CHAPTER 1

Introduction

1.1 Quantum dots (QDs)

The semiconductor quantum dots (QDs) are inorganic fluorescent nanocrystals over the size range of 1 – 20 nm mead up of compounds from group II to VI and III to V such as Ag, Cd, Hg, Ln, P, Pb, Se, Te and Zn. The specific optical and electronic properties dependent on their surface to volume ratio and on the phenomenon (strict confinement of electrons and holes when the nanoparticle radius is below the exciton Bohr radius). QDs have size-tunable emission, strong light absorbance, bright fluorescence with narrow symmetric emission band, high photosensitivity, physical stability, brightness and long fluorescent lifetime. QDs consists of a core and a shell which covers the core. Core are usually composed of elements from group II and VI or III and V such as zinc, cadmium, CdS, CdSe, CdTe, InP, GaP, InAs, GaAs, GaN, CuCl and others. Shell is the coat of ZnS, ZnSe, ZnO, PbS, CdS, CdS, CdSe or ZnTe surrounds the semiconductor core for improving its optical properties. Among them, the II-VI and IV-VI QDs have been extensively studied because of their good optical properties although they consist of toxic elements like cadmium (Cd), mercury (Hg), lead (Pb), selenium (Se), tellurium (Te) and arsenic (As). According to the materials chemistry viewpoint, it is worthy challenge to develop novel and less toxic alternatives for replacement of using Cd- and Pb-based QDs. Recently, heavy metal-free QDs of I-III-VI_2 such as $CuInS_2$, $CuInSe_2$ and $AgInS_2$ exhibit unique optical properties with less toxicity [1-6].

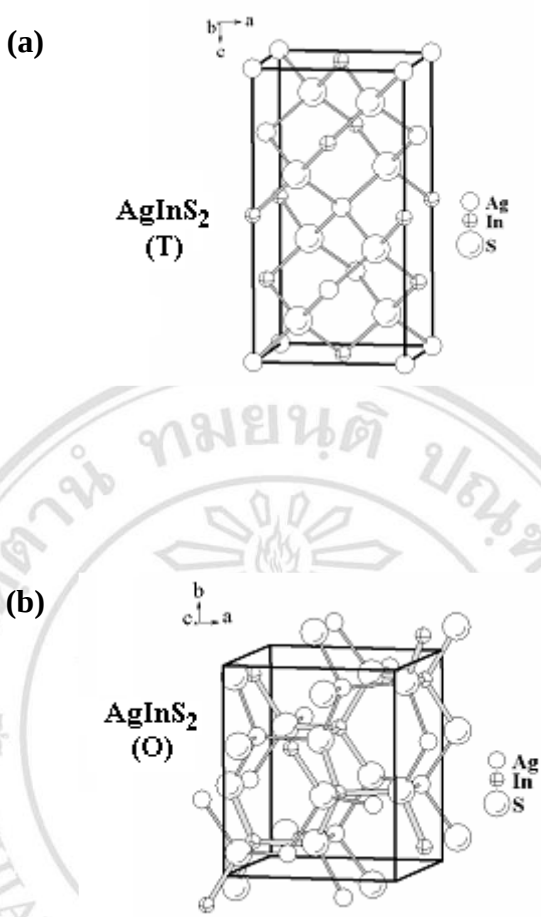

Figure 1.1 Structure of $AgInS_2$: (a) tetragonal and (b) orthorhombic [7].

1.2 Silver indium sulfide ($AgInS_2$)

The ternary I-III-VI_2 QDs is silver indium sulfide ($AgInS_2$) that is able to be crystallized in two different phases known as tetragonal (chalcopyrite) and orthorhombic structures. The tetragonal is a low-temperature phase and is stable below 620 °C. For the high-temperature phase, it is orthorhombic and is stable above 620 °C [8]. Tetragonal and orthorhombic phases have two direct band gap energies in the range of 1.86 – 2.04 eV. In addition, both Ag^+ and S^{2-} precursors are much more stable than Cu^+, Se^{2-} and Te^{2-}. Thus, $AgInS_2$-based QDs is able to be produced directly in air and may provide excellent band gap in the visible region. Unfortunately, the as-produced naked I-III-VI_2 QDs usually exhibit weak photoluminescence emission [5, 9].

1.3 Synthesis

$AgInS_2$ QDs can be produced by different methods: aqueous synthesis [6], one step reaction at low temperature [9, 10], hot-injection method [11], microwave method [12, 13], microwave hydrothermal method [14] and solvothermal method [15, 16]. Hydrothermal method is heterogeneous reaction in the presence of aqueous solvents or mineralizers under high pressure and temperature conditions to dissolve and recrystallize materials that are relatively insoluble under ordinary condition. This method has some advantages: water is environmental benign and cheaper than other solvents. Water is able to volatile at low temperature and can be removed from the product very easily. It is nontoxic, nonflammable, no carcinogenic, no mutagenic and thermodynamically stable [17, 18].

Figure 1.2 Autoclave for hydrothermal synthesis [17].

The method is able to be used to synthesize large crystals of high quality. It is possible to control particle size and morphology. The final size and structure of nanomaterials are controlled by the salt and surfactant additive, reactant concentration, reaction temperature and solvent condition used during the synthesis. Many inorganic phases are unstable under high temperature. Another advantages of hydrothermal method for mixing water-soluble nanoparticles should be mentioned [17, 18].

1.4 Polysaccharides

Polysaccharides are regarded as the most popular polymeric materials used to prepare nanoparticles for drug delivery systems. Due to the presence of various derivable groups on molecular chains, polysaccharides can be easily modified chemically and biochemically, resulting in many kinds of polysaccharide derivatives. Carboxymethyl cellulose (CMC) is a water-soluble polysaccharide possessing carboxylate and hydroxyl groups that considered as green, natural, inexpensive polysugar, stable, non-toxic, biodegradable and biocompatible [19, 20]. Thus, it can be used as passivation agent for producing biocompatible nanoparticles. Furthermore, CMC is also applied for drug delivery system to control drug release and considered as a promising material for biomedical application [21, 22].

CMC has a negative charge polysaccharide. This negatively charged CMC can then be attracted with the positively charged drugs such as doxorubicin (DOX) *via* electrostatic interaction [23]. More importantly, the carbohydrates also exhibit cancer-cell specific capability, leading to use them as targeting vector for cancer treatment [24].

1.5 Characterization

1.5.1 X-ray diffraction (XRD)

X-ray diffraction (XRD) is a technique importance in many industrial application on account of its wide availability and prevalence. XRD technique is used to study qualitative and quantitative phase identification and analysis, determination of crystallinity, micro identification, lattice-parameter determinations, high-temperature studies, thin film characterization, and crystal structure analysis. Powders are the samples for analysis. This technique can be analyze several materials consist of inorganic, organic, polymers, metals or composites and the potential applications cover almost all research fields, e.g. metallurgy, pharmaceuticals, earth sciences, polymers and composites, microelectronics and nanotechnology. [25, 26]

Figure 1.3 X-ray diffractrometer. [26]

1.5.2 X-ray photoelectron spectroscopy (XPS)

Figure 1.4 X-ray photoelectron spectrometer. [27]

X-ray photoelectron spectroscopy (XPS) is a technique the standard instrument for analyzing the surface chemistry of a material. XPS can be used to analyze the elemental composition, empirical formula, chemical state and electronic state of the elements within a material. The physical and

chemical interactions can be solve by XPS technique, it obtained at the surface or at the interfaces of a material's layers. The spectrum shows atoms emitting electrons of a particular characteristic energy. The energies and intensities of the photoelectron peaks enable identification and quantification of all surface elements (except hydrogen). [28]

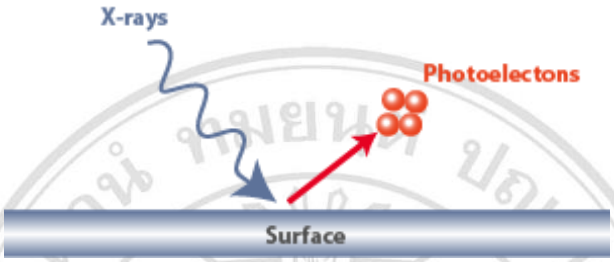

Figure 1.5 Ejection of photoelectrons. [28]

1.5.3 Tranmission electron microscopy (TEM)

Figure 1.6 Tranmission electron microscope. [29]

Tranmission electron microscopy (TEM) is a technique used to analyze materials characterization by electron beam interacts and passes through a specimen. The tranmission electron microscope shows in Figure 1.7. The electron beam is limited with the two condenser lenses, passes the condenser aperture and “hits” the sample. The electrons that are elastically scattered consist the transmitted beams, which pass through the objective lens. The objective lens forms the image exhibit and the following apertures, the objective and selected area aperture are used to choose of the elastically scattered electrons that will form the image of the microscope. Finally, the image displayed on a fluorescent screen or in monitor or both and is printed on a photographic film. [30]

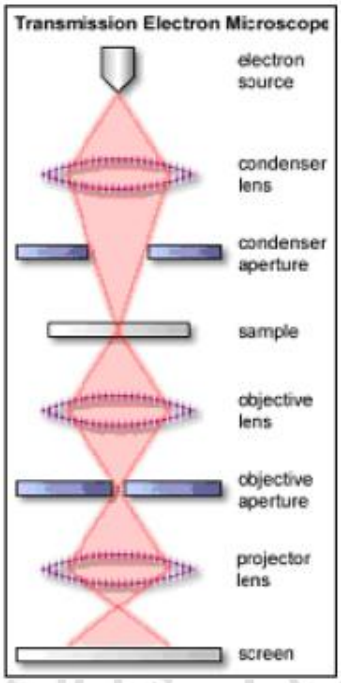

Figure 1.7 Transmission electron microscope with all of its components. [30]

1.5.4 Dynamic light scattering (DLS)

Dynamic light scattering (DLS) or Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering is a technique used to measure the size of particles in the term hydrodynamic diameter. [31]

Figure 1.8 Dynamic light scattering. [32]

DLS obtained the hydrodynamic size by Brownian motion. Brownian motion is the random movement of particles due to the attack by the solvent molecules. Normally DLS is concerned with measurement of particles suspended within a liquid. The larger the particle will be slow the Brownian motion than smaller particles. In the measure, temperature is necessary for DLS technique because of the temperature significant to the viscosity. [31]

The Hydrodynamic Diameter, the size of a particle is calculated from the translational diffusion coefficient by using the Stokes-Einstein equation [31];

$$d(H) = \frac{kT}{3\pi\eta D}$$

where d(H) = hydrodynamic diameter
D = translational diffusion coefficient
k = Boltzmann's constant
T = absolute temperature
η = viscosity

1.5.5 Fourier transform infrared spectroscopy (FTIR)

Figure 1.9 Fourier transform infrared spectroscope. [33]

Fourier transform infrared spectroscopy (FTIR) is a technique used to determine qualitative and quantitative features of IR-active molecules. Sample are analyzed consist of solid, liquid and gas in an atmospheric environment. [34]

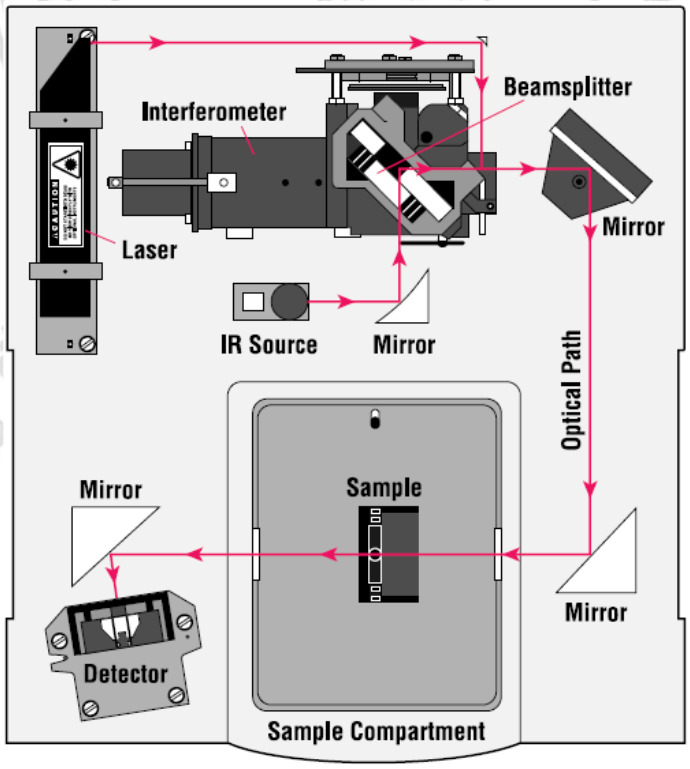

Figure 1.10 Fourier transform infrared spectroscope layout. [34]

Infrared radiation was passed through a sample (solid or liquid or gas). Some infrared radiation was absorbed by the sample and some it was passed through. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. [34]

1.5.6 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is a technique used to study changes in physical and chemical properties of materials, its weight increases or decreases when the temperature increases. TGA instrument consists of a sample pan that is supported by a precision balance. That pan resides in a furnace and is heated or cooled during the experiment. The mass of the sample is monitored during the experiment. A sample purge gas controls the sample environment. This gas may be inert or a reactive gas that flows over the sample and exits through an exhaust. [35]

Figure 1.11 Thermogravimetric analysis. [36]

1.5.7 UV-Visible spectrophotometry

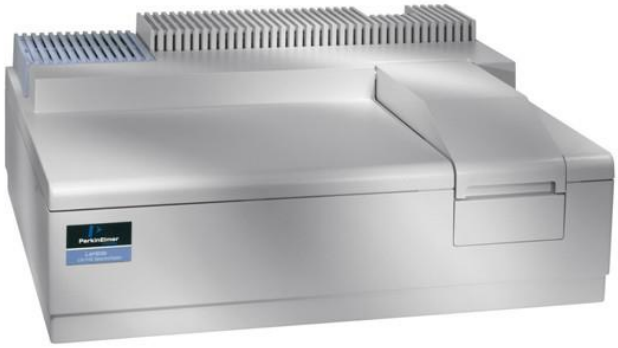

Figure 1.12 UV-Visible spectrophotometer. [37]

UV-Visible spectrophotometry is a technique used to measure the amount of ultraviolet or visible radiation absorbed by a substance in solution. UV-Visible spectrophotometry is simple, rapid, moderately specific and applicable to small quantities of compounds. In qualitative analysis, the sample can be identified by use of spectrophotometer and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. The quantitative spectrophotometric analysis is the Beer -Lambert law. [38]

Beer's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration.

Lambert's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness.

A combination of these two laws yields the Beer-Lambert law.

Beer-Lambert law: When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur. Mathematically, Beer-Lambert law is expressed as [38]

$$A = \varepsilon bc$$

Where A = absorbance or optical density

ε = absorptivity or extinction coefficient

b = path length of radiation through sample (cm)

c = concentration of solute in solution.

Both b and ε are constant so a is directly proportional to the concentration c.

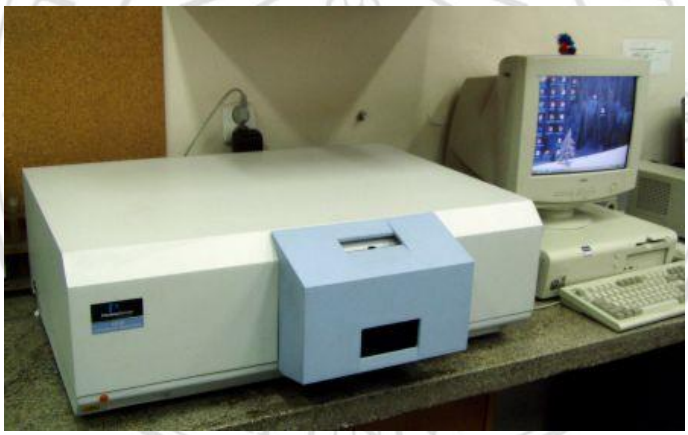

Figure 1.13 Photoluminescence spectroscope. [39]

1.5.8 Photoluminescence spectroscopy (PL)

Photoluminescence spectroscopy is a technique used to measure the emission of light from a material under optical excitation. The excitation energy and intensity are chosen to probe different regions and excitation concentrations in the sample. Features of the emission spectrum can be used to identify surface, interface, and impurity levels and to gauge alloy disorder and interface roughness. The intensity of the PL signal provides information on the quality of surfaces and interfaces. PL technique can be analyze very

fast, making it useful for characterizing the most rapid processes in a material. [40]

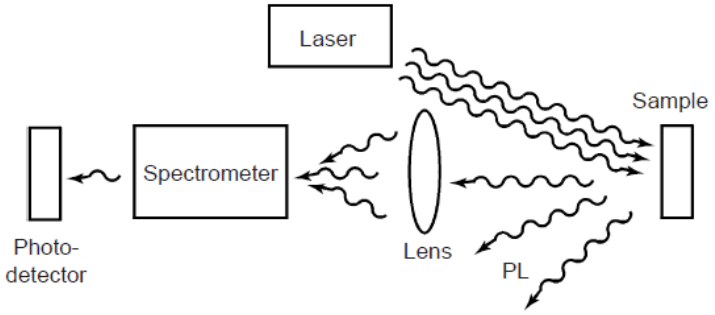

Figure 1.14 Typical experimental set-up for PL measurements. [40]

1.5.9 Fluorescence microscopy

Fluorescence microscopy is a tool analyzing in biology. The use of an array of fluorochromes has made it possible to identify cells and submicroscopic cellular components and entities with a high degree of specificity amid nonfluorescing material. The fluorescence microscope can reveal the presence of a single fluorescing molecule. [41]

Figure 1.15 Fluorescence microscope. [42]

1.5.10 Flow cytometry

Flow cytometry is a technique used to measures and analyzes multiple physical characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light. The properties measured include a particle's relative size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence. [43]

In the flow cytometer, particles are carried to the laser intercept in a fluid stream. Any suspended particle or cell from 0.2 – 150 micrometers in size is suitable for analysis. Cells from solid tissue must be disaggregated before analysis. The portion of the fluid stream where particles are located is called the sample core. When particles pass through the laser intercept, they scatter laser light. Any fluorescent molecules present on the particle fluoresce. The scattered and fluorescent light is collected by appropriately positioned lenses. A combination of beam splitters and filters steers the scattered and fluorescent light to the appropriate detectors. The detectors produce electronic signals proportional to the optical signals striking them. [43]

Figure 1.16 Flow cytometer. [44]

The data are collected and stored in the computer. This data can be analyzed to provide information about subpopulations within the sample (Figure 1.17).

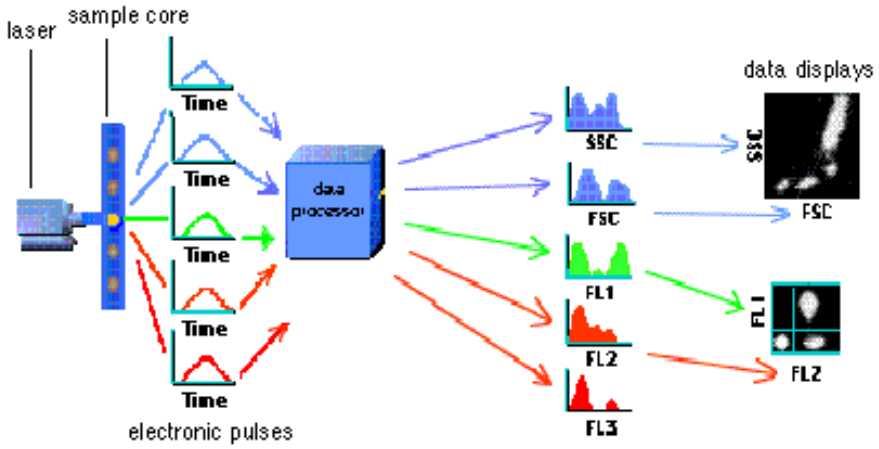

Figure 1.17 Scattered and emitted light signals are converted to electronic pulses that can be processed by the computer. [43]

1.6 Applications

These nanocrystals have several applications like light-emitting diodes, thermoelectric materials, sensors, solar cells and nanomedicine.

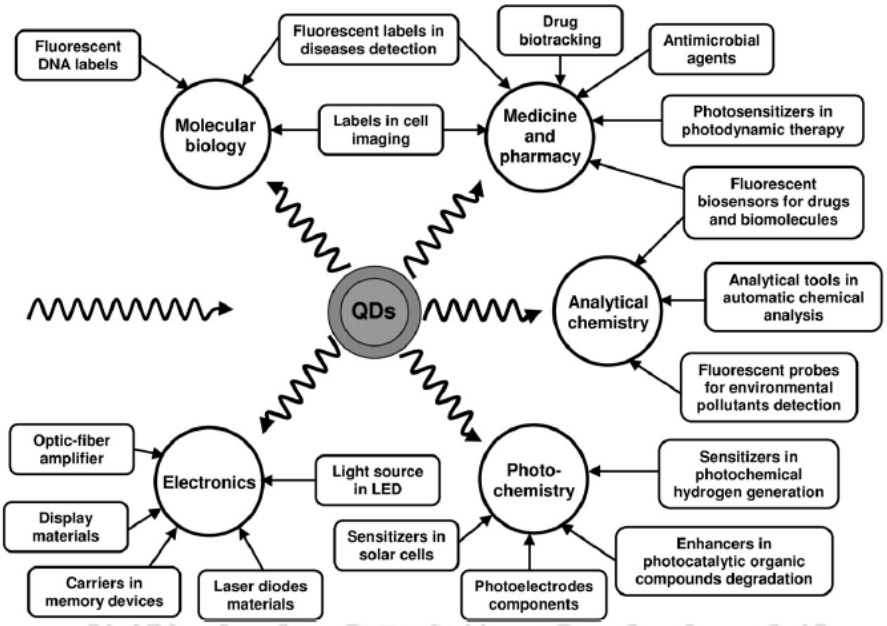

Figure 1.18 The application in different branches of science of quantum dots. [1]

Presently, biomedical is an interesting application for nanomedicine. Thus the application of nanotechnology is focused on medicine, particularly with its promise of improved therapy and diagnostic and reduction inside effect. The unique combination of optical properties has led to intense interest, particularly for biomedical applications. A major challenge in this field is the transfer of QDs to water. To evaluate functionalizing schemes for water solubilization, three key requirements are required: the QDs should be monodisperse and not aggregate, the QDs suspension should be stable for at least several days, and there should be minimum attenuation in the quantum yield. These applications are used to nanodiagnostic and imaging, targeted drug delivery and controlled release, and photodynamic therapy [1, 3, 45].

1.7 Literature Review

In the field of biomedical, nanotechnology offers potential development in pharmaceuticals, medical imaging and diagnosis. The most exciting applications are the ability of nanoparticles for imaging and of fluorescent inorganic nanocrystalline quantum dots for loading. I-III-VI_2 ternary quantum dots have been synthesized in organic solvents [7, 15, 16]. The final size and structure of these quantum dots are

controlled by the surfactant additives, reactant concentrations, reaction temperatures and solvent conditions used during the process. This method can be readily employed to prepare other ternary quantum dots. It is able to be used with organic solvents which may have been contaminated the products because the organic solvent removal could be difficult. The synthesis of these materials requires the use of organic solvents which can seriously affect protein stability. The limitations are to position the quantum dots in living cell. The quantum dots may kill the cells due to the aggregation, metabolism and excretion which are unknown, accumulation in body tissues and led to cytotoxicity. Therefore, it is necessary to improve the surface area at the synthesis stage. quantum dots can protect drugs from being degraded in the body before quantum dots reach the target. Enhance drug absorption into tumors and the cancerous cells themselves can lead to better control over the timing and distribution of drugs to the tissue. It is easier for oncologists to assess how well they work and prevent drugs from interacting with normal cells, thus avoiding side effects.

The main parameters that need to be considered for the synthesis of quantum dots for biomedical application are optical core, shell, surface modification and bioconjugation. The core has been synthesized by precipitation of the metal with the hydroxide in the presence of colloidal stabilizer. The core is covered with a shell. All quantum dots surface chemistries are designed to provide reactive group such as amine ($-NH_2$), carboxyl (-COOH) or mercapto (-SH) groups for direct conjugated to biomolecules.

Deng *et al.* [5] have synthesized water-soluble quaternary Zn-Ag-In-S quantum dots (ZAIS QDs) for tumor cell-targeted imaging. These highly luminescent ZAIS QDs are less toxic due to the absence of highly toxic cadmium. The GSH-capped ZAIS QDs are particularly attractive for bioimaging and biolabeling applications, as a potential alternative for Cd-based QDs.

Regulacio *et al.* [6] have synthesized $AgInS_2$-ZnS quantum dots (ZAIS QDs) in aqueous media in the presence of polyacrylic acid (PAA) and mercaptoacetic acid (MAA) as stabilizing and reactivity-controlling agents. These water-dispersible ZAIS QDs are particularly attractive for biological applications because of their long fluorescence

lifetimes (>100 ns), excellent optical and colloidal stability in the physiological pH range, and very low cytotoxicity. They have demonstrated that these ZAIS QDs, when attached to baculoviral vectors, can efficiently enter and label the cells.

Liu *et al.* [46] have synthesized luminescent near-infrared $AgInS_2$ nanocrystals as optical probes for in vivo applications. Pluronic F127 was used to encapsulate the nanocrystals and made them dispersible in aqueous solution. By employing a whole body small animal optical imaging setup, they were able to use the $AgInS_2$ nanocrystals formulation for passive targeted delivery to the tumor site. The ultra-small crystal size, near-infrared emitting luminescence, and high quantum yield make $AgInS_2$ nanocrystals an attractive candidate as a biological contrast agent for cancer sensing and imaging.

In recent years, researchers look for overcome these limitations by physically or chemically anchoring biocompatible polymers on the surfaces of nanodiagnostic and imaging, targeted drug delivery and controlled release and photodynamic therapy. The surface modification of nanoparticles is by using hydrophilic polymers such as polyethylene glycol (PEG). In addition, the surface ornamentation of nanoparticles with hydrophilic polymers may minimize recognition by proteins and cells in the body, allowing the nanomedicine to revolve in the blood for a longer period of time and increasing the possibility that it will reach the target site. Self-assembled nanoparticles comprised of hydrophobically modified polysaccharides have been extensively studied as drug carriers, because of their excellent biocompatibility and comfort of preparation. These polysaccharides are natural, water-soluble polymers that are inherently biocompatible and biodegradable. The core-shell structure of self-assembled hydrogels is considered as a potential system for effective delivery of hydrophobic drugs. The combination of tumor targeting, therapy, and imaging in an all-in-one system could provide a useful multi-modal strategy for the battle against cancer, opening up a new field in which nanotechnology has set the stage for an evolutionary in the therapy and diagnosis of human cancer.

Sivakumar *et al.* [47] have synthesized a multifunctional biocompatible nanovector based on magnetic nanoparticle and carboxymethyl cellulose (CMC). The nanovector

was targeted to a folate-receptor tumor marker that was overexpressed in cancer cell. 5-FU was encapsulated as a model drug for delivering cytotoxicity, and the sustained release of 5-FU was demonstrated. The carboxymethyl cellulose magnetic nanoparticles (CMC MNPs) could induce significant cell death when an alternating magnetic field was applied. These results indicate that the multifunctional CMC MNPs possess a high drug loading efficiency and high biocompatibility with low cell cytotoxicity. They can be considered to be promising candidates for CMC-based targeted drug delivery, cellular imaging, and magnetic hyperthermia (MHT).

Ma *et al*. [48] have synthesized carboxymethyl chitosan-coated cadmium telluride quantum dots (CMC-CdTe QDs) *via* the electrostatic interaction between amino groups in the carboxymethyl chitosan polymeric chains and carboxyl groups of the CdTe QDs. The experimental results indicate that the CMC-CdTe QDs possess favorable cell compatibility, good sensitivity and selectivity for intracellular sensing. They are promising candidates for cellular imaging and sensing in prostate cancer cells.

In addition, the CMC coating on the QDs surface effectively reduced the potential toxicity, enhanced their biocompatibility and stability. Therefore, our research will focus on the synthesis of CMC modified $AgInS_2$ QDs by hydrothermal method for biomedical application.