Miray
ÜNLÜ

INVESTIGATION OF INTERACTION BETWEEN NANOCRYSTAL
QUANTUM DOT FILMS AND ESCHERICHIA COLI

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INVESTIGATION OF INTERACTION BETWEEN NANOCRYSTAL QUANTUM DOT FILMS AND ESCHERICHIA COLI

A Master's Thesis

By
Miray ÜNLÜ
July 2017

ABDULLAH GÜL
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INVESTIGATION OF INTERACTION BETWEEN NANOCRYSTAL QUANTUM DOT FILMS AND ESCHERICHIA COLI

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By
Miray ÜNLÜ
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SCIENTIFIC ETHICS COMPLIANCE

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Miray ÜNLÜ

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M.Sc. thesis titled “Investigation of Interaction Between Nanocrystal Quantum Dot Films and Escherichia Coli” has been prepared in accordance with the Thesis Writing Guidelines of the Abdullah Gül University, Graduate School of Engineering and Science.

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M.Sc. thesis titled “Investigation of Interaction Between Nanocrystal Quantum Dot Films and Escherichia Coli” was prepared by Miray ÜNLÜ, which has been accepted by the jury in the Advanced Materials and Nanotechnology Graduate Program at Abdullah Gül University, Graduate School of Engineering and Science.

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ABSTRACT

INVESTIGATION OF INTERACTION BETWEEN NANOCRYSTAL QUANTUM DOT FILMS AND ESCHERICHIA COLI

Miray ÜNLÜ
M.Sc. in Advanced Materials and Nanotechnology
Supervisor: Assist. Prof. Evren MUTLUGÜN

July 2017

Semiconductor nanocrytals also known as quantum dots (QD) with high photoluminesce quantum yield (PLQY), size tunability and favorable optical characteristics occupy a significant area in display technology, solar energy conversion and biotechnology. Size tuning feature of QDs allows peak emission wavelength ranging from ultraviolet to infrared spectral region. In literature, QD based studies have been performed in visible spectral range by employing mostly cadmium, being a toxic heavy metal. Recently, the search for less toxic alternatives revealed the cadmium free compounds, particularly InP. Cadmium free semiconductor nanocrytals' potential to be used as fluorescent probes in biodetection and biolabeling area has been proved over the past decades.

Pathogens threaten life particularly via water sources like rivers, reservoirs and groundwater. Increasing demand for managing the 'contamination of drinkable water by pathogenic bacteria' problem needs a broad perspective about pathogens and their membrane characteristics which are integral part of microorganism detection platforms.

Bacteria are categorized mainly upon their membrane properties which are gram negative and gram positive. Extra wall called as peptidoglycan layer in gram positive bacteria makes them more resistant to external forces. Gram negative bacteria with wavy wall is relatively more prone to their environment. One of the most known pathogenic bacteria, E. Coli, have damaged and destroyed many lives throughout the world. High growth rate enables this microorganism to spread around large areas in short time. Therefore, accurate and definite detection of this bacteria in water is crucial.

The main frame of this research depends on QD based biodetection of bacteria. First of all, organic based QDs (50% PLQY) containing triocytlyphosphine-sulfur ligand were synthesized and via successful phase transfer, QDs in aqueous solvent with 20% PLQY were achieved. Although surface is damaged during ligand exchange procedure, QDs in aqueous solvent with high PLQY were obtained. SiO_2 was covered with QDs thanks to the attraction between their NH_2 group and carboxylic ends, respectively. In the final step, this hybrid structure was encapsulated with SiO_2 and silica coated QDs (SCQD) were formed. In order to utilize SCQDs in bacteria detection, fluorescent agents were embeded in polymeric films which were formed by spin coating. As a result, SCQD facilitates the attachment of negatively charged bacteria onto the surface of the films.

Appropriately grown DH5 alpha (E. Coli strain) expressing green fluorescent protein (GFP) was used as pathogen in the detection part. SCQD thin films were treated with water containing E.Coli DH5 alpha. Positively charged SCQD attracted negatively charged bacteria and the conjugation between them was analysed with time resolved spectroscopy and monitored with fluorescence microscope. Thus, usage of QDs as biosensor in pathogen detection could provide an insight in the future studies.

Keywords: biodetection, E.coli, quantum dots, semiconductors, silica coated quantum dots, indium phosphate, InP QD

ÖZET

NANOKRİSTAL KUANTUM NOKTA FİLMLER İLE ESCHERICHIA COLİ ARASINDAKİ ETKİLEŞİMİN İNCELENMESİ

Miray ÜNLÜ
İleri Malzemeler ve Nanoteknoloji Anabilim Dalı Yüksek Lisans
Tez Yöneticisi: Doç. Dr. Evren MUTLUGÜN

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Kuantum noktacık olarak adlandırılan yarı iletken nanokristaller yüksek fotolüminesans verimi, değiştirilebilir boyut ve üstün optik özellikleri ile ekran teknolojileri, güneş panelleri ve biyouygulamalarda önemli bir yere sahiptir. Emisyon dalga doyu spektrumu ultraviyole bölgeden kızılötesi bölgeye kadar uzanmaktadır. Literatürde QD tabanlı çalışmalar görünür spektral alanda, özellikle toksik ve ağır bir metal olan kadmiyum kullanılarak gerçekleştirilmektedir. Düşük toksisiteye sahip alternatiflerin arayışı InP gibi kadmiyum içermeyen bileşikleri ortaya çıkarmıştır. Kadmiyum içermeyen kuantum noktacıkların biyoalgılama ve biyoişaretleme alanlarında floresan ajanlar olarak kullanım potansiyeli son yıllarda yapılan çalışmalarla ortaya konmuştur.

Patojenler canlıları su üzerinden özellikle nehir, yeraltı kaynakları ve rezervler yoluyla tehdit etmektedir. Patojenik bakteriler tarafından yapılan kontaminasyon tüm dünyayı endişelendiren bir olgu haline gelmiştir. Bu probleme yönelik çözüm arayışları, bakteriler ve onların tespiti için kritik öneme sahip olan membran özelliklerinin geniş bir perspektifte ele alınmasını gerektirmektedir.

Bakteriler genellikle membran yapılarına göre (gram pozitif ve gram negatif) sınıflandırılmaktadır. Peptidoglikan tabaka olarak da adlandırılan ekstra hücre duvarı gram pozitif bakterileri çevresine karşı daha dirençli yapmaktadır. İnce bir duvarla çevrili olan gram negatif bakteriler ise dışarıdan gelen etkilere daha açıktır. En iyi bilinen patojenik bakteri türlerinden biri olan E. Koli şimdiye kadar dünya çapında birçok yaşamı tehdit etmiş ve zarar vermiştir. Yüksek çoğalma kapasitesi bu mikroorganizmanın kısa sürede büyük alanlara yayılmasına imkan vermektedir. Bu sebeplerden dolayı su kaynaklarındaki bakterilerin doğru ve kesin tespiti elzemdir.

Bu çalışmanın ana iskeletini bakterilerin kuantum noktacık bazlı biyoalgılaması oluşturmaktadır. Öncelikle triositilfosfin-sülfür ligandına sahip kuantum noktacıklar (50% ışıma verimi) sentezlendi ve başarılı bir faz transferi ile su bazlı (20% ışıma verimi) kuantum noktacıklar hazırlandı. Ligand değişimi sırasında ortaya çıkan yüzey hasarına rağmen, yüksek verimle ışıyan su bazlı kuantum noktacıklar elde edildi. NH_2 kaplı SiO_2, yüzey etkileşimi yaratan karboksilik ligandlara sahip kuantum noktacıklar ile kaplandı. Bu parçacıkların üzerine tekrar SiO_2 kaplanarak silika içeren kuantum noktacık yapıları oluşturuldu. Bu yapıların bakteri tespitinde kullanılması floresan ajanların dönel kaplama yöntemiyle hazırlanan polimerik filmlerin içerisine gömülmesiyle sağlandı.

Uygun bir şekilde büyütülen yeşil floresan protein sentezleyen DH5 alfa (E. Koli ırkı) algılama için patojen olarak kullanıldı. Silika kaplı hibrit yapıyı içeren polimerik filmler içerisinde E. Koli DH5 alfa bulunduran su ile muamele edildi. Pozitif yüklü hibrit yapı ile negatif yüklü bakteri arasındaki etkileşim zaman çözünürlüklü spektroskopi ve floresan mikroskobu ile analiz edildi. Böylece, kuantum noktacıkların patojen biyoalgılamada sensör olarak kullanılabileceği gösterilmiş ve gelecek çalışmalara ışık tutması amaçlanmıştır.

Anahtar kelimeler: biyoalgılama E.koli, kuantum noktacık, yarıiletkenler, silika kaplı kuantum noktacıklar, indiyum fosfat, InP QD

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Chapter 1

Introduction

Quantum dots (QD), also known as semiconductor nanocrystals are building blocks in nanoscale dimesion (2-20 nanometer). QD indicates promising approachs in solar cells [1], in vivo imaging [2], photodetection [3] due to having narrow and size tunable emission characteristics with broad absorption and excitation spectrum.

Discovery of QD dates back to 1980s [4]. The breakthrough phenomenon called as *quantum size effect* of semiconductors in optic spectral level boosted up the studies on nanocrystals (NCs) [4] [5]. Glass matrices were used to compile optic data of QD. However, adjustable physical and chemical characteristic, uniformity, size tunability of QDs took a decade. In 1993, the demonstration of hot injection technique by Bawendi group enabled production of highly monodispersed cadmium based QDs [6]. Understanding the photophysical properties of QDs provides a comprehensive approach about the energy states, electron hole pair generation, and quantum confinement related semiconductor nanocrystals issues. Lowest and highest unoccupied energy levels form the conduction and valence bands. Discrimination of bands is provided by *band gap*.

Chemical synthesis of nanocrystals was developed by anionic and cationic precursors with a stabilizing agent in aqueous media. Similar to precipitation reaction, a stabilizing agent is needed to restrict the growth process of semiconductor nanocrystals. Initial studies included vesicle formation by amphibilic molecules and nonsoluble materials in chemical reactions leaded the utilization of similar technique to obtain CdS nanocrystals as well as PbS [7], ZnS [8] QDs. Firstly, Cd^{2+} and S^{2-} reaction was the main QD synthesis type, then other anionic precursors changed the source of sulfur (H_2S gas). Phosphate, styrene copolymer, polyphosphates were proceeded to use as stabilizing agents. Thereafter, chelatin peptide, thiol molecules and DNA, RNA were included as stabilizers into this study. Thiol ligands were one of the best for NCs due to its monodisperse QD formation and applicability to various QD types including cadmium, lead, silver, and mercury [9]. Improvements in early studies allowed expansion of synthesis techniques from CdS QDs to CdSe and CdTe QDs with narrower band gaps. Tuning capacity characteristics of CdTe revealed the potential applications of different color emitted QDs. Ligand transfer of QDs allowed the detection and monitoring of biomolecules. Rogach team transferred QDs in organic solvent into aqueous ones with the attachment of mercaptoethanol ligand. In addition, other achievements were done with 18% and 38% PLQY by thiol groups (MPA and TGA groups) [10] [11] respectively. The increase of PLQY depends on firmly binding of thiol groups onto surface of CdTe NCs (Cd/SR binding). Therefore size and nuclei growth are notably regulated. Aqueous synthesis of CdTe QDs became more crucial because of its utilization in biosensing and optoelectronic applications.

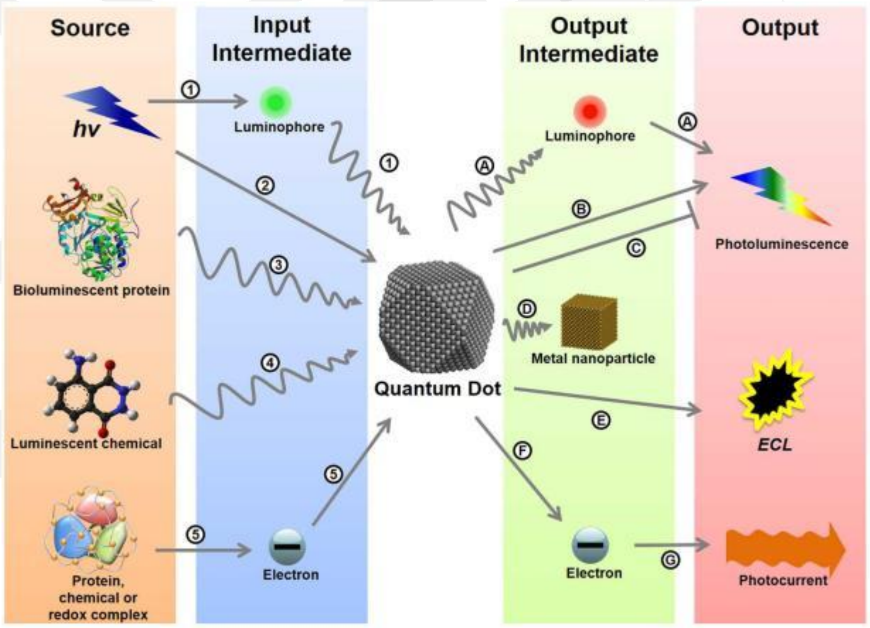


Figure 1.1 Illustration of QD energy transfer schema [12]

Carboxyl groups at the surface of crystal offer reactive sites for biolabeling. Further studies demonstrated that PLQY of TGA and MPA capped CdTe QDs could be rise to 60% by changing parameters (precursor and ligand ratio, pH) [13]. In addition to size tunability capacity, TGA and its derivatives were also used to determine the shape of CdTe QDs as nanorods [14], nanowires[15], nanosheets. On the other hand, MPA had the capacity to shift the emission of NCs into infrared region by increasing size of them up to 6 nm with keeping up the PLQY around 70-80% [13].

Nowadays, pathogens affect the water quality of rivers and streams in many parts of the world. According to geobiological research suggestions, bacteria were emerged more than 3 billion years ago [16]. The hypothesis about bacteria capability of living in communities is related with the surface, membrane, which allows protection from the terrestrial environment, cell growth, attachment to particular surfaces [17].

Adherence to the surface with special organelles such as flagellum and uptake the nutrients facilitates the bacterial growth. Shewanella like bacteria grow on metal surfaces and use them for respiration [18]. Extracellular organelles promote the ionic transportation between cell and membrane surface. For instance, an outer membrane protein complex provides electron bridge in Shewanella oneidensis [19]. Bacterial attachment to the surface usually takes place as biofilms providing protective barrier. Biofilms also have role in antibiotic resistance [17]. In a study surface related resistance was explained by two mechanisms: reducing the negative charge on the cell and improve the stability of membrane [20].

Lifted up levels of E. Coli in United States (*Watershed Assessment*, *Tracking & Environmental Results*. Washington DC: USEPA) demonstrated that understanding the variation mechanism of these kind of pathogens is essential for diminishing the contamination of water. With new improvements in technology, studies have pointed out the great diversity of E. Coli strains in the environment [21]. Up to now several methods were tried to detect the pathogens in water (Figure 1.2) but nontoxic material usage during the detection is other essential part of the researches.

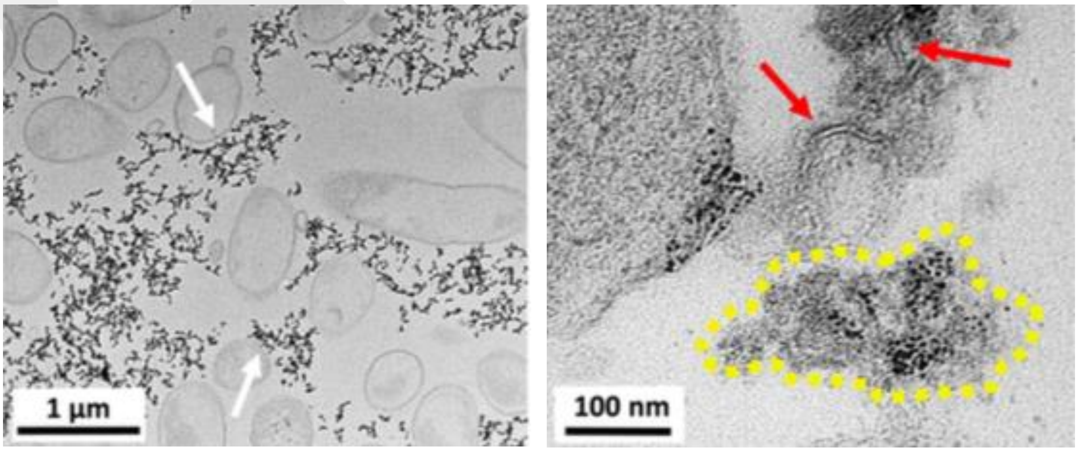


Figure 1.2 TEM image of Shewanella treated with MPA-gold nanoparticles. Dashed line shows bacterial cytoplasm including gold nanoparticles [22]

Biocompatible, inert and aqueous solubility properties of silica dots intensified the researches into silica coating QDs [23]. On top of providing colloidal stability, diminishing cytotoxicity, silica shell enables functionalization of QD surface ligands [24]. The classical approaches to cover QDs with silica shell diminish the PLQY of nanocrystals and cannot stabilize PL in harsh environment [25]. Only 57% of initial PL was protected for silica capped core shell CdTe QDs [26] Subsequently studies proceeded to electrostatic enhancement between silica particles and CdSe/CdS QDs [27] and in situ growth of QDs on mercapto-silica microspheres [28]. Although relatively high OY were developed from these novel approaches, harsh environment stability could not overcome fully.

A sandwich like method was developed by a group: silica/CdTe QD/silica nanoparticles (SQMS). This approach depends on electrostatic assembly between CdTe QDs including 3-mercaptopropionic acid (MPA) ligand and amino-capped SiO_2 nanosphere. Composite structure retains 80% of initial PL of CdTe QDs and in highly concentrated saline environment. Chemical stability of semiconductor nanocrystal is maintained by ligand exchange to MPA [29].

1.1 Colloidal Semconductor Nanocrystals

Quantum dots with less than 20 nm sized hallmark are defined as a piece of matter that confine electrons and holes in 3D dimension. Non-aqueous surfactant such as organic solvents are comprised in colloidal synthesis. The term nanoparticle demonstrates the size of approximately 1-100 nm.

Colloidal synthesis of semiconductor nanocrystals are based on high and low temperature route. While hot injection technique, a kind of high temperature process, maintains temperature approximately at 300 °C for QD growth, low temperature method keeps temperature relatively at 90 °C. High temperature technique results in better PLQY efficiency with highly monodispersed QD particles compared to low temperature route [30] [31].

Molecular precursor introducing into the reaction mixture initiates the monomer formation which culminate in nucleation [32]. Growth process continues with the flowing of monomers onto nanocrystal surface. Ostwald ripening [33] may take place in case of monomer concentration depletion by dissolving of small dots to form larger ones (Figure 1.1.1). These steps could change with the introduction of stabilizers which adhere to the dot surface and give chemical and colloidal stability to NCs.

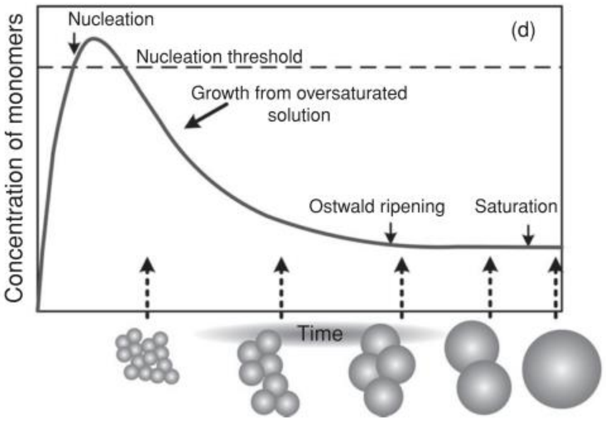


Figure 1.1.1 Colloidal quantum dots. a. Illustration of NC synthesis regarding concentration of the monomers [34]

Upon reaching the nucleation threshold, fast nucleation occurs with the hot injection method which induce rapid production of monomers. Optimal precursor theory explains the bacis principles of hot injection tecnique [5].

As nucleation rate is defined at *dN/dt:*

$$\boldsymbol{dN/dT = Aexp}(-\Delta \boldsymbol{G^{N}/RT}) \qquad (1.1.1\text{-}1)$$

ΔG^N is the activation energy related to $16\pi\gamma^3 V_m{}^2/3(RT \ln S)^2$, γ is the surface energy, V_m is the molar volume, S is the supersaturation. S = [Monomer] C^O . Highly reactive precursors are required in a short period of time at a specific temperature. Metal compounds such as PbSnTe [35], Fe_2O_3 [36], cheaper, less air sensitive Cd chalcogenide [37] ; non-metal precursors such as trimethylsilyl, alkylphophine related chemicals are also used for efficient NC production. Capping ligands (myristic acid [38], trioctylphosphine [39]) are stabilizers of the reaction, also can perform as coordinating solvent such as hexadecylamine [40].

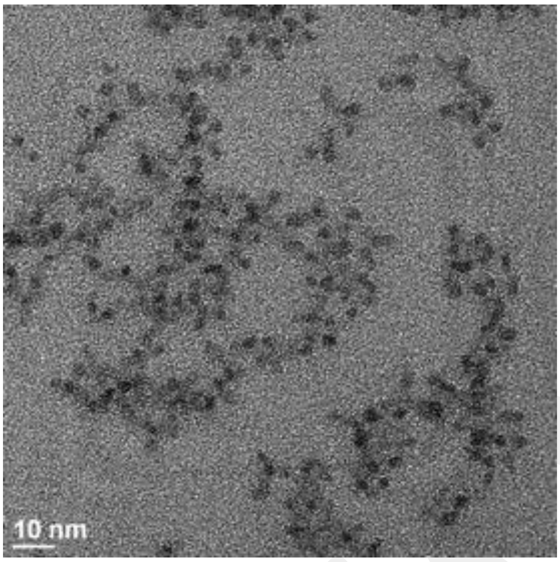


Figure 1.1.2 a) TEM image of colloidal InP/ZnS nanocrystals in Mutlugun laboratory

1.1.1 Surface chemistry

Photophysical characteristic of inorganic NCs makes explicit response to surface passivation and defective problems so proper ligand selection is one of the fundamental issue in light driven processes in QDs. Donor and acceptor dependent resonance energy transfer (RET) efficiency was studied by removing surface ligands with an insulating shell. Drastic quenching of RET were observed in that research [41].

In addition to crucial effect on nucleation and growth, adherence of surface ligands to the surface supports the interparticle attraction such as electrostatic interaction and dissolving in certain solvents. Mostly used ligands in the formation of NCs are the organic hydrocarbon molecules. Even as providing high chemical flexibility to crystals, these long chain molecules mostly block the charge transport [42]. On the other hand, total removal of ligands cause destruction of surface dangling bonds which provides carrier trapping in NCs [43]. Thus, accessing the target NCs is possible via slightly removal of ligands and ligand exchange methods nowadays.

1.1.2 Organic capping ligands

Surfactants as initial capping includes anchoring chemical groups such as amino, sulfonato, thio and alkyl or aromatic based ones [44]. Instead, steric stabilization of II-IV semiconductor NCs (CdTe, HgTe) is constructed with a thiol and a charged group. High luminescence emission of QDs with charge stabilizer has so far limited to thiolgycol based ones [45]. Binding mechanism of NC and ligand status has not been completed yet due to missing part of analytical tools to discover the surface atomic structure of these crystals. In metal ligand materials and salts binding type is known. For example, olate like anionic material bind cationic surfaces [46] (Cd^{2+}).

1.1.3 Ligand exchange molecules

Initial long chain ligand exchange procedure depends on two stages: removal of initial capping molecules and introducing new short chain ligand into the solution. Ligand exchange-phase transfer process is limited with the diminishing of colloidal stability and emission capacity. Nanoparticle aggregation is also another drawback of this kind of transfer strategy. To enhance the quality of NC which is embedded into film in several applications, amine- based or pyridine type solutions are used by soaking of CdSe or PbSe QD films into the solution [47], [48]. The improvement of photoconductivity (10^{12}-10^{13} Jones) by PbS QD film in is maintained butylamine solution by decreasing inter NC spacing [49]. In addition, capping ligands polythiophene [50] like electrically conjugated polymers enhance the electrical characteristics of NCs.

1.1.4 Inorganic capping ligands

Metal chalcogenide complexes (MCCs) such as SnS_4, In_2Se_4 can behave like surface capping ligands. The terminal chalcogenide atoms have high chemical affinity to electron deficient surface of NCs. Transfer of MCCs by removing organic ligands from the NC surface, provide high chemical stability and solubility in formamide and hydrazine like polar solvents with high dielectric constant. In a study, halide (CI^-, Br^-, I^-) ligands were used to passivate the NCs with alkylphosponate following treating NC films with alkylamonium halides. Stable colloidal QDs were obtained [51].

1.1.5 Bare NC surfaces

Ligand free NCs have many drawbacks such as crystal aggregation, contamination. In a research study, $NOBF_4$ was introduced as removing of surface ligands of NCs [52]. Positively charged and stable dispersed NCs were obtained. The applicability of $NOBF_4$ is limited since its oxidative and acidity ion level characteristics [51].

1.2 Optical Characteristics of Quantum Dots

In this part, introduction will be done with material types and basic concepts about QDs and zero dimensional quantum confinement.

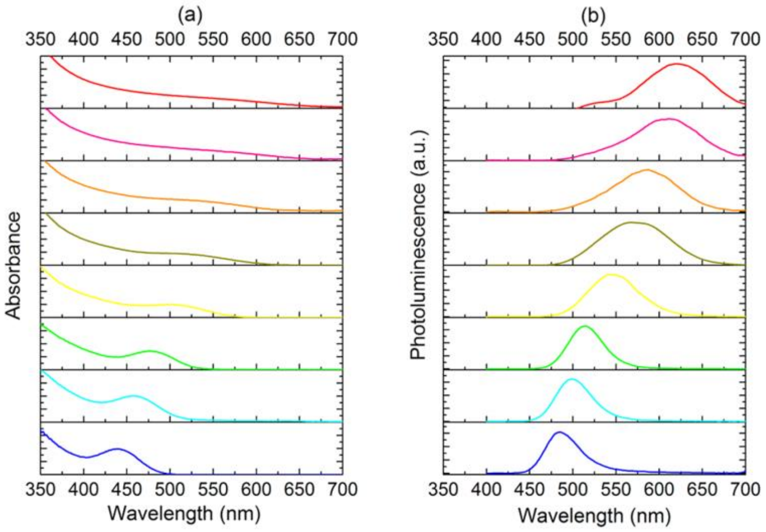


Figure 1.2.1 Absorption and emission spectra of InP/ZnS QD in different sizes in Mutlugün laboratory [53]

These inorganic nanoparticles exhibit broad absorption spectra like bulk nanocrystals. Furthermore narrow and high capacity fluorescence efficiency is similar to molecular dyes (Figure 1.2.1). Tunability of optical properties is controlled by the size of QDs. Space between energy levels decreases as the QD size increases. These unique properties boosted up the potential usage of QDs in optoelectronic applications.

1.2.1 Quantum Dot Energy States

Two aspects as molecule like- and bulk crystal like- conduct to understand the approach of quantum dot energy states. In the *bottom-up* perspective, ordered individual atoms construct the core of QD. With the improvements in computational based area, this approach achieved a great importance by providing calculations on QDs [54].

Besides, *top-down* approach explains the contribution of finite sized QDs to characteristics of larger particles. Similarities of this perspective with quantum mechanical models serve as to understood well of fundamentals of QD photophysics.

In *top-down* approach, optical and electronic framework of QD is modeled based on bulky crystal properties [34]. Accordingly, it is essential to understand well of atomic orbitals in bulk structure in terms of energy state distribution (Figure 1.2.1.1).

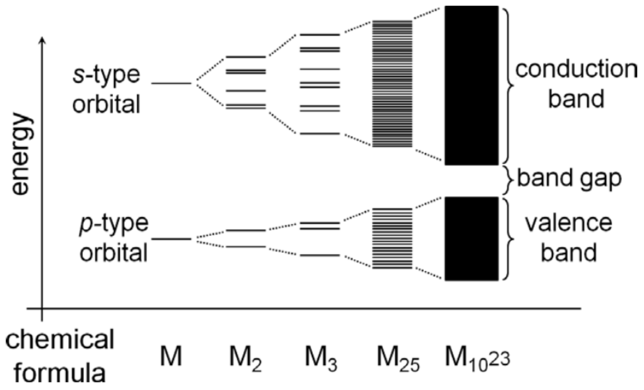


Figure 1.2.1.1 Increasing energy levels between bands as the number of atom in material decreases. Loss of discrete energy levels in bulky species and only continuous band observation [34]

Conduction and valence bands are formed by the lowest and highest unoccupied energy levels. As illustrated in figure 1.2.1.1, bands separation is provided by a specific gap known as *band gap* E_g that comprise no energy state, therefore, discrimination of bands is promoted. In the ground state, valence band loaded with electron is exposed to an outcoming energy. If energy is equal or greater than band gap, electron in valence band moves up to conduction band. This process gives rise to production of two charge carriers. In conduction band, negatively charged electron (e^-) is formed as positively charged hole (h^+) in valence band.

Quantum confinement effect is not substantial for all semiconductor nanocrystals, merely become essential in case of QD size border on electron and hole's length level. This level (a_B) also called as Bohr radius refers to e^- Bohr radius in H atom. Counting radius of H atom as a_0, dielectric constant as ε, carrier effective mass as m^*, formula is given as:

$$\alpha_B = \varepsilon \, (m_0 / m^*) \, \alpha_0 \tag{1.2.1-1}$$

Moreover, confinement of electron and hole together give in a new term exciton. It's Bohr radius is calculated as:

$$m_{exc}^{*-1} = m_e^{*-1} + m_h^{*-1} \tag{1.2.1-2}$$

1.2.2 Experimental proofs describing the quantum confinement effect

Absorbance measurement is a kind of technique to detect different sized QDs. These semiconductor nanocrystals are excited with a stimulator between the ranges of 300-700 nm and give particular picks in response to size of crystals in discrete wavelengths.

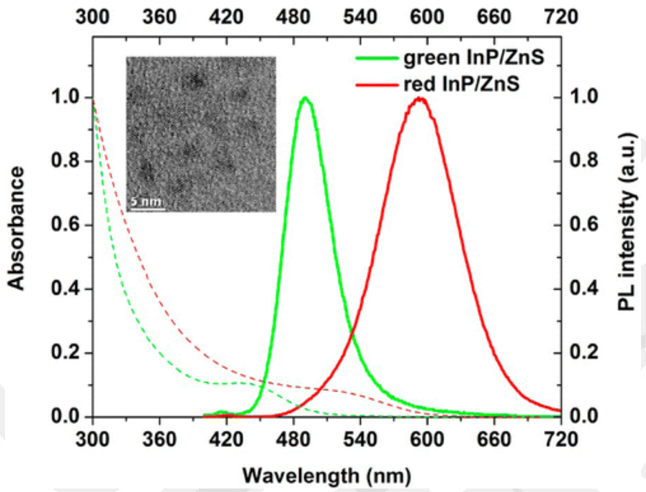


Figure 1.2.2.1 a) Absorbance (dashed line) and emission (solid line) spectra of green and red fluorescence emitting InP/ZnS QDs [55]

Highly monodisperse QDs could be detected by the absorption and emission spectra (Figure 1.2.2.1). Narrow full width at half maximum (FWHM) value in PL emission spectra demonstrate highly monodisperse QDs. PL of core quantum yield (QY) is calculated in a range 10-50% by comparing with a reference sample QY. Post modification such as adding capping agents or shell could promote the efficiency [56], [57]. From experimental point of view, there is a restriction part in absorbance spectroscopy on detection of multiple transitions due to QD energy gaps. Prevailing on this problem, techniques as photoluminescence excitation (PLE) were introduced [58].At a fixed emission value, excitation wavelength is scanned. Other strategy is using fluorescence line narrowing (FLN) spectroscopy [59]. FLN instrument is capable of exciting smallest sized QDs.

Optimization of colloidal QDs properties includes epitaxial growth of a second layer called as shell on top of the presynthesized core particle. Surface atom passivation is provided by inorganic materials due to high ratio of surface to core atoms. Delocalized electrons and holes in the core of nanocrystal are capable of locate on energetically trap charge carriers because of defective passivation by organic materials.

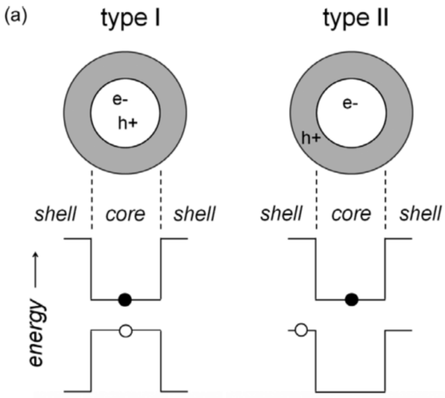


Figure 1.2.2.2 a) Band alignment in type I and type II core/shell QDs. Electron and hole confinement preserved in type I core QD; one of the carrier remains in core, other one localized in the shell structure. [34]

Classification of core shell QDs depends on their band energy comparison in core and shell, for instance in type I the conduction band energy is lower in the shell than in core one. In type II, it's vice versa. Alignment between core and shell affects the photo excited electrons and holes; while photo excited electrons quickly relax to their minimum conduction band states, photo excited hole relaxation occurs toward the maximum valence band state (Figure 1.2.2.2). CdSe/ZnS QD (type I) is constructed by a thin shell layer of ZnS with a bulk bandgap of 3.6 eV [60]. Finite potential difference between core and shell is approximately 1 eV which diminishes the penetration of exciton wave function into the shell. Moreover surface passivation provided by core/shell/shell QD increase the PL intensity up to 5-10 fold [60]. A reported PL intensity 80% [61] with high brightness and surface protection make use of these type of QDs in biological applications [2]. Most studies base on cadmium chalcogenide materials which is relatively simple to synthesize with great optical quality [6]. By virtue of highly toxic characteristics, massive amount usage of material containing cadmium in technological area and toxicity level in vivo applications conducted researchers to investigate new materials with reduced toxicity with cost efficient productions. The concern shifted to Cd-free alternatives particularly InP QDs [62]. Cd-free synthesis depends on phosphorus precursor $(TMS)_3P$ [63], PCI_3[64], P_4, [65] Trioctylphosphine (TOP) [66]. Widely used $(TMS)_3P$ in optimized protocols give QDs with 40-60 FWHM but its highly toxic gas (non-environmental) and expensive price properties make large scale synthesis difficult [38]. $(DMA)_3P$ as an alternative phosphorous precursor (relatively safer and cheaper) used to produce QDs with several combinations of InP/Zn- QDs (P:DMA dimethlyamino, DEA diethylamino; InX as InCI, InBr, InI; ZnS and ZnSe) [67]

1.3 Experimental

1.3.1 Materials

InP/ZnS (contains TMSP): Indium(III) acetate (99.99%), myristic acid (99%), 1-octadecene (90 %), 1-dodecanethiol (98%), zinc stearate (purum), tris (trimethylesilyl) phosphine (95%) were purchased from Sigma Aldrich.

InP/ZnS (contains TOP): Indium(III) chloride (99.999 %), indium(III) bromide (99.999 %), indium(III) iodide (99.998 %), zinc(II) chloride (99.999 %), tris(dimethylamino)phosphine (97 %), and zinc stearate (technical grade), oleylamine (80-90 %), trioctylphosphine (97 %), Octadecene (technical 90 %), mercaptosuccinic acid, ammonium hydroxide, TEOS, APTES and sulfur powder were purchased from Sigma Aldrich.

Organic and other solvents: Absolute ethanol , acetone (99.5%), methanol (99.8) and hexane (95%) were purchased from Merck.

1.3.2 Characterization Techniques

QD synthesis was done under vacuum/argon gase based manifold system. Absorbance and photoluminescence spectrum results were recorded on Genesys 10S UV-Vis Spectrometer (Mutlugun Lab) and Cary Eclipse Fluorescence Spectroscopy (Mutlugun Lab) respectively. Photoluminescent quantum yield (PLQY) was calculated by formula given below:

$$\Phi_{\text{flu}}^{\text{sample}} = \Phi_{\text{flu}}^{\text{reference}} \left(\frac{F^{\text{sample}}}{F^{\text{reference}}} \right) \left(\frac{\eta^{\text{sample}}}{\eta^{\text{reference}}} \right) \left(\frac{\text{Abs}^{\text{reference}}}{\text{Abs}^{\text{sample}}} \right) \quad \textbf{(1.3.2-1)}$$

QY for all fluorescent samples was calculated as in equation above. (Φ: Quantum yield, F: integrated emission area, η: refractive index o solvent, Abs: absorbance value of selected wavelength) [68] Rhodamine 6G (QY: 95%), sulforhodamine (QY:90%) dissolved in absolute ethanol were used as reference to determine the QY of samples. All optical densities at particular wavelengths were retain below 0.1 to prevent internal filtering effects.

Time resolved photoluminescence spectroscopy (Picoquant Fluotime 200-AGU) was used to measure life time of particular samples (measurement was completed upon reaching 1000 photon). Best emission wavelength was determined by excitation at 450 nm. To analyse fluorescence decay, Fluofit software was utilized.

Transmission electron microscope (TEM) and scanning electron microscope (NanoSEM) -BILKENT UNAM- were used to determine the surface and structural analysis of samples. While magnification with SEM allows to observe object at micron level, more detailed results at nanometer scale could be illustrated by TEM. Dimpling and ion milling processes are integral part of TEM specimen preparation. Diluted samples were dropped onto grids and pilko tab held the grid in proper position. After drying of sample solution in an hour, grid was placed into TEM component and analysis started.

Hydrodynamic size distribution of nanocrystals were recorded by Malvern Zetasizer. This instrument measures size of particle in nanometer scale. Proper dilution level is essential to acquire good results because of colloidal QD particle size is nearly picometer scale. Polydispersed particles or aggregated ones have potential to change the precision of measurement.

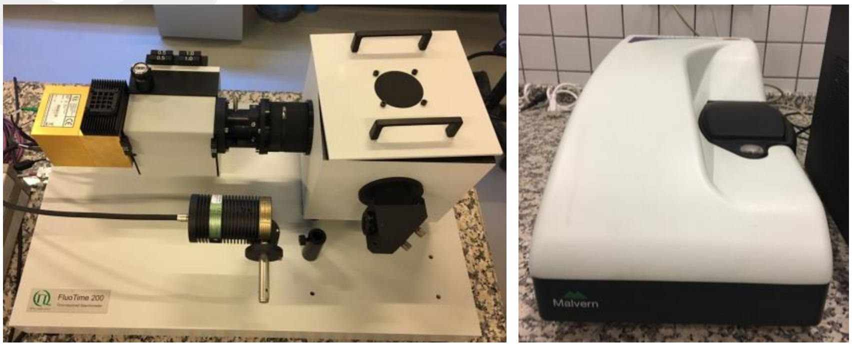


Figure 1.3.2.1 Time resolved photoluminescence spectroscopy and Malvern Zetasizer

1.3.3 Synthesis Procedure

Alloyed-Core synthesis of InP/ZnS QD (using TMS$_3$P as phosphor precursor)-1X volume

0.1 mmol InAc$_3$ and 0.3 mmol MA were mixed with 8 ml ODE in triple neck flask. Under vacuum, the solution was stirred for 1 hour at 95 °C. To obtain a clear solution, mixture was kept going to stirred at 120 °C under Ar gas for 20 min. After cooled down to 25 °C, 0.1 mmol zinc stearate and 0.1 mmol dodecanethiol were added into clear solution and on the back of heating up the mixture to 230 °C under gas, 0.1 mmol TMSP in 1 ml ODE was quicly injected into solution. Following increasing temperature to 285 °C , alloyed-core QDs were generated at 285 °C after waiting for 10 min and then cooled down to 25 °C

Figure 1.3.3.1 Synthesis Setup

Shell synthesis of InP/ZnS QD (using TMS$_3$P as phosphor precursor)-1X volume

For shell coating, 0.2 mmol zinc stearate was added into solution at 25 °C and heating up temperature to 230 °C under gas started the passivation and growth of shell on top of alloyed core. After 2 hours, 0.4 mmol dodecanethiol in 1 ml ODE was injected into mixture. The synthesis was completed with 1 hour wait at 230 °C and solution was cooled down to 25 °C. (QD cleaning was described in detail under the cleaning procedure title)

(2x volume of QDs core synthesis: 0.2 mmol $InAc_3$, 0.6 mmol MA, 0.6 mmol zinc stearate, 0.2 mmol DDT, 0.125 mmol TMSP/ shell: 0.4 mmol zinc stearate, 0.8 mmol DDT)

(5x volume of QDs core synthesis: 0.6 mmol $InAc_3$, 1.8 mmol MA, 20 ml ODE, 2 mmol zinc stearate,0.25 mmol DDT, 0.125 mmol TMSP/ shell: 1 mmol zinc stearate, 2 mmol DDT)

Core synthesis of red fluorescence light emitting InP QDs (using TOP as phosphor precursor):

QD synthesis method was modified from the article of Tessier et al [67]. 0.45 mmol $InCI_3$ and 2.2 mmol $ZnCI_2$ were mixed with 6 ml oleylamine solvent in triple neck flask. Under vacuum, the mixture was stirred at 120 °C for 1 hour. Then, under inert atmosphere, the system heated up to 180 °C. Upon reaching to specified temperature, 250 µl tris(dimethylamino)phosphine was quickly injected into solution. Allied with the phosphorous precursor injection for 20 min at 180 °C, core stage of nanoparticles accomplished (main solution).

Core synthesis of orange fluorescence light emitting InP QDs (using TOP as phosphor precursor):

Same protocol was followed for the synthesis of orange light emitting QDs except 0.45 mmol indium(III) bromide substituded for indium(III) chloride.

Core synthesis of green fluorescence light emitting InP QDs (using TOP as phosphor precursor):

Same protocol was followed for the synthesis of green light emitting QDs except 0.90 mmol indium(III) iodide substituded for indium(III) chloride.

Shell synthesis of InP QD (using TOP as phosphor precursor)

After achieving core structured nanocrystals as outlined above, passivative and protective layer called as shell was sythesized onto core nanocrystals. Primarily, 2.2 M stock solution (0.72 g Sulfur in 10 ml TOP) was prepared in glovebox. 1ml TOP-S taken form stock solution was slowly added into triple neck and at 40 minutes the system was heated up to 200 °C. At 60 minutes, readily prepared 4 ml Zn-Stearate/ODE

(2g Zn-Ste in 7 ml ODE in triple neck under Ar gas at 170 °C) was injected into main solution containing core QDs and the temperature heated up to 220 °C. At 90 minutes, 1ml TOP-S at 240 °C was injected into colloidal solution. At 110 minutes, 2 ml Zn-Ste/ODE was added at 260 °C. At 130 minutes, 0.7 ml TOP-S (2.2 M) was slowly loaded at 280 °C for 20 min. At 150 minutes, 2 ml Zn-Ste/ODE was injected and after 15 min the system was cooled down to room temperature.

1.3.4 Cleaning procedure& Phase transfer

Properly synthesized QDs were cleaned with a process to remove excess ligands on the surface of QD. At first, QDs in triple neck were transferred to a falcon and half volume of hexane organic solvent was added, vortexed for 1 min and centrifuged at 5000 rpm 15 min. Supernatant (stock solution) was transferred to another falcon. This step was repeated to discard unreacted (pellet) chemicals from QD solution. To remove excess ligand, acetone as double in volume was added into stock solution and centrifuged at 5000 rpm for 5 min. After removing liquid, pellet including QD in precipitate form could be seen obviously. This part is the general cleaning procedure. Following steps were performed for InP/ZnS (contains TOP). Afterwards, pellet QDs dried at room temperature (overnight) was weighted (0.25 g) and a second washing treatment was done to prepare QDs for phase transfer.

Most of organic ligand removing makes attachment of water based ligand easier. For second washing, acetone washing process was repeated. After centrifugation, pellet QDs were dissolved in 10 ml hexane and put into 100 ml glass bottle. 15 ml ammonium hydroxide and 40 ml water also added into solution and mixture was stirred on hot plate at room temperature for 3 hours. Then, 0.75 g MSA was put into mixture and overnight stirring helped to attach the MSA onto QD surface. In case of no passage to water phase of some QDs (WQD), these ones could be washed with acetone and mixed with MSA again. For precipitation of water based QDs, ethanol is added into mixture -QD:ethanol 4:1 (v/v)- 5000 rpm for 10 min was sufficient to precipitate QDs in aqueous solvent which are then dissolved in water for further application.

1.3.5 Polymeric films

Polymer 1g PMMA was dissolved in 10 ml anisole or toluene (10% w/v). Mixture was stirred overnight at 50 °C to obtain a clear polymer solution. Then QDs prepared in toluene were added into polymer for mixing (2 hour). At the end, QD polymers were dropped onto clear glass surface.

1.4 RESULTS AND DISCUSSIONS

InP/ZnS (containing TMSP) synthesis procedure was used to achieve highly efficient alloy shell semiconductor nanocrystals with low FWHM. Figure 1.4.1 shows the alloy/shell QDs after hot injection synthesis.

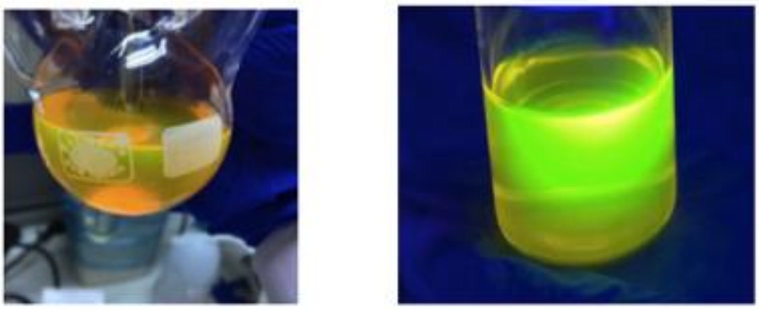


Figure 1.4.1 InP/ZnS QDs in triple neck and in vial a. before cleaning b. after shortage of ligands with acetone

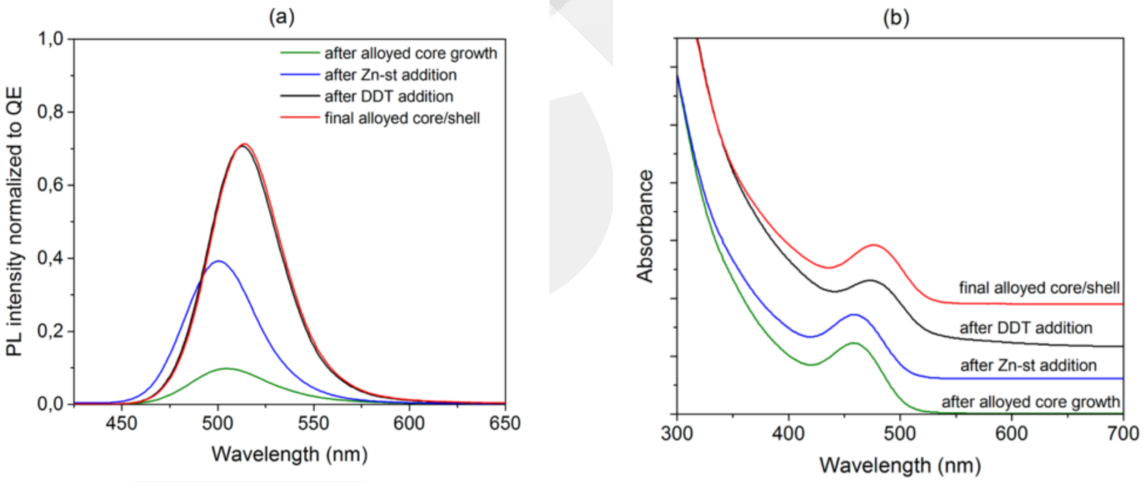


Figure 1.4.2 Figure InP/ZnS QDs (containing TMSP) during formation of nanocrystals a. PL spectra b. absorption spectra [53]

Distinctive characteristics of alloyed core QDs are related to their alloyed structure. Precursors (Indium and myristic acid) and shell materials (zinc stearate and DDT) were fused simultaneously to generate alloyed core framework. This arrangement of alloyed core/shell QDs allowed high surface passivation of nanocrystals due to zinc stearate and low FWHM relevant to DDT. PL intensity of alloyed core is lower compared to alloyed core/shell QDs. Furthermore, shell capping shifts the emission wavelength to red side due to size increment capability of zinc stearate. Absorbance spectra (peak point) also shift toward red side (Figure 1.4.2).

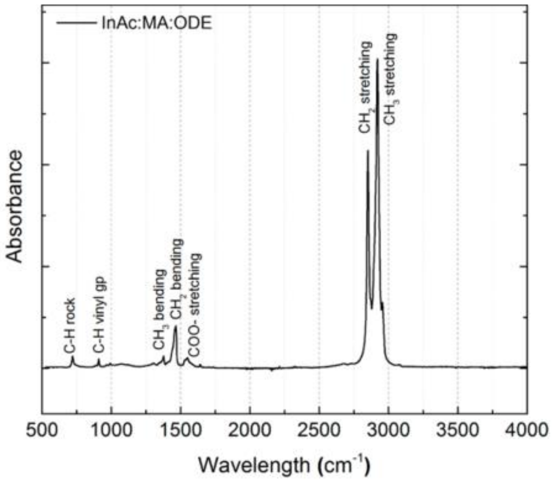


Figure 1.4.3 FTIR spectrum of InP/ZnS QDs including myristic acid and ODE [53]

The bands at 2800 and 2920 cm^{-1} refer to CH_3 and CH_2 stretching bonds respectively. Vinyl group at 900 cm^{-1} and CH rock bonds at 730 cm^{-1} demonstrate the formation of QDs with myristic acid and DDT ligands (Figure 1.4.3).

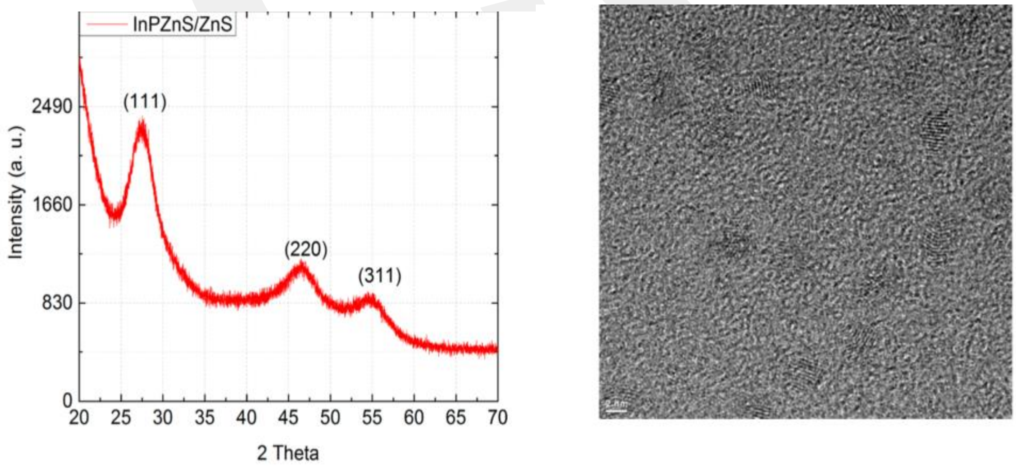


Figure 1.4.4 InP/ZnS QDs characterization a.XRD spectrum b. TEM image (scale bar 2 nm) [53]

X-Ray diffraction (XRD) determines the structure of sample crystalline. Peaks shown in figure refer to cubic zinc blende pattern. TEM also showed the size of QDs around 3.5 nm (Figure 1.4.4).

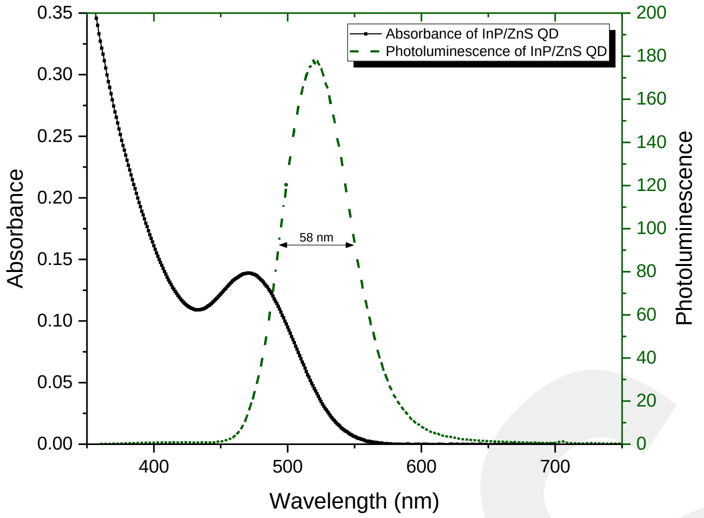


Figure 1.4.5 PL and absorption spectrum of InP/ZnS QDs

Scale up synthesis of InP/ZnS QDs was done to obtain high amount of QD at the same time. As seen in Table 1.4.1, 1x and 2x volumes have the same FWHM but 5x volume has lower FWHM. PLQY for all fully synthesized samples were range over 85. As expected, PLQY of 5X volume-core is lower than alloyed core/shell QDs. 10 nm emission shifting was a result of shell capping.

Table 1.4.1 Table Scale up synthesis of InP/ZnS QD (containing TMSP) and their characterizations (Emission wavelength, FWHM and QY)

Sample	Emission (nm)	FWHM (nm)	PLQY
1x volume	528	54	88
2x volume	511	54	96
5x volume (only core)	498	49	76
5x volume (core+shell)	508	50	87

InP/ZnS QDs (using TOP as phosphor precursor)

As mentioned in the experimental part, core generation is provided by $InCI_3$ and $ZnCI_2$ precursors. Oleylamine contributed to synthesis as solvent and ligand, which facilitate to reaching relatively high temperature (340 °C) for shell formation properly. Size dispersity of nanocrystals is controlled and decreased by $ZnCI_2$. Although precursors listed above are needed, $(DMA)_3P$ as phosphor source relatively occupy the crucial role for core development. Low price and toxicity compared to TMSP like phosphorus sources arouse interest for this chemical.

Shell development is maintained by sulphur dissolved in TOP (TOP-S). As an alternative to dodecanethiol, TOP-S does not stimulate blue shifted emission. Each addition of TOP-S and $ZnCl_2$ into the synthesis mixture following the rise of temperature provides a protection of oxidation layer of InP QDs. Formation of shell layer onto core structure of QD increase the diameter of nanocrytals which shift the emmision wavelength. Possible explanation for this event is the lattice mismatch of InP and ZnS. In addition to low price (total synthesis), other key point is the chemical yield. The alteration of $P:InX_3$ ratio from 1:1 to 3.6:1 boosted up the chemical yield from 30% to 85%.

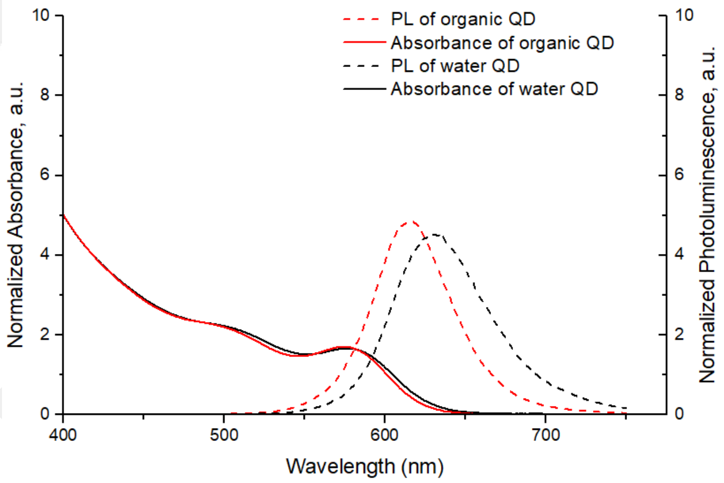


Figure 1.4.6 Absorption and emission spectra of organic and water based InP/ZnS QDs

As seen in Figure 1.4.6, organic InP/ZnS QDs have lower FWHM and higher PLQY compared to QDs in aqueous solvent . Shifting of emission wavelentgh of aqueous QD is caused by phase transfer in which MSA attached to QDs after removing most of TOP-S ones. This alteration also affected QY negatively due to incomplete surface passivation capacity of MSA. Dimension of QD in hexane and water was 6.38 nm and 5.21 respectively (Figure 1.4.7).

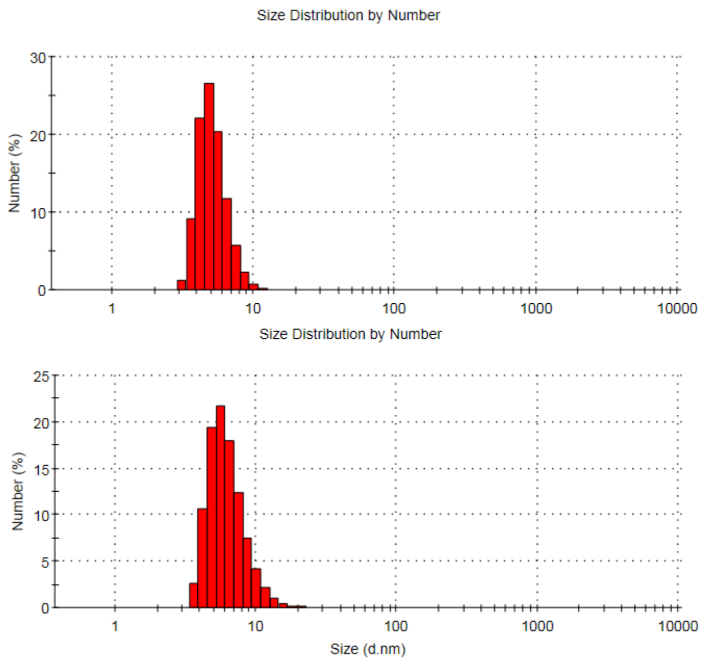


Figure 1.4.7 Size distribution by number of QD a. organic b. water based QD

Figure 1.4.8 shows the vibration peaks of QDs in organic solvent functionalized with C-H (stretch bond) groups at 2820 cm^{-1} and C-C groups at 3223 cm^{-1}.Furthermore, the stretch bands at 1620 cm^{-1}, 1457 cm^{-1} refers to C=C vibrational ,C-C stretching bands (vinyl group) respectively. Functional groups mentioned above indicates the presence of TOP ligands for QDs in organic solvent . Aquoeus QD spectra with ligand MSA includes carboxylic and sulfur related group. O-H, S-H stretch bonds at 3326, 2551 cm^{-1} and C=O, C-O, C-S stretch band at 1553, 1377, 715 cm^{-1} respectively are associated with the successful ligand exchange procedure.

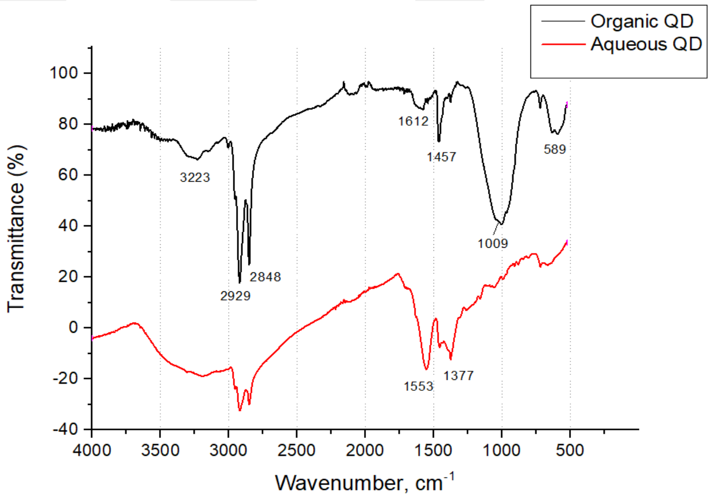


Figure 1.4.8 FTIR analysis of organic and water based QDs

QD emitting at different wavelengths was obtained by size tuning property of these nanocrystals. By altering In halide precursor (InX; X:CI, Br, I) emission wavelengths were measured at 530, 590, 620 roughly. Shifting emission to less than 500 or more than 630 is restricted by precursor solubility in oleylamine (Figure 1.4.9).

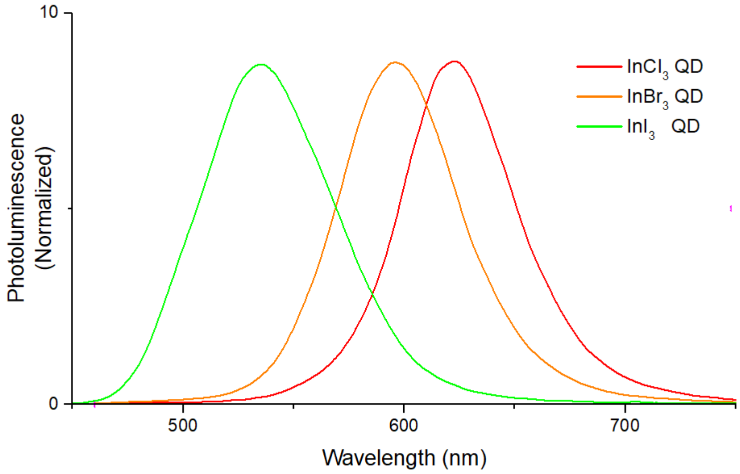


Figure 1.4.9 Halide effect on PL spectra of QDs

PDMA as phosphor precursor was injected into synthesis solution in different concentrations. Best FWHM and PLQY were obtained by using 250 ul PDMA. Concentration changes also affected the emission wavelength of QDs (Figure 1.4.10).

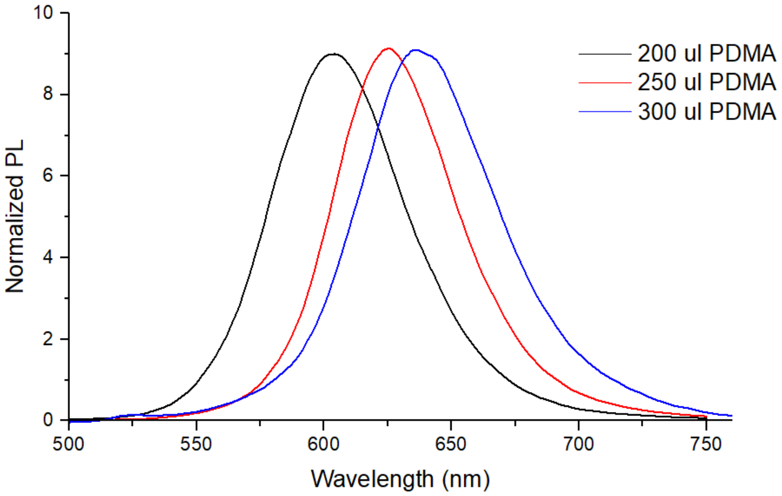


Figure 1.4.10 PDMA effect on PL spectra of QDs

Time resolved spectroscopy analyses the excited photon time delay of samples. The delay times are plotted according to occurrence of emission throughout the time following excitation process. As seen in graphic 1.4.11, QDs in aqueous solvent have a bit different line compared to QDs in organic solvent . According to table, there are three components of each of plotted line. Percentages of component 1 (T1) demonstrate the difference between organic and water based QD 20.60% and 25.56% respectively. T2 and T3 also indicate the alteration of component percentages due to phase transfer (ligand alteration). Average time relapse is the same for both of the samples.

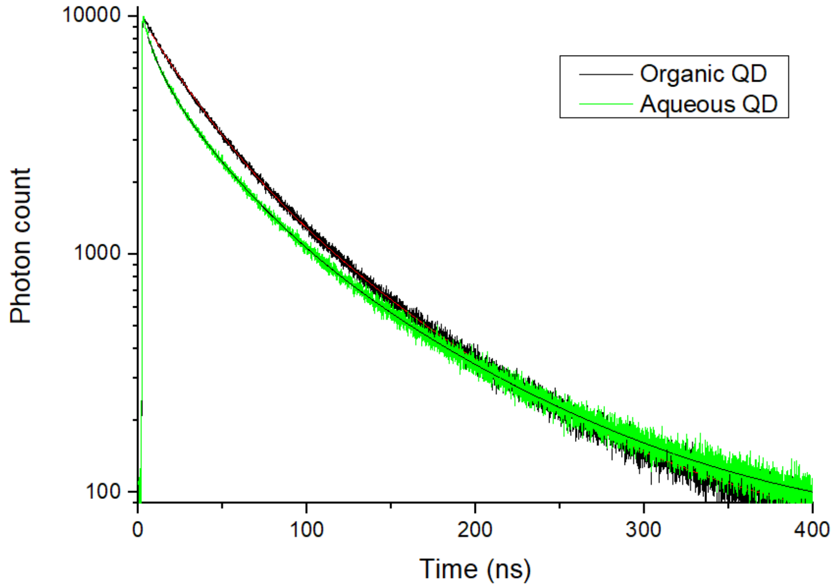


Figure 1.4.11 Time resolved spectra of organic and aqueous quantum dots

Table 1.4.2 The lifetime components of organic and aqueous quantum dots

Sample	A1	T1 (ns)	A2	T2 (ns)	A3	T3 (ns)	Tavg
QD in organic solvent	1909.3 ± 20.3	94.760 ±0.665 **20.60%**	5688.7 ±51.4	32.677 ±0.405 **61.38%**	1670 ±102	14.65 ±1.04 **18.02%**	42.218
QD in aqueous solvent	2053.6 ±19.9	94.760 ±0.665 **25.56%**	4109.9 ±52.6	32.677 ±0.405 **51.15%**	1871 ±132	8.062 ±0.696 **23.29%**	42.813

QDs in organic and aqueous solvents were also compared according to their emission time capacity (TRPL). As demonstrated in Table 1.4.2, life time of both samples have three components in which the percentages of each of them differs. Organic QDs T1,T2 and T3 values were recorded as 20.60, 61.38, 18.02 % respectively. On the contrary, percentages in QDs (aqueous solvent) was seen as 25.56, 51.15, 23.29 %. Despite similar average T values, changes in life time components showed the difference between organic and aqueous QDs.

Polymeric films

Properly embedded QDs into polymeric films is essential in display technology, biosensor applications. In this part, it was studied on dissolving QDs in particular polymers and preventing the decrease of QY after embedding.

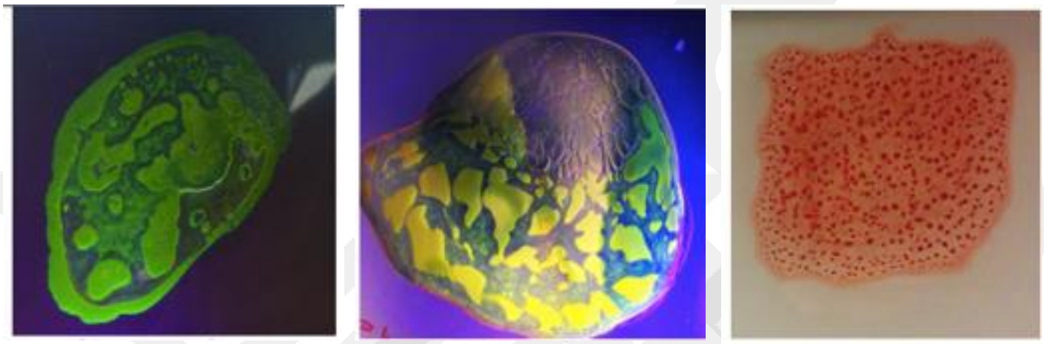


Figure 1.4.12 a. Green emitting InP/ZnS QDs in PMMA-anisole polymer (10% w/v) b. green and red emitting InP/ZnS QDs in PMMA-anisole polymer (10% w/v) c. red emitting InP/ZnS QDs PMMA-toluene polymer (10% w/v)

As seen in figure 1.4.12, QDs in the film were not dispersed homogeniously and not dried very well. Polymer concentration (10%) might affect the disperse ratio. In addition, QDs cleaning procedure may result in aggregation of these crystals inside the polymer. Anisole and toluene solvents of polymer might prevent the dispersion of QDs properly.

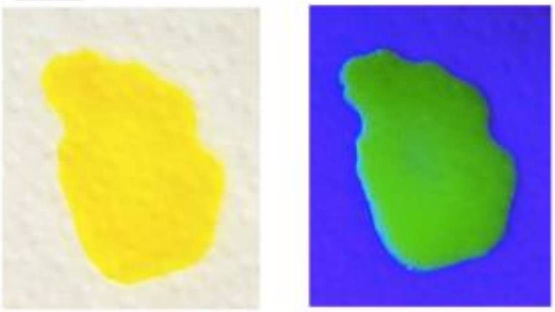


Figure 1.4.13 InP/ZnS QDs PMMA-toluene a. films under normal light and b. UV light (5% w/v)

PMMA polymer was dissolved in toluene and anisole to detect the best solvent for QDs. Polymer concentration was decreased to 5%. Monodispersion of green QDs was clearly observed but a dried in PMMA film could not be obtained.

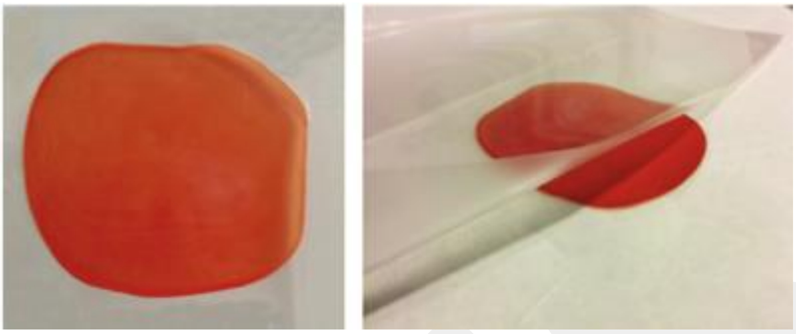


Figure 1.4.14 Red emitting InP/ZnS QDs PMMA-anisole polymer (10% w/v)

Red emitting QDs were sythesized with TOP ligand which allowed QDs better drying capability but PMMA-toluene polymeric film was not suitable for embedding procedure. Therefore, red emitting InP/ZnS QDs were dissolved in PMMA-anisole polymer and as seen in figure 1.4.14 laminated QDs barely losed the QY (18% reduction). Dispersion quality depends on mixing time of polymer and QDs. Short time stirring blocks the dispersion of QDs inside polymer. Other critical point is the QDs ligand shortage process. Too long or short ligands diminish the polymer and QDs attraction. The last point is the interaction of surface material with drying polymer. Polymeric lamination material was used to cover two sided of QD film. FWHM value was even stable after lamination.

Chapter 2

Biodetection

2.1 Aqueous based semiconductor nanocrytals

Initiated from 1980s by great effort of studies [47] [48], aqueous synthesis of colloidal QDs has drawn attention over three decades [71] [72] [73]. CdS QDs were firstly perfect model to understand the size dependent optical characteristics of colloidal nanocrytals. This routes shifted to the synthesis of other types nanocrytals such as zinc, cadmium, mercury [13] [74]. The critical phenomena, quantum confinement effect, triggered the detailed discovery of the colloidal NC applications in biomedical and optoelectronic scopes [75].

Nonaqueous hot injection' method developed by researchers [76] [77] [78] gave rise to highly florescence efficient and structurally uniformed QDs. High temperature organic synthesis approach derives lower defects and enable to several ligand selection [79] [80].Ligand transfer facility of these QDs serve as attachment of biomolecules in order to detect the microorganisms by mineralization approach [81] [82] Furthermore, nucleic acid conducted QD synthesis approach is a robust method due to targeting of NCs with specific DNA and protein which are related to particular diseases [83], [84].

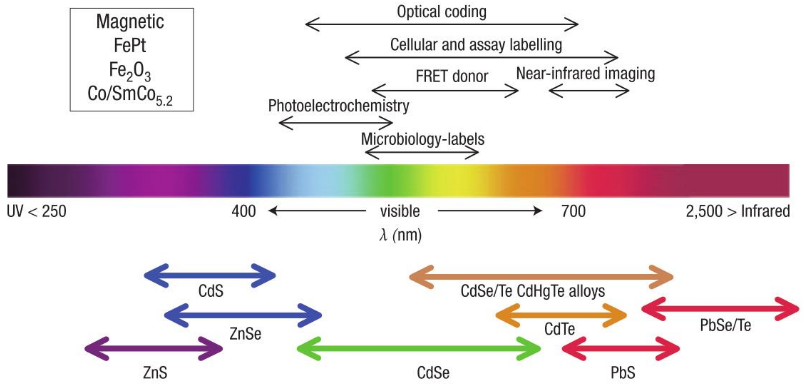


Figure 2.1.1 Illustration of QD core structure usage area in terms of emission wavelength. Biological interet is mostly focused on visible and near infrared regions [76]

In optoelectronic devices, long alkyl chains have very limited solubility in water. Therefore, aqueous based QDs with short ligand are preferred since diminishing dielectric barrier. TiO_2 like colloidal QDs containing ligand with mercapto acid and thiol derivates are favorable to produce thin dense films [72] [85]

A notable and stable water based NC synthesis was done by Rogach team by using mercaptoethanol ligand. Effective binding of thiol ligands onto surface of CdTe NCs formed the dots with 3 % QY 13. Other constituent achievements with high colloidal stability were done by thioglycolic acid (TGA) capping onto CdTe NCs with 18% QY [10]; mercaptopropionic acid (MPA) with 38% QY [11].

2.2 Inorganic & Biological Hybrids

Inorganic material and biomolecule combination provides particular spectral characteristics from nanoparticle and cellular attachment functionality from biomolecule. Finite size capability of QD allows simultaneous binding to various proteins. Acting as scaffold surface for protein, hybrid structure of nanocrystals with biomolecules construct multifunctional pattern [76]. Maltose binding proteins conjugates with 6 nm sized QD decrease the binding capacity to nanocrystals if their recognition site is lost [86].

Protein attachment can be classified in three main areas: EDC treatment of carboxyl groups of QD to surface of amines; direct conjugation of QD to particular protein by thiol capped peptides; noncovalent interaction of QD by using engineered proteins. Poor QD stability in pH alteration of EDC applications causes unwanted intermediate accumulation. Capsulation of QD with a polymer shell could culminate in nonspecific binding of targeting molecule onto nanocrystal surface [76].

Direct attachment consists mainly on surface-protein interaction. A study carried by Weiss group inludes phytochelatin peptide covering of CdSe/ZnS QDs. This comprehensive study includes not only surface passivation and phase transfer, but also biochemical alteration [87],[88]

Other approach is related to adherence of biomolecules onto target surface. Positively charged engineered proteins self-accumulate onto surface of negatively charged QDs due to electrostatic interaction. This procedure is practical in different areas including protein G binding to IgG site of an antibody [89], nonspecific protein adsorption onto QDs [90].

2.3 Silica Nanospheres

QDs with fascinating optical characteristics have attracted large scale area such as nanosensors and bioimaging. However, II-VI semiconductor NCs could deteriorate and lose chemical stability due to harsh conditions (pH, oxygen, solution with metal ions, quenchers) [73]. To prevent unstable PL intensity of QDs, several ideas were put forward. Conjugation of QD with a fluorophore was studied [91] but fluorescence fluctuations caused many turmoil in quantitative analysis results. In addition cytotoxic heavy metal ion forestalled the utilization of QD in interested applications [73]. Therefore, appropriate techniques were investigated to overcome the PL and cytotoxicity problems such as polymer spheres [92] and silica dots [93] (Figure 2.3.1).

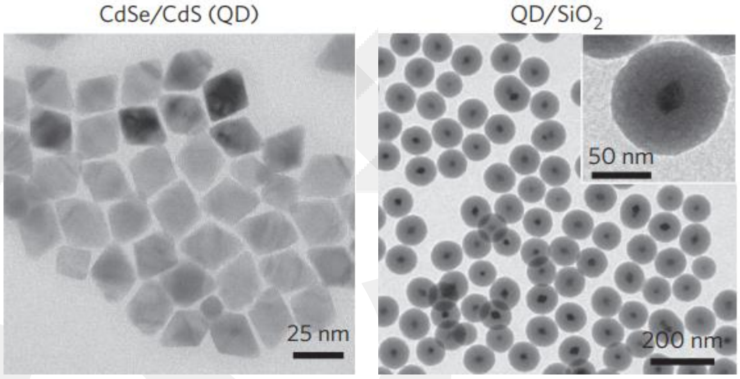


Figure 2.3.1 Schematic illustration of silica coating stages (top) and TEM images (bottom) a. Cd QD b. Quantum Dot/ SiO_2 nanoparticles [94]

On top of providing colloidal stability, diminishing cytotoxicity, silica shell enables functionalization of QD surface ligands. However, Stöber procedure and reverse microemulsion technique not only plunge the PL of QD, but also cannot maintain QD from harsh microenvironment [25]. Approximately 50% of initial CdTe QD PL is lost after CdTe/SiO_2 nanoparticles processed by reverse microemulsion technique [26]. Besides this kind of approaches, instability in harsh microenvironment problem could not solved.

A remarkable process (double silica coated) was applied by a group [29] This approach is called as sandwich model that SiO_2/CdTe QD/ SiO_2 composite structure was constructed respectively. Mercaptopropionic acid capped QDs with carboxylic group electrostatically attract amino-functionalized silica dots. Composite material preserves nearly 80% of initial QY of CdTe QD. Furthermore stability of the composite retains in solutions with pH from 2 to 8.

2.4 Bacteria detection

Nowadays, pathogens affect the water quality of rivers and streams in many parts of the world. Lifted up levels of E. Coli in United States (*Watershed Assessment, Tracking & Environmental Results*. Washington DC: USEPA) demonstrated that understanding the variation mechanism of these kind of pathogens is essential for diminishing the contamination of water. With new improvements in technology, studies have pointed out the great diversity of E. Coli strains in the environment [21]

These variations have been defined by the impact of genotypic and phenotypic characters of pathogens. Surviving and growth mechanism of bacteria is related with the surface feature such as lipopolysaccharides (LPS), hydrophobicity and net charge.

E. coli surface on account of peptidoglycan and LPS on cell wall is negatively charged [95]. The habitat of pathogens determines the magnitude of surface charge and hydrophobicity [96] which affects the attraction and repulsion of particles. Ca^{2+} and Mg^{2+} cations could raise the hydrophobicity by binding to membrane proteins of pathogen [97].

Extracellular polymeric substances (EPS) produced by bacteria construct the outer cell surface and mainly consist of polysaccharides, proteins and DNA, lipid in small amount. EPS by providing heterogeneity actively contribute to biofilm production, cell adhesion, protection from microenvironment [98].

Mechanism of bacterial attachment is based on motile and unmotile types of microorganism. Motile pathogens occupy different depth of microenvironment: liquid with no effect from surface, near surface with hydrodynamic effect, surface with effect of electrostatic forces [99]

Location of pathogen in fluid region is related to flagella which is the motion organelle of bacteria. E. Coli with flagella have the capacity to move through different depths in liquid. Upon contacting target surface of liquid, attachment of cellular components occurs in two ways. Reversible attachment is a kind of hydrodynamic and electrostatic interaction like polystyrene bead contact to the surface [100].

Bacteria mostly with negative surface charge particularly in stationary phase are affected by positively charged surfaces. Upon sensing quorum, E. Coli raises up the negative charge on the membrane during biofilm generation [101].

2.5 Experimental

2.5.1 Materials and Characterization Techniques

Ammonium hydroxide, TEOS, APTES and sulfur powder, polystrene were purchased from Sigma Aldrich. E. Coli DH5 Alpha strain expressing green fluorescent protein (GFP) was prepared by Synhetic biosystem research group (Urartu Şeker). Characterization techniques and instruments used in this part were described in chapter 1. In adddition, fluorescence spectrophotometer (Bilkent-UNAM) was utilized to illustrate the biosensing images. Two different filters allowing the particular flow of light were used. Exciting of sample at approximately 470 nm and 540 nm resulted in emission of GFP at 530 nm and InP QD at 630 nm, respectively.

Time resolved photoluminescence spectroscopy (Picoquant Fluotime 200-AGU) on the right side was used to measure life time of particular samples (10000 photon count/ maximum emission values of samples at 450 nm PL spectra). To analyse fluorescence decay, Fluofit software was utilized.

Transmission electron microscope (TEM) and scanning electron microscope (NanoSEM) were used to determine the surface and structural analysis of samples. After dropping diluted samples onto grids, pilko tab hold the grid in proper position. With drying of sample solution in an hour, grid is placed into TEM component and analysis starts.

SEM specimen preparation is needed an extra step before analysis. Non-dense material composition generates too low signal for surface scanning images. Therefore, an extra coating with gold provides a strong signal to visualise depth with 3D appearance. QD samples were dropped onto pin mount stand (as seen on the right) and waited for drying for an hour. Then, samples on the pin was coated with gold and observed with SEM.

2.5.2 Silica dots

15 ml ammonium hydroxide and 125 ml ethanol were mixed in a 100 ml glass bottle. After stirred on hot plate for 5 min (40 °C), 5 ml TEOS was added and silica dots formed with overnight stirring process. With 1 hour time interval, 2.5 ml TEOS and 0.2 ml APTES were added into mixture. Incubation for 6 hour helped to form ligand attached silica dots. 3 times washed silica dots (ethanol/water 1:3 v/v centrifuged at 5000 rpm for 5 min) were weighted at last washing process. Approximately 1.5 g silica dot was obtained. Then, they were dissolved in water and to separate the aggregated dots sonicator was used.

2.5.3 Silica coated QDs

50 mg WQD and 1g silica dot were dissolved in 100 ml deionized water and stirred on hot plate for 2 minutes. Negative and positive charge attraction caused an adherance of aqueous QDs onto silica surface. New product SQ was precipitated with ethanol/water 1:2 (v/v) at 5000 rpm for 5 min. For extra silica layer covering, precipitated SQ was dissolved in 20 ml di-water and 66 ul APTES, 1600 ul TEOS, 30 ml ethanol were added into mixture. Following stirring for 4 hour at 25 °C, product called as SCQD was washed with ethanol/water (1:2 (v/v) at 5000 rpm for 5 min). Dried SCQD particles were stored at +4 °C for further applications.

2.5.4 Polymeric film & Bacteria detection

Polymeric film was prepared by dissolving 10 g polystyrene (PS) in 100 ml chloroform (10%). PS solution was stirred overnight at room temperature. For QD film formation, 200 mg Silica-QDs was mixed with 4 ml PS polymer and then 400 ul taken from this mixture was poured onto glass.

Spin coating process was applied to obtain thin films (at 1000 rpm for 30 sec) by spin coater as seen on the right side. Stock of DH5 Alpha strain producing GFP protein was dissolved and grown in petri dishes with Luria broth nutrient rich media at 30 °C (overnight).

2.6 RESULTS AND DISCUSSIONS

After phase transfer, QDs in aqueous solvent were prepared for bio sensing. Before phase transfer process, red emitting InP/ZnS QDs were washed with acetone twice to shorten ligand of nanocrystals that facilitate binding of water based ligands, MSA (Figure 2.6.1).

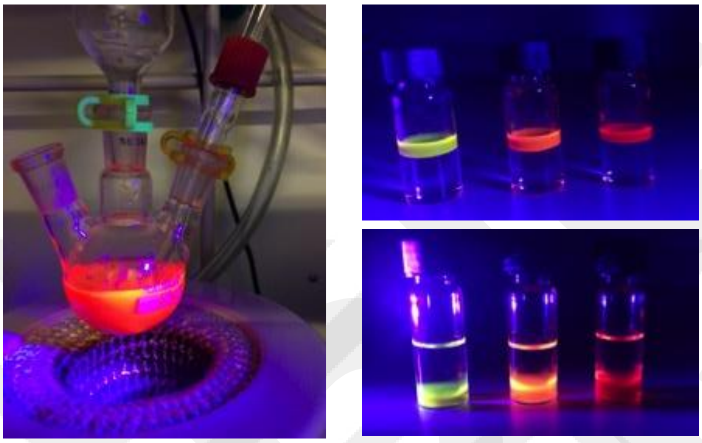


Figure 2.6.1 InP/ZnS QDs (including TOP ligand) a. red emitting under UV light soon after synthesis b. QDs in hexane c. QDs in water

Table 2.6.1 InP/ZnS QDs in organic and aqueous form including PLQY, FWHM and emission wavelength

	Green QD		Orange QD		Red QD	
	Organic	Aqueous	Organic	Aqueous	Organic	Aqueous
Emission(nm)	557	560	606	614	631	635
FWHM (nm)	68	72	59	69	59	63
PLQY %	45	18	48	20	50	18

For biosensing application red emitting QD type was selected due to its large-scale excitation characteristics. Other essential point is the GFP related green emitting DH5 alpha bacteria. For efficient result in fluorescence microscope, fluorescence emission types of samples were critical. 20% red emitted water based QDs with 63 nm FWHM were prepared for integration into polymer.

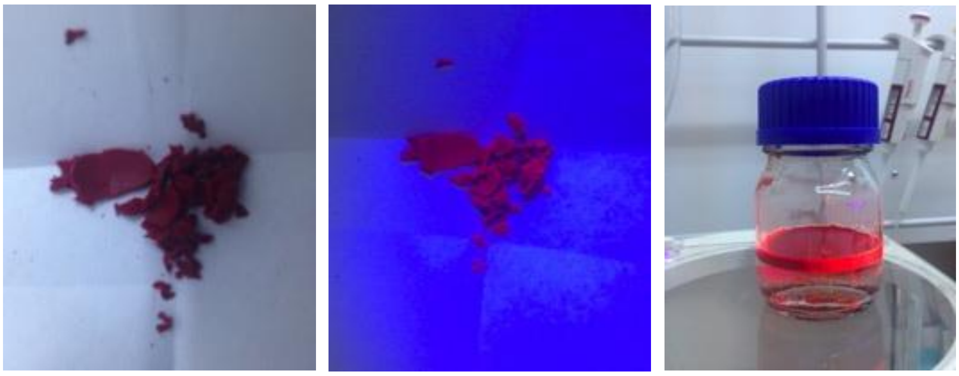


Figure 2.6.2 InP/ZnS QDs a. solid QDs after synthesis (2 times washed with acetone) b. red emitting QDs under UV c. organic based QD during phase transfer (water:bottom solution, hexane:top solution)

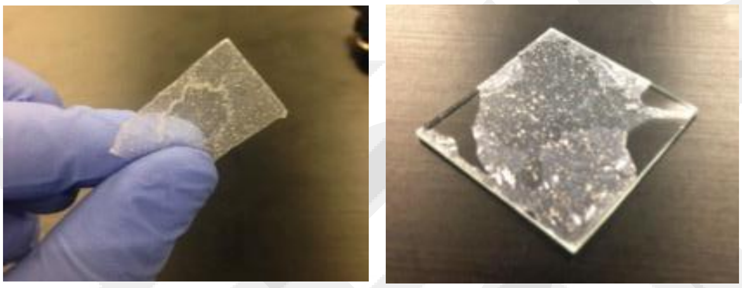


Figure 2.6.3 InP/ZnS QDs in PS polymeric film a. embedded QDs into polymer b. thin SCQD structure

In this part of study, silica capped QD generation process was applied and SEM images showed the scan of the PS film with magnifying (10.000x) of SCQD structure. Each round shape illustrates the silica dots with 500 nm sized approximately (Figure 2.6.4).

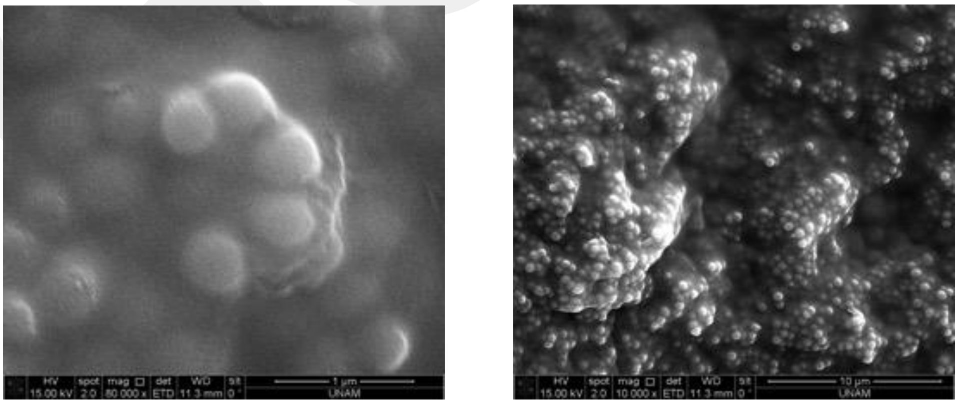
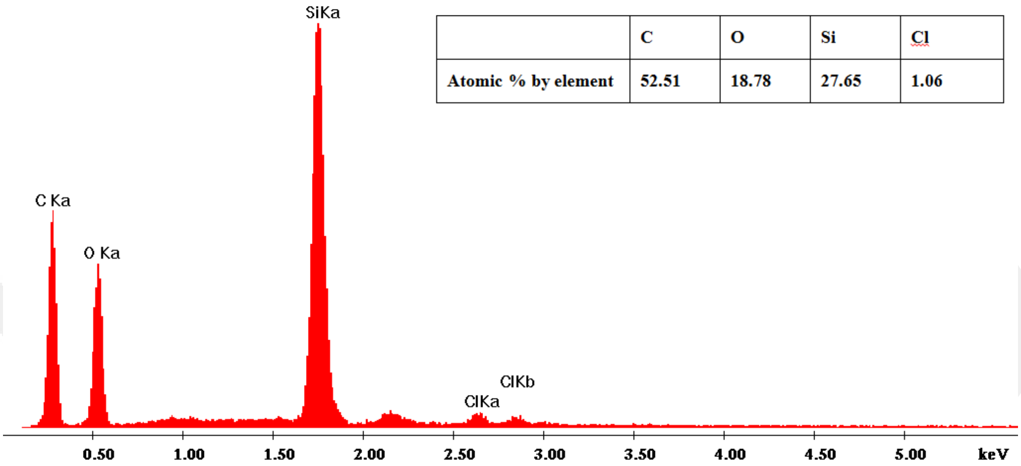


Figure 2.6.4 SEM image demonstrating the silica QDs on PS film

To understand the proper covering of QDs with SiO_2, SEM elemental analysis was done.As illustrated in figure 2.6.5, atomic percentages on the table show that Si, C and O are the fundamental elements for silica generation. Furthermore, CI element is core material of QDs. SEM analysis only indicated silica dots but QDs in aqueous solvent could not be seen due to their small size compared to silica dots.



	C	O	Si	Cl
Atomic % by element	52.51	18.78	27.65	1.06

Figure 2.6.5 Elemental distribution of SCQD in SEM

To confirm the proper attachment of QDs onto silica dots, TEM was performed (Figure 2.6.6). Firstly, aqueous InP/ZnS QDs were detected in 5 nm and 20 nm scale bars. 3.4 nm sized QDs in aqueous solvent were clearly detected with 5nm scale bar.

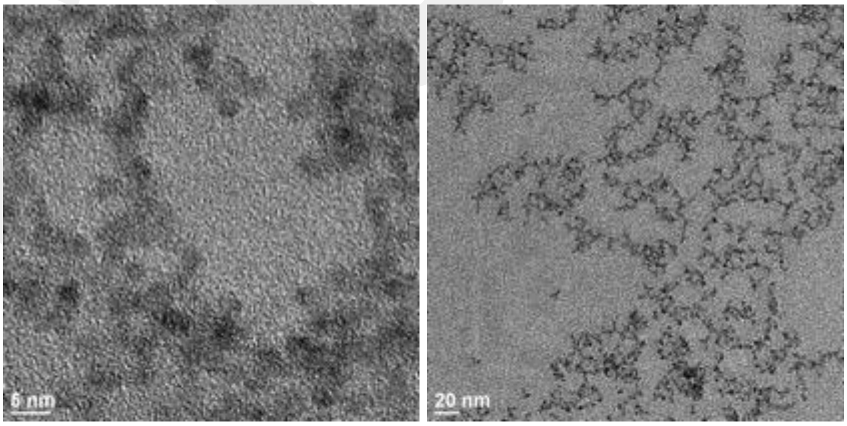


Figure 2.6.6 TEM images of QDs in aqueous solvent with 5 nm and 20 nm scale bars

Attached QDs onto SiO_2 was seen clearly with TEM instrument. Silica dot size is around 350 nm. In 10 nm scale bar image, small dots on the silica dot surface indicate very well conjugation of QDs onto silica material (Figure 2.6.7).

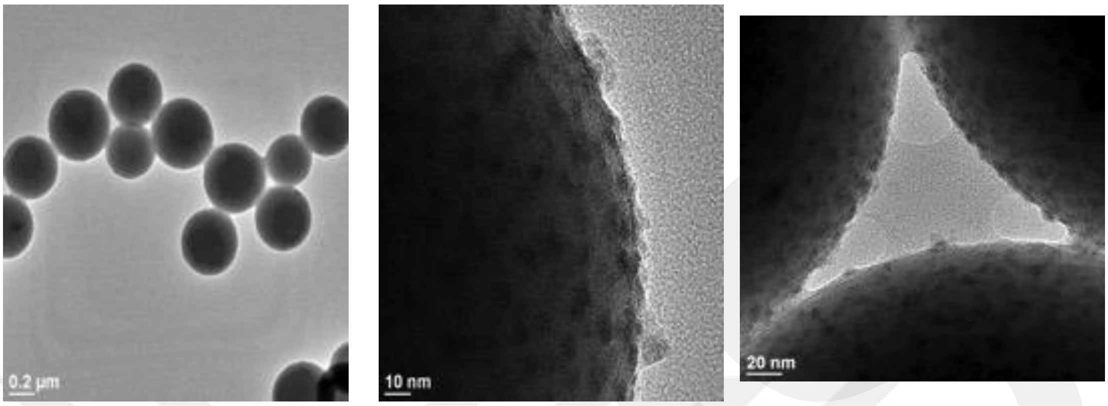


Figure 2.6.7 TEM images of silica capped QDs

After silica capped QD (SCQD) structure was well defined, bacteria detection with SCQD on film was performed. To understand the conjugation of bacteria onto SCQD in film, time resolved analysis was performed. Fluorescence time of SCQD film with emission at 620 nm was quite changed by attachment of E. Coli onto SCQD spheres (Figure 2.6.8).

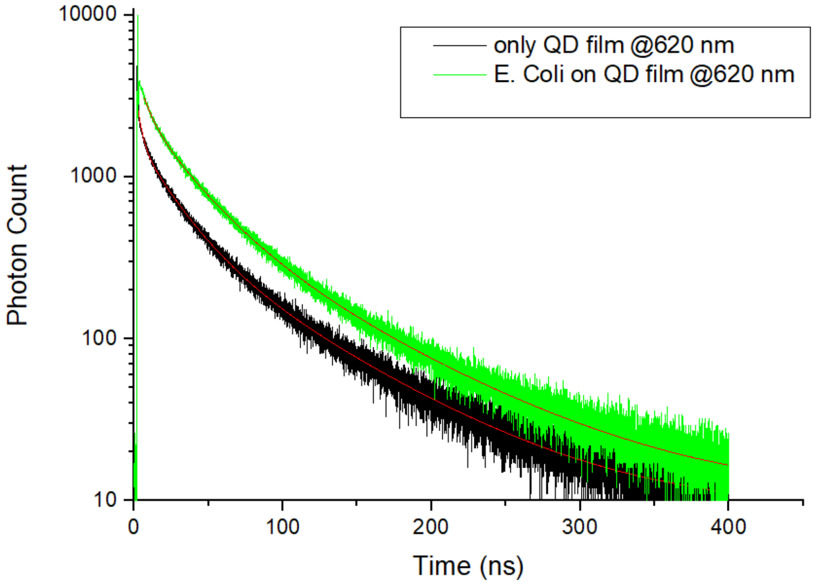


Figure 2.6.8 Time resolved spectra of SCQD film and E. Coli attached onto SCQD film

Table 2.6.2 The lifetime components of SCQD film and E. Coli attached SCQD film

Sample	A1	T1 (ns)	A2	T2 (ns)	A3	T3 (ns)	Tavg (ns)
SCQD film	1161.1 ±31.8	29.607 ±0.517 **44.38%**	994 ±119	6.945 ±0.879 **38.00%**	460.9 ±10.3	75.47 ±1.20 **17.62%**	29.077
SCQD film +E. coli	1647.6 ±31.8	29.607 ±0.517 **53.53%**	811.8 ±84.2	6.945 ±0.879 **26.37%**	618.7 ±11.5	84.32 ±1.06 **20.10%**	34.628

SCQD film and attached E. Coli were compared according to their emission time capacity (TRPL) which is related to measure the fast charge carrier dynamics. As demonstrated in Table 2.6.2, life time of both samples have three components in which the percentages of each of them differs. SCQD film T1,T2 and T3 values were recorded as 44.38, 38, 17.62 % respectively. On the contrary, percentages in SCQD attached E. Coli was seen as 53.53, 26.37, 20.10 %. Average T values were also different, changes in life time components showed the difference between SCQD and bacteria attached SCQD film. Time dependent photon delay time goes up to 34.628 ns. This mechanism could be explained by Förster Resonance Energy Transfer (FRET). This distance dependent physical interaction allows passage of energy from donor to acceptor molecule. GFP expressed DH5 alpha strain have emission capacity around 530 nm and SCQD emits red color at 620 nm (Figure 2.6.9). Assuming that GFP with high energy could donate its energy into QD with relatively lower energy as acceptor. Therefore, this interaction might alters the delay time of QDs embedded into silica film.

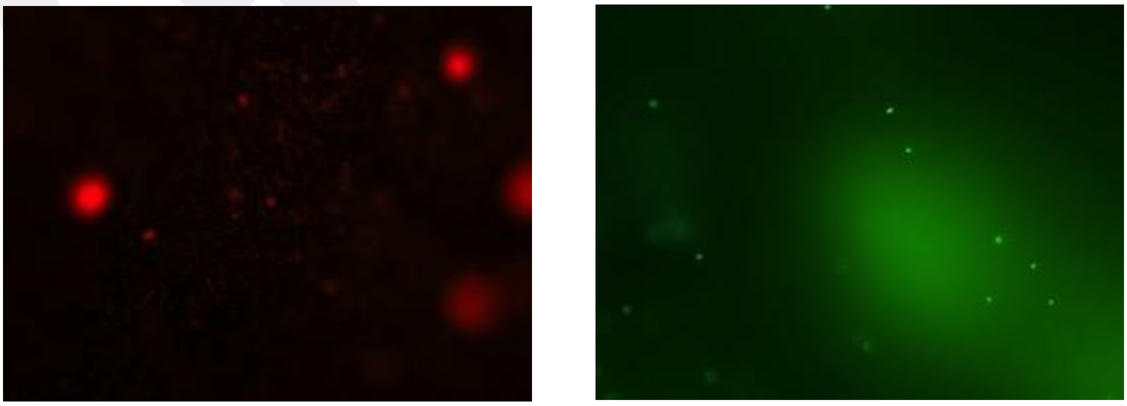


Figure 2.6.9 Fluorescence microscope image of a. SCQD film prepared by polyvinylalcohol (PVA) and water b. GFP expressed E. Coli on SCQD film (PVA and water)

Polymer of SCQD firstly made by PVA and water which is environmental and easily disposable. However its endurance to bacteria environment is rather low and dissolves in a short time. Therefore, a water resist polymer was needed for biosensing approach. For the further detection, polystyrene was prepared in chloroform and QDs were embedded into this film (Figure 2.6.10).

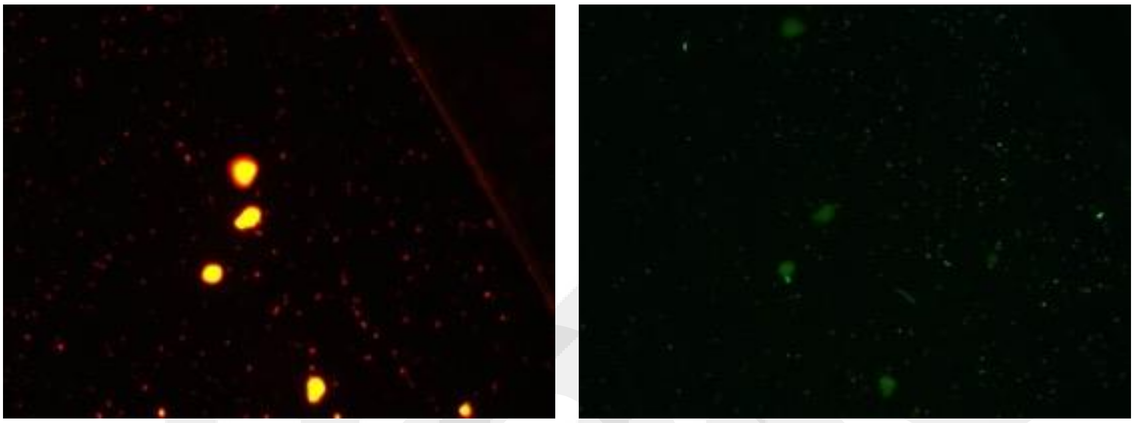


Figure 2.6.10 Fluorescence microscope image of a. SCQD film (prepared by PS and chloroform) with emission at 620 nm b. GFP expressed E. Coli on SCQD film with 530 nm emission wavelength

As seen in Figure 2.6.7, attraction of E. Coli from SCQD was clearly observed by fluorescence microscope. E. Coli with negative charge attracted positively charged SCQD. Van der waals forces are essential for the assembling of this pathogen onto SCQD film (The image was taken from the same position by only changing excitation wavelength to detect both QDs and DH5 alpha).

Chapter 3

CONCLUSION and FUTURE PERSPECTIVE

The main target of this thesis is the rapid and simple paper based detection of bacteria by using silica covered QDs. Nowadays pathogen toxicity in water threatens lives in cities and villages. Therefore, quick detection of toxic and harmful substance have critical role in rescue and raise the quality of life.

Biosensing approach by using high fluorescence emitting QDs was investigated. Currently, many studies in bio field are based on formation and application of environmental and less toxic materials onto biosystems. Therefore, cadmium free materials are essential factor in bioapplications. These semiconductor nanocrystals have size tuning capability so halide precursor changing results in differences in emission wavelength. In addition, high and relatively more stable PLQY compared to biological dyes gave an opportunity to QDs entering into biotreatments.

In this study best QY (50%) and FWHM 56 (nm) were obtained in red emitting InP/ZnS QDs due to excitation with variable energy states and also red QD stability directs many studies currently. As mentioned in result part, QDs synthesized with interested PLQY and FWHM and chemical yield were then functionalized for the utilization of QDs on bacteria detection. Following phase transfer, PLQY was decreased to 20% owing to insufficient surface passivation by removing TOP-S ligands. However, stability was maintained by covering aqueous QDs with SiO_2. With the TEM analysis approval, SCQD generation was proved. As a sample pathogen, DH5 Alpha expressing GFP was used. SCQD QDs with NH_2 groups on the surface attracted negatively charged gram negative E. Coli pathogen with having LPS on its membrane. It is expected that this study can pave the way for rapid and efficient detection of pathogens.

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