

Quantum Dots as Biosensors in the Determination of Biochemical Parameters in Xenobiotic Exposure and Toxins

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Quantum dots (QDs) have been exploited for a range of scientific applications where the analytes can be expected to have significant photoluminescent properties. Previously, the applications of QDs as nanosensors for the detection of toxics in biospecimens, especially in cases of poisoning, have been discussed. This review focuses on the applications of QDs as biosensors for the detection of phytotoxins, vertebrate and invertebrate toxins, and microbial toxins present in biospecimens. Further, the role of QDs in the measurement of biochemical parameters of patient/victim as an indirect method of poison detection is also highlighted.

Keywords Quantum dots, biosensor, toxin, clinical toxicology, biochemical parameters

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1 Introduction

The toxicants or poisons produced by artificial processes are called toxics, whereas toxins or biotoxins are biologically produced poisons for the purpose of predation and self-defense.¹ In routine chemical analytical procedures in toxicology, extraction and purification of xenobiotics from complex biological specimens is a major step.² Clinical as well as forensic toxicological analysis is usually carried out on blood,

urine, viscera, and gastric aspirate and the approach may vary from case to case, but it mainly depends on the robustness and availability of analytical methods, instrumentation and time with respect to the specimen as well as the analyte of interest.³ Most conventional analytical toxicological methods require pretreatment, viz., sample preparation and processing of complex biomatrices, extraction of the analytes of interest and purification prior to analysis, and a few of these methods are robust, reliable and reproducible even while utilizing the biological fluids and tissues as such.⁴ For the analysis of



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Table 1 Various types and characteristics of QD-based biosensors used in the determination of biochemical parameters and toxins

Analyte	QD	Receptor/active component of the sensor	Sensor/working principle	LOD	Ref.
Small analytes molecules					
pH/H ⁺ ion	CQD	Terpyridine	FL Turn-On	pH 6.5 – 9.0	16
	CdSe/ZnS modified SiO ₂	SiO ₂	QDs assisted electrolyte insulator semiconductor	pH 2 – 12	16
Blood glucose	CQD	Boronic acid	EIS	1.5 µM	17
Biothiol	Au@CQD	AuNPs	FL Turn-On	50 nM	19, 21
	CQD /Hg(II)	Hg(II)	FL Turn-On	0.06 µM	20
GSH	VS ₂ /MnO ₂	MnO ₂	FL Turn-On	0.31 µM	23
	N-CQD/ Hg(II)	Hg(II)	FL Turn-On	40 nM	24
	CQD/MnO ₂	MnO ₂	FL Turn-On	300 nM	25
ACh	CQD@RGO	RGO	PL Turn-Off	30 µM	26
	CdSe/ZnS	<i>p</i> -Sulfonato calix-4-arene	FL Turn-Off	1.0 mM of ACh gives 50% quenching	27
Large analyte molecules					
Ricin	SdAb /QD bioconjugate	DHLA	FL signal amplification	16 ngmL ⁻¹	32
	Streptavidin modified QD	Streptavidin		156 ng mL ⁻¹	32
Digoxin	GQDs	Digoxin aptamer	FL Turn-On	7.95 × 10 ⁻¹² mol L ⁻¹	34
Amanita	MIP-coated CQD	<i>N</i> -acetyl tryptophan	FL Turn-Off	15 ng mL ⁻¹	38
Scorpion venom	GQDs	Screen printed electrode	GQDs constructed on the surface carbon screen-printed electrodes	0.55 pg mL ⁻¹	46
Bee venom	CdSe/ZnS	Trypsin/Cy-3 labelled mellitin	FL Turn-Off	NA	49
Tetradotoxin or TTX	QD nanobeads	TTX-mAb conjugated AuNF	FL Turn-On	0.2 ng mL ⁻¹	53
Bacterial strains	CdTe	NA	Electron transfer	NA	59
2,6-pyridine carboxylic acid (Anthrax biomarker)	CdS	Eu ³⁺	FL Turn-On	0.2 µM	60
Botox	Carboxy QDs	BoNT/E recognition peptides	PL Turn-On	0.02 ng mL ⁻¹	63
	CdSe/ZnS	Single-chain variable fragments chromophore	FL Turn-Off	20 – 40 pM	64
<i>E. coli</i>	QD nanobeads/mAb	Magnetic separation	Separation of analyte using magneticnanobeads-mAb and then measure remaining nanobeads using QDs	3.98 × 10 ³ CFU mL ⁻¹ (pure culture), 6.46 × 10 ⁴ CFU mL ⁻¹ (ground beef)	65
Salmonella typhimurium	QD-anti-Salmonella/ <i>E. coli</i> antibody conjugates	<i>S. typhimurium</i> antibody	Separation of analyte using magneticnanobeads-mAb and then measure remaining nanobeads using QDs	10 ⁴ CFU mL ⁻¹	66
Aflatoxin B-1	QD-mAb	Antigen	FL Turn-Off	0.80 µg kg ⁻¹	70
	QB-mAb	Antigen		0.30 µg kg ⁻¹	
	TRFN-mAb	Antigen		0.04 µgkg ⁻¹	
Aflatoxin	QD-mAb	Antigen	FL Turn-Off	1.4 pg mL ⁻¹ (rice), 2.9 pg mL ⁻¹ (peanut)	71
Zearalenone	CQD	Silica based matrix	FL Turn-Off	0.02 mg mL ⁻¹	74
Aflatoxin B1	CdSe/SiO ₂ (QDNBs)-mAb	Antigen	FL Turn-Off	10 pg mL ⁻¹	76
Zearalenone				80 pg mL ⁻¹	
Deoxynivalenol				500 pg mL ⁻¹	
Fuminosin B	MPA-CdTe	Catalase regulated fluorescence quenching	FL Turn-Off	0.33 ng mL ⁻¹	77
	QD-mAb	Antigen	FL Turn-Off	2.8 mg L ⁻¹	78
Fuminosin B1	QDNBs-mAb	Antigen	FL Turn-Off	12.66 ng mL ⁻¹	79
Deoxynivalenol				2.97 ng mL ⁻¹	
Zearalenone				0.87 ng mL ⁻¹	

Abbreviations: QD, quantum dot; LOD, limit of detection; CQD, carbon quantum dots; FL, fluorescence; N-CQD, nitrogen doped carbon quantum dots; EIS, electrolyte insulator semiconductor; RGO, reduced graphene oxide; GSH, glutathione; ACh, acetyl choline; PL, photoluminescence; SdAb, single domain antibody; DHLA, dihydrolipoic acid; GQD, graphene quantum dot; MIP, molecularly imprinted polymer; mAb, monoclonal antibody; NA, not available; BoNT/E, Botulinum neurotoxin E; CFU, colony forming units; QB, quantum nano bead; TRFN, time resolved fluorescence nanobead; QDNB, quantum dot nano bead; MPA, 3-mercaptopropionic acid.

xenobiotics in biological samples, modern toxicological setups are equipped with highly sensitive, sophisticated instruments and advanced techniques, for example, GC/LC-HRMS, GC/LC-MS-MS, GC/LC-TOF-MS, LC-Orbitrap-MS, UHPLC-MS/MS, *etc.*⁵ However, these methods usually require extensive sample processing prior to analysis, and are expensive in terms of both time and resources. Furthermore, their limits of detection, especially with low quantities of analytes in the biospecimens as well as unprocessed or highly contaminated samples, are poor. There is an urgent need for the development of versatile analytical procedures that overcome the limitations of conventional testing protocols for xenobiotics, which are also eco-friendly, simple and inexpensive.

Sensor based analysis has become an unavoidable alternative in chemical and biological analysis, owing to its easy handling and real time output with excellent sensitivity and reproducibility. Quantum dots (QDs) or zero-dimensional (0-D) materials are a novelty in sensor technology. Their size is within the range of 1 to 10 nm.⁶ QDs have been adopted for all kinds of light-based applications due to their excellent optical properties.⁶ The photoluminescence (PL) of QDs is dependent on their size and surface structure. Therefore, when different ions or molecules are adsorbed, QDs undergo size and superficial changes that are reflected as PL enhancement or quenching.⁶ The observed PL changes can be interpreted for the detection of xenobiotics, or for measurement of biochemical parameters. Types of QDs include plain QDs like CdTe, carbon, and graphene, or core/shell QDs like CdSe/ZnS and CdTe/ZnS QDs.^{7,8} Among various types of QDs, graphene quantum dots (GQDs) and carbon atom-based quantum dots (CQDs) have attracted tremendous interest,⁸ since they can be prepared from natural carbonaceous sources and are distinctly non-toxic in nature.⁹

QD-based biosensors are usually wreathed with multilayers of biomolecules, *viz.*, macromolecules of amino acids, oligosaccharides, enzymes, antibodies, *etc.* and have great biocompatibility and negligible toxicity.¹⁰ QDs as biosensors have been utilized in the analysis of a variety of chemical as well as biological molecules, including toxics, toxins, and biothreat agents (bacteria and viruses).¹¹ QDs have also been used in cellular and tissue imaging analysis for biological detection.¹² In addition, these QD-based sensors have also been utilized in the detection of biomarkers of inflammation, cancers, *etc.*¹³

A systematic forensic toxicological analysis of biological specimens and crime scene articles mainly focuses on two tasks, i) detection of xenobiotics as such, toxics as well as toxins ii) determination of biochemical parameters. QDs based chemosensors and biosensors play an important role in clinical and forensic toxicological analysis. The applications of QDs as chemosensors in the determination of toxics due to gaseous, anionic, phenolic, metallic, pesticide and drug-overdose poisonings have already been discussed in detail.¹⁴

In this review, the applications of QDs as modern luminescent biosensors in the determination of biochemicals in xenobiotic exposure, and also in the analysis of toxins are summarized. This review aims to provide a better perspective for developing many more QDs based biosensors for analytical toxicology. In Table 1, the various types and characteristics of QD-based biosensors available in the literature for the determination of biochemical parameters and toxins are summarized.

2 Determinations of Biochemical Parameters

QD-based biosensors have tremendous potential for a wide

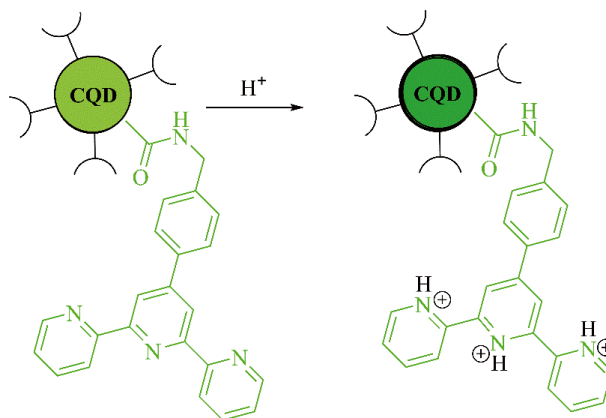


Fig. 1 Diagrammatic illustration of H^+ ions interacting with CQDs.

variety of applications, including qualitative as well as quantitative analysis of blood glucose, pH, electrolyte, blood gases, plasma enzymes, cholinesterase activity, and erythrocyte.¹⁰ These biochemical parameters are very useful in clinical toxicology cases in which the patient is alive and the poison has to be identified quickly.¹⁵ The applications of QDs as sensors in determining biochemical parameters of toxicological interest are summarized here.

2.1 pH of biospecimen

The measurement of pH has been the primary procedure in the preliminary analysis of any biochemical sample. For instance, in case of poisoning by ingestion of acids, alkalis or substances such as cyanide and phosphine, the pH of the biospecimen is a crucial clue. In this context, QDs have been found very useful in the determination of pH of the given samples.¹⁶ For example, using CQDs functionalized with terpyridine as the proton receptor, Tian and coworkers monitored the physiological pH of the solution by means of change in the emission intensity.¹⁶ The CQDs possess -OH and -COOH groups, which can be used for the covalent linking with 4'-(aminomethylphenyl)-2,2':6',2''-terpyridine (TPY) molecules. The CQD-TPY exhibits fluorescent emissions in the visible region (440 – 650 nm), when excited at 800 nm. The fluorescence (FL) intensity was found to continuously increase with the H^+ ion concentration from pH 9 to 6.5. Upon adsorption/desorption of H^+ ions, the TPY receptor molecules present on the surface of CQDs induce variations in the FL intensity (Fig. 1).¹⁶ The CQD sensor was also successfully applied for the biosensing of intracellular pH.

Maikap and coworkers devised an electrolyte insulator semiconductor (EIS) structure for the sensing of hydrogen ions concentration in a broad pH range of 2 to 12 using SiO_2/Si membrane coated with chaperonin GroEL protein and then with core-shell CdSe/ZnS QDs.¹⁶ QDs possess a larger surface area to volume ratio and show stable and better response towards pH changes than the bare SiO_2 sensors.

2.2 Blood glucose

The determination of blood glucose is routine in the clinical toxicology for the cases of coma from suicidal, accidental, malicious administration of insulin or oral hypoglycemic agents.² Hypoglycemia can also occur in the case of liver damage due to overdose of paracetamol and a blood glucose level below 40 mg/100 mL, along with a suspicious case history, could be indicative of poisoning.² A QD-based non-enzymatic glucose sensing system has been reported in the literature.¹⁷

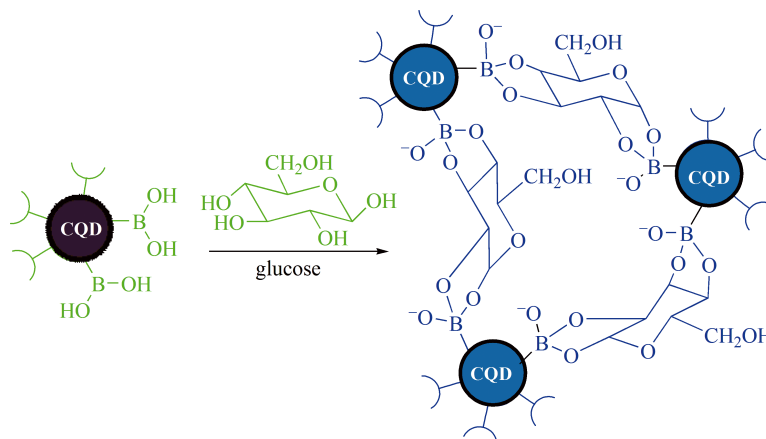


Fig. 2 Diagrammatic illustration of the interaction of glucose with CQDs.

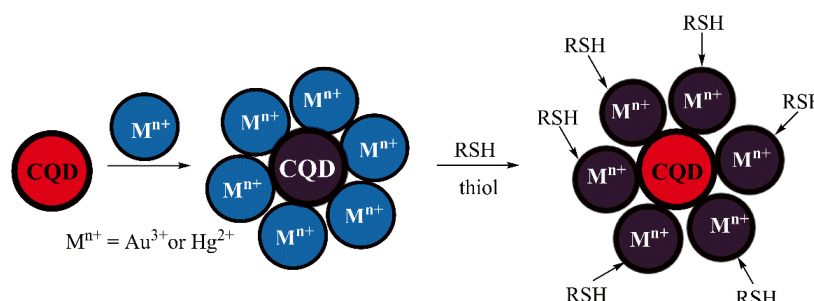


Fig. 3 Diagrammatic illustration of the interaction of CQDs with biothiols.

For example, CQDs prepared from phenylboronic acid as the sole precursor used for the estimation of blood glucose levels with a limit of detection (LOD) of 1.5 μM . The boronic acid receptors present on the as-prepared CQDs induce the intercalation of CQDs with added glucose molecules. Consequently, the interaction between phenylboronic acid and glucose leads to the aggregation of CQDs, which is accompanied by FL quenching (Fig. 2).¹⁷

2.3 Biothiols

The detection of biothiols (sulfhydryl compounds) such as, amino acids, peptides, proteins, and enzymes, has been an important research interest with in clinical toxicology.¹⁸ Sulfhydryl amino acids like glutathione (GSH) are involved in the antioxidative and detoxification processes in the body, and support detoxification to produce more GSH and metallothionein, respectively. Softer metal ions like Ag^+ , Hg^{2+} , *etc.*, prefer to bind with soft anions such as S^{2-} ion (hard and soft acids and bases-HSAB principle). Upon interaction with the most insidious toxic sulfhydryl-reactive metals, which include Hg, Cd, Pb, and As, GSH and Cys are depleted from the body. Thus, in cases of heavy metal poisoning, the measurement of sulfhydryl amino acids is a useful tool to indirectly estimate exposure to toxic metals. In this context, Mandani *et al.* reported thiol containing amino acids a Au@CQDs probe based on FL “on-off-on” approach with a LOD of 50 nM.¹⁹ The CQDs were subjected to form a thin layer surrounding Au nanoparticles (AuNPs) to obtain the Au@CQDs nanocomposite. Upon interaction with AuNPs, FL of the CQDs was quenched (Fig. 3). In the presence of thiol containing

biomolecules, the Au@CQDs probe underwent ligand exchange with thiol moiety to form AuNP-thiol clusters resulting in the release of CQDs from the AuNP surface to recover the quenched FL.¹⁹ A novel FL “on-off-on” sensor using the CQDs-Hg(II) system was reported for the detection of GSH, Cys and histidine (His).²⁰ The FL of CQDs was quenched by the addition of Hg(II). However, the addition of GSH, Cys or His recovered the FL due to their stronger affinity with Hg(II) (Fig. 3).²⁰ Another sensor system containing AuNPs surrounded by a shell of CQDs was used to detect Cys by disaggregation method.²¹ Similar works towards the detection of thiol containing compounds using QDs in biological samples, have also been found in the literature.²² Examples include VS_2 QDs for GSH in human serum,²³ CQDs for Hg (II) and GSH in living cells.²⁴

Fluorescence resonance energy transfer (FRET) is a non-radiative energy transfer between two light-sensitive molecules in which one of them acts as an excited donor, while the other one act as an acceptor. This energy transfer resulted in the enhancement of FL signal of acceptor chromophore. QDs as FRET donor have been extensively studied for sensing applications. For instance, Cai *et al.* reported a rapid FL “switch-on” assay to detect trace amounts of GSH with a LOD of 300 nM, using CQDs- MnO_2 nanocomposites.²⁵ When QDs were reacted with Mn-nanosheets, the FL was quenched off due to FRET from QDs to Mn-nanosheets. However, on the addition of GSH, the MnO_2 nanosheet was reduced into Mn^{2+} , recovering the FL.²⁵

2.4 Acetylcholine

The well-known neurotransmitter acetylcholine (ACh)

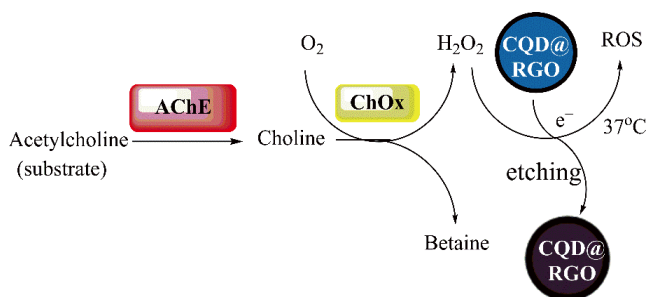


Fig. 4 Diagrammatic illustration of the interaction of ACh with CQDs@RGO.

undergoes hydrolysis as a substrate in the presence of acetylcholinesterase (AChE) during the process of termination of impulse transmission at cholinergic synapses to form acetate and choline. Moreover, the estimation of AChE activity is a routine process in clinical toxicology laboratories, in the cases of poisoning by its inhibitors such as, organophosphate or carbamate pesticides. The measurement of ACh can be very useful in the estimation of AChE activity.²⁻⁴ Wang *et al.* developed a photoluminescence based “turn-off” sensor system for the detection of ACh with a LOD of 30 pM using reduced graphene oxide (RGO) decorated CQDs (CQDs@RGO).²⁶ ACh, in the presence of AChE usually undergoes hydrolysis to form choline, which in turn undergoes oxidation catalyzed by choline oxidase (ChOx) to produce H₂O₂ and betaine. H₂O₂ in the presence of RGO at 37°C generates reactive oxygen species (ROS) such as HO• and O₂^{•-}. The as-generated ROS quenches fluorescent intensity of the CQDs@RGO by an etching process. The fluorescent intensity of the CQDs@RGO is inversely proportional to the concentration of ACh.²⁶ Jin and coworkers prepared water soluble core-shell CdSe/ZnS QDs capped with trioctylphosphineoxide, surface-modified with tetrahexyl ether derivatives of *p*-sulfonatocalix[4]arene for the optical detection of acetylcholine.²⁷ Similarly, a nitrogen-doped CQD (N-CQD) based ratiometric fluorescent biosensor was also devised for the estimation of AChE activity and OPCs (Fig. 4).²⁸

3 Detection of Toxins

The term toxin was first coined by Ludwig Brieger for the poison produced by living cells or organisms.²⁹ The classification of toxins based on their sources includes phytotoxins, invertebrate and vertebrate toxins, and microorganism toxin (bacterial toxins, algal toxins, mycotoxins). Since, these toxins are extremely active at very low concentrations, sensitive and effective analytical techniques such as immunoassay have been developed to detect their presence.³⁰

The present methods available for the detection of toxins include radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), fluorescent ELISA (FELISA), fluorescence-linked immunosorbent assay (FLISA), and lateral-flow immunochromatography (LFIC). Among them, FLISA methods generally consume fewer antigens and antibodies and require less manipulation than traditional ELISA, and significantly decrease the time required for antibody immobilization in the conventional ELISA assay. QDs as fluorophores have a broad excitation spectra and narrow emission spectra. In addition, QDs also provide stronger and photobleaching-resistant fluorescence that can be easily checked under a UV microscope.

As QD-based biosensors are highly sensitive and selective, they have also been utilized for the detection of toxins.³¹

3-1 Phytotoxins

Most of the plant poisoning cases received in the clinical toxicology laboratory are due to the ingestion of active principles of certain plants such as oleander, *Ricinus communis*, foxglove (digitalis, digoxin and digitoxin), *Gloriosa superba*, *Cleistanthus collinus*, *Strychnos nux-vomica*, *Abrus precatorius*, and *Semecarpus anacardium*. Detection of these plant poisonings involves the utilization of different highly laborious analytical procedures. Analysis assisted by sensors such as QDs have been extremely supportive in dealing with the analysis of plant toxins.

3-1-1 Ricin

The seeds of *Ricinus communis*, a castor oil plant, contain a highly potent toxin called ricin which is a lectin (a carbohydrate-binding protein). This toxic plant protein was detected by fluoroimmunoassay and surface plasmon resonance using QDs as sensors. Anderson *et al.* reported the application of Llama-derived single domain antibodies (sdAb)-QDs bioconjugates in both fluorometric and surface plasmon resonance (SPR) immunoassays for the detection of ricin. Anti-ricin sdAb were self-assembled on dihydrolipoic acid (DHLA)-capped CdSe-ZnS core-shell QDs. The QD-based fluorophore in fluoroimmuno assay provided an equivalent LOD of ricin, compared with a conventional fluorophore, whereas the bioconjugate exhibited 10-fold signal amplification in SPR assay. Thus, DHLA-sdAb-QDs conjugate with a LOD of 16 ng/mL is more sensitive than the unconjugated sdAb reporters in SPR assay. In addition, the authors also studied the commercially prepared streptavidin-modified polymer-coated QDs and observed a 4-fold greater signal amplification of the SPR signal than the sdAb-DHLA-QDs conjugate, but with a lower LOD of 156 ng mL⁻¹ in the detection of ricin.³²

3-1-2 Digitalis toxin

Digitalis toxicity can be studied by measuring digoxin together with other information such as renal function, electrolyte balance and cardiac status.³³ Elmizadeh *et al.* fabricated a nanobiosensor using reduced graphene quantum dots (rGQDs) as an optical probe and digoxin (DX) aptamer as a sensing material for the fluorometric determination of digoxin (DX) in biological fluids with a LOD of $7.95 \pm 0.22 \times 10^{-12}$ mol/L.

The fluorescence intensity of rGQDs functionalized with amine labeled DX aptamer was first turned off using oxidized carbon nanotubes (CNTs) through a fluorescence resonance energy transfer (FRET) mechanism (oxidized CNTs as electron acceptor and rGQDs as electron donor). Next, the addition of the target molecule (DX) and its interaction with aptamer inhibited the connection between CNTs and rGQDs-aptamer, hence the fluorescence of rGQDs was recovered (Fig. 5).³⁴

3-1-3 Oleandrin

Three methods, specifically fluorescence polarization immunoassay, digoxin immunoassay (Digoxin III) and LC-MS/MS, have generally been used for detecting oleandrin in the biospecimens. Oleandrin, the oleander glycoside, has a structural similarity to cardiac glycoside digoxin and is known to cross react with various digoxin immunoassays. In fact, oleandrin and its deglycosylated congener oleandrigen were found to have high cross reactivity for digitoxin in a fluorescence polarization immunoassay but have minimal cross reactivity in amonoclonal chemiluminescent assay.³⁵ Thus, the presence of oleandrin toxins can be studied with the help of digoxin assay and the role of QDs needs to be explored for specific detection of oleandrin toxins.

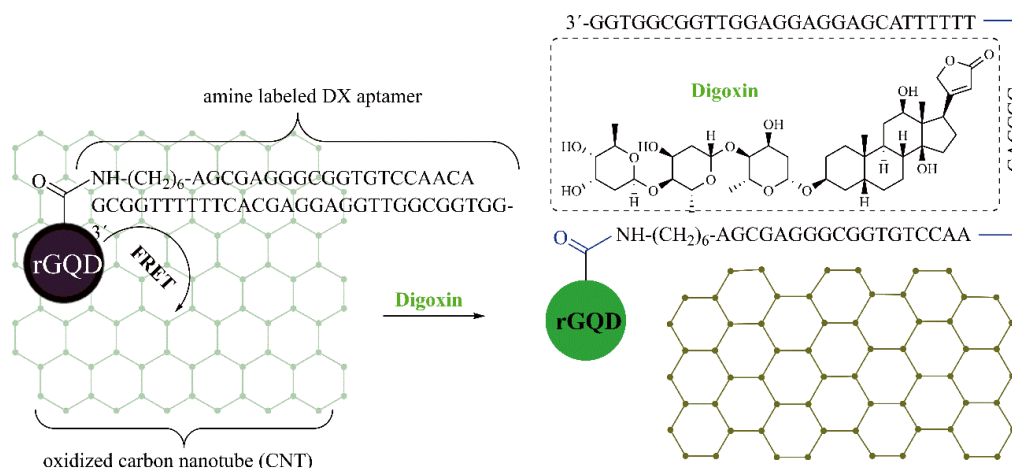


Fig. 5 Diagrammatic illustration of the interaction of digoxin with rQDs.

3-1-4 Amanita toxins

Amanita toxins, found in *Amanita* mushrooms such as the fly agaric, are classified into amatoxins, phallotoxins and virotoxins according to their amino acid composition and structure. Among them, α -amanitin, a cyclic peptide of eight amino acids, is possibly the most deadly of all the Amanita toxins.³⁶ At present, some direct detection methods are available for detection of α -amanitin, such as color reaction, paper layer chromatography (PLC), thin-layer chromatography (TLC), high efficiency liquid chromatography (HPLC), radio immune assay (RIA), capillary zone electrophoresis (CZE), LC-MS/MS/MS and enzyme-linked immunosorbent assay (ELISA).³⁷

Feng *et al.* reported selective QDs based detection of α -amanitin with a LOD of 15 ng/mL in serum without any pretreatment. The sensor was devised using microimprinted polymer (MIP) coated with CQDs and was found to be highly selective towards α -amanitin over its structural analogues, tryptophan, glutathione, *N*-acetyltryptophan and BSA.³⁸ The molecularly imprinted polymers (MIPs) were prepared through co-polymerization of template molecules (*N*-acetyl-tryptophan) and functional monomers, 4-vinyl pyridine (4-Vpy) and methacrylic acid (MAA), followed by removal of the template. The MIP was then coated with CQDs. The MIP-coated CQDs exhibited strong FL at the excitation wavelength of 380 nm. Upon addition of the analyte α -amanitin, the FL of the CQDs was quenched off due to the adsorption of the analyte particles into the template.

3-2 Vertebrate and invertebrate toxins

For the detection of animal toxins,³⁹ including aquatic toxins⁴⁰ and insect toxins,⁴¹ the clinical toxicologists are usually provided with a portion of the skin bite and heart blood samples of the patient/deceased. For predicting the animal toxin and in order to differentiate between the toxin protein and non-toxin protein, the properties of protein, such as amino acid composition, protein length, molecular weight, hydrophobicity and charge, have been found to be highly useful.⁴² Based on these properties, it is possible to perform surface functionalization on QDs so as to ensure high selectivity and sensitivity.

3-2-1 Snake venom

The conventional methods of approaching snake bite cases have major drawbacks like waiting for the symptoms to exhibit and identification of the snake responsible for the bite, in order to decide which antivenom to administer to the envenomed

victim and more importantly, administration of polyspecific antivenoms that can lead to side effects. Many analytical methods have been applied for the detection of snake toxins.⁴³ Gao *et al.* reported an immunofluorescence method for the detection of snake venom with a LOD of 5 – 10 ng/mL under a UV microscope using microscale polystyrene beads coupled with QDs as fluorescence label.⁴⁴

3-2-2 Scorpion toxin

Scorpion toxins have been detected by using many methods such as ELISA.⁴⁵ Mars *et al.* reported GQDs coated on the surface carbon screen-printed electrodes as sensor for the detection of scorpion venom toxin. A hydroquinone/H₂O₂/peroxidase system was used to amplify the current in order to achieve a LOD of 0.55 pg/mL.⁴⁶

3-2-3 Bee venom

The venom of honeybee (*Apis mellifera*) contains melittin, a basic peptide composed of 26 amino acids, as the major pain producing component. For the detection of bee venom, many methods have been developed including HPLC-MS/MS⁴⁷ and voltammetry.⁴⁸

Dang *et al.* reported QD-based sensor system to monitor the proteolytic activity of trypsin using Cy-3 labeled melittin as the substrate. Trypsin will hydrolyze melittin and destroy the interaction between DLPC-TOPO-QDs and Cy3-melittin, demolishing the FRET between QDs and Cy3. The specific and high affinity interaction between melittin and phosphocholine provides a stable assembly and hence is chosen to construct the QD-based FRET system. CdSe/ZnS core/shell QDs, encapsulated with the phosphocholine layer, provides a sphere scaffold for further binding with Cy3 labeled melittin.⁴⁹

3-2-4 Marine toxin

Marine toxins can be enriched in shellfish tissues through the food chain, and the human ingestion of toxin-contaminated seafood can cause poisoning symptoms in different system organs. The representative toxins include saxitoxin, okadaic acid, tetrodotoxin, palytoxin, brevetoxin, domoic acid, dinophysistoxin, cylindrospermopsin, and ciguatera toxin.⁵⁰ The present detection methods available for marine toxins include HPLC-fluorimetric, HPLC-ultraviolet LC-mass spectrometry (LC-MS), LC-tandem mass spectrometry (LC-MS-MS), and immuno-based assays.⁵¹

Tetrodotoxin or TTX is a powerful neurotoxin found mainly in puffer fish and also in some octopuses, flatworms, sea stars, angelfish, toads, and newts.⁵² A lateral flow test strip coupled

with QD-based fluorescence sensing was reported for the detection of small molecules such as TTX, using TTX monoclonal antibodies with a LOD of 0.2 ng/mL.⁵³ As the emission spectrum of QDs nanobeads (QDNBs) at 604 nm and the absorption spectrum of gold nanoflowers (AuNFs) at 600 nm have a large overlap, there exists FRET between them which leads to the reduction of the FL intensity of energy donor QDNBs. In the competitive LFICS test, AuNFs were conjugated with TTX-mAb (antibody) and QDNBs were conjugated with BSA (antigen). First, the mixture QDNBs-BSA and TTX-BSA, was allowed to competitively react with AuNF-mAb which 'turned off' the fluorescent signal of QDNBs by FRET. However, when the sample containing TTX was added, AuNFs were captured and only a few AuNFs were available to interact with QDNBs and hence the FL of QDs was "turned on".

3-3 Microorganism toxins

For the detection of toxins due to microbes, *viz.*, bacteria, archaea, fungi, protozoa, algae, and viruses, sensors should be sensitive enough, rapid, and robust with long operational lifetime.⁵⁴ Until now, a significant number of detection methods have been developed using the optical, electrochemical, biochemical, and physical properties of the microorganisms.⁵⁵ However, these methods are nonspecific, respond not only to bacteria but to all types of microorganisms present in the water sample, and are time consuming because they are based on microorganism growth. Hence, methods like the ones using QDs with a high degree of selectivity towards specific microorganisms will be highly desirable.

3-3-1 Bacteria

Bacteria such as *Clostridium botulinum*, *Mycobacterium marinum*, *Shigella* sp. and *Salmonella typhi* and *Escherichia coli* have the ability to produce toxins responsible for different diseases like botulism, chronic glaucomatous, shigellosis, typhoid, and gastroenteritis, respectively.⁵⁶ Direct instrumental assays rely on sophisticated equipment, such as high-performance liquid chromatography (HPLC), HPLC with tandem mass spectrometry (HPLC-MS/MS) and matrix-assisted laser desorption ionization time-of-flight mass (MALDI-TOF MS), to decipher the toxin profile. Detection of bacterial toxins by antibody-based assays or immunoassays has also been a successful approach for decades and still gains much attention due to the inherent advantages, such as simplicity, speed and cost-effectiveness.⁵⁷ Bacteriophages or phage organisms tagged with fluorophores can be employed as recognition elements to detect deadly bacteria such as *E. coli* and *Salmonella*.⁵⁸

Dumas *et al.* reported the detection of bacterial strains, namely two gram-negative strains (*Escherichia coli* ATCC 11775 and *Pseudomonas aeruginosa* ATCC BAA-47) and two gram-positive strains (*Staphylococcus aureus* methicillin-resistant clinical isolate and *Bacillus subtilis* ATCC 23857), using QDs and found that all four strains tested bind CdTe to a reasonable degree, but gram-positives more than gram-negatives and also found that direct electron transfer occurs only from CdTe to gram-positive strains, resulting in changes in CdTe fluorescence lifetimes.⁵⁹

Anthrax infection is caused by a spore-forming, gram-positive bacterium *Bacillus anthracis*. Very recently, Li *et al.* reported the detection of an anthrax biomarker, 2,6-pyridinedicarboxylic acid (DPA), using QDs. It was first established that DPA had a strong interaction with Eu^{3+} and thus can be detected using QDs based on the turn-on fluorescence method with a LOD of 0.2 μM . The addition of Eu^{3+} quenched the fluorescence emission intensity of QDs at 650 nm when excited at 460 nm and then the presence of DPA reduced the quenching effect of

Eu^{3+} toward CdS QDs. As the DPA concentration increases, the color of the probe was found to change from colorless to red. The authors determined the DPA released from bacteria spores when using this QD-based nanoprobe with the LOD of 3.5×10^4 CFU/mL.⁶⁰ There are many other recent reports available for the detection of an anthrax biomarker.⁶¹

Botulinum toxin (Botox) is a deadly neurotoxic protein produced by the bacterium *Clostridium botulinum*.⁶² It prevents the release of the neurotransmitter ACh from axon endings at the neuromuscular junction and thus causes flaccid paralysis. Infection with the bacterium causes botulism poisoning. A tiny dose of Botox freezes muscles into place minimizing wrinkles and hence the contracted muscles are unable to relax. Wang *et al.* reported a nanobiosensor for the detection of botulinum neurotoxin serotype E (BoNT-E), based on FRET between QDs and dark quencher-labeled peptide probe, with the LOD of 0.02 and 2 ng/mL for BoNT-E light chain (the catalytic domain of the toxin) and holotoxin (the form of the toxin that possesses the capability to cause botulism in humans).⁶³ The QD is employed as the signal reporter in this nanobiosensor, and a novel peptide probe labeled with a dark quencher chromophore as a FRET acceptor contains a specific cleavage site for active BoNT-E and when the peptide probe is cleaved, was used to indicate toxin presence and quantity. Using this approach, only proteolytically active forms of BoNT-E are detected. The nanobiosensor reported here is the first to detect and quantify active holotoxin of BoNT-E; this is also the first time a nanobiosensor has been used to simultaneously detect and discriminate three BoNT serotypes in a single experiment. The authors also reported a FRET-based nanobiosensor using QDs that detects and discriminates between BoNT-A and -B.⁶³ Lee *et al.* reported a ratiometric QD-based sensor for the detection of BoNT serotype A1 with a LOD of 20 – 40 pM.⁶⁴ In order to create a stronger interaction between FRET donor and acceptor in immunological assay, the authors used single-chain variable fragments (scFvs) instead of large sized fluorophores like antibodies, to reduce the distance (usually <10 nm). The scFvs chromophores were conjugated with red-emitting CdSe/ZnS core/shell QDs and a deep-red organic dye (Dylite 650) that acted as FRET donor (termed BOT1) and acceptor (termed BOT2), respectively. Upon introduction of BoNT HCR to a solution containing the two scFvs, a FRET that causes reduction in QD PL with a commensurate increase in acceptor emission was observed.⁶⁴ Zhao reported nanobead-based magnetic separation and QD-labeled fluorescence measurement for the detection of *E. coli* O157:H7 with the LODs of 3.98×10^3 and 6.46×10^4 CFU/mL in pure culture and ground beef samples, respectively.⁶⁵ Yang *et al.* reported QD-based detection of *Escherichia coli* O157:H7 and *Salmonella typhimurium* with a LOD of 10^4 CFU/mL.⁶⁶ The biotinylated QDs were conjugated with antibodies of *Escherichia coli* O157:H7 and *Salmonella typhimurium* and allowed to react with target bacteria, which were separated from the samples using antibody coated magnetic beads. Other QD-based detection of bacterium toxin including *E. coli* have also been reported.⁶⁷

3-3-2 Mycotoxins

Mycotoxins are naturally occurring toxic compounds produced by certain types of molds.⁶⁸ Molds that can produce mycotoxins grow on numerous foodstuffs, such as cereals, dried fruits, nuts and spices. Most mycotoxins are chemically stable and survive food processing. Long term effects on health of chronic mycotoxin exposure include the induction of cancers and immune deficiency. Chromatographic techniques, such as HPLC coupled with various detectors like fluorescence, diode array, UV, LC-MS and LC-MS/MS, are powerful tools for

analyzing and detecting major mycotoxins. In addition, immune affinity-based detection techniques, proteomic and genomic methods, molecular techniques, electronic nose, aggregation-induced emission dye, quantitative NMR, hyperspectral imaging, etc. have also been explored for the detection of mycotoxins in foods. Some of them have proven to be particularly effective in not only the detection of mycotoxins, but also in detecting mycotoxin-producing fungi.⁶⁸

Aflatoxins (AFTs) are a group of toxic secondary metabolites, mainly produced by *Aspergillus flavus* and *A. parasiticus*. Among these, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, and aflatoxin M2 have been recognized as human carcinogens.⁶⁹ Wang *et al.* developed three different fluorescence immunochromatography assays (FICAs), time-resolved fluorescent nanobeads (TRFN), quantum dots nanobeads (QDNB) and QD-based FICAs, and compared them for the quantitative detection of aflatoxin B1 (AFB1) in six grains (corn, soybeans, sorghum, wheat, rice and oat) with the LODs of 0.04, 0.30 and 0.80 µg/kg, respectively. TRFN-FICA exhibits the best performance with the least immune reagent consumption, shortest immunoassay duration and lowest LOD. In the direct competition reaction, the rabbit anti-chicken IgY-IgG was immobilized on C line containing the antigen TRFN-chicken IgY, and exhibited a constant C line fluorescence signal. Then, the coating antigen AFB1-CMO-BSA was immobilized on T line to capture the fluorescence probes (QD-mAb, QB-mAb and TRFN-mAb). However, when free AFB1 molecules are present, the fluorescence intensity of T line decreased with increased concentration of AFB1, as the probes flow past both T and C lines without binding with the coating antigen present at the T-line.⁷⁰ Ouyang *et al.* reported antibody-antigen interaction based detection of aflatoxin using quantum dot nanobeads with the LODs of 1.4 and 2.9 pg/mL for rice and peanut samples, respectively.⁷¹ Other QD-based Immunochromatographic assays have been widely employed in the analysis of AFTs (mainly for AFB1).⁷²

Zearalenone (ZEA) also known as RAL and F-2 mycotoxin is a macrocyclic β -resorcylic acid lactone and a non-estrogenic mycotoxin produced by some *Fusarium* and *Gibberella* species. Various instrumental methods, including TLC, ELISA, and HPLC have been developed for ZEA detection with poor selectivity or sensitivity in complex biomatrices.⁷³

Shao *et al.* reported the determination of zearalenone with a LOD of 0.02 mg/L using CQDs encapsulated on molecularly imprinted fluorescence quenching particles (MIFQPs) such as silica-based matrix. The fluorescence intensity of MIFQP, upon addition of ZEA was quenched and found inversely proportional to the amount of ZEA. The quenching was attributed to the FRET from the acceptor ZEA and donor CQDs in MIFQPs.⁷⁴ Beloglazova *et al.* reported QD-based FLISA for zearalenone detection with a LOD of 0.03 ng/mL.⁷⁵

Li *et al.* reported the simultaneous detection of three mycotoxins, aflatoxin B1 (AFB1), zearalenone (ZEN) and deoxynivalenol (DON), with the LODs of 10, 80 and 500 pg/mL with the help of fluorometric lateral flow immunoassay (LFA) using CdSe/SiO₂ quantum dot microbeads.⁷⁶ Fumonisin (FMs) are mycotoxins mainly produced by *Fusarium* species growing on agricultural commodities in the field, at the harvest or during storage. There are different forms of fumonisins and among these, Fumonisin B1 (FMB1) is the most common naturally occurring form, followed by FMB2 and FMB3. Lu *et al.* reported a competitive fluorescence enzyme-linked immunosorbent assay (cFELISA) for the detection of fumonisin B (FB) based on the catalase (CAT)-regulated fluorescence quenching of mercaptopropionic acid-modified CdTe quantum dots (MPA-

QDs) with a LOD of 0.33 ng/mL.⁷⁷

Di Nardo *et al.* reported fluorescent competitive immunochromatographic strip test (ICST) based on the use of QDs for the detection of fumonisin B1 with a LOD of 2.8 mg/L. The fluorescence signal of ICST in the dipstick format was qualitatively estimated by the naked eye under a UV light at 365nm at room temperature.⁷⁸

Very recently, Hou *et al.* reported a fluorescent immunochromatographic assay for simultaneous determination of fumonisin B1 (FB1), deoxynivalenol (DON) and zearalenone (ZEN) using quantum dot nanobeads as immunosensor. Compared with traditional ELISA, the immunosensor was found to be more sensitive with IC₅₀ of 12.66 ng/mL (FB1), 2.97 ng/mL (DON), and 0.87 ng/mL (ZEN), respectively.⁷⁹

3.4 Multi-analyte detection

The use of QDs has also been extended to immunoassay-based multi-analyte detection of a variety of toxins. The screening of multiple analytes in a single assay offers many advantages including higher throughput compared with single-target systems, decreased sampling errors, easy inclusion of internal controls, savings in reagents and consumables, as well as stability in many environments for long periods of time. Fluoroimmuno assays using QD-based fluorophores can be multiplexed for screening multiple analytes simultaneously.⁸⁰ For example, Golden *et al.* carried out multi-analyte detection in a single sandwich immunoassay for the detection of four protein toxins, cholera toxin, ricin, shiga-like toxin 1, and Staphylococcal enterotoxin B, simultaneously in single wells of a microtiter plate. The authors prepared CdSe-ZnS core-shell QDs capped with DHLA and then conjugated with antibodies, rabbit anti-CT, rabbit anti-ricin, pool of monoclonals and rabbit anti-SEB, which emit strong FL at 510, 555, 590 and 610 nm, respectively, in this multiplex assay for four toxins.⁸¹ Yu *et al.* reported a duplex competitive flow cytometric immunoassay for the detection of the cyanobacterial toxin microcystin-LR and the polycyclic aromatic hydrocarbon compound benzo[a]pyrene using antibody-coated QD detection probes and antigen-immobilized microspheres.⁸²

4 Conclusions

Quantum dots based biosensors have increasingly become important in the determination of biochemical markers as well as toxins. QD-based sensors can detect analytes in a short time and in most of the cases, the colour change can be seen with the naked eyes. Therefore, qualitative analytical procedures can be reliably and accurately performed without actually requiring sophisticated instruments. It is noted in the literature that advanced biosensing techniques, like FRET and FLISA using antigen-antibody recognition, have been applied for the detection of toxins using QDs as fluorophore. Since CQDs and GQDs have very low toxicity and high surface modification properties, they can be indispensable replacements for heavy metal based QDs. The usage of QDs in direct qualitative toxicological analysis of biospecimens such as blood, urine and visceral organs is not explored much presently; there is immense scope for further experimentation, especially on the mechanism of interaction between QDs and toxins, and subsequent routine application after surface modification of QDs using suitable receptor molecules. Utilization of QDs in the detection of phytotoxins in biospecimens is scarce when compared to microbial or animal origin toxins; therefore, it is important to focus on this unexplored avenue.

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