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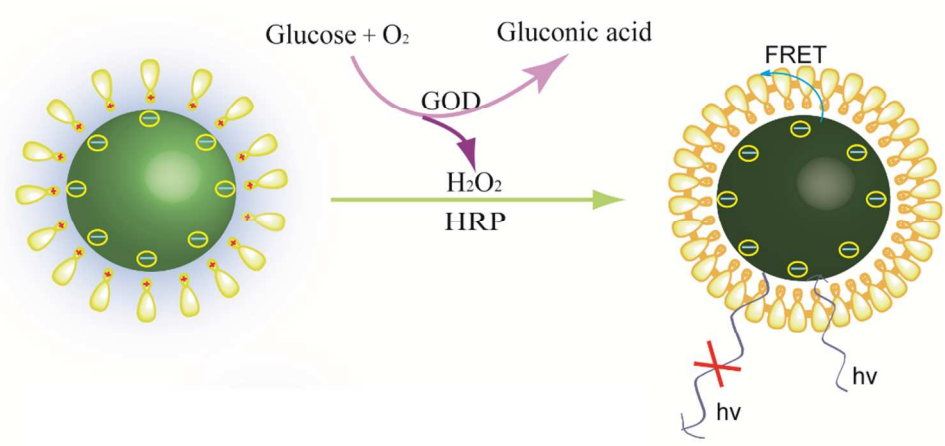
A simple and highly sensitive carbon quantum dots based sensing platform for glucose and hydrogen peroxide.



In Situ Polymerization of Aniline on Carbon Quantum Dots: A New Platform for Ultrasensitive Detection of Glucose and Hydrogen Peroxide

Yanmei Zhang, Xinjian Yang, and Zhiqiang Gao*

Department of Chemistry, National University of Singapore, Singapore 117543.

Abstract

A simple and ultrasensitive platform for the detection of glucose and hydrogen peroxide based on fluorescence resonance energy transfer between carbon quantum dots (CQDs) and polyaniline is described in this report. Briefly, during enzymatic oxidization of glucose, hydrogen peroxide is generated. The generated hydrogen peroxide, in turn, initiates oxidative polymerization of aniline on the surface of the CQDs in the presence of horseradish peroxidase. And the formation of a thin polyaniline layer efficiently quenches the fluorescence of the CQDs. It was observed that the quenching of the florescence is directly associated with the concentration of glucose and ultrasensitive detection of glucose down to submicromolar levels was achieved. In addition, since the formation of polyaniline on the CQD surface is directly associated with hydrogen peroxide, it was found that traces of hydrogen peroxide can be detected in a range of 0.5–50 μM with good selectivity.

*Corresponding author. Tel.: 65163887, Fax.: 67791691. E-mail address: chmgaoz@nus.edu.sg (Z.Q. Gao).

1. Introduction

The accidental discovery of carbon quantum dots (CQDs) during the purification of carbon nanotubes in 2004 has triggered extensive research to exploit the physicochemical properties of CQDs and created a new class of viable nanomaterials.[1-5] CQDs are typically quasi-spherical nanoparticles comprising of amorphous to nanocrystalline cores with predominantly graphitic or turbostratic carbon (sp^2 carbon) or graphene and graphene oxide sheets fused by diamond-like sp^3 hybridised carbon insertions.[2-5]Among the electronic and physicochemical characteristics of CQDs, their optical properties and their fluorescence emissions in particular have attracted increasing interest in recent years. For many years, semiconductor quantum dots have extensively been investigated for their strong and tunable fluorescence emission properties, which enable a wide variety of technical applications.[6] However, semiconductor quantum dots possess certain limitations such as high toxicity due to the use of heavy metals in their production.[7-9] The applications of semiconductor quantum dots are therefore restricted by virtue of toxicity of heavy metal elements.[10-12] On the other hand, in addition to their comparable optical properties and fluorescence emissions in particular, CQDs have the desired advantages of simple synthetic routes, low toxicity, good chemical and photochemical stability, environmental friendliness, and low cost. Furthermore, surface passivation and functionalization of CQDs allow for the control of their physicochemical properties.[10,11] Therefore, CQDs have recently emerged as potential competitors to conventional semiconductor quantum dots and various technical applications. An interesting application of CQDs is in the field of chemical and biological sensing such as the detection of metal ions, small biological molecules, proteins, and nucleic acids.[2] For example, one of the first attempts of utilizing CQDs in chemical sensing is the selective detection of Hg^{2+} in aqueous solution[12,13] and live cells.[14] It was demonstrated that the fluorescence emissions of both CQD solution and CQDs immobilized in sol-gel are sensitive to the presence of Hg^{2+}.[12] It was also

observed that the fluorescence intensity of the CQDs is efficiently quenched by micromolars of $Hg^{2+}$and the quenching provoked by Hg^{2+} is probably due to static quenching arising from the formation of a stable non-fluorescent complex between CQD and Hg^{2+}. A substantial improvement in the sensitivity down to nanomolars was later realized by replacing the CQDs with nitrogen doped CQDs.[13] It was suggested that the presence of nitrogen element in the nitrogen doped CQDs, most probably –CN groups on the nitrogen doped CQDs surface, is responsible for the much improved performance of Hg^{2+} sensing.[13] Along with the development of sensitive metal ion assays, CQDs have also found applications in the development of bioassays for dopamine,[18-20] and ascorbic acid.[18] For instance, Qu and co-workers synthesized highly fluorescent CQDs using dopamine as carbon source and applied them to label-free detection of dopamine. In contrast to the metal ion assays, dopamine effectively recovered the fluorescence of the already quenched Fe^{3+}-CQD complex. It was shown that the enhancement of the fluorescence is proportional to the dopamine concentration in the range of 0.1–10 μM. Apart from metal ion detection, CQDs have also been used to determine hydrogen peroxide and glucose.[22-25] For instance, Sadhukhan et al. used formic acid as the precursor for microwave assisted synthetizing CQDs, which were then employed for fluorescent or electrochemical determination of hydrogen peroxide[23]. Jiang and co-workers employed 3-aminophenylboronic (APBA) functionalized CQDs for glucose detection[24]. Acting as an acceptor, the APBA functional group could bind with glucose to form a less fluorescent complex. Although promising, it is still one challenging to develop more simple sensors for glucose concentrations monitoring.

Herein, we proposed a simple, rapid, highly sensitive and selective platform for ultrasensitive detection of glucose and hydrogen peroxide by employing highly fluorescent CQDs. By selectively and efficiently depositing a thin polyaniline layer on the CQDs, ultralow levels of glucose and

hydrogen peroxide can be unambiguously detected through the significant change in the fluorescence intensity of the CQDs.

2. Experimental Section

2.1. Materials and reagents

Aniline, glucose oxidase (GOx), horseradish peroxidase (HRP), ethylenediamine, glucose, hydrogen peroxide, citric acid, and galactose were obtained from Sigma-Aldrich. Agarose, fructose and sucrose were from Alfa Aesar. Synthetic blood samples were purchased from Roche Diagnostics. All other reagents were of analytical grade and used without further purification. Ultrapure water with an electric resistance of 18.3 MΩ/cm was used for all solution preparations.

2.2. Apparatus and CQD characterization

All UV-vis absorption experiments were conducted on an Agilent Cary 60 UV–Vis spectrophotometer using a 10-mm path length quartz cuvette. Cary Eclipse Fluorescence Spectrophotometer with a 10-mm path length quartz cuvette was used to study the fluorescence properties of the CQDs, the kinetics of the polyaniline deposition process, and the detection of glucose and hydrogen peroxide. The FT-IR spectra of the CQDs and polyaniline synthesized at different pHs were recorded on Alpha FT-IR spectrophotometer. Transition electron microscopic (TEM) images of the CQDs and polyaniline were carried on a JEOL JEM 2010F field emission transmission electron microscope. Atomic force microscopic (AFM) experiments of the CQDs were conducted on an XE-100 atomic force microscope.

2.3. Preparation of the CQDs

The highly fluorescent CQDs with a quantum yield of ~80% were prepared using a hydrothermal

method published elsewhere.[26] In a typical experiment, citric acid (0.426 g) and ethylenediamine (536 μL) were dissolved in 10 ml water. The solution was then transferred to a poly (tetrafluoroethylene)-lined autoclave (30-mL) and heated at 200 ℃ for 5 h. After the reaction, the autoclave was naturally cooled down to room temperature. A red-brown and transparent CQD solution was obtained, which was used in all experiments without further purification.

2.4. Optimization of pH

0.34 μL of the CQD solution, 5.0 μL of 8.0 mg/ml GOx and 4.0 μL of 1.0 mg/ml HRP were added into 500 μL of 10 mM phosphate buffer solution with different pHs. The fluorescence spectra of these solutions were recorded. Subsequently, the fluorescence intensities of the above solutions in the presence of different amounts of glucose were recorded, respectively.

2.5. Detection of glucose

The detection of glucose was achieved as follows: 0.34 μL of the CQD solution, 5.0 μL of 8.0 mg/ml GOx, 4.0 μL of 1.0 mg/ml HRP and different amounts of glucose were added to 500 μL of pH 3.0 or pH 7.0 10 mM phosphate buffer solutions, respectively. The florescence intensities of the solutions were recorded by the fluorescence spectrophotometer after 18 min, respectively.

2.6. Detection of hydrogen peroxide

To investigate fluorescence quenching efficiency by hydrogen peroxide, the fluorescence intensity of CQDs in the presence of hydrogen peroxide was examined at pH 7.0. In a typical experiment, 0.34 μL of the CQD solution, 4.0 μL of 1.0 mg/mL HRP and different amount of hydrogen peroxide were added into 500 μL of pH7.0 10 mM phosphate buffer solution containing 25 mM aniline. All the reactions were monitored by the fluorescence spectrophotometer after 18 min.

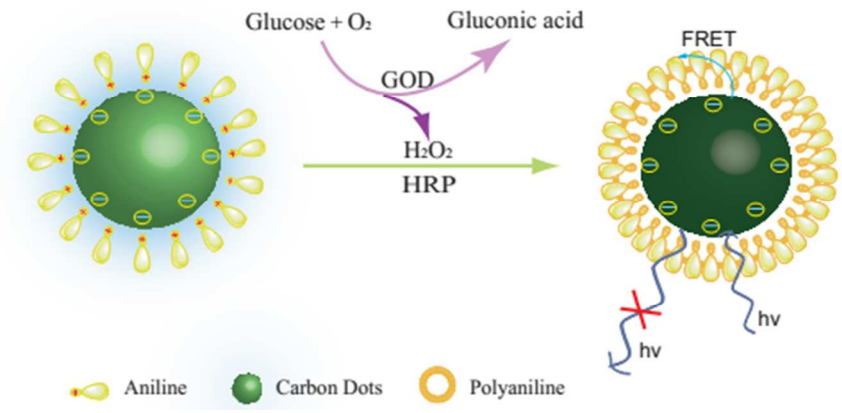


Figure 1. Glucose sensing principle based on the in situ polymerization of aniline on the CQDs.

3. Results and Discussion

3.1. Sensing principle

The glucose sensing principle is illustrated in Figure 1. Aniline is able to selectively absorb onto the CQDs with rich carboxylic groups on their surface by electrostatic interaction.[26] More importantly, it has been demonstrated that peroxidases, such as HRP, effectively catalyze the polymerization of aniline in the presence of hydrogen peroxide under very mild conditions.[27-28] As a result, aniline is concentrated on the surface of the CQDs and polymerization of aniline exclusively occurs on the surface of the CQDs in the presence of catalytic amounts of hydrogen peroxide and HRP. The high fluorescence of the CQDs is quenched due to fluorescence resonance energy transfer (FRET), in which the generated polyaniline on the CQD surface acts as an acceptor. Similarly, hydrogen peroxide can be produced during the enzymatic oxidation of glucose in the presence of GOx. Consequently, based on FRET between the highly fluorescent CQDs and polyaniline an ultrasensitive glucose assay can be developed by engaging GOx in the system. To further confirm the generation of polyaniline in our system, we have conducted the FT-IR spectra of polyaniline synthesized at different pHs (Figure S1). At pH 3, the peaks located at 1570 cm^{-1} and 1460 cm^{-1} implied the quinonoid ring and benzenoid ring in the product.[29,30] The same

observations were obtained at pH 7 but with a little red shift. Furthermore, at pH 3, a band located at 1160 cm^{-1} indicating the conductivity of polyaniline. According to the report by MacDiarmid et al.,[31] the band located at 1160 cm^{-1} was an 'electronic-like band', a characteristic peak of polyaniline conductivity. While at pH 7, the band disappeared, suggesting that the product had no conductivity, which was consistent with the characteristic of polyaniline.[32]

3.2. Feasibility study

The CQDs were synthesized following a published procedure with little modification.[26] The morphology and structure of CQDs were conducted by TEM and AFM (Figure S2 and S3). As shown in the AFM image, the size of the CQDs was ~4 nm. The surface groups of the CQDs were investigated by FT-IR (Figure S4). Our results showed the existence of hydroxyl and carbonyl groups on the CQDs surface. Furthermore, agarose gel electrophoresis was used to illustrate the electrical property of the CQDs. As shown in Figure S5, the CQDs migrated toward the positive electrode in an electrical field, thus demonstrating that the CQDs were negatively charged. Next, the fluorescent spectra of the CQDs under different excitation wavelength were investigated (Figure S6). It was found that the strongest fluorescence intensity of the CQDs was observed at 446 nm when excited at 340 nm. Moreover, UV-vis spectra (Figure S7) of the CQD aqueous solutions further confirmed that the optimal excitation wavelength was ~340 nm.

Because the polymerization of aniline is hydrogen peroxide concentration dependent, the fluorescence intensity reduction is directly correlated to the concentration of hydrogen peroxide. Therefore, the system discussed above could be used for hydrogen peroxide detection. Figure 2 shows a typical correlation between hydrogen peroxide concentration and the fluorescence intensity change at pH 7.0 where as low as 0.20 μM hydrogen peroxide can be detected with a

linear range from 0.50 to 50 μM (R^2= 0.998).

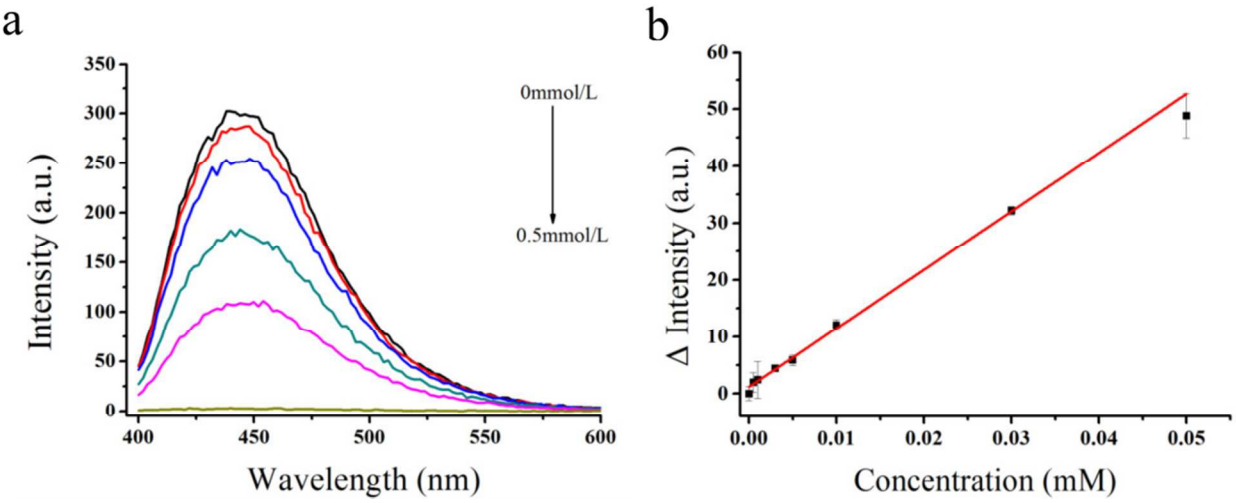


Figure 2.(a) Fluorescence response of the CQDs after the addition of hydrogen peroxide and (b) the calibration curve for hydrogen peroxide.

To rule out any possible quenching effect of hydrogen peroxide on the fluorescence emission of the CQDs as florescence quenching is the sole analytical signal in the detection of glucose, we conducted a series of experiments in solutions containing the CQDs and different amounts of hydrogen peroxide. Our experiments confirmed that hydrogen peroxide shows no obvious effect on the fluorescence emission of the CQDs (Figure S8). Therefore, the change in fluorescence intensity is attributed to the formation of a thin polyaniline layer on the CQDs surface and the FRET between CQDs and polyaniline. To demonstrate the existing of FRET procedure in our system, UV-vis spectra of polyaniline produced at different pH were tested. As shown in Figure 3, a gradual increase of absorption was observed with the increase of pH at 445 nm which significantly overlaps with the fluorescence emission spectrum of the CQDs centered at 440 nm. This result suggests that with the increase of pH, polyaniline absorbed more energy from CQDs through FRET which in turn results in a higher quenching efficiency.

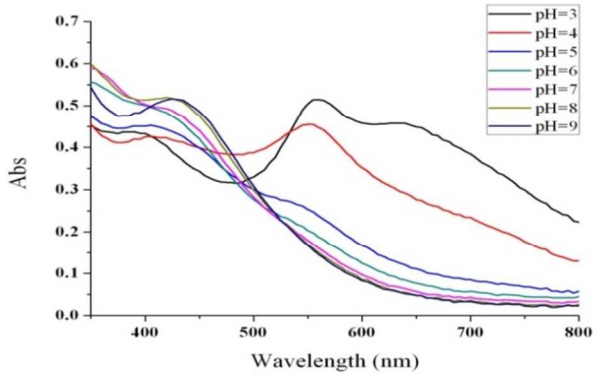


Figure 3.UV-vis spectra of polyaniline at different pH.

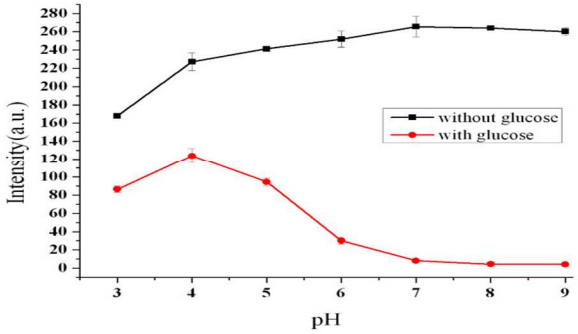


Figure 4. The effect of glucose on the fluorescence of the CQDs at different pH.

3.3. Optimization

In order to achieve best performance for glucose detection, we first thoroughly investigated the effect of pH in the absence and the presence of a fix amount of glucose. Figure 4 illustrates the effect of pH on the fluorescence intensity of CQDs. According to Figure 4, the fluorescence intensity changes ($\Delta I = I_{without\ glucose} - I_{glucose}$) at different pHs were compared. It can be seen that ΔI levels off at 7.0. This finding suggested that pH 7.0 can be selected as the optimal pH for the detection of glucose. On the other hand, there was another option at pH 3.0 because polyaniline at pH 3.0 gave an obvious color change in the presence of hydrogen peroxide, which provided us with an additional way to detect glucose – colorimetric detection of glucose, an appealing way for detecting glucose by the naked eye (Figure 5). As shown in Figure 5, an obvious color change

from almost colorless to brown-grey appeared at pH 3.0 with the increase of glucose concentration, whereas the color change was much less pronounced at pH 7.0. These phenomena are likely due to the formation of different forms of polyaniline at different pHs. According to previous research, the oxidative polymerization of aniline results in a number of intrinsic redox states at different pHs, which display different colors.[33,34]

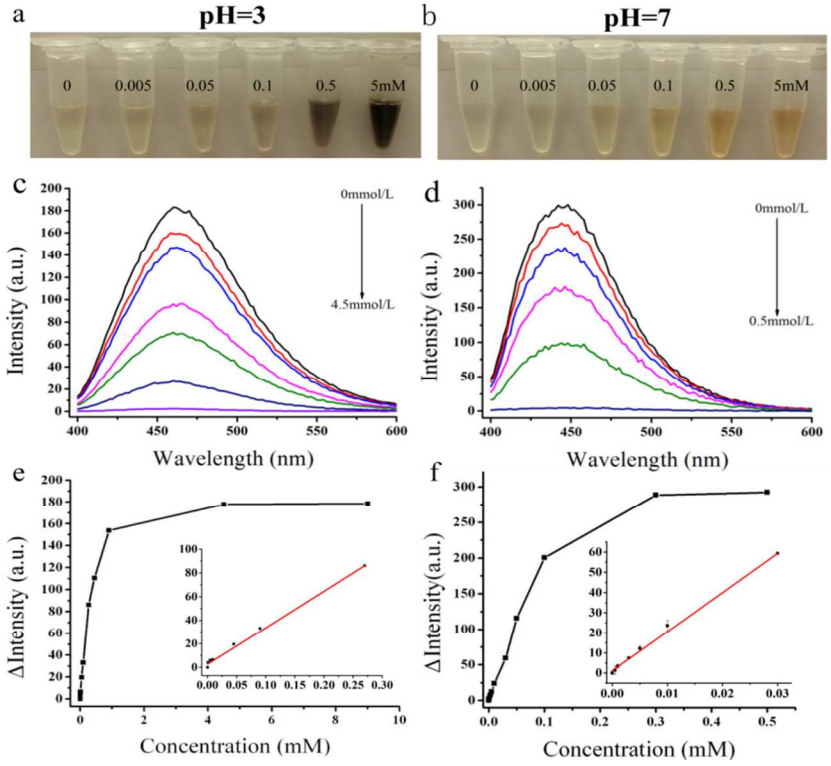


Figure 5. Image of CQDs solutions with different concentrations of glucose at (a) pH 3.0 and (b) pH 7.0; the fluorescence spectra representing the quenching effect of glucose–GOx system with different concentrations of glucose on the fluorescence of CQDs at (c) pH 3 and (d) pH 7; dose-response curves for glucose detection using the CQDs at (e) pH 3 and (f) pH7. Insert: The linear range of the calibration curves for glucose.

3.4. Glucose sensing performance

As we know, glucose can be oxidized by O_2 in the presence of GOx by the following reaction:

$$\text{glucose} + O_2 + H_2O \longrightarrow \text{gluconic acid} + \text{hydrogen peroxide} \quad (1)$$

As seen in equation (1), a stoichiometric amount of hydrogen peroxide is produced during the

GOx-catalyzed oxidation of glucose; a simple one-step method for the detection of glucose can be conveniently constructed by using the CQDs. Typical fluorescent responses for glucose analysis at pH 3.0 and pH 7.0 are shown in Figure 5. As seen in Figure 5, the detection limit was found to be 0.90 μM and the linear range is from 0.90 to 270 μM (R^2= 0.999) at pH 3.0. While better performance was obtained at pH 7.0 – the linear range is from 0.5 to 30 μM with a limit of detection of 0.20 μM (R^2 = 0.999).

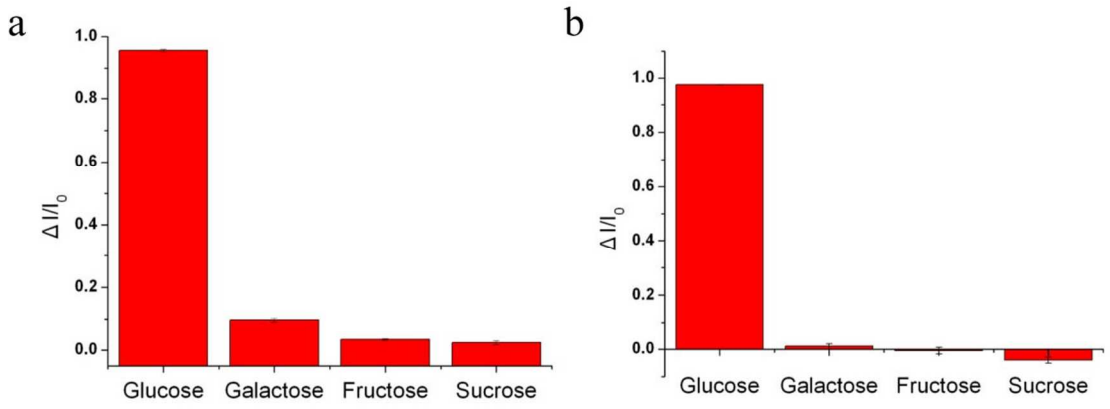


Figure 6. Selectivity of the glucose assay at (a) pH 3.0 and (b) pH 7.0.
From left to right: 0.50 mM glucose, galactose, fructose, and sucrose, respectively.

High selectivity in the detection of glucose is essential in glucose assay. Some interfering substances such as fructose, galactose, sucrose, and other biological sugars that co-exist with glucose in blood and many other biological samples may interfere with the detection of glucose. To investigate whether our CQD-based platform is highly selective to glucose, the influence of other sugars commonly found in biological samples on the fluorescence intensity of CQDs was tested under optimal conditions for the detection of glucose (e.g. excitation and emission wavelength, etc). Figure 6 shows the selectivity of the CQD-based platform in the detection of glucose. Because of the high specificity of GOx in the enzymatic oxidation of glucose, the addition of glucose to a blank solution caused an obvious decrease in fluorescence intensity, while other sugars, such as galactose, fructose, and sucrose had no noticeable effect on the fluorescence

emission under the same experimental conditions. Moreover, the coexistence of these sugars did not adversely affect the sensing performance for glucose in terms of both detection sensitivity and selectivity. Therefore, the proposed platform offers an attractive alternative for the detection of ultralow levels of glucose in biological samples.

3.5. Sample analysis

To verify its applicability, the newly developed platform was applied to determine the concentration of glucose in real samples. Due to biosafety and strict regulations, commercial control solutions resembling to human blood (synthetic blood) for glucose meters that contain basic components of blood were used in this report instead. Test results were listed in Table 1. It can been seen that glucose contents measured by the proposed platform were in good agreement with the reference values of the control solutions, thus suggesting the potential of the proposed platform in the development of glucose assay for practical applications.

Table 1. Determination of glucose in synthetic blood samples.

Sample	Measured（mM）	Reference（mM）	Relative Derivation（%）
1	3.12±0.30	2.9	7.6
2	5.22±0.21	5.1	2.4
3	9.63±0.26	9.4	2.5

4. Conclusions

We have developed a, simple and ultrasensitive sensing platform for glucose and hydrogen peroxide based on FRET between the CQDs and polyaniline. The highly efficient FRET between

the CQDs and polyaniline offered a powerful and yet flexible means to significantly improve the sensitivity of the platform. The simplicity and high sensitivity greatly improved the suitability in the detection of traces of glucose and hydrogen peroxide with minimal or no sample pretreatments. The engagement of GOx and/or HRP ensured a complete removal of possible interference from other sugars, thus producing an ultralow detection limit and an extended dynamic range. The simplicity, high sensitivity, and the freedom from sample preparation are some of the interesting features for the development of a simple, robust, low cost, and highly sensitive and selective glucose and hydrogen peroxide detection platform.

Acknowledgements

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