Biosensors and Bioelectronics 24 (2009) 2665–2670

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Development of a novel, sensitive amperometric-FIA glucose biosensor by packing up the amperometric cell with glucose oxidase modified anion exchange resin

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ARTICLE INFO

Article history:
Received 31 October 2008
Received in revised form
29 December 2008
Accepted 22 January 2009
Available online 2 February 2009

Keywords:
Packed amperometric cell
Enzyme reactor
Porous electrode
Layer-by-layer electrostatic self-assembling
Glucose biosensor
Flow injection analysis

ABSTRACT

In this work, the anion exchange resin (AER) was modified with a layer of glucose oxidase (GOD) and poly(diallyldimethylammonium chloride) (PDDA), respectively, via layer-by-layer electrostatic self-assembling strategy. The PDDA and GOD modified AER (PDDA/GOD/AER) was then packed into a home-made amperometric cell for flow injection analysis (FIA) of glucose. This design simplified the setup by integrating the enzyme reactor into the amperometric cell. And the AER in the cell behaved bifunctional, it was not only the support of enzymes, but also an anti-interference tool due to its retention effect toward ascorbic acid (AA) and uric acid (UA). A platinum modified porous titanium (Pt/PTi) electrode was utilized in the cell as the working electrode (WE), due to its large effective surface area it could increase the response by 8.3 times as compared with the planar pure platinum electrode. The proposed biosensor was very sensitive (22.4 $\mu A\,cm^{-2}\,mM^{-1}$) in glucose quantification, and the linear range was from 1 $\mu mol\,L^{-1}$ to 2 $mmol\,L^{-1}$ with the detection limit of 0.8 $\mu mol\,L^{-1}$. The biosensor was used for serum glucose determination, and the result obtained was satisfying. This work may have provided a reference design of the amperometric cell which could be adopted in other enzymatic-FIA biosensors.

1. Introduction

Flow injection analysis (FIA) is a dynamic process that produces a well-defined and highly reproducible concentration transient at the detector site. Its combination with the enzymatic derivatization strategy can even provide a highly selective detection. Thus a number of strategies have been adopted for introducing enzymes into the flow system. One common strategy is the homogeneous derivatization, which means the enzyme solution and sample solution are separately pumped into a reactor and get mixed there (Hirata et al., 2005; Potter et al., 1983). This successfully introduces the specificity of enzymatic reaction. Unfortunately it suffers from some drawbacks such as an increased complexity in the setup and a large waste of the expensive enzymes. In this respect, a significant improvement is represented by the use of immobilized enzyme reactor (IER) (Eva et al., 1984; Franchini et al., 2008; Ho et al., 2007; Ling et al., 2000; Matos et al., 2006; Matsumoto et al., 1988), thus the goals of simplifying the experimental setup and reducing the analysis cost could be reached. And in order to modify more enzymes with no distinctive band-broading, beads of small-size particles are usually utilized (Franchini et al., 2008; Ling et al., 2000; Matos et al., 2006; Matsumoto et al., 1988). Another substitute is to immobilize the enzymes directly onto the working electrode (WE) of the amperometric cell (Álvarez-Romero et al., 2004; Guerrieri and Palmisano, 2001; Liu and Lin, 2006a,b; Palmisano et al., 2000; Tsiafoulis et al., 2005). Compared with the IER, this further simplifies the setup and brings no dead volume, however, it suffers disadvantages as the difficulty in immobilizing large amount of enzymes onto a limited planar surface and the transient enzymatic reaction time restricts the detection sensitivity.

As its importance in biology, clinic and nutrition, the detection of β-D-glucose has been the subject of considerable efforts (Lu et al., 2007; Magner, 1998; Wang, 2008). Typical biosensor for glucose is based on its enzymatic reaction with the glucose oxidase (GOD), which in the presence of oxygen converts glucose to gluconic acid and hydrogen peroxide (H_2O_2). Then the quantification of glucose can be achieved via electrochemical detection of the enzymatic produced H_2O_2 (Álvarez-Romero et al., 2004; Ho et al., 2007; Liu and Lin, 2006a; Wu et al., 2007; Zhou et al., 2007). The direct electrochemical detection of H_2O_2 is usually accomplished at anodic potentials of 0.6 V (versus Ag/AgCl) or above (Álvarez-Romero et al., 2004; Matsumoto et al., 1988). However, many substances normally present in biological fluids, such as ascorbic acid (AA) and uric acid (UA), are easily co-oxidized at similar potentials and

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doi:10.1016/j.bios.2009.01.037

may cause interfering responses. Consequently, the strategies to avoid interfering responses have attracted widespread attention. For example, the utilization of ascorbate oxidase modified pre-reactor is a common technique (Hayashi et al., 2003; Matsumoto et al., 1988). Unfortunately, it has disadvantages as complicating the setup and requiring of expensive enzymes. An effective strategy to overcome these limitations is the permselective film, which as a kind of electrode modification material could diminish or eliminate the electro-activity of the interferents. However, it usually entails a decrease in reproducibility and sensitivity of the biosensor (Cano et al., 2008; Lu et al., 2007). As the simplest technique to eliminate the interference, a lower operation potential at which the interferents have little or no electro-activity is chosen for biosensing, but it subtracts the signal as well (Ho et al., 2007; Lu et al., 2007; Wu et al., 2007).

The ion exchange resin is cheap, abundant, mechanically resistant, chemically stable, non-toxic, non-pollutant and easily regenerated after use. Thus it is a kind of good support for enzymes. Both the immobilization of enzymes onto the ion exchange resin and the properties of the immobilized enzymes on it have been extensively studied (Marquez et al., 2008; Saroğlu et al., 2001; Tomotani and Vitolo, 2006). Moreover, there are some reports on the enzyme-immobilized ion exchange resin used for biosensing (Eva et al., 1984; Franchini et al., 2008; Ling et al., 2000; Matos et al., 2006). However, as far as we know, there is no report on the layer-by-layer electrostatic self-assembling modification of it for fabrication of biosensors.

In this work, the anion exchange resin (AER) is modified with a layer of GOD and then poly(diallyldimethylammonium chloride) (PDDA), respectively, via layer-by-layer electrostatic self-assembling strategy. The PDDA/GOD/AER is then packed into the home-made amperometric cell for glucose sensing. Due to the novel design of the amperometric cell, the setup is simplified by integrating the enzyme reactor into it, and the interferences are avoided with no subtraction of the sensitivity. In combination with the large effective surface area of the porous WE, the resultant biosensor is very sensitive in glucose quantification. This work may have provided a reference design of the amperometric cell which could be adopted in other enzymatic-FIA biosensors.

2. Experimental

2.1. Instrumentation and reagents

Amperometric measurements were performed using an electrochemical analyzer CHI 631B connected to a personal computer. In the FIA system, a home-made amperometric cell based on a sandwich-like style (Fig. 1) was used. It comprised mainly a column (6 mm in inner diameter and 7 mm in length) packed with the PDDA/GOD/AER and two platinum modified porous titanium (Pt/PTi) electrodes (8 mm in diameter and 1 mm in thickness) contacted with each end of the column and served as the WE and counter electrode (CE), respectively. Each electrode was embedded in a PVC block and attached to the packed column by screws with a Teflon membrane (TM, 0.2 mm in thickness) inserted to keep no leakage of the solution. And each TM had a hole (6 mm in diameter) at the center for solution traveling. Up on the TM at the WE side only, a micropore filtration membrane (MFM) was set. This was necessary because beads on the WE may partly block the access of the electroactive compounds. It was worth noting that the solution flowed through the CE and WE. A home-made Ag/AgCl (3 mol L^{-1} KCl) reference electrode (RE) was located at the side of the column, and all potentials were referred to it. The supporting electrolyte was powered by a HPLC pump (LC-10AT, Shimadzu) equipped with a 20 μL sample loop. Commercially available PEEK tubing (0.25 mm in inner diameter) and standard fingertight fittings were purchased from Upchurch Scientific Inc. (Oak Harbor, WA). The scanning electron microscopy (SEM) pictures were taken on a LEO-1530 scanning electron microscope (Germany). And the UV–vis absorption spectra were recorded on a DU 7400 UV–vis spectrophotometer (Beckman Co., USA).

Porous titanium plate with the thickness of 1 mm and the average pore diameter of ~50 μm was acquired from Yonglian Rare Metal Co, Ltd. (Baoji, China) and cut to the desired shape before use. AER (201 × 7 WQ-400, ~200–400 mesh) with high exchange capacity was purchased from Nankai University (Tianjin, China) and dipped in water for 24 h before use. GOD (EC 1.1.3.4; type II from *Aspergillus niger*, activity ≈100,000 U g^{-1}) was purchased from Amresco (USA). PDDA and glucose was bought from Sigma–Aldrich (USA) and Guoyao Chemicals Co., Ltd. (Shanghai, China), respectively. H_2O_2, AA and UA were used as received. All chemicals were analytical grade or better. Phosphate buffer solution (PBS, pH 6.98, 12 mmol L^{-1} KH_2PO_4 + 18 mmol L^{-1} K_2HPO_4 + 0.1 mol L^{-1} K_2SO_4) was used as the supporting electrolyte. For the experiment of serum glucose determination, the pre-analyzed human serum sample was obtained fresh from the Hospital of Xiamen University (Xiamen, China). All experiments were carried out at room temperature (25 ± 2 °C) and ultrapure water was used throughout the experiments.

2.2. Self-assembling of GOD onto the AER

After being dipped in water for 24 h, the AER was filtered, washed with water, and subsequently dried its surface water with filter paper (we defined the AER after these treatment as "wet AER"). Then 0.6 g wet AER was stirred with 6 mL 0.4 mg mL^{-1} GOD in Tris–HCl buffer solution (pH 8.0) for about 4 h. The isoelectric point of GOD was about 4.2 (Simpson et al., 2007), thus the negatively charged GOD got adsorbed onto the AER surface via electrostatic effect. The amount of immobilized GOD was determined according to the absorbance change of the GOD solution at 278 nm (see Fig. 1S in the Supplementary data) after adsorption (Lv et al., 2003). The result of 1.05 ± 0.02 mg GOD was obtained for 1 g wet resin in three repeated assays. And it was 1.81 ± 0.03 mg for dried resin. The GOD/AER was filtered and washed with water. Then using the same procedure, a layer of positively charged PDDA was adsorbed in a 0.5 mol L^{-1} NaCl solution containing 1 mg mL^{-1} PDDA to prevent leakage of the GOD. We found in the pre-experiments that the reproducibility and stability of the biosensor was improved when with this PDDA layer. By alternative self-assembling of GOD and PDDA, multilayer modification could be realized. And the sensitivity of the biosensor could be increased by about 35% when more than three bilayers of PDDA/GOD were modified (compared with only one). However, the modification process was too time-consuming and the manipulation was labor-intensive. Thus in the present work, only one bilayer was chosen. The sketch of the PDDA/GOD/AER is shown in Fig. 1 too. Then 0.18 g wet PDDA/GOD/AER was packed into the amperometric cell. Thus the immobilized GOD in the cell was calculated to be 0.189 mg.

2.3. Chemical deposition of Pt onto the PTi electrode

The chemical deposition of Pt onto the PTi electrode was described in detail elsewhere (Ding et al., 2008), and it was introduced briefly in the Supplementary data. The whole Pt/PTi electrode was electrochemically treated in H_2SO_4 solution and its real surface area (A_r) was determined to be 4.98 ± 0.03 times (in three repeated assays) of the planar pure Pt electrode which had the same shape (Papadimitriou et al., 2008; Sarapuu et al., 2008).

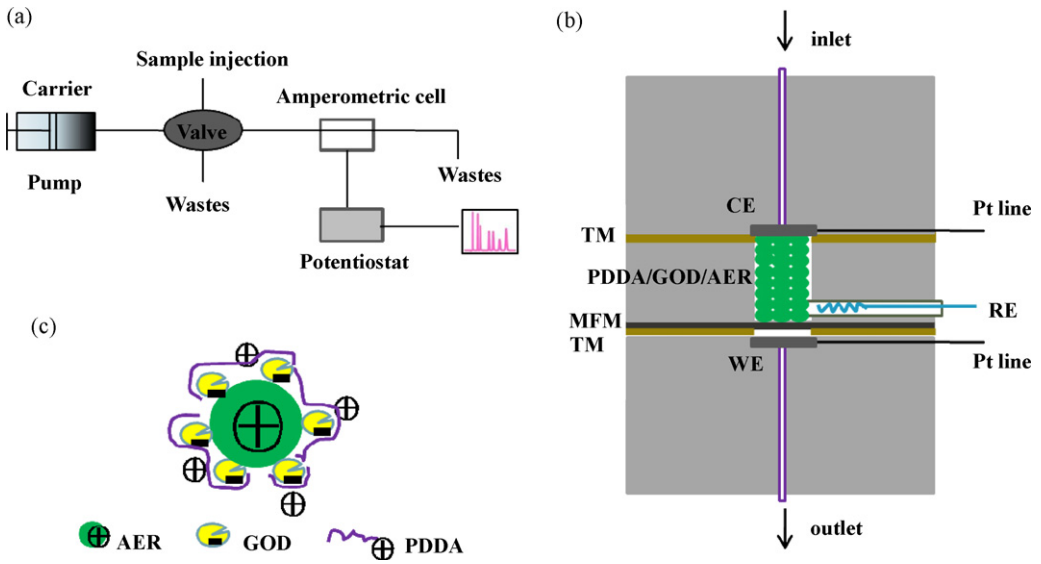

Fig. 1. Schematics of the amperometric-FIA sensing system (a), the home-made amperometric cell (b) and the PDDA/GOD/AER (c).

2.4. Real-sample analysis

Fresh human serum (20 μL) was diluted with 180 μL pH 6.98 PBS and centrifuged at 2000 × *g* for 5 min. Twenty microliters were taken directly from the supernatant and injected via the valve. After its response at 0.4 mL min^{-1} was recorded for 50 s, the flow rate was changed to 4.0 mL min^{-1} for fast elution of AA and UA. Then after the current was stable, the flow rate was changed back to 0.4 mL min^{-1} and another sample was injected. The potential was set at 0.6 V throughout the whole experiment. And the glucose concentration was quantified according to its peak current (i_p). The result obtained using the proposed biosensor was compared with that of a clinically used glucose analyzer (Beckman CX5 automated biochemical analyzer).

3. Results and discussion

3.1. Electrocatalytic activity of Pt/PTi electrode for H_2O_2

The functioning principle of the glucose biosensor was based on the amperometric detection of the enzymatic produced H_2O_2. The Pt/PTi electrode was subjected to cyclic voltammograms in a 5-mL PBS with different concentrations of H_2O_2, and it showed significant electrocatalysis toward both the oxidation and reduction of H_2O_2. In order to determine the optimum potential, hydrodynamic voltammetric studies were carried out with 0.1 mM H_2O_2 in the 5 mL electrochemical cell. The electrode was operated at a desired potential (between 0.1 and 0.7 V, 0.1 V potential step), and the transient current after the addition of H_2O_2 was allowed to decay to a steady-state value. The response reached a plateau around 0.5–0.7 V. Therefore, in the following work 0.6 V was chosen as the operating potential for the amperometric-FIA of glucose.

3.2. Amperometric-FIA performance of the biosensor

We studied the pH effect of the supporting electrolyte, it showed stable and larger responses were obtained between pH 6 and 8, and the signal was decreased with the pH shifted toward both acidity and basicity. This was mainly attributed to the decrease of the enzymatic activity at more acid or basic environment (Simpson et al., 2007; Zhou et al., 2007). Besides destruction of the layer structure which led to leakage of GOD may also play an important role when in acid solution (Liu and Lin, 2006a). So a PBS with the pH of 6.98 was chosen as the supporting electrolyte in this paper.

As known, the flow rate had a great effect on FIA. In order to optimize this, the responses of 2 mmol L^{-1} glucose with or without coexisting of both 1 mmol L^{-1} AA and UA were recorded at different flow rates, as shown in Fig. 2. It is seen that at the injection of only 2 mmol L^{-1} glucose, the biosensor responses more quickly and the peak becomes sharper as the flow rate increasing, while the i_p decreases since the enzymatic reaction time is subtracted. At the injection of 2 mmol L^{-1} glucose + 1 mmol L^{-1} AA + 1 mmol L^{-1} UA, it is very interesting that three peaks are obtained. After further study, we found they were attributed to glucose, AA and UA, respectively, from left to right. This was due to their different charge in the pH 6.98 PBS. As AA and UA were negative charged, they could be retained and separated by the positively charged AER (Gjerde et al., 1979; Small et al., 1975); while glucose (and its enzymatic product, H_2O_2) was electro-neutral, it had no retention and then got the response much faster. As seen from the figure, the three peaks are separated better as the flow rate decreasing, however, more time is needed. UA has little interference on the peak of glucose at all of the flow rates, while AA affects it seriously at large flow rates. When at 1.0 mL min^{-1}, due to the serious overlapping of the AA peak, the i_p of glucose increases by 50.8%. And at this flow rate, about 1000 s is needed in total for one mixture sample of glucose, AA and UA. When the flow rate gets to 0.5 mL min^{-1}, the i_p of glucose only increases by 5.0%, but 1600 s is needed. While at 0.1 mL min^{-1}, the peaks of glucose and AA are almost separated (the resolution is 1.0), thus AA nearly has no interference on glucose, whereas it takes 6500 s in total (the peak of UA is not shown). Obviously, the peaks of glucose and AA could not be separated completely under these conditions. In order to improve their resolution, we changed the concentration of the PBS, and even replaced it with a pH 7.0 BR buffer, but little improvement was obtained. Nevertheless, this did not matter much, since the interference of AA could be omitted at flow rates smaller than 0.5 mL min^{-1}, as mentioned above. In consideration of both the sensitivity and analysis time, 0.4 mL min^{-1} was chosen in the following work for glucose biosensing. And at this flow rate, about 2000 and 144 s were needed for one glucose sample with or without coexisting of AA and UA, respectively.

For further subtraction of the analysis time, the strategy of gradient elution could be adopted. After the injection of 2 mmol L^{-1} glucose + 1 mmol L^{-1} AA + 1 mmol L^{-1} UA, the response at 0.4 mL min^{-1} was recorded only for 50 s, at which the peak of

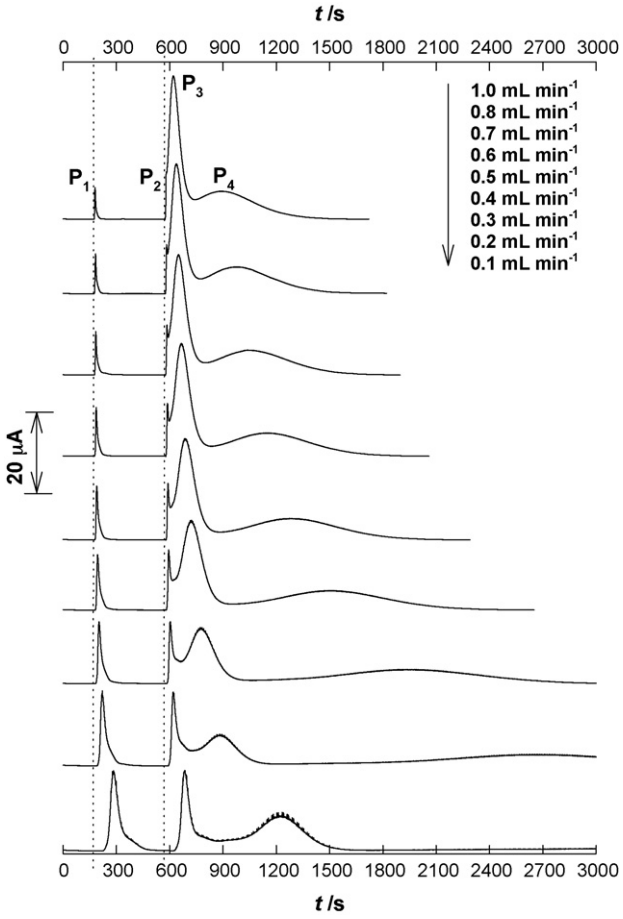

Fig. 2. Amperometric responses of the biosensor to 2 mmol L^{-1} glucose and then 2 mmol L^{-1} glucose + 1 mmol L^{-1} AA + 1 mmol L^{-1} UA at different flow rates. Potential, 0.6 V; P_1–P_4 represent the peaks of glucose, glucose, AA and UA, respectively; the dashed lines indicate the injection times of the two samples.

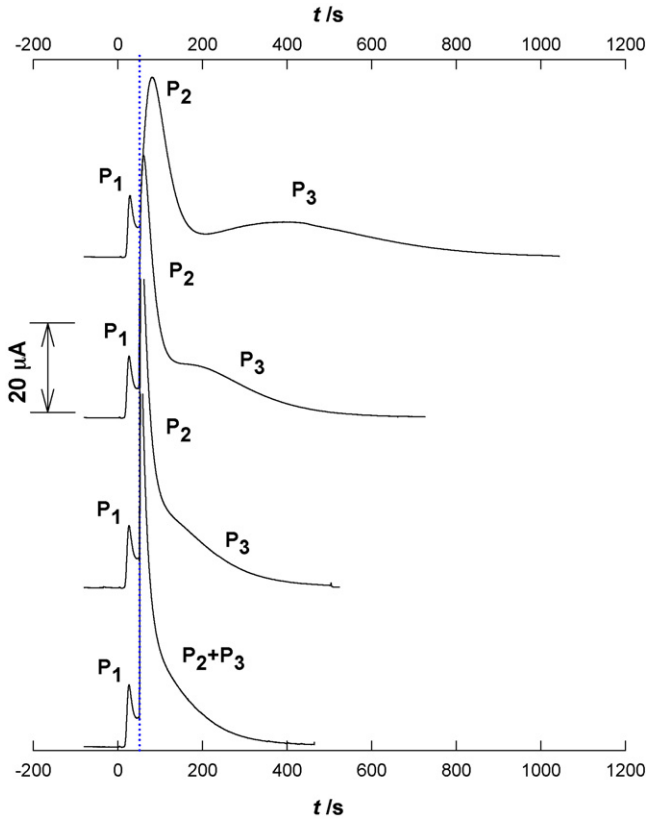

Fig. 3. Amperometric responses of the biosensor to 2 mmol L^{-1} glucose + 1 mmol L^{-1} AA + 1 mmol L^{-1} UA under gradient elution. Potential, 0.6 V; P_1–P_3 represent the peaks of glucose, AA and UA, respectively; the flow rate on the left of the dashed line is 0.4 mL min^{-1} for each curve, and on the right is 1.0, 2.0, 3.0 and 4.0 mL min^{-1} respectively, from top to bottom; the sample was injected at time zero.

AA would start to overlap onto the glucose, then the flow rate was changed to a larger value, for example 1.0 mL min^{-1}, for fast elution of AA and UA. As shown in Fig. 3, the analysis time gets much reduced with the above manipulation. It takes about 1000, 650, 500 and 420 s in total if AA and UA were eluted at 1.0, 2.0, 3.0 and 4.0 mL min^{-1}, respectively. The response of 2 mmol L^{-1} glucose at 0.4 mL min^{-1} was recorded both before and after each gradient elution (results not shown), their consistent values showed the good reproducibility of the biosensor under these gradient elution manipulations.

To illustrate the retention effect of AER toward AA and UA more clear, we packed up the amperometric cell with PDDA/GOD/AER, unmodified AER and unmodified cation exchange resin (CER, with the same size as AER), and tested their respective response to 1 mmol L^{-1} AA + 1 mmol L^{-1} UA, the results are shown in Fig. 4. In the case of unmodified AER packed amperometric cell, two peaks are obtained, which is very similar to that of the PDDA/GOD/AER. This was due to the separation effect of the AER toward AA and UA, since in the pH 6.98 PBS AA and UA were negatively charged, while the AER itself was positively charged. And this was just the separation mechanism of the anion exchange chromatography (Gjerde et al., 1979; Small et al., 1975). The small differences between unmodified AER and PDDA/GOD/AER in this figure (curves b and c) may due to the change of the AER surface after its modification. While for the CER, because it was negatively charged, it had no retention toward AA and UA, thus only one sharp and very high peak (note the current is 20× reduced) appears soon after the sample injection

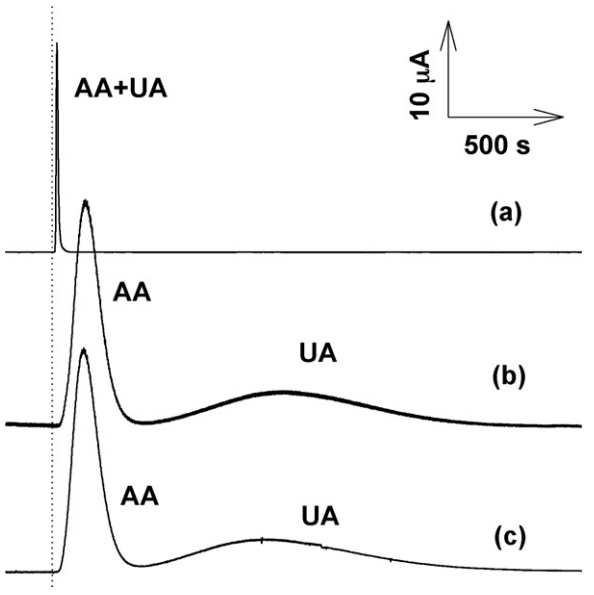

Fig. 4. Responses of the home-made amperometric cell packed with unmodified CER (a), unmodified AER (b) and PDDA/GOD/AER (c) to the injection of 1 mmol L^{-1} AA + 1 mmol L^{-1} UA. Potential, 0.6 V; flow rate, 0.4 mL min^{-1}; the current in case (a) is 20× reduced for drawing clarity; and the dashed line means the injection time of the sample.

(curve a). The dead volume of the amperometric cell was calculated to be 127 μL according to the dead time as indicated in Fig. 4(a). This value was acceptable since an enzyme reactor was embodied in the cell. And it could be reduced by modulating the dimensions of the reactor if needed.

Therefore, the PDDA/GOD/AER packed column behaved bifunctional. It served as both the enzyme reactor and anion separation column which could eliminate the interferences from AA and UA. This anti-interference strategy avoided many shortages that others may had, such as complicating of the setup and wasting of the expensive enzymes (Hayashi et al., 2003; Matsumoto et al., 1988), or decreasing the sensitivity of the biosensor (Cano et al., 2008; Ho et al., 2007; Lu et al., 2007; Wu et al., 2007). On the other hand, compared with the commercial available anion separation column, this was much cheaper and its operation pressure was much lower.

Fig. 5 shows the typical amperometric responses of the sensor to the successive injections of various concentrations of glucose. The value of i_p is increasing with the increase of the glucose concentration, and the resulting calibration curve displays linearly from 1 μmol L^{-1} to 2 mmol L^{-1}. The linearity equation obtained

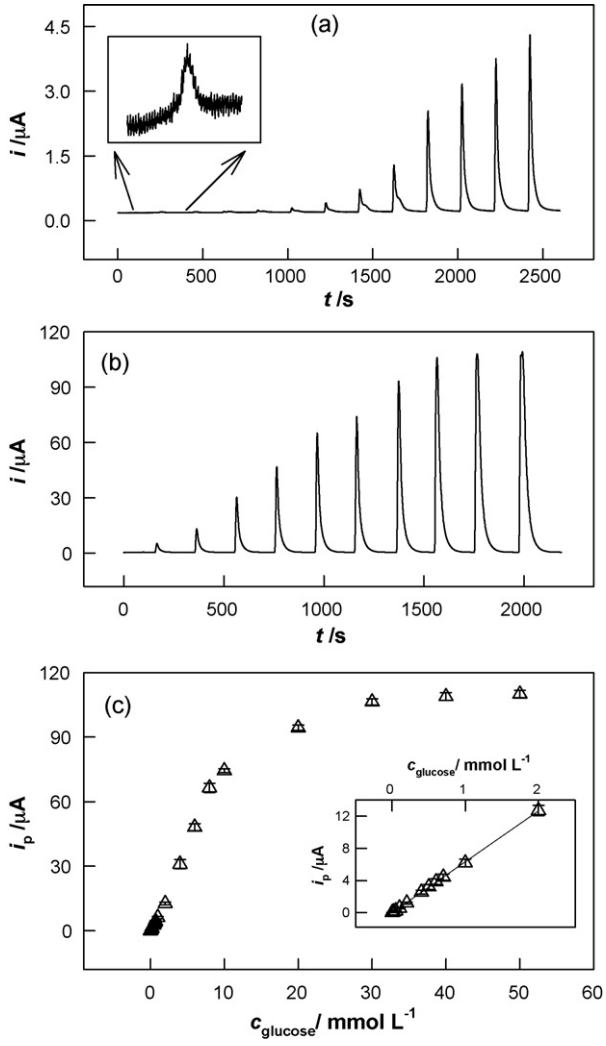

Fig. 5. Amperometric responses of the biosensor to the injections of different concentrations of glucose (a and b), and the calibration curve obtained with three parallel assays (c). The concentrations of injected glucose are 1×10^{-3}, 2×10^{-3}, 5×10^{-3}, 1×10^{-2}, 2×10^{-2}, 5×10^{-2}, 1×10^{-1}, 2×10^{-1}, 4×10^{-1}, 5×10^{-1}, 6×10^{-1}, 7×10^{-1}, 1, 2, 4, 6, 8, 1×10^{1}, 2×10^{1}, 3×10^{1}, 4×10^{1}, 5×10^{1} mmol L^{-1}, respectively. Other conditions are the same as in Fig. 4.

with three parallel assays is expressed as i_p (μA) = $6.33c_{glucose}$ (mmol L^{-1}), and the correlation coefficient is 0.9954. The sensitivity of this sensor was 22.4 μA cm^{-2} mM^{-1}, and the detection limit was estimated to be 0.8 μmol L^{-1} at a signal-to-noise ratio of 3. This sensitivity was larger than most of the reported glucose biosensors constructed via layer-by-layer modification (Liu and Lin, 2006a; Sun et al., 2008; Yang et al., 2006; Zhang et al., 2004a,b), despite a little smaller than the GOD, gold nanoparticle and carbon nanotube (each nine layers) modified Pt electrode under batch conditions (Wu et al., 2007). And the detection limit was lower than all of these by about one order of magnitude (Liu and Lin, 2006a; Sun et al., 2008; Wu et al., 2007; Yang et al., 2006; Zhang et al., 2004b), except the case of six bilayers of GOD/poly(allylamine) modified electrode, which was by 3.8 times (Zhang et al., 2004a).

The reproducibility was measured by six successive injections of 2 mmol L^{-1} glucose, and the R.S.D. of 3.5% was obtained. On the other hand, the responses of three biosensors from different batches were investigated, and the R.S.D. for inner- and inter-day was 4.3% and 5.7%, respectively. The stability of the biosensor, which was stored at 4 °C, was studied with its responses to 2 mmol L^{-1} glucose as well. After 10 days the biosensor showed no decrease of its initial sensitivity. With the experiment prolonged, the decrease of the response was observed. However, it could retain 80% of the original response after storage for 45 days (see Fig. 3S in the Supplementary data). The biosensor showed a comparative reproducibility and stability with some layer-by-layer fabricated ones (Wu et al., 2007; Yang et al., 2006), but worse than others especially in the stability. They could retain 80% of the original responses after stored for 2–3 months (Zhang et al., 2004a,b).

The biosensor was used for measuring of the serum glucose. In this study, glucose was determined in human serum from adult by using the proposed system (Ho et al., 2007). Prior to analysis, the sample serum was 10× diluted with the pH 6.98 PBS and centrifuged at 2000 × *g* for 5 min. Twenty microliters were taken directly from the supernatant and injected into the system. The glucose concentration was tested to be 5.77 ± 0.05 mmol L^{-1} in three repeated assays, with 4.2% relative error and 0.87% R.S.D. to the value of 5.54 mmol L^{-1} that obtained clinically by the Beckman CX5 automated biochemical analyzer in the Hospital of Xiamen University.

3.3. *Comparative performance study when replacing the Pt/PTi WE for a planar Pt electrode or use the home-made amperometric cell only as an enzyme reactor*

As illuminated above, the biosensor was very sensitive. It was mainly due to the large amount of immobilized GOD and no subtraction of the sensing signal for anti-interference manipulation. Besides, we believed the large effective surface area of the WE could be an important factor as well. After replacing the Pt/PTi WE in the amperometric cell for a planar Pt electrode, we compared their responses to 1 mmol L^{-1} glucose, as shown in Fig. 6. Since the solution could not flow through the planar Pt electrode, the amperometric cell with it was a little changed to the flow-onto design (see Fig. 4S in the Supplementary data). The results showed the i_p of the Pt/PTi electrode was 8.3 times larger, and only 8% of the signal decreased if change the amperometric cell with the Pt/PTi WE to the flow-onto design as well. As mentioned above the A_r of the Pt/PTi electrode was only 4.98 times of the planar Pt electrode, thus their larger difference in the response must be attributed to the porous character of the Pt/PTi electrode. It approved the immersion and flow-through of the solution, and thus the effective surface area was enlarged.

In order to further illuminate the good sensitivity of the biosensor, we chose the Dionex ED50 amperometric cell as a model and linked it to the home-made amperometric cell which served

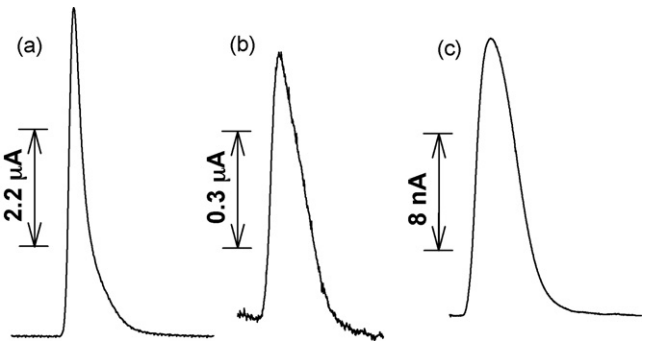

Fig. 6. Amperometric responses of the home-made amperometric cell with the Pt/PTi WE and based on the flow-through design (a) or with the planar Pt WE and based on the flow-onto design (b) to the injection of 1 mmol L^{-1} glucose. And the response of the Dionex ED50 amperometric cell to the injection of 1 mmol L^{-1} glucose (c). Other conditions are the same as in Fig. 4.

only as an enzyme reactor for glucose sensing. This experiment was carried out on the Dionex ICS-2500 ion chromatography controlled by its own software, and its Pt electrode was chosen as the WE. The resultant biosensor was tested with 1 mmol L^{-1} glucose, and the result is shown in Fig. 6 as well. The linear range of this biosensor was from 10 μmol L^{-1} to 5 mmol L^{-1}, and the calibration curve (conducted with three parallel assays) was expressed as i_p (μA) = $1.96 \times 10^{-2} c_{glucose}$ (mmol L^{-1}), with the correlation coefficient of 0.9958 obtained. Because of the low noise level, the detection limit was calculated to be 7 μmol L^{-1}. Thus compared with this biosensor, the sensitivity of the proposed one was 7.97 times higher as calculated with the current density, and its detection limit was about one order of magnitude lower.

4. Conclusions

In this work, the AER was modified with a layer of GOD and then PDDA, respectively, via layer-by-layer electrostatic self-assembling strategy. The PDDA/GOD/AER was then packed into a home-made amperometric cell for FIA of glucose. This design simplified the setup by integrating the enzyme reactor into the amperometric cell. And the AER in the cell could function as not only the support of enzymes, but also the tool for eliminating the interferences. Besides, the porous WE in this cell increased the response greatly due to its large effective surface area. The proposed biosensor was very sensitive in glucose quantification, but its stability was not very satisfying. This work may have provided a reference design of the amperometric cell which could be adopted in other enzymatic-FIA biosensors.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.01.037.

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