Ultrasensitive Detection of Neurotransmitter in Neurological Disorders

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Abstract

Psychological or neurological disorders have worldwide prevalence of 10-13 %. In Pakistan the situation is equally alarming affecting 10-16% of the population. Among all the neurological diseases the most prevalent one in Pakistani population are: depression (6%), schizophrenia (1.5%) and epilepsy (1-2%). Dopamine, a critical neurotransmitter plays an important role in onset and progression of aforementioned disorders. Normal dopamine levels in human blood are 0 to 30 pg/mL (195.8 pmol/L). A little variation in its levels can cause onset of depression and various neurological diseases. Conventional dopamine detection techniques are laborious and time consuming that's why less commonly practiced in Pakistani clinics. We propose an immunoassay chip offering optical detection of different log dilutions (1/10, 1/100 and 1/1000) of dopamine using CdSe/ZnS quantum dots (QDs). Elucidation of fluorescence response of different dilutions of quantum dot conjugated dopamine antibody revealed maximum fluorescence intensity at 1/10 dilution. This demonstrates the efficiency and sensitivity of the current method. The current study would be revolutionary in proposing a timely diagnosis of dopamine levels to patients suffering from neurological problems. Similarly, facilitating timely diagnosis and personalized treatment for patients with neurological disorders.

Keywords: CdSe/ZnS QD; dopamine; fluorescence; immunoassay chip; optical.

Introduction

The brain is the body's main controlling center that controls behavioral changes under the external and internal influences of the body. Behavioral changes are the result of neuronal networks in the body. Neurons communicate with each other through neurotransmitters [1]. Neurotransmitters are chemical messengers between neurons; connecting nerve cells by exciting and inhibiting them. Among fifty neurotransmitters, the most commonly studied and identified are categorized as excitatory (acetylcholine and nor epinephrine) and inhibitory (GABA, dopamine and serotonin) [2]. Any variation in the levels of these neurotransmitters can affect the mental health of the individual. Neurological disorders have 10-13 % prevalence globally while affecting 10-16% of the population in Pakistan. Among all neurological diseases, the most prevalent ones in the Pakistani population are: depression (6%), schizophrenia (1.5%), and epilepsy (1-2%). Among all the neurotransmitters, dopamine is a catecholamine that has a pivotal role in various biological systems such as the central nervous system, cardiovascular, gastrointestinal, hormonal and renal [3-4]. In the brain, dopamine is involved in multiple functions like motivation, movement, memory, cognition, sleep, memory, and learning. It can also act as a precursor in the biosynthesis of other neurotransmitters like epinephrine and nor epinephrine [5]. Dopamine levels play an important role in the body and have a normal range of 0–30 pg/mL (195.8 pmol/L) [6]. Any variation from the mentioned range can cause problem, like level below this range can cause cardiotoxicity [7], and higher levels of dopamine can cause various psychological issues like: Parkinson's disease [8], Schizophrenia [9], Alzheimer's disease [10], and depression [11]. It is very important for clinical psychologists to measure the levels of dopamine in the blood of patients with any neurological issue. Conventional dopamine level detection techniques are: enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC) and mass spectrometry (MS). However, these techniques are time consuming, laborious, and require skilled personnel [12-13].

With the advent of nanotechnology, it has revolutionized every field of science, especially the biomedical science/healthcare sector. Nanotechnology-based dopamine detection techniques offer robust, timely, and easy-to-use diagnosis. Several studies have reported dopamine detection using different nanomaterials [15] such as optical, colorimetry, surface enhanced Raman spectroscopy (SERS), and electrochemical sensors [14-16]. Electrochemical detection of dopamine has been reported in various studies as they give improved selectivity and sensitivity. Major drawback of electrochemical detection is laborious electrode preparation step [17] Colorimetry technique given poor sensitivity is unable to detect lower concentrations of dopamine [14]. SERS based detection involves trained personnel and extensive step of preparation of Raman reporter [22]. Optical detection method is only technique which requires low amount of sample, little preparation, gives faster turnaround and does not require any trained personnel. Among all nanoparticles, quantum dots are more preferred for detection due to sensitivity, photo stability and biocompatibility [18]. Considering the advantages of optical detection methodology, the current study was designed. Current research elucidates optical response of nanoparticles (quantum dot) conjugated with dopamine antibody followed by detection of different log dilutions of dopamine in buffer.

Materials & Methods

Chemicals

Dopamine rabbit polyclonal antibody (200µl) was purchased from Abcam (Cambridge, UK). MAA (99%) from Sigma-Aldrich. Polyclonal digoxin Ab was purchased from Abcam, and 100 mM phosphate buffer saline (PBS) pH 7.4 ± 0.1 was obtained from bioWORLD. Phosphate buffer saline (PBS) pellets, N-(3- Dimethylaminopropyl)-N′- ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), dopamine hydrochloride, thioglycolic acid/ mercaptoacetic acid (MAA), chloroform, bovine serum albumin (BSA), ethyl alcohol, isopropanol, and potassium chloride were purchased from Sigma-Aldrich (United states, St. Louis). CdSe/ZnS quantum dots with an emission wavelength of 665 nm were purchased from PlasmaChem GmbH (Berlin, Germany).

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Experiments

A- Biofunctionalization of QDs:

CdSe/ZnS dots (1-2mg, 250 µl, 6 nm) dispersed in chloroform were stirred with 1 ml MAA (1M) overnight. Twenty-four hours later, phosphate buffer saline (PBS) buffer (pH 7.2) (1:1 by volume) was added to the solution of MAA-coated dots, which were then separated and purified using centrifugation at 3000 rpm for 30 min. The purified dots were then dispersed in PBS.

B- Conjugation of MAA-coated dots with the antibody

MAA-coated dots in buffer were incubated with EDC/NHS (pick 75µl, 0.1 M) add 150µl MAA coated QDs for 2 h incubation using carbodiimide crosslinking chemistry.

After 2 h of incubation, the antibody dilutions (75µl, 1: 10, 1:100 and 1:1000 dilution in PBS) were prepared and left overnight for conjugation.

C- Carboxyl activation of Quartz slides:

For detection on immunoassay chips, quartz slides were cleaned in ethanol solution using ultrasonication. Followed by cleaning, slides were coated with MAA for carboxyl activation followed by coating with the antibody, as mentioned by Chaudhry et al. 2022.

D- Preparation of Dopamine dilutions

Different log dilutions of dopamine were prepared in PBS buffer from the stock dopamine solution (1 mg per ml in distilled water). Dilutions were prepared Dilutions (1ml) were 1:10, 1:100, and 1:1000 in PBS buffer (1 mL, 1X, pH 7.2).

E- Detection on quartz slides or immunoassay chips

Incubation of slides or chips with different dopamine concentrations in buffer. Washing with PBS followed by incubation with antibody-conjugated QDs.

Characterization

Optical Fluorescence

Optical fluorescence is an imaging technique that is highly sensitive and reliable. The light used in the optical microscope was ultraviolet light. Optical fluorescence images were acquired using an optical microscope (Olympus, Model BX61) equipped with a Texas Red filter.

Photoluminescence (PL)

Light strikes a sample; it is absorbed by imparting its excess energy to the material by a phenomenon known as photoexcitation. It is a nondestructive method for analyzing samples. The light source used in it is He-Cd laser of wavelength 325 nm.

Results & Discussions

The current study involves detection of different dilutions of dopamine using biofunctionalized QDS.

Biofunctionalization of QDs

CdSe/ZnS QDs dispersed in chloroform were made water soluble after coating with MAA (Figure 1). QDs were then finally conjugated with dopamine antibody using crosslinking chemistry.

Optical Fluorescence

Optical fluorescence images of the bare and MAA-coated CdSe/ZnS QDs are shown in Figure 2. QDs were of 645 nm wavelength, emitting red color. There is no obvious color variation in bare and MAA coated dots. MAA coated dots were then conjugated with different antibody dilutions like 1/10, 1/100, and 1/1000. Significant variation in fluorescence of QDs can be seen after conjugating with dopamine antibody (Figure 3 (a), (b) & (c)) and chemical dopamine (Figure 4 (a), (b) & (c)).

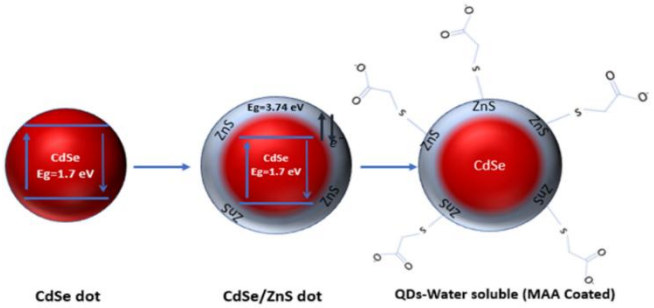


Figure 1: Scheme of bio functionalization of QDs.

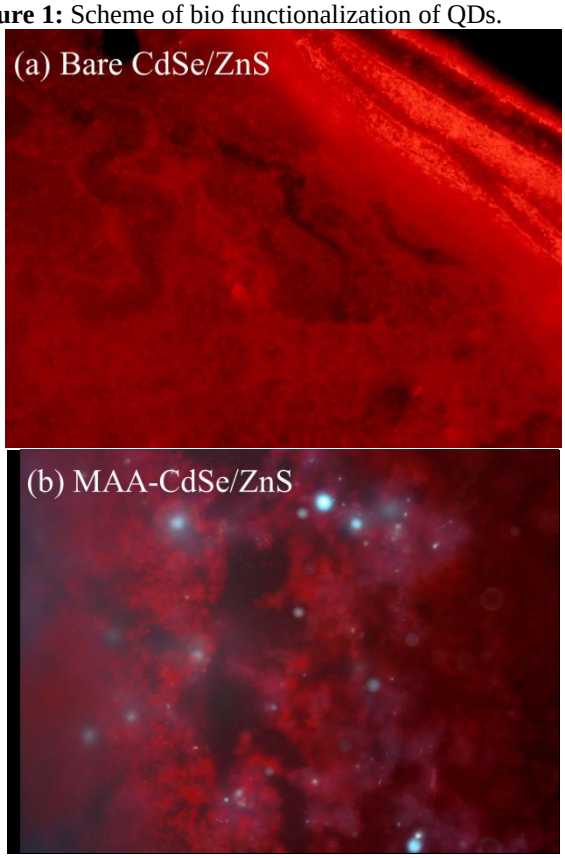


Figure 2: Optical fluorescence images of (a) bare and (b) MAA coated QDs.

PL spectroscopy

The attachment of MAA to QDs was also confirmed through photoluminescence spectroscopy (PL) Figure 5. The PL spectrum showed the emission peak of bare CdSe/ZnS QDs in chloroform, which was at 645 nm and blue-shifted to 640 nm for MAA-coated QDs. There is a shift to lower wavelengths termed the blue shift; there no obvious variation in color of dots (Figure 2 (b)). This apparent blue shift appears to be due to a decrease in surface charges and passivation of surface defects due to MAA coating on the surface of QDs [19-21].

Another possible reason for this blue shift could be the decrease in the size of the QDs after coating with MAA. Bare QDs are coated with TOPO, and after coating with MAA; MAA replaces TOPO, and its size is less than that of TOPO. This could also be a reason for the decrease in the size of the dots, which ultimately leads to blue fluorescence emission and a shift in PL.

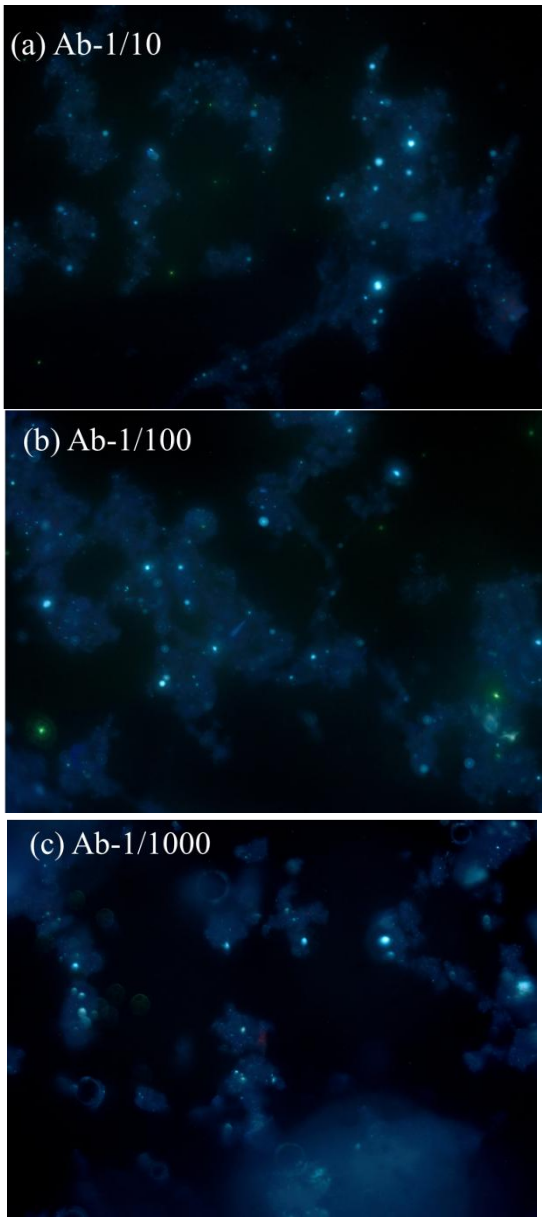


Figure 3: Optical fluorescence images of different antibody dilution shows (a) 1/10 (b) 1/100 and (c) 1/1000 at 20x.

There is a shift in wavelength from red to blue as TOPO coated dots replaced by MAA. According to quantum dot theory, size of the dot is directly proportional to the wavelength. Larger size quantum dots emit larger wavelengths (red) as compared to smaller size emitting lower wavelengths of light. [18][23]This blue shift originated from the quantum dot confinement effect as the sizes of nanoparticles decreased below Bohr's radius. [22-24]

The energy band differences of bare CdSe/ZnS QDs and pure MAA with respect to the normal hydrogen electrode (NHE) scale are 1.74 eV and 1.66 Ev [21]. This clearly demonstrated that the attachment of MAA would result in a reduced effective band gap of QDs. It should be noted here that the HOMO level of MAA lies above the VB edge of CdSe/ZnS QDs, which acts as a Lewis base or electron-donating center. On the other hand, the LUMO level lies below the CB edge of CdSe/ZnS QDs, which acts as a Lewis acid or an electron acceptor. [22-24]

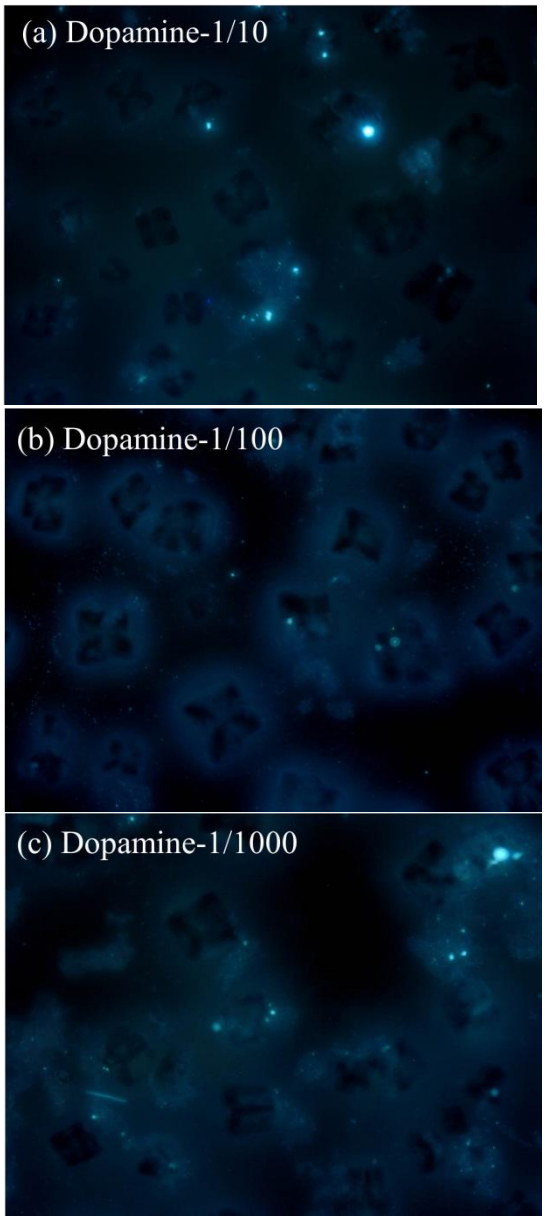


Figure 4: Optical fluorescence images of different dopamine dilutions shows (a) 1/10 (b)] 1/100 and (c) 1/1000 at 20x.

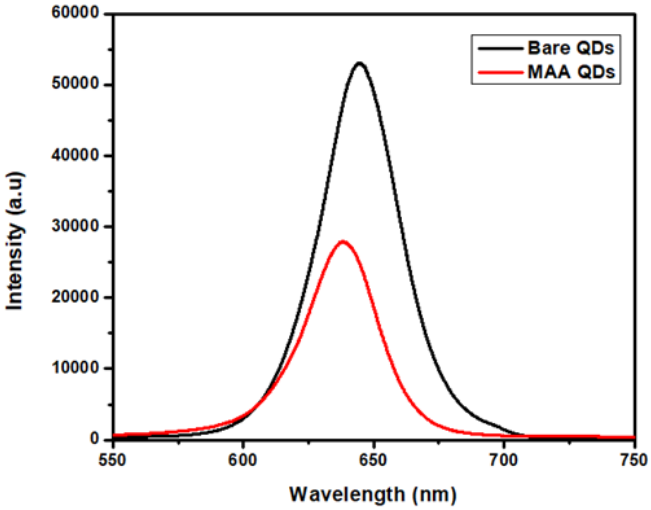


Figure 5: PL Spectra of bare and MAA coated QDs

Figure 6 (a) shows the PL spectra of QD-conjugated antibody dilutions like 1/10,1/100 and 1/1000 and (b) comparison with MAA-coated QDs. Both spectra (a) and (b) show that maximum fluorescence was obtained using 1/10 dilution of the antibody. Figure 6 (b) also delineates that there is a decrease in the PL of the QD-conjugated antibody with increasing dilutions. [20-22] After antibody conjugation of MAA-coated dots, there is a blue shift of MAA-coated dots from 640 nm to 635 nm for Ab 1/10 dilution followed by a decrease in intensity. For the remaining Ab dilutions of 1/100 and 1/1000, there is a red shift in the wavelength 641 nm for Ab 1/100 and 645 nm for 1/1000 nm) with a further decrease in PL. The aforementioned phenomenon could be due to the energy transfer process during the hybridization of the antibody with MAA-coated dots. Because of the biofunctionalization or hybridization of dots, luminescence and intensity are quenched, as shown in Figure 6[23].

Antibody 1/10 dilution with maximum fluorescence is used for detection of different log dilutions of the dopamine chemical on an immunoassay chip. Figure 7 shows the increasing PL trend with increasing dopamine concentration. This could be due to the passivation of surface defects created during Ab conjugation or the charge transfer mechanism between the antibody and the dopamine molecule. [18,21-24]

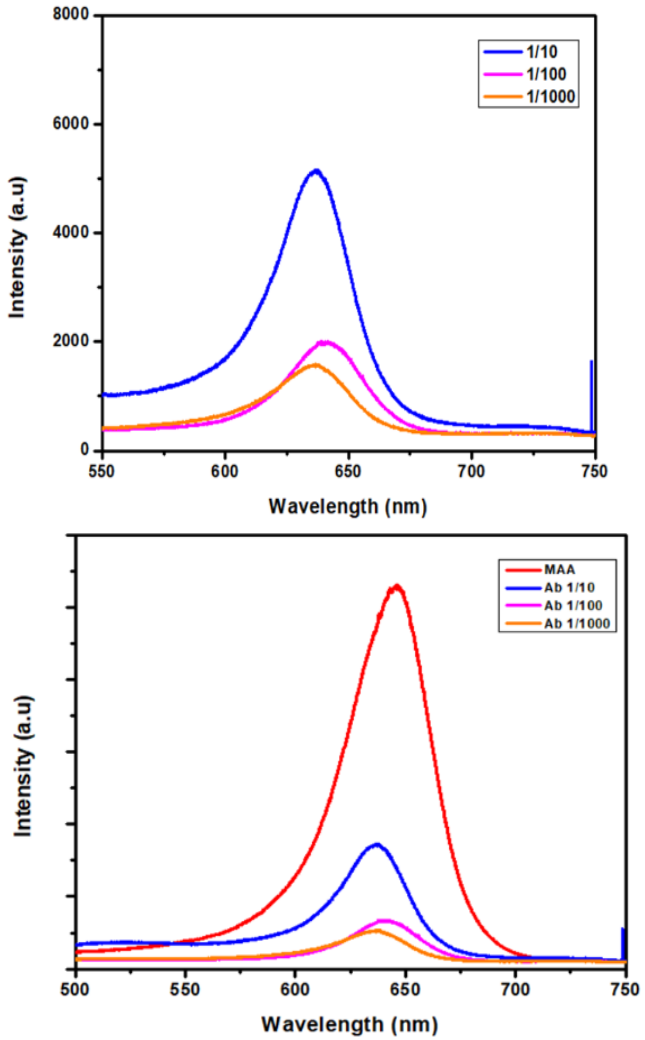


Figure 6: PL spectra of (a) different log dilutions of dopamine antibody (Ab) after conjugation with QDs, (b) comparison of intensities of log dilutions of dopamine with MAA coated or biofunctionalized QD.

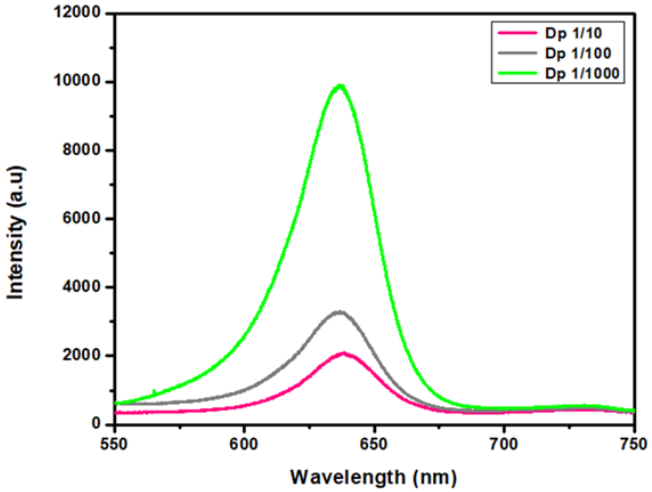
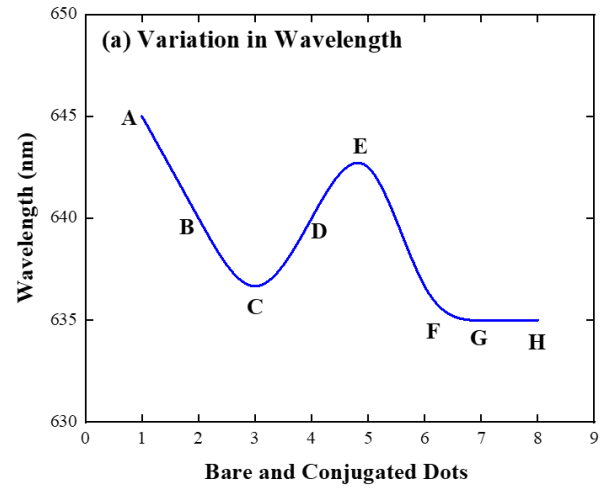


Figure 7: Detection of different log dilutions of dopamine chemical spiked in buffer using QD conjugated 1/10 dilution of dopamine antibody

Exploration of the chemical phenomenon occurring on the surface of the dots is shown in Figure 8 (a, b &c). Conjugation of bare CdSe/ZnS dots with MAA revealed a blue shift in wavelength, as shown in Figure 6 (a), along with a decrease in PL of MAA coated dots. In the case of full width half maximum (FWHM), there is an increase in the width of MAA-coated dots due to aggregation caused by crosslinking of the stabilizer molecules with themselves and the QDs as well [24].The similar trend was observed by Zhu et al., 2014, after coating CdSe/ZnS dots with thiol they observed increasing trend in FWHM of dots within a period of 2 hours. [24]

On conjugation with various dilutions of Ab, there is a further blue shift of MAA-coated dots from 640 to 636 nm. However, for the Ab 1/100 and 1/1000 dilutions, a red shift in wavelength was observed at 645 nm for the Ab 1/1000 dilution, as shown by the bare dots. This could be due to attachment of Ab molecules to the QD surfaceand increasing surface defects... Followed by the decrease in FWHM; Peng et al., 2023 also found that on increasing concentration of polymer, there is decrease in FWHM possibly due to re-absorption effect on surface of dot. [22] The decreasing trend in intensity and FWHM continues for all Ab dilutions, as shown in Figure 8 (b). However, as soon as the Ab-coated dots were coated with different dopamine dilutions, we observed a blue shift in intensity that remained constant for all dopamine dilutions (Figure 8(a)). Following the blue shift, there was a slight improvement in the PL intensity, as shown in Figure 8(b), with maximum fluorescent intensity at 1/1000 dilution of dopamine. Another important phenomenon to notice is the width of dots; which increases drastically after MAA coating and then decreases after Ab coating but is still less than the width of the bare dots. However, after dopamine coating, there is an increase in the width, similar to bare dots. This figure shows the interaction of charge carriers with the CdSe/ZnS dots. This increase in the width of the dots after conjugation with dopamine is the same as that of the bare dots. It justifies that defects created after MAA coating are stabilized with dopamine coating or it could be as quoted earlier that for 1/1000 dilution, there is detachment of biomolecules from the surface of dots. [22-24].



KEY:
A-CdSe/ZnS
B-CdSe/ZnS-MAA
C-CdSe/ZnS-MAA-Ab (1/10)
D-CdSe/ZnS-MAA-Ab (1/100)
E-CdSe/ZnS-MAA-Ab (1/1000)
F-CdSe/ZnS-MAA-Ab-Dp (1/10)
G-CdSe/ZnS-MAA-Ab-Dp (1/100)
H-CdSe/ZnS-MAA-Ab-Dp (1/1000)

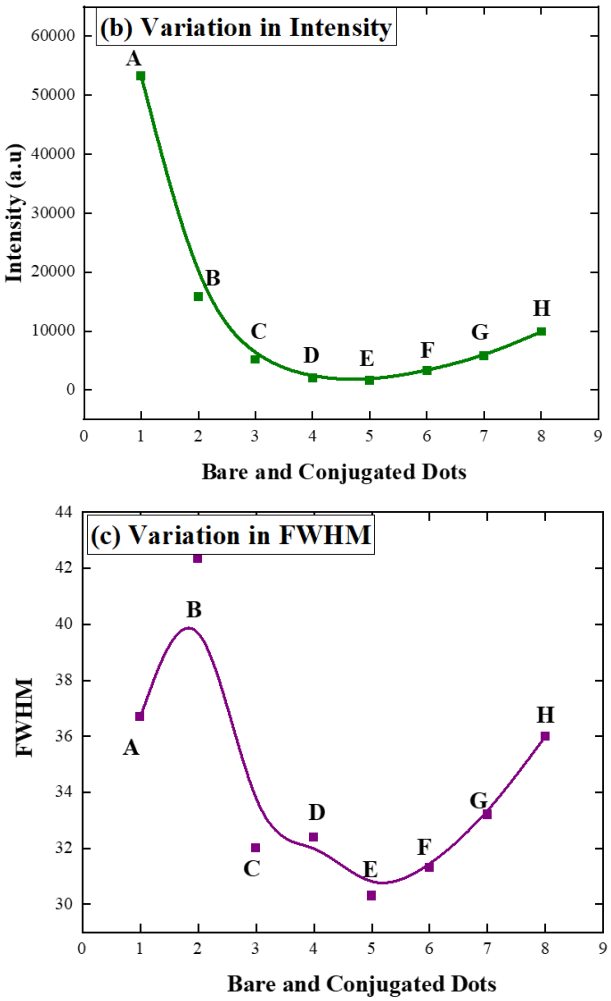


Figure 8: Graphs showing variation in (a) Wavelength, (b) Intensity and (c) Fill width half maximum of bare and biofunctionalized QDs.

Conclusion

Psychological disorders are the most prevalent in Pakistan with no immediate diagnosis. In current stud an immunoassay chip offering on chip detection of dopamine has been proposed. Water soluble CdSe/ZnS dots are biofunctionalized with different log dilutions of dopamine antibody. After finalizing 1/10 dilution exhibiting maximum fluorescence; different log dilutions of dopamine were detected on immunoassay chip. An increasing trend in fluorescence along with concentration of dopamine has been observed detecting lowest 0.1 dilutions. The QDs based detection offers more sensitivity as compared to conventional techniques. Current study envisages importance of development of an immunoassay chip for on spot diagnosis of neurotransmitters especially dopamine in patients with neurological issues.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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