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Superior long-term stability of a glucose biosensor based on inserted barrel plating gold electrodes

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ABSTRACT

Disposable one shot usage blood glucose strips are routinely used in the diagnosis and management of diabetes mellitus and their performance can vary greatly. In this paper we critically evaluated the long-term stability of glucose strips made of barrel plating gold electrodes. Compared to other glucose biosensing platforms of vapor deposited palladium and screen printed carbon electrodes, the proposed glucose biosensor was found to show the best stability among the three biosensing platforms in thermal acceleration experiments at 40 °C for 6 months with an average bias of 3.4% at glucose concentrations of 5–20 mM. The precision test of this barrel plating gold glucose biosensor also showed the best performance (coefficients of variation in the range of 1.4–2.4%) in thermal acceleration experiments at 40 °C, 50 °C and 70 °C for 27 days. Error grid analysis revealed that all measurements fell in zone A and zone B. Regression analysis showed no significant difference between the proposed biosensor and the reference method at 99% confidence level. The amperometric glucose biosensor fabricated by inserting two barrel plating gold electrodes onto an injection-molding plastic base followed by immobilizing with a bio-reagent layer and membrane was very impressive with a long-term stability up to 2.5 years at 25 °C. Overall, these results indicated that the glucose oxidase/barrel plating gold biosensing platform is ideal for long-term accurate glycemic control.

1. Introduction

Exact and rapid *in situ* glycemic control is of great importance in the diagnosis and management of diabetes mellitus. This global, multibillion dollar market is based on modified electrodes where the manufacturing of disposable one shot usage strips is attractive due to the low cost and ability to mass produce. The performance of biosensors is electrochemically interrogated by reagent composition and electrode behavior and should be evaluated by sensitivity, linearity, precision and long-term stability (Sarma et al., 2009). So far, most enzymatic biosensors are developed with suitable surface modification to result in faster electron relay and lower applied potential (Bianco, 2002). Very few long-term stability studies, however, were attempted primarily due to the time consuming process of traditional life data analysis involving the analysis of times-to-failure data under normal operating conditions. In general, to allow efficient design and optimal working performance of biosensor, the underlying electrode must hold/or exhibit favorable electrochemical properties such as fast electron transfer and good reproducibility. Additionally, the lifetime of the enzyme electrode and the interaction between the enzymatic redox reaction and electrode are critical to this interdisciplinary research. Precise coupling of the catalytic enzyme with electrode improves the biosensing stability and hence results in improved accuracy.

Glucose oxidase (GOx) is extensively used in portable glucose biosensors and it is well known that the tertiary structure of GOx with the active site embedded in thick binding-domains hinders the direct electron transfer from catalytic site to sensing electrodes (Wilson and Turner, 1992; Hecht et al., 1993). In order to facilitate electron relay, strategies for immobilizing the GOx on various methodologies have been explored (Albareda-Sirvent et al., 2000; Miscoria et al., 2006; Jia et al., 2007; Renedo et al., 2007; Kuwahara et al., 2008). Commonly employed electrochemical transducers are either inert metals (such as platinum and gold) or carbonaceous materials (Sotiropoulou et al., 2003; Lim et al., 2005).

Recently we have reported a simple process for fabricating a new type of amperometric glucose biosensor by inserting two barrel plating gold electrodes (BPAuEs) onto an injection-molding plastic base followed by immobilizing with a bio-reagent layer and membrane (Hsu et al., 2009a). The proposed biosensor was found to be suitable for alternative-site testing (e.g., fingertip, palm and arm) and free of interference effect (Wu et al., 2008; Hsu et al., 2009b). This one-step process of manufacturing

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electrodes for injection-molding biosensors can greatly simplify the manufacture step to mass produce. Note that almost all of the commercially available amperometric type self-monitoring blood glucose (SMBG) biosensors are produced via screen printed carbon electrode (SPCE, e.g., Super Glucocard II/Arkray; Onetouch Ultra/LifeScan), vapor deposited palladium electrode (VDPdE, e.g., Accu-Chek Advantage/Roche) and barrel plating gold electrode (BPAuE, e.g., Rightest/Bionime). To shorten the time required obtaining times-to-failure data, the term accelerated life testing has been used to describe all such practices (ReliaSoft Publishing, 2007). The objective of this study is to characterize different glucose biosensing platforms with a view to determining which sensors provide optimal results in long-term stability by accelerated life testing. Our study is provided such that researchers can make informed decisions in biosensor design and development.

2. Experimental

GOx (EC 1.1.3.4, >180 U/mg) from *Aspergillus niger* was purchased from Amano Enzyme Inc. (Nagoya, Japan) and stored at −20 °C. Potassium ferricyanide and β-D(+)-glucose were obtained from MP Biomedicals (Solon, OH, USA). Unless stated otherwise, all chemicals were of analytical grade, and all solutions were prepared with de-ionized water (18 MΩ cm). Standard glucose solutions were prepared in 0.1 M, pH 7.4 phosphate buffer solution and the concentrations were calibrated by YSI 2300 STAT Plus glucose and lactate analyzer (Yellow Springs, OH, USA).

The test strip was fabricated by an one-step coating of reagents prepared from a mixture of 48.5% GOx, 8.5% potassium ferricyanide and 43% nonreactive ingredients without any additional modification on the BPAuE (diameter = 1.145 mm). The fabrication process of BPAuE was reported earlier (Hsu et al., 2009a) (Supplementary 1). After dried at 45 °C for 20 min, the test strip was covered with a hydrophilic film, between which a tiny space was used for electrochemical reaction. The biosensor constructed thereon requires only 1.4-μL blood sample and can report a plasma-equivalent value in 8 s.

Two other types of glucose biosensors made of pyrroloquinolinequinone (PQQ)-glucose dehydrogenase (GDH)/VDPdE and GOx/SPCE, as shown in Fig. 1, were applied for the thermal-accelerative precision test at 40 °C, 50 °C and 70 °C. Two meters was used in each type of sensor, and the measurements were repeated for ten times on each meter. Venous blood having glucose level ranging from 5.0 mM to 5.5 mM was used in the whole experiments. For long-term stability test, the thermal acceleration experiments were carried out at 40 °C for 6 months and the performance of which was supposed to be equivalent to that at 25 °C for 24 months according to Arrhenius equations (British Standard, 2002). Standard glucose solutions (pH 7.4) equivalent to 5 mM, 10 mM and 20 mM were prepared freshly before conducting the experiments. Triplicate measurements were performed at each concentration level, and the mean values were plotted as the dependent variable on the graph. The day-to-day bias was determined as compared to the initial measurement, and the overall bias was an average of absolute value of day-to-day bias. Meanwhile, the proposed biosensor was also examined at 25 °C and 40 °C for 2.5 years, using 3 mM, 5 mM, 10 mM and 20 mM standard glucose solutions.

A total of 164 patients were enrolled at the outpatient clinic of Chung-Shan Medical University Hospital for 7 successive days. Two lots of test strips, lot no. 117C171(21A) and 117C173(22A), were used to assess the clinical performance of the proposed biosensor. The study protocol was approved by the Institutional Review Boards (CS07087), and informed consent was received from all participants. Capillary blood samples were collected from the fingertip of each subject by experienced technicians and licensed nurses. In addition, venous blood was immediately drawn into a tube with heparin anticoagulant for comparative hexokinase assay, using the Olympus AU2700 system.

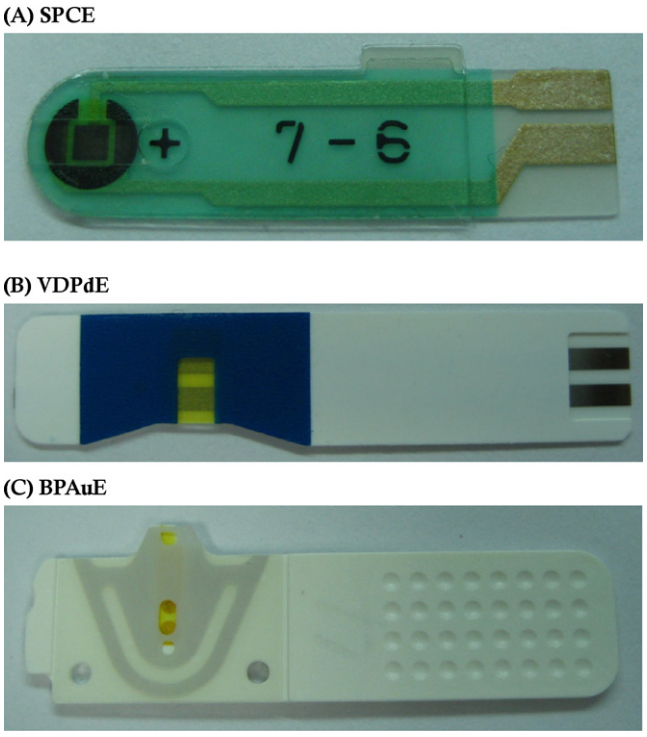

Fig. 1. Pictures of test strips made of (A) SPCE, (B) VDPdE, and (C) BPAuE.

Systematic accuracy was evaluated on the Clarke error grid (Clarke et al., 1987), which is divided into five zones by assigning specific SMBG errors with different clinical significances. Zone A is regarded as lack of treatment error, of which an increase in clinical risk is associated with zones B–E. Regression analysis was performed with least-square method (Linnet, 1993) and the standard deviation of residual ($S_{y/x}$) as well as the 99% confidence interval of slope and intercept was reported. The Pearson's correlation coefficient (r) was used as a measure of the strength of linearity.

3. Results and discussion

The physical and chemical properties of the materials used in the construction of biosensors, especially the underlying electrode used to modify the enzyme, have significant influence on their performance. Consequently, beside the proposed BPAuE, we have also explored the characteristic behavior of two other different types of glucose biosensors made of VDPdE and SPCE and fabricated by different manufacturing and immobilizing methods. Note that previous report compared platinum, gold, palladium and glassy carbon as electrode materials in the design of biosensors for glutamate (O'Neill et al., 2004); similar issue for the choice of electrode material for enzyme immobilization and electrochemical detection was addressed in this study.

3.1. Long-term stability

A main issue addressed is the choice of electrode material for enzyme immobilization and electrochemical detection. A suitable material should be given to less variation in the measurements for sufficiently long period. In thermal-accelerative precision tests directed at 40 °C, 50 °C and 70 °C for 27 days, we found that there was no obvious change in the coefficients of variation (CVs) of

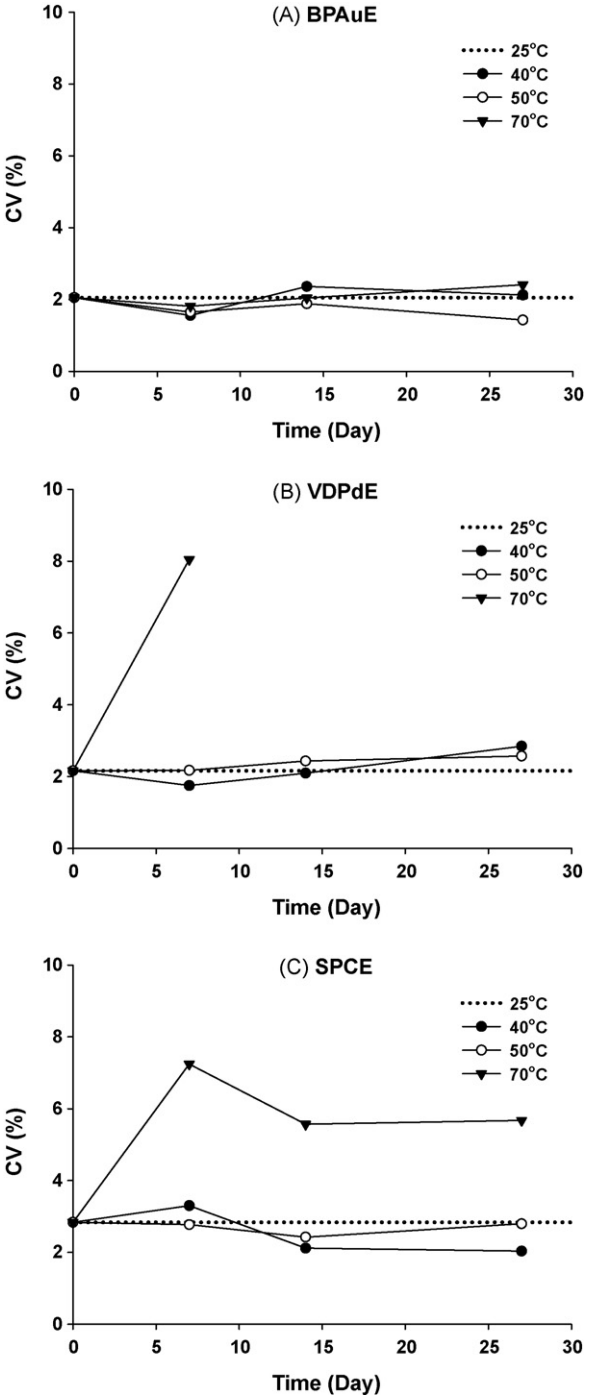

Fig. 2. Thermal-accelerative precision test at 40 °C, 50 °C and 70 °C for test strips made of (A) BPAuE, (B) VDPdE, and (C) SPCE.

BPAuE (Fig. 2A). The BPAuE showed very small variation (CVs: 1.4–2.4%) in all testing condition, indicating strong integration of GOx with gold electrodes and reliable performance after remote transportation without air conditioning. The CVs of VDPdE and SPCE were also stable at 40 °C and 50 °C. However, when the temperature was elevated to 70 °C, the precision of VDPdE and SPCE decreased tremendously (Fig. 2B and C). The CV of VDPdE and SPCE was found to increase from 2.2% (Day 0) to 8.0% (Day 7) and 2.8% (Day 0) to 5.7% (Day 27), respectively. Possible reasons to result in inferior precision in high-temperature circumstances include deterioration of reagent composition and plastic base, instability of electrode materials, inactive hydrophobicity and so forth. The superior precision of the BPAuE may also be attributed to the smooth and fine surface structure of electrodes to reduce the environmental interference, like temperature, humidity or other physical damage due to improper handling. Surprisingly, after exposure to 70 °C for 2 weeks, the blood could not be drawn into the test strips of VDPdE by capillary action and hence the precision test would not be carried out thereafter.

For the purpose of evaluating the accuracy of these biosensors, another comparative study among BPAuE, VDPdE and SPCE was carried out at 25 °C for at least 6 months. The overall bias at 5–20 mM glucose solution for BPAuE, VDPdE and SPCE were observed as 3.7%, 4.0% and 6.3%, respectively (Table 1). In other words, there was no obvious difference in glucose measurements for these biosensors at any concentration levels. As the temperature was increased to 40 °C, the stability of SPCE was found to decrease noticeably from 5.9% to 18.2% and 7.3% to 19.3% for 10 mM and 20 mM glucose, respectively (Fig. 3B and C). The stability of the VDPdE was also affected under this elevated temperature except in a relatively mild manner, *i.e.*, from 5.5% to 6.9% in 20 mM glucose (note that a larger deviation at Day 87 was the intrinsic variety of the strip or temperature fluctuation). The stability of the BPAuE, on the other hand, remained constant and robust in response at higher temperature (Fig. 3). The difference is therefore very obvious at 40 °C and the overall bias at 5–20 mM glucose solution for BPAuE, VDPdE and SPCE were found to be 3.3%, 5.4% and 16.2%, respectively (Table 1). The decrease in stability of the SPCE at 40 °C may suggest the loss of enzyme activity possibly due to the deterioration of binding reagents in the printing ink and therefore the fracture of electrode structure upon reagent dissolution. Besides, the high pressure during the printing process might also impair the integrated structure of electrodes. As to the VDPdE, the decline in stability might be related to poor electrochemically integration between glucose dehydrogenase and Pd electrode at higher temperatures. The fact that the BPAuE possesses the most stable performance over a period of 6 months may be attributed to the excellent collaboration of GOx and Au electrodes. Indeed gold electrodes have been used previously in most of the long-term stability studies (Ozoemena et al., 2001; Xiao et al., 2003; Pan et al., 2005). Gold seems to represent an excellent electrode material to provide flexible platforms for immobilizing enzymes thereon because of its intrinsic stability and chemical inactivity (Zhang et al., 2009). Gold nanoparticles were also reported to allow proteins to retain their biological activity upon adsorption and are able to reduce the insulating effect of the protein shell for direct electron transfer (Doron et al., 1995). It is thus not surprised that the BPAuE exhibits the most stable electrochemical properties with GOx-immobilization and also for electrochemical detection.

In order to assess the long-term stability of the proposed biosensor, the study was carried out for 2.5 years through measuring standard glucose concentration at four different levels. As illustrated in Fig. 4A, a survey of the glucose concentration of 3–20 mM exhibited steady and consistent performance up to 911 days at 25 °C. No measurements were deviated from the criteria of ±20% as compared with the initial values under low-glucose condition

Table 1
Overall bias at 25 °C and 40 °C using standard glucose solutions of 5 mM, 10 mM and 20 mM.

[Glucose] (mM)	25 °C			40 °C		
	BPAuE	VDPdE	SPCE	BPAuE	VDPdE	SPCE
5	4.1%	3.1%	5.9%	2.5%	6.4%	11.0%
10	4.0%	3.4%	5.9%	3.7%	3.0%	18.2%
20	3.0%	5.5%	7.3%	3.8%	6.9%	19.3%
Total	3.7%	4.0%	6.3%	3.3%	5.4%	16.2%

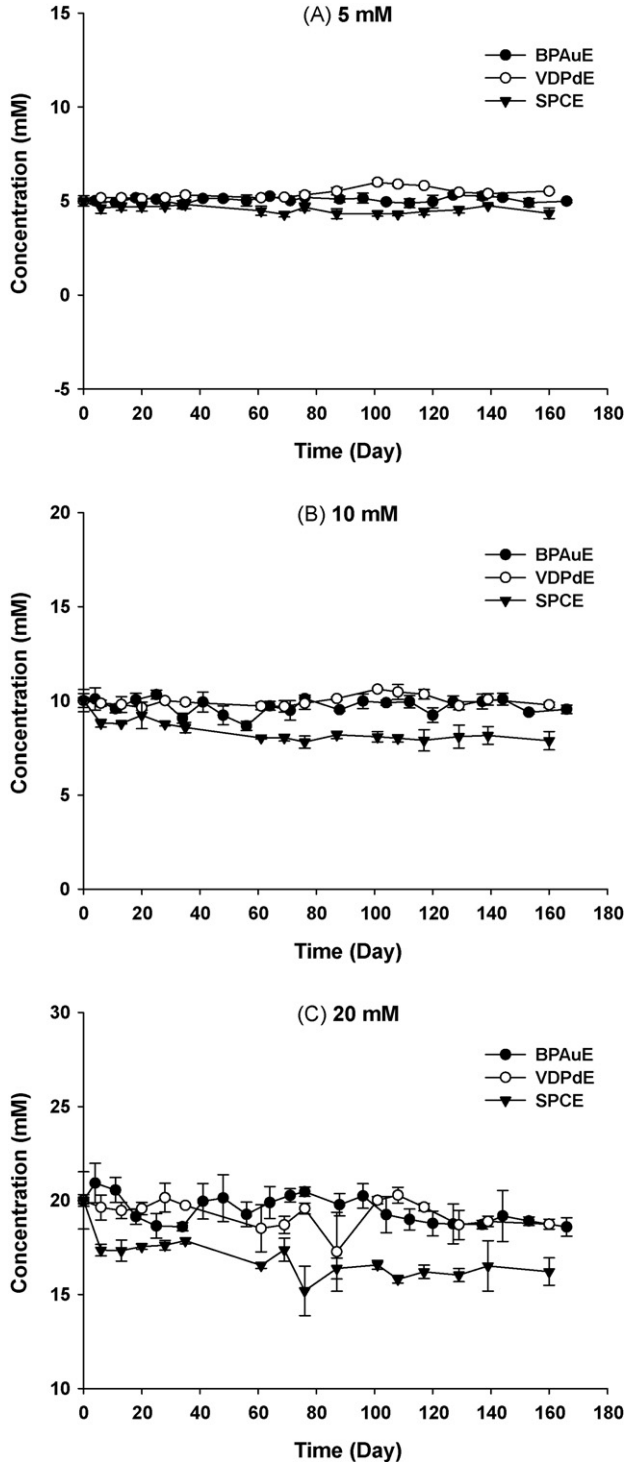

Fig. 3. Comparison of storage stability at 40 °C using standard glucose solutions of 5 mM, 10 mM and 20 mM. All data points are normalized to Day 0 and error bars on the plot represent the standard deviation (SD). The relative standard deviations (RSD) of BPAuE, VDPdE and SPCE was in the range of 0.1–2.9%, 0.2–12.2% and 0.4–11.1%, respectively.

(3 mM), nor were the readings abnormal from ±10% under normal- and high-glucose conditions (5–20 mM). The excellent long-term stability of the BPAuE at 40 °C was verified as well with overall bias at 25 °C and 40 °C measured as 2.5–5.2% and 2.4–5.6%, respectively (Fig. 4B). These results indicate that the biosensor made of GOx-immobilized BPAuE was suitable for long-term glycemic control at 25–40 °C. Long-term stability is affected by various factors, such as reagents composition, electrode materials, interferences and ambient circumstances. As reported previously, precision is a vital factor to accurate measurement of glucose concentration and thus only high precision can result in superior accuracy of biosensors (Krouwer, 2001; Wu et al., 2008). Interference effect and ambient circumstances can also influence the accuracy of biosensors (Hsu et al., 2009a,b). The excellent stability should be attributed to the simple, one-step immobilization process without deteriorating the enzyme activity.

3.2. Clinical evaluation

An assessment on the systematic error further demonstrates that the biosensor made of GOx-immobilized BPAuE possesses high accuracy with any strip lots. In error grid analysis, 98.8% (lot 21A) and 98.2% (lot 22A) of 164 paired points was assigned to zone A, and 100% was distributed in zone A + B (Fig. 5). Regression analysis showed that the 99% confidence intervals of slope (0.96–1.01 for lot 21A; 0.96–1.01 for lot 22A) and intercept (−0.24 to 0.32 for lot 21A; −0.02 to 0.56 for lot 22A) comprised one and zero, respectively (for lot 21A, $y = 0.99x + 0.04$ mM, $S_{y/x} = 0.75$ mM; for lot 22A, $y = 0.99x + 0.27$ mM, $S_{y/x} = 0.76$ mM). The correlation coefficients (r) were larger than 0.99, indicating a strong linear relationship between the proposed biosensor and the Olympus system.

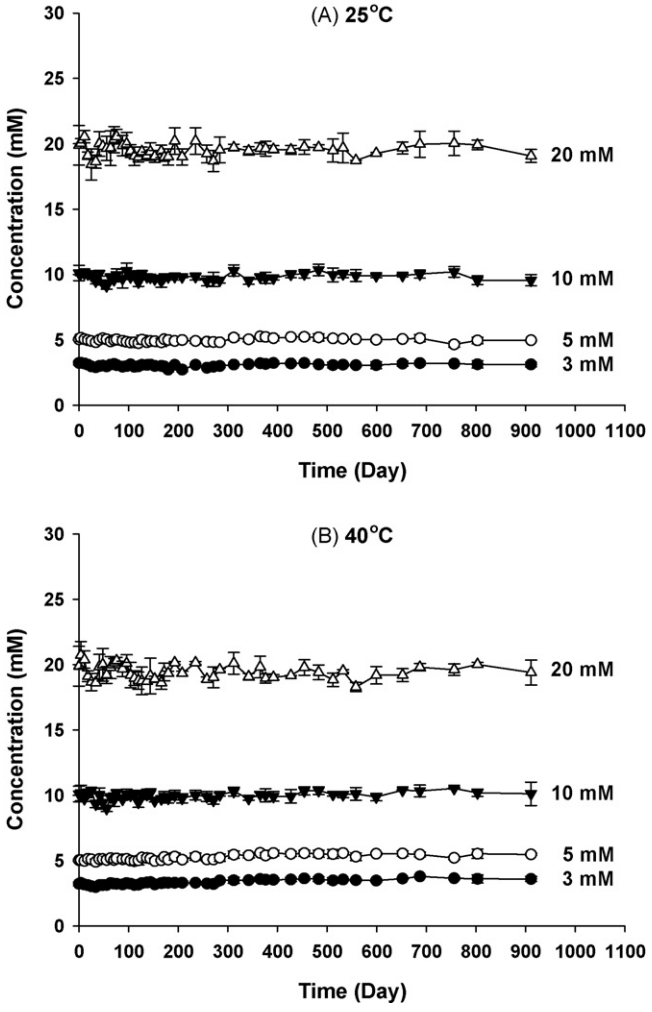

Fig. 4. Long-term stability of the GOx-immobilized BPAuE at 25 °C (A) and 40 °C (B). The standard glucose solutions of 3 mM, 5 mM, 10 mM and 20 mM were used for the test. Error bars on the plot represent the SD, and the RSD of all measurements was in the range of 0.01–3.3%.

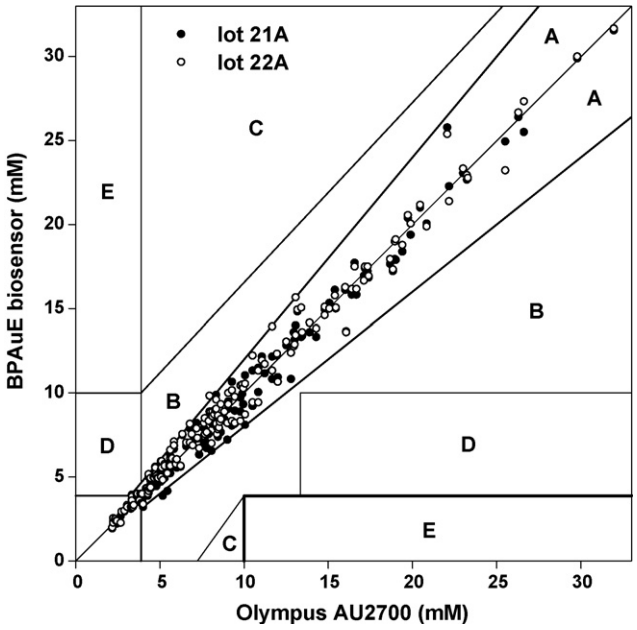

Fig. 5. Error grid analysis on the clinical performance of GOx-immobilized BPAuE biosensor. Measurements ($N = 164$) from the venous plasma were used as references. For lot 21A: $y = 0.99x + 0.04$ mM, $S_{y/x} = 0.75$ mM; for lot 22A: $y = 0.99x + 0.27$ mM, $S_{y/x} = 0.76$ mM.

These results demonstrate that the analytic error in the biosensor made of GOx-immobilized BPAuE was statistically insignificant.

It is shown that modification of reagents or electrodes can enhance the stability of biosensors (Bianco, 2002; Gouda et al., 2002). Therefore, various studies had been carried out to improve the stability of test strips, but only few of them were successful in extending the testing period for several months (Liu et al., 1997; Schulz et al., 1999; Chen et al., 2002; Nakamura et al., 2007). To the best of our knowledge, it was state-of-the-art that the stability test had been accomplished for 2.5 years, and the most intensive and extensive research allows us to evaluate the systematic accuracy in a prolonged time scale. A 2.5-year working lifetime of the GOx-immobilized BPAuE at 25 °C is verified in this study, so are the test strips kept at 40 °C (Fig. 4). The overall bias was <6% in all of the testing conditions.

4. Conclusions

In this study, an amperometric type self-monitoring blood glucose biosensor fabricated by a one-step coating process without any additional modification on the BPAuE was tested for long-term stability. In thermal acceleration experiments, the GOx/BPAuE shows better precision and stability than either PQQ-GDH/VDPdE or GOx/SPCE. The biosensor exhibits a steady and consistent performance for as long as 2.5 years and shows acceptable relevance to the comparative method. These convincing evidences support the conclusion that the inserted barrel plating gold electrodes-based biosensor is very suitable for long-term glycemic control.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.07.028.

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