

# Dual role of CdSe quantum dots for simultaneous separation and spectrofluorimetric ultrasensitive determination of heparin

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## ABSTRACT

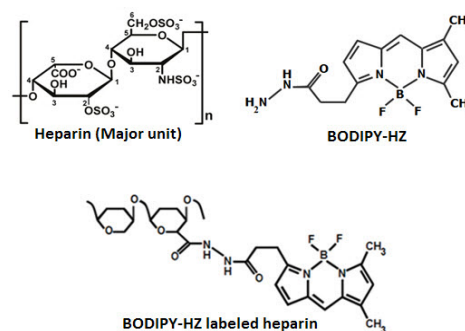
The present study offers a new method based on CdSe quantum dots (QDs) for simultaneous separation and determination of trace levels of heparin (Hep) in human serum samples. In this technique, CdSe QDs perform two different functions in Hep analysis process. Mercaptoacetic acid-capped red CdSe QDs ( $\lambda_{\text{ex}}=690$  nm) are conjugated to Hep and the Hep-QD conjugation is then used as an extraction tool from microencapsulation of real samples. For quick release of the analyte from conjugation, the microcapsules are irradiated using an intensive near infrared wavelength as the controllable releasing agent. The L-cysteine-capped green CdSe QDs ( $\lambda_{\text{ex}}=480$  nm) play the additional role of Hep spectrofluorimetric detection. By labeling the appropriate chromophore, the extracted Hep can effectively quench green CdSe QD fluorescence which is quenched relative to Hep concentration. In comparison with other available methods, the newly-developed assay has a low detection limit (0.3 nmol L<sup>-1</sup>), wide linear range (1 to 8500 nmol L<sup>-1</sup>), good accuracy and high selectivity, that make it a good candidate for Hep separation and monitoring of complex physiological samples.

**Keywords:** Heparin, Quantum dot, Determination, Human serum, Near infrared

Heparin (Hep) is a linear, highly-charged, anionic polysaccharide that belongs to the glycosaminoglycan family and consists of a variably-sulfated repeating disaccharide unit (Fig. 1) [1–4]. Hep plays many important roles in physiological and pathophysiological processes. It is common on cell surfaces and inside cells, as well as the extracellular matrix. It is also a potent anticoagulant agent administered systemically following vascular surgery to prevent acute thrombosis [5].

Studies have established the specificity of Hep and its derivative interactions with chemokines, cytokines and growth factor receptors [6–8]. These interactions play pivotal roles in cell adhesion, proliferation, motility, differentiation, viral and bacterial infection, cancer and inflammation [9–13]. Recently, potential pharmaceutical applications for Hep have been reported for diseases including senile dementia [14], angiogenesis

[15], diabetic nephropathy [16] and cancer treatment [17].



**Fig 1.** Chemical structures of heparin, BODIPY-HZ, and labeled heparin by BODIPY-HZ.

To investigate Hep function in biological interactions and understand its relationship with disease treatment, quantitative analysis of Hep is essential.

Hep is extremely difficult to analyze because of its high negative charge, polydispersity and microheterogeneity. During surgery or postoperative therapy, Hep is commonly monitored indirectly in plasma samples by determination of activated clotting time or chromogenic factor Xa assay. Despite their wide use, these assays are affected by factors other than Hep [18]. As a result, they are inaccurate certain disease states. There is significant interest in developing a fast, simple and reliable test with the potential for Hep trace level monitoring in real complicated matrices, such as in point-of-care detection of Hep in clinical serum and plasma samples during dialysis.

Some separation techniques, including high-performance liquid chromatography [19–21], gel permeation chromatography [22–24], polyacrylamide gel electrophoresis [25–27] and capillary electrophoresis [28–30] have been used to prepare Hep disaccharides and oligosaccharides to solve complex structures. Most of these methods are not sufficiently specific and sensitive to quantify trace amounts of Hep in physiological samples. Separation methods are commonly coupled with detection techniques such as electrochemical methods, quartz crystal microbalance analysis, mass spectrometry, tandem mass spectrometry (MS-MS) and luminescence [31–35] to improve Hep quantification efficiency. Electrochemical methods such as polymeric membrane-based ion selective electrodes or ion-selective field effect transistors avoid the inaccuracy caused by indirect assays, but the response is intrinsically dependent on the activity of other ions in the sample (chloride, bicarbonate), making sensor calibration difficult. Other disadvantages are the irreversibility of detection (for polymer membrane electrodes) and potential drifts (for field effect transistors). Although quartz crystal microbalances, including protamine-absorbed surfaces, have been successfully applied to Hep detection, they suffer from a long incubation period to reach steady state conditions [36]. Tandem mass spectrometry has become increasingly important for the analysis of Hep oligosaccharides with the development of the soft ionization methods of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) [37,38]. Although MS-MS technology is a sensitive technique, it is costly and not routinely accessible as a tool in every lab.

A wide variety of luminescent and colorimetric methods have been established for Hep sensing [35, 39–42]. Most methods have been used to detect Hep in an extracted solution without a separation step. The samples must be diluted before determination to eliminate disturbance from the luminescence of interfering species [41, 42]. This can decrease the detection limit (DL) and sensitivity of the method and increase the probability of error.

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Some luminescence assays employ fluorescence quenching of QDs for room temperature fluorescence (RTF) probes [35, 39, and 41]. The rationale behind fluorescence-based methods is to determine the concentration of Hep using labeling by a special fluorophore. Synthetic fluorophores, such as tripodalboronic acids, polycationic calyx arenes, polymethinium salts and chromophore-tethered flexible copolymer, have been used as Hep indicators. One promising fluorescent label that offers high yields (through a more reactive hydrazide group) and enhanced sensitivity of fluorescence detection of Hep is 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid hydrazide (BODIPY-HZ) (Fig. 1) [43]. The extinction coefficient of this fluorophore is significantly higher ( $\epsilon = 71000 \text{ M}^{-1} \text{ cm}^{-1}$ ) at the optimum extinction wavelength of 513 nm.

A successful combination of simple and reliable separation methods and spectrofluorimetric detection techniques provides critical advantages during analysis of Hep. In a previous study [44], a new method based on carboxylated cadmium selenide quantum dots (CdSe QDs) was applied for preconcentration and determination of retinoic acid (RA) isomers in whole rudimentary embryo cultures in rats. CdSe QD modification using suitable chemicals such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), sulfonated N-hydroxysuccinimide (sulfo-NHS), and phenylenediamine (PDA) give RA the ability to conjugate. In the study, RA isomers were extracted from samples by conjugation to an appropriate amount of CdSe QDs. For quick release of the analyte, separated RA-QD conjugations were irradiated using intensive near infrared wavelength (NIR). This method has good accuracy, a low detection limit, high preconcentration factor, excellent precision and sensitivity for the target analytes, which indicate its potential for trace analysis of physiological samples with complex matrices. The present study developed a new technique for simultaneous separation and determination of Hep in human serum using conjugation to red CdSe QDs (modified by mercaptoacetic acid) and quick release by NIR-intensive irradiation before high sensitive spectrofluorimetric measurement using green CdSe QDs (modified by L-cysteine).

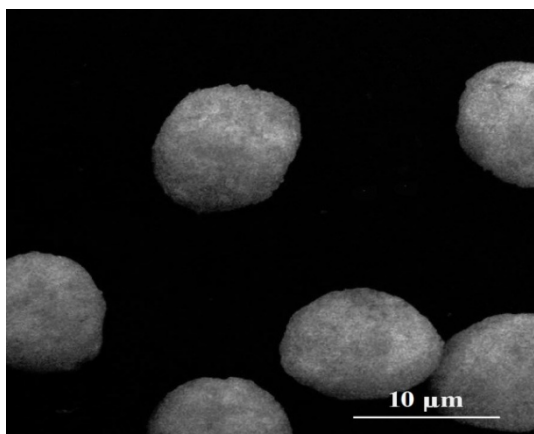
## MATERIALS AND METHODS

### Apparatus

All fluorescence measurements of Hep in standard stock and serum samples were made using a RF-5301 spectrofluorophotometer (Shimadzu, Japan) equipped with a 150 W xenon lamp and 1 cm quartz cell. The excitation wavelength was set at 480 nm and the fluorescence intensity was measured at 520 nm. Detector sensitivity was adjusted to the fluorescence intensity of the sample. NIR irradiations were achieved using an AM1.5G solar simulator (ABET Technologies, Sun 3000). All scanning electron microscopy (SEM) images were obtained using a Hitachi, S-2600N scanning electron microscope (Hitachi, Japan).

## Materials

All reagents were used without further purification. Analytical grade methanol, ethylene glycol, dichloromethane and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). Poly(D, L-lactide-co-glycolide) (PLGA), PDA, EDC, sulfo-NHS, poly( $\epsilon$ -caprolactone) (PCL) (Mw 110000 g/mol), poly(vinyl alcohol), phosphate buffered saline and heparin sodium salt were purchased from Sigma-Aldrich (St. Louis, MO, USA). The molecular weight of the heparin was calculated as a repeating disaccharide unit (644.2 g/mol; Fig. 1), and 1  $\mu$ m equals 0.12 U mL<sup>-1</sup> [42]. Surface-modified CdSe QDs were obtained from Evident Technologies (Troy, NY, USA). QDs used for separation purposes were mercaptoacetic acid (MAA)-capped red CdSe with a primary excitation of 690 nm. QDs used for spectrofluorimetric detection were L-cysteine (L-cyst)-capped green CdSe with a primary excitation of 480 nm and emission of 520 nm. Deionized water was purified using a Milli-Q system (Millipore, USA). BODIPY-HZ was obtained from Molecular Probes (Eugene, OR, USA).



**Fig 2.** SEM image of conjugated red CdSe quantum dot-heparin microcapsules.

## Sample preparation

Venous blood samples were obtained from a clean vein puncture into a serum separator and allowed to clot for 30 min, then centrifuged at 1800 rpm for 15 min. The serum sample was then shock frozen and stored at -25°C [45]. At the time of assay, frozen serum was homogenized on ice and, after EDC activation, was used to analyze the Hep. Stock solutions of Hep were freshly prepared.

## Modification

A stock solution of 1  $\mu$ mol L-1 Hep was prepared by dissolving Hep in deionized water for storage at 4°C. This solution was diluted as needed to provide standard stock solution.

It was necessary to conjugate Hep to red CdSe QDs to separate the Hep from the sample; the conjugation was then microencapsulated. For conjugation purposes, the analyte and QD were activated by an EDC activation reaction. This reaction occurs for molecules that have reactable carboxylate groups on their surfaces. Hep has a natural carboxylate group at the end of every repeating disaccharide unit. MAA-capped red CdSe QDs with carboxyl groups were used for conjugation purposes.

The pH of all standard stock and samples were adjusted to 8.2 by the addition of 0.1M Tris-HCl buffer solution.

## Separation and desorption

To provide Hep-conjugated red CdSe QDs, 2 mL of the 200 mg L-1 carboxylated red QDs in aqueous solution was added to 2 mL of 0.1-900 nmol L-1 Hep solution and 20 mg EDC, 2 mg sulfo-NHS and 2 mg PDA were added to the solution. After 5 minutes of gentle stirring, QD-PDA-Hep conjugation was achieved.

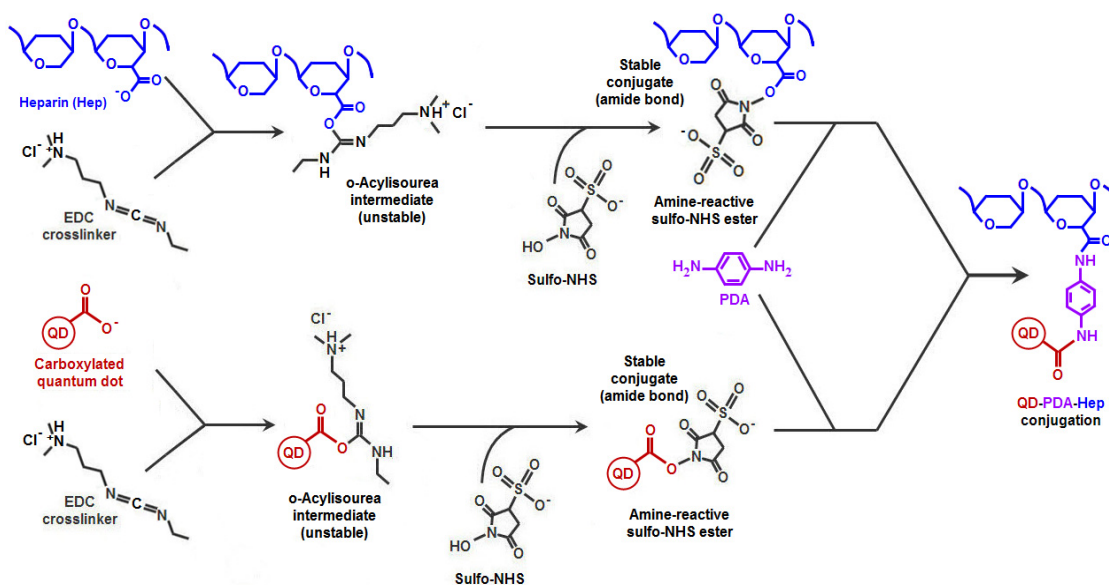
It was necessary to encapsulate the QD-PDA-Hep conjugation before irradiation to prevent the release of Hep from conjugation. Microencapsulation of QD-PDA-Hep was performed by emulsifying the mixture in 8 mL solution of 100 mg PLGA and 100 mg PCL in dichloromethane while vortexing for 5 minutes at room temperature (25 $\pm$ 1). The microcapsules were separated from the mixture by centrifuging, decantation and washed with methanol. Finally, 1 mL methanol was added to the microcapsules. The morphology of micro-spheres was examined by SEM (Fig 2).

The microcapsules were exposed to NIR irradiation to release Hep from conjugation. The resultant solution was centrifuged at 1500 rpm for 2 minutes and filtered (0.2 $\mu$ m pore size) to remove any microcapsules. Then, 1 mL methanol containing BODIPY-HZ was added to the solution for labeling prior to spectrofluorimetric determination.

## BODIPY-HZ labeling and spectrofluorimetric determination

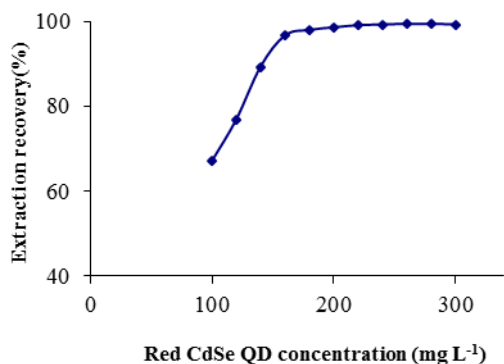
The BODIPY-HZ and Hep mixture solution achieved in the separation step was stirred for 2 minutes and reacted at 25°C. The labeled sample was stored in darkness for up to one month at -80°C without evidence of degradation. The samples degraded after approximately 3 hours at room temperature (25 $\pm$ 1) and were, thus, protected from exposure to direct light.

Green CdSe QD exhibits intensive fluorescence ( $\lambda_{max}$ =520 nm) at maximum absorbance of 480 nm. Based on this emission, green CdSe QD can be applied as a suitable indicator of Hep, with the intensive fluorescence emission quenching in the presence of BODIPY-HZ-labeled Hep. As mentioned, BODIPY-Z has a maximum extinction coefficient of  $\lambda$ =513 nm.



**Fig 3.** Schematic illustration for conjugation of red CdSe quantum dot–PDA–heparin. Addition of sulfo-NHS to EDC crosslinking reactions, increases efficiency of EDC activation.

To explore the potential of CdSe QDs for spectrofluorimetric detection of Hep, RTF spectroscopic measurement was performed in an aqueous buffer solution by separately adding and mixing 2 mL green aqueous CdSe QD solution ( $300 \text{ mg L}^{-1}$ ), 1 mL Tris–HCl buffer solution (0.1M) and a series of different concentrations of Hep (from 1 to  $8 \times 10^3 \text{ nmol L}^{-1}$ ) solution in 5 mL calibrated test tubes. The mixtures were subsequently diluted to the mark with water and mixed thoroughly for 5 min at room temperature before detection. The fluorescence spectra were recorded in the 520 nm emission wavelength at an excitation wavelength of 480 nm.



**Fig 4.** Relationship between heparin recovery and concentration of red CdSe quantum dot. The NIR irradiance intensity was kept in  $300 \text{ mW/cm}^2$  at 15 min.

## RESULTS AND DISCUSSION

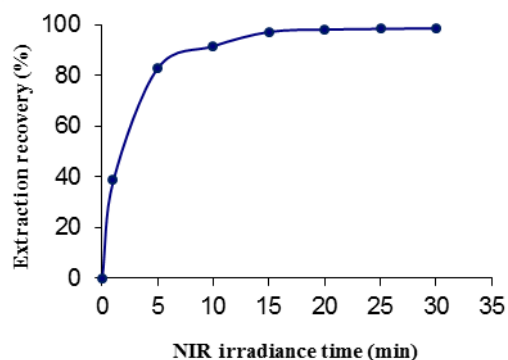
### Separation step

#### 1. Conjugation mechanism

Nanoparticle conjugation to biological molecules and cells has been applied for purposes such as bio-labeling, bio-imaging and bio-sensing [46–47]. Since bioconjugation systems can be prepared from different organic and inorganic materials, their fabrication procedures are different. Surface modification is needed to conjugate different particles to molecules and macromolecules. This is essential to the development of the assays, probes and sensors and can show common chemistry. Surface modification of inorganic nanoparticles, such as QDs, by carboxylate group is done to prevent their agglomeration and to increase their selectivity towards the analyte. The most common ligands used to coat QDs are small thiol molecules with terminal carboxylate groups, such as MAA, that coordinate directly with the QDs surfaces [46].

After surface modification, CdSe QDs were initially activated using an activators such as EDC and sulfo-NHS. Sulfo-NHS is used to prepare amine-reactive esters of carboxylate groups for chemical labeling, cross linking and solid-phase immobilization applications. Carboxylates ( $-\text{COOH}$ ) react with sulfo-NHS in the presence of a carbodiimide such as EDC, resulting in a semi-stable sulfo-NHS ester that can then react with primary amines ( $-\text{NH}_2$ ) to form an amide crosslink. Although sulfo-NHS is not required for carbodiimide reactions, its application greatly enhances coupling efficiency.

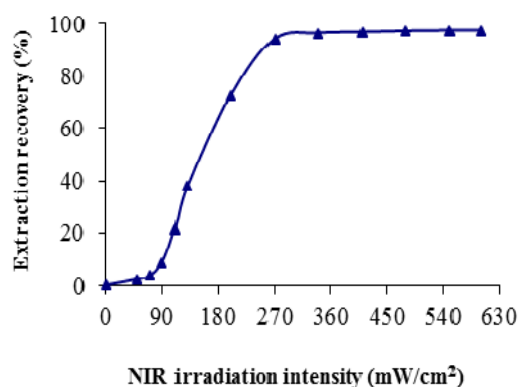
Furthermore, the use of sulfo-NHS makes it possible to perform a two-step reaction where PDA can bind to both EDC-activated Hep and CdSe QD through its two NH<sub>2</sub> groups (Fig. 3).



**Fig 5.** Relationship between heparin recovery and NIR Irradiation time. Red CdSe quantum dot concentration in this experiment was adjusted in 200 mg L<sup>-1</sup> and the NIR Irradiance intensity were kept in 300 mW/cm<sup>2</sup>.

## II. Effect of quantity of red QDs

The influence of the amount of QDs on Hep separation was studied by varying the concentration of CdSe QDs from 100 to 300 mg L<sup>-1</sup> (Fig. 4). Increasing QD concentration in the mixture to 160 mg L<sup>-1</sup> improved Hep separation efficiency. Because of the presence of other compounds in the physiological matrix and their potential conjugation to QDs, a concentration of 200 mg L<sup>-1</sup> was selected as an optimal for further study.



**Fig 6.** Relationship between heparin recovery and NIR irradiance intensity. Red CdSe quantum dot concentration in this experiment was adjusted in 200 mg L<sup>-1</sup> and the NIR radiation time were kept at 15 min.

## III. Release mechanism

Light is the most appropriate trigger to release Hep from conjugation because of its controllable exertion. The use of NIR beams for provocation of QDs allows the release of Hep in physiological samples with no cleavage in the analyte chain. Although CdSe QDs show strong absorption in NIR region, very few organic chromophores are absorbed in this region and even fewer are capable of converting the absorbed energy into a chemical or thermal response. These QDs absorb light efficiently because of the coherent oscillations of metal conduction band electrons in strong resonance with visible and infrared frequencies of light. Photo excitation of metal nano-structures results in the formation of a heated electron gas that cools rapidly within 1 ps by exchanging energy with the nanoparticle lattices. The nanoparticle lattices, in turn, rapidly exchange energy with the surrounding medium on a timescale of 100 ps, causing localized heating [48]. Spontaneous local heating to temperatures well above hundreds of degrees °C induces significant thermal and mechanical stress in the system [49, 50] and ruptures the conjugation, causing rapid release.

## IV. Effect of duration of NIR exposure

The effect of NIR irradiation time on the extraction efficiency was investigated under optimum conditions from 0 to 30 min at constant irradiation intensity (Fig. 5). The results indicated that Hep separation recovery increased as the irradiation when time increased from 1 to 15 minutes. After 15 minutes, recovery did not increase significantly. The duration of exposure was thus adjusted to 15 minutes for the upcoming experiments.

## V. Effect of intensity of NIR irradiation

The effect of NIR irradiation intensity on Hep release was investigated at 0 to 600 mW/cm<sup>2</sup> (Fig. 6). Hep release at intensities less than 90 mW/cm<sup>2</sup> was very slow, but NIR irradiance at intensities greater than 90 mW/cm<sup>2</sup> caused quick release. The RA release rate did not change noticeably at intensities greater than 270 mW/cm<sup>2</sup>.

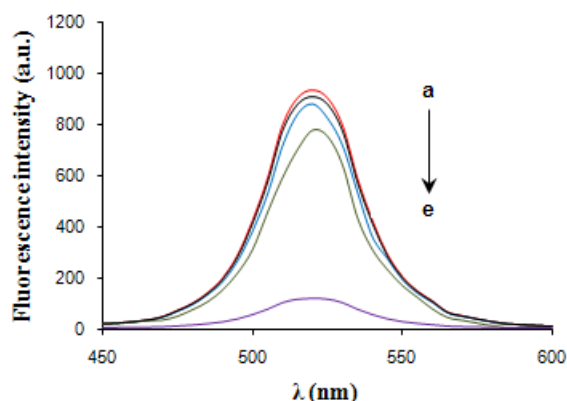
## Determination step

### I. BODIPY-HZ labeling

Hep is a polysaccharide and has a carboxylate group at the end of every repeating disaccharide unit. There is also a reactable amine group in the structure of BODIPY-HZ. In the presence of BODIPY-HZ, the separated Hep was labeled by formation of a carbonyl group at the carboxylate end. It is known that BODIPY-HZ shows high relative efficiency for Hep-labeling and can be assumed to be a labeling agent specific to this

analyte [43]. This is related to the strongly electronegative N-sulfate group that tends to withdraw electrons from the anomeric carbon center, making it more susceptible to nucleophilic attack by the nitrogen of the label.

BODIPY-HZ has a high extinction coefficient at the maximum emission wavelength of green CdSe QDs ( $\lambda_{\text{max}}=520$  nm) and causes an appreciable increase in quenching effect of Hep at the fluorescence intensity of green CdSe QDs. Fig. 7 illustrates the key role of BODIPY-HZ in determination of Hep. Figs. 7b and 7c show that BODIPY-HZ and Hep separately were not tangible quenchers for green CdSe QDs fluorescence; however, when BODIPY-HZ was attached to Hep, quenching ability increased strongly (Fig.7e). Fig. 7d shows that human serum had no significant quenching effect on green CdSe QDs fluorescence.

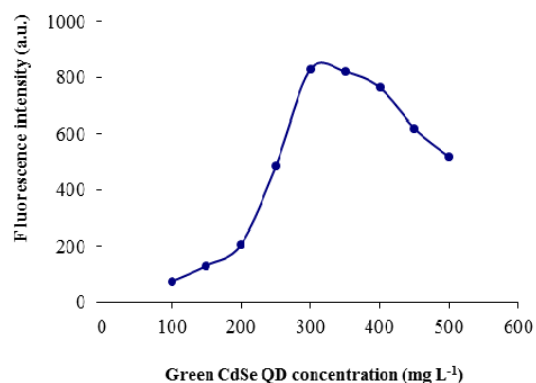


**Fig 7.** Fluorescence emission spectra of (a) green CdSe QDs (b) green CdSe QDs in presence of  $10 \mu\text{mol L}^{-1}$  BODIPY-HZ (c) green CdSe QDs in presence of  $10 \mu\text{mol L}^{-1}$  Hep (d) green CdSe QDs in presence of 1 mL human serum (e) green CdSe QDs in presence of  $10 \text{nmol L}^{-1}$  BODIPY-HZ labeled Hep.

## II. Effect of concentration of green CdSe QDs

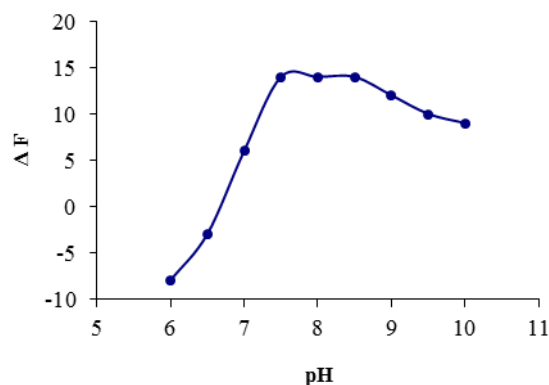
It was found that the concentration of QDs affected the intensity of fluorescence and the sensitivity of the assay (Fig. 8). The low concentration of aqueous L-cyst-capped green CdSe QDs decreased the intensity of fluorescence and may have resulted in a narrow linear range; however, when the concentration of aqueous CdSe QDs was too high, self-quenching of the QD fluorescence caused a significant decrease in sensitivity. Based on these results,  $300 \text{mg L}^{-1}$  of aqueous green CdSe QDs was chosen as the optimum amount. III. Effect of buffer solution

The quenching effect of Hep on fluorescence emission of green CdSe QD was monitored for different buffered aqueous solutions.  $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ , Tris-HCl and  $\text{H}_3\text{BO}_3\text{-Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$  were used as buffers at constant pH values and concentrations. The results showed that Tris-HCl buffer significantly influenced the intensity of fluorescence.



**Fig 8.** Effect of green CdSe quantum dot concentration on fluorescence intensity. This experiment was carried out in absence of heparin.

It is known that QDs are pH sensitive. The fluorescence intensity of a series of Tris-HCl buffer solutions at different pH (F) values and the corresponding blank solution ( $F_0$ ) were measured at  $\lambda_{\text{ex}}/\lambda_{\text{em}} = 480/520$  nm. It was shown that pH had a strong effect on the intensity of fluorescence of the system (Fig. 9) and that  $\Delta F$  is negative ( $F_0 > F$ ) at  $\text{pH} \leq 6.5$ . The results showed that intensity of fluorescence reached a maximum at pH values from 7.5 to 8.5. An optimum pH of 8.0 was thus selected for  $0.10 \text{mol L}^{-1}$  Tris-HCl buffer solution. The dosage of the buffer solution had little effect on the intensity of fluorescence of the system; thus, 1.0 mL was chosen for the present experiments.



**Fig 9.** pH of Tris-HCl buffer solution affect the spectrofluorimetric detection of heparin.  $\Delta F$  is difference between the fluorescence intensity of green CdSe quantum dot in Tris-HCl buffer solution (F) and corresponding blank solution ( $F_0$ ).

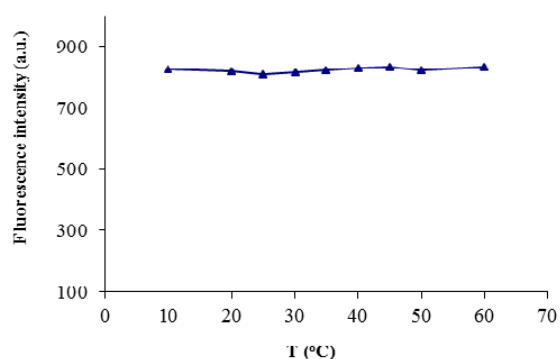
**Table 1.** Figures of merit in different methods for heparin determination.

Method	Matrix	Linear Range	RSD %	DL	Ref.
MIP potentiometry	Injection solutions	0.003 – 0.7 <sup>a</sup>	—	1 <sup>d</sup>	33
Danofloxacin– terbium probe	Injection solutions	0.1 – 1.5 <sup>b</sup>	1.15–3.19	24.62 <sup>c</sup>	39
Cu InS <sub>2</sub> QD nanosensor	Bovine serum	0.05 – 15 <sup>a</sup>	1.03–2.31	12.46 <sup>d</sup>	41
PEI–Mn–ZnS QD phosphorescence probe*	Human serum	1 – 4 <sup>a</sup>	2.2	50 <sup>d</sup>	42
CdSe QD dual role	Human serum	0.001 – 8.5 <sup>a</sup>	0.9–1.7	0.3 <sup>d</sup>	This study

NR: Not Reported; MIP: Molecularly Imprinted Polymer; QD: Quantum Dot; PEI–Mn–ZnS: polyethyleneimine capped Mn–doped ZnS; a:  $\mu\text{mol L}^{-1}$ ; b:  $\mu\text{g mL}^{-1}$ ; c:  $\text{ng mL}^{-1}$ ; d:  $\text{nmol L}^{-1}$ ; \*: The reported data is belong to QD1 with hydrodynamic diameter of 122.4 nm.

#### IV. Effect of temperature

The relationship between temperature and fluorescence intensity is shown in Fig. 10. Although the intensity of fluorescence changed slightly as temperature changed, when the temperature was above 45°C, the fluorescence intensity of the system was unstable. Minimal instability of fluorescence intensity was reached when the solution was incubated at about 25°C, so the testing was performed at room temperature.



**Fig 10.** Effect of temperature on fluorescence intensity of green CdSe quantum dot.

#### Analytical features of the method

The analytical features of the proposed method were studied to evaluate its usefulness for quantitative analysis. The calibration curves of the analytical signal versus concentration of the standard solutions were recorded (RSD) was in the range of 0.9% to 1.7%. The limit

of detection of the method was defined as the lowest concentration of analyte in the physiological matrices that could be analyzed using the proposed method while generating a signal with an S/N ratio of 3. The DL was 0.3  $\text{nmol L}^{-1}$ . Table 1 compares the characteristics of the proposed method with those reported in literature.

**Table 2.** Recovery tests of heparin determination in human serum samples

Sample	Added Con.*	Found Con.&	Recovery (n=5)	RDS (n=5)
1	1	0.98	98.10	5.6
2	10	9.91	99.17	1.3
3	100	103.24	103.24	2.1
4	1000	994.82	99.48	1.2
5	5000	5006.88	100.14	2.0

\*Added concentration: ( $\text{nmol L}^{-1}$ ), &Found concentration: ( $\text{nmol L}^{-1}$ )

#### Applications to human serum

To investigate possible practical applications for the proposed assay for Hep detection in biological media, 0.5 mL human serum samples were assayed under optimal conditions. Briefly, 0.2 mg sulfo-NHS and 0.5 mL of carboxylated red QD aqueous solution ( $200 \text{ mg L}^{-1}$ ) were added to the sample and then 0.5 mg PDA was subjoined to the mixture under vortexing. After microencapsulation of the QD-PDA-Hep, the RA was separated from the microcapsules under NIR beams. Finally, the sample was cooled to 4°C, centrifuged at 1500 rpm for 3 minutes and filtered to remove any microcapsules. The residual solution below the filter was evaporated under reduced pressure and the total Hep was re-dissolved in 0.2 mL of methanol. The resulting

solution was subjected to spectrofluorimetric assay after labeling with 0.2 mL BODIPY-HZ (20  $\mu$ mol L<sup>-1</sup>).

The human serum was spiked with Hep to validate the proposed method. The recovery test results of five experimental replications are listed in Table 2 that are acceptable results with a recovery of 98% to 103% standard measurements and RSD values of less than 5.6%.

This paper proposes a novel approach for analysis of highly sensitive determination of Hep using CdSe QDs. The method was then used to monitor Hep concentrations in human serum. A low detection limit, wide linear range, good accuracy and high selectivity were achieved using the proposed method, indicating its potential for analysis of Hep in biological samples with complex matrices. The more important features of the proposed method are the dual role of CdSe QDs for the separation and spectrofluorimetric detection of an analyte.

#### ACKNOWLEDGEMENTS

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