10 and −0.30 V, which were attributed to the redox processes of MG adsorbed onto SWNTs (black curve).[2d] The addition of $Bmim^+NAD^-$ into the buffer clearly results in an increase in the oxidation peak current and a decrease in the reduction peak current at −0.10 V (red curve). Since $Bmim^+NAD^-$ itself was electrochemically inert, the change in the current responses was indicative of the occurrence of glucose oxidation under the present conditions, revealing that the synthetic $Bmim^+NAD^-$ retains its bioactivity as the cofactor to assist GDH to catalyze the oxidation of glucose. This property further validates the strategy for formation of the multifunctional gel electronic transducer by integrating all elements necessitated for electronically transducing process, as described below. Note that, since NAD cofactor is well water-soluble and difficult to be stably confined onto electrode surface, almost all kinds of the dehydrogenase-based electrochemical biosensors reported so far have been prepared by dissolving such NAD into the solution or confining NAD onto electrode surface through covalent immobilization[13] or entrapment into different matrix such as carbon paste,[14] membrane,[15] polymers,[16] and assembled layer.[17] This property, on one hand, renders difficulties in applying this kind of biosensors for rapid and cost-effective analysis and, on the other hand, increases the environmental burden.

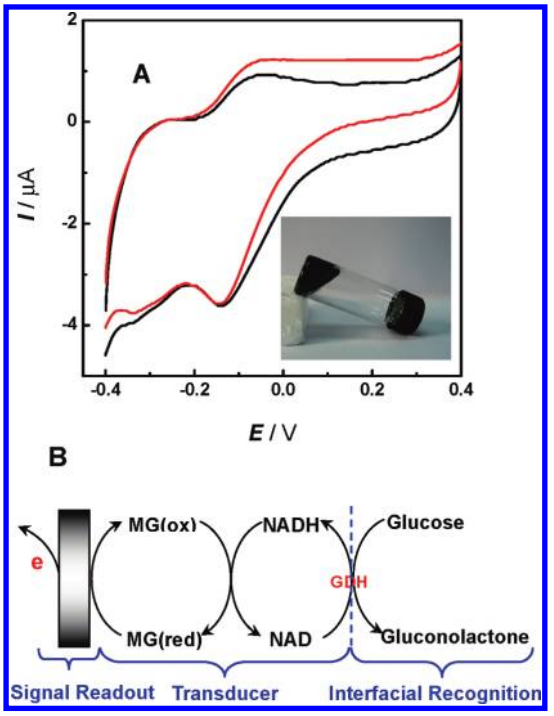


Figure 2. (A) Bioelectrocatalytic oxidation of glucose at the multifunctional gel based biosensor in 0.10 M phosphate buffer (pH 7.0) in the absence (black curve) and presence (red curve) of 40 mM glucose. Scan rate, 1 mV/s. Inset, digital picture of the prepared multifunctional gel. (B) Illustration of the reaction schemes involved in the bioelectrocatalytic oxidation of glucose at the multifunctional gel based biosensor.

Electrochemical Biosensing Properties of Multifunctional Gel-Based Biosensor. With rationally designed components as mentioned above, the multifunctional gel was one-step-formed by simply grinding the mixture containing SWNTs, $Bmim^+NAD^-$, $Bmim^+Gly^-$, and MG, as displayed in Figure 2A (inset). With the as-prepared gel as the electronic transducer to transduce the GDH-catalyzed oxidation of glucose into current signal, the biosensor was simply fabricated by first mounting the gel onto GC electrode by polishing the electrode on the gel and then overcoating GDH onto the gel-mounted electrode. It is worth noting that the present strategies for formation and fabrication of electronic transducer and biosensors, respectively, not only greatly simplify the procedures for biosensor fabrication but also are envisaged to minimize the biosensor-to-biosensor deviation as described below. This property substantially enables the as-prepared biosensors to meet well the pressing need for rapid and reliable in situ/on-spot analysis in various fields.

Figure 2A depicts the typical CVs for bioelectrocatalytic oxidation of glucose at the multifunctional gel based biosensor in 0.10 M phosphate buffer (pH 7.0). The biosensor itself exhibits one pair of redox wave at ca. −0.10 V (vs Ag/AgCl), which was ascribed to the redox process of MG electrocatalyst encapsulated into the gel transducer. The addition of 40 mM glucose into the buffer solution leads to the increase in the oxidation current at ca. −0.10 V, indicative of the occurrence of glucose oxidation on the biosensor under the bioelectrocatalysis of GDH. This property eventually suggests that the rationally designed and one-step-formed multifunctional gel prepared by

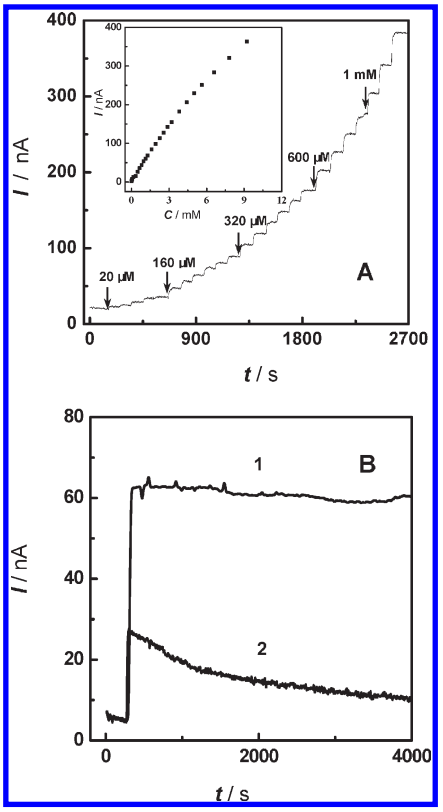


Figure 3. (A) Typical amperometric response obtained at the multifunctional gel based biosensor in 0.10 M phosphate buffer (pH 7.0) toward successive addition of glucose with the concentration indicated in the figure. Inset: plot of current response versus concentration of glucose. (B) Typical amperometric response of glucose (2 mM) obtained at the biosensors: curve 1, with the multifunctional gel as the electronic transducer; curve 2, with the composite of natural NAD dissolved in the $Bmim^+Gly^-$/SWNT bucky gel as the electronic transducer. Potential applied, +0.20 V.

encapsulating all elements into the IL/SWNT bucky gel well serves as the electronic transducer to efficiently transduces the GDH-catalyzed glucose oxidation into physically measurable current signal through the sequential chemical and electrochemical reactions shown in Figure 2B.

In addition to its good catalytic activity toward glucose oxidation and simple fabrication, the biosensor with the multifunctional gel as the electronic transducer was very responsive toward glucose, as shown in Figure 3A. In 0.10 M phosphate buffer (pH 7.0), well-defined current response was obtained toward the successive addition of glucose into the buffer when the biosensor was polarized at a constant potential of +0.20 V (vs Ag/AgCl). The current response was linear with the concentration of glucose within a range from 20 μM to 3.8 mM with a linear coefficient of 0.999. All these results suggested that the strategy demonstrated here to simplify the biosensor fabrication through rational design, one-step formation, and smart use of the multifunctional gel as the electronic transducer essentially paves a straightforward route to rapid fabrication of dehydrogenase-based electrochemical biosensors.

The multifunctional gel based biosensors were relatively stable and reproducible for glucose measurements, as shown in Figure 3 B. The current response remains unchanged after continuously running the measurements for more than 60 min (curve 1). The good stability of the biosensor may be benefited from the

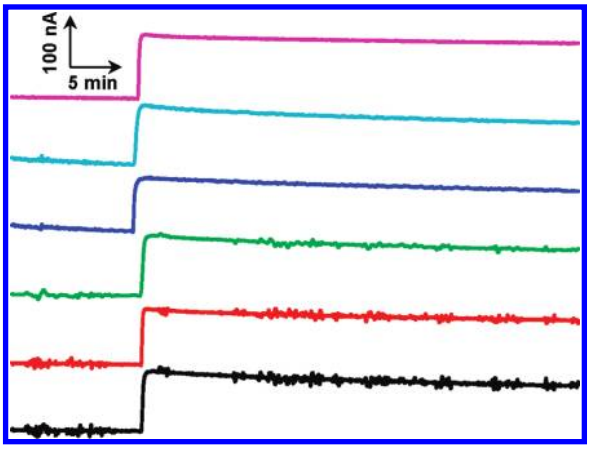


Figure 4. Typical amperometric responses of glucose (5 mM) in 0.10 M phosphate buffer (pH 7.0) obtained at the six different multifunctional gel based biosensors prepared by the same method. The electrodes were poised at +0.20 V.

synergic interactions among the synthetic $Bmim^+NAD^-$, $Bmim^+Gly^-$, MG, and SWNTs in as-prepared multifunctional gel, which eventually lead to the stable encapsulation of each components in the gel, even though both $Bmim^+NAD^-$ and MG were water-soluble. This was illustrated by comparing the stability of the multifunctional gel based biosensor with that of the biosensor with the electronic transducer prepared by first dissolving the natural NAD into $Bmim^+Gly^-$ containing about 20% water and then grinding the mixture with SWNTs to form a black gel. As depicted in Figure 3B, the current response of the as-prepared biosensor for the glucose gradually decreases as a function of time (curve 2), suggesting the poor stability of the electrochemical biosensor prepared with this method. This comparison further validates the strategy demonstrated in this study to design rationally and to form in one step the electronic transducer for simple fabrication of the electrochemical biosensors with a high stability. In addition to the high stability, the multifunctional gel based biosensor possesses a higher sensitivity toward glucose than the biosensor with the composite of natural NAD dissolved in the IL/SWNT gel as the electronic transducer (Figure 3B).

More importantly, the biosensors with the synthetic multifunctional gel as the electronic transducer show good reproducibility both for the repeated measurements with one electrode (Supporting Information, Figure S2) and for the same measurements with different electrodes prepared with the same method (Figure 4). These properties were illustrated by the small relative standard deviations (RSD) obtained both with one electrode for the repeated measurements of glucose (3.30%, n = 7) and with the different electrodes prepared with the same method for the parallel measurements of glucose with the same concentration (4.70%, n = 6). The latter property suggests the good reproducibility for biosensor fabrication by using the multifunctional gel as the electronic transducers for the biosensors. These excellent properties strongly demonstrate that the uses of rationally designed and one-step-formed multifunctional gel to transduce efficiently the biorecognition events into physically readable current signal greatly simplify the biosensor fabrication, prolong the stability of the biosensors, and, more remarkably, minimize the biosensor-to-biosensor deviation. These properties of the multifunctional gel based biosensors substantially enable them to meet well the pressing need for rapid and in situ/on-spot

measurements, such as for environmental monitoring, food analysis, clinical detection, and so forth.

CONCLUSIONS

In summary, a novel strategy has been demonstrated to simplify the fabrication of electrochemical biosensors and thus minimize the biosensor-to-biosensor deviation through rationally designing and one-step formation of a multifunctional gel integrating all elements necessitated for electronically transducing the biorecognition events into physically readable signal. The electrochemical biosensors with the multifunctional gel as the electronic transducer are very responsive toward the target, with a simple fabrication procedure, good stability and reproducibility, and less biosensor-to-biosensor deviation. These excellent properties of the multifunctional gel based biosensors substantially enable them to be very attractive for practical applications, for example, environmental monitoring, food analysis, and clinical diagnoses. Moreover, the strategy demonstrated here may also be versatile for greatly simplifying the fabrication of other kinds of molecular bioelectronic devices, such as biofuel cells and bioreactors.

ASSOCIATED CONTENT

Supporting Information. Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

This research was financially supported by the NSF of China (Grant Nos. 20975104, 20935005, 90813032 for L.M. and 20805050 for P.Y.), the National Basic Research Program of China (973 program, 2007CB935603, and 2010CB33502), and The Chinese Academy of Sciences (KJCX2-YW-W25 and Y2010015).

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