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Quantum dots and p-phenylenediamine based method for the sensitive determination of glucose

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ABSTRACT

By introducing p-phenylenediamine (PPD) to the hybrid system of Mn-doped CdS/ZnS quantum dots (QDs) and glucose oxidase (GOD), a sensitive label-free method was proposed for direct detection of glucose. With glucose and PPD as substrates, 2,5-diamino-N,N′-di-(4-aminophenyl)-2,5-cyclohexadiene-1,4-diimine (DDACD) that intensively quenches the fluorescence of QDs can be produced by the catalysis of GOD. A detection limit as low as 3.2 μM was obtained with the high-efficient fluorescence quencher. Two linear ranges, from 5.0 μM to 1000 μM and from 1.0 mM to 10.0 mM, were identified between time-gated fluorescence intensity and the concentration of glucose. It is shown that the newly proposed methods have high selectivity for glucose over other saccharides and coexisting biological species in serum. The method can be used directly to determine glucose in normal adult human serum without any complicated sample pretreatments. The recovery rate and repeatability of the method were also shown to be satisfactory.

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1. Introduction

As a main source of energy, glucose plays a critical role in normal human metabolism [1,2]. Glucose concentration in human blood is used as a clinical indicator of diabetes. Deviation of concentration of glucose can be harmful to human health [3,4]. Hence, rapid and accurate detection of glucose in human blood is of great importance in diagnosing metabolites.

In the literature, various glucose sensors were designed based on quantum dots (QDs) and glucose oxidase (GOD) catalyzed reactions [5–8]. Semiconductor QDs, also called semiconductor nanocrystals, are semiconducting particles with a diameter of a few nanometers. They are preferable due to their size dependent optical and physical properties relative to organic dyes [9]. Interest in fluorescent QDs also derives from their broad absorption, narrow emission, large Stokes shift, excellent light stability, and good biological compatibility [10]. As the fluorescence intensity of QDs can be influenced by H_2O_2 or gluconic acid produced in a GOD catalyzed oxidization process of glucose, glucose can be determined by measuring fluorescence intensity [11–14]. However, both H_2O_2 and acidity change are not intensive quencher, resulting in the unsatisfactory sensitivity of these methods. For sensitive detection of glucose, several strategies were designed in recent studies. An alternative approach for glucose sensing was developed by conjugating GOD onto fluorescent QDs. Proper immobilization of enzyme can stabilize the enzyme against interaction with inhibitors or inactivation reagents, and even improve enzyme activity [15–17]. Moreover, the immobilization can bring GOD closer to QDs, improving the interaction between QDs and the produced H_2O_2 or gluconic acid. These methods provided new possibilities to improve the sensibility for fluorescence sensing of glucose [11–13]. Fe_3O_4 magnetic nanoparticles were also used to improve the sensitivity of QDs–GOD based methods for glucose. With Fe_3O_4 nanoparticles as peroxidase mimetic catalyst, H_2O_2 was decomposed into radical that can quench the fluorescence of QDs more efficiently [18,19].

Electrochemical sensors using phenylenediamine have also been proposed for the sensitive detection of glucose [20–22]. In the hands of the electrochemical sensors, phenylenediamine is used as a monomer that can chemically or electrochemically polymerize into conductive or non-conductive polymers, depending on the conditions of the polymerization. These obtained sensors exhibited sufficient sensitivity for the practical determination. However, to the best of our knowledge, fluorescent sensor using phenylenediamine for glucose monitoring has not been reported in the literature.

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In this study, p-phenylenediamine (PPD) was introduced to the GOD–QDs system and a sensitive detection method for glucose was proposed. Triggered by the produced H_2O_2 during the oxidation process of glucose, PPD can be oxidized to 2,5-diamino-N,N′-di-(4-aminophenyl)-2,5-cyclohexadiene-1,4-diimine (DDACD). DDACD can intensively quench the fluorescence of QDs and improve the sensitivity of the method. Mn-doped QDs with long lifetime fluorescence were used as an indicator to eliminate the autofluorescence of biological samples. The proposed method provided improved sensitivity and satisfactory selectivity for glucose sensing. In addition, this method has a simple experimental procedure and favored biological applications in real human serum samples without any complicated sample pretreatments.

2. Experimental section

2.1. Instruments and reagents

The GOD used in this study was purchased from J&K Chemical Technology Co. Ltd. (Shanghai, China). It was dissolved in PBS buffer (10 mM, pH 7.0) and stored at 4 °C. 3-mercaptopropionic acid (MPA, 99+%) was purchased from Sigma-Aldrich. The ultrapure water (18.3 MΩ cm) used in the experiments was produced by a Millpore water purification system. All the chemicals were of analytical grade and were used as received without further purification. The real human serum was obtained from the Affiliated Hospital of Hunan University. Time-gated fluorescence intensities were measured using a Perkin-Elmer LS-55 spectrofluorimeter (United Kingdom) in the phosphorescence mode.

2.2. Synthesis of water-soluble Mn-doped CdS/ZnS core/shell QDs

Mn-doped CdS/ZnS core/shell QDs (Mn:CdS/ZnS QDs) were synthesized according to the method reported by Norris and Yang [23,24]. The poor water solubility of the prepared QDs limited their application in aqueous solutions. In order to make the QDs applicable in aqueous medium, the water-soluble QDs were prepared using MPA [25]. MPA (1.0 mL, 99%) was dissolved in 15 mL of 2.4 mM KOH aqueous solution. Subsequently, 1.0 mL of the prepared MPA solution was added via a syringe to a suspension of QDs (1.0–2.0 mg) in 1 mL chloroform. The mixture was stirred overnight at room temperature (25–28 °C). Then, 2.0 mL of methanol was added to the solution and mixed uniformly. The QDs were precipitated with acetone, and centrifuged at 4000 rpm for 5.0 min. The supernatant was decanted to remove the organic solvent. The as prepared Mn:CdS/ZnS core/shell QDs were suspended in ultrapure water and stored at 4 °C.

2.3. H_2O_2 sensitivity measurement of the PPD–QDs system

Sensitive detection of H_2O_2 is critical to accurate determination of glucose levels. In order to measure the H_2O_2 sensitivity of the PPD–QDs system, a series of concentrations of H_2O_2 (0–600 μM) were tested. Samples containing different concentrations of H_2O_2 were prepared by adding various amounts of H_2O_2 to PBS buffer (0.1 M, pH 7.0) to reach a final volume of 600 μL. A PPD–QDs mixture was added to the samples and mixed uniformly. The final concentration of PPD was 1.0 μmol L^{-1}. Fluorescence intensities were measured and recorded by the spectrofluorimeter.

2.4. Assay procedures of the proposed method

The assay procedure of the proposed method was as follows: GOD (11.6 U) was added to the samples and mixed uniformly. The mixture was incubated at 35 °C for 10 min. Then, 100 μL of 6.0 μM PPD and 20 μL of QDs suspension were added to the mixture. The mixture was incubated for 3 min at room temperature (25–28 °C). Finally, the time-gated fluorescence spectra of the mixture were monitored and recorded. The excitation wavelength and emission wavelength were set as 400 nm and 609 nm, respectively. The excitation and emission slits were both 15 nm. The delay time was 0.1 ms. For all reactions and tests, the experiments were repeated three times to ensure the accuracy of the measurements.

2.5. Glucose sensitivity and selectivity measurement of the proposed method

In the sensitivity experiment, the relationship between fluorescence intensity and the concentrations (0 μM, 5.0 μM, 50 μM, 100 μM, 200 μM, 400 μM, 600 μM, 800 μM, 1.0 mM, 3.0 mM, 5.0 mM, 7.0 mM, and 10.0 mM) of glucose was investigated. Different types of carbohydrates and other coexisting species in biological fluids, such as amino acid and relevant ions, were studied in the selectivity experiment. 1.0 mM of glucose was added to each sample and the other foreign species were added subsequently. Then the samples were treated and tested following the assay procedures. A mixed sample containing 1.0 mM of glucose and 10.0 mM of all the saccharides mentioned above was also be tested. The foreign species investigated in this section were maltose, D-fructose, galactose, sucrose, mannose, xylose, K^+, Na^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, bilirubin, glycine, arginine, L-phenylalanine, lysine, tyrosine, L-cysteine, and cholesterol. The concentrations of K^+, Na^+, Mg^{2+}, Ca^{2+}, and Zn^{2+} were adopted as the maximum tolerant in human serum. The concentrations of other interferential species were 10.0 mM.

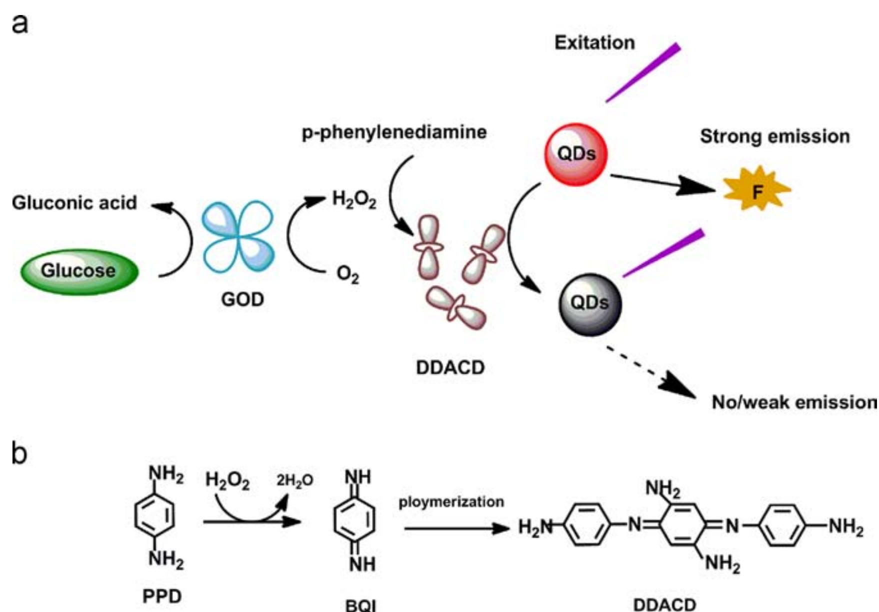
2.6. Application experiments in serum samples

In the application experiment, a standard addition recovery method was used. No other sample pretreatments were employed except for dilution. Glucose of 1.0 mM was added to a series of diluted human serum samples (10, 100, and 1000 times diluted). The concentrations of glucose in human serum and spiked samples were derived from the assay procedures illustrated in Section 2.4 and the regression equation. The recovery rate was calculated using the formula of $R=(C_1-C_2)/C_3$, where C_1 was the concentration of glucose in spiked samples, C_2 was the concentration of glucose in serum, and C_3 was the concentration of the added glucose. The relative standard deviation (RSD) was obtained from three duplicate measuring of the same serum samples.

3. Results and discussion

3.1. Experimental principle of the proposed method

For the sensitive detection of glucose, PPD was introduced into the GOD–QDs system. The assay procedures and principle of the proposed method are shown in Scheme 1. To verify that PPD can increase the sensitivity for the determination of H_2O_2, a comparison between the quenching efficiency of H_2O_2 on QDs and that on the PPD–QDs hybrid system was given. The fluorescence quenching efficiency was calculated using the equation of $I=(F_0-F)/F_0$, where F and F_0 were the fluorescence intensities with and without H_2O_2, respectively. The results are shown in Fig. 1. It can be seen that the quenching efficiency increased with increasing H_2O_2 concentration. However, the quenching to the fluorescence of QDs without PPD was very weak under low concentrations of H_2O_2. Only about 4.6% fluorescence was quenched with 200 μM H_2O_2. When PPD is used, the quenching efficiency became much higher. When the solution contained 1.0 μM PPD, 200 μM H_2O_2 was enough to quench the fluorescence completely.



Scheme 1. (a) Schematic diagram of the glucose sensor based on p-phenylenediamine and long lifetime quantum dots. In the presence of glucose oxidase, glucose is oxidated, and H_2O_2 is derived from deoxidation of O_2. The p-phenylenediamine was immediately oxidized to 2,5-diamino-*N*,*N*′-di-(4-aminophenyl)-2,5-cyclohexadiene-1,4-diimine by the produced H_2O_2. Based on the quenching effect of 2,5-diamino-*N*,*N*′-di-(4-aminophenyl)-2,5-cyclohexadiene-1,4-diimine on the QDs, the glucose is detected by monitoring the change of the QDs fluorescence. (b) Reaction of p-phenylenediamine with H_2O_2.

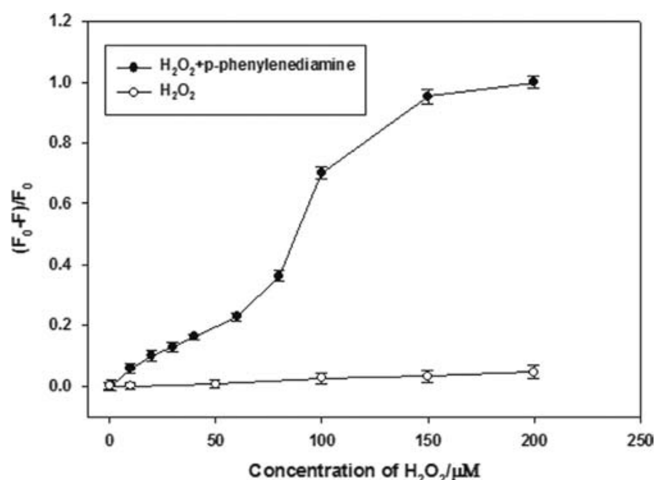


Fig. 1. Quenching effect of H_2O_2 on QDs and QDs–p-phenylenediamine. The concentration of p-phenylenediamine was 1.0 μM.

It was reported that H_2O_2 could result in irreversible decrease of QDs fluorescence intensity by oxidizing the surface or destroying the lattice structure of QDs [26,27]. The results of this experiment show that the destruction by H_2O_2 may be very weak when the concentration of H_2O_2 is in micromolar range. Due to the presence of PPD, the increased quenching efficiency may be caused by the new product formed in the oxidization process of PPD. According to the test results reported by Jiao, PPD can be oxidized by H_2O_2 and finally produced DDACD [28]. The conjugated $\pi-\pi$ system and conjugated π–p system in DDACD can absorb energy from the excimer of QDs, leading to intensive fluorescence quenching. Although PPD can also slightly quench the fluorescence of QDs, the quenching efficiency of PPD is negligible compared with that of DDACD (Fig. S1).

3.2. Optimization strategy for an assay system

In the optimization experiments, four experimental conditions were optimized. First, in order to quench the fluorescence of QDs intensively, the effect of PPD concentration on the quenching efficiency was studied. As shown in Fig. 2a, the fluorescence quenching efficiency enhanced dramatically with the increase of PPD concentration when it was at low levels (<1.0 μM). The quenching efficiency reached its maximum value when the concentration of PPD was 1.0 μM, and then decreased slowly at higher concentrations. This was mainly because higher concentrations of PPD can slightly quench the fluorescence of QDs. Based on the experiment results, 1.0 μM was selected as the optimum concentration of PPD. As the pH value can strongly affect the activity of GOD, its influence on the fluorescence of QDs is investigated. A pH range from 4.0 to 10.0 was studied here. As shown in Fig. 2b, the quenching efficiency reached the maximum at the pH value of 7.0. Hence, phosphate buffer (0.1 M) with pH value of 7.0 was used for all experimental steps. The incubation time was also studied and the results are shown in Fig. 2c. The quenching efficiency increased with the incubation time and then tended to stabilize after 8.0 min. On the basis of this test result, 8.0 min was chosen as the optimum incubation time. To find out the optimum oxidization time of PPD, the fluorescence intensity was measured every minute after PPD and QDs were added into the enzyme reaction system. The results show that the quenching efficiency increased rapidly in the first 3.0 min, and then tended to increase slowly (Fig. 2d). To save time and ensure the distinguishability of the detection, 3.0 min was selected as the oxidization time. Furthermore, because the most suitable temperature for GOD is 35 °C, 35 °C is selected as the incubation temperature in the following experiments.

3.3. Sensitivity of the proposed fluorescence method

Based on the optimized assay conditions and the standard procedures described in the experiment section, different concentrations of glucose were introduced to the assay system to evaluate the sensitivity of the proposed method. The experiment results are shown in Fig. 3. With increase of the glucose concentration, the time-gated fluorescence intensity decreased gradually. Two calibration curves were found in ranges from 5.0 to 1000 μM and from 1.0 mM to 10.0 mM, as shown in Fig. 4. The regression equations

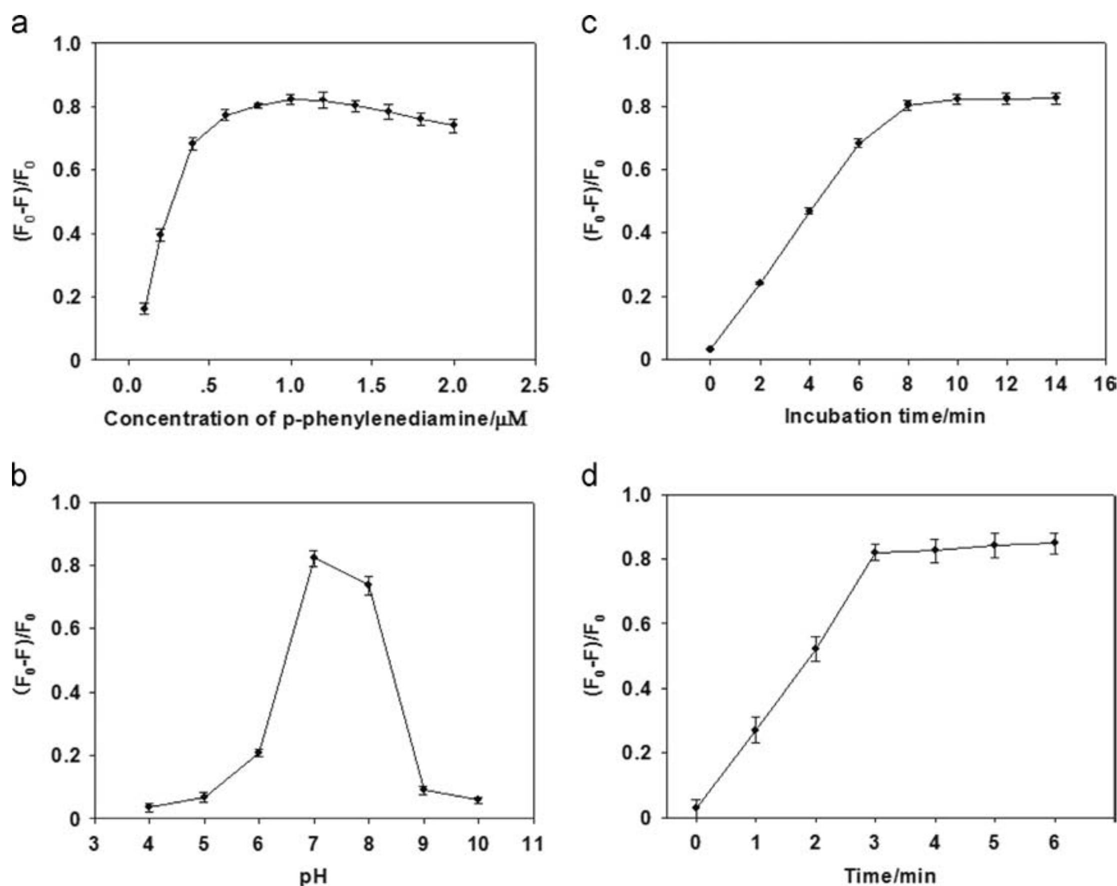


Fig. 2. Optimization experiments of (a) concentration of p-phenylenediamine, (b) pH, (c) incubation time, (d) oxidation time of p-phenylenediamine. Herein, the concentration of glucose was 10.0 mM in the optimization experiments. Each data point is the mean of three measurements. The error bars are the standard deviation.

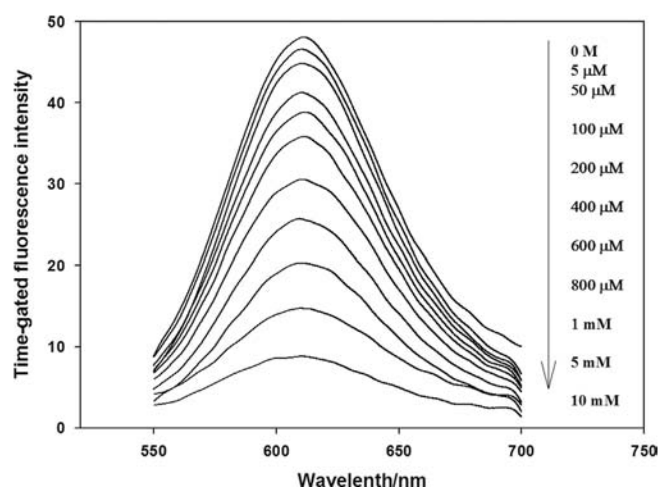


Fig. 3. Time-gated fluorescence emission spectra with different concentrations (0–10.0 mM) of glucose in phosphate buffer (pH 7.0).

were

$$y = -0.024x + 47.27 \quad (1)$$

and

$$y = -0.0016x + 24.47 \quad (2)$$

where x was the concentration of glucose, and y was the time-gated fluorescence intensity. The correlation coefficient was 0.9977 and 0.9887 for Eqs. (1) and (2) respectively. Using the standard deviation of 12 parallel blank signals and Eq. (1), a detection limit was estimated to be 3.2 μM. The detection limit is lower than that of the previously reported label-free sensors [6,8,14,29].

3.4. Effects of coexisting biological species on the proposed method

In order to determine the selectivity of the protocol for glucose detection, the effects of various coexisting species were examined. The results are shown in Table 1. It can be seen that the foreign carbohydrates showed negligible influence on the detection results even at 10 times higher concentrations than that of glucose. The good selectivity for glucose over other carbohydrates is mainly due to the good specificity of GOD. Some metal ions with the maximum tolerable concentrations in serum, such as K^+, Na^+, Mg^{2+}, Ca^{2+}, and Zn^{2+}, also did not obviously disturb the glucose determination. The influence of some typical amino acids was also investigated. The experiment results showed that no significant interference on the detection results was recorded at millimolar levels except L-cysteine. Interference from L-cysteine usually blocks application of the oxidant dependent analytical methods. In the proposed method, the interference can be eliminated by diluting samples to lower concentrations. According to the literature, the normal level of L-cysteine is 50–300 μM in human plasma and the average level of glucose in human serum is 4.4–6.6 mM [30,31]. Because the applicable concentration of this protocol is in the micromolar range and this method can be used to determine glucose in diluted serum samples, such interferences may be ignored. The test result for the mixed sample illustrated that the fluorescence intensity of the mixture was close to that of the sample only containing glucose.

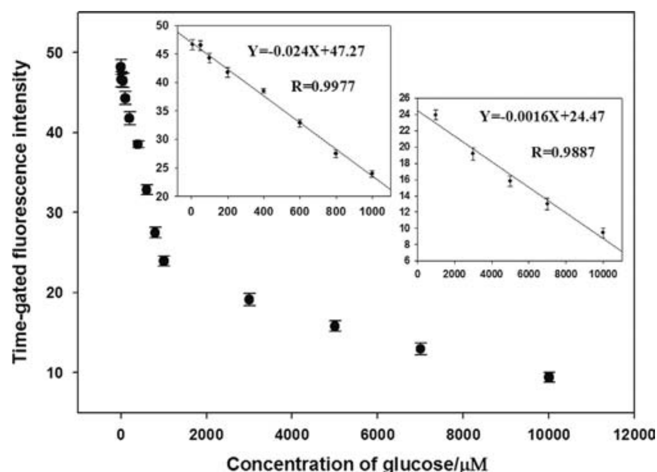


Fig. 4. Relationship between fluorescence intensity and the concentration of glucose. The inset shows a linear region. The concentrations of glucose were 0 μM, 5.0 μM, 50 μM, 100 μM, 200 μM, 400 μM, 600 μM, 800 μM, 1.0 mM, 3.0 mM, 5.0 mM, 7.0 mM, and 10.0 mM. Each data point is the mean of three measurements. The error bars are the standard deviation.

Table 1
Influence of various coexisting biological species.

Saccharides/ions	Concentration (mM)	Relative error (%)
Sucrose	10.0	2.3
Maltose	10.0	−0.9
Galactose	10.0	0.8
D-fructose	10.0	1.8
Xylose	10.0	1.2
Mannose	10.0	2.3
K^+	10.0	−1.9
Na^+	150.0	5.3
Ca^{2+}	2.0	3.1
Mg^{2+}	1.0	2.2
Zn^{2+}	0.02	0.6
Mixture	–	8.4

Table 2
Recovery experiments of glucose in serum samples.

Dilution multiple	Found[a] (mM)	Skiped[b] (mM)	Recovered[c] (mM)	Recovery (%)
10	0.55 ± 0.05	1.0	1.51 ± 0.43	97.42
100	$5.38 \pm 0.45 \times 10^{-2}$	1.0	1.12 ± 0.33	106.28
1000	$5.52 \pm 1.73 \times 10^{-3}$	1.0	1.03 ± 0.28	102.35

[a] Concentrations of glucose found in serum samples using this proposed method.
[b] Concentrations of added glucose.
[c] Concentrations of glucose in spiked samples obtained using this proposed method.

3.5. Detection of glucose in human serum samples

In order to verify the applicability of the proposed method, glucose concentration in three different multiple diluted human serum samples was tested using this method. A standard addition method was used in this experiment. If a certain amount of glucose is added into the serum samples and it can be found out using this method, this method is considered to be applicable in the serum samples. The results are shown in Table 2. The satisfactory recovery rate revealed that the sensitivity and selectivity of this method were not influenced by complicated components in human serum samples after 10 times dilution. The results demonstrated the good applicability of this method. The good applicability may be due to two factors. One is that the high sensitivity of this method makes the glucose detectable in diluted samples, eliminating the influence of high levels of interfering substances. The other one is that the long lifetime fluorescence of Mn:CdS/ZnS QDs reduces the background noises caused by auto-fluorescence from biological samples.

4. Conclusion

A sensitive method for glucose detection was developed by introducing PPD to the QDs–GOD hybrid system. Using this newly proposed method, glucose can be successfully analyzed in a wide range of concentration. The detection limit was shown to be 3.2 μM. The experiment results demonstrated that the proposed method has good selectivity for glucose to the other saccharides. In addition, it can be used for the detection of glucose in real human serum samples. The proposed assay method may be a promising tool for clinical diagnose of glucose and other fields. This system may also be extrapolated to other oxidase used biosensors.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.04.050.

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