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Short communication

Optical colorimetric sensor strip for direct readout glucose measurement

Xu-dong Wang[a], Hai-xu Chen[a], Ting-yao Zhou[a], Zhi-jie Lin[a], Jing-bin Zeng[a], Zhao-xiong Xie[c], Xi Chen[a,b,*], Kwok-yin Wong[d], Guo-nan Chen[e], Xiao-ru Wang[a]

[a] The Key Laboratory of Analytical Sciences of the Ministry of Education, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China
[b] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China
[c] State Key Laboratory for Physical Chemistry of Solid Surfaces & Department of Chemistry, Xiamen University, Xiamen 361005, China
[d] Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hunghom, Kowloon, Hong Kong, China
[e] The MOE Key Laboratory of Analysis and Detection Technology for Food Safety, and Department of Chemistry, Fuzhou University, Fuzhou, Fujian, 350002, China

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ABSTRACT

A novel direct readout colorimetric optical glucose sensor strip was constructed based on a three-layer film, including a green-emitted CdTe/CdS quantum dots (QDs) layer as a stable color background, a red-fluorescent platinum-porphyrin oxygen-sensing layer and a glucose oxidase layer. The sensor achieved high resolution (up to 0.2 mmol L^{-1}) glucose determination with a detection range from 0 to 3.0 mmol L^{-1}. A "glucose ruler" which acts as a glucose standard colorimetric card was obtained. Glucose concentration could easily be directly readout using the "glucose ruler", which made the glucose determination rapid, convenient and easy. The effects of pH, salinity and temperature were systematically investigated. The prepared sensor was finally applied for glucose sample analysis, compared with the "glucose ruler", accurate results could be directly readout.

1. Introduction

Glucose sensors have wide applications in life science (Carling, 2004; Porterfield, 2007), biology (Rolland et al., 2006, 2001), chemistry, clinical analysis (Heller and Feldman, 2008; Scognamiglio et al., 2004; Reach and Wilson, 1992), agriculture and the food industry (Terry et al., 2005). Since their first appearance (Clark and Lyons, 1962), glucose sensors have been investigated for about 5 decades, and many kinds have been constructed. These include enzyme based electrochemical, enzyme based optical, non-enzyme based electrochemical, and boronic acid derivative based optical glucose sensors (Park et al., 2006; Wang, 2008; Borisov and Wolfbeis, 2008; Moreno-Bondi et al., 1990; Wolfbeis, 2000, 2002, 2004, 2006, 2008; Moschou et al., 2004; Fang et al., 2004; Pickup et al., 2005). However, almost all of them are based on the calibration curve method, and involve time-consuming, complicated data-collection and processing procedures, which further require sophisticated scientific instruments and professional operators. This makes them costly and limits their extensive applications. The colorimetric approach shows promise in solving these problems due to its simplification, convenience, and even more good ability for high throughput analysis and continuous monitoring, which has become a trend in rapid and semi-quantitative determinations. Several groups (Nakayama et al., 2003; Badugu et al., 2006; Nakabayashi et al., 2006) have attempted to detect glucose using this colorimetric method, but it still suffers from low resolution which severely limits its application. Here, we constructed a reversible colorimetric glucose sensor based on glucose oxidase and an indirect oxygen determination method, and we achieved direct readout of glucose concentration with high resolution and an extended detection range.

2. Experimental

2.1. Materials and reagents

Glucose oxidase (EC 1.1.3.4 from *Aspergillus niger*) with a specific activity of 210 units per milligram of lyophilized solid, 3-aminopropyl-trimethoxysilane (APTMS), tetramethoxysilane (TMOS), polyvinyl alcohol (PVA) and Glucose Assay Kit (GAGO20) was purchased from Sigma–Aldrich and used as received. Dimethyldimethoxysilane (DiMe-DMOS) was obtained from Fluka AG (Buchs, Switzerland). [meso-tetrakis (pentafluorophenyl) porphyrinato] platinum (II) ($PtF_{20}TPP$), the oxygen-sensing indicator, was synthesized and purified in the laboratory of the Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University. A buffer solution of pH 7.0 was prepared

* Corresponding author at: The Key Laboratory of Analytical Sciences of the Ministry of Education, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China. Tel.: +86 592 218 4530; fax: +86 592 218 4530.
E-mail address: xichen@xmu.edu.cn (X. Chen).

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using 0.5 mol L^{-1} Na_2HPO_4 and KH_2PO_4. Glucose injections were acquired from local hospital. Beer samples were bought from local supermarket. Human serum samples were obtained from the Fuzhou University Hospital (Fujian, China). Glucose injections and human serum samples were diluted using the above-mentioned phosphate buffer solution. All other chemicals used in this study were analytical reagent grade and all solutions were prepared with deionized distilled water.

2.2. Construction of the glucose sensor

The glucose sensor was prepared layer by layer based on our previous research (Wang et al., 2008a,b; Lin et al., 2006). Detailed descriptions are as follows.

2.2.1. QDs layer

10 mL APTMS methanol solution with a molar ratio of 1:50, 100 μL tetraethoxysilane (TEOS), and 2 mL distilled water were mixed and stirred for 1 h to promote hydrolysis. The mixture was kept in a 30 ± 0.2 °C incubator for 48 h, after which 1 mL thiolglycolic acid-capped CdTe/CdS core/shell QDs solution ($\lambda_{em,max}$ = 552 nm) and 0.3 mL of Cd precursor solution were added. The mixture was stored in the incubator for 4 h, and then 60 μL of the mixture was daubed evenly onto a glass-slide (48 mm × 12.4 mm × 0.9 mm) which had been soaked in concentrated nitric acid for 12 h and washed with distilled water and ethanol to obtain a QDs layer. The prepared QDs layer was dried in darkness in the same incubator (Wang et al., 2008a,b; Li et al., 2004; Li and Murase, 2004).

2.2.2. Oxygen-sensing layer

1.0 mL TMOS, 1.8 mL DiMe-DMOS and 1.5 mL 0.01 mol L^{-1} HCl were mixed, and stirred in a 60 °C water bath for about 1.5 h until an emulsion formed. Then 0.4 mL emulsion and 0.1 mL 2.0 mg mL^{-1} $PtF_{20}TPP$ (Amao et al., 2001; Lai et al., 2004; Lee and Okura, 1997) in tetrahydrofuran (THF) were mixed, and vigorously stirred for 20 min to ensure homogenization. 40 μL of the mixture was dip-coated onto the surface of the QDs layer to form an oxygen-sensing layer (Wang et al., 2008a,b; Lin et al., 2006).

2.2.3. Glucose oxidase layer

Organically modified silicate (ORMOSIL) was prepared by mixing TMOS, DiMe-DMOS and 0.01 mol L^{-1} HCl (1:1.2:1, v/v). The mixture was stirred in a 60 °C water bath for about 4 h. Then 500 μL of ORMOSIL was mixed with 500 μL 5% (w/w) PVA. After leaving for 30 min, 200 μL of the ORMOSIL–PVA mixture and 200 μL of the glucose oxidase buffer solution were blended and stirred for 1 min, and then the mixture was applied evenly as a third layer. The prepared glucose sensor was dried at room temperature for 12 h, and then stored at 4 °C (Lin et al., 2006; Wang et al., 2008b; Wolfbeis et al., 2000).

2.3. Characterization of the prepared sensor

Fluorescence profiles were obtained from a Hitachi F-4500 fluorometer (Hitachi Co. Ltd., Japan). An ultra-bright 395 nm LED was used to excite the QDs and $PtF_{20}TPP$. A 1 cm cuvette holder with a 450 nm filter was used as the main imaging system (Ocean Optics Inc., USA). A compact charge coupled device (CCD) camera (Canon Co. Ltd., Japan) was used for recording color images. Parameters of the CCD camera were set as follows: white balance: cloudy; focus: macro-mode; aperture: F = 4.5; ISO sensitivity: ISO 100; shutter speed: 1/10 s.

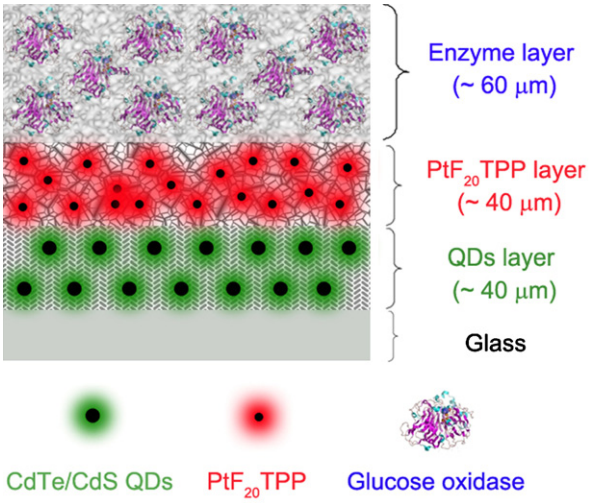

Fig. 1. Schematic diagram of the sensing layers in the colorimetric glucose sensor.

3. Results and discussion

3.1. Colorimetric determination of glucose

Fig. 1 shows a schematic diagram of our proposed colorimetric glucose sensor, which consisted of a colorimetric oxygen-sensor strip (including a $PtF_{20}TPP$ layer and a QDs layer as described in our previous paper (Wang et al., 2008a), and a glucose oxidase layer. The glucose oxidase was immobilized in a sol–gel hybrid PVA matrix and deposited on the oxygen-sensor strip. During glucose determination, glucose oxidase reacts with molecular oxygen, catalyzing the oxidation of glucose, and causing a variation in oxygen concentration. The oxygen concentration was measured using the colorimetric oxygen-sensor strip (Wang et al., 2008a), which directly reflects the concentration of glucose.

Fig. 2 shows the response of the glucose sensor towards various concentrations of glucose buffer solution (pH = 7.0). The sensor displayed distinguishable colors from green to red with a resolution up to 0.20 mmol L^{-1}, which could easily be identified with the naked eyes or a CCD camera. The sensor strip also acted as an optical "glucose ruler" for colorimetric determinations. The detection range shown in Fig. 2 was from 0 to 3.0 mmol L^{-1}.

Based on our previous research about the colorimetric oxygen sensor (Wang et al., 2008a), the CdTe/CdS QDs layer was stable and insensitive towards oxygen and served as a green-colored background. The oxygen-sensing layer (the $PtF_{20}TPP$ layer) emits a red color in the absence of oxygen and the red luminescence can be quenched when increasing the oxygen concentration (Wang et al., 2008a). Therefore, the apparent color of the glucose sensor represented green color in the absence of glucose because of the quenching of red emissions in the presence of large amount of oxygen. When increasing the glucose concentration, the oxygen was consumed in the glucose enzymatic reaction, resulting in the increase of the intensity of red emission (see supporting information Fig. SI-1). The apparent color of the sensor then changed gradually to red (Fig. 2).

Glucose determination using glucose oxidase has its origin in 1962. Designed by Clark and Lyons (1962), it is a classic and efficient indirect sensing method; used throughout the entire range of glucose sensing measurement. The calibration curve method is always used in such glucose measurements (Li and Walt, 1995; Wolfbeis et al., 2000; Yang et al., 2006). Fig. SI-1 shows a typical calibration curve for the fluorescence intensity of the glucose sensor in various concentrations of glucose. It was found that there was a linear relationship between glucose concentrations (from 0 to 0.6 mmol L^{-1})

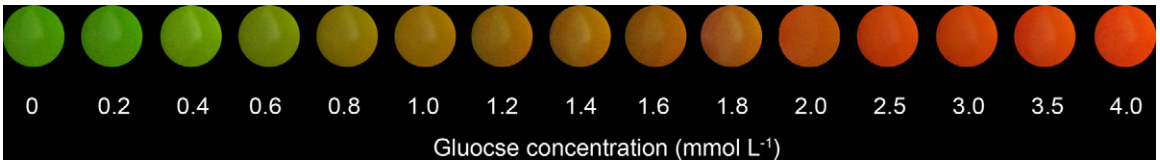

Fig. 2. Apparent colors of the prepared sensor in different concentrations of glucose buffer solution at 35 °C forming a "Glucose ruler" for the colorimetric determination method.

and fluorescence intensity (Fig. SI-1 insert), the detection range was much smaller and limits the extent of its applications. A partitioned calibration curve method has been used to extend the detection range (Wang et al., 2008b), but it was still complicated, and suffered from time and labor consumption. As for the colorimetric method, the glucose sensor presented distinguishable color in glucose concentrations from 0 to 3.0 mmol L^{-1}, which could be used for high glucose concentration measurements. Additionally, our colorimetric glucose sensor revealed glucose concentration by direct readout, making it convenient, easy, and labor saving.

3.2. Sensor stability

Sensor stability was investigated using intensity based determination, results revealed that the glucose sensor presented good stability with a relative standard deviation smaller than 5% in 20 continuous measurements in 0.8 and 2.0 mmol L^{-1} glucose buffer solutions, which assured the accuracy and reproducibility of the data acquired.

3.3. Effect of pH, salinity and temperature

The activity of enzymes is always influenced by the ambient environment, such as pH, salinity and, especially, temperature, and so their effects were systematically investigated in 0.8 mmol L^{-1} glucose solution. Our results showed that the change of salinity did not have any obvious influence on the performance of the glucose sensor (Fig. SI-2 4c 1st row). As for the effect of pH, as shown in Fig. SI-2 4a and 4c 2nd row, only a slight color change was found in the pH range 6.1–7.7. Temperature had a conspicuous influence both on the fluorescence intensity and on the apparent color of the glucose sensor (Fig. SI-2 4b and 4c 3rd row). In the temperature range 5–45 °C, with temperature increase, both the enzyme activity and the diffusion rate of the substrate increased, resulting in the increase of fluorescence intensity and the apparent color change from green to yellow-red, with a resolution up to 5 °C. This result also indicated a potential for fabricating a colorimetric temperature sensor. When the temperature was higher than 50 °C, the fluorescence intensity plunged quickly, and beyond 70 °C, glucose oxidase completely lost its activity. In order to guarantee the enzyme activity and for long-term usage, 35 °C was chosen during the entire investigation. This reflects the fact that temperature influence should be taken into consideration during glucose measurements.

3.4. Chosen of reaction time

The reaction time, counted from when the sensing layer was submersed into a sample solution, directly affected the fluorescence intensity of $PtF_{20}TPP$, and further determined the sensitivity, resolution and detection range of the glucose sensor. Images of different glucose concentrations (from 0 to 5.0 mmol L^{-1} with an interval of 0.2 mmol L^{-1}) at different reaction time (from 1 to 10 min with an interval time of 1 min) were recorded. After comparison, a 5 min reaction time was chosen due to its higher resolution.

Table 1
Sample analysis using colorimetric method.

Sample	Spectrometric method [a] (mmol L^{-1})	Color
5 % Glucose NaCl injection [b]	2.48	
	1.19	
	0.46	
5 % Glucose injection [b]	2.51	
	1.27	
	0.50	
Beer [c]	0.28	
Human serum 1 [d]	1.41	
Human serum 2 [d]	2.02	

[a] Samples were determined using Sigma–Aldrich Glucose Assay Kit (GAGO20).
[b] Glucose injection samples were diluted for different times (100-, 200-, and 500-folds, respectively).
[c] Beer sample was detected without dilution.
[d] Human serum samples were diluted for 5 times.

3.5. Sensor reproducibility

Our previous research proves that the construction process used for preparing uniform oxygen-sensing films is reproducible (Wang et al., 2008a). The glucose sensor presented good reversibility due to the excellent reversible property of $PtF_{20}TPP$ and, after being continuously used for 4 h, 92.8% of the fluorescence intensity in 3.0 mmol L^{-1} glucose buffer solution could be recovered. After being stored in the refrigerator at 4 °C for 2 months, 89.7% of the fluorescence intensity in 3.0 mmol L^{-1} glucose buffer solution could be retrieved, and no obvious color variation in 0.8 mmol L^{-1} glucose buffer solution occurred.

3.6. Sample analysis

Finally, the glucose sensor was applied to the analysis of glucose samples. Two kinds of diluted glucose injection, beer and diluted human serum samples were detected using both spectrometric method (Sigma–Aldrich Glucose Assay Kit, GOGA20) and the colorimetric method. As shown in Table 1, glucose concentration could be directly determined by comparing the color with the "glucose ruler". Compared with the spectrometric method, the results obtained from the colorimetric method were accurate and the analytical performance of the colorimetric glucose sensor was satisfied. It is also found that the colorimetric method possesses extra advantages, such as rapid, convenient, easy, and time and labor-saving.

4. Conclusions

In summary, we fabricated a direct readout colorimetric optical glucose sensor strip. The sensor achieved high resolution (up to 0.2 mmol L^{-1}) glucose determination with a detection range from 0 to 3.0 mmol L^{-1}. Glucose concentration could easily be directly readout using the "glucose ruler", which made the glucose determination rapid, convenient and easy. Based on this, direct readout glucose determination with high resolution has potential applications in the early diagnosis of diabetes, food freshness assessment, continuous monitoring of glucose levels in urine (Moreno-Bondi et al., 1990), and investigations of metabolism. The sensor can easily be miniaturized and constructed into arrays to achieve high throughput glucose analysis. Furthermore, we also provided a method for fabricating colorimetric biosensors which consisted of three layers: a recognition unit (such as oxidases, aerobic dehydrogenases, oxygenases and microorganisms), an optical signal transducer (e.g. oxygen indicators, organic fluorophors and fluorescent nanoparticles), and a colored background (e.g. fluorescent nanoparticles, organic dyes, and multicolor light emitting diodes). This method could easily be applied for constructing high resolution colorimetric biosensors.

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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.2009.05.018.

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