Volume 53 Number 6
June 2010 1225–1458

ISSN 1674-7291 CN 11-5839/O6

中国科学：化学

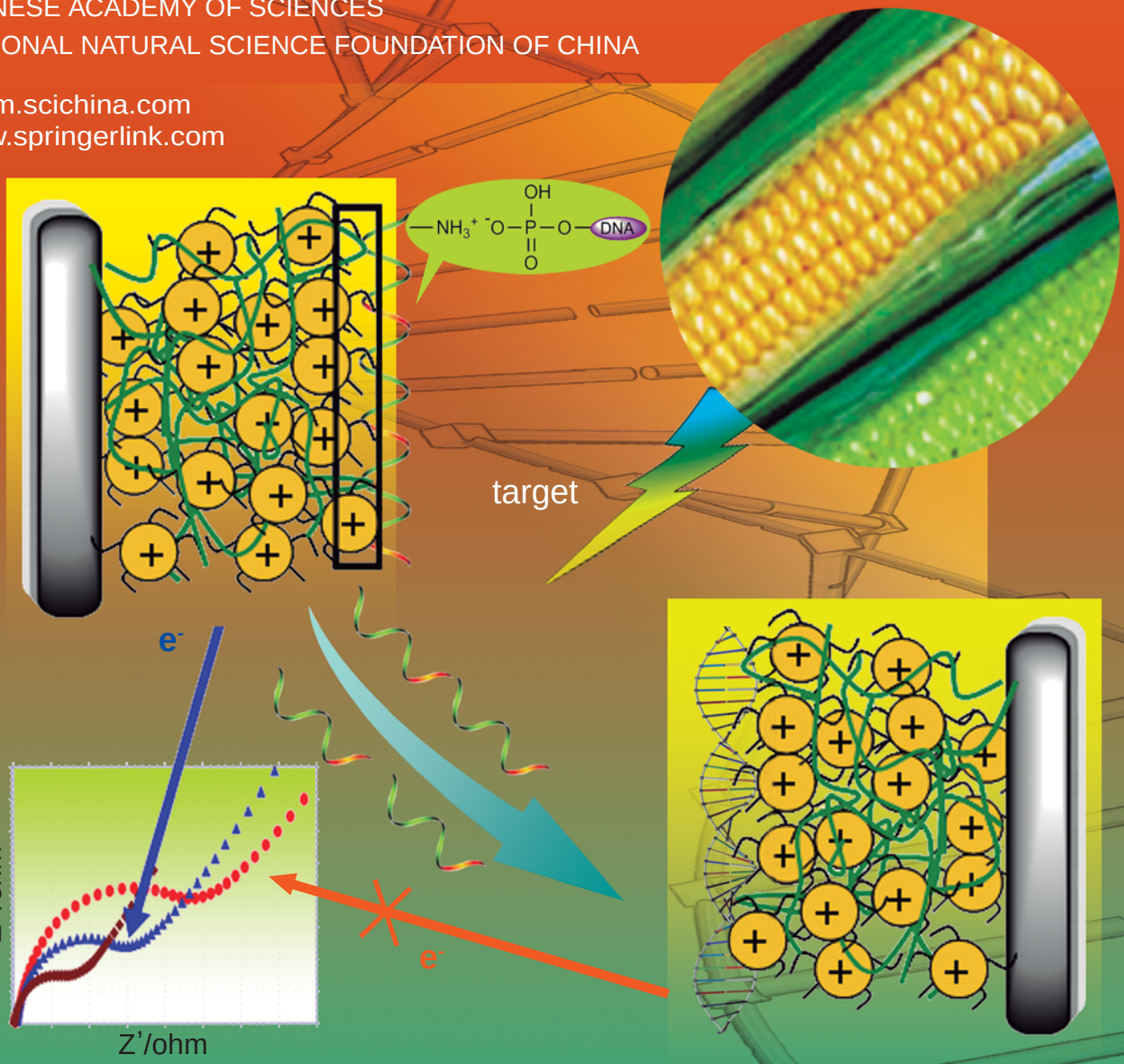
SCIENCE CHINA

Chemistry

(Formerly *Science in China Series B: Chemistry*)

Sponsored by
CHINESE ACADEMY OF SCIENCES
NATIONAL NATURAL SCIENCE FOUNDATION OF CHINA

chem.scichina.com
www.springerlink.com



SCIENCE CHINA PRESS
Springer

中国科学 化学
SCIENCE CHINA Chemistry
Vol. 53 No. 6 Jun. 2010 1225–1458

SCIENCE CHINA
Chemistry

• ARTICLES •

June 2010 Vol.53 No.6: 1385–1390
doi: 10.1007/s11426-010-3161-6

Extended detection range for an optical enzymatic glucose sensor coupling with a novel data-processing method

GUO GuangMei[1], WANG XuDong[2], ZHOU TingYao[2] & CHEN Xi[2*]

[1]*College of Sciences, Hebei University of Science and Technology, Shijiazhuang 050018, China*
[2]*Department of Chemistry, College of Chemistry and Chemical Engineering, Xiamen University; Key Laboratory of Analytical Sciences of Xiamen University, Xiamen 361005, China*

Received November 12, 2009; accepted December 23, 2009

A new data-processing method was established and applied for optical enzymatic glucose sensing, in which oxygen and glucose were simultaneously consumed. The oxygen level remaining in the detection system, which was equal to the difference between the initial and consumed oxygen concentrations, could be measured using fluorescent oxygen indicators immobilized in the sensing layer. It was deduced that the ratio of I_0 and I was inversely proportional to glucose concentration, where I_0 is the maximum fluorescence intensity in various glucose solutions, and I is the fluorescence intensity at various concentrations of glucose. Using the new data-processing method, the detection range of the calibration curve method was extended from 0 to 1.2 mmol L^{-1}, which was enlarged about 2–3 folds over that in ordinary approaches. The prepared glucose sensor could be directly applied to detect high concentrations of glucose.

optical glucose sensor, glucose oxidase, extended detection range

1 Introduction

Enzyme catalyst reactions in glucose determination have been widely applied in the Fasting Plasma Glucose Test and Oral Glucose Tolerance Test detections due to their high efficiency and specificity, but these methods are all with high cost and inconvenience since the enzyme is generally consumed in the determination. Sensing approaches are suitable for solving the problem. Enzyme based glucose sensors have extensive applications in the diagnosis of diabetes [1–3]. Numerous enzymatic glucose sensors have been constructed, including enzyme based electrochemical [4, 5], enzyme based optical [6–8], and boronic acid derivative based optical glucose sensors [9]. In these glucose sensors, the enzyme is immobilized on a membrane (bio-film) placed in close contact with an amperometric oxygen electrode or an optical oxygen sensor, which relies on measuring the enzymatic oxidization rate in the bio-film.

In the fluorescence based glucose sensor, glucose oxidase is applied to oxidize glucose into gluconic acid. In this process, oxygen is simultaneously consumed, and the change of dissolved oxygen (DO) in the glucose sensor causes a quenching fluorescent intensity. Compared with the electrochemical glucose sensor based on the Clark oxygen electrode [10], an optical glucose sensor based on fluorescence intensity quenching has generated much interest since it provides high sensitivity and little or no damage to the host system. The optical glucose sensor is ready to be miniaturized, and has the ability of remote monitoring and measurement, no requirement for reference cells and is inert against sample flow rates and stirring. However, optical glucose sensors always suffer from limited detection range [11–13], and some of them could not even be directly used for samples with higher glucose levels, for example, glucose in urine (normal concentration: 0 to 0.8 mmol L^{-1}) [7],

*Corresponding author (email: xichen@xmu.edu.cn)

© Science China Press and Springer-Verlag Berlin Heidelberg 2010

which is the easiest method for early diabetes diagnosis. Dilution of samples is always used in such circumstances, but data inaccuracy frequently occurs. Several methods have been employed to extend the detection range, including minimizing the sensor size, capsulation of an enzyme into micro beads, different sensing film configuration, and the employment of an engineered enzyme [14–16], but these methods remain complex and decrease the sensitivity of the prepared glucose sensor because they affect the activity of enzymes and prolong the response time. Although a partitioned calibration curve method has been applied to extend the detection range, for example, in our previous study [17], the glucose optical sensor had linear ranges from 0.00 to 0.50 mmol L^{-1} ($Y = 358.25X - 2.93$, $R^2 = 0.9954$) and 0.50 to 3.00 mmol L^{-1} ($Y = 430.12X - 46.43$, $R^2 = 0.9972$), it remains complex and suffers from time and labor consumption. In this study, we tried to extend the detection range of the prepared glucose sensor. From a systematic study of two different glucose sensors based on different oxygen indicators, we found that the ratio of I_0 and I was inversely proportional to glucose concentration. Using this new data-processing method, we obtained a good linear range between I/I_0 and glucose concentration, and the sensor exhibited satisfactory stability in continuous measurements. The proposed approach was applied to the glucose determination in glucose injection samples, and all results revealed that the approach is suitable for optical enzymatic glucose measurement and the determination of high concentrations of samples.

2 Experimental

2.1 Chemicals and instruments

Glucose oxidase (EC 1.1.3.4 from *Aspergillus niger*) with a specific activity of 210 units per milligram of lyophilized solid, tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) bis (perchlorate) complex ($Ru(dpp)_3^{2+}$, shown in Figure 1), tetramethoxysilane (TMOS) and polyvinyl alcohol (PVA) were purchased from Sigma-Aldrich and used as received. Dimethyldimethoxysilane (DiMe-DMOS) was obtained from Fluka AG (Buchs, Switzerland). [*meso*-tetrakis (pentafluorophenyl) porphyrinato] platinum (II) ($PtF_{20}TPP$, shown in Figure 1) 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 using 0.5 mol L^{-1} Na_2HPO_4 and KH_2PO_4. Glucose injection samples were acquired from a local hospital and other samples were bought from a local supermarket. All the other chemicals used in this study were of analytical reagent grade and all solutions were prepared with deionized distilled water.

Fluorescence spectral characteristics of the glucose sensor were obtained using a Hitachi F-4500 fluorometer (Hitachi Co. Ltd., Japan). The temperature was controlled at 35 °C with a precision of ± 0.1 °C using a Julabo F12-ED Refrigerated/Heating Circulator (Julabo Inc., Germany).

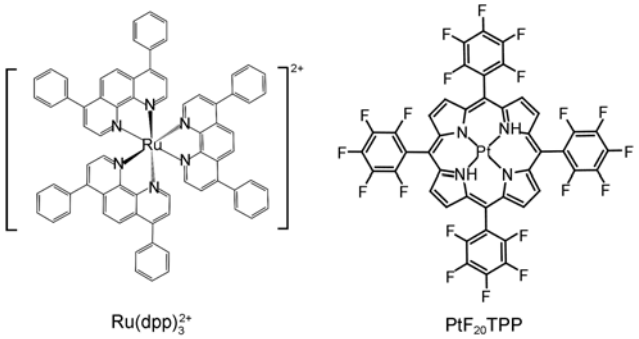


Figure 1 Chemical structures of the selected oxygen indicators.

2.2 Construction of the glucose sensor

The glucose sensor consisted of an oxygen sensing layer and a glucose oxidase layer, and was prepared layer by layer based on our previous description [17–19].

2.2.1 The oxygen sensing layer

Typically, 1.0 mL TMOS, 1.8 mL DiMe-DMOS and 1.5 mL of 0.01 mol L^{-1} hydrochloric acid were added into an open vial. After the mixture was magnetically stirred for approximately 1 min, it was heated and stirred for about 3 h in a 60 °C water bath. An emulsion formed during this step. After 10 min of still placement, 0.8 mL of the gel solution and 0.2 mL of 2.0 mg mL^{-1} $PtF_{20}TPP$ in tetrahydrofuran were blended. The mixture was then vigorously stirred for 20 min to ensure its homogenization. Films were prepared by pipetting 60 μL of the mixture onto a glass slide (48 × 12.4 × 0.9, mm). The resulting oxygen sensing films were left undisturbed under ambient conditions for 0.5 h, then thermally cured for 24 hours at 80 °C, and finally cooled to room temperature ready for use.

2.2.2 The glucose oxidase layer

Typically, 2 mL TMOS, 2.4 mL DiMe-DMOS and 2 mL 0.01 mol L^{-1} HCl were mixed together. The mixture was stirred at 60 °C for about 4 h to yield an organically modified silicate (ORMOSIL). 500 μL ORMOSIL was mixed with 500 μL 5% (*w*/*w*) PVA to form a sol-gel hybrid PVA matrix. After laying aside for 30 min, 200 μL ORMOSIL-PVA mixture and 200 μL buffer solution with 250 units of glucose oxidase at pH 7.0 were blended. After vigorously stirring for 1 min, the mixture was daubed onto the optical oxygen-sensing film produced previously. The glucose sensing films were dried at room temperature for 24 hours and stored in a pH 7.0 phosphate buffer solution at 4 °C before use.

2.3 Measurement methods

The glucose sensor was placed into a quartz cuvette, and a 2.5 mL glucose sample solution was added. Its spectral responses were then recorded using an F-4500 fluorometer. Glucose oxidase reacted with molecular oxygen to catalyze the oxidation of glucose, resulting in the consumption of both glucose and oxygen in the solution. As the oxygen content decreased, the fluorescence intensity of $PtF_{20}TPP$ immobilized in the sensing layer increased correspondingly. After the measurement, the solution was poured out, and the glucose sensor was washed with phosphate buffer solution and placed in an ambient environment for 1 min to recover its baseline signal. Standard glucose solutions with a concentration range 0 to 1.80 mmol L^{-1} were progressively determined to chart a calibration curve.

3 Results and discussion

3.1 Data processing method and calibration curve

Figure 2 shows that the fluorescence intensity gradually increased with the increase in glucose concentration. It is hard to find a linear relationship between glucose concentration and the corresponding fluorescence intensity, even in the low concentration of glucose (from 0 to 0.6 mmol L^{-1}). The obtained data exhibited an upward curve and only existed in a limited linear range (Figures 2 and 3). However, if all the data was divided by the maximum intensity and transformed into I_0/I, a well-fitted linear relationship between I_0/I and glucose concentration was found (Figure 2(b)). Additionally, the linear range was largely extended.

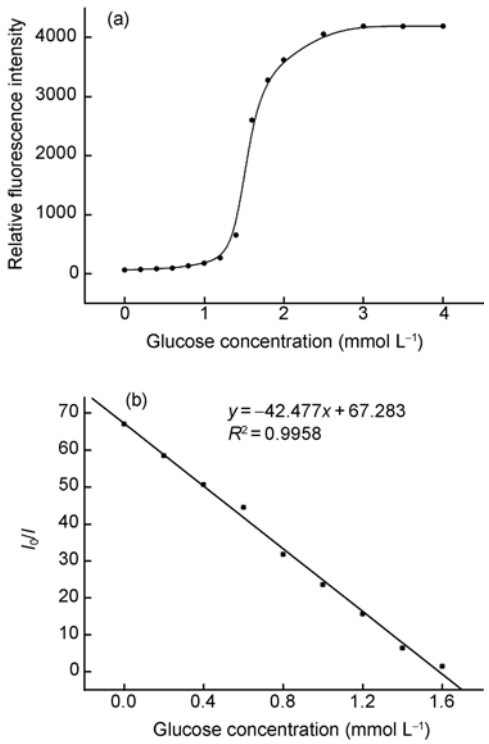


Figure 2 (a) Response of the glucose sensor towards various concentrations of glucose buffer solutions; (b) linear relationship between the intensity ratio I_0/I and glucose concentration.

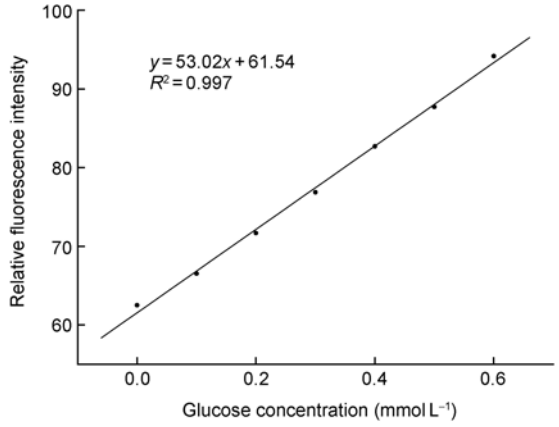


Figure 3 Linear relationship between fluorescence intensity and glucose concentration.

In the glucose oxidase based glucose determination, glucose and oxygen were simultaneously consumed, resulting in the gradual increase of fluorescence intensity of the oxygen indicator. It is usually regarded that there is a linear relationship between glucose concentration and fluorescence intensity. However, our results revealed that the ratio of I_0 and I was inversely proportional to glucose concentration, which is more suitable for optical glucose sensing determination.

As shown in Scheme 1, in this glucose oxidase based enzymatic reaction, glucose concentration is related to the consumed oxygen concentration [6]. The fluorescence intensity of the oxygen indicator reflects the remaining oxygen concentration, and could be calculated using the difference between the initial and consumed oxygen concentrations. The initial oxygen concentration could be considered as a constant at a certain temperature and atmosphere pressure. Substituting it into the Stern-Volmer equation, we deduced that the ratio of I_0 and I was inversely proportional to glucose concentration. It is apparent that oxygen consumption in the enzymatic reaction is small, which induces the change of fluorescence intensity I limited. As shown in Figure 2(a), the fluorescence intensity change in the low concentration of glucose is very small which makes the linear range much smaller. However, the intensity of saturated glucose concentration, I_0, is very large. Deducing I from I_0, the signal change would be magnified and further enlarge the linear range for glucose detection.

To further test and verify this data-processing method for a glucose sensor immobilizing different types of oxygen quenching probes, $Ru(dpp)_3^{2+}$ and PtOEP were employed.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

Scheme 1 Glucose enzymatic reaction.

As shown in Figure 4, for the $Ru(dpp)_3^{2+}$ probe, a linear range between I/I_0 and glucose concentration from 0 to 1.2 mmol L^{-1} was obtained, which was enlarged about 2–3 folds over that in previous reports [11, 17]. For PtOEP (Figure 5), it is apparent that there was a bent curve between I/I_0 and glucose concentrations from 0 to 1.2 mmol L^{-1}. These results confirmed that a good linear relationship between I_0/I and glucose concentration with extended detection range could be readily obtained using our data-processing method, and this approach is suitable for optical enzymatic glucose measurement and enables the direct monitoring of high glucose levels in samples.

3.2 Selection of reaction time

The reaction time directly affected the fluorescence intensity of the oxygen indicator, and further determined the sensitivity and detection range of the glucose sensor. When the sensing layer was submersed into a glucose solution, fluorescence responses of different concentrations of glucose (from 0 to 1.5 mmol L^{-1} with an interval concentration of 0.20 mmol L^{-1}) at different reaction times (from 1 to 10 min with an interval of 1 min) were recorded. After comparison, the 5 min reaction time was selected because the sensor exhibited the optimal detection range at this time.

3.3 Response towards oxygen

Oxygen plays a crucial role in the oxidase enzyme based glucose sensing measurements. Before exploring the performance of the glucose sensor, it was necessary to investigate the response of the glucose sensor towards different concentrations of oxygen. This could ensure that the glucose sensor worked in the linear oxygen range during all the following glucose determinations. Figure 6 shows that the prepared glucose sensor exhibited a good linear response towards oxygen concentrations from 0 to 20%, which is much larger than the amount of dissolved oxygen in aqueous media.

3.4 Sensor stability and reproducibility

Our results showed that the construction process used for preparing uniform oxygen sensing films was reproducible. The prepared glucose sensor exhibited very good stability with a relative standard deviation (RSD) of 4.7% in 20 continuous measurements in 0.8 mmol L^{-1} glucose buffer solution. The glucose sensor exhibited good reversibility due to the excellent reversible property of the oxygen indicator $PtF_{20}TPP$. After it was continuously used for 4 hours in different concentrations of glucose buffer solutions, 96.7% of the fluorescence intensity in 1.0 mmol L^{-1} glucose buffer

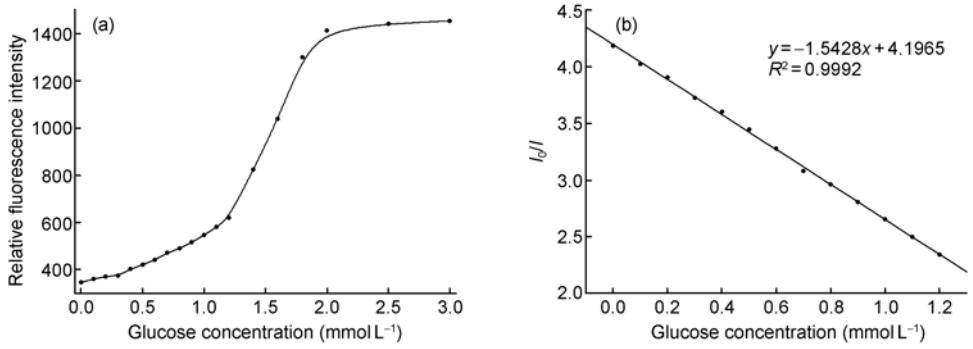


Figure 4 (a) Response of the $Ru(dpp)_3^{2+}$ based glucose sensor towards various concentrations of glucose buffer solutions; (b) linear relationship between the intensity ratio I_0/I and glucose concentration.

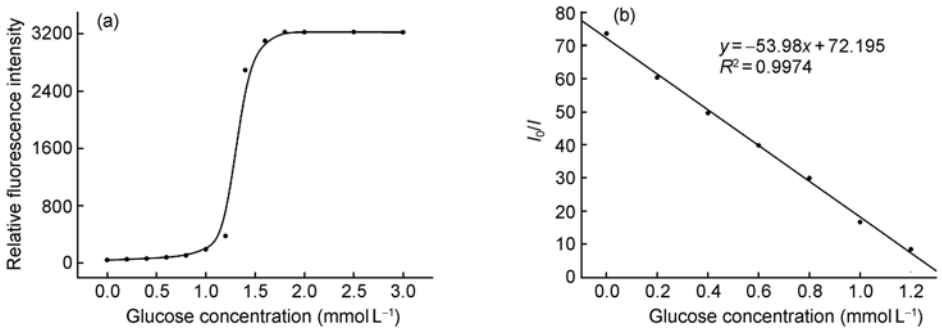


Figure 5 (a) Response of the PtOEP based glucose sensor towards various concentrations of glucose buffer solutions; (b) linear relationship between the intensity ratio I_0/I and glucose concentration.

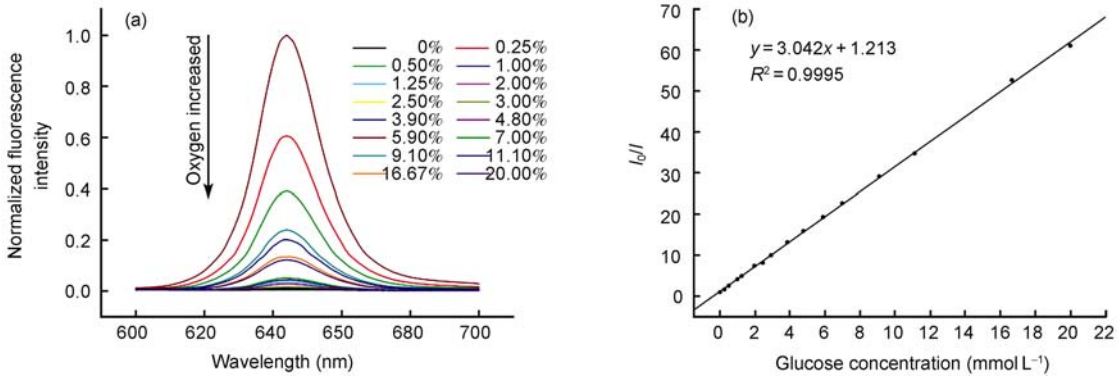


Figure 6 (a) Response of the $PtF_{20}TPP$ based glucose sensor towards various concentrations of oxygen; (b) the linear relationship between I_0/I and oxygen concentration.

solution could be recovered. After it was stored in the refrigerator at 4 °C for 2 months, 91.3% of the fluorescence intensity in 1.0 mmol L^{-1} glucose buffer solution could be retrieved.

3.5 Sample analysis

The prepared glucose sensor was subsequently used for glucose sample analysis. Glucose injection samples were selected and detected directly. As summarized in Table 1, in the determination of low concentrations of glucose samples such as in alcohols and beers, the glucose sensor exhibited good recovery from 86.46% to 102.94%, and for the measurement of the glucose level in glucose injections, the sensor showed good accuracy with RSD smaller than 5%. This demonstrated that the sensor could be used for the analysis of glucose samples and has potential applications in directly monitoring samples with high glucose levels.

4 Conclusions

The ratio of I_0 and I was found to be inversely proportional to glucose concentration by systematically studying two types of glucose sensors based on different oxygen indicators. A new data-processing method for optical enzymatic glucose sensing determination was developed. This method was further applied to sample analysis, which was demonstrated to be convenient and precise. Using the new data- processing method, the detection range of the calibration curve method could be readily extended and the prepared glucose sensors have potential applications in direct glucose determination and monitoring high concentrations of glucose in samples.

Table 1 Sample analysis using the modified calibration curve method

Sample	Labeled (mmol L^{-1})	Detected (mmol L^{-1})	Added (mmol L^{-1})	Found (mmol L^{-1})	Recovery (%)
alcohol 1	–	N.D.	0.60	0.508	87.46
			0.90	0.778	86.46
alcohol 2	–	N.D.	0.60	0.604	100.67
			0.90	0.926	102.94
beer	–	N.D.	0.60	0.593	98.89
			0.90	0.875	97.19
5% G.I.	0.51	0.52 ± 0.02	–	–	–
	1.06	1.08 ± 0.08	–	–	–
5% NaCl G.I.	0.51	0.50 ± 0.01	–	–	–
	0.96	0.92 ± 0.08	–	–	–

*G.I. = glucose injection, N.D. = not detected.

The authors express their great thanks for the support from the Science and Technology Project of Hebei Province (07246751D) and the National Science Fund for Fostering Talents in Basic Research of the National Natural Science Foundation of China.

1 Heller A, Feldman B. Electrochemical glucose sensors and their applications in diabetes management. *Chem Rev*, 2008, 108: 2482–2505
2 Scognamiglio V, Staiano M, Rossi M, D'Auria S. Protein-based biosensors for diabetic patients. *J Fluoresc*, 2004, 14: 491–498
3 Reach G, Wilson GS. Can continuous glucose monitoring be used for the treatment of diabetes? *Anal Chem*, 1992, 64: 381A–386A
4 Park S, Boo H, Chung TD. Electrochemical non-enzymatic glucose sensors. *Anal Chim Acta*, 2006, 556: 46–57
5 Wang J. Electrochemical glucose biosensors. *Chem Rev*, 2008, 108: 814–825
6 Borisov SM, Wolfbeis OS. Optical biosensors. *Chem Rev*, 2008, 108: 423–461
7 Moreno-Bondi MC, Wolfbeis OS, Leiner MJP, Schaffar BPH. Oxygen optrode for use in a fiber-optic glucose biosensor. *Anal Chem*, 1990, 62: 2377–2380
8 Wolfbeis OS. Fiber-optic chemical sensors and biosensors. *Anal Chem*, 2008, 80: 4269–4283
9 Fang H, Kaur G, Wang B. Progress in boronic acid-based fluorescent glucose sensors. *J Fluoresc*, 2004, 14: 481–489
10 Clark LC, Lyons C. Electrode systems for continuous monitoring in cardiovascular surgery. *Ann N Y Acad Sci*, 1962, 102: 29–45
11 Yang X, Zhou Z, Xiao D, Choi MMF. A fluorescent glucose biosensor based on immobilized glucose oxidase on bamboo inner shell membrane. *Biosens Bioelectron*, 2006, 21: 1613–1620
12 Scully PJ, Betancor L, Bolyo J, Dzyadevych S, Guisan JM, Fernández-Lafuente R, Jaffrezic-Renault N, Kuncová G, Matějec V, O'Kennedy B, Podrazky O, Rose K, Sasek L, Young JS. Optical fibre biosensors using enzymatic transducers to monitor glucose. *Meas Sci Technol*, 2007, 18: 3177–3186
13 Manesh KM, Santhosh P, Gopalan A, Lee K. Electrospun poly(vinylidene Xuoride)/poly(aminophenylboronic acid) composite nano Wbrous membrane as a novel glucose sensor. *Anal Biochem*, 2007, 360: 189–195
14 Yamazaki T, Kojima K, Sode K. Extended-range glucose sensor employing engineered glucose dehydrogenases. *Anal Chem*, 2000, 72: 4689–4693
15 Wolfbeis OS, Oehme I, Papkovskaya N, Klimant I. Sol-gel based glucose biosensors employing optical oxygen transducers, and a method for compensating for variable oxygen background. *Biosens Bioelectron*, 2000, 15: 69–76
16 Wolfbeis OS, Schäferling M, Dürkop A. Reversible optical sensor membrane for hydrogen peroxide using an immobilized fluorescent probe, and its application to a glucose biosensor. *Microchim Acta*, 2003, 143: 221–227
17 Wang X, Zhou T, Chen X, Wong K, Wang X. An optical biosensor for the rapid determination of glucose in human serum. *Sens Actuators B*, 2008, 129: 866–873
18 Lin L, Xiao L, Huang S, Zhao L, Cui J, Wang X, Chen X. Novel BOD optical fiber biosensor based on co-immobilized microorganisms in ormosils matrix. *Biosens Bioelectron*, 2006, 21: 1703–1709
19 Wang X, Chen X, Xie Z, Wang X. Reversible optical sensor strip for oxygen. *Angew Chem Int Ed*, 2008, 47: 7450–7453