EFFECT OF ASCORBIC ACID ON CdTe QUANTUM DOTS AND INVESTIGATION OF DIFFERENT CHEMICAL SENSORS ON CdTeHg QUANTUM DOTS

by

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ABSTRACT

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The objective of my thesis is to investigate the effect of ascorbic acid on CdTe quantum dots, which is an antioxidant. It was observed that the quantum dots recovered their luminescence intensity after the interaction of the ascorbic acid, where the intensity was quenched by the photosensitizer. The luminescence intensity increased gradually for 0.1mM-0.5mM concentration of ascorbic acid and remained stable with higher concentrations of the antioxidant. Also, it was observed that under some reaction conditions like pH, temperature and concentration, ascorbic acid had a great influence on its photoluminescence.

In the second part of the research project, I studied the influence of different chemicals on the luminescence intensity of CdTeHg (NIR) quantum dots. The interaction of certain chemical agents like bleach, dimethyl methyl phosphonate (DMMP), with these quantum dots had quenching effect on the luminescence intensity, whereas with the common background chemicals like ethanol could not quench the luminescence intensity of the quantum dots. Studies

were carried out to find out the reasons behind this quenching and whether it is the singlet oxygen generated by these chemicals.

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CHAPTER 1

INTRODUCTION

1.1 Nanoscience and Nanotechnology

In last few years, Nanotechnology has been a buzzword among science and technology but actually what is “nanoscience and nanotechnology” and what possibilities it has in the advancement of our lives? [1]

Nanoscience and nanotechnology signifies mainly about the advancement of technologies at the nanoscale level, where the distinctive sizes are in the range of 1-100 nanometer, which is less than 1/1000 the diameter of a human hair. This perception allows the establishment of these nano-structures and devices that have innovative properties and functions because of their size. Recent technology in the field of science and engineering, have resulted in amazing new opportunities for research because of the potential applications of this nano-scale. The advancements in nanotechnology showed a great progress in the prevention, detection, diagnosis and treatment of diseases. It also provides different strategies to investigate the interior of individual molecules, whereas the other technologies require the analysis of large numbers of molecules purified away from the cells and tissues in which they usually function.

The awareness of this idea of analyzing things at the atomic, molecular and macromolecular level which turned out to be Nanotechnology which was proposed by Richard Feynman in 1959 who delivered a speech in the American Physical Society meeting. [1,3].

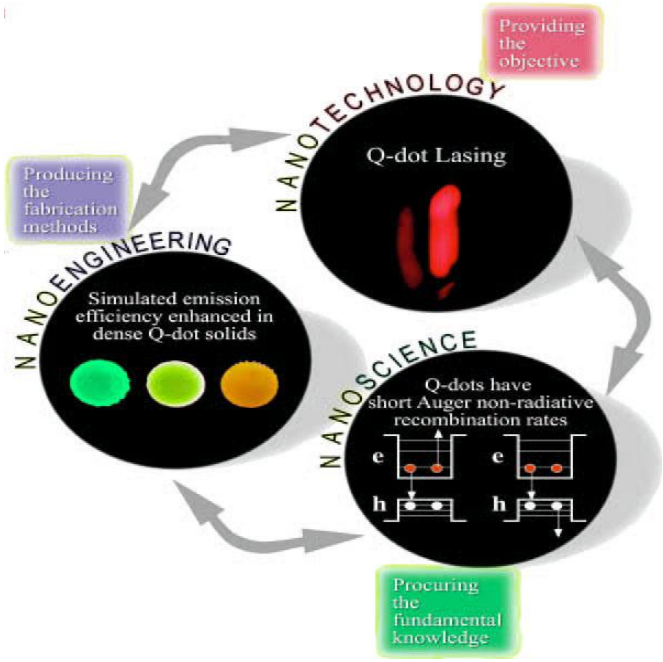

Figure 1.1- Relation of Nanoscience, Nanoengineering and Nanotechnology [3]

1.2 Applications of Nanotechnology

The potential of nanotechnology and nanoscience is helpful in improving the quality of human life, with wide applications in developing manufactured materials for aircraft and automobiles, nano devices and electronics with higher speed, more efficient security devices, and targeted drug delivery systems. The most stimulating application of nanoscience and nanotechnology is the diagnosis and therapy of diseases. This biomedical opportunity for nanotechnology and nanoscience will include many other applications discussed below:

1. Biomaterials and Tissue engineering- Biomaterials have the ability to recover the injured tissue with the help of nanotechnology. They also have the ability to oversee any acute clinical issues and interfaces for electrical stimulation. These biomaterials would not have inflammatory properties which can damage the surrounding tissues, instead they can withstand to the exposure

of inner tissue fluids. Also, they are biodegradable which can actively interfere with the host cells and later break up into non-toxic particles that can be easily excreted after the use. The fabrication of biomaterials and scaffolds with uniform structure and potential function to the human tissues and processes are in the possible area for research pathways which are now becoming obvious.

2. Targeted drug delivery- The functioning of cellular pathways mostly is still unknown though the awareness of these pathways related to disease has recently flourished. The potential to deliver the drug to the targeted site and to control the release in action to the intracellular responses reduces some consequences though it interferes with the damaged tissues. This theory can also be used to deliver drugs for diagnosis and therapy of *in vivo* imaging, and also for monitoring of various diseases and early detection.

3. Imaging biological processes and the effects of disease-The present imaging techniques offer vast knowledge on the morphology and structure of *in vitro* molecules and also anatomical data with great resolution. Nevertheless, it is necessary to know the dynamic living systems, and their outcomes by diseased pathways and for that we should be aware of imaging natural processes *in vivo* in real time without ruining them [2,3].

1.3 Quantum Dots

Semiconductor Quantum dots (QDs) are nanoscale materials with excellent optical and electronic properties compared to other bulk semiconductor crystals. Quantum dots are mostly in the range of Bohr exciton radius, which is the distance between the electron-hole pair (exciton). Electron-hole pair is formed when there is excitation of an electron from the valance band to the conduction band and exciton is produced by this bound electron and hole pair which are generally attracted by the electrostatic force. The band gap energy transition between the

molecular orbitals i.e., from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) is the function of quantum dot diameter [4]. Hence, this HOMO-LUMO transition energy between molecular orbitals determines the peak wavelength of optical absorption and luminescence of the semiconductor quantum dots, which range from UV to infrared region.

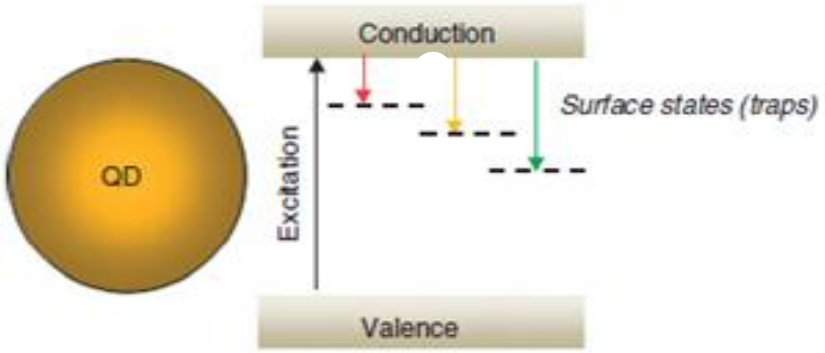

Figure 1.2- Electronic energy states localized within QDs [4].

Advantages of Quantum Dots over other organic dyes-

1. Quantum dots are 20 times brighter than other flurophores, because of high quantum yield
2. QDs have symmetrical and narrow emission spectra with broad excitation bandwidth.
3. QDs are more resistant to photobleaching than other organic dyes.
4. They are highly stable to photochemical degradation
5. Wavelength range is adjustable according to the size.
6. QDs have high extinction coefficients

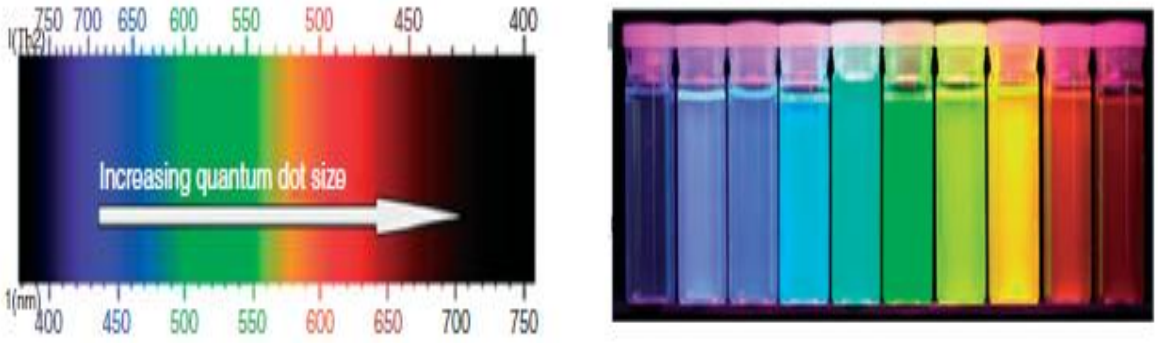

Figure 1.3- Schematic showing changes in optical properties of nanoparticles [4]

In quantum state, semiconductor nanomaterials exhibit some unique electrical and optical properties due to some quantum effect. As shown in figure 1.3,we can say that the wavelength shifts according to the size change. This effect is due to quantum size confinement [5].

It is discussed in more detail below:

1.3.1 Quantum Size Confinement

Semiconductor quantum dots, because of their extreme small size result in various physical, chemical and electronic modifications in the properties. These sudden changes occur due to the quantum effects. The most popular phrase in the present nanotechnology world is "quantum confinement" which explains about the electronic properties of how the organization of energy levels into which electrons can rise and fall and also how the optical properties change when the material is sampled in sufficient quantities. Specifically, the phenomenon results from electrons and holes being forced into a dimension that approaches a critical quantum measurement, called the exciton bohr radius, which was explained previously.

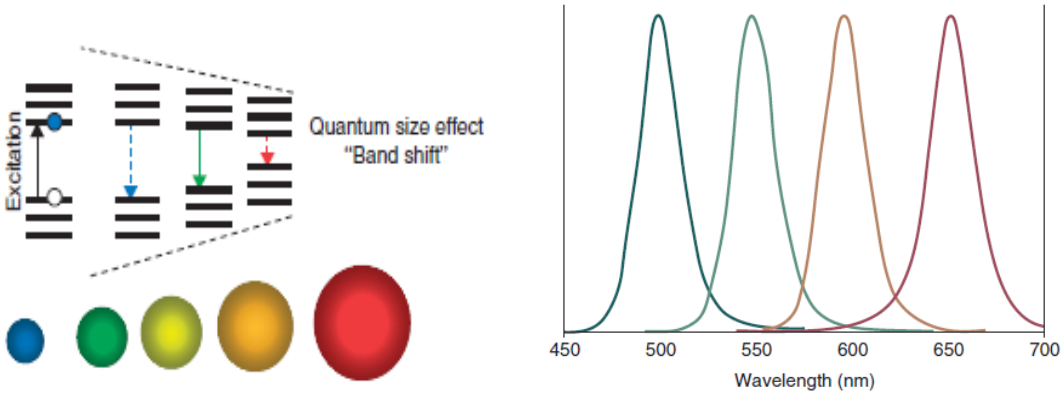

Figure 1.4- Electronic structure of QDs with blue shift due to quantum confinement [4]

Semiconductor nanocrystals are classified with different names according to their shapes. A quantum well which is said to be confined in one dimension, is a thin film of macroscopic width and length which is enough to be visible to the naked eye. Quantum wires, which are confined in two dimensions, can be thought of as nanometer-sized cylinders that can measure up to several microns in length [5, 6]. Finally, the quantum dot is a unidirectional nanometer-sized sphere, confined in all three dimensions. In figure 1.5, the electronic density of states in different dimensions is shown.

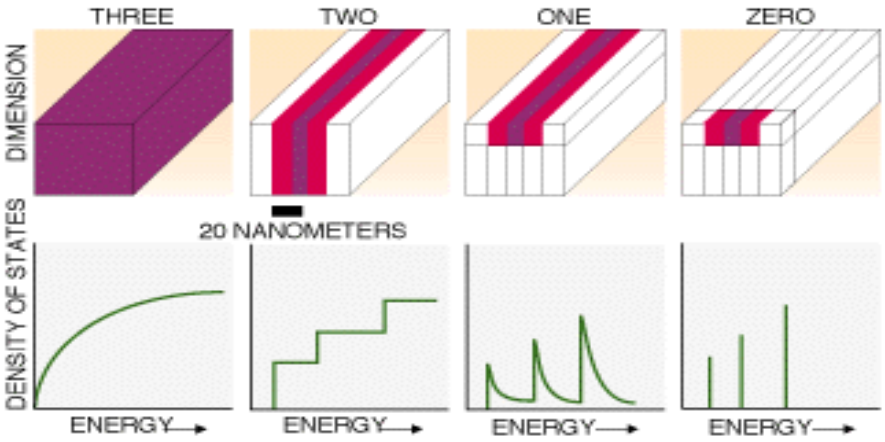

Figure 1.5- Electronic density of states as a function of dimensionality (D) of active layer.

1.4 Applications of Quantum Dots

As discussed earlier, quantum dots have a wide range of applications over other fluorophores because of their amazing properties like photostability, high quantum yield, and tunable wavelengths with respect to the size, narrow emission and broad excitation spectra. All these unique optical properties of quantum dots make them more beneficial and highly applicable in nanotechnology. Some of the main applications of quantum dots are discussed below:

1. Quantum dots in Biology and Medicine- Most of the research of quantum dots is being done for investigating applications for molecular and cellular biology, in DNA technology, as biosensors, immunofluorescence assays and in vivo imaging. This intense research of quantum dots in biomedical applications is because they can be imaged with higher resolutions than other organic dyes. Inspite of the developing research, some other concerns like narrow size distributions are still being studied for *in vitro* and *in vivo* applications. The size plays an important role as it can affect the biodistribution by controlling the receptors which have to be targeted. Thus, to make the semiconductor quantum dots more suitable for biomedical use, they are classifies into two categories based on the specificity. The first group is target-specific, in which the semiconductor quantum dots are conjugated to some ligands to obtain a core shell structure, which can be targeted to the specific injured site. After exposure to radiation, the targeted quantum dots at that particular site will be bright and the diseased site can be easily located from that of a healthy tissue. Hence, we can say that the quantum dots can be used in biomedical applications especially for *in vivo* and *in vitro* imaging, though there are some concerns to be considered like cytotoxicity, size distribution and surface chemistry.

2. Quantum Dots in Tissue and cell labeling- For testing the biocompatibility of the nano composites, *in vitro* and in vivo studies are done. The nanomaterials need to cross the cell membrane, the major barrier made of a lipid bilayer. This is carried out by different processes which are discussed below:

a) by nonspecific endocytosis where nanoparticles are not targeted to the appropriate site,

b) by microinjection of nanomaterials which is relevant only for few cells,

c) electroporation where the movement of nanomaterials across membranes consumes charges

d) the last most flexible process is targeted uptake which depends on surface modification. Therefore, for the nanomaterials to be biocompatible with the body, they are coated and conjugated to exhibit some properties like hydrophilicity, bio-inert, less aggregation, holding the role of nanomaterials and certifying the compatibility of nanocomposites in advance and decrease the clearance rate from the bloodstream. Recent observations said that, some of the issues faced by quantum dots can be improved by modifying the hydrophobic surface with amphiphilic polymers to avoid quenching of fluorescence, aggregation of nanoparticles, increase colloidal stability and reduce surface defects. By, continuous progress in the field of nanotechnology, it is acceptable to believe that the quantum dots coated with a biocompatible material, will be applicable in cell and tissue labeling, imaging and clinical trials.

3. Quantum dots and nanocomposites in Cancer diagnosis and therapy- Bioconjugated quantum dots act as distinctive markers because of their high photostability and multicolor emission, for detection of cancer cells *in vivo* especially during metastasis (major issue in the development of applicable treatments for cancer). In general, early diagnosis of cancer cells can be treated with effective drug delivery to the targeted site. The NIR quantum dots are more beneficial than other quantum dots as they are less invasive as the tissue absorbs less light. They allow less photons to

penetrate through tissue and limit the effect of tissue autofluorescence, which is the main application for discovering cancer lesions in complicated samples. Thus, quantum dots act as photosensitizers and radiosensitizers for cancer treatment [4, 21, 23]. Nevertheless, considering all the above applications, there are many issues to be considered especially the toxicity and degradation of quantum dots, which is the major problem, targeting with high specificity, designing high quality quantum dots. By looking at recent growth in research, the novel nanocomposites bioconjugated with surface modified quantum dots will probably knock out all the problems.

1.5 Toxicity of Quantum Dots

As mentioned earlier, quantum dots are preferred over other fluorophores because of their astonishing properties. Inspite of having a wide range of applications, they are facing a key issue of toxicity. Most of the quantum dots, currently synthesized are made of heavy metal atoms like cadmium, mercury, lead and arsenic which can be cytotoxic and more research is in progress for finding out their long term effects. It is still uncertain, whether surface coating of polymers onto these hydrophilic capped metal ions, will have any influence. In one of the quantitative analysis on the biodistribution of quantum dots, it is said that amount of cadmium in the liver and kidney was steadily rising over the period of 28 days, after injecting cadmium based quantum dots . While, the naturally aggregating sites of ions are liver and kidney, this redistribution of cadmium however indicates the degradation of the quantum dots. These particular results emphasize on the size and nonspecific protein interaction of the quantum dots, which has to be reduced to allow renal filtration and minimize the degradation in the tissues and organs of reticuloendothelial system. Though, at present it is still vague about the renal filtration limits for metal based nanoparticles and also the degradation of some organic capping. Therefore, it is a critical

requirement to investigate more about the toxicity, renal clearance, half-lives of quantum dots in human body, because these nanoparticles in future will be of great importance [4, 28].

1.6 Aim of the Research Project

The main focus of my thesis was on applications of CdTe and CdTeHg quantum dots in various applications of nanotechnology and nanomedicine. The two projects which were carried out towards achieving these goals are:

1. To study the effect of ascorbic acid on the photoluminescence of CdTe quantum dots that can be used for imaging applications.
2. Investigating the influence of different chemical sensors with CdTeHg (NIR) quantum dots and detection of singlet oxygen ($^1O_2$).

CHAPTER 2

INFLUENCE OF ASCORBIC ACID ON CdTe QUANTUM DOTS

2.1 Introduction

As discussed in the previous chapter, quantum dots have the ability and efficiency to develop into a new class of fluorescent probes for many biological applications because of their unique optical properties. Because of their narrow emission spectra, broad excitation spectra and tunable wavelength according to the size, these quantum dots have wide applications. Also, their brightness and endurance to photo bleaching allows the use of lower intensity from laser over longer durations. This novel and exciting property makes them more applicable for live-cell imaging of thick tissues and cells for extensive period of time. Quantum dot based fluorescent sensors are being more focused in recent investigations. Mainly, turn-on sensors are beneficial than turn off sensors as they have high sensitivity and less background signal. This idea was revealed by quantum dot based sensors through signal quenching.

Ascorbic acid (vitamin C) is an antioxidant which is one of the important substance required by the body. It has many functions as an antioxidant and as a nutrient. It also has a role of a cofactor in neurotransmitters, enzymes which essential for preventing many diseases. Many investigations have been done for sensing ascorbic acid in biological fluids. Recent studies show that fluorescent probes like quantum dots were used for detection as they seemed to be more useful due to their high sensitivity. CdTe quantum dots were used as turn-on fluorescent sensors for probing the antioxidant in biological fluids [11, 19].

From previous research, photosensitizers like protoorphyrin IX (PPIX) has a quenching effect on the quantum dot luminescence [10]. Here, we study the effect of ascorbic acid on CdTe quantum dots with protoporphyrin (photosensitizer) and explore whether there is any recovery of fluorescence induced by the photosensitizer. Also some fluorescence studies of ascorbic acid were carried under different reaction conditions like pH, temperature, concentration and reaction time.

2.2 Effect of Ascorbic Acid on photoluminescence of CdTe Quantum Dots

CdTe quantum dots stabilized with TGA were used to investigate the effect of porphyrins on these quantum dots. Here, the porphyrin used was protoporphyrin (PPIX) which can be used as a photosensitizer in the application of photodynamic therapy. The CdTe quantum dots used in this study have wavelength in the visible region, where emission wavelength is around 613nm and excitation wavelength around 490nm.

2.2.1 Experimental Section

PPIX was used in two different concentrations of 0.35mM and 0.5mM and their respective stock solutions were prepared. CdTe quantum dots were then interacted with PPIX at different concentrations and their respective photoluminescence was measured. Then the ascorbic acid (antioxidant) samples were prepared at 0.3mM, 0.4mM, 0.5mM, 0.7mM and 1mM concentrations respectively. These standards of ascorbic acid at various concentrations were then added to CdTe quantum dots mixed with PPIX to study their influence on the luminescence intensity of the quantum dots. The photoluminescence was measured and compared to previous results before addition of the antioxidant. In figure 2.1, we can observe the samples before addition of ascorbic acid and also after addition with different concentrations under normal and UV light.

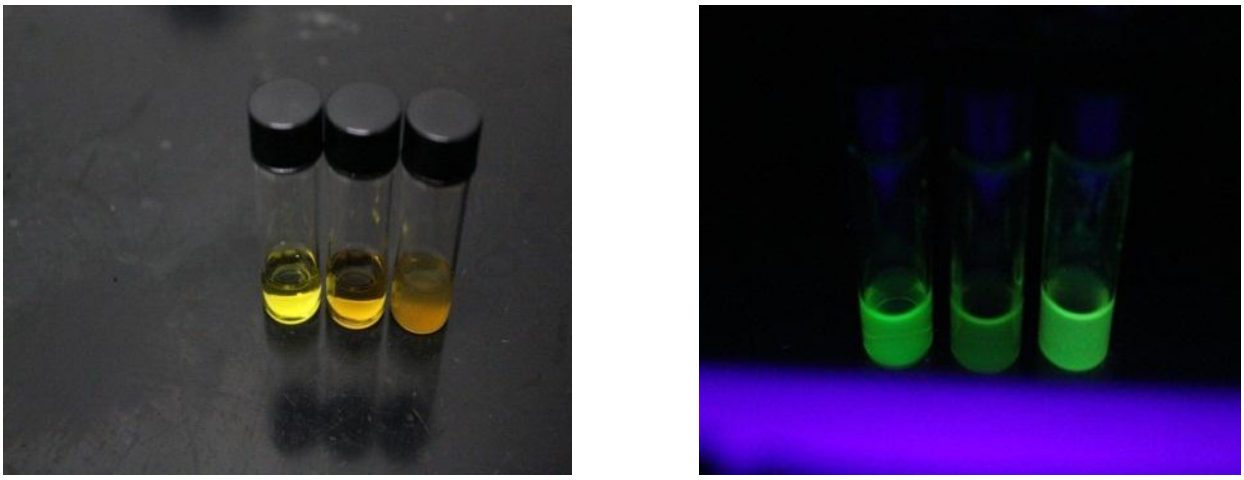

Figure 2.1- Left to right: CdTe, CdTe with PPIX, CdTe with PPIX after adding ascorbic acid respectively under normal light and same samples under UV light

2.2.2 Results and Discussion

The luminescence intensity was quenched with the interaction of porphyrin on CdTe quantum dots for both the concentrations. According to previous literatures, the mechanism behind this quenching was because of the oxidation of the quantum dots due to generation of singlet oxygen by photosensitizer (PPIX) [10]. Energy transfer cannot be possible as there would be no formation of chemical bonding as the porphyrin was just added to the quantum dots. Therefore, it was expected that the size of quantum dots was affected because of surface defects due to which it also resulted in photobleaching of these quantum dots [11].

When ascorbic acid was added to the quantum dots mixed with protoporphyrin, there was an enhancement in the luminescence intensity. This recovery was due to the action of ascorbic acid in reducing the oxidized states on the surface which helped in quantum dot passivation. From previous research, it was said that certain antioxidants consume the oxidized surface atoms resulting in the fluorescence restoration [11].

So, as the concentration of ascorbic acid increased, it induced recovery of the luminescence intensity. This increase in the intensity was observed only till 0.5mM concentration and from 0.7mM the intensity almost remained the same with no rise. This step function is because ascorbic acid at higher concentrations acts as a pro-oxidant according to previous research [42].

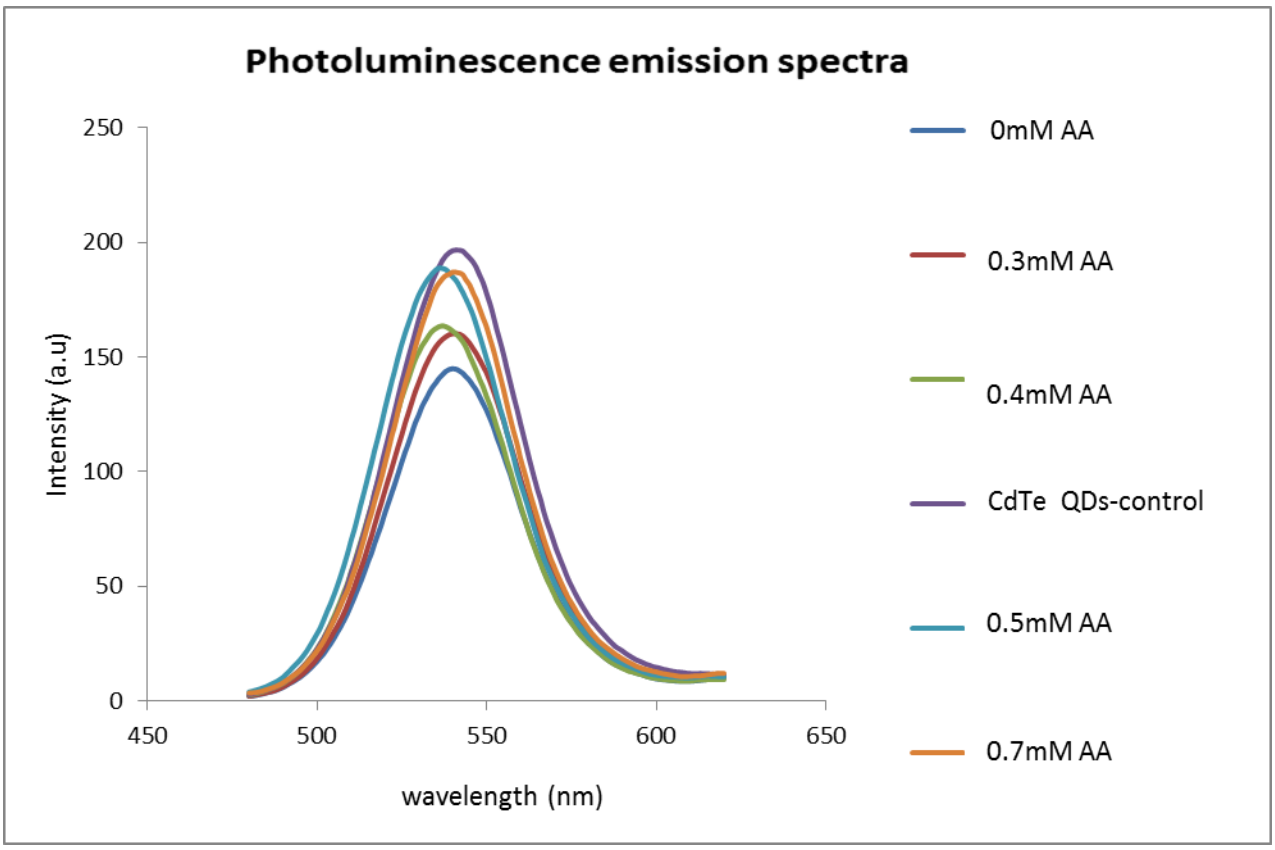

Figure 2.2- Effect of different concentrations of ascorbic acid on PPIX-CdTe QDs (AA-Ascorbic acid)

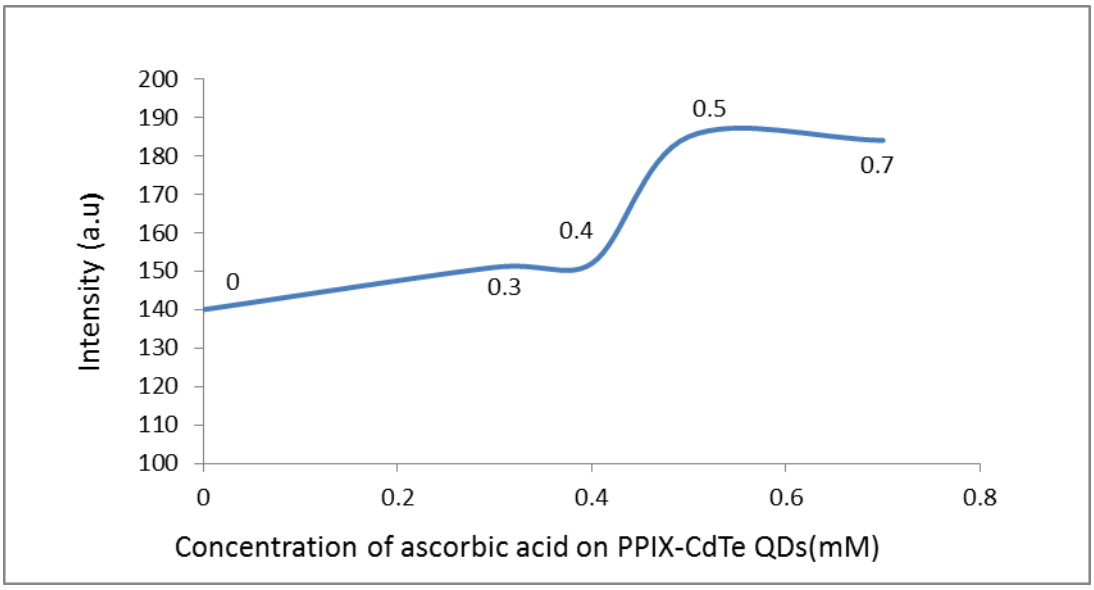

Figure 2.3- Fluorescence Intensity with change in concentration of ascorbic acid

2.3 Photoluminescence of Ascorbic Acid

In previous section, we observed that there was a recovery of fluorescence intensity in CdTe quantum dots due to the interaction of ascorbic acid. After observing this, further studies have been done to investigate more about ascorbic acid and its luminescence. Here, ascorbic acid with different pH values was studied to study effect of luminescence.

2.3.1 Experimental Section

Materials used here were ascorbic acid, DI water, NaOH and HCl for making the desired samples. Ascorbic acid samples were prepared at different pH values from 2 to 12. Ascorbic acid had a pH value in the range of 3-3.5, so the chemicals used here to adjust pH were HCl to decrease pH and NaOH to increase the pH value. The samples of ascorbic acid with pH 2-12, which were prepared, are then measured for fluorescence and also pictures were taken in normal light and under UV light.

2.3.2 Results and Discussion

In figure 2.4 it was observed that, samples with pH greater than 7, showed some luminescence and after 1 day heating there was an increase in the intensity. This was because the ascorbic acid reacts with NaOH to form metal ascorbates with Na. The formation of these complexes resulted in the photoluminescence as reported from previous reviews [41]. Also from figure 2.5, the luminescence spectra of the samples before heating are observed. The ascorbate undergoes auto-oxidation in the presence of metal ions [13]. So, as the concentration of NaOH increase ascorbate oxidation increases with increase in the luminescence. And later the samples were heated for 1 day at 40°C and again the fluorescence was measured after heating. From figure 2.6, the photoluminescence measurements are observed where the luminescence intensity increased drastically after heating for 1 day with increase in pH. This shows that even temperature has a strong influence on the ascorbate formation. From previous literature, it was said that pH has a strong effect on the sensitivity and oxidation of ascorbic acid, and also temperature and concentration improves the formation of metal ascorbate complexes [13, 41]. All these factors result in the enhancement of luminescence intensity. The temperature also was selected as 40°C to be suitable for biological applications. If it was greater than 45°C then it cannot be used for biological purposes [12, 13].

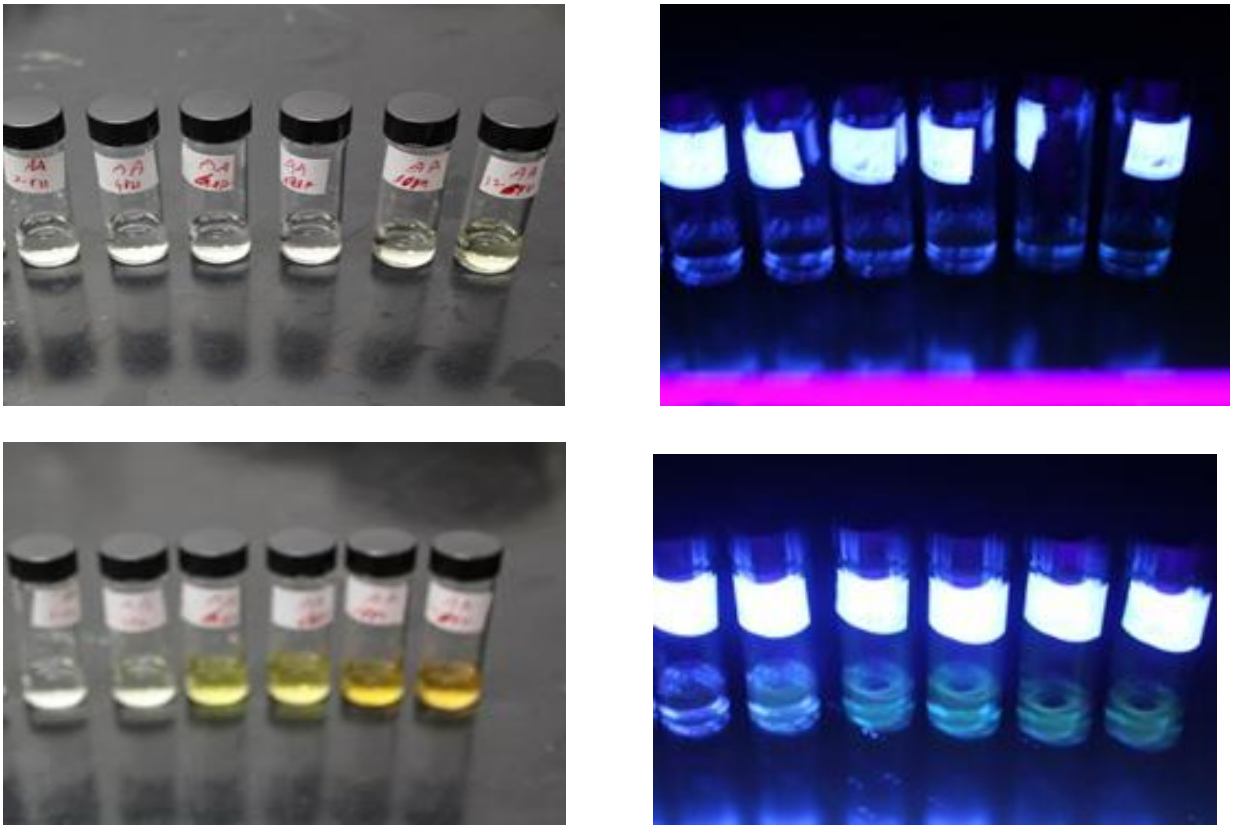

Figure 2.4- Top to Bottom: Ascorbic acid samples from 2pH to 12pH under normal and UV light respectively and same samples after 1 day heating at 40°C under normal and UV light.

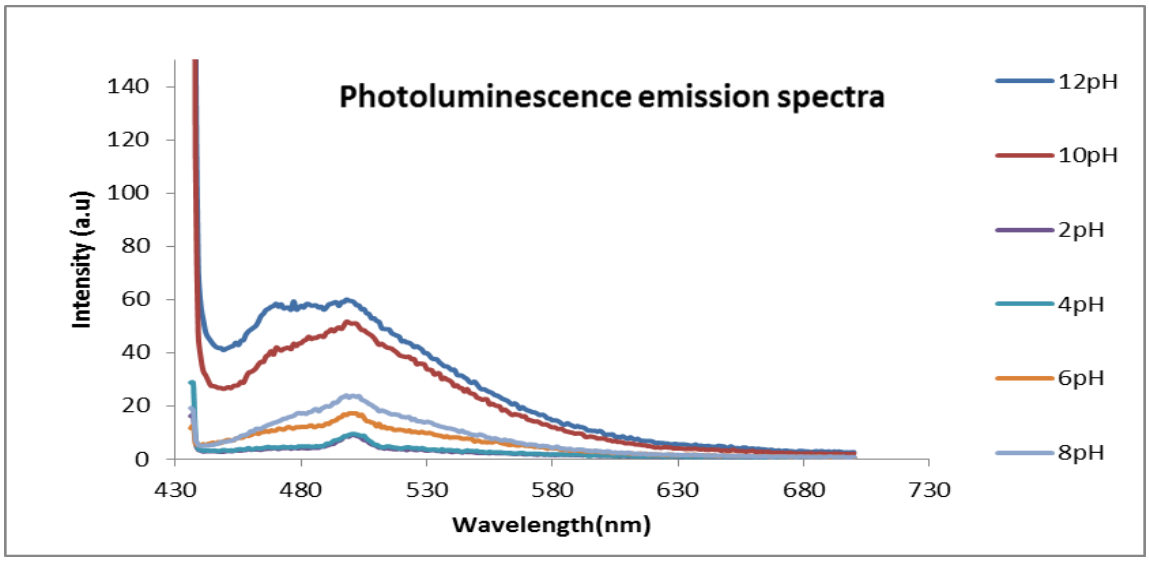

Figure 2.5- Photoluminescence measurement of ascorbic acid samples of different pH values.

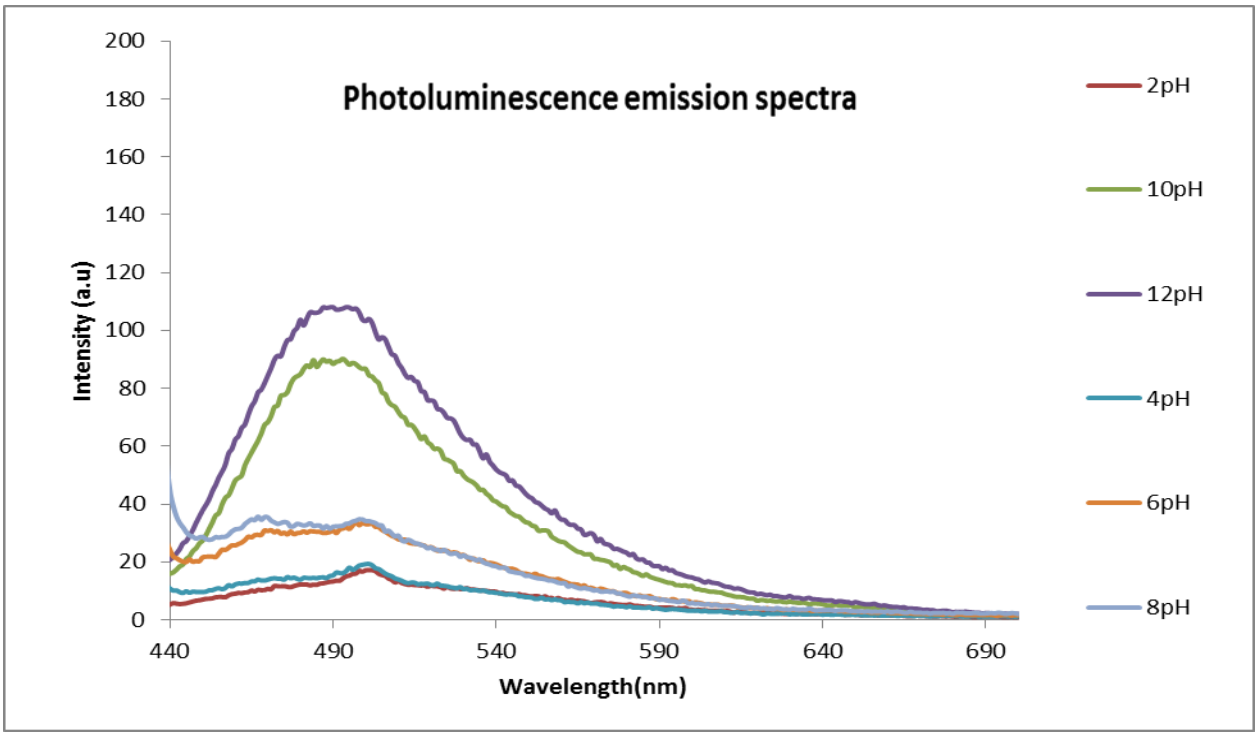

Figure 2.6- Photoluminescence measurement of ascorbic acid samples of different pH values after 1 day heating at 40°C.

2.4 Conclusion

In this section, we observed that ascorbic acid had a huge impact on the photoluminescence of CdTe quantum dots. For CdTe quantum dots intensity that quenched, when reacted with the photosensitizer (PPIX), the quenched intensity was restored when ascorbic acid was added to it. This was because ascorbic acid helped in quantum dot passivation by reducing the oxidized states. But this recovery was only for the concentrations from 0.1mM to 0.5mM ascorbic acid, as for higher concentrations of ascorbate the oxidation effect will reduce. It was also investigated that, fluorescence of ascorbic acid was a function of pH, temperature and also concentration. As the pH value increased from 2 to 12, the luminescence intensity of ascorbic acid also increased. This was due to the formation of metal ascorbate complexes, where

NaOH was used as base to adjust the pH. Here, NaOH reacted with ascorbic acid to form these metal ascorbic acid complexes, which are responsible for the luminescence. The formation of these compounds depends on temperature and concentration. Hence after heating the same samples for 1 day at 40°C, there was a drastic improvement in the intensity.

CHAPTER 3

EFFECT OF DIFFERENT CHEMICAL SENSORS ON CdTeHg QUANTUM DOTS FOR DETECTING SINGLET OXYGEN

3.1 Introduction

The photosensitizers are favourably confined in the tissue, which generate singlet oxygen, some toxic species and other free radicals that will be responsible for destroying the cells, mutations, photooxidation of proteins and DNA. Singlet oxygen has a very short half-life, and they can also be generated by some proteins in the UV and IR region. It is not a free radical, on the contrary is highly reactive substance which is an excited form of dioxygen molecule [15]. The photosensitizers that have been accepted for clinical trials fall in visible spectral region (<700nm), which prevents light from deeper penetration and hence, restricted to superficial tumors and skin disorders. Researchers are trying to overcome these limitations by developing photosenstizers which can absorb in the NIR region, having high capability of penetration. Also, they are trying to focus on the efficiency of singlet oxygen generation in the pathological atmosphere of the tissue which would help in minimizing the photosensitizer dosage [15, 16].

3.2 NIR Quantum Dots

Most of the previously developed fluorescence imaging systems in visible region. Recently, investigators have mainly concentrated on developing quantum dots for imaging in near infrared (NIR) region as they have more advantages. The photon penetration into the interior of the tissue in visible region is not possible because of the scattering and absorption coefficients of body fluids like fats, oxyheamoglobin, deoxyheamoglobin and water is maximum.

Whereas, NIR spectral region acts as diagnostic window for imaging due to less autofluorescence by tissue and also the absorption coeffecients of the tissue components is lower. This allows the deeper penetration of photons into the tissue and it is used in diagnosis of many tumors. Hence, NIR quantum dots have overcome the limitations of low penetration depth and tissue autofluorescence of other fluorophores [16, 34].

Here, CdTeHg quantum dots of NIR range were synthesized from CdTe quantum dots. By increasing the size, the emission wavelengths could be adjusted to produce the quantum dots in this infrared region, which is not an effective approach though. In this experiment, we prepared near infrared quantum dots from by previously used methods to decrease the bandgap and increase the emission wavelength [38]. In brief, with 140µL of $Hg(ClO_4)_2$ was added to 3mL CdTe quantum dots stabilized with TGA. This solution was store in refrigerator for 1 week to shift the emission peak. When the luminescence was measured, there was a red shift observed without any fall in the intensity. This was because of the Hg^{2+} ions replacing the Cd^{2+} ions, which results in the surface defects of the quantum dots. Hence, CdTeHg quantum dots which are NIR counterpart of CdTe quantum dots were obtained. The spectrum obtained was had a peak emission at 873nm with an excitation of 590nm.

3.3 Interaction of various sensors with CdTeHg Quantum dots

As we know that, NIR quantum dots would be more useful in *in vivo* imaging with deeper penetration of light and lower tissue autofluorescence. Here we studied whether some chemicals/sensors have any influence on the photoluminescence of CdTeHg quantum dots. So, in this study we have chosen some common background chemicals like ethanol, and other chemical agents like bleach and dimethyl methyl phosphonate (DMMP).

3.3.1 Experimental section

CdTeHg with common background chemicals- First, interaction of ethanol (common background chemicals) with NIR quantum dots was studied. Here, 3ml of CdTeHg quantum dots was mixed with gasoline of 0.01mM, 0.03mM, 0.05mM, 0.07mM, 0.1mM and 0.125mM concentrations (1%, 3%, 5%, 7%, 10% and 12.5%). Each concentration of ethanol was added to 3mL of CdTeHg quantum dots and their photoluminescence was measured. The photoluminescence was measured for all the concentrations of ethanol.

CdTeHg with other chemical sensors- Here, other chemical sensors like bleach and dimethyl methyl phosphonate (DMMP) were added to CdTeHg quantum dots. 3mL CdTeHg quantum dots was added to various concentrations of bleach and DMMP (0.01mM, 0.03mM, 0.05mM, 0.07mM, 0.1mM and 0.125mM) to study their influence on the fluorescence intensity of the quantum dots. All the samples were excited at 590nm wavelength at 3nm slit width using a Shimadzu RF-5301PC fluorescence spectrophotometer with an NIR set up (Ocean Optics kit with NIR probe). The results obtained will be discussed in the next section. The photoluminescence measurements were taken, for ethanol, bleach and DMMP including DI water for comparing the results.

3.3.2 Results and Discussion

The photoluminescence results of NIR quantum dots interacted with various chemicals are discussed in this section. NIR quantum dots were first mixed with common background chemicals i.e., ethanol and its influence on the photoluminescence of quantum dots was examined. The results show that that there was no much quenching of the fluorescence intensity with the interaction of this type of chemicals. Even at higher concentrations of these chemicals,

there was barely any impact on the intensity of CdTeHg quantum dots. The peak emission wavelength was in the range of 870-892nm at excitation of 590nm. The mechanism behind this is that in the main additives involved are antioxidants. And we know, from previous literature that most of the antioxidants help in passivating the surface of quantum dots by reducing the oxidized states [11]. Since, ethanol is the major component in gasoline and sometimes used as an alternative for gasoline, we expect that it will show no quenching effect on the luminescence intensity of the quantum dots [17]. As seen in figure 3.1, the photoluminescence measurements of different concentrations of ethanol can be observed. In figure 3.2 it shows the relation between the concentration and intensity of ethanol. With increase in concentration of ethanol, the intensity had very less influence.

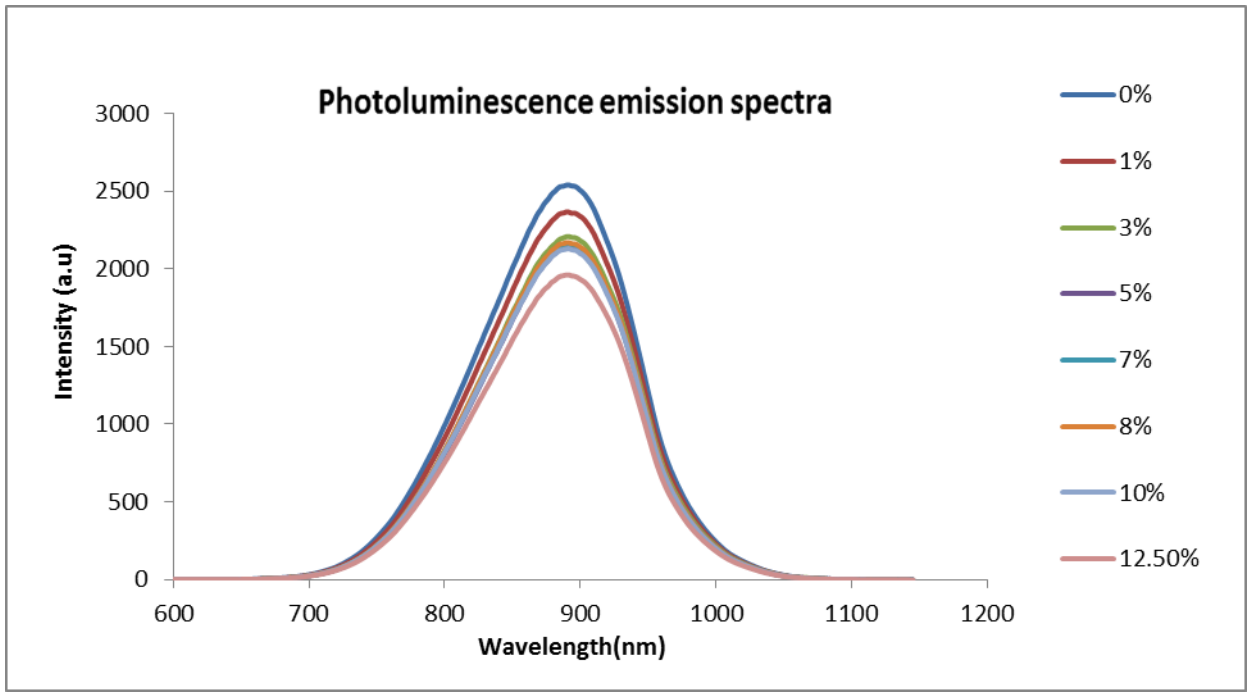

Figure 3.1- Photoluminescece measurements of different concentrations of ethanol interacted with CdTeHg quantum dots.

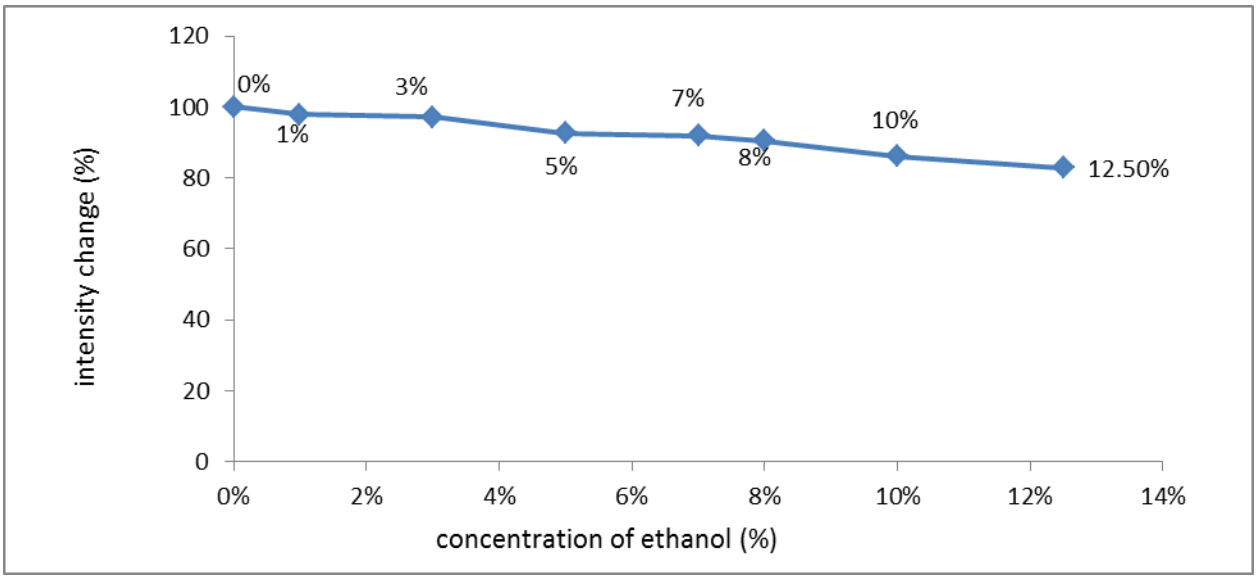

Figure 3.2- Graph showing intensity change of NIR quantum dots Vs concentration of ethanol

For other chemicals i.e. dimethyl methyl phosphonate (DMMP) and bleach, the results showed a quenching effect in the photoluminescence intensity of the NIR quantum dots unlike the common background chemicals. When these chemicals were added to CdTeHg quantum dots, the luminescence decreased with the increase in concentration of the compoumds. As we know that, bleach is a chemical that undergoes oxidation in eliminating the color. In this study also, we observe that addition of bleach to the quantum dots reduces the intensity. There is a tremendous decrease observed with higher concentrations of bleach. This quenching corresponds to the increased number of lattice defects in the quantum dots resulting in further nonradiative recombination pathways also known as quenching states [18]. Some of these defects are not permanent and are vanished which result in the intensity variations, whereas permanent defects result in complete quenching of the fluorescence. In case of DMMP, it is known that DMMP is very sensitive to most of the fluorophores but there was no strong reason for the quenching effect. It might be because of the oxidation, the surface defects can occur by which the intensity

decreases with increase in concentration of DMMP. In figures 3.3 and 3.5, the photoluminescence measurements of different concentration of DMMP and bleach can be observed. In figures 3.4 and 3.6 shows the relation between the concentration and intensity of both DMMP and bleach respectively. Here, we can interpret that these chemicals have a quenching effect on the luminescence intensity of NIR quantum dots.

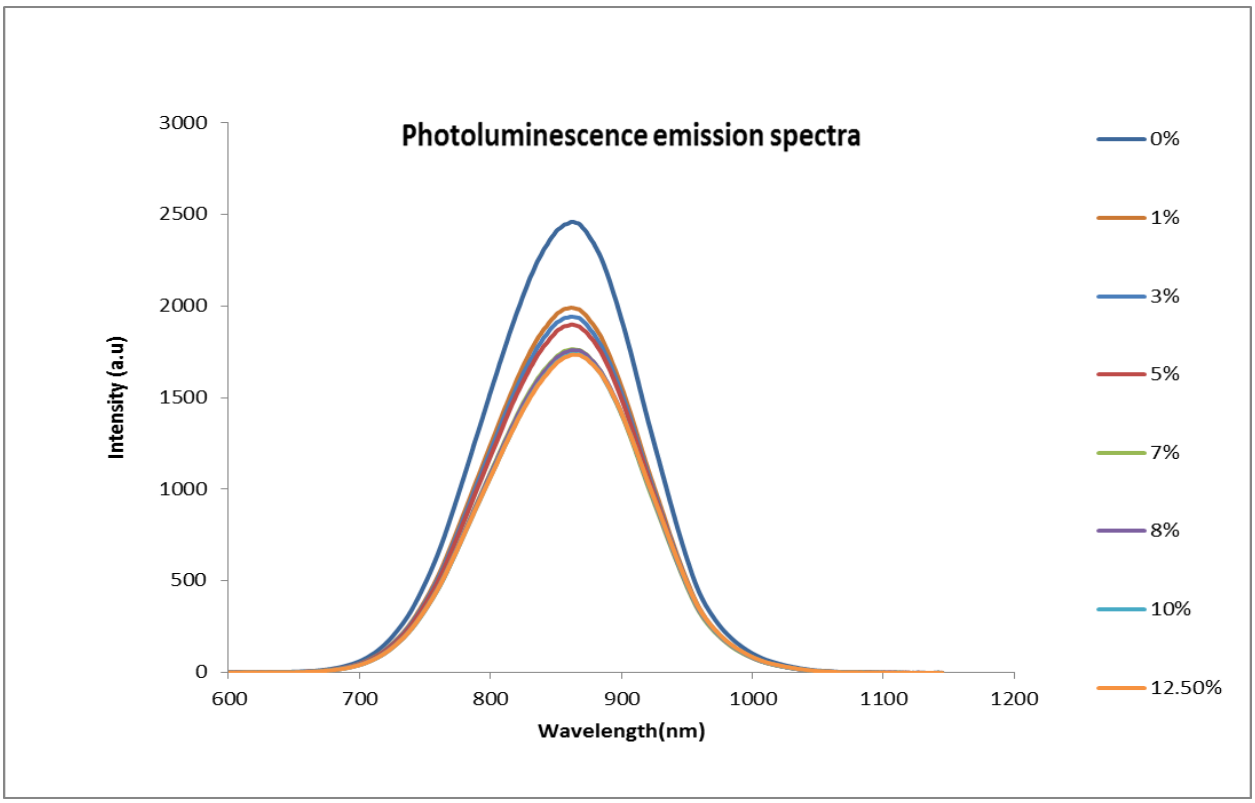

Figure 3.3- Photoluminescence measurements of different concentrations of DMMP interacted with CdTeHg quantum dots.

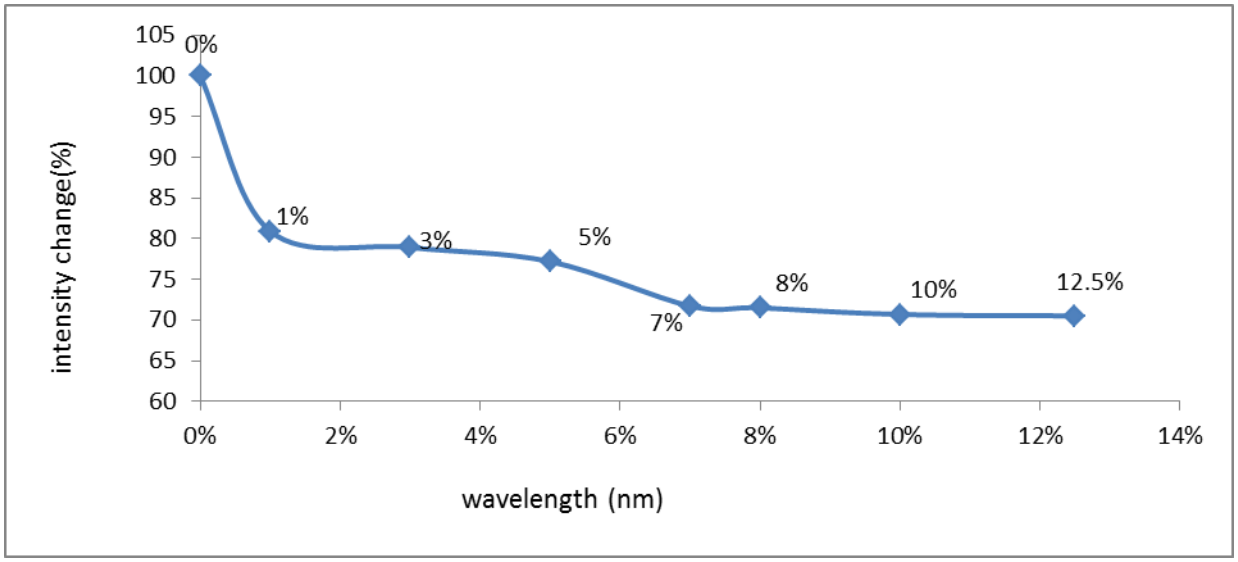

Figure 3.4- Graph showing intensity change of NIR quantum dots Vs concentration of DMMP

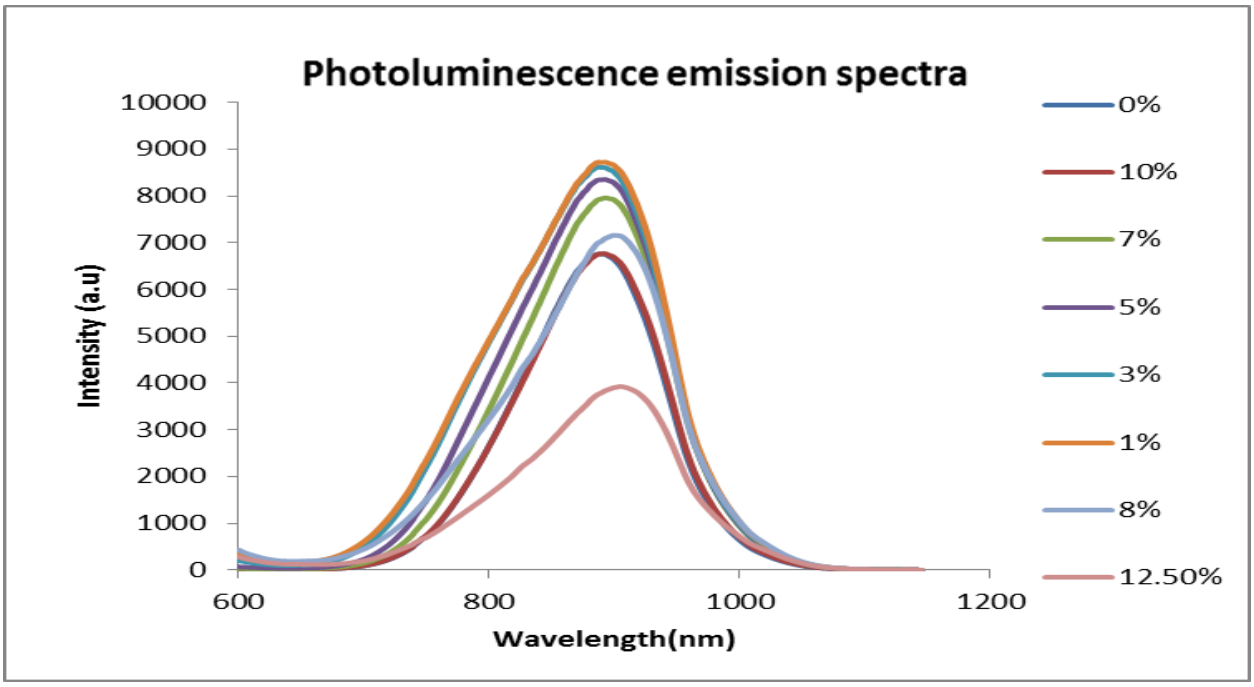

Figure 3.5- Photoluminescece measurements of different concentrations of bleach interacted with CdTeHg quantum dots.

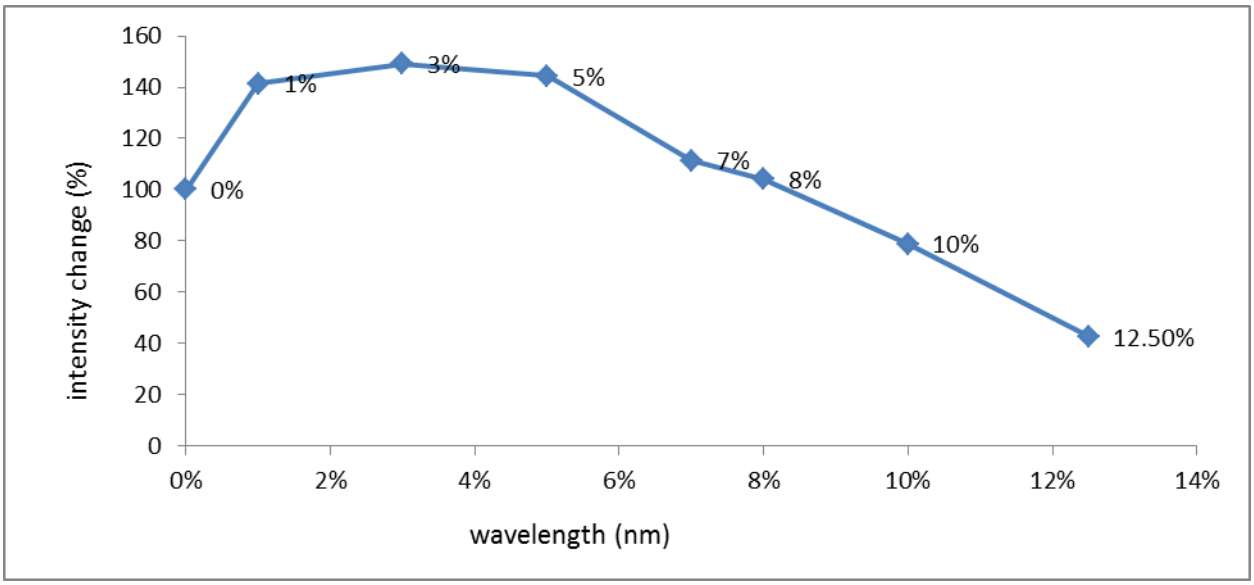

Figure 3.6- Graph showing intensity change of NIR quantum dots Vs concentration of Bleach.

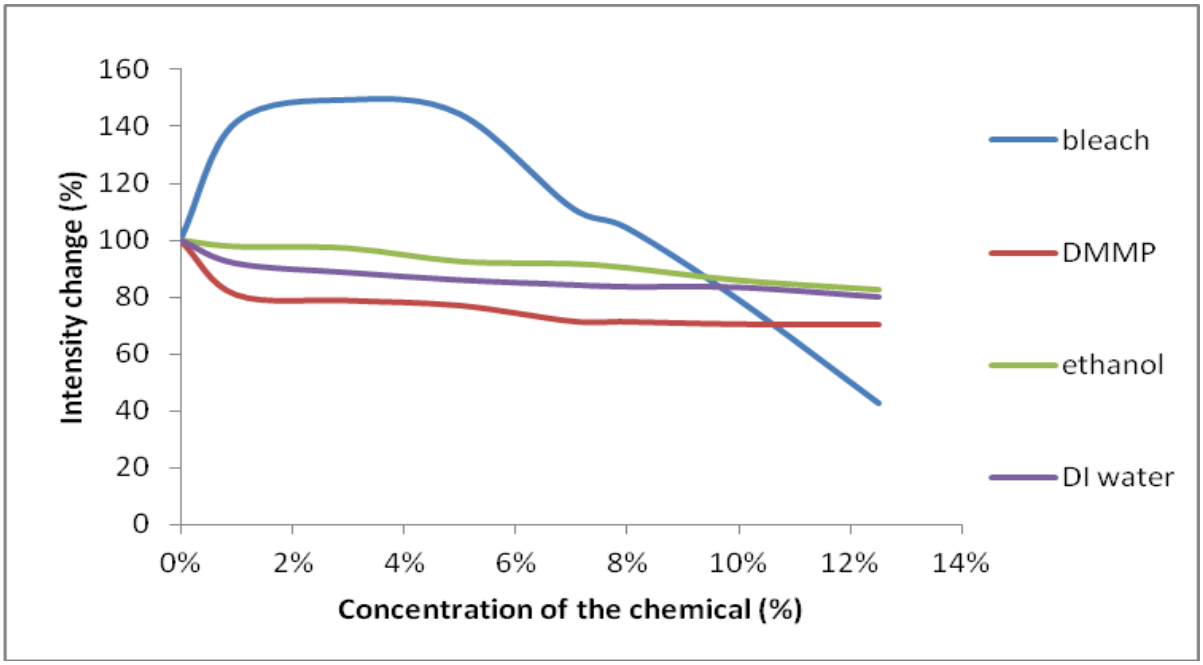

Figure 3.7- Comparison of luminescence intensity of all chemicals with DI water.

In figure 3.7., all the photoluminescence results of bleach, ethanol, DMMP are shwon and compared with DI water. It was observed that DMMP, ethanol, have almost similar spectra of DI water. While, the only chemical which showed a very high quenching effect with NIR quantum dots was bleach.

3.4 Singlet Oxygen Detection

In the previous section, from the photoluminescence results we observed that with chemical sensors like bleach and DMMP, the fluorescence intensity of CdTeHg quantum dots decreased with increase in the concentration of chemicals. This was interesting and seems like they have the properties of a photosensitizer, where it gets excited when irradiated and generates singlet oxygen. It was interesting to know the reason behind the quenching for some chemicals like bleach, DMMP. So here, some studies have been carried out to investigate the presence of singlet oxygen, as oxidation might be the reason for quenching. Other quenching mechanisms being energy transfer, chemical reactions which are impossible here, as seen from the luminescence data.

3.4.1 Singlet Oxygen and its generation

Singlet oxygen is the most reactive form of oxygen molecule in its excited state. Many experiments were carried out to study its physical and chemical properties, as it was found to be participating in most of the biological reactions. It was later confirmed that singlet oxygen reacted with many biomolecules like DNA, proteins and lipids and it was proved to be the main intermediate product in oxidation of these biological molecules. The lifetime of singlet oxygen varies according to the solvent used.

Singlet oxygen is generally generated by both physical and chemical methods. The two methods will be discussed briefly below:

1. Physical methods- The most used physical method to produce singlet oxygen is by the use of photosensitizers useful in photodynamic therapy. There are two different types of mechanisms involved in this process. In the first type radicals are produced by reaction the between sensitizer and substrate molecule. These radical ions formed by the electron transfer interactions generate superoxide ions when reacted with oxygen. In the second type of mechanism the photosensitizer is excited to triplet state when illuminated and the absorbed energy is then used to convert the adjacent oxygen molecule to its singlet state. The cytotoxic singlet oxygen produced in this process, is responsible for damaging the diseased tissue and has become the root for photodynamic therapy.

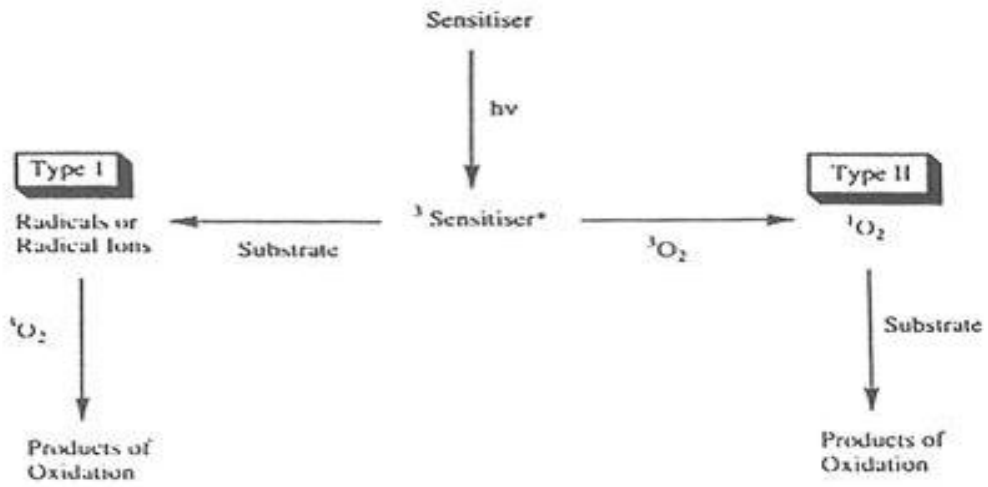

Figure 3.8- Schematic showing the two mechanisms involved in PDT, where type I generates radicals and type II generates singlet oxygen. [24].

Other physical method is where radio or microwaves are used to produce singlet oxygen. The chemical methods will be briefly discussed below.

2. Chemical methods- Apart from physical methods, some chemicals are also used to generate singlet oxygen. When hydrogen peroxide and hypochlorite react during phagocytosis and also

during thermolysis of some aromatic hydrocarbons and organic compounds, this reactive singlet oxygen molecule can be formed [24].

3.4.2 Methods for detecting singlet oxygen

Singlet oxygen is detected in many different ways. Some of the ways are discussed briefly below:

1. By scavengers- They can prevent the reaction which depends on singlet oxygen production. Examples are azide, carotene, ascorbate and many more.

2. By heavy water- Using heavy water (D_2O) presence of singlet oxygen can be detected, as the lifetime of singlet oxygen is longer in heavy water than normal light water. So, the reaction in D_2O will be highly dependent on singlet oxygen.

Other ways for detecting singlet oxygen are by luminescence, calorimetry, photon ionization and ESR [24, 25, 26].

3.4.3 Experimental Section

Materials used were Singlet oxygen sensor green (SOSG) from Invitrogen Corp, CA, USA, Sodium azide (NaN_3), Ethanol and Dimethyl methyl phosphonate (DMMP) was purchased from Sigma-Aldrich, and the other chemicals bleach and gasoline.

According to the instruction in the manual, the stock solution of Singlet oxygen sensor green (SOSG) was prepared [35]. One vial from the package which was stored at - 20 ℃ was taken to make the solution of 5mM concentration. To this vial, 33μL of methanol and 792μL of deionized water was added to make a stock solution of 200μM concentration. After the stock is prepared, the solutions which have to be investigated are prepared. 3mL of sample to be tested is taken and 20μL of stock solution of SOSG was added to make the final solution to be in the detection range (1-10μM). These samples were then measured for the photoluminescence.

Later, sodium azide which is the chemical responsible for singlet oxygen quenching is added to the above samples to compare them to results before adding it. So, 200μL of sodium azide was added to these samples and mixed thoroughly. Then photoluminescence measurements were taken and compared with previous measurements to study whether any singlet oxygen can be detected.

3.4.4 Results and Discussion

The samples were prepared and the photoluminescence was measured to study the detection of singlet oxygen. The samples of SOSG with bleach, ethanol and DMMP were measured to observe the change between the common background chemicals (ethanol) and other chemicals (bleach and DMMP). As the photoluminescence of CdTeHg quantum dots with bleach and DMMP decreased, there might be singlet oxygen produced. If, that is the case then CdTeHg quantum dots can be used as the singlet oxygen sensors because of the release of singlet oxygen by these photosensitizers.

Here, the singlet oxygen sensor used is SOSG (Singlet Oxygen Sensor Green), which is a fluorescent reagent. When this is reacted with singlet oxygen, it shows some luminescence at excitation wavelength of 504nm. Many investigations are still being carried out to find the cheapest and most efficient way for the detection of singlet oxygen. Here, SOSG stock solution was prepared in the detectable range of 1-10μM and the samples were made and investigated. The working excitation of SOSG according to the manual is 504nm and the emission peak observed at that excitation was at 536nm. Though the singlet oxygen sensor cannot provide with any quantitative information of the amount of singlet oxygen generated, it just indicates the presence of singlet oxygen. According to the previous research, the life time of singlet oxygen is very short i.e. in microseconds, and SOSG is unable to show any data related to its persistence.

The luminescence intensity of SOSG will keep increasing till the sample has lost its potential to generate singlet oxygen. Therefore, it is very difficult to obtain the concentration of singlet oxygen corresponding to the intensity of SOSG [10, 25].

In this study, sodium azide a singlet oxygen quencher is used. This when added to the samples is expected to quench the fluorescence intensity and sometimes even reaches zero depending on the duration. Here, SOSG when reacted with DMMP before adding sodium azide showed slightly higher intensity than for the one where sodium azide was added. For bleach, the luminescence intensity quenched suddenly when sodium azide was added. This quenching might be due to the singlet oxygen produced by bleach and DMMP and sodium azide is responsible for quenching the singlet oxygen. For DMMP, the quenching was not much may be because the amount of sodium azide was not sufficient for the singlet oxygen to quench whereas for bleach the quench almost reached zero, which shows the sodium azide had quenched most of the singlet oxygen generated.

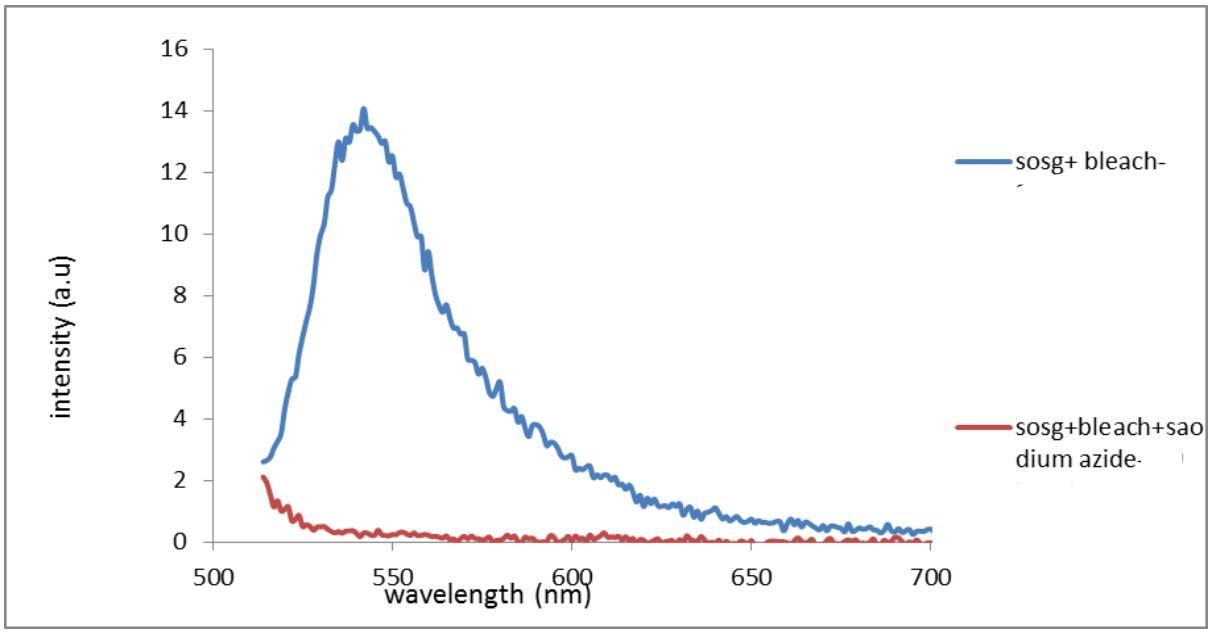

Figure 3.9- Fluorescence of bleach with SOSG before and after addition of NaN_3

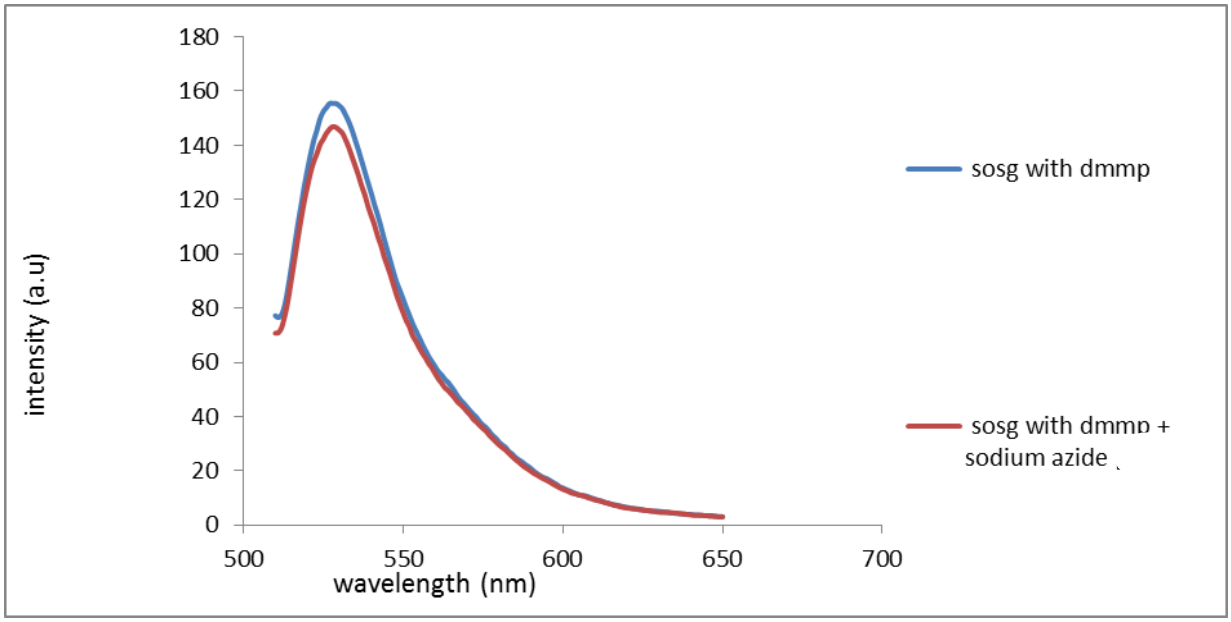

Figure 3.10- Fluorescence of DMMP with SOSG before and after addition of NaN_3

But for the chemicals like ethanol, there was no drop in the luminescence intensity was observed after adding sodium azide to the samples. For ethanol, the addition of sodium azide increased the intensity instead of quenching it, which implies there was no chance of singlet oxygen production in this sample. Therefore, sodium azide had increased the luminescence intensity after reacting with SOSG sample with ethanol. In figure 3.11, it can be seen that there was no quenching of singlet oxygen even after addition of sodium azide. So, the spectrum when ethanol interacted with SOSG might be due to other chemical reaction taking place and there is no singlet oxygen produced with ethanol.

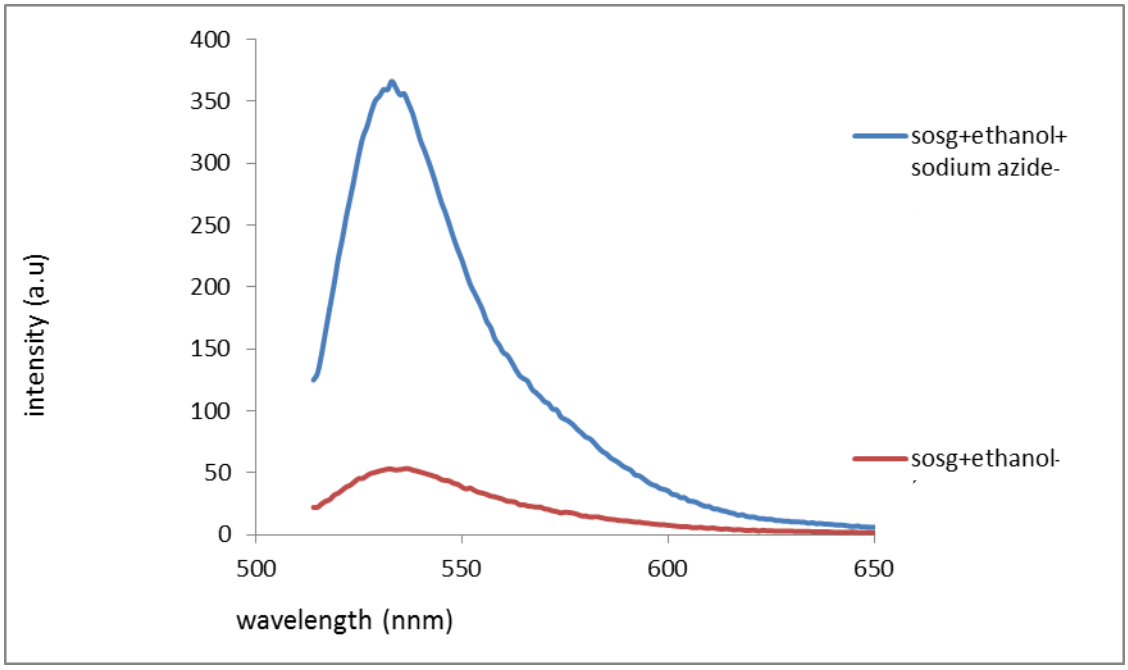

Figure 3.11- Fluorescence of ethanol with SOSG before and after addition of NaN_3

3.5 Conclusion

The common background chemicals i.e., ethanol had no effect on the luminescence intensity of CdTeHg quantum dots, whereas other chemical sensors like bleach had a quenching effect on the photoluminescence. This shows that bleach act as photosensitizers with interaction of these NIR quantum dots. Though after reacting with SOSG the response was as expected on the luminescence intensity, there was no evidence to show that the quenching of singlet oxygen was because of sodium azide. Therefore, after keen observation, we can conclude that bleach might be regarded as photosensitizer, but further investigations have to be carried out for confirming the singlet oxygen production. Also, sodium azide cannot provide information about the amount of singlet oxygen produced and hence, it is not considered to be a valid way for detection of singlet oxygen.

CHAPTER 4

SUMMARY AND FUTURE WORK

Recent research conveys that quantum dots have the ability and efficiency to develop into a new class of fluorescent probes for many biological applications because of their unique optical properties. CdTe quantum dots were used as turn-on fluorescent sensors for probing the antioxidant in biological fluids. So in my first part of the project, it was observed that the antioxidant ascorbic acid has luminescence. CdTe quantum dots lose their luminescence efficiency when interacted with protoporphyrin (PPIX). This quenched luminescence intensity was recovered by the addition of ascorbic acid to the PPIX coated quantum dots. This recovery was only observed for 0.1-0.5mM concentration of ascorbic acid and higher concentration than 0.5mM, resulted in steady declining of the intensity. The fluorescence of ascorbic acid was affected by various conditions like pH, temperature and concentration. There was an enhancement in the photoluminescence with increase in pH and temperature. Aging also showed an impact on the intensity of the antioxidant. Hence, ascorbic acid would be of great help in imaging applications as a fluorescent probe.

Most of the previously developed fluorescence imaging systems were in visible region of the electromagnetic spectrum. Recently, investigators have mainly concentrated on developing quantum dots for imaging in near infrared (NIR) region as they have the ability to penetrate more deeply into the tissue. As in this region, the tissue is said to be transparent and penetration

of light through skin will be easier. So, my second part was mainly focused on the investigation of various chemicals on CdTeHg quantum dots whose emission is in the near infrared window. Here, both common background chemicals like ethanol and other chemicals like DMMP, bleach were used to compare the results. The interaction of ethanol on CdTeHg quantum dots had no impact on the luminescence intensity. Whereas, DMMP and bleach had a great influence on NIR quantum dots, as they showed a decrease in the luminescence intensity when reacted with these semiconductor quantum dots. Hence, a singlet oxygen sensor (SOSG) was used to detect if any singlet oxygen is produced which would be applicable for photodynamic therapy. Sodium azide was used, which was a singlet oxygen quencher. But the results showed that further investigations are necessary to confirm the production of the singlet oxygen from the photosensitizer. Also, the concentration of singlet oxygen produced was unknown and other quantitative information was not available with the singlet sensor used in this study (SOSG).

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BIOGRAPHICAL INFORMATION

Yamini Chitrala pursued her Bachelor in Biomedical Engineering from the University College of Engineering at Osmania University, Hyderabad, India and graduated in June 2009. In January 2010, she joined University of Texas at Arlington for pursuing her Masters in Bioengineering. Her research interest being Nanomedicine and Nanotechnology, she worked as research assistant in Nano-biophysics lab under the supervision of Dr. Wei Chen. Her work mainly focused on the application of quantum dots for treatment of various cancers.