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Optical detection of CA 15.3 breast cancer antigen using CdS quantum dot

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Abstract: The present study focus on optical sensing of breast cancer antigen 15.3 (CA 15.3) using cadmium sulphide quantum dot (CdS-QD) in saline and serum samples spiked with antigen. The surface of CdS-QD was modified by cysteamine capping followed by tagging of CA 15.3 antibody. The samples were characterised using UV-visible absorption spectroscopy (UV-VIS Spectroscopy), Fourier transform infrared spectroscopy (FTIR), high-resolution transmission electron microscopy (HRTEM) attached with energy-dispersive X-ray spectroscopy, phase contrast inverted epi-fluorescence microscopy and photoluminescence (PL) spectrophotometry (EDS). The CdS-QD showed a mean diameter of 3.02 ± 0.6 nm. The complex formed after antigen-antibody interaction resulted in distinguishable optical and fluorescence intensity with respect to varying concentration of antigen. The PL study revealed that CA 15.3 antibody labelled CdS QD can detect CA 15.3 tumour marker even at very low concentration of 0.002 KU/L with a constant response time of 15 min. This study clearly indicates that detection of CA 15.3 at low concentration is possible using surface modified CdS QD in serum samples and can find immense applications in biosensor development for detection of breast cancer marker similar to various automated detection kits available in market.

1 Introduction

Breast cancer is the second leading cause for cancer related death in India and it is a major health problem affecting women in both developed and developing countries [1, 2]. The global burden of breast cancer is expected to cross 2 million by the year 2030, with growing proportions from developing countries [3]. Early diagnosis of cancer increases the chance of survival which strongly depend upon stage at which diagnosis is being done [4, 5]. During cancer, specific protein molecules are found in the body tissues and blood serum which are called as tumour markers [6]. These tumour markers are used to detect the presence of cancer, to analyse the cancer behaviour and to check the response to various anti-cancer treatments including recurrence. There are various types of serum markers reported for breast cancer namely, cancer antigen (CA) 15.3, carcinoembryonic antigen, CA 27.29, tissue polypeptide antigen and HER2 [7]. American Society of Clinical Oncology Panel, National Academy of Clinical Biochemistry and European Group on Tumour Markers Panel have recommended the use of cancer antigen 15.3 (CA 15.3) for monitoring progress of therapy in patients with advanced breast cancer [7, 8]. CA 15.3 is a large mucin glycoprotein coded by MUC1 gene and its epitope is present on episialin [9]. This MUC 1 gene is expressed on the ductal cell surface of glandular epithelia resulting in the production of CA 15.3. Mucin proteins play an important role in cell protection and lubrication. Under normal conditions, the CA 15.3 concentration in blood is reported to be 25–30 U/ml. However, during breast cancer, the CA 15.3 level in blood serum increases depending upon the stage of malignancy [10, 11]. During malignancy, cell polarity is lost so that the proteins are expressed on the entire cell surface and is shed into the circulation [10, 11]. The variation in CA 15.3 content in serum beyond its normal level indicates the progression of disease and its response to the therapy [7]. Since CA 15.3 has been found to have a lead time of nine months before detection of relapse, it becomes necessary to monitor the CA 15.3 biomarker level that is increasing slowly and continuously after treatment. However to perform such assessment, a highly sensitive and selective detection assay is needed. At present, two monoclonal antibodies DF3 and 115D8 have been used for CA 15.3 assay [10]. DF3 is generated against a membrane extract which is directed to an epitope in the region DTRPAGS, whereas 115D8 is generated against milk fat globulin membranes directed to a peptide carbohydrate epitope on the SAPDTRPA region [12]. The automated kits and its detection limits (given in bracket) for CA 15.3 antigen are as follows; Access 2 (0.02 KU/L), ADVIA Centaur (0.19 KU/L), ARCHITECT i2000 (0.37 KU/L), AxSYM (0.12 KU/L), IMMULITE 2000 (0.15 KU/L), VITROS ECi (0.005 KU/L) and Elecsys (0.09 KU/L) [13]. Although the sensitivity of current detection methods is high, a further increase in sensitivity can yield better understanding of the disease progression.

In cancer diagnostics, fluorescent nanoparticles have been studied for their multiple simultaneous profiling of tumour biomarkers in serum and tissue [14–16]. Among the various nanoparticles, quantum dots (QD) play a vital role in chemical and biological sensing [17, 18]. QD are inorganic semiconductor nanoparticles having unique optical property which makes them suitable for highly sensitive bioassays. QD are used as an alternative fluorescent probes for detection of cancer markers, imaging, protein trafficking and other clinical as well as biosensor applications due to their small size, high surface area, fluorescence stability, resistance to photobleaching, large molar extinction coefficients and narrow emission and excitation wavelength [14, 19–21]. QD are also widely employed for target specific biomarker detection due to the possibility of easy manipulation of surface by conjugating with antibody, protein, or other biomolecules and by tuning its optical properties [22]. Compared with other time consuming, expensive and labour intensive bioassays, QD based optical detection is rapid, easy and economical. Despite of all these applications and advantages, QD faces some disadvantage like toxicity and hydrophobicity. These issues need to be solved in order to apply QD effectively in diagnosis and drug deliver application [14]. Cadmium sulphide quantum dot (CdS-QD) has been widely used to detect analytes like proteins, DNA and metal ions [23, 24].

In this study, we have developed CA 15.3 antibody conjugated CdS-QD for the detection of CA 15.3 antigen in saline and spiked serum with an aim to increase the sensitivity of the detection method. The synthesised CdS-QD was characterised using suitable techniques like UV-visible (UV-VIS) absorption spectroscopy, Fourier transform infrared spectroscopy (FTIR), high-resolution transmission electron microscopy (HRTEM) attached with energy-dispersive X-ray spectroscopy (EDS), phase contrast inverted epi-fluorescence microscopy and photoluminescence (PL) spectrophotometry. The detection limit of the developed CA 15.3 antibody conjugated CdS-QD was deduced and compared.

2 Experimental

2.1 Chemicals and solutions

All chemicals used were of analytical grade and used without further purification. Cadmium chloride monohydrate (98%) and glycine were purchased from Merck (Mumbai, India). Monoclonal CA 15.3 antibody and antigen were purchased from MP-Bio (India), Sodium sulphide (97%), cysteamine hydrochloride (C_2H_7NS•HCl), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) (98%) and N-hydroxyl sulphosuccinimide (NHS) (97%) were obtained from Sigma-Aldrich, USA. Sodium hydroxide and bovine serum albumin (BSA) were obtained from Hi-Media, India. Ultrapure water obtained from a Millipore water purification system (Milli – Q, Millipore, Bangalore, India) was used throughout the experiment. Phosphate buffer saline (PBS) (pH 7.4) was prepared using sodium chloride, potassium chloride, disodium hydrogen phosphate and sodium dihydrogen phosphate purchased from Hi-Media (India).

2.2 Synthesis of cysteamine capped cadmium sulphide quantum dots (Cys-CdS QD)

Synthesis of Cys-CdS QD nanoparticles was carried out according to Noipa *et al.* [25]. In brief, 9.35 mmol of cadmium chloride and 46.75 mmol of cysteamine hydrochloride were dissolved in 25 ml milli-*Q* water separately. Further, cysteamine hydrochloride solution was added in cadmium chloride under constant stirring condition for 30 min. The pH of the reaction mixture was carefully adjusted to 6.5 by adding 0.1M NaOH followed by addition of 2.5 ml of 9.35 mmol sodium sulphide solution. The reaction mixture was refluxed at 75°C for 1 h. All the reactions were performed under constant nitrogen flow.

2.3 Tagging of CA 15. 3 antibody with Cys-CdS QD (Ab-Cys-CdS QD)

CA 15.3 antibody was tagged onto Cys-CdS QD according to Wang *et al.* [26]. In brief, a known volume of Cys-CdS QD solutions was diluted up to 0.5 ml using PBS followed by addition of 15 µl of EDC and 20 µl of NHS and various concentrations of antibody (2, 4, 6, 8 and 10 U/ml). After convulsing vigorously, the solution was incubated at 37°C for 2 h. Finally, 5 µl of 0.01 mol/l glycine was added to it and kept overnight at 4°C. The mixture was purified using Amicon ultra centrifugal filter (Ultra-15) MWCO 30 kDa. The unbound spaces in the Ab-Cys-CdS QD were blocked using BSA by incubating the filtrate in BSA (2 mg/mL) at 37°C for 1 h.

2.3.1 Determination of CA 15.3 antigen binding onto Ab-Cys-CdS QD in PBS and spiked serum: Various concentration of CA 15.3 antigen (2, 4, 6, and 8 U/ml) was added to the PBS buffer containing a known concentration of Ab-Cys-CdS QD, respectively, and incubated at 37°C for 15 min. Similar to PBS, experiments were also carried out using CA 15.3 antigen spiked serum. In brief, spiked serum was prepared by separating serum from blood collected from control volunteered female donors. The collected whole blood was allowed to clot and further centrifuged at 2000 rpm for 15 min. The supernatant (serum) was collected and maintained at 2–8°C till further use. The serum was spiked with various concentration of CA 15.3 antigen to it. The binding efficiency of CA 15.3 antigen onto Ab-Cys-CdS QD in PBS and spiked serum was evaluated using UV-VIS spectroscopy and PL spectrophotometry.

2.4 Characterisation methods

All the samples prepared were characterised using the following techniques. UV-VIS spectra of the samples were obtained using T90+ UV/Vis spectrophotometer over a wavelength range from 300 to 800 nm with PBS as reference. The FTIR spectroscopic analysis was carried out using IR Affinity FTIR spectrometer (Shimadzu, Japan) at a scan range of 800–4000 cm^{-1}. The TEM images were acquired with JEOL-JEM 2100, Japan, at an accelerating voltage of 200 kV for QD and 80 kV for antibody tagged QD followed by elemental analysis using EDS (Oxford Instruments, UK). TEM specimens were prepared on a 3 mm carbon coated copper grid and subsequently dried at room temperature for 15 min before analysis. PL spectra were measured at room temperature using PL spectrophotometer (Shimadzhu Sluro Max, Japan) for quantum dot, antibody tagged and antigen conjugated QD. PL studies were performed at the excitation wavelength of 350 nm and emission scan were measured from 360 to 600 nm.

3 Results and discussions

The process of CA 15.3 detection using CdS QD is illustrated in (Fig. 1). Cys-CdS QD and Ab-Cys-CdS QD were successfully synthesised as per the given protocol (Fig. 2) and were characterised for its properties. Cysteamine was used as a capping agent since it has bifunctional ligands such as thiol and hydrophilic amine groups. The thiol group can strongly bind with Cd^{2+} on the QDs surface and amine group is responsible for achieving water-compatibility of the QDs [27].

3.1 UV-VIS spectroscopy

The UV-VIS spectrum of CdS-QD exhibited absorption edge at 470 nm which is relatively shifted towards blue spectra than that of bulk CdS particles (515 nm) [28]. The excitation wavelength at 360 nm coincided with Noipa *et al.* [25] (Fig. 3*a*). The shift in the absorption peak indicates proper tagging of antibody to Cys-CdS QD. CA 15.3 antibody tagged Cys-CdS QD shows peak shift from 360 to 290 nm, indicating the surface modification of Cys-CdS QD by antibody. Likewise, CA 15.3 antigen conjugated to antibody tagged Cys-CdS QD showed further peak shift from 290 to 260 nm confirming the antigen interaction with antibody [29]. Similar peak shifts were reported by Dwarakanath *et al.* [30] and Zekavati *et al.* [31]. As evident from Fig. 3*b*, antibody (2, 4, 6, 8 and 10 U/ml) tagged with Cys-CdS QD showed gradual increase in peak intensity at 290 nm with increase in the concentration of antibody and substantial quenching of QD emission (Fig. 3*b* insert) indicating possible interaction between Cys-CdS QD and CA 15.3 antibody. Further, on addition of different concentrations of antigen (2, 4, 6 and 8 U/ml), the peak intensity increased with increase in antigen concentration (Fig. 3*c*). When the concentration of antigen was increased, the protein sequence of antigen and QD absorbs more energy leading to increase in absorbance [32].

3.2 FTIR analysis

The surface functional groups involved in the tagging of antibody to Cys-CdS QD were identified using FTIR spectroscopy (Table 1). The Cys-CdS QD showed the characteristic peak at 1640 cm^{-1} corresponding to C = O stretching of amide group present on the surface of the QD (Fig. 4). Similarly, Ab-Cys-CdS QD peak at 1640 cm^{-1} remained unchanged indicating that the C = O in amide group is not involved in the tagging process. The peak shift from 1531 cm^{-1} in Cys-CdS QD to 1547 cm^{-1} in Ab-Cys-CdS QD indicated NH_2 scissoring mode. New peaks obtained in Ab-Cys-CdS QD at 1551 and 1523 cm^{-1} indicated the formation of amide bond. The peak shift from 1489 to 1493 cm^{-1} showed the formation of C-H bond for the Ab-Cys-CdS QD. The above results

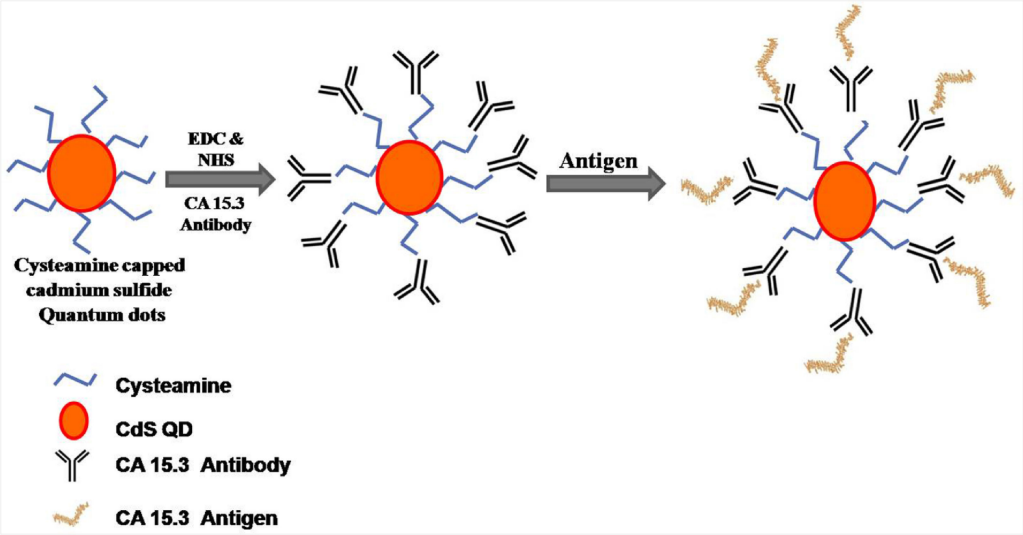

Fig. 1 *CdS-QD for optical detection of CA 15.3*

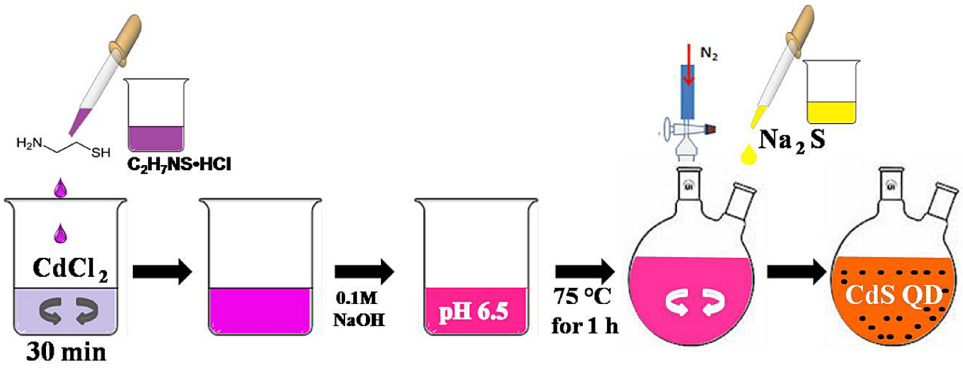

Fig. 2 *Schematic representation of CdS quantum dot synthesis*

confirm the formation of amide bond between Cys-CdS QD and Ab-Cys-CdS QD.

3.3 Microscopic analysis

Fig. 5 shows TEM image of synthesised Cys-CdS QD and Ab-Cys-CdS QD. The shape of the Cys-CdS QD particles was close to spherical and the particle size distribution of QD were found to be relatively uniform (Fig. 5*a*). The average particle size was calculated to be 3.02 ± 0.6 nm. Initially, slight aggregation of CA 15.3 antibody tagged Cys-CdS QDs due to inter particle interactions was observed (Fig. 5*b*). Hence the antibody tagged nanoparticles were sonicated for 2 min before use to avoid such aggregations. The phase contrast microscopic images (Fig. 6) indicated that higher is the antigen concentration, greater is the fluorescence. This may be due to the internal fluorescence of protein molecules bound to the QD [30].

3.4 Determination of CA 15.3 antigen binding onto Ab-Cys-CdS QD in PBS

PL spectra of control Cys-CdS QD (Fig. 7) showed excitation peaks at 404 nm, 437 nm, 546 nm. When CA 15.3 antibody was tagged to Cys-CdS QD, the spectrum exhibited additional peaks at 432 and 457 nm with reduction in peak intensity. The presence of new peak could arise from the defect state appearing between the conduction and valence band that could quench the fluorescence of QD [33]. Once antigen-antibody interaction was induced, the PL spectra showed additional peaks along with peak broadening at 455, 467 and 493 nm. The peak at 546 nm disappeared after antigen antibody interaction. Antigen interactions to Ab-Cys-CdS QD were monitored at different concentration. The fluorescence intensity was found to increase as a function of concentration of antigen. This may be due to the aromatic amino acids present in the amino acid sequence of antigen leading to enhanced fluorescence [34]. The spectrum of Ag-Ab-Cys-CdS QD (2 U, 4 U and 6 U/ml of antibody) in Figs. 8*a–c* showed no uniformity in peak shift. Although it showed drastic differences in the peak positions, the intensity of fluorescence emission was observed to be increasing with increase in antigen concentration. In case of Ag-Ab-Cys-CdS QD (8 U and 10 U/ml of antibody) (Figs. 8*d* and *e*) fluorescence intensity was found to increase with concentration of antigen uniformly. Ag-Ab-Cys-CdS (10 U/ml of antibody) showed broadening of peak along with high intensity according to increase in the antigen concentration. From these results, it can be concluded that an optimised concentration of 10 U/ml of antibody on CdS QD is required to detect CA 15.3 antigen at a concentration ranging from 2–8 U/ml in saline buffer.

3.5 Determination of CA 15.3 antigen binding onto Ab-Cys-CdS QD in spiked serum

In continuation of saline based experiments, the detection of spiked CA 15.3 antigen in serum samples was carried out using CA 15.3 antibody tagged CDS QD. From the fluorescence study, 10 U/ml antibody tagged CdS QD showed optimum emission capacity and hence was chosen for further study in serum. Ab-Cys-CdS QD was allowed to react with CA 15.3 spiked serum samples for 15 min. The PL intensity of the Ag-Ab-Cys-CdS QD in spiked serum is given in Table 2. The fluorescence emission increased with increase in CA 15.3 concentration in serum. The result also indicates that there is a minimum interference of the serum components on the emission profile. At higher antigen concentration (8 U/ml) the fluorescence emission was distinct. This study revealed that Ab-Cys-CdS QD can detect CA 15.3 tumour marker even at very low concentration of 0.002 KU/L. Our results are superior to the previous literatures in terms of sensitivity (Table 3). In addition, CA 15.3 biomarker detection was validated

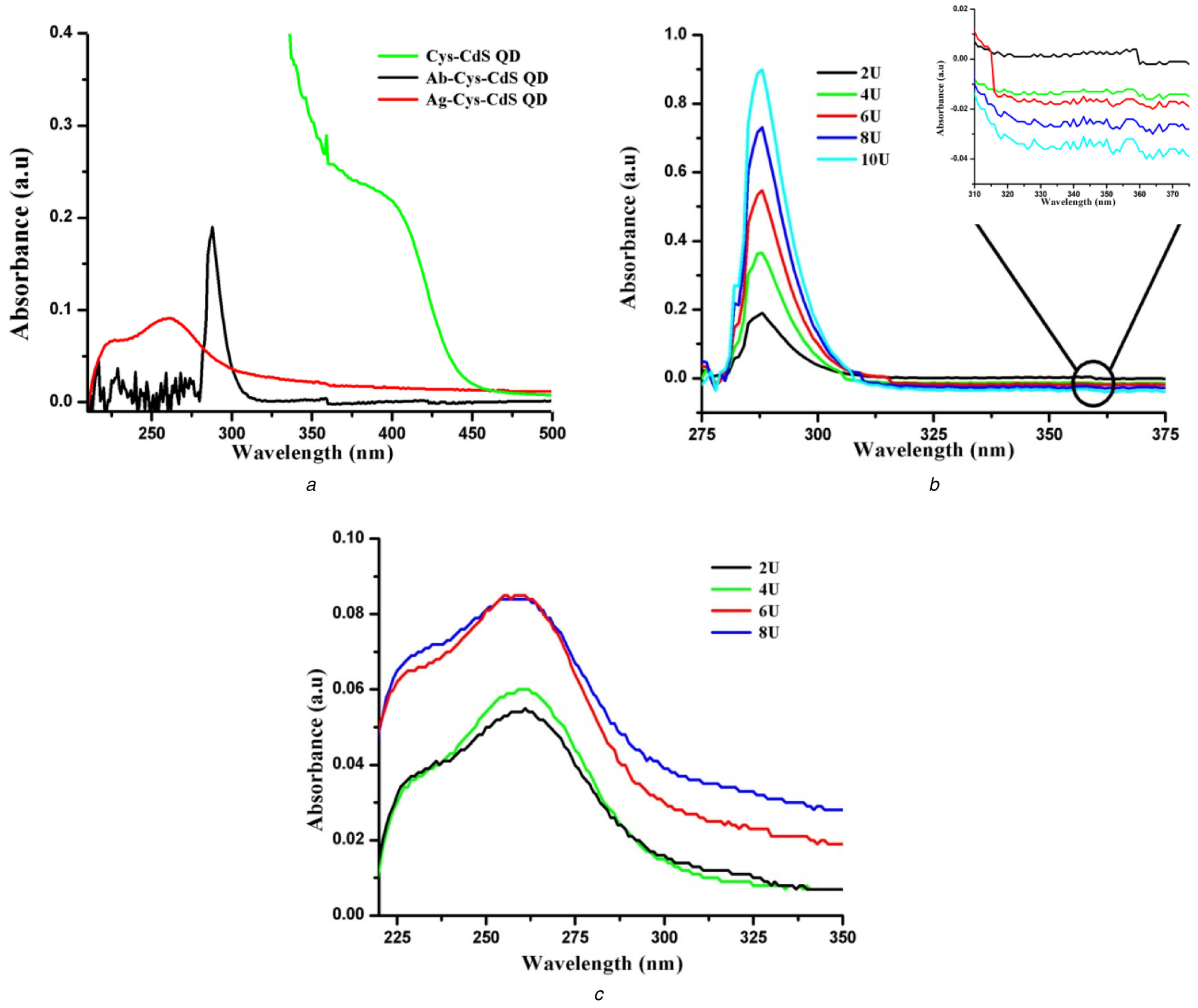

Fig. 3 *Absorbence spectra of*
(a) Synthesised Cys-CdS QD, Ab-Cys-CdS QD and Ag-Ab-Cys-CdS QD, ***(b)*** Ab-Cys-CdS QD with five different concentration of antibody from 2 U/ml to 10 U/ml, ***(c)*** Ag-Ab-Cys-CdS QD with four different concentration of antigen from 2 U/ml to 8 U/ml

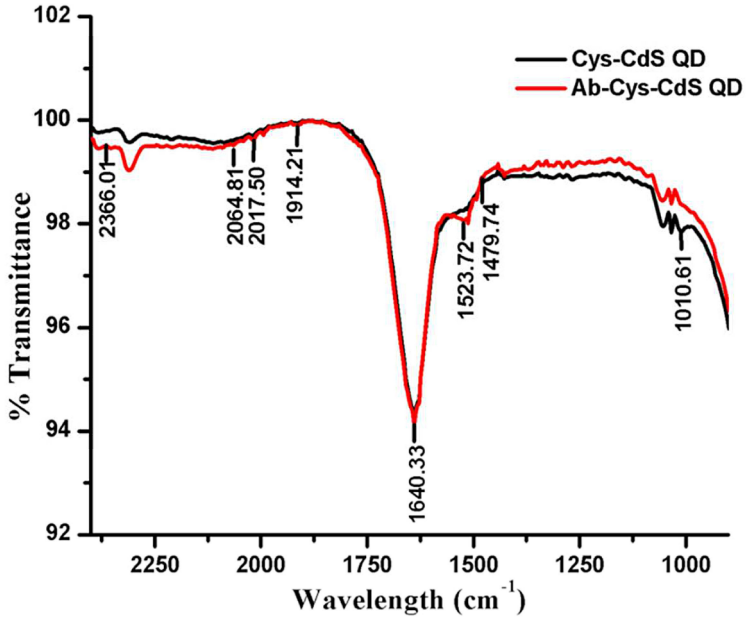

Fig. 4 *FTIR spectra of Cys-CdS QD and Ab-Cys-CdS QD at 10 U/ml concentration*

with respect to healthy individuals and cancer patients. The concentration of CA 15.3 for normal and cancer patients for the detection were fixed based on available literature data (normal individuals: 25–30 U/ml; cancer patients: above 40 U/ml in serum) [10, 11]. The results (Fig. 9) showed high linearity during CA 15.3 detection using Ab-Cys-CdS QD in both normal individuals (R^2: 0.999) and in cancer patients (R^2: 0.993) indicating that this QD based detection method is viable for practical applications. For better understanding of the methodology developed in our study

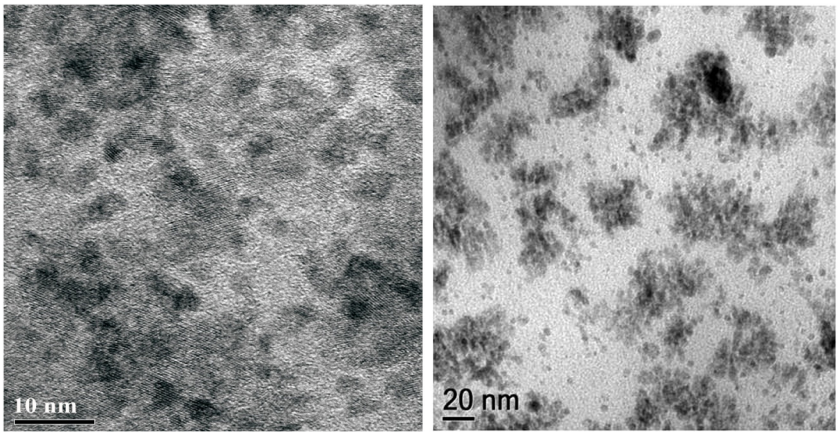

Fig. 5 *TEM images of*
(a) Cys-CdS QD and, ***(b)*** Ab-Cys-CdS QD at 10 U/ml concentration

Table 1 FTIR peak assignment for CysCdS QD and Ab-CysCdS QD

Peak frequency, cm^1		Peak assignment
Cys-CdS QD	Antibody tagged Cys-CdS QD	
1640	1640	C = O
2016	2017	
	1913	
	1551	N-H bend
1531	1547	N-H bend
	1523	N-H bend
	1493	H-C-H bend
1010		C-O stretch
1489	1493	amide bond

using QD, a comparative illustration has been provided with already reported automated kit method (Fig. 10).

4 Conclusion

A QD based assay was developed for optical detection of CA 15.3 biomarker in buffer and spiked human serum. This study revealed that Ab-Cys-CdS QD can detect CA 15.3 biomarker even at very low concentration of 0.002 KU/L. The developed QD based assay exhibited higher sensitivity and linear detection response towards spiked CA 15.3 antigen with high stability and reproducibility. The proposed study can be used as a simple, low cost and technically undemanding assay which can be an effective replacement to expensive automated assays.

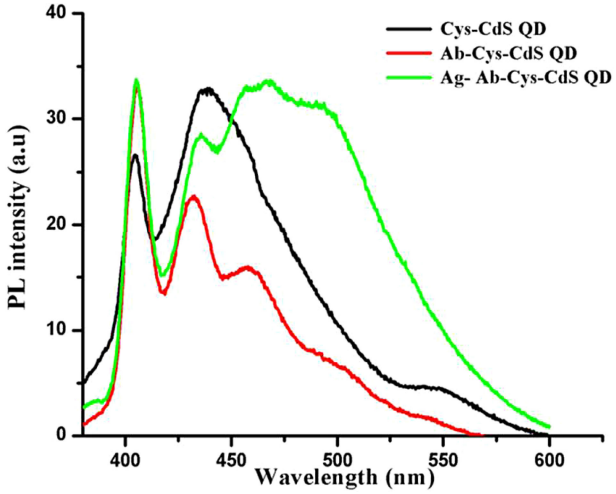

Fig. 7 *PL spectra of Cys-CdS QD, Ab-Cys-CdS QD at 10 U/ml antibody and Ag-Ab-Cys-CdS QD at 10 U/ml of antibody and 8 U/ml of antigen*

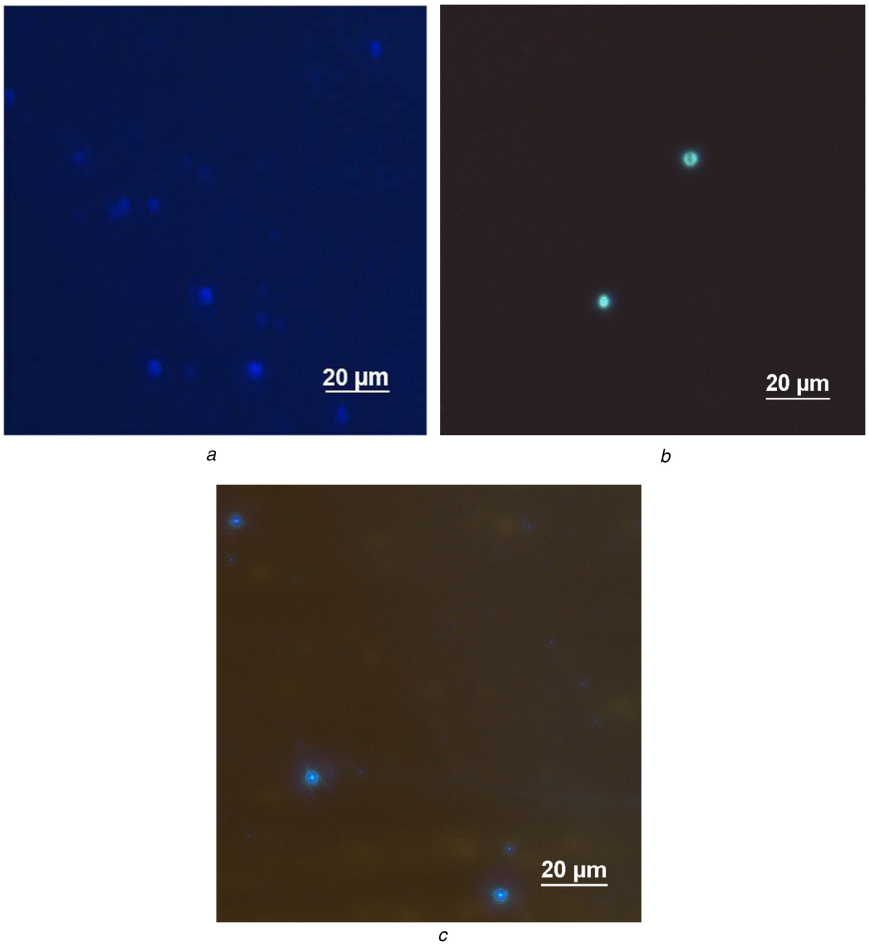

Fig. 6 *Phase contrast inverted epi fluorescence microscopic images of*
(a) Cys-CdS QD, ***(b)*** Ab-Cys-CdS QD at 10 U/ml and, ***(c)*** Ag- Ab-Cys-CdS QD at 10 U/ml of antibody and 8 U of antigen

Table 2 PL intensity of CA 15.3 spiked serum sample at different concentrations

Sl. no.	Sample	PL intensity, a.u
1	control serum	98.00 ± 0.577
2	serum + 2 U/ml	147.59 ± 1.509
3	serum + 4 U/ml	155.91 ± 0.214
4	serum + 6 U/ml	177.31 ± 0.878
5	serum + 8 U/ml	318.71 ± 1.154

Table 3 Comparison of CA 15.3 detection limit with earlier literature

Sl. no	Nanoparticle	Sensor	Detection limit	Reference
1	gold nanoparticle	electrochemical sensor	0.01- 0.200 KU/L	[35]
2	gold nanoparticle	optical sensor	0.01-0.5 KU/L	[36]
4	magnet core/ shell NiFe2O4/ SiO2 nanoparticles	electrochemical	0.5 KU/ml	[37]
5	gold nanoparticle	nanochannel-based immunoassay	52 U /ml	[38]
6	CdS QD	optical sensor	0.002 KU/L	current study

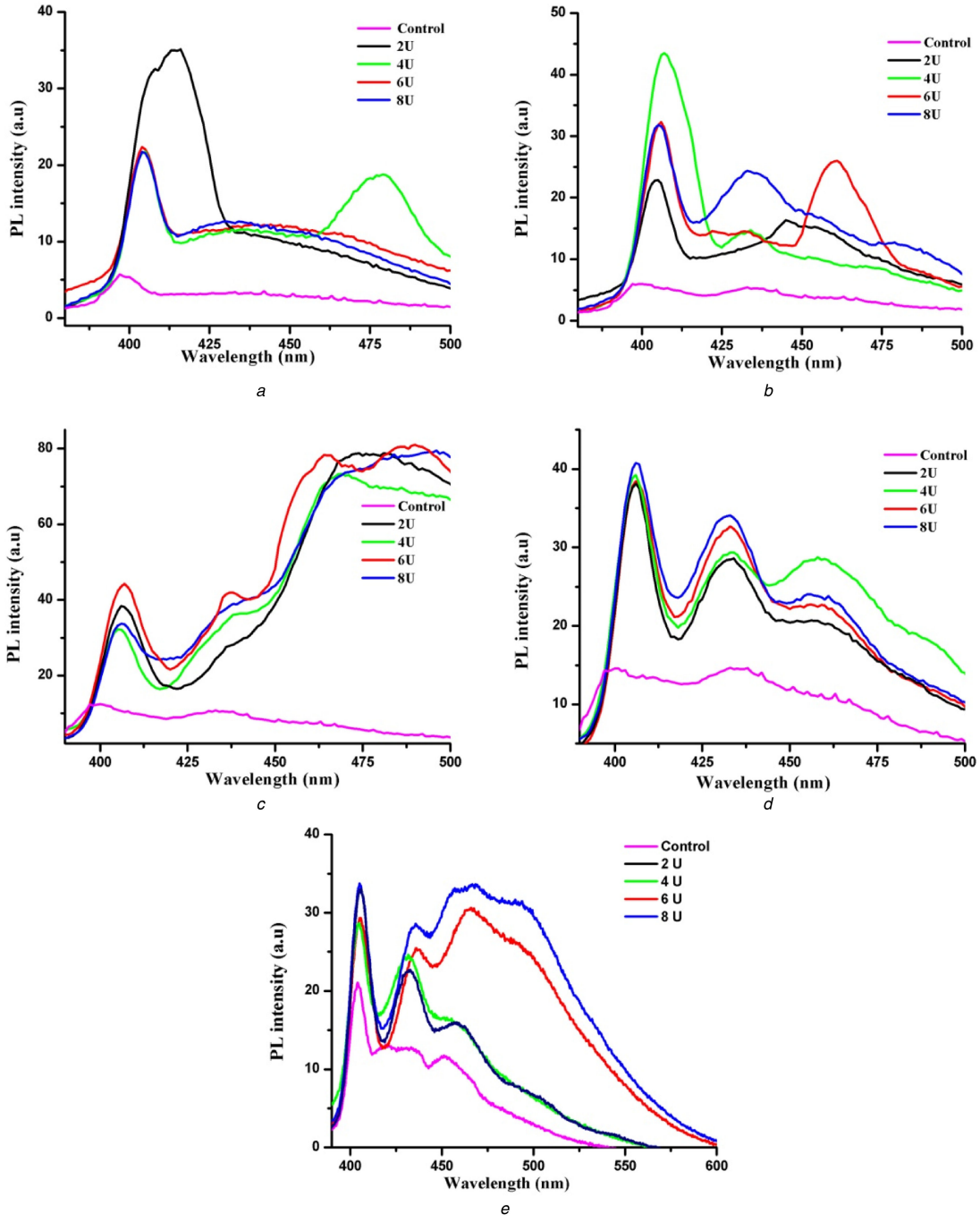

Fig. 8.

Fig. 8 *PL spectra of*

(a) Ab-Cys-CdS QD (2 U/ml antibody) with different concentration of antigen, ***(b)*** Ab-Cys-CdS QD (4 U/ml antibody) with different concentration of antigen, ***(c)*** Ab-Cys-CdS QD(6 U/ml antibody) with different concentration of antigen, ***(d)*** Ab-Cys-CdS QD (8 U/ml antibody) with different concentration of antigen, ***(e)*** Ab-Cys-CdS QD (10 U/ml antibody) with different concentration of antigen

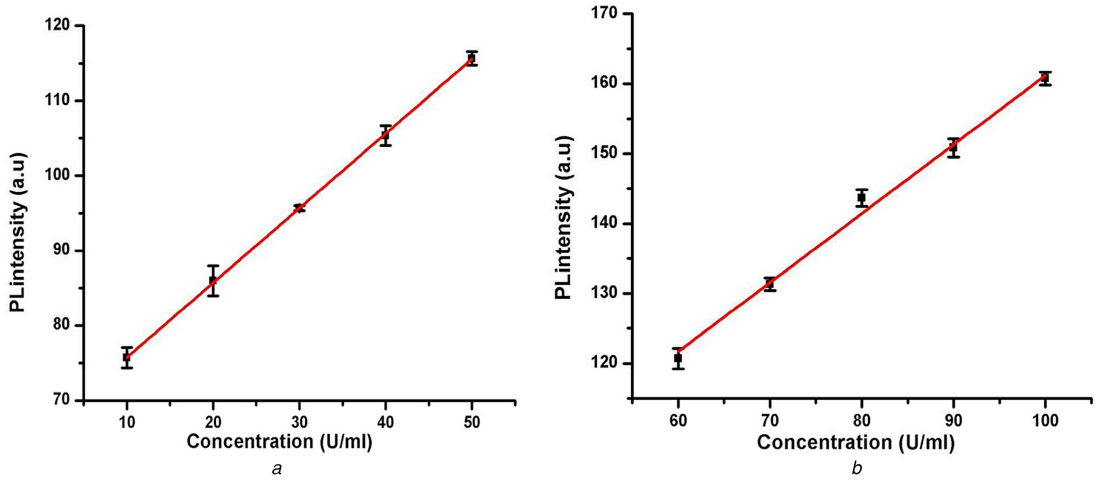

Fig. 9.

Fig. 9 *Validation of QD based detection using CA 15.3 concentration range present in* ***(a)*** *healthy individuals,* and ***(b)*** cancer patients

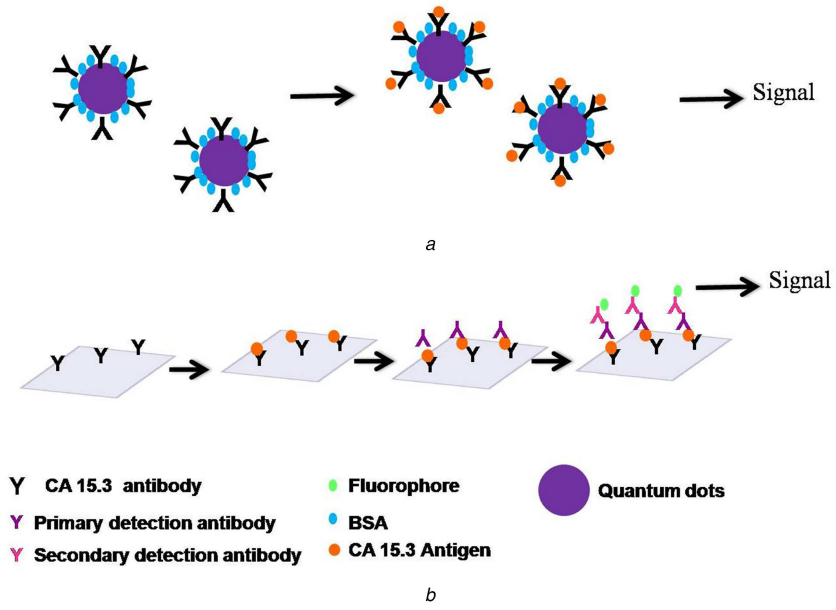

Fig. 10.

Fig. 10 *Comparison between QD*

(a) and automated kit, ***(b)*** for the detection of cancer biomarkers

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