Surface Modification of Cellulose Paper for Quantum Dot-based Sensing Applications

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Cellulose paper specimens with and without surface modification were compared in order to study their physicochemical compatibility with quantum dots (QDs) for biochemical sensing applications. Silane and chitosan modification methods were applied. The distribution of QDs deposited on untreated paper and papers modified with silane and chitosan were investigated in order to understand the interaction between QDs and fibre. Modified papers were shown to significantly reduce the undesirable redistribution of QDs during paper drying. The retention ability and thermal resistance of QDs to the loss of fluorescence on modified papers were also studied for the purpose of determining the most suitable paper surface modification for developing QD-Paper-based analytical devices (QD-PADs). Furthermore, chitosan-modified paper was used to design QD-PADs to quantify glucose concentration in aqueous samples; the quenching effect of the enzymatic product on the fluorescent emission of QDs was used as the indicator system. The change of fluorescence of QDs was measured by a simple in-house constructed fluorescence imaging system. The detection limit of glucose was 5 mg/dL, which is comparable with other reported paper sensors for detection of glucose.

Keywords: Cellulose Paper; Surface Modification; Quantum Dots; Paper-based Analytical Devices; Glucose Sensing

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INTRODUCTION

In recent years cellulose paper has attracted the interest of those working in the field of developing paper-based analytical devices (PADs) for diagnostics applications (Balu *et al.* 2009; He *et al.* 2013). Made of cheap and widely available cellulose fibre (Hubbe *et al.* 2008), paper is a suitable material for manufacturing analytical sensors on a large scale to meet the demand of diagnostics from human populations living in developing regions. The rapid advancement of PADs research can be attributed to the unique physicochemical properties of paper including: compatibility with biological samples (Tan *et al.* 2012); easy surface modification to immobilize proteins, DNA, and other molecules (Xiao and Huang 2011; Araujo *et al.* 2012); and controllable wettability for transporting fluids (Tian *et al.* 2010). Historically, concepts of using paper for chemical assay tests have been explored from as early as 1937 (Yagoda 1937). Since 2007, paper-based diagnostics have been re-discovered and pursued by many research groups globally; a trend initiated by the Whitesides Group (Martinez *et al.* 2007).

During the development of PADs, several techniques have been reported for qualitative or semi-quantitative sensing applications, such as colorimetric (Xiao *et al.* 2013), electrochemical (Dungchai *et al.* 2009), chemiluminescent (Liu *et al.* 2013), and fluorescent methods (Ma *et al.* 2012). At the same time, a variety of sensor probes, whose properties change when they interact with analytes, have been adapted to these methods and used to indicate the functions of paper sensors (Li *et al.* 2012; Liu *et al.* 2013). The performances of probes applied in PADs are extremely important for obtaining accurate assay results. Quantum dots (QDs) have been considered to have great potential as fluorescence probes for biosensing (Hai *et al.* 2013; Chen *et al.* 2013). In particular, the comparable dimensions of QDs and biomolecules, such as proteins and DNA, enable the preparation of biocompatible QD-bioconjugates with special recognition functions (Li *et al.* 2011). Their excellent properties such as photo-stability and broad absorption spectra, together with high surface activity, make QDs advantageous probes in the development of biochemical sensors (Li *et al.* 2011).

Recently, a few studies have been published about the combination of QD probes with cellulose paper for sensing applications (Niu *et al.* 2011; Yuan *et al.* 2012; Noor *et al.* 2013). Yuan *et al.* reported the use of polymer QD-enzyme hybrids on PADs to detect the presence of a substrate with corresponding enzymes (Yuan *et al.* 2012). Noor *et al.* proposed a paper-based solid phase nucleic acid hybridization assay using immobilized QDs as donors in fluorescence resonance energy transfer (Noor *et al.* 2013). The QDs and QD conjugates were immobilized onto the surface of paper through imidazole ligands modification chemistry. These works successfully demonstrated applications of PADs using QDs as sensing probes. In this study, we further investigated the physicochemical compatibility between cellulose paper and QDs. Different surface modifications of cellulose paper were studied to evaluate their effects on QD particles distribution, retention ability, and thermal stability for sensing applications. A simple fluorescence imaging system has been constructed in-house in order to obtain the fluorescence characteristics of the QDs on cellulose paper. Furthermore, QD-Paper-based analytical devices (QD-PADs) based on these studies have been successfully developed for glucose detection with fluorescence intensity change of QD probes.

EXPERIMENTAL

Reagents and Apparatus

Chitosan (medium molecular weight) and 3-aminopropyltrimethoxysilane (APTMS, >97%) were purchased from Sigma Aldrich. Phosphate buffer solution (PBS) tablets and Tween 20 were obtained from Sigma Aldrich. Tween-20 (0.5%, v/v) was spiked into 0.01 mol/L pH 7.4 PBS solutions as wash buffer (PBST). Ultra-pure water (>18.0 MΩ) was obtained from a Milli-Q integral water purification system. All reagents were used without further purification. Carboxyl group functionalized CdS_xSe_{1-x}/ZnS alloyed quantum dots (QDs) (λ_{em} = 630 nm) were acquired from Sigma Aldrich.

Whatman No.1 filter paper (diameter = 20 mm) was obtained from Sigma Aldrich; this grade of filter paper is made of pure cellulose fibres. The paper was cut into different sizes for further use. K1050X Plasma Asher (Quorum Emitech, U.K.) was used for plasma treatment. The vacuum level for the treatment was 0.6 mbar and the paper was placed in the center of the chamber for 30 s of treatment.

Surface Modification of Cellulose Paper

Silane Modification

The preparation procedures for silane modified paper can be summarized as follows (Li *et al.* 2010; Koga *et al.* 2011; Jin *et al.* 2011, 2012): a volume of 3 mL 2.5% (v/v) 3-aminopropyltrimethoxysilane (APTMS) was prepared using 80% (v/v) ethanol solvent, and thus was hydrolyzed to form reactive silanol groups. Then 20 pieces of paper squares (1 cm × 1 cm), after plasma treatment, were immersed in the resulting solution for 2 h, followed by thorough washing with ethanol. Subsequently, the solvent was evaporated in the fume hood at room temperature for 10 min. The obtained paper squares were thermally treated at 110 °C for 3 h.

Chitosan Modification

To prepare chitosan modified paper, a volume of 10 mL 0.1% (w/v) chitosan was freshly prepared using a weak acid solution. Then 20 µL of chitosan solution were uniformly deposited on 1 cm × 1 cm paper squares, and then dried at room temperature for 1 h. Subsequently, each paper square was washed three times with 20 µL of ultrapure water, and then dried at room temperature. The chitosan retention was reported to be strong, due to the opposite charges of the chitosan and cellulose with the former being cationic and the latter being anionic (Wang *et al.* 2012; Xiao *et al.* 2013).

Preparation and Characterization of QDs on Cellulose Paper

Fluorescence and SEM characterization

Papers with APTMS- and chitosan-modification were prepared following the above procedures in Section "Surface Modification of Cellulose Paper". Untreated filter paper, APTMS- and chitosan-modified filter papers were cut into 1 cm × 1 cm squares for further QDs compatibility studies. Then 4 µL of 0.02 mg/mL QDs were dropped into the center of each paper square and allowed to dry at room temperature for 20 min.

For the purpose of collecting the fluorescence signals of QDs on the paper, a simple fluorescent imaging system (Fig. 1) was constructed in-house to capture the fluorescence signals.

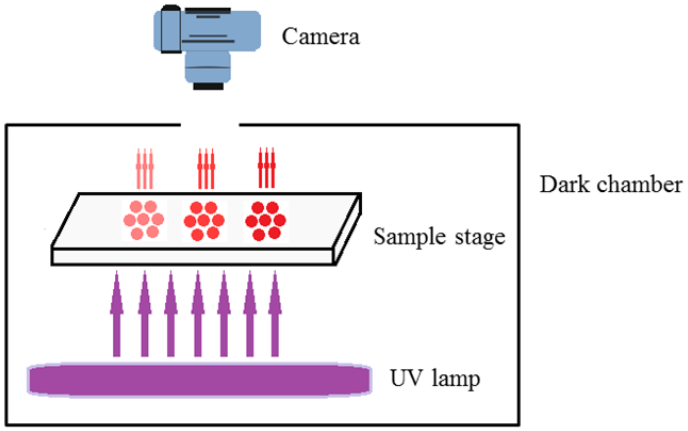

Fig. 1. Schematic diagram of the fluorescence imaging system assembled in-house.

The in-house built fluorescent imaging system was comprised of a 365 nm emission UV-lamp, a sample stage, and a digital camera. The UV-lamp, used as an excitation light source, was placed at the bottom of a dark chamber and the sample stage was fixed above

the UV-lamp. After turning on the light source, the UV light passed through the optically transparent sample stage and excited the fluorescence of the samples. The fluorescence signals emitted from the samples were captured by the camera right on the top of the sample stage. Before each fluorescence test, the light source was turned on and warmed up for 15 min to allow the stabilization of the radiation intensity.

The cellulose papers with and without QDs were further investigated by a MECM Nova NanoSEM scanning electron microscope (SEM) in order to observe the morphology of cellulose paper and the distribution of QDs on the cellulosic structure of paper. A volume of 4 µL 1 mg/mL, instead of 0.02 mg/mL QDs, was dropped onto each paper square for preparing samples for SEM study.

Retention tests by washes with aqueous solution

The retention ability of QDs on APTMS- and chitosan-modified cellulose papers was also investigated following the procedures below: three aliquots of 40 µL 0.5% PBST solution were introduced onto each testing paper, while standard blotting papers (drink coaster blotting, 280 g/m^2) were used to remove liquid during the washing process. The fluorescence of QDs on paper squares before and after washing were measured using the above in-house built fluorescence imaging system.

The quantitative analyses were carried out following the description in Section "The Quantitative Analysis Technique".

Temperature treatment of QDs on cellulose paper

Immediately after preparation, three groups of paper squares loaded with QDs were treated at temperatures of 4 °C, 25 °C, and 45 °C. The fluorescence signals of these paper squares were respectively measured after 0 h, 24 h, 36 h, and 60 h of treatment. The quantitative analyses were performed following the procedures described in Section "The Quantitative Analysis Technique".

Development of QD-PADs for Glucose Detection

Chitosan-modified paper squares (0.5 cm × 0.5 cm) were employed to make QD-PADs for detecting glucose. Five hundred microliters of QD suspension (0.04 mg/mL) was mixed with 500 µL of glucose oxidase (100 U/mL). A volume of 2 µL of the above suspension was dropped onto the center of each chitosan-modified paper square and allowed to dry for 15 min under room temperature (Gill *et al.* 2008). Then several 1.2 µL volumes of glucose solution, with concentrations of 0, 5, 10, 20, 50, 100, 150, and 200 mg/dL, were respectively introduced onto each paper square, and then allowed to react for 15 min under a controlled high humidity.

The fluorescence images of all the paper squares were captured by the in-house built fluorescence imaging system. Quantitative analyses were performed following the descriptions in Section "The Quantitative Analysis Technique".

The Quantitative Analysis Technique

The test paper squares with QDs fluorescence images were imported into Adobe Photoshop. The mean red intensity of the reaction spot was obtained using the histogram function of the software. Error bars (standard deviation) were obtained from five repeats of all the tests.

RESULTS AND DISCUSSION

Characterization of QDs on Cellulose Paper

Fluorescence characterization

The fluorescence emission performance of QDs on cellulose paper, with and without modification, was investigated using the above system (Fig. 1). Figure 2 shows the fluorescence images of untreated paper (Fig. 2, b1), APTMS- (Fig. 2, b2), and chitosan-modified paper (Fig. 2, b3). The blue color in these images was the background UV light source, and the red color is the fluorescence of QDs. In Fig. 2, b1, an extremely weak red color spread around the edge of the paper surface and could be easily observed by the naked eye. This was because QD nanoparticles dried on the untreated cellulose paper tend to migrate with the solvent phase (PBS solutions). In contrast to this, clear red fluorescence signals of QDs were captured in the center of both APTMS- (Fig. 2, b2) and chitosan-modified papers (Fig. 2, b3), which indicates that QDs were well restricted within the center range of modified papers. Whatman #1 filter paper used in this study was made of pure cellulose fibres with no additives such as strengthening or whitening agents. The basic structure of untreated paper is composed of cellulose fibers that are firmly bond together through hydrogen bonding (Sahin and Arslan 2008). This kind of paper has inactive, slightly anionic surface with a low, negative, surface-charge density (Wang *et al.* 2012). In this situation, negative-charged COOH─QDs particles have a weak tendency to strongly attach to the paper surface by the physical immobilization method. Therefore, when QDs particles were dropped onto the untreated cellulose paper, QDs will be carried by the mobile phase (PBS solution) wicking front and spread to the edge of the paper. However, the surface chemistry property of paper will change after modification processes due to the introduction of active functional groups, which enhance the charge interaction between QDs particles and the paper surface. Hence, QD particles will be left behind from the solvent phase wicking front and immobilized in the center area of paper (Fig. 2, b2 and b3).

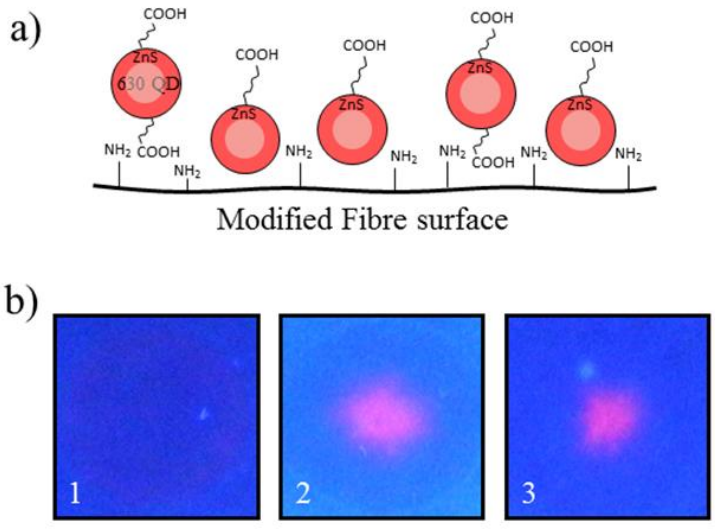

Fig. 2. a) Possible schemes of the QDs captured by modified cellulose paper' b) Fluorescence images of QDs on cellulose paper (1) without modification, (2) with APTMS-modification, (3) with chitosan modification

It was observed that the average size of the formed colored QD spots on APTMS-modified paper was larger than that on chitosan-modified paper. Additionally, the size of QDs on APTMS-modified papers was less uniform than that on chitosan-modified papers, and thus the reproducibility of QD spot sizes on APTMS-modified papers was not as consistent (not presented). The APTMS modification method in this study applied a silane coupling technique through the reaction between silanol groups (Si─OH) of APTMS and groups (mainly hydroxyl groups C─OH) of cellulose paper.

The relatively low reactivity of existing groups (mainly C─OH groups) on paper will greatly affect the amount of introduced groups with ATPMS treatment processes (Pelton 2009). However, as a physical modification approach, the chitosan modification method can be applied reproducibly with uniform coating on the surface of paper. As a result, the outsourcing active groups in the cellulosic network of APTMS-modified paper are less densely distributed compared to that of chitosan-modified paper. Therefore, QD particles have a stronger interaction with chitosan modified paper; this difference is the main reason for the difference of QD spots between the two modified papers.

SEM Characterization

The porous fibre network structures of papers remained after modification with APTMS and chitosan (Fig. 3). Figure 3 shows the SEM images of untreated papers, papers with APTMS-modification, and papers with chitosan-modification loaded with QDs. QD particles show different distributions on non-modified and modified papers (Fig. 3). Abundant QD particles were observed around the edges of untreated paper (Fig. 3, a2) while very a small quantity of particles could be observed around the center of the paper (Fig. 3, a1). However, the distribution of QDs was the opposite in the cases of APTMS- and chitosan-modified papers; most QD particles gathered around the center of the modified papers (Fig. 3, b1 and c1). The results are consistent with the above fluorescence characterization studies, indicating that surface modification can result in immobilization of QD particles to any point of interest on paper (*e.g*. to the center area of the paper square in this study).

In addition, particles tend to attach to the cellulose fibres rather than stay in pores of fibre network (Fig. 3, b1 and c1), suggesting that most of the active groups of APTMS and chitosan have been modified onto the fibres. Furthermore, QD particles have been found to be more closely packed on chitosan-modified paper (Fig. 3, c1) than that on APTMS-modified paper (Fig. 3, b2). The results illustrate that the charge interaction between QD particles and a chitosan-modified fibre surface was stronger than their interaction with a fibre surface modified with APTMS. The former captured more QD particles at points of interest.

QD particle aggregates were observed on modified paper. Their sizes were greater than separate QD particles (about 6 nm), and they lose the ability to produce fluorescence signals (Liu *et al*. 2012). One reason for the formation of large particles could be that a part of the original aqueous QD suspension had started to form aggregates, which has been observed and reported previously (Noh *et al.* 2010). Another reason, which is more likely, is that the high concentration of QD suspension applied to the paper surface for SEM tests had aggregated during the drying process.

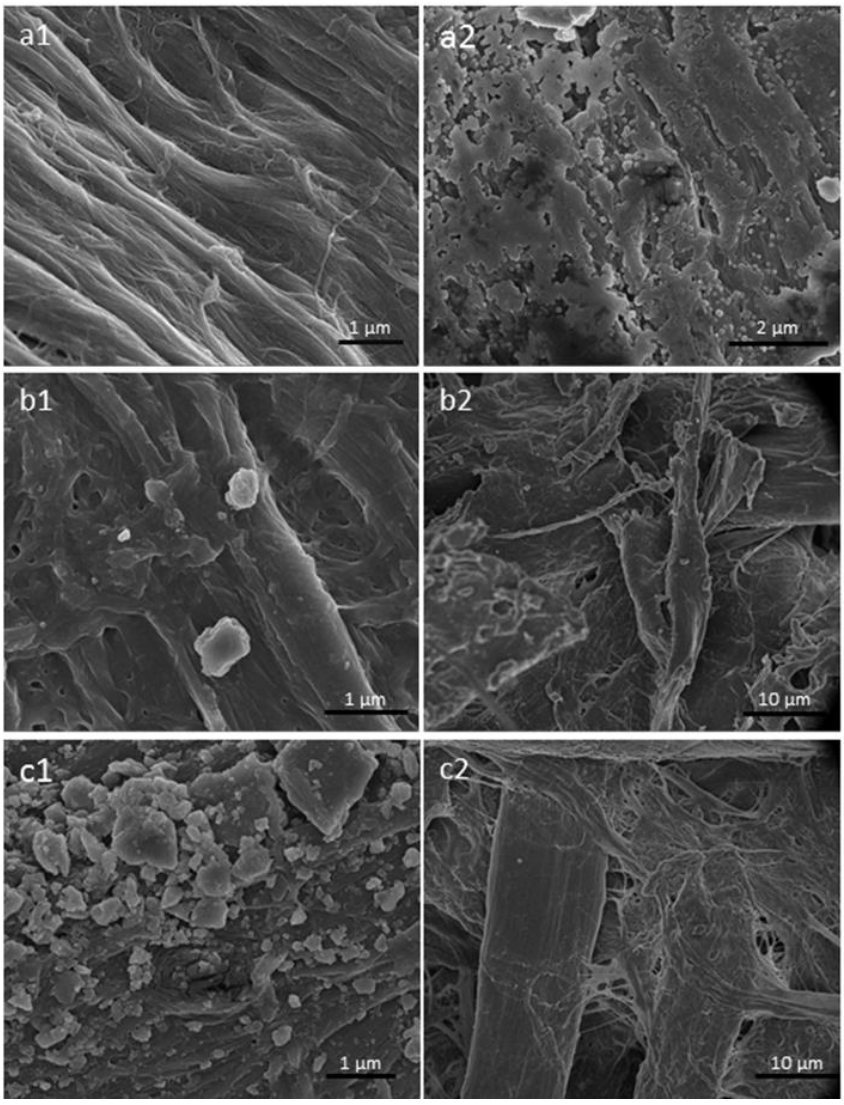

Fig. 3. SEM images of the (a1) center and (a2) edge areas of untreated cellulose paper; (b1) center and (b2) edge areas of APTMS-modified paper; (c1) center and (c2) edge areas of chitosan-modified paper. All the papers were loaded with QDs particles for SEM tests.

Retention of QDs on Modified Cellulose Paper

The retention ability of QD probes on a paper substrate is critical to the performance of QD-PADs. This is because when an analyte solution is introduced onto the detection point of the sensor, QD probes with weak bonding to fibres could be eluted away from the detection point, leading to signal distortion in the detection zone. To study the retention of QDs on the two modified papers, 0.5% PBST solutions were applied to wash modified papers loaded with QDs. The more QD particles retained after washing means the stronger interaction between QDs and paper. In this study, the red intensity measured from the QD spots is the fluorescence intensity of QDs. Figure 4 shows the fluorescence intensity changes of modified papers with QDs after washing. The fluorescence intensity decreased by 26.5% on APTMS-modified paper (Fig. 4, left), while for QDs on chitosan-modified paper (Fig. 4, right), the intensity dropped by 18.2%. This result indicates that the retention ability of QDs on chitosan-modified paper was greater than that on APTMS-modified

paper, which was further evidence that the interaction between QDs and chitosan-modified paper is stronger.

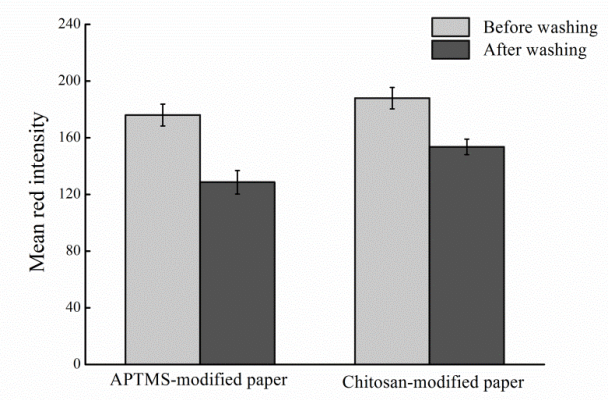

Fig. 4. The fluorescence intensity of QDs on APTMS-modified (left) and chitosan-modified (right) cellulose paper before and after washing with PBST buffer solutions

Thermal Stability of QDs on Modified Paper

Since thermal stability is a very important performance indicator of PADs for sensor applications (Guan *et al.* 2014), both APTMS- and chitosan-modified papers loaded with QDs were aged at different temperatures to test their thermal stability. Thermal stability was evaluated by measuring the red intensities of QDs spots formed in the center of the paper before and after aging. Figure 5 shows little intensity change of QDs bounded to both modified papers after aging at 4 °C; in contrast to this, a significant decrease in fluorescent intensity was observed when the papers were treated at 45 °C. Intensity decrease of QD spots on papers also occurred with a treatment temperature of 25 °C, however, it was less than that with the treatment temperature of 45 °C. The results indicate that QDs fixed in a cellulosic network could remain relatively stable at low temperatures, but a rise of temperature will lead to partial loss of fluorescence. Moreover, the results showed that the intensity decrease of QDs on APTMS-modified paper (Fig. 5a) was much greater than that on chitosan-modified paper (Fig. 5b) after aging at 45 °C. It is therefore most likely that the temperature-driven fluorescence loss of QDs on chitosan-modified paper is slower than that on APTMS-modified paper because of the stronger interaction between QD particles and the former paper.

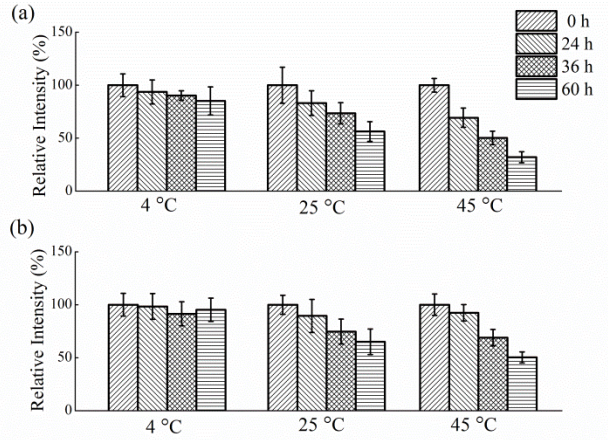

Fig. 5. The fluorescence intensity of QDs on (a) APTMS-modified and (b) Chitosan-modified cellulose paper after at 4 °C, 25°C, and 45 °C temperature treatment with different time

Development of QD-PADs for Glucose Detection

To design practical QD-PADs, the primary consideration is immobilizing QDs in the detection zones of the paper to reduce QD migration. However, QDs deposited on untreated cellulose filter paper will migrate with the solvent and mostly gather around the edge of the paper. These migrated QDs were no longer suitable for any sensor applications. Yuan *et al.* (2012) attempted to solve this problem by encapsulating QDs with opposite-charged polymer. The present study shows that suitably modified papers could well solve the migration problem of QDs. Therefore in this study, chitosan-modified paper was selected to fabricate QD-PADs for demonstrating sensor application.

QDs have been reported as a sensitive probe for hydrogen peroxide, and further used in the detection of glucose with glucose oxidase as the catalyst to produce hydrogen peroxide (Wu *et al.* 2010). The possible mechanism that stimulates the fluorescence quenching of QDs is the oxidation of S^{2-} surface states, which presumably yields Zn^{2+} surface traps for the electrons (Gill *et al.* 2008). Figure 6a shows the fluorescence quenching effect of the QDs on the chitosan-modified paper by different concentrations of glucose. The calibration curve (Fig. 6a) represents the quenching effect of glucose yields ${I_0}/{I} = 1.139 + 0.0069C_{glucose}$ $(r^2 = 0.95)$, where I_0 is the mean red intensity of QD-PADs when the concentration of glucose is 0, and I is the measured mean red intensity for the target sample. The detection limit of glucose is 5 mg/dL and the detection linear range is between 20 mg/dL and 200 mg/dL. Compared with the previous methods utilizing the glucose oxidase and QDs-polymer co-immobilization method in normal filter paper (Yuan *et al.* 2012), a lower detection limit of glucose was obtained in this study. Also in comparison to the solution-based method, this study largely reduces the usage of QDs and other reagents.

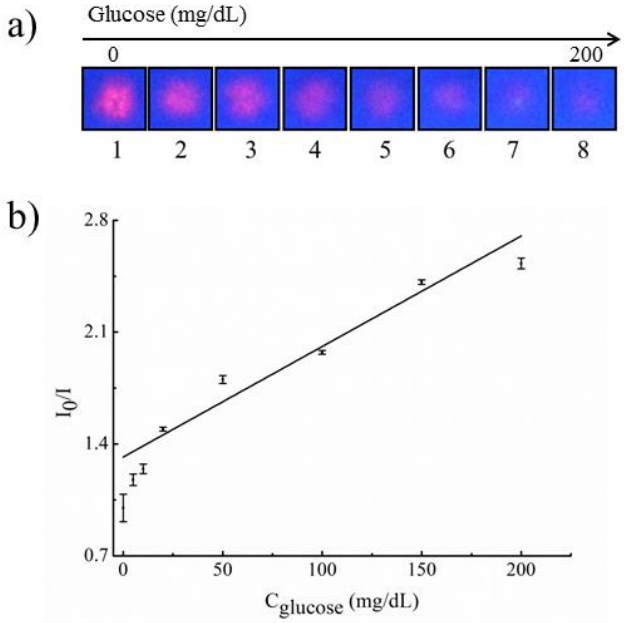

Fig. 6. a) Response of QD-PADs using chitosan-modified paper to different concentrations of glucose 0, 5, 10, 20, 50, 100, 150, 200 mg/dL (from 1 to 8); b) corresponding calibration curve

CONCLUSIONS

1. This study investigated the physicochemical compatibility between QD particles and cellulose papers for the purpose of developing reproducible QD-PADs. The migration problem of QDs moving with the solvent phase in the fibre network structure of paper was greatly reduced by modifying the surface of cellulose. The retention ability and thermal stability of QD probes on paper have been studied as critical performance indicators of QD-PADs.
2. QD-PADs using chitosan-modified paper was demonstrated for glucose detection and the detection limit is comparable with reported paper-based glucose sensors.
3. This work provides an important framework for the development of QD-PADs and it is expected that QD-PADs can lay a foundation for further developing practical and stable sensor for diagnostic and analytical applications.

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