Advances in Nanoparticles, 2016, 5, 1-8
Published Online February 2016 in SciRes. http://www.scirp.org/journal/anp
http://dx.doi.org/10.4236/anp.2016.51001

Biotoxicity of CdS/CdSe Core-Shell Nano-Structures

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Received 3 August 2015; accepted 1 February 2016; published 4 February 2016

Abstract

The cytotoxicity of hydrophobic QDs CdS/CdSe was tested assigning MTT assay on Human Embryonic Kidney cells (HEK-293), breast cancer cells (MCF-7) and Enrichlish Ascitices Cells (EAC). Approximately 65% bio-toxicity was observed in MCF-7 for the core-shell QDs. These QDs may also find effective applications in other optoelectronic devices. CdS/CdSe core-shell hetrostructure quantum dots (QDs) were generated by chemical reaction between the respective chalcogens and cadmium metal salt. Sulphur powder was utilized for CdS core preparation while selenium was extracted from an organoselenium compound to impart CdSe shell layer at a temperature between 150˚C - 200˚C. So-prepared core-shell QDs showed good optical properties. The particle size was found to be in the range of 3 - 4 nm with spherical morphology and cubic crystal structure.

Keywords

Quantum Dots, Metal Chalcogenides, Bio-Toxicity, Cell Lines

1. Introduction

Amongst II-VI QDs, CdS, ZnSe, CdSe and core-shell type e.g. ZnSe/CdS, CdS/CdSe etc. are documented to be important for photonics and biomedical applications because of their inorganic nature which offers good environmental and photostability as well as chemical inertness. Such QDs are useful because of their tunable optical properties in the entire visible light spectrum. Because of their suitability for biological as well as for photonics

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How to cite this paper: Bhanoth, S., Kshirsagar, A.S., Khanna, P.K., Tyagi, A. and Verma, A.K. (2016) Biotoxicity of CdS/CdSe Core-Shell Nano-Structures. *Advances in Nanoparticles*, **5**, 1-8. http://dx.doi.org/10.4236/anp.2016.51001

application, synthesis of tailor made particles with amphiphilic (hydrophoibic as well as hydrophilic) surfaces are often advantageous [1]-[4]. Application of QDs in bio-medicine is expected to bring great advantage in diagnosis and treatment of disease. Potential areas that may get advantage by utility of quantum dots including drug delivery, clinical diagnostics, diet industry and improved biocompatible materials. Semiconductor QDs are future tools that can offer better viability and control targets. In pursuit of the use of QDs, researchers have maintained importance of the synthesis, dispersion, bio-solubilisation and bio-conjugation of fluorescent QD with a core-shell type of nano-structures. A nano-structure with core composed of CdS or CdSe material and shell of a semiconductor material helps to stabilize the core which further helps to improve optical properties as well as bioavailability of the material. In last few decades good efforts were taken by various research groups and scientists for the synthesis of various types of good quality QDs (including core-shell nano-structures) in terms of their dispersion in organic as well as aqueous medium and making them typically hydrophilic or hydrophobic. Cadmium chalcogenide QDs are very useful in the variety of *in vitro* and *in vivo* applications because of their versatile surface chemistry and superior optical properties [5]. The application of such QDs in bio-imaging attracted attention of the researchers because of good simultaneous coupling with biological molecules or ligands [6]. Fluorescence efficiency can be optimized via inorganic or organic coatings on QDs because of dangling bonds. Water dispersibility and bioactivity of QDs can be enhanced by coatings of biocompatible materials or polymer. For utility of QDs in biology functionalization is required to recognize molecules such as proteins, peptides and nucleic acids, forming bio-conjugates [7]. It has been documented that size-tunable CdSe QDs have great potential in diagnosis of cancerous cells. However, pharmacokinetic and toxicology studies require further detailed understanding and analysis [8]. Additionally, the surfactants such as long chain carboxylic acids, alkyl or aryl amines and low molecular weight polymers normally are employed to improve the quality of quantum dots for a variety of applications. Another option to alter the surface is by coating of same group QDs as a shell upon inorganic core called core-shell structures such as CdSe/CdS [3], CdSe/ZnS [4], CdSe/CdTe [9], CdSe/ZnSe [10], CdTe/CdSe [11]. We have recently reported synthesis and characterization of ZnSe/CdSe core-shell quantum dots and its relation with size and optical properties [12].

Size, surface chemistry, charge and monodispersity are important properties required to facilitate the biological internalization of QDs. Cadmium and selenium are essentially toxic to cells and living organisms leading to apprehension over their potential toxicity for *in vivo* applications. Several researchers in the past have demonstrated cytotoxic studies of quantum dots e.g. Derfus *et al*. [13] studied the cytotoxicity of CdSe QDs and pointed out that without presence of coating, the cytotoxicity of CdSe core can be correlated with the liberation of free Cd^{2+} ions due to deterioration of QDs lattice. According to them the liver is the primary site of acute injury *in vivo* caused by cadmium ions even when the concentration of Cd^{2+} ion is reduced to minimum level. It has been opined that quantum confinement effect could further lead to cytotoxicity because QDs within certain diameters may be of similar size to certain cellular components and proteins bypassing natural mechanical barriers, thus leading to adverse tissue reactions [14]. Similarly, highly luminescent QDs such as core/shell CdSe/ZnS and core/shell/shell CdSe/ZnS/TEOS type were developed by Dabbousi *et al*. [15] and Selvan *et al*. [16] and have been tested for their biototoxicity. Chenbo *et al*. [17] [18] studied change in cellular biomechanics with exposer of carbon nanotubes and cytotoxicity effect of MWCNT (Multiwall carbon nanotubes) on human lung epithelial cells. Similarly, Seigrist *et al*. [19] reported genotoxicity caused by MWCNT. Zhang *et al*. [20] studied effect of nitrogen doped carbon QDs on living (Hela) cell and as selective probe for Fe. In the current work we assigned MTT assay on MCF-7, HEK-293 and EAC cell lines and evaluated internalization as well as cellular uptake induced biotoxicity by use of CdS/CdSe QDs.

Previously our group reported synthesis of CdSe, ZnSe and ZnSe/CdSe QDs by using cyclohexeno-1,2,3-selenadiazole where in it was shown that cyclohexeno-1,2,3-slenadiazole can be used effectively for synthesis of various types of chalcogenide based QDs including core-shell nano-structures [12]. In order to use QDs in various fields of optoelectronic, biological and medical fields, the cellular uptake, internalization and toxicity mechanism of QDs need to be evaluated. As part of our on-going studies, we herein present a simpler method for synthesis of CdS/CdSe core-shell and its cytotoxic studies. This paper describes a synthetic method that avoids use of toxic TOPSe for inverted core-shell structures CdS/CdSe. Well known organometallic selenium-N heterocyclic compound cyclohexeno-1,2,3-selenadiazole has been exploited as selenium precursor along with cadmium acetate as cadmium precursor and oleic acid as capping agent. The reaction was performed at much lower reaction temperature than those documented in the literature. Additionally, internalization of cellular in-

duced biotoxicity assigning MTT assay on anti cancer HEK-293, MCF-7 and EAC cell lines was studied.

2. Materials and Methods

2.1. Chemicals

Cadmium acetate dihydrate, 1,2,3-selenadiazole [21], sulphur (99.9%), Diphenyl ether (98%), Oleic acid (98%), paraformaldehyde, n-hexane, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, dimethylsulfoxide (DMSO) and methanol were purchased from Aldrich and used without further purification. UV-Visible spectrum was recorded at room temperature using analytik jena SPECORD@210 PLUS spectrophotometer. Similarly, photoluminescence (PL) spectrum was taken on Cary-Eclipse Fluorescence spectrophotometer (Agilent Technology) at an excitation wave length of 350 nm. X-ray diffraction (XRD) measurement was done with a Bruker D8 advance diffractometer with Cu-Ka radiation (1.5405 Å). Transmission electron microscopy (TEM) was done on FEI Tecnai G2 with 30 KV.

2.2. Preparation of Hydrophobic Core-Shell Quantum Dots

Core CdS hetero nano-structures were initially prepared via customized literature method developed by our group [12]. Cd $(Ac)_2$ or CdO was dissolved in a mixture of oleic acid (OA) and diphenyl ether by maintaining temperature between 100˚C - 150˚C. Pre-dissolved sulphur in diphenyl ether was injected in to the reaction mixture in the temperature range of 150˚C - 200˚C and stirred for some time at the same temperature. For over growth of CdSe shell cyclohexeno-1,2,3-selenadiazole and Cd-precursor were added in the same mixture and the reaction was continued for 2 - 3 hrs. CdS/CdSe QDs were precipitated by addition of methanol and collected via centrifugation followed by simultaneous washings with ethanol and finally dried in vacuum oven at room temperature.

2.3. Cell Culture and Cytotoxicity Studies of Quantum Dots

The *in vitro* evaluation was done on Human Embryonic Kidney cell line HEK-293 and Human breast adenocarcinoma MCF-7 cell line (American Type Culture Collection, Rockville, MD) [22]. Confluent flasks were subcultured and maintained at 36˚C in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) supplemented with 10% fatal calf serum (Himedia), and antibiotic containing 50 U/mL of penicillin (Sigma) and 50 mg/mL of streptomycin (Sigma, USA) under a humidified atmosphere (5% CO_2). Briefly, 5×10^3 cells/well of HEK-293, MCF-7 and EAC cells were plated in 96-well microtiter plates. CdS/CdSe QDs were then added to the cells at defined concentrations (10 µg/mL, 5 µg/ml and 2.5 µg·µg/mL) and incubated for 24 hours and 48 hours. After incubation, 20 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; (5 mg/mL) was then added per well and the plate was incubated for four hours in an incubator. After incubation, the media was discarded carefully without disturbing the formazan crystals in the wells and 120 µl of dimethyl sulfoxide was added to solubilize the formazan crystals formed and reading were taken in a (Synergy HT, Biotek, USA) using a 540 nm filter [23]. All measurements were done in triplicates. Percentage viability of the cells was calculated as the ratio of mean absorbance of triplicate readings with respect to mean absorbance of control wells.

Cell viability = $(I_{control} - I_{sample}/I_{control}) \times 100$

2.4. Cellular Uptake

For understanding the internalization of nanoparticles cellular uptake was assessed in a fluorescence microscope as per our published protocols [24]. Briefly, MCF-7, HEK-293 and EAC cells were cultured on coverslips till 90% confluence was attained. The cells were further incubated with 10 µg/ml of CdS/CdSe QDs. The cells were fixed with 4% paraformaldehyde and visualized in a fluorescence microscope (Eclips 90i, Nikon) at Mag. 20×.

3. Results and Discussion

Growth of a CdSe shell around the core CdS resulted in an increase in particle diameter which was monitored via absorption spectroscopy. As shell grows around CdS core both absorption and emission wavelength show systematic red-shifts *i.e.* shifts towards the longer wavelength, indicative of formation of larger particle possibly due to clustering. This red-shift can be correlated with decrease in band gap of CdSe due to the formation of CdS/CdSe core-shell. Such observations are quite handy in termination of chemical reaction during the synthesis

and assuming that the right optical quality has been obtained for the QDs that can be exploited for biological as well other electronic applications including optical sensor applications. **Figure 1** shows UV-visible and photoluminescence spectrum of as-synthesized core-shell nanostructures for present use. The band gap based on absorption value is estimated to be about 2.78 eV and that based on emission wavelength is ~2.21 eV. The crystal structure and the particle diameter of the sample was studied by use of the powder XRD analysis which confirmed cubic crystal structure with diameter of about 4 - 5 nm. The narrow peak width in emission spectrum indicated that the particles are uniformly distributed within a given solution. An average particle size of about 3 - 4 nm was observed from TEM.

Cellular Uptake Induced Biotoxicity of CdS/CdSe

Cytotoxicity was assessed by assigning the MTT assay on anti cancer cell lines. In our studies CdS/CdSe treated MCF-7, HEK-293 and EAC cells were fixed and visualized in a fluorescence microscope (Eclips 90i, Nikon) in **Figure 2**. The release of free cadmium has often been reported by researcher during the study of their biocytotoxicity e.g. King-Heiden *et al*. [25] have reported release of free Cd and Se from ZnS-capped CdSe QDs when tested on to the zebrafish embryo Daniorerio. It is opined that size dependent intracellular routing enables QDs to reach organelles that are otherwise inaccessible to metal ions. It has been proposed by Parak *et al*. [26] that QDs uptake by a cell is packaged into small intracellular vesicles and transported from the cell periphery to the perinuclear region. There is also a report that cadmium ions are predominantly located in the cytoplasm, where they are sequestered by metallothione [27].

The cytotoxicity response against CdS/CdSe QDs was shown by all the cell lines and it was found that the response was more in MCF-7 cell lines while incubated for 24 hours and 48 hours with 2.5 µg, 5 µg and 10 µg concentration as compared to the other cell lines HEK-293 and EAC. Around 63.422%, 46.11% and 44.81%

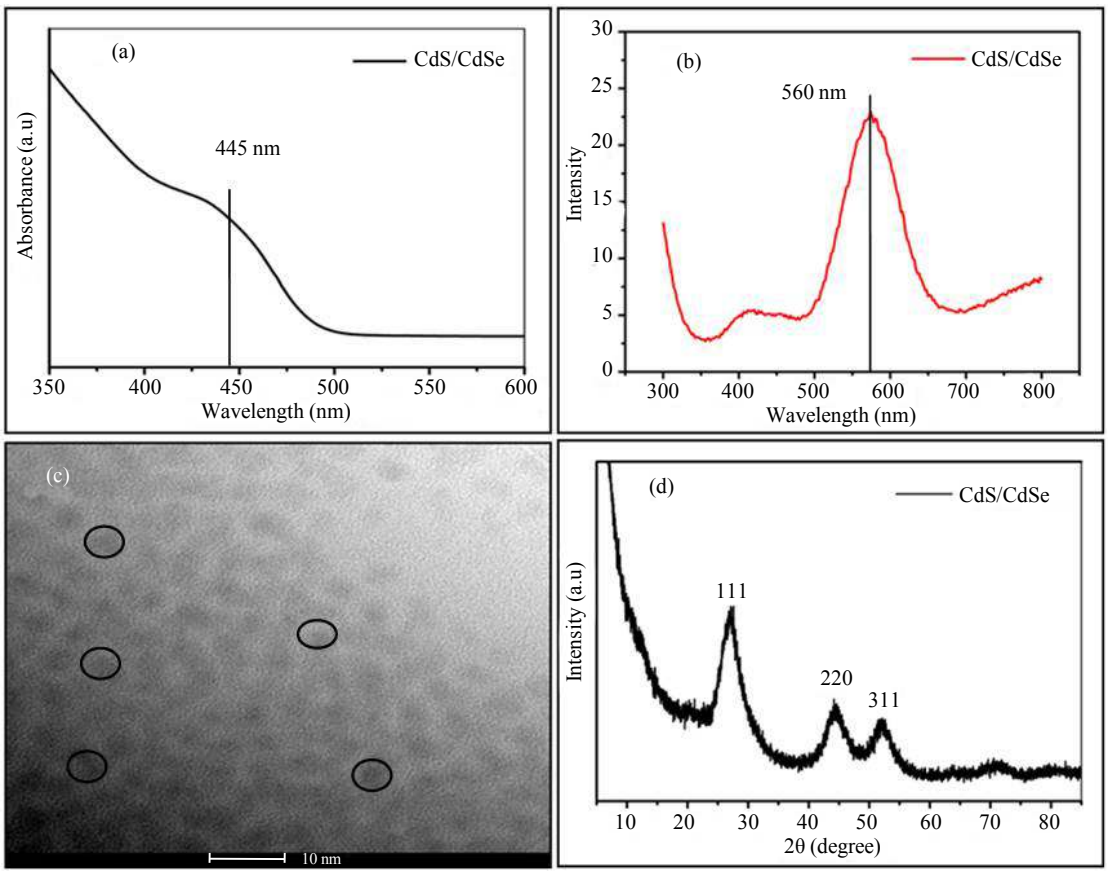

Figure 1. (a) UV-Visible absorption spectrum; (b) photoluminescence spectrum (λ_{ex} = 350 nm); (c) TEM; (d) XRD of core-shell CdS/CdSe QDs.

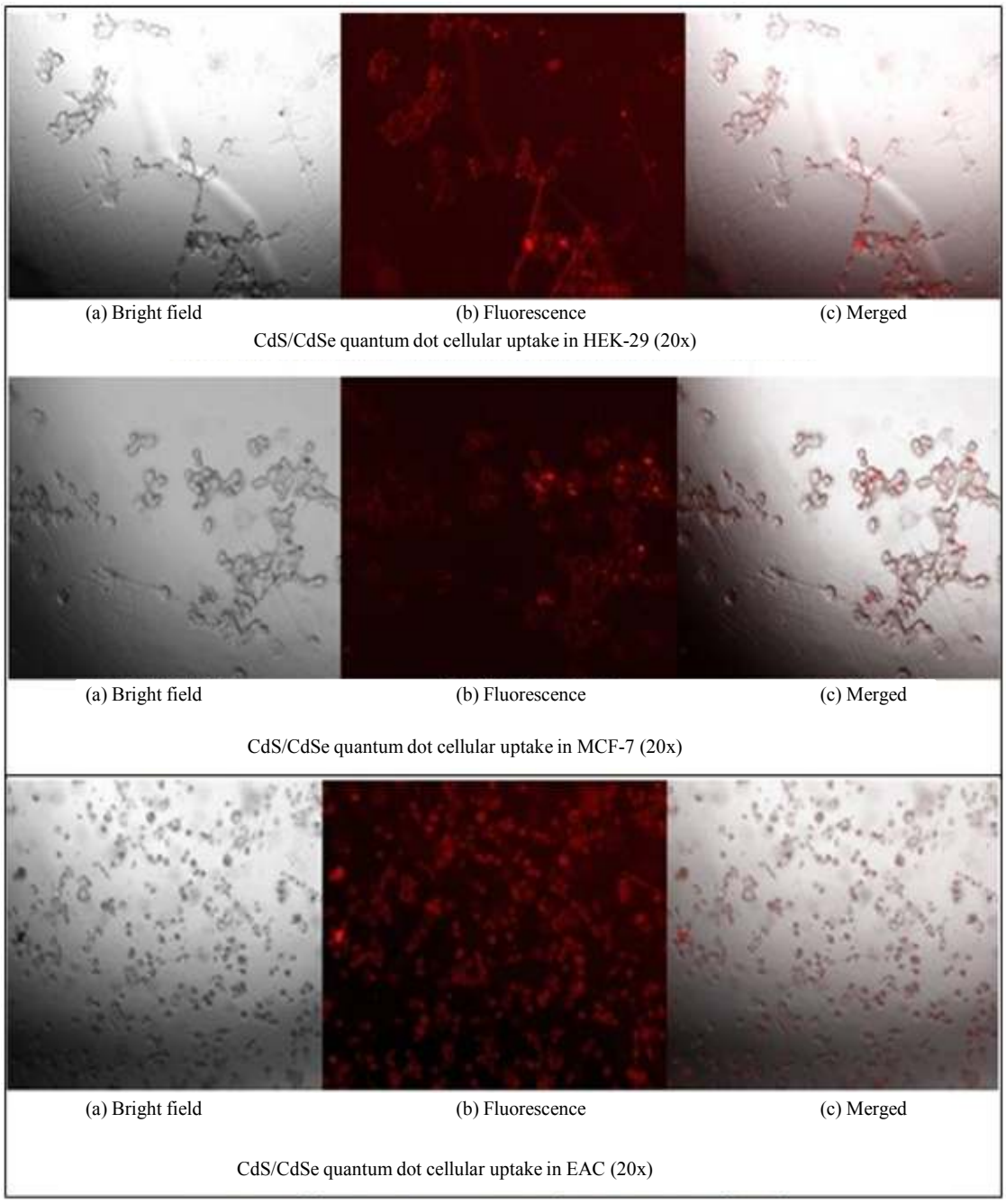

Figure 2. HEK-293, MCF-7 and EAC cells were treated with 10 µg/ml of CdS/CdSe quantum dots, incubated for 3 hrs, then fixed with 4% para-formaldehyde and viewed under microscope [Nikon 90i] at Magnification 20×.

cytoyoxicity was observed subsequently in MCF-7, HEK-293 and EAC cell lines at a concentration of 10 µg in 48 hours (**Table 1**). 20% less toxicity was shown by EAC cell lines in 48 hrs. But only 14% cytotoxicity was indicated in 24 hrs in EAC cell lines. The observed biotoxicity towards hydrophobic core-shell CdS/CdSe QDs in all cell lines is shown in **Figure 3**.

Our experimental study shows the QDs are more cytotoxic in MCF-7 compared to HEK-293, and EAC cells. Time dependent cellular up take induced biotoxicitywas observed to bemaximum post 48 hrsofincubation with 10 µg conentration in MCF-7 cell lines. After performing the experiments, we observed that cytotoxicity, cellular uptake, internalization are concentration and time dependent as shown in **Figure 3**. The cytotoxicity of QDs

Table 1. Percentage of cytotoxicity of CdS/CdSe on cell lines.

Sample	Cell lines					
CdS/CdSe	HEK-293		MCF-7		EAC	
Conc.	24h	48h	24h	48h	24h	48h
10 µg/ml	15.4595	46.1148	16.6317	63.4224	14.0830	44.8163
5 µg/ml	6.6747	7.6486	9.874	39.7930	0.7658	9.8283
2.5 µg/ml	3.6721	0.2637	3.2647	11.2726	-	-

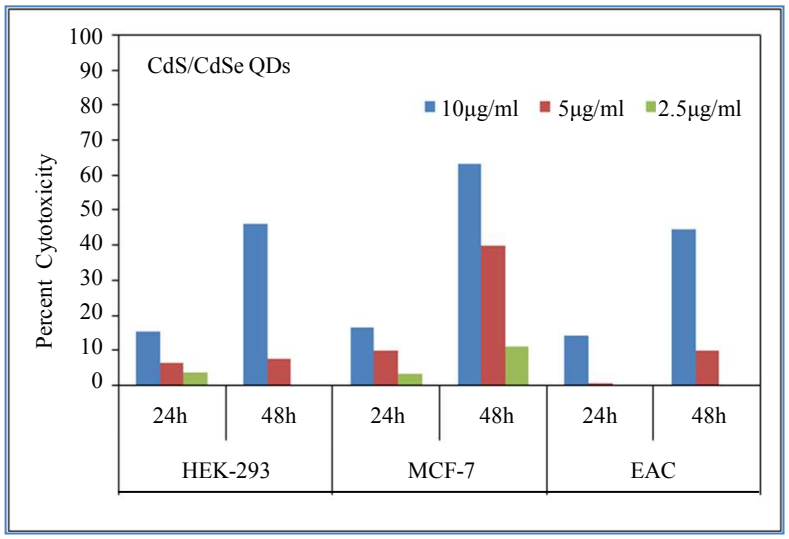

Figure 3. Time and dose dependent cytotoxicity of CdS/CdSe QDs in HEK-293, MCF-7 and EAC cell lines.

may be related to their physicochemical states as observed by Bruchez *et al*. [28]. The observed major QDs toxicity is directly proportional to the release of metal ions (e.g. Cd^{2+}) which is in agreement with the earlier reports [29] [30]. However, according to some other reports, QDs are efficient energy donors and hence can be able to transfer energy to the oxygen molecules leading to the formation of reactive oxygen radicals/species which can promote cell death. The composition of the core and outer capping also plays vital role in QD toxicity. Thus formation of free radicals of the cadmium and selenium upon photo or air oxidation initiates the cytotoxicity [31] [32]. In the present case CdS/CdSe nano-structure contains cadmium and therefore likely to increase cadmium radicals leading to the cytotoxicity.

4. Conclusion

CdS/CdSe core-shell quantum dots were synthesized using 1,2,3-selenadiazole as selenium precursor and so-generated QDs were studied for their bio-toxicity of three human cell lines viz HEK-293, MCF-7, EAC cells. Results confirm that QDs are toxic to all of them. The increase in concentration of QDs leads to higher toxicity response with time. The hydrophobic quantum dots were studied as they are less likely to disintegrate in bio-fluids present in the cells.

Acknowledgements

This work was supported by DIAT and Department of Science and Technology (DST), GOI program (SR/S1/PC-39/2010). SB and ASK thanks DIAT for fellowship. AT acknowledges UGC for her Junior Research Fellowship.

Conflict of Interest

The authors declare no competing financial interest.

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