Glucose sensor for flow injection analysis of serum glucose based on immobilization of glucose oxidase in titania sol–gel membrane

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Abstract

An novel amperometric glucose sensor was constructed by immobilizing glucose oxidase (GOD) in a titania sol–gel film, which was prepared with a vapor deposition method. The sol–gel film was uniform, porous and showed a very low mass transport barrier and a regular dense distribution of GOD. Titania sol–gel matrix retained the native structure and activity of entrapped enzyme and prevented the cracking of conventional sol–gel glasses and the leaking of enzyme out of the film. With ferrocenium as a mediator the glucose sensor exhibited a fast response, a wide linear range from 0.07 to 15 mM. It showed a good accuracy and high sensitivity as \(7.2 \, \text{mAM} \cdot \text{cm}^{-2} \cdot \text{mM}^{-1}\). The general interferences coexisted in blood except ascorbic acid did not affect glucose determination, and coating Nafion film on the sol–gel film could eliminate the interference from ascorbic acid. The serum glucose determination results obtained with a flow injection analysis (FIA) system showed an acceptable accuracy, a good reproducibility and stability and indicated the sensor could be used in FIA determination of glucose. The vapor deposition method could fabricate glucose sensor in batches with a very small amount of enzyme.

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1. Introduction

During the past 30 years numerous attempts have been made to fabricate sensitive, selective, reliable and low cost glucose sensors because of the clinical significance of measuring blood glucose levels. Among these sensors, amperometric electrodes based on the immobilized glucose oxidase (GOD) have attracted considerable interest due to their simple alternative analytical systems (Cosnier, 1999). Effective immobilization of GOD is one of the key features for successful application. Many methods such as physical adsorption (Battaglini et al., 2000), cross-linking (Cass et al., 1984; Senillou et al., 1999; Burmeister and Gerhardt, 2001), self-assembly (Gooding et al., 1998; Murthy and Sharma, 1998), incorporation in carbon paste (Kulys et al., 2001; Moscone et al., 2001), polymers (Palmisano et al., 2000) and hydrogels (Binyamin and Heller, 1999), have been employed to immobilize GOD on the electrode surface. However, some of these immobilization methods are relatively complicated, require expensive reagents or environmentally unattractive solvents and result in relatively poor stability, which leads to relatively slow acceptance of glucose biosensing technology (Myler et al., 2002). In order to gain wider acceptance and indeed practical application of glucose biosensor, new immobilization schemes and advanced materials are highly desired to improve its analytical capabilities regarding specificity, stability, sensitivity, accuracy and reproducibility and to meet the challenges posed by complex clinical samples.

Since Braun et al. (1990) first reported the possibility of protein immobilization in a sol–gel silica matrix, this technique has extensively been used to immobilize enzymes (Dave et al., 1994; Lev et al., 1995; Wang, 1999), antigen (Wang and Pamidi, 1998) and cells (Carturan et al., 1998). In the sol–gel network the catalytic activities of immobilized enzymes can be retained to a large extent (Wang et al., 1993; Chen et al., 1998; Wang et al., 1998). This kind of biocompatible...
materials has also been widely used to immobilize GOD for the construction of glucose amperometric biosensors (Li et al., 1999; Tian and Zhu, 2002). However, the silica sol–gel derived matrix is fragile and easy cracking (Lev et al., 1995; Wang, 1999) and easy to desquamate from the electrode surface. Some surface-active agents (Lev, 1992; Li et al., 1996) and grafting copolymers (Wang et al., 1998, 1999) have been doped to overcome these problems. Obviously, the surfactant is detrimental to the enzyme.

Another key feature is the diffusional barrier of the membrane to the enzyme substrates and electron transfer mediators (Myler et al., 2002), which lowers the magnitude of sensors responses (Iwuoha et al., 1999) and results in the longer response times (Wang et al., 1999) and limitation of on-line analysis for glucose. The thin film-based sol–gel technique has been used to decrease the diffusion barrier (Wang et al., 1999). A better porous material than silica sol–gel matrix will reduce the barrier.

Some researches have been carried out to seek new sol–gel materials, which can overcome the shortcomings caused by the silica sol–gel for enzyme immobilization in glucose biosensor construction. Lev designed a glucose biosensor based on a highly conductive and porous sol–gel vanadium pentaoxide (Glezer and Lev, 1993). Deng et al. developed a glucose biosensor by immobilizing GOD in sol–gel alumina matrix on a porous platinum particle platinized glassy carbon electrode (Liu et al., 1999). These sensors display a good stability and very sensitive response to glucose. Although glucose sensors have been well-studied (Tatsu et al., 1992; Narang et al., 1994; Schmidtke and Heller, 1998; Vidal et al., 1998; Yao and Takashima, 1998), few works have fully quantified their analytical performance such as the stability, affinity and characterization of immobilized enzyme, and the response time, sensitivity, detection limit, accuracy, interference and reproducibility of sensor for glucose assay. On the other hand, the amperometric glucose biosensor have now been commercially available from several manufactures and accepted by individuals for blood glucose monitoring, however, few reports has introduced their application to the on-line determination of glucose by using a flow injection analysis (FIA) system, and no sensor with a low cost, stable, sensitive and on-line glucose monitoring suitable for clinical application has been reported. Our previous work has demonstrated the titania sol–gel material prepared with a vapor deposition method to be a suitable matrix for enzyme immobilization (Yu and Ju, 2002). In this paper, we try to use this membrane to immobilize GOD for meeting the challenges posed by complex clinical samples and the need for on-line determination of serum glucose.

Vapor deposition technique provides ambient conditions for preparation of titania sol–gel film and immobilization of enzyme in neutral medium, thus is very advantageous to the fabrication of biosensors. The results obtained from scanning electron microscopy (SEM), FTIR, XPS, FIA and electrochemical studies indicate that titania sol–gel film is porous and highly homogeneous and shows a very low mass transport barrier to both substrate and ferrocenium, an electron transfer mediator. GOD can be effectively entrapped in the film with a regular dense distribution and a higher bioactivity than that of native GOD in solution. With this method the sensor can be fabricated in batches with a very small amount of enzyme and a low cost. The presented FIA procedure needs a small sample volume of 0.2 ml and a short analysis time. The obtained glucose sensor possesses a very high sensitivity, good reproducibility and fast response, and is stable enough for FIA of the serum glucose level.

2. Materials and methods

2.1. Reagents

GOD (EC 1.1.3.4, 35.3 U mg$^{-1}$). Type II from Aspergillus niger) was purchased from Sigma and used as received. Titanium isopropoxide (Ti(i-PrO)$_4$), ferrocenium hexafluorophosphate (FcPF$_6$) and Nafion solution (5.0 wt.%) were obtained from Aldrich. All other chemicals were of analytical grade and were used without further purification. All solutions were made up with twice-distilled water. Serum standard sample was provided by Zhongsheng Biology Engineering Co. (Beijing) and prepared according to the instructions.

2.2. Apparatus

Electrochemical measurements were performed with a BAS-100B electrochemical analyzer connected a PA-1 preamplifier (Bioanalytical Systems Inc., USA). A three-electrode system comprising the enzyme electrode as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference was employed for all electrochemical experiments. All the potentials given here were relative to SCE. Cyclic voltammetric experiments were carried out in a static electrochemical cell at 25 ± 0.2 °C, while amperometric experiments were carried out in a stirred cell with a successive addition of glucose standard solution to the solution by applying a potential step of 300 mV to the enzyme electrode.

Scanning electron micrographs of titania sol–gel and GOD doped titania sol–gel (GOD/titania) membranes were obtained with a Hitachi X-650 scanning electron microscope (Hitachi Ltd., Japan) undertaken at an acceleration voltage of 20 kV. Fourier transform infrared spectra of the KBr disc supported films were
recorded on a Vector 22 Fourier transform infrared spectrometer (Bruker). Twenty scans were collected and averaged for each spectrum. X-ray photoelectron spectroscopic analysis was carried out using a VG Scientific ESCALAB MK2 spectrometer with a Mg Kα radiation. Survey spectrum, in the range of 0–1000 eV, was recorded with a pass energy of 50 eV and corrected for sample charging using the C1s peak at 284.6 eV as the internal reference.

2.3. Preparation of enzyme electrode

Prior to modification, glassy carbon electrodes (diameter of 4 mm) were polished with 1.0, 0.3 and 0.05 μm alumina slurry (Beuhler), respectively, and rinsed thoroughly with doubly distilled water between each polishing step. The electrodes were then successively sonicated in 1:1 nitric acid, acetone and doubly distilled water, and then allowed to dry at room temperature.

Titania sol–gel and GOD/titania membranes were prepared as described previously (Yu and Ju, 2002). A GOD solution was first obtained by dissolving 2.0 mg GOD in 1.0 ml 0.02 M pH 7.0 phosphate buffer solution (PBS). PBS or 10 μl GOD solution (0.7 U GOD) was dropped onto the surface of a pretreated glassy carbon electrode. The electrode was then suspended vertically above titanium isopropoxide in a sealed flask kept at 25 °C for 6 h. This resulted in absorption of saturate titanium isopropoxide vapor by the solution and slow formation of a titania sol–gel or GOD/titania membrane through hydrolysis of titanium isopropoxide on the surface.

Nafion/GOD/titania electrode was prepared by dropping 10 μl of Nafion solution (0.5 wt.%) on the surface of GOD/titania and drying at 4 °C for 10 h. Prior to electrochemical experiments, the electrodes were rinsed thoroughly with doubly distilled water and kept in PBS at 4 °C.

3. Results and discussion

3.1. Morphologies of titania sol–gel and GOD/titania films

The morphologies of titania sol–gel and GOD/titania films were characterized by SEM. The SEMs of titania sol–gel membrane display a chemically clean three-dimensional uniform porous structure. The aggregates of the titania sol–gel matrix on electrode surface are well distributed and the pores in the network can be clearly observed (Fig. 1a). This uniform porous structure results in a high enzyme loading. When GOD is immobilized in the titania sol–gel matrix, the SEMs display a uniform and regular dense distribution of the bright particles for the GOD domains with a good reproducibility (Fig. 1b). The porous structure of the titania sol–gel film makes the immobilized enzyme easy to be accessed by its substrate and brings a good performance of the modified electrode.

3.2. Cyclic voltammetric behavior of enzyme electrode

Fig. 2 shows the cyclic voltammograms of the sensor in different solutions. The response of the enzyme electrode in 0.02 M pH 7.3 PBS displays a low background current (Fig. 2a). When PBS contains 0.4 mM FcPF₆, the cyclic voltammogram gives a couple of peaks attributing to the redox process of ferrocenium with a one-electron transfer at the enzyme electrode (Fig. 2b). The peak currents are proportional to the square root of scan rate and the peak potentials keep at constant values in the scan rate range from 50 to 500 mV s⁻¹, showing a typical diffusion-controlled electrochemical behavior. The peak currents show a slight decrease at the enzyme electrode in comparison with those at bare and titania sol–gel film coated electrodes (shown as inset in Fig. 2), which is due to the diffusional barrier of the film. However, the diffusional barrier does not lower the magnitude of sensors responses as reported previously (Iwuoha et al., 1999), indicative of a very low mass transport barrier of GOD/titania film to ferrocenium. The redox peak potentials of 222 ± 3 and 147 ± 2 mV are the same as those at both bare and titania sol–gel film coated electrodes. Thus, ferrocenium can exchange the electron with the electrochemical sensing sites on glassy carbon surface without any obstacle, and both titania sol–gel and GOD/titania films are of a porous structure. The addition of glucose to the solution containing FcPF₆ results in a dramatic increase in the oxidation current and a decrease in reduction current of ferrocenium at the enzyme electrode (Fig. 2c–e), showing a typical shape for electrocatalytic process, while no any change is observed at both bare and titania sol–gel coated electrodes in the studied potential range.

3.3. Optimization of enzyme electrode preparation

Titanium isopropoxide is much more active than tetraethyl orthosilicate. In case of its touching with water, the precipitate of titanium dioxide is formed immediately. So a vapor deposition method was proposed to decrease the hydrolysis rate and to prepare titania sol–gel matrix (Yu and Ju, 2002). The performance of the enzyme electrode mainly depends on the preparation temperature and the amount of enzyme dropped on the electrode surface. The temperature directly dominates the vapor pressure of titanium isopropoxide, which controls the hydrolysis rate. The experiments show that the enzyme electrode gets the best performance at the preparation temperature of 25 °C at which hydrolysis rate matches water volatilization rate.
The amperometric performance of enzyme electrode is related to the concentration of 10 µl enzyme solution dropped on the electrode surface for GOD/titania film preparation. The amperometric response to glucose increases with an increasing concentration of enzyme and reaches a plateau at an enzyme concentration of 2.0 mg ml\(^{-1}\) (0.7 U GOD, Fig. 3). This indicates the enzyme loading capacity of titania sol–gel film has been saturated. When the concentration is more than this value, excess GOD will be leached in the rinsing procedure.

3.4. XPS analysis of GOD/titania film on sensor surface

The surface chemical composition was evaluated with XPS analysis. On the titania sol–gel modified electrode surface, the O1s and Ti2p peaks are detectable at the binding energy of 533.70 and 461.95 eV. This film does not display the C1s peaks, so the hydrolysis of titanium isopropoxide under the selected conditions is complete. The film obtained with 2.0 mg ml\(^{-1}\) GOD solution for the hydrolysis of titanium isopropoxide also gives the O1s and Ti2p peaks, at the same time it shows other peaks at 288.55 eV for C1s and 403.60 eV for N1s (as shown in Fig. 4). The C1s and N1s peaks result from the GOD immobilized in the sol–gel film. This illustrates that the GOD is successfully immobilized in GOD/titania film.

3.5. FTIR analysis of sol–gel and enzyme entrapped sol–gel films

FTIR spectroscopy was used to describe the composition and structure character of the films. The FTIR spectrum of titania sol–gel film shows a peak at 1640 cm\(^{-1}\) and two wide peaks centered at 3380 cm\(^{-1}\) and below 650 cm\(^{-1}\) (Fig. 5a). The peaks at 1640 and 3380
3.6. Condition optimization for glucose sensing

The response of the glucose sensor depends on the activity of immobilized GOD, which is related to solution pH. The sensor shows electrocatalytic responses to 0.75 mM glucose in presence of 0.4 mM FeC₆PF₆ at the pH ranging from 5.6 to 10.0 with a maximum value at pH 7.3 (inset a in Fig. 6). The response decreases at pH more than 7.3 is due to enzyme denaturation at high pH. GOD retains its maximum activity and catalysis efficiency at pH 7.3, which is selected to obtain the best sensitivity for glucose sensing.

This work uses ferrocenium hexafluorophosphate as a mediator for glucose sensing. With increasing FeC₆PF₆ concentration the electrocatalytic current, the difference between the oxidation peak currents of the cyclic voltammograms of ferrocenium in presence and absence of glucose, increases quickly and reaches a maximum value at the FeC₆PF₆ concentration of 0.4 mM (inset b in Fig. 6). At the concentrations larger than 0.4 mM the oxidation peak current of the reduced form of ferrocenium increases, but i_{p,cat} remains at the maximum value. So 0.4 mM FeC₆PF₆ in 0.02 M pH 7.3 PBS is chosen as the detection solution of glucose.

The amperometric response of the sensor depends on the applying potential. Electrocatalytic oxidation of glucose is already observed at around 100 mV. With increasing the applied potential from 100 to 450 mV, the steady-state current increases and approaches a plateau at 300 mV (Fig. 6). The increase in amperometric response is due to the increased driving force for the fast oxidation of ferrocenium. 300 mV is selected as the working potential for amperometric detection of glucose.

3.7. Amperometric response and calibration curve

Fig. 7 shows a typical current–time plot for the sensor at 300 mV on successive addition of 1.0 mM glucose in 0.02 mM pH 7.3 PBS containing 0.4 mM FeC₆PF₆. When an aliquot of glucose is added into the buffer solution, the oxidation current rises steeply to reach a stable value. The presence of dissolved oxygen does not affect the response current. The sensor achieves 95% of steady-
Fig. 7. Typical steady-state response of the enzyme electrode in 0.02 M pH 7.3 PBS containing 0.4 mM FcPF$_6$ at 300 mV on an increasing concentration of glucose in 1.0 mM steps. Inset: (a) calibration curve and (b) Lineweaver–Burk plot.

state current in less than 6 s. Such a short response time indicates a fast mass transfer of both ferrocenium and the enzyme substrate across the film and a fast electron exchange between GOD and its substrate as well as between the reduced form of GOD and ferrocenium, proving further that the titania sol–gel matrix is promising for the construction of biosensors due to its porous structure.

The calibration curve of the sensor under the optimized conditions shows a linear range from 0.07 to 15 mM for glucose response with a correlation coefficient of 0.9971 and a sensitivity of 7.2 μA cm$^{-2}$ mM$^{-1}$ (inset a in Fig. 7). The detection limit is 0.07 mM at 3σ. The sensitivity is 1.5 times that reported for glucose sensor based on copolymer modified silica sol–gel (Wang et al., 1998) and higher than those of 6.25 (Ohara et al., 1993), 5.2 (Chara et al., 1994), 6.91 (Csöreg et al., 1994), 1.7 (Lumley-Woodyear et al., 1995) and 0.28 μA cm$^{-2}$ mM$^{-1}$ (Vidal et al., 1998).

At high glucose concentrations a platform response is observed, showing a characteristic of the Michaelis–Menten kinetic mechanism. The apparent Michaelis–Menten constant ($K_{\text{app}}$), a reflection of the enzymatic affinity, is calculated to be $6.34 \pm 0.21$ mM according to the Lineweaver–Burk equation (inset b in Fig. 7) (Kamin and Wilson, 1980). This value is lower than 27 mM for native GOD in solution (Rogers and Brandt, 1971), 20 mM for GOD bound to self-assemble monolayer electrode (Murthy and Sharma, 1998), 20 mM for GOD cross-linked to poly(vinylimidazole) (Chara et al., 1994), 33 mM at the GOD-DMFc-CPE (Amine et al., 1993), 10.3 mM at the Pt/PB/GOD-Pan (Garjonyte and Malinauskas, 2000), 25.3 mM at the GOD-polypyrrole (Vidal et al., 1998) and 22 mM for GOD entrapped in copolymer modified silica sol–gel (Wang et al., 1998), indicative of a higher affinity of GOD doped in titania sol–gel to glucose.

3.8. Interferences

The influence of chemical interferences on amperometric determination of glucose was evaluated by successively adding these interferences up to their normal blood concentrations (Cass et al., 1984) to 0.02 M pH 7.3 PBS containing 0.4 mM FcPF$_6$ and 6.0 mM glucose. Similar to Cass’s and Dong’s results (Cass et al., 1984; Wang et al., 1998), addition of 0.5 mM uric acid, and 0.1 mM $p$-acetaminophenol did not cause any observable interference to the sensor response to glucose, and only ascorbic acid at the concentration of 0.1 mM caused a mean increase in current of about 9.0% (shown in Fig. 8A). The titania sol–gel film is of a porous structure. Ascorbic acid can diffuse through the film to the electrode surface and be oxidized there.

In order to eliminate the interference from ascorbic acid, a Nafion/GOD/titania electrode was prepared by dropping Nafion solution on GOD/titania modified electrode to form a Nafion film on the surface. Fig. 8B shows the response of the Nafion/GOD/titania electrode to glucose and these interfering substances at their blood concentrations. All interferences from uric acid, $p$-acetaminophenol, particularly from ascorbic acid are omissible. The catalytic currents remain at the same magnitude as those at the GOD/titania electrode. Thus, the presence of Nafion does not affect the enzymatic activity.

3.9. Flow injection analysis for glucose determination

The practical application of the developed sensor in the on-line assay for glucose is studied by establishing a FIA system with a thin-layer cell. The flow rate used for glucose measurements is an important parameter since the process involves the enzymatic reaction kinetics and the diffusion of both glucose and ferrocenium and their products through the sol–gel film. An optimal flow rate of 1.2 ml min$^{-1}$ was obtained by evaluating the

![Fig. 8. Current response of the GOD/titania electrode (A) and Nafion/GOD/titania electrode (B) in 0.02 M pH 7.3 PBS containing 0.4 mM FcPF$_6$ at 300 mV to (a) 0.5 mM uric acid injection, (b) 0.1 mM $p$-acetaminophenol injection, (c) 0.1 mM ascorbic acid injection and (d) glucose injections with 2.0 mM concentration step.](image-url)
analytical performance of the sensor, peak width and the measurement reproducibility. Typical flow injection response for glucose at GOD/titania modified electrode with an applying potential of 300 mV is shown in Fig. 9. With an injection time of 10 s and a period of 30 s, the sensor gives a steady base-line. The current responses to glucose concentrations in the range of normal serum glucose levels (Fig. 9a) show a good linear relation with a linear regression equation of \( y = -0.29(\pm 0.17) + 1.04(\pm 0.03)x \) and a correlation coefficient of 0.9992. The sensitivity is 8.3 \( \mu A \) cm\(^{-2}\) mM\(^{-1}\), which is slightly larger than that obtained with the current–time technique. The analysis time for each sample is 30 s. The analysis time for a FIA process is much less than that of 20 min at silica sol–gel derived sensor (Tatsu et al., 1992), and also less than 1.6 min at a miniature needle-type sensor (Wang and Zhang, 2001). The total sample volume for each glucose detection is 0.2 ml, which is less than those of 4 ml (Tatsu et al., 1992). The sample volume is also less than 0.5 ml for the sensor based on GOD cross-linked to a redox mediator, Os(4,4'-methoxy-2,2'-bipyridine)\(_2\) poly(1-vinylimidazole) Cl\(_2\) (Heller et al., 1998). In fact a smaller sample volume can be controlled in practical assay procedure.

3.10. Detection and fabrication reproducibility

The reproducibility is ascertained by monitoring the current response for ten replicate injections of 8.0 mM glucose (Fig. 9b). The relative standard deviation (R.S.D.) is 4.1%, indicating a good reproducibility and stability of the sensor for FIA. Thus the enzyme does not leak out of the film in the process of FIA, and the sensor can repeatedly be used for FIA or on-line determination of glucose.

The fabrication reproducibility of six sensors, independently constructed based on the same bare electrode, shows an acceptable reproducibility with a R.S.D. of 2.3% for the steady-state current obtained at a serum glucose concentration. Thus, vapor deposition technique provides a cheap method for reproducible preparation of the sensor in batches.

3.11. Sample determination

The FIA of glucose in serum sample was performed on the sensor utilizing a calibration method. The sample was diluted to its half concentration by mixing it with 0.02 M pH 7.3 PBS containing 0.8 mM FcPF\(_6\). Five parallel determinations were carried out. The glucose level was determined to be 8.64 mM, close to 8.74 mM by spectrophotometry, showing a good accuracy. The recoveries for the assays of 1.0–8.0 mM glucose were between 98 and 101% for ten measurements.

3.12. Stability of the sensor

After an 80-day storage period in PBS at 4 °C, the sensor retained 96% of its initial current response. When the biosensor was stored in air at 4 °C for 80 days, it can retain the same activity as that stored in PBS. If its current response was detected once per 10 days, it retained 91% of its initial current response after the intermittent use over the 80-day period. Thus, titania sol–gel derived glucose sensor is stable enough for the clinical application. This further demonstrates that titania sol–gel film is very efficient for retaining the enzyme activity. Good long-term stability is attributed to two aspects: a mild process for sol–gel formation and enzyme immobilization and a beneficial environment for preventing the enzyme from leaking out of the film. The immobilization does not involve in the chemical modification of the enzyme molecules and provides a biocompatible microenvironment around the enzyme molecules to stabilize its biological activity, which maintains the biological activity of enzyme to a large extent (Chen et al., 1998). Large quantities of hydroxyl groups in titania sol–gel hybrid material can form strong hydrogen bonds with the enzyme molecules, which prevents the enzyme from leaking out of the film (Wang et al., 1999).

4. Conclusions

The titania sol–gel film is a suitable kind of material for enzyme loading. The formed GOD/titania film displays a regular dense distribution of the GOD and is of a uniform porous structure and a very low mass transport barrier. The immobilized GOD retains its native structure. The sensor exhibits a high affinity to glucose, an acceptable accuracy for determination of serum glucose level, and a variety of good performance characteristics such as high sensitivity, fast response, low interference and long-term stability. This sensor can be repeatedly used and produced in batches. The estab-
lished FIA system shows a short analysis time, a small volume of sample solution and a good reproducibility, which provides a simple, rapid and acceptable method for the determination of glucose. To be pointed out, the analytical procedure needs ferrocenium hexafluorophosphate as a mediator for glucose sensing. The mediator cannot be co-immobilized in the titania sol–gel film due to the porous structure of the film. However, mixing the sample solution with the pH 7.3 PBS containing 0.8 mM FcPF$_6$ in the same volume can easily solve this problem. The slight increase in the complexity and the cost of determination process can be acceptable in clinic application due to the low cost of the sensor and the convenient supply of pH 7.3 PBS containing 0.8 mM FcPF$_6$ by packing it in the assay kit.

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