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High-performance amperometric biosensors and biofuel cell based on chitosan-strengthened cast thin films of chemically synthesized catecholamine polymers with glucose oxidase effectively entrapped

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ABSTRACT

Rapid oxidation of dopamine (DA) or L-noradrenaline (NA) by $K_3Fe(CN)_6$ yields poly(DA) (PDA_C) or poly(NA) (PNA_C) with glucose oxidase (GOx) effectively entrapped, and such an enzyme-entrapped catecholamine polymer is cast on an Au electrode followed by chitosan (CS) strengthening for biosensing and fabrication of a biofuel cell (BFC). The optimized glucose biosensor of CS/PDA_C–GOx/Au displays an extremely high sensitivity up to 135 μA mM^{-1} cm^{-2}, a very low limit of detection of 0.07 μM, a response time of <3 s, good suppression of interferents, striking thermostability (lifetime of 3 weeks at 60 °C and over 2 months at 30 °C), and high resistance to urea denaturation. The biosensor also works well in the second generation biosensing mode with *p*-benzoquinone (BQ) or ferrocene monocarboxylic acid (Fc) as an artificial mediator, with greatly broadened linear detection ranges (2.0 μM–48.0 mM for BQ and 2.0 μM–16.0 mM for Fc) and up to mA cm^{-2}-scale glucose-saturated current density. The good permeability of artificial mediators across the enzyme film enables the quantification of the surface concentration of immobilized GOx on the basis of a reported kinetic model, and UV–Vis spectrophotometry is used to measure the enzymatic activity, revealing high enzymatic activity/load at CS/PDA_C–GOx/Au. A BFC is also successfully fabricated with a bioanode of CS/PDA_C–GOx/Au in phosphate buffer solution containing 100 mM glucose and 4.0 mM BQ and a carbon cathode in Nafion-membrane-isolated acidic $KMnO_4$, and its maximum power density of 1.62 mW cm^{-2} is superior to those of most BFC hitherto reported.

1. Introduction

Catecholamines, e.g. dopamine (DA) and L-noradrenaline (NA), are important hormones and neurotransmitters, and their intriguing redox properties have been so widely studied (Chen and Peng, 2003; Hawley et al., 1967; Lakshmi et al., 2009; Schwarz and Hauser, 2003). The polymers prepared by DA and NA electrooxidation (PDA_E and PNA_E) have been recognized as excellent biocompatible matrices to immobilize enzymes for highly sensitive and selective biosensing (Chen et al., 2009; Li et al., 2006a). Interestingly, electropolymerization of DA and NA can yield electrode-supported films that are much thicker than many other insulating polymer films owing to their special melanin-like porous structure, despite of the insulating nature of DA and NA polymers (He et al., 2005). The proven properties of PDA_E and PNA_E are quite useful in entrapping biomacromolecules at high load/activity (Chen et al., 2009). In contrast, the studies on the chemical polymerization of DA and NA and the resultant polymers (PDA_C and PNA_C) are rather limited to date. Recently, Messersmith et al. have proposed and developed DA self-polymerization as a novel and important protocol for mussel-inspired multifunctional coatings of thin, surface-adherent DA polymer films onto a wide range of inorganic and organic materials (Lee et al., 2007). Caruso et al. have fabricated the assembly of DA polymer films by the spontaneous oxidative polymerization of a DA solution onto silica particles to form robust capsules (Postma et al., 2009). The DA self-polymerization is virtually driven by oxidation of dissolved O_2 in DA solution. However, the saturated concentration of O_2 in water is only ca. 1.3 mM (Wilhelm et al., 1977), thus the low solubility of O_2 in neutral aqueous media makes the DA self-polymerization a

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relatively slow process, though the DA-polymerization rate is positively relevant to solution pH. Hence, we hope that the chemical oxidation of DA and NA by an appropriate coexisting oxidant can accelerate their polymerization for time-saving immobilization of biomacromolecules in neutral media.

The tremendous potential of enzymes as highly efficient catalysts is proverbially recognized, but the poor thermostability of enzymes greatly limits their practical applications in many cases (Alexander and Klibanov, 2001; Amine and Kauffmann, 1992; Karyakin et al., 2002; Wang et al., 1997; Zhang et al., 1996). Advantages of employing high temperature lie in higher process rates, lower viscosity of the medium, fewer diffusion limitations, decreased bacterial contamination and thermodynamic equilibrium shift in endothermic reactions (Iyer and Ananthanarayan, 2008). As one of the most important model enzymes, glucose oxidase (GOx) is of considerable commercial importance for its advantages in removing glucose, oxygen and bacteria from food products without safety problems (Ahmad et al., 2001; Rauf et al., 2006). The extensive utilization of GOx in fermentation media and feed additives as well as function as an antibiotics and anticoccidiosis agent highly requires its long-term thermostability. Unfortunately, native GOx is known to be denatured in solution above 55 °C (Cass et al., 1984; Wang et al., 1997), and its half-life at 60 °C is only 22 min (Fortier et al., 1992). Researchers have exerted great efforts on improving the thermostability of enzymes by exploring various protein-stabilization mechanisms (Ahmad et al., 2001; Daniel, 1996; Vieille and Zeikus, 1996). Highly hydrophobic materials have been utilized to immobilize GOx at improved thermostability (Appleton et al., 1997; Wang et al., 1997), but the hydrophobic materials are detrimental to the bioactivity of GOx. Obviously, it is interesting and important to design suitable support matrices and new immobilization protocols for more effectively retaining the high enzymatic activity at elevated temperatures (Dong and Wang, 2002; Lee et al., 2005; Patel et al., 2006).

The third-generation amperometric biosensor at which the redox center of GOx can directly exchange electrons with the electrode is of great importance in bioscience and biotechnology (Khan et al., 1996; Palmisano and Zambonin, 2002). However, it is very difficult to simultaneously realize high enzymatic and electrochemical activities of the immobilized GOx (Su et al., 2008; Wang, 2008) and mediated electrochemistry is thus often used for GOx (Wen et al., 2007). When the natural mediator (O_2) is used as the electron acceptor (first-generation biosensors), an obvious limitation is the dependence of sensor response on O_2 concentration (Fu et al., 2009). To solve this problem, second-generation biosensors based on artificial mediators have been introduced, which exhibit improved performance such as higher current output and wider linear detection range (LDR) (Hale et al., 1989; Kajiya et al., 1991).

Herein, we report on a novel and high-performance biosensing platform based on entrapment of GOx in chemically oxidized catecholamine polymers (PDA_C and PNA_C) and cast of the resultant enzymatic composites on an Au electrode with chitosan (CS) as a binder. The thus-prepared thin film amperometric biosensor exhibits high sensitivity/thermostability and works well both in the first and second-generation biosensing modes. The enzyme film is also demonstrated as an excellent anodic material in biofuel cell (BFC) construction.

2. Experimental

2.1. Instrumentation and chemicals

All electrochemical experiments were conducted on a CHI660C electrochemical workstation (CH Instrument Co., USA), and a conventional three-electrode electrolytic cell was used. A 3.0-mm diameter Au disk electrode, a KCl-saturated calomel electrode (SCE), and a carbon rod were used as the working, reference, and counter electrodes, respectively. All potentials here are cited versus SCE (vs. SCE). Scanning electron microscopy (SEM) pictures were collected on an EM-6700F field emission scanning electron microscope.

GOx (EC 1.1.3.4; type II from *Aspergillus niger*, activity ≈ 150 kU g^{-1}) was purchased from Sigma. DA and NA were purchased from Fluka and used as received. Ferrocene monocarboxylic acid (Fc) and *p*-benzoquinone (BQ) were purchased from Suzhou Time-Chem Technologies Co., Ltd. (Suzhou, China) and Tianjin Rainda Chemical Co., Ltd. (Tianjin, China), respectively. Phosphate buffer solution (PBS) consisting of 0.10 M KH_2PO_4–K_2HPO_4 + 0.10 M K_2SO_4 (pH 7.4) served as the supporting electrolyte. 0.50 wt% CS solution was prepared in 0.10 M acetate buffer solution (pH 5.4). All other chemicals were of analytical grade or better quality. Milli-Q ultrapure water (Millipore, ≥18 MΩ cm) was used throughout.

2.2. Procedures

The fabrication of GOx biosensor with DA as the model monomer is described in Scheme 1. A stirred PBS containing 30.0 mM DA and 1.50 mg mL^{-1} GOx was subject to chemical polymerization by adding 5.0 mM $K_3Fe(CN)_6$ (**I**). Visible PDA_C–GOx precipitates were obtained after ca. 30 min, and mild electromagnetic stirring (ca. 500 rpm) was kept for 2 h (**II**) to achieve reaction equilibrium. 5.0 μL of the prepared PDA_C–GOx suspension was cast on a clean Au electrode (**III**), and 2.0 μL of 0.50 wt% CS solution was immediately cast to strengthen the enzyme film (**IV**) and then air-dried. The SEM pictures of the enzyme–polymer composite film ($\mathbf{III}_{SEM}$, also air-dried, with much porous polymer granules) and the CS strengthened enzyme film ($\mathbf{IV}_{SEM}$, obviously glued by CS gel) are also shown for clarity. For comparison, PDA_E–GOx electrodes were also fabricated by cyclic voltammetry (CV, here −0.50 to 0.50 V, 20.0 mV s^{-1}, 20 cycles) in unstirred PBS solution containing 30.0 mM DA + 3.5 mg mL^{-1} GOx as reported previously (Chen et al., 2009). When not in use, the prepared biosensors were stored in PBS at 4 °C unless otherwise noted.

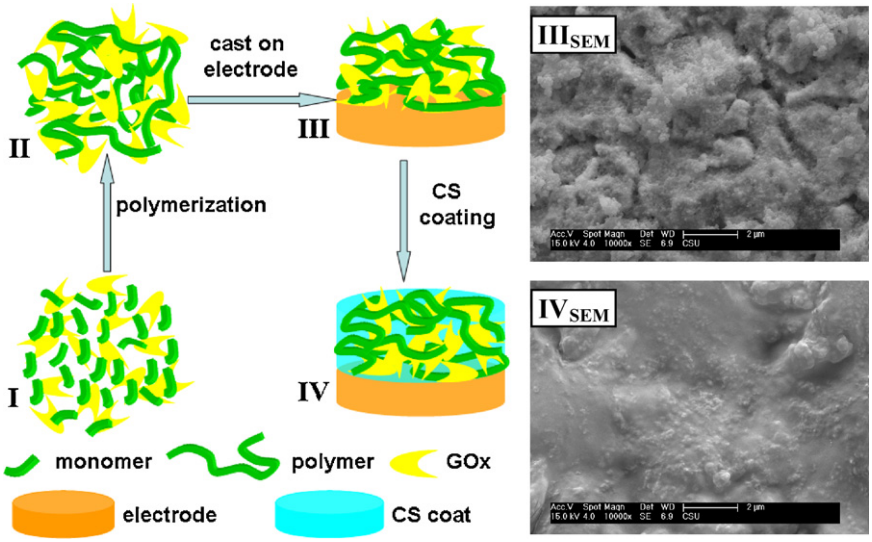
In the first-generation biosensing mode, the prepared enzyme electrodes were tested by potentiostating at 0.70 V in stirred PBS to detect oxidation of enzymatically generated H_2O_2. In the second-generation biosensing mode, the enzyme electrodes were tested by potentiostating at 0.40 V in N_2-bubbling stirred PBS containing 4.0 mM artificial mediator (Fc or BQ). In each run the response current was marked with the change value between the steady-state current after adding glucose and the initial background current without the substrate. The thermostability of the biosensors was evaluated by storing the enzyme electrodes in an oven and measuring the response for the corresponding substrate at regular intervals by removing the electrode from the oven (Wang et al., 1997).

A monopolar glucose BFC with a CS/PDA_C–GOx/Au enzyme electrode as the bioanode in 20 mL PBS (pH 7.4) containing 4.0 mM BQ and 100 mM glucose and a carbon rod as the cathode in Nafion-membrane-isolated 0.40 M $KMnO_4$ + 0.60 M H_2SO_4 aqueous solution, and the cell voltage (U_{cell}) and current density (j_{cell}) of the BFC at varying external resistance loads (R_e) were dynamically monitored with the electrochemical noise module of an Autolab PGSTAT30 electrochemical workstation (Netherlands) (Tan et al., 2008, 2009, 2010).

3. Results and discussion

3.1. Biosensing performance in the first-generation biosensing mode

To obtain the best sensitivities for glucose assay, various conditions, including the concentration of GOx, film-thickness, detection



Scheme 1. Procedures for preparing an enzyme electrode (left) and the SEM pictures of PDA_C–GOx/Au (III_{SEM}) and CS/PDA_C–GOx/Au (IV_{SEM}) (right). The enzyme films in the DA case were constructed under optimized conditions using 5.0 μL of (30.0 mM DA + 1.50 mg mL^{-1} GOx + 5.0 mM $K_3Fe(CN)_6$) and 2.0 μL CS.

potential and pH values were investigated via variation of the examined one while others fixed. We found that the biosensor gave maximum response at GOx concentration of 1.50 mg mL^{-1}, and similar peak-type response to GOx concentration was also reported previously (Razola et al., 2002; Vreeke et al., 1992). The cast volumes of prepared PDA_C–GOx suspension and 0.50 wt% CS were optimized as 5.0 and 2.0 μL, respectively. CS as a polysaccharide biopolymer was selected here mainly due to its excellent film-forming ability, high water permeability, and good adhesion (Gong et al., 2007; Kafi et al., 2008; Zhao et al., 2003). Solution of pH 7.4 and an applied potential of 0.70 V versus SCE with the maximum current response were selected as reported previously (Chen et al., 2002b; Garjonyte and Malinauskas, 2000a).

The calibration curves of CS/PDA_C–GOx/Au, PDA_C–GOx/Au, and CS–GOx/Au under optimized conditions are comparatively shown in Fig. 1(left), and the former exhibits the highest sensitivity to glucose and is thus focused below. The CS/PDA_C/GOx/Au electrode gives an LDR of 2.0 μM–3.0 mM, with a sensitivity (S_{G1}) as high as 135 ± 8.5 μA mM^{-1} cm^{-2} and a limit of detection (LOD) as low as 0.07 μM ($S/N = 3$). The Michaelis–Menten constant (K_m) is calculated to be 2.1 mM from the Lineweaver–Burk bireciprocal plot (Dixon, 1953). It also exhibited excellent anti-interferents ability, as shown in Fig. 1S, since the polymer film showed good permselectivity toward H_2O_2 but efficient rejection of ascorbic acid and uric acid (insert). The potential of practical usage of the biosensor was validated with a series of serum samples and the values measured at CS/PDA_C–GOx/Au agreed well with those obtained in hospital on glucose automatic analyzer (Table 1S).

The effect of temperature on GOx was examined by amperometric detection of enzymatically generated H_2O_2 at various temperatures (Fig. 2S). Both solution-state GOx and GOx entrapped in PDA_E thin film showed the maximum current response at about 40 °C, and a higher temperature decreased the enzymatic activity as reported (Pan et al., 2004; Wang et al., 1997). In contrast, GOx immobilized in CS, PDA_C, and CS/PDA_C could well withstand higher temperatures and showed the highest response at 60 °C. Fig. 2 comparatively displays a study of the thermodeactivation kinetics of GOx in solution as well as GOx entrapped in PDA_E, CS,

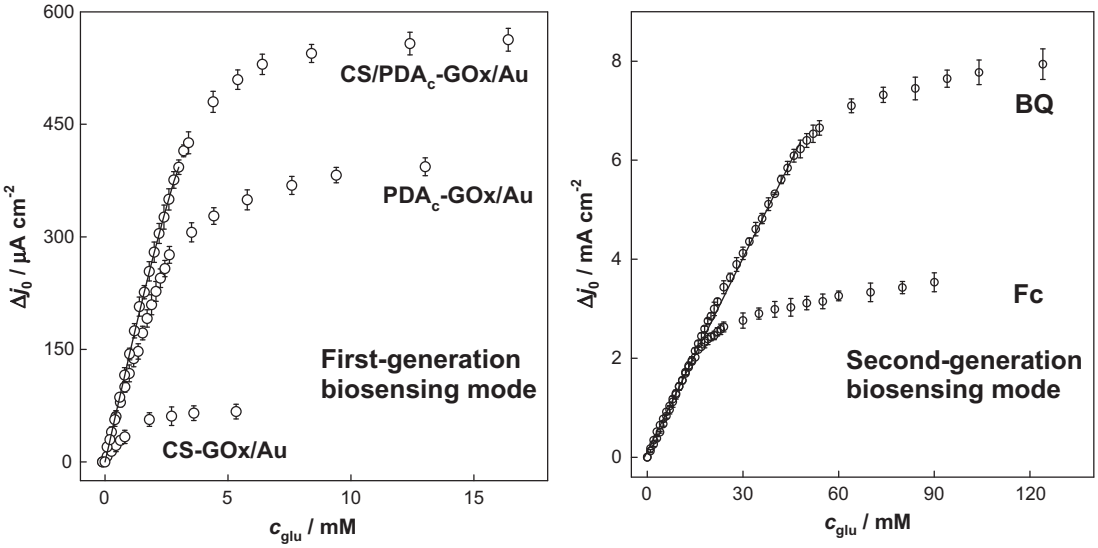


Fig. 1. The calibration curves at CS/PDA_C–GOx/Au, PDA_C–GOx/Au, and CS–GOx/Au obtained in 0.10 M PBS (pH 7.4) in the first-generation biosensing mode (0.7 V vs. SCE, left panel) as well as at CS/PDA_C–GOx/Au obtained in 0.10 M PBS (pH 7.4) containing 4.0 mM Fc or BQ in the second-generation biosensing mode (0.40 V vs. SCE, right panel).

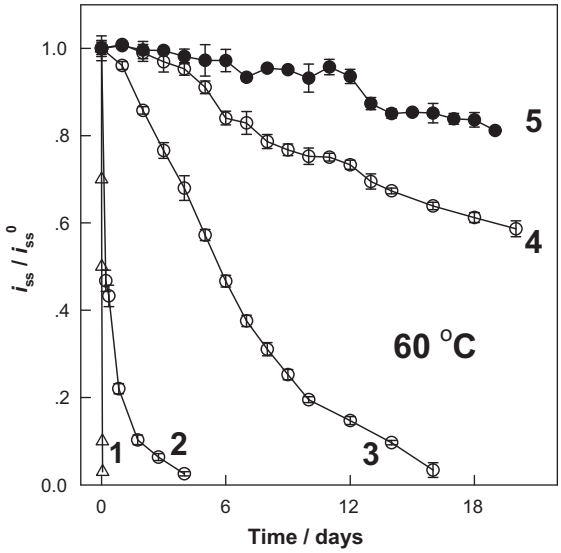


Fig. 2. Thermodeactivation kinetics of solution-state GOx (1), or GOx entrapped in PDA_E (2), CS (3), PDA_C (4), or CS/PDA_C film (5) at 60 °C. Applied potential: 0.70 V versus SCE.

PDA_C and CS/PDA_C at 60 °C. The solution-state GOx lost 50% activity after 22 min and 100% activity after 90 min, the PDA_E-entrapped GOx lost 90% activity after 48-h incubation at 60 °C, demonstrating that both the solution-state GOx and PDA_E-entrapped GOx rapidly lost their activity under this thermal stress. In contrast, GOx at CS/PDA_C–GOx/Au just lost 5% activity even after 12 days and 20% activity after 20 days at 60 °C, and it also only lost 20% activity even after 60-day incubation at 30 °C (not shown).

The urea denaturation of free and immobilized GOx was also investigated. The solution-state GOx experimentally became fully denatured after incubating in 6.0 M urea solution at 4 °C for 1 h, and many immobilized GOx also did so (Akhtar et al., 2002; Rauf et al., 2006). In contrast, here GOx in CS/PDA_C–GOx/Au negligibly lost its bioactivity even after treatment with 20.0 M urea solution for 30 h (not shown), indicating a less chemical susceptibility of GOx in CS/PDA_C–GOx/Au.

For comparison, we prepared a series of enzyme electrodes by replacing the DA monomer with NA, *o*-aminophenol (*o*AP), *o*-phenyldiamine (*o*PD), indole, or aniline. As listed in Table 1, the biosensors fabricated similarly by chemical polymerization/CS-strengthened cast coating exhibited a sensitivity order of DA ≈ NA > *o*AP > *o*PD > indole > aniline, demonstrating that the two catecholamine monomers are the best among the examined monomers, due perhaps to the special melanin-like porous nature of their polymers by which GOx can be entrapped at higher load/activity (Chen et al., 2009; Li et al., 2006b). The sensitivities of the DA-/NA-based biosensors are also higher than many other biosensors hitherto reported (Chen et al., 2002a; Fu et al., 2008; Garjonyte and Malinauskas, 2000a; Garjonyte and Malinauskas, 2000b; Guan et al., 2005; Hodak et al., 1997; Karyakin et al., 2002; Lu et al., 2007; Njagi and Andreescu, 2007; Wang et al., 1997, 2006). Since the electropolymerization protocol is so widely used for fabricating various enzyme electrodes, we also prepared several enzyme electrodes by electropolymerization using the same monomers. We also found the same sensitivity order, which validates again that the DA and NA monomers perform the best amongst the tested monomers. In addition, the sensitivity value of the biosensor prepared by electropolymerization is notably smaller than that prepared from chemical polymerization for each monomer, which can be explained by the facts that a thinner enzyme film and a more seriously blocked electroactivity of the substrate electrode should occur in each electropolymerization case due to the insulating nature of each polymer, i.e. electrodeposition of an insulating polymer should preferentially occur on every bare electrode sites but coating of a chemically synthesized polymer may leave more electroactive sites on the substrate electrode unoccupied for subsequent amperometric biosensing.

3.2. Biosensing performance in the second-generation biosensing mode and BFC construction

Cyclic voltammograms at CS/PDA_CGOx/Au in 0.10 M PBS (pH 7.4) containing 4.0 mM Fc (or BQ, or $K_3Fe(CN)_6$) and 0 (or 0.10) M glucose are shown in Fig. 3. BQ and Fc are validated here as good artificial mediators for GOx, and the maximum catalytic current densities of 2.45 mA cm^{-2} for Fc and 7.2 mA cm^{-2} for BQ were obtained after additions of 0.10 M glucose. However, in the $K_3Fe(CN)_6$ case almost no enzymatically catalyzed current was observed as reported, (Calvo and Etchenique, 1996) demonstrating that $Fe(CN)_6^{3-}$ cannot efficiently mediate the catalytic cycle of the GOx confined in the enzyme film despite its acceptable permeability inside the enzyme film (little change in its electroactivity before and after glucose addition).

The second-generation biosensing modes were also examined. The calibration curves on CS/PDA_C–GOx/Au to glucose in the presence of 4.0 mM Fc or BQ are presented in Fig. 1(right) (also see Table 1). The addition of Fc or BQ greatly broadened the LDR and increased the glucose-saturated current (j_{max}) at CS/PDA_C–GOx/Au, i.e. in the BQ case the LDR is 2 μM–48 mM with a sensitivity as high as 135 ± 4.4 μA mM^{-1} cm^{-2}, and the j_{max} is as high as ca. 8.0 mA cm^{-2}, being one of the biggest ones hitherto reported for glucose biosensors; in the Fc case, the LDR is 2 μM–16.0 mM with the same sensitivity as in the BQ case, and the j_{max} is 3.5 mA cm^{-2}. The different performances of the two mediators may result from the difference in redox state (Fc is in reduced form and BQ is in oxidized form) and in number of electrons transferred (n) (n = 2 for BQ but n = 1 for Fc).

The reactions after glucose addition to the detection solution containing Fc can be described below (Bourdillon et al., 1993;

Table 1
Performance of several enzyme electrodes prepared through chemical polymerization/CS-strengthened cast coating and conventional electropolymerization protocols[a].

Monomer		DA	NA	*o*AP	*o*PD	Indole	Aniline
Chemical polymerization/ CS-strengthened cast coating	S_{G1} (μA mM^{-1} cm^{-2})	135 ± 8.5	134 ± 6.7	102 ± 3.5	64 ± 6.9	61 ± 3.4	47 ± 3.7
	LDR_{G1} (mM)	0.002–3.0	0.002–3.0	0.002–2.0	0.002–1.7	0.001–3.0	0.002–1.7
	$S_{G2\text{-}BQ}$ (μA mM^{-1} cm^{-2})	135 ± 4.4	134 ± 5.2	105 ± 7.0	67 ± 7.2	55 ± 6.4	47 ± 6.2
	$LDR_{G2\text{-}BQ}$ (mM)	0.002–48	0.002–46	0.002–35	0.002–19	0.002–30	0.002–28
Electropolymerization	S_{G1} (μA mM^{-1} cm^{-2})	10.5 ± 0.8	9.8 ± 1.2	4.0 ± 0.2	3.0 ± 0.1	0.4 ± 0.05	0.2 ± 0.02

[a] Concentrations: 1.50 mg mL^{-1} for GOx, 30.0 mM for DA, 10.0 mM for NA, indole and *o*AP, and 100 mM for *o*PD and aniline. Biosensing measurements were conducted in pH 7.4 PBS containing 0 (S_{G1} and LDR_{G1}, conducted at 0.7 V versus SCE in the first generation biosensing mode) or 4.0 ($S_{G2\text{-}BQ}$ and $LDR_{G2\text{-}BQ}$, conducted at 0.4 V versus SCE in the second generation biosensing mode) mM BQ at 30 °C.

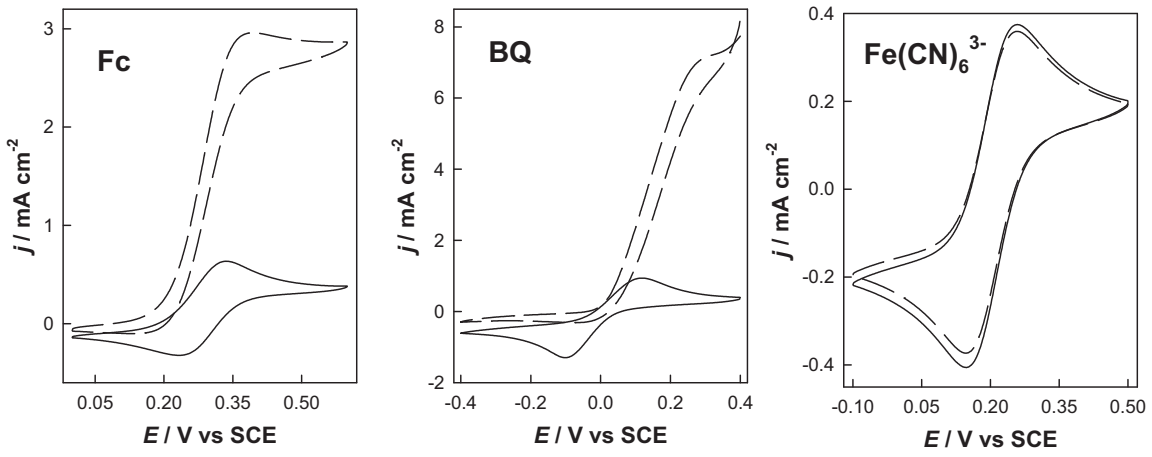


Fig. 3. Cyclic voltammograms at CS/PDA$_C$-GOx/Au in 0.10 M PBS (pH 7.4) containing 4.0 mM Fc (or BQ, or $K_3Fe(CN)_6$) and 0 (solid curves) or 0.10 (broken curves) M glucose. Scan rate: 50 mV s^{-1}.

Ferreyra et al., 2004),

$$Fc \rightleftharpoons Fc^+ + e^-$$

$$FAD + G \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} FADG \xrightarrow{k_2} FADH_2 + GL$$

$$FADH_2 + 2Fc^+ \xrightarrow{k_3} FAD + 2Fc$$

where the Fc and Fc$^+$ are the reduced and oxidized forms of Fc; $FADH_2$ and FAD represent the reduced and oxidized forms of the active center of GOx; and G and GL are β-D-glucose and gluconoD-lactone, respectively. The plateau catalytic current density from the mediator-mediated cyclic voltammograms, i_{cat}, obeys Eq. (1) in the absence of mass transport limitations in the framework of the above catalytic reaction mechanism (Anicet et al., 1998; Calvo and Etchenique, 1996; Deng et al., 2007; Muguruma et al., 2005),

$$\frac{1}{i_{cat}} = \frac{1}{2Fk_3\Gamma_E^0}\frac{1}{[M]_0} + \frac{1}{2F\Gamma_E^0}\left(\frac{1}{k_2} + \frac{1}{k_{red}[G]}\right) \quad (1)$$

where F is the Faraday constant (F = 96485.33 C mol^{-1}), Γ_E^0 in mol cm^{-2} is the surface concentration of immobilized active enzyme, $[M]_0$ in M is the mediator concentration, and $[G]$ in M is the glucose concentration in solution. On the basis of Eq. (1) and the known rate constant values for Fc (k_2 = 700 s^{-1}, $k_3 = 1.2 \times 10^7$ M^{-1} s^{-1}, $k_{red} = k_1k_2/(k_{-1} + k_2) = 1.1 \times 10^4$ M^{-1} s^{-1}), the concentration of immobilized active GOx (Γ_E^0) on the enzyme electrode surface can be determined (Zhang et al., 2005). Here, i_{cat} is derived from the experimental data by simply subtracting the current at 0.40 V obtained in PBS solution containing 4.0 mM Fc in the absence of glucose from that obtained with glucose present in the solution. A slope of 5.46 M cm^2 A^{-1} is obtained by linearly regressing $1/i_{cat}$ versus $1/[G]$ and the Γ_E^0 is thus calculated to be 8.63×10^{-11} mol cm^{-2}. Γ_E^0 for the present enzyme electrode corresponds to ca. 51 times of an enzyme monolayer (1.7×10^{-12} mol cm^{-2}) and 21 times of that previously reported ($(4.1 \pm 0.1) \times 10^{-12}$ mol cm^{-2}) (Zhang et al., 2005), indicating a high enzyme load here.

The enzyme specific activity (ESA) for GOx was evaluated with UV–Vis spectrophotometry based on chromogenic oxidation of *o*-dianisidine by the O_2 produced by the oxidation of GOx-generated H_2O_2 in the presence of horseradish peroxidase (Chen et al., 2009; Fu et al., 2008) and calculated according to $ESA = 0.1 \times 60/(20tE_{436}\Delta m_{GOx})$, where t in s is the time for an absorbance increase at 436 nm by 0.10 in the initial stage of the enzymatic reaction, the factor "1/20" means a calibration of the total glucose molar quantity used in E_{436} determination to 1.0 μmol, and "60" means a calibration of the enzymatic reaction time to 60 s. The t was measured to be 28.5 s and Δm_{GOx} was 0.94 μg as demonstrated above (8.63×10^{-11} mol cm^{-2} and MW_{GOx} = 154,000 g mol^{-1}) at CS/PDA$_C$–GOx/Au, so ESA for the immobilized GOx (ESA_i) is calculated to be 78.9 kU g^{-1}. For solution-state GOx, we experimentally used Δm_{GOx} of 0.3 μg, and measured E_{436} to be 0.142 and t to be 88 s, thus the ESA for GOx in solution (ESA_n) is calcu-

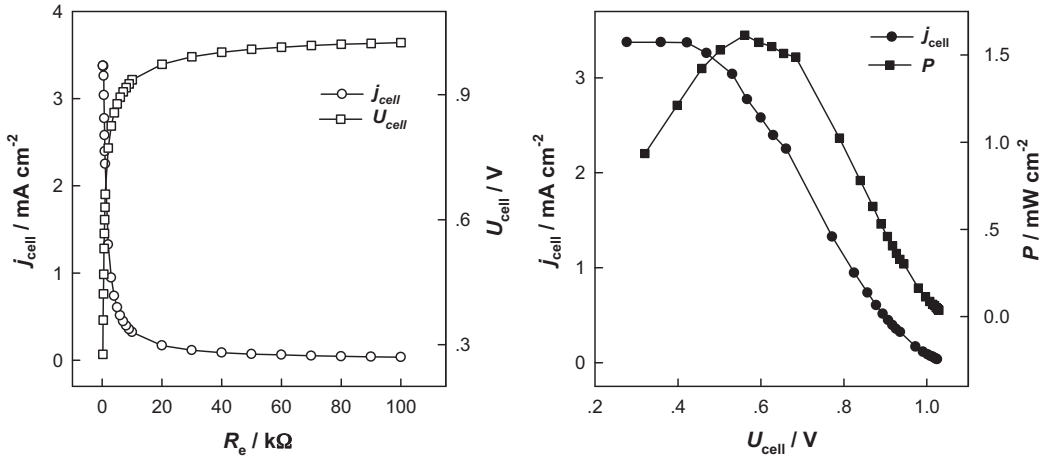


Fig. 4. j_{cell} and U_{cell} as functions of R_e as well as j_{cell} and P as functions of U_{cell} for the monopolar BFC fabricated as detailed in the text.

lated to be 80.0 kU g^{-1}. The enzymatic relative activity ($ERA = ESA_i/ESA_n$) is thus 98.6%. The above results should demonstrate that we have immobilized GOx on CS/PDA_C–GOx/Au at high load/activity.

A monopolar BFC based on the prepared enzyme film was fabricated as above. As shown in Fig. 4, it exhibited an open-circuit potential of 1.09 V, a short-circuit current of 3.38 mA cm^{-2}, and a maximum power density (P_{max}) of 1.62 mW cm^{-2}. This P_{max} is much larger than most reported values (Bunte et al., 2010; Hubenova et al., in press; Katz et al., 2005; Tamaki and Yamaguchi, 2006; Tan et al., 2010; Tasca et al., 2008), indicating the enzyme film here is an excellent anodic material for BFC development.

4. Conclusions

In summary, we have described that chemically synthesized PDA_C and PNA_C with GOx effectively entrapped can be used for fabricating CS-strengthened cast thin film biosensors with outstanding sensitivity and thermostability and a high-performance BFC. The two catecholamine polymers, either chemically or electrochemically synthesized, are proven to behave obviously better than many other commonly used polymers for biosensing. The high load/activity of the immobilized GOx at CS/PDA_C–GOx/Au have been proven by a reported kinetic model and UV–Vis spectrophotometry. The catecholamine polymers-based system is simple and efficient, which may lead to new thermoresistant enzyme reactors with good performance.

Acknowledgments

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Appendix A. Supplementary data

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