

POLYMERIC COATED CdSe QUANTUM DOTS

Dissertation submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY AND BIOINFOMATICS

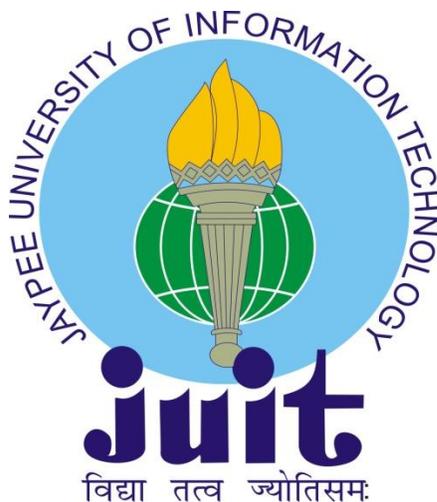
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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B-Tech. thesis entitled “**Polymeric Coated Cadmium Selenide Quantum Dots**”, submitted by **Bandana Sharma** and **Rimple Chaudhary** at **JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT, INDIA** is a bonafide record of their original work carried out under my supervision.

This work has not been submitted elsewhere for any other degree or diploma.

(Signature of Supervisor)

Dr. Udaybhanu M.

Senior Professor

Date

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Needless to say, errors and omissions are ours.

Bandana Kumari (141819)

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LIST OF ABBREVIATIONS

ABBREVIATION	EXPANDED FORM
QD	Quantum Dots
PCL	poly-caprolactone
Cdse4	cadmium selenide
XRD	x-ray diffraction
PVA	polyvinyl alcohol
FTIR	Fourier-transform infrared spectroscopy
MTT	methyl-thiazolyl-tetrazolium
SEM	scanning electron microscope
TEM	Transmission electron microscopy

SUMMARY

Nanotechnology is an upcoming technology which is used in various subjects like: physics, chemistry, biotechnology, Medical etc. it is study of small nanoparticles i.e particles having size below 10nm. Quantum dots are the small nanoparticle or we can say small semiconductors having size between 2nm-10 nm. These quantum dots have wide range of applications in medical, biotechnology, solar lights, LED etc. as they are fluorescent in nature which made them useful for medical purposes. They emit light of particular wavelength. Some examples of quantum dots are: silicon QDs, copper QDs, gold QDs, CdSe4 QDs, etc.

Our project is on CdSe4 quantum dots as they have very bright and broad range of emission. But the main problem or we can say main disadvantage of using these QDs is that they are toxic in nature. Due to which they are not biocompatible for humans and have toxic effects in human cells. So to use these CdSe4 QDs in medical purposes we used biocompatible polymer i.e PCL (Polycaprolactone), which is biodegradable in nature. By coating this polymer on our CdSe4 quantum dots we can make our quantum dots biocompatible. So that its cytotoxicity get reduced and we can use these quantum dots for human body analysis. We can also use another biocompatible polymer instead of PCL eg: PEG,

But we have used PCL because there is no previous research done by using this polymer.

CHAPTER 1

INTRODUCTION

Science and technology is so much incorporated in our life today that there are enormous modifications is done in all the areas of scientific knowledge. These new changes develop fresh opportunities as they cross over to practical applications. Physical science came into popularity in twentieth century, while twenty first century was thought to be a century for life sciences. But a new stream of science called nanoscience is emerging which is associated with both physical science as well as life science which inspire our concept on science and technology in this new century. This new science and technology is called nanoscience and nanotechnology, which is based on the ability to manipulate measure and organize matter in the nanoscale. This nanoscale is billionth of meter. This science is the management and reconstruction of matter at the level of atoms and molecules to generate novel materials, devices and functional system.

Recently it become one of the most interesting and a great field in Chemistry, Physics, Engineering and most important in Biology. Nanotechnology display the great potential for giving us in the near future with many discoveries that will modify the direction of technological advances in a broad range of applications. This technology is considered to be one of the fast emerging technologies, many new researches and development will become possible with the help of this technology in future. In nanotechnology 100nm dimension is necessary, as under this limit we can precisely measure the new properties of matter. At present it is the main field, on which research and study are going on vast scale.

It has enhanced and developed many areas of life including the cleaner, safer and faster production, lowering down the use of injurious resources and enhanced life cycle of products. We can manufacture many new products and devices which are mainly used by man like medicine, electronics, energy production etc with the help of nanotechnology. In the coming future it is only due to nanotechnology that we will buy rapid computers, tiny mobile devices and other small electronics devices. it will give us better built, cleaner, lenth lasting ,safer and smarter materials which are favorable for communications, medicines, agriculture, homes,

transportation, industry etc. Nanotechnology consist of unit with dimensions calculated in nanometers ($1\text{nm}=10^{-9}\text{m}$). These particles are known as nanoparticles which exist in real world and are produced as a result of man activities. Due to their small or submicroscopic size, they have rare material characteristics and produced nanoparticles may discover practical applications in the different areas which includes engineering, catalysis, medicine and environmental remediation.

From the beginning of human development, starting of production and construction of clay elements and separation of metals to design microprocessors, material sciences have developed excellently by supplying a large amount of products. Furthermore there is endless or continuous seeking for better materials and this chase has been motivated by expanding large demands. Taking examples from vacuum tube to micro and biotechnology based semiconductor devices, these are the continuous growth of electronic devices in terms of efficiency, dimensions, compatibility and speed. With the growing demand of fast and efficient electronic devices elaboration in manufacturing techniques of semiconductor devices is essentially required. As a result semiconductor nanotechnology has come out as a aspect and discovery for materials scientists and engineers. Nanoparticles consist of a branch which is known as semiconductor nanotechnology which with deals with fabrication, synthesis, manipulation, characterization and applications of semiconducting materials at nanoscale ($1\text{nm}= 1$ billionth of 1m). These both compound and elemental semiconductors have some properties with is depend on their size unlike metal nanoparticles which display shape dependent magnetic, electrical and optical properties. Semiconductor Compounds present in the group II-VI, III-V and IV-V has become interesting topic or subject for researchers due to their different types of application. Due to the different properties inherited by semiconductors nanostructures, this technology assure to construct more useful electronic devices and their devices are expected to change traditional devices due to their various advantages. Any material at nanoscale is considered as nanoparticles. Quantum dots are known as nanoparticles which are made of any semiconductor material for example: cadmium sulfide, indium arsenide, silicon, cadmium selenide etc. These quantum dots capable to raise the efficiency of solar cells. With the help of quantum dots in solar cell we can modify and improve its efficiency or increase its use in electric purposes. As fluorescence of quantum

dots depend on its size so we can use this idea of different fluorescence in light market. When UV light is given to these dots, these dots get illuminated. These dots are semiconductor nanoparticles that glow and give bright fluorescence at particular color wavelength. This color depends on the size of nanoparticle. In this our project we used these Quantum dots for the medical use. These QDs have many medical applications which include: drug delivery, bioimaging, diagnosis purpose, cancer detection etc. We have used Cadmium selenide for our project which is a semiconductor nanoparticle. This cadmium selenide has broad emission field and emit light at greater wavelength compare to another quantum dots. So we used cadmium selenide in our project. But the main drawback of this cadmium selenide is that is made up of a toxic metal which make this quantum dots more toxic to humans. Cadmium and selenide both are toxic metals and are considering as carcinogen to humans. So before using these quantum dots for medical purposes firstly, we have to reduce their toxicity. So the main purpose of project is to reduce the toxicity which can be done by coating technique. In which we will coat our CdSe4 Quantum dots with a polymer, which is biodegradable and biocompatible in nature .

These polymers are composed of large number of macromolecules having repeated subunits. Polymers are of two types: synthetic and natural. DNA, proteins, wool silk etc are some examples of natural polymers. These are derived from plants or which are naturally present in our nature. While some polymers like: polypropylene, polystyrene, polyvinyl chloride, phenol etc are some examples of synthetic polymer which man made or which not present naturally. We used Polyvinyl chloride as polymer to coat our CdSe4 QD. As this polymer has ore degradation time (about 2-3 years) compare to other polymers so we select this polymers for our coating procedure. Also any research work related to the PCL coated CdSe4 is not done previously. This polymer is non toxic to human body and cells as they are biodegradable in nature. Miscibility of this polymer is quite high. Its mechanical compatibility is also high for many polymers. Adhesion power of this polymer with other substrate is good than compare to other polymers. This polymer has melting point of 60degree Celsius. It can be reheated and reshaped again and again. This polymer basically used for prototyping or model making process. It is non hazardous in nature, this has elasticity of about 440 MPa along with tensile strength 16 MPa. PCL is resistant to oil, solvent, water and chlorine.

CHAPTER 2

REVIEW OF LITERATURE

Semiconductors are those material that has an electrical conductivity between insulator and conductor. Semiconductor has occupied energy bands, one is valence band which is completely filled with electrons and the another one is the conduction band which is empty or half filled. Resistivity of these semiconductor can be altered upto 10 orders of magnitude by various mehods. This can be done by external biases or doping. The conductors which has very low resistivities, their resistance are very difficult to change or modify. Also their greater occupied energy band are somewhat filled with electrons and insulators are of higher resistivities. It is quite challenging to alter the resistivity with the help of external fields or doping because of the presence of large or high gap between the conduction band and valence band. By the flow of electrons, the current is carried in metallic conductors. While in case of semiconductors current is carried by the flow of positively charged holes and by flow of electrons in the electron structure of material. Nanotechnology after microtechnology is not only the future step of research and development, also for this technology technological problems to study the natural architectures and to mimic them is less (Chan,2006).

It is the basic future of advances in biological world for Research through the use of these interesting materials i.e semiconductors nanoparticles, nanotechnology. Recent advances and researches in the field of these unique nanoparticles such as CdTe, quantum dots (QDs), copper nanoparticles,PLA, gold nanoparticles (GNPs) etc. these have gained a lot of interest in the biological research as well as in medical line. This is because of their unique properties of broad spectral and optical properties (Bruchez et al., 1998; Grieve et al., 2000; Aoyagi and Kudo, 2005; Hutter and Maysinger, 2011).

At nanometer size when the size of particles is modified , then its properties varies as compare to those of other bulk particles made up of same material. This will attribute to the changes in electronic orbitals properties and bonding energy of the atoms present in the particles .These optical properties of the particles may be different from other particles with respect to the nature of nanoparticle such as : metals , semiconductors . By the confinement of electrons in these particles band structure of these particles may remarkably change.

Discretize density state of electronic orbitals of nanoparticles is the result of this quantum confinement effect which leads leaving a great separation or band gap between valence band and conduction band. However, in particles made up of same material, partial fulfillment of conduction band with electrons is the result of continuous density states (Hosokawa et al., 2007).

Nanoparticles has an important role and application in biosensors and biomedical background because of there unique photoluminescence properties, by improving its sensitivity and performance of biosensors (Vo-Dinh et al., 2001; Haruyama, 2003; Jain,2003; Jianrong et al., 2004). In result, applications of these different nanoparticles, such as CdTe, CdSe₄, copper nanoparticles, gold nanoparticles, magnetic nanoparticles and carbon nanotubes etc can be used in biosensor development .

Around 10 years ago,nanomaterials which has a diameter in range between 1 to 20 nm, has become a main area for research work(Bal krishan1,menu rani garg,2015).This paper aims to study the concept of quantum dots. The review discusses the basic introduction of quantum dots(QDs) , QDs compared with with atoms , gate voltage characteristics , stability diagram of QDs, phenomenology of QDs and quantized charge tunnelling . practical applications of Qds in various fields has also been studied. Various present applications as well as the future scope of QDs also has been discussed .This paper also targets the study of various important parameters of QDs such as resonance amplitude versus magnetic field, conductance versus gate voltage .

Luminescent QDs are one of the semiconductor fluorescent molecules having size between 2 to 10 nm and these are the product of few hundred atoms. These molecules are the part of II-VI,III-V and IV-VI group elements of the periodic table. This grouping is done on the basis of the diameter of the particles on the order of the compound's exciton bohr radius (Chan et al., 2002; Pathak et al.,2007).

The strong confinement of excited electrons and comparable holes called excitons in their structures is possible due to their unique optical and electronic properties (Alivisatos, 1996; Murphy and Coffey, 2002). These unique and extraordinary properties of QDs are not related to conventional fluorophores, which make QDs an interesting and useful tool for biosensing.

QDs are used over conventional fluorescent labels such as fluorescent dyes in terms of high quantum yield, long photostability, narrow and stable emission spectra, broad absorption spectra, color codes for multiplexed detection and size tunable fluorescent spectrum (Zhang et al., 2009). The combination of all these cited properties made the researchers to study about these QDs in qualitative and quantitative analysis (Grieve et al., 2000; Aoyagi and Kudo, 2005; Bakalova et al., 2006).

QDs have been prepared exclusively and made use to target the cellular labeling, drug delivery system, molecular therapeutics, to detect site directed mutagenesis (Mamedova et al., 2001; Willard et al., 2001; Wargnier et al., 2004; Ma et al., 2005), cancer cell imaging with QD immunoconjugates (Kaul et al., 2003), imaging (Medintz et al., 2005), real time detection of electron tunneling (Lu et al., 2003)

There are many methods for chemical synthesis methods for the synthesis of QDs in which aqueous method is always chosen over others. For example: in production of CdTe and CdSe for biosensor applications, although production of water soluble QDs the main primary principle (Li et al., 2007).

With the help of colloidal synthesis which has many advantages such as it gives mono disperse, perfect size nanoparticles also the optical properties of these particles is appropriate for biosensor and biomedical applications. We should keep in mind the biocompatibility power before joining the QDs with biomolecules. As except those reactions which involves lipids, most of the biological reactions are hydrophilic in nature. For the Capping method they use materials which contain thiol group such as, mercaptoethane sulfonate, thioglycerol, mercaptoacetic acid, (Liang et al., 2004; Jin et al., 2005) along with the incorporation of cysteine with peptides, as for an organic surfactant on QDs for dispersal in aqueous solution

these terminal amino acid or peptides containing cysteine groups which being used (Tsay et al., 2005, 2006; Ding et al., 2006).

Hybrid materials which is the result of these QDs conjugated with various biomolecules contain the unique magnetic, electronic and optical properties of nanoparticles. This have the selective and specific binding behavior of biomolecules (Wang et al., 2002; Ramadurai et al., 2006; Vinayaka et al., 2009). This combining of QDs with biomolecules may provide an observation into the survey of phenomena of energy transfer. It also clears a channel for the study of nano-biomolecular interactions derived from the move in the gap energy present in the bands of QD as a function of quantum confinement. This absorption spectral studies on combination of nanoparticles with biomolecules which give information regarding nano-biomolecular interactions, as biomolecules and QDs were capable to join a dipolar interactions between donor or acceptor which appear like FRET phenomenon (Mamedova et al., 2001; Willard et al., 2001; Ma et al., 2005).

Colloidal QDs are used for both *in vivo* and *in vitro* studies Hasegawa et al., 2005; Vinayaka et al. (Wang et al., 2002; Derfus et al., 2004; al., 2009). The toxicity of these QDs is still unknown till date especially during *in vivo* survey (Derkus et al., 2004). Also, the adverse Effects, safety issues, and environmental impacts connected with these QDs are of public interest. Some heavy metals are toxic for human body or cells and this toxicity occurred due to the possible joining of these QDs with biomolecular within the cell which has to be addressed before their use and applications for both *in vivo* and *in vitro* studies. Physicochemical properties of these particles along with the degree of toxicity, will have variations in the organism due to the diversity of chemicals involved in the synthesis of these QDs. Also, QDs which has applications for biosensor also transmit toxic effects in the soil and ecosystem which is due to the improper sample post treatment and disposal.

For *in vivo* studies, joining of these particles with biomolecules is important such as production of QDs by endocytosis. Bakalova et al. (2004) in his work he recorded the power and efficiency of bioconjugated QDs as sensitizers of cell just to encourage the photodynamic

activity of the photosensitizers which is used during cancer therapy of leukemia cells. Bentzen et al. (2005)

to find an evolution of respiratory syncytial virus (RSV) infection, scientist noted the uses and applications of QDs(Mohammed Abedalwafa, Fujun Wang, Lu Wang and Chaojing Li,2012)

For biomedical applications, Biodegradable polymers has been used specially during tissue culture or engineering, due to great biological and physical properties of the polymer.

Poly- caprolactone (PCL) is one of that biodegradable polymers, that has a high or great time for degradation. In some of the biomedical applications such as bone tissue engineering its mechanical properties, biodegradability and biocompatibility cannot match up with the demand. For that lot of researchers have found just to concentrate on the alteration of this polymer i.e PCL. Distinct effects on the fabrication of PCL is given in this review for specific field of tissue culture or engineering. Tissue engineering is used for merging of different PCL with other polymers, their micro-porous structures and surface modifications are represented in the results. Adding to this, effect on their properties was attainable by the separation of PCL in distinct organic solvents. However , these biological and physical properties of this polymer PCL for various type of tissue culture or engineering applications are gettable.

CHAPTER 3

MATERIALS AND METHODS

3.1 Synthesis of CdSe4 Quantum dots

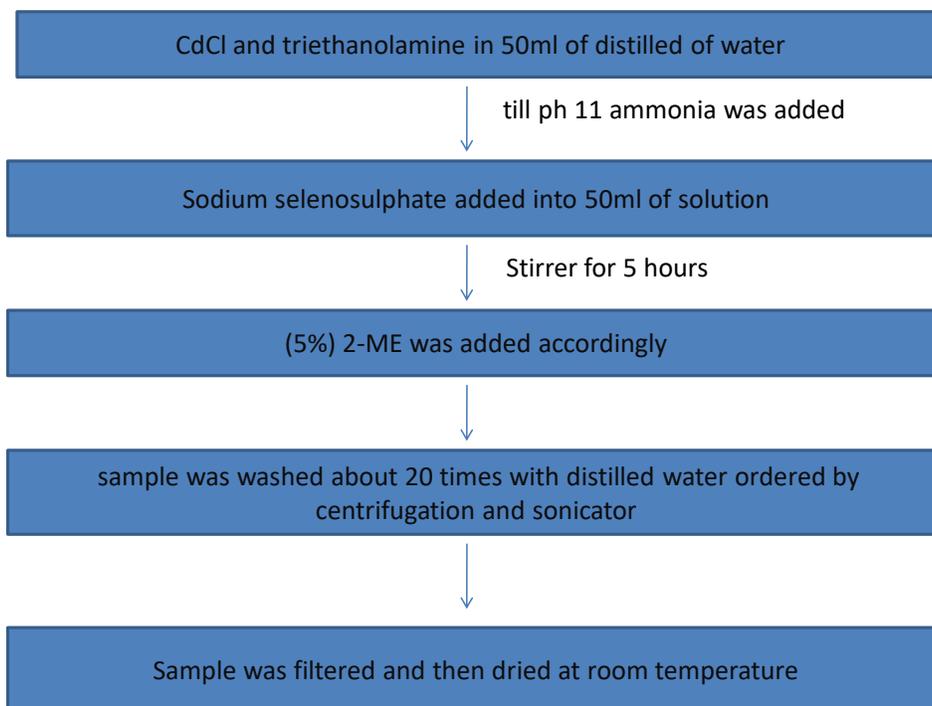


Figure 3.1 Flow chart of synthesis of CdSe4 QDs

By this method i.e wet chemical route quantum dots was synthesized at 70°C, taking water as a solvent. For the cadmium source, cadmium chloride is used. Also Triethanolamine and sodiumselenosulphate is used as the selenium source which is in 3:1.37:1 molar ratio. In this firstly, mixing of CdSe and triethanolamine was done in 50 ml of distilled water. Then . Till the pH reach at 11 add ammonia continuously to it. Then in the 50ml of prepared solution sodiumselenosulphate was added in it and do stirring continuously for 5 hour. Now, instantly stabilizing agent was added that is (5%)2-ME just to control the particle size during the production of CdSe QDs. After this, 2- ME in 50 ml of distilled water of different concentration was used to synthesize different sized QDs. Then, by using 1 ml, 2 ml, 4 ml and 6 ml of 2-ME separately we will prepare CdSe1, CdSe2, CdSe4 and CdSe 6 QDs. In the end washing of sample was done so many times with distilled water followed by the

sonication and centrifugation to remove extra by products from mixture. For this the probe sonicator was used. After all the steps collect washed samples, filtered them and in the end dried them at the room temperature.

3.2 Synthesis of CdSe4 coatedd with Polymer(PCL)

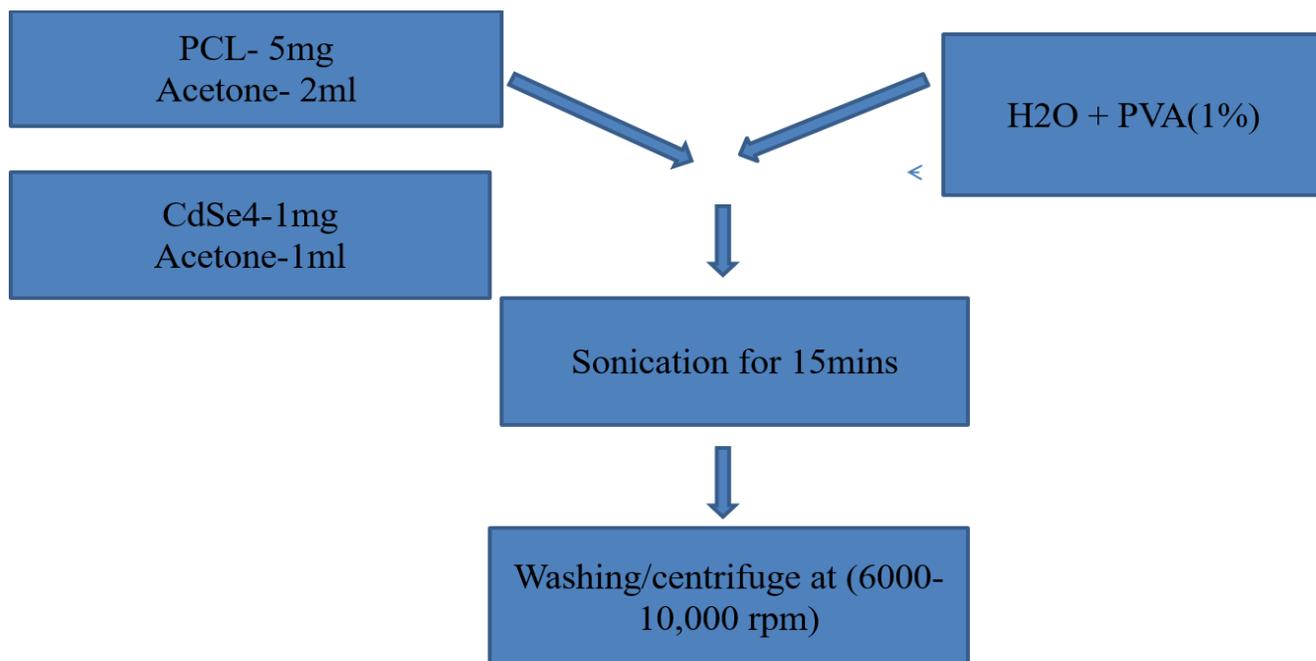


Figure 3.2 Flow chart of synthesis of coated CdSe4 QDs with PCL

In this method :

Take 5mg PCL(polycaprolactone) as a polymer. Mix it with 2ml of Acetone as solvent. Label it as “A”. And then take 1mg of CdSe4(quantum dots) and mix it with 1 ml of Acetone. Label it as “B”. After labeling “A and “B” Mix both the solution “A” & “B”. Now sonicate the mixture(A+B) for 5 mins. After mixing that, Add Water + 1% PVA in both solutions. Then after that again sonicate it for 15 minutes and then finally centrifuge it at 6000-10,000 rpm. And after that collect the pellet after wash.

CHAPTER 4

RESULTS & DISCUSSION

We have characterization results of the sample with us by using different specialty techniques just to study the different properties of ready QDs.

4.1 FLUORESCENCE MICROSCOPIC IMAGE

It is a merger of both biological microscope and fluorescence incident illumination equipment. It's structure includes a focusing knob, a revolver for switching objective lenses and XY stage handler for positioning specimens.

It is based on the Stoke's law principle: which states that fluorescent materials are the material that absorb a specific wavelength of light (excitation light) and after absorbing the particular wavelength of light they emit light of a longer wavelength (fluorescence).

This microscope have all of the components required to induce the fluorescence and capture the resulting image.

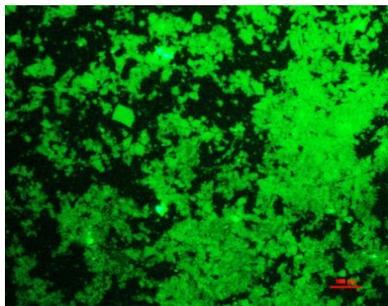


Figure 4.1 Microscopic image of CdSe4 at 20X

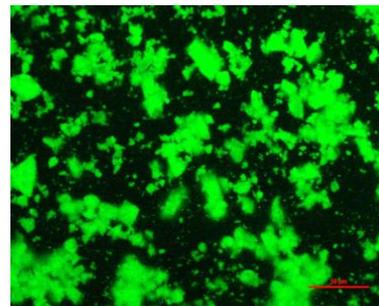


Figure 4.2 Microscopic image of CdSe4 at 40X

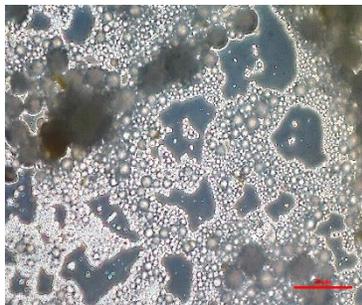


Figure 4.3 PCL-blank nanoparticles (40x)

Strong fluorescence in the visible region is noted for the CdSe quantum dots. Fluorescence is defined as the emission of a photon from a molecules. After emission an electron got excited which results in the movement of that electrons from an excited state to a ground state. Energy difference between these states decides the color of the emission. Reason for the coloration of QDs is the “quantum confinement effect” which is directly related to the level of their energy.

Their optoelectronic properties changes with the change of both size and shape.

QDs of large size(radius of 5–6 nm) give fluorescence or light of longer wavelengths which result in emission of light of different colors such as orange or red etc.QDs having smaller size (radius of 2–3 nm) emit shorter wavelengths which results in emission of light of different colors like blue and green, different colors and sizes varies according to the composition of the QD.

We have taken the fluorescent images of nanoparticles with fluorescent microscope(NIKON-model no.).

Green light reflects the presence of quantum dots in it.

Image 1 has fluorescent image of quantum dots coated with polymer at 20x.

Image 2 has fluorescent image of quantum dots at 40x.

Image 3 has blank nanoparticles (without quantum dots) in it ,to get basic idea to make Q.Ds. Blank image is taken at 40X.

Hence it means quantum dots present in the nanoparticles got coated with the biocompatible polymer that is –PCL(polycaprolactone). Now this encapsulated quantum dots has many characteristics which can be measured by certain tests. So that we assure that that toxicity of that quantum dots has reduced and is biocompatible for the normal human cells.

4.2 X-RAY DIFFRACTION

XRD is being used for Structural analysis, and in x-ray diffractometer its XRD patterns were noted with the help of radiations to analyze the structure of its crystal and size of the particles present in the prepared sample.

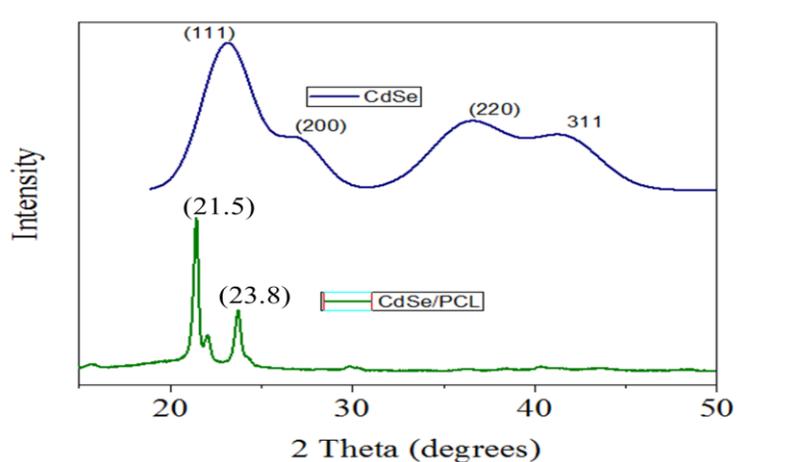


Figure 4.4 XRD results of CdSe₄ above one and CdSe₄/PCL below one

Results shows that CdSe₄ QD without polymer coating diffract light at different angles(i.e (111), (200), (220) while QDs coated with PCL have two main peaks at diffraction angles (21.5) and (23.8).

Structural determination of different size CdSe₄ quantum dots were made possible by the spectra obtained by XRD technology. This technology is said to be one of the most important tools which did not cause destruction and is used to determine the nature of different kind of particle present in powder, fluid and crystals. This is an vital method for the characterization starting from research to the end production and engineering, and quality control of the material . This techniques was used to identify the quantitative phase and crystalline phases

of different materials. atomic structure of crystalline solids is better to explain by X-ray diffraction technology is the three-dimensional. On their crystal structures functions and properties of materials is dependant. This techniques is therefore, been widely used in development, research and production of materials science. XRD follows the Bragg equation, according to this $n\lambda = 2d\sin\theta$. Which is the basic principle of understanding X-ray diffraction.

In this equation,

n is an integer,

λ is the characteristic wavelength of the X-rays,

d is the interplanar spacing atoms, and

θ is the angle of the X-ray beam.

According to this, X-rays were diffracted by the atoms present in the sample in the plane of a periodic structure. angle θ , is the angle where diffraction take place by the particles in the sample. Or we can say that this method have different a set of diffracted intensities and the angles at which they are studied. By comparing this diffraction pattern to a database of known patterns This diffraction pattern can be used to identify performance of particle.

4.3 TRANSMISSION ELECTRON MICROSCOPY (TEM)

The shape of CdSe QDs was determined by Transmission electron microscopy. To obtain results of their particle size this technique is used. Samples of Transmission electron microscopy is prepared by Sonication method in sonicator. Results of TEM images of CdSe4 show that these QDs without coated with PCL are spherical in shape and does not collected into mass. TEM image results is used to find the average diameter of CdSe4 QDs. IMAGE- J –SOFTWARE is used to acquire results of TEM and in result we found that it has a value around 1.9 nm. These CdSe4 without coating with polyer has size prediction which is less than Bohr excitonic radius.

After polymer encapsulation on CdSe4 there is no special effect clearly noted in polymer encapsulated CdSe4 TEM image.

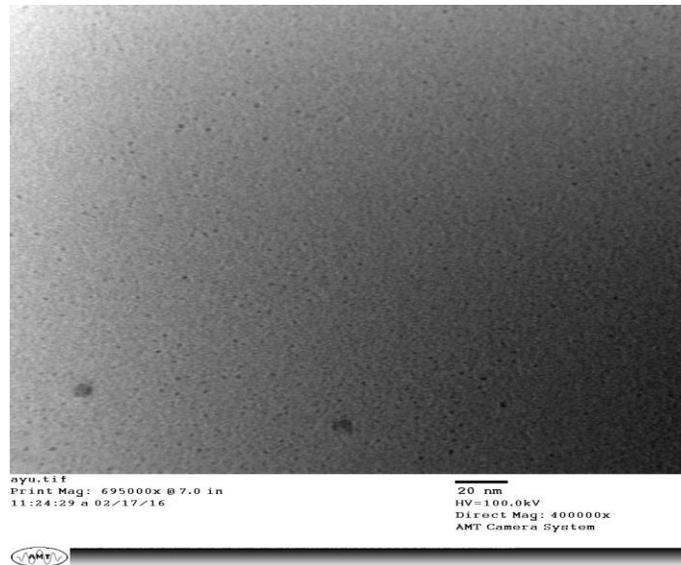


Figure 4.5 TEM image of CdSe4 without coating with PCL

For the material science field it is considered one of the strongest tool. Through the sample high energy beam of electrons is incorporated, and we examine the interactions between the electrons and the atoms just to observe the features such as the crystal structure. Chemical analysis is also achieved by this technique. This is also used to examine their layers, composition of the particles and defects present in the semiconductor. Also to examine the quality of material, density of quantum wells, shape of particles, size of particles and dots, we can use this technique at high resolution.

The TEM follows basic principles same as the light microscope. It uses electrons instead of light, as light is used in light microscope. Results acquired by TEM images is much better than that of the images acquired from a light microscope because the wavelength of light in light microscope is much bigger than that of electrons of TEM. Thus, TEMs can show the deep information and details of internal structure of quantum dots. From the results taken by IMAGE- J-SOFTWAR size of the quantum dots without coating with polymer is approximately 2 nm(1.9nm) and shape is almost spherical.

4.4 SEM (SCANNING ELECTRON MICROSCOPY)

It is another type of electron microscope construct the images of particles present in a sample. Communication between electrons and atoms present in the sample is done with a focused electron's beam. This can be possible by scanning the surface of sample, which produce distinct signals which has the data regarding topography and composition of sample's surface. Scanning of the beam of electrons is done in a pattern which follows raster scan system, and the position of electron beam is merged with the signal which is detected. This is done to produce an SEM image as a result. SEM image is taken at 10um.

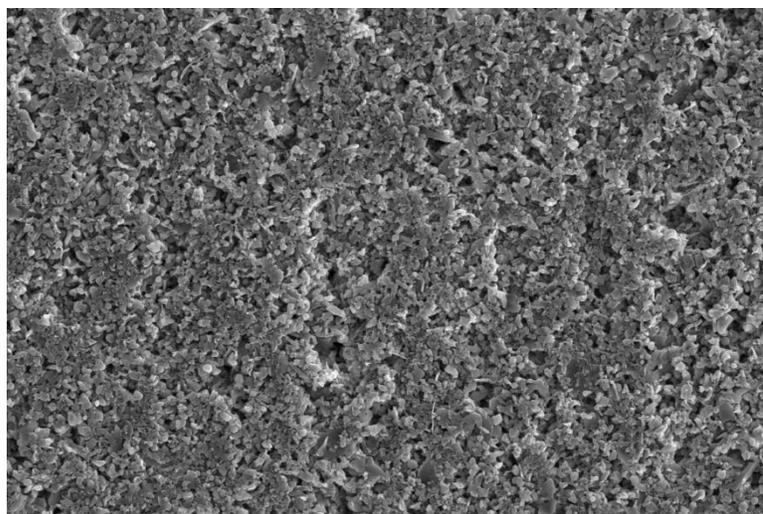


Figure 4.6 SEM image of CDSE4 coated with PCL

It has an application- to make a distinct type of signals at the surface of solid specimens with help of focused beam of high-energy electrons. The electron-sample interactions give signals which reveal information regarding the sample which includes crystalline structure, chemical composition of particles or sample, physical or outer morphology (texture), , and orientation of materials present in the sample. Data was collected in a selected area of the sample surface as its applications,. And variations in these properties occur due to the generation of a 2-dimensional image. In the result , image shows 2 dimensional image of CdSe4 QDs coated with PCL polymer.

4.5 FTIR (FOURIER-TRANSFORM INFRARED SPECTROSCOPY)

FTIR spectra of CdSe4 QDs without coated with PCL and CdSe4 coated with PCL were studied in range between 4000 to 600 per cm. CdSe4 have peak which show that there is presence of OH group at 3346 per cm present in prepared sample. Another peak which is present around 1630 per cm show that there is presence of NH group while around 1462 per cm give proof for the CH₂ bending. There is presence of thiol group due to which weak bands appears around 2200 cm⁻¹. C-O stretching Peak appeared between 1067–1041 cm⁻¹. Due to some impurities ,some extra peaks get appeared

After encapsulation of CdSe4 with polymer PCL, it shows an intense bright peak at around 3000cm⁻¹ that indicates the presence of CH group.

Aqueous route is used to prepared the luminescent QDs by directly affixing them to bio molecules. Due to their hydrophilic nature they affixed with great strength to the surface ,as QDs were prepared at huge temperature by the synthesis of organic route. This possess surfaces which is hydrophobic in nature and require treatment after biocompatibility and bioconjugation of biomolecules with QDs. Molecules that are found normally as functional groups on biomolecules are-, -C=O, -NH, -CHO,OH, and -SH etc.

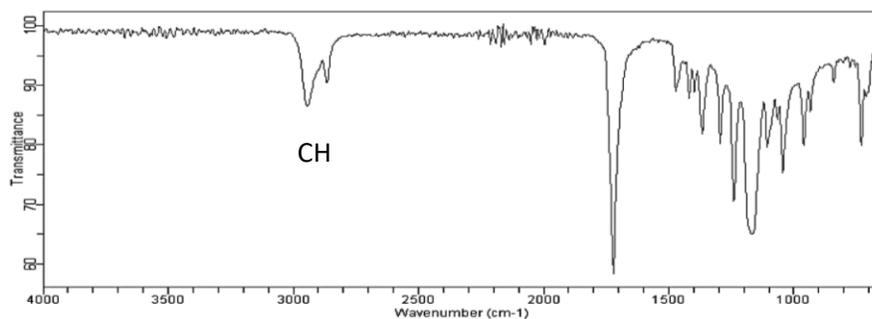


Figure 4.7 Standard peak of PCL polymer C=O results

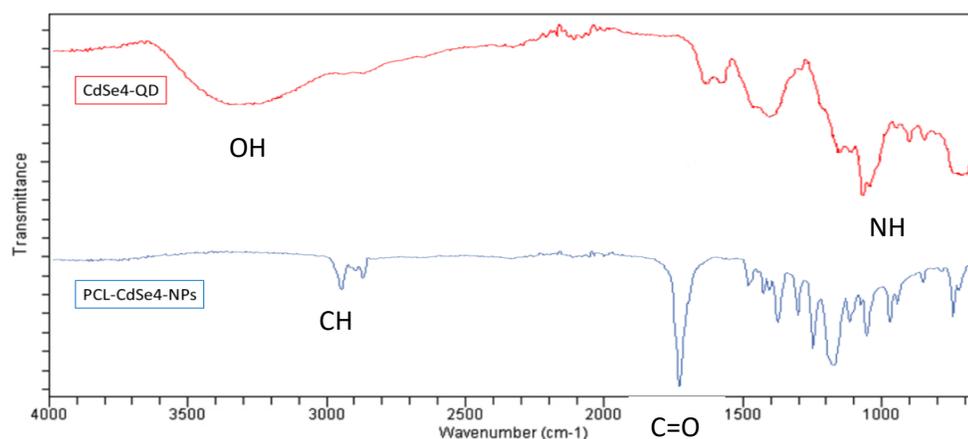


Figure 4.8 FTIR results of CdSe4 in above and CdSe4/PCL below

This technique is applied to find the nature of organic and inorganic materials. With the help of sample material versus wavelength graph we can measure the absorption of infrared radiation. Molecular components and structures of material is determined by the infrared absorption bands. First image is the standard image of FTIR indicates the functional groups present polymer(PCL) to compare the results with second image. Second image is the difference of results between CdSe4 without coating with polymer and CdSe4 coated with PCL polymer. Difference in the presence of various function group at specific wavelength indicates the presence of polymer on CdSe4 QDs.

4.6 MTT(3-(4,5-DIMETHYLTHIAZOL-2-YL)-2,5 DIPHENYLTETRAZOLIUM BROMIDE)

It is an assay which is used to determine the metabolic activity of cell with the help of colour indication. Under defined conditions it uses NAD(P)H-dependent oxidoreductase cellular enzymes, that show and indicate the number of live cells present in the sample. In this assay enzymes are being used which have capacity to reduce the tetrazolium dye MTT into insoluble formazan, which is represented by purple in color.

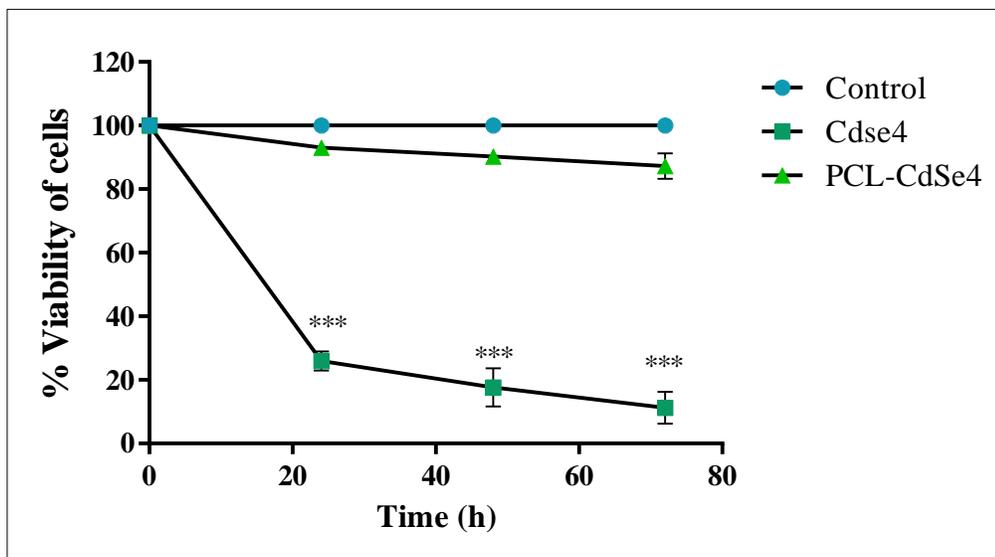


Figure 4.9 Graphically representation of MTT in cell containing CdSe4 and CdSE4/PCL

This image is graphically representation of viability of cells containing firstly control, second containing CdSe4 QDs without coated with polymer and in third cells containing CdSe4 coated with PCL polymer. We observe the metabolic activity of cells with different respective time i.e 24 hrs, 48hrs, and 72 hrs. in results we observe that cells containing CdSe4 QDs did not get killed or have less toxic effect of CdSe4 on cells in 72hrs.

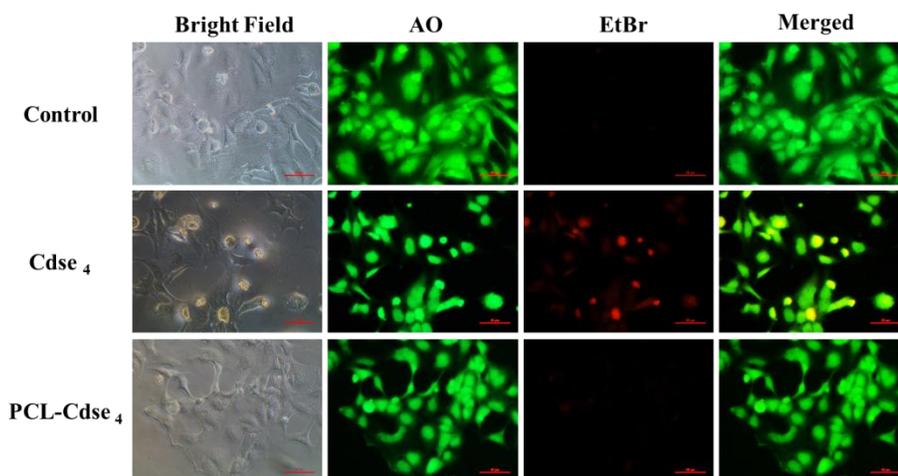


Figure 4.10 MTT images represent the viability of cell containing CdSE4 and CdSe4/PCL in 24 hrs

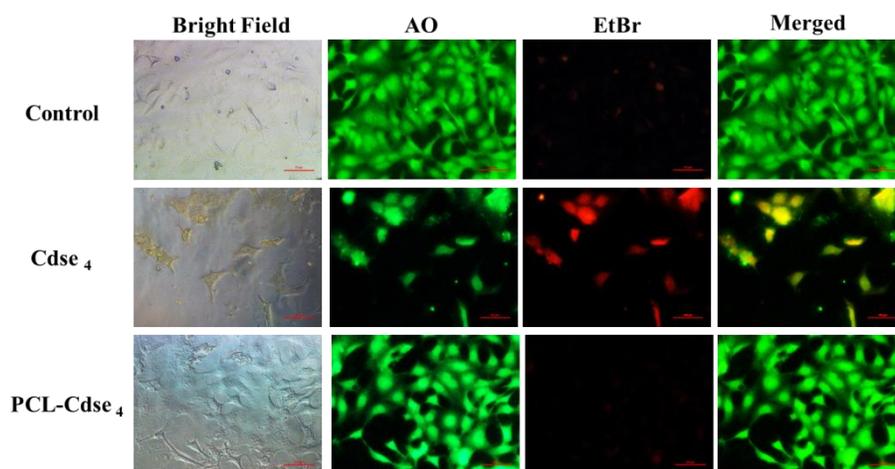
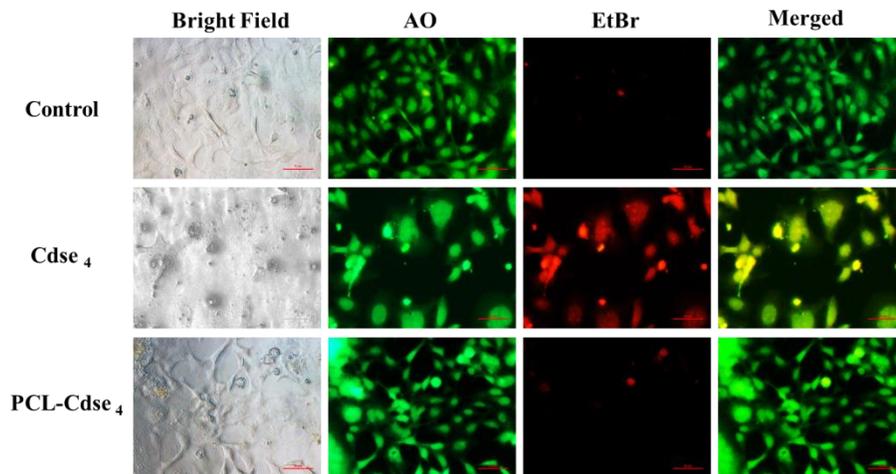


Figure 4.11 MTT image represent the viability of cell containing CdSE4 and CdSe4/PCL in 48 hrs



4.11 MTT images represent the viability of cell containing CdSE₄ and CdSe₄/PCL in 72 hrs

In this blue one the control and light green one is the PCL coated Cdse₄ and the dark green is the Cdse₄. This graph represents that the Cdse₄ is more toxic than the formulated PCL Cdse₄. Secondly these three images represent that AO is the Acridin Orange stain and EtBr (ethidium bromide) stain. Acridin orange stains both the cells dead cells as well as live cells, whereas EtBr specifically stains only a dead cell and then there is a merged image to depict that the area is the same. Then this experiment will be performed for 24 hours, 48 hours and 72 hours. In the same manner as this performed in MTT. In this mitochondrial dehydrogenase with that the tetrazolium salt that converts into formazan. Initially MTT is in yellow form then converted into purple color with the help of DMSO. That insoluble purple crystals get converted into soluble crystals with the help of UV where the cells are live or not. From this result we can say that the toxicity of CdSe₄ QDs gets reduced by coating it with PCL polymer.

CHAPTER 5

CONCLUSION

CdSe4 is made up of heavy toxic metals i.e cadmium and selenide which is toxic in nature. But it has various application in medical fields . so to reduce the toxicity of QDs we coated it with the biocompatible polymer i.e PCL. From results after coating it with this polymer we concluded that the toxicity of CdSe4 get reduced.

QDs have much potential to be used in the applications of biomedical and biotechnology. Also along with their unique fluorescence properties and the capability to become biocompatible by coating with biocompatible polymer and conjugation specifically with various biomolecules, it can have wide applications in medical and biotechnology field. They can also be used in various immune based techniques and in assays of molecules to detect different pathogens biomarkers, in more than one strategies.

Also, in disease outbreak areas, testing with care is the testing field which is supported. QDs is substantially increasing because of it's commercial availability and a various successful research of clinical utility has already done in this field. These factors have great concern and focus on the unique and applicative role of QDs in the coming of bio world. Thus, applications of CdSe4 quantum dots in medical and biotechnology world is only possible when the cytotoxicity of these QDs get reduced and become biocompatible for human body.

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