SYNTHESIS OF Ag2S QUANTUM DOTS AND THEIR BIOMEDICAL APPLICATIONS

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In the last years Quantum dots have been studied from various perspectives from basic science such as physics to nanotechnology and biotechnology with its possible applications in different areas such as sensors, biosensors, lasers, NEMs, semiconductors, green energy, environmental and biomedical applications. This review briefly describes the properties, confinement and synthesis of QDs as well as the performance of QDs in different biomedical applications.

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1. Introduction

Materials science is an interdisciplinary field of science and engineering that studies and manipulates the composition and structure of materials on different dimensional scales in order to control, modify or design materials with specific properties through their synthesis and processing [1]. Biomaterials are natural or synthetic materials designed, manufactured, applied, studied, analysed and developed to interact with biological systems. These materials could be used as a prostheses, biosensors, implants, devices and equipment for detection and diagnosis of diseases [2]. The study of biomaterials involves both the diagnosis and the treatment of different diseases and includes the collaborative work of diverse sciences such as biology, chemistry, physics, engineering and medicine [2]. Nowadays a great variety of materials and devices have been manufactured for biomedical purposes from ceramic materials for dental prostheses to the small confined materials based on semiconductors for biosensors.

Nanometric materials are a group of materials with a very small size within the nanoscale that includes structures ranging from 1 to 100 nm [3]. If the size of the nanoparticles is smaller than the physical size of the Bohr exciton, the structure is confined. The confined materials are those that present the intrinsic quantum confinement effect due to their extremely small size, smaller than 2 to 10 nm in either one, two or three dimensions [4]. Quantum confinement is a mechanical phenomenon that causes a profound variation in the energy of the electronic spectrum of confined materials [5]. During this effect a large increase occurs in the strength of coloumbic interactions increasing to magnitudes close to the Broglie wavelength. When the energy of hollow-electron interactions increases, a type of quasi particles called excitons is formed. The excitons are present in confined materials such as quantum dots and have shown excellent optoelectronic of photoluminescence and laser effect [5].

Confined materials may have different degrees of confinement. This is due to the number of dimensions (x, y, z) that are confined. If only one of the three dimensions of the material has a physical size between 2 and 10 nm, a quantum well is obtained; If two of the dimensions are confined then it is produced a quantum wire and if the three dimensions of the material present this feature then it is produced quantum dots (QDs) [6]. Quantum dots are semiconductor crystalline materials with dimensions below the Bohr radius [7]. Bohr's radius is the distance between the gap

and an electron in the conduction band when an exciton is formed. The structure of the quantum point is formed by a core that can be coated (or not) by a layer that help to increase its properties or protect the nucleus (Figure 1). The properties of QDs includes absorption of white light and re-emittion of light with a specific color in many nanoseconds depending on the band gap of the material. That is, they have phosphorescence [4]. According with their chemical composition, they are composed of semiconductor elements of groups II-VI, III-V and IV-VI [7, 4].

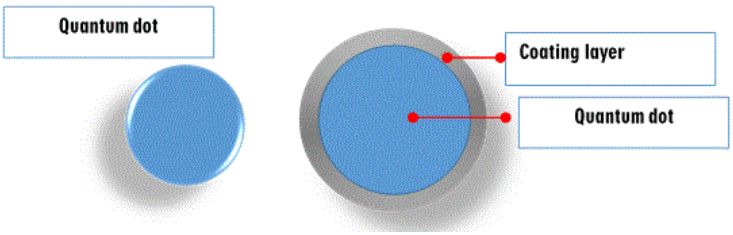

Fig. 1. Quantum dot structure.

2. A brief history of quantum dots

The QDs were discovered by the Russian physicist Alexei Ekimov when he was studying the color formation in doped glasses with activated semiconductors of CdS, CdSe, CuCl and CuBr and he observed the presence of nanocrystals in the studied material [8]. Thereafter, Ekimov and Alexander Efros demonstrated that the semiconductor nanocrystals in the glass had a much shorter wavelength than they expected and that the optical properties of the nanocrystals were dominated by their size [9]. On the other hand, while Luis Brus worked with colloidal nanocrystals of CdS, he could control the size of the crystals to 4.5 nm. This permit that he could derived the relation that exists between the size of the nanocrystal and its energetic gap. Also, he found that the absorption spectrum of the novel nanoparticles had a substantial change in the blue of the spectrum compared to the CdS in a bulk [10].

In those years, these materials were still not known as quantum dots and were referred only as nanocrystalline materials formed by semiconductors. In 1987, it was study the discrete electronic states of zero-dimensional semiconductors [11]. Until that time, two different methods had been developed to synthesize zero-dimensional nanocrystals: the wet path in which colloidal nanocrystals were obtained and the physical method to form epitaxial nanocrystals ("The many aspects of quantum dots," 2010). In 1998, it was proposed a recipe for obtaining QDs of CdS, CdSe and CdTe, through of a colloidal synthesis of semiconductor nanocrystals [12]. On the other hand, the physical epitaxial method for the synthesis of nanocrystals, (known as top-down approach), began with the synthesis of 2D nanostructures or quantum Wells [13] and subsequently with quantum wires or 1D materials until eventually the production of 0D materials ("The many aspects of quantum dots," 2010). As it was previously mentioned, Mark Reed was the first person to called them quantum dots [11]. Nevertheless, it was until 1998 that QDs were used for first time in luminescence tests for targeting biomedical applications [14].

Quantum dots have been studied from various perspectives from basic science such as physics to nanotechnology and biotechnology with its possible applications in different areas such as sensors, biosensors, lasers, NEMs, semiconductors, green energy, environmental and biomedical applications. During a search, in Elsevier database it was found for the keywords "quantum dots for biomedical applications" documents since 2001 and for “applications related to cancer detection” it was until 2008. In the field of the biomedical application, one of the first researches about quantum dots was developed using CdSe (QDs-CdSe) coated with ZnS and bioconjugates with immunoglobulins G (IgG). This research was developed to carry out immunoassays for the specific capture of antigens based in the method of fluorescence image analysis [15]. Subsequently, other research was published about the luminescent properties of quantum dots and how they can be focused on bioimaging applications and for detection of active biomolecules [7]. QDs-CdSe with sizes from 2 to 7 nm functionalized with tert-butyl N- (2-

mercaptoethyl) carbamate were synthesized for optical determination of cyanide ions, a carcinogenic component present in seeds of peach, apricot, plums and others [16]. It was reported the development of fluorescence contrast agents in the near infrared spectrum of QDs-CdMnTe / Hg coated with bovine serum albumin (BSA) for use in tissue imaging [17]. QDs of CdS were developed in luminescent tests for the determination of spironolactone, a medicine used as a diuretic [18] and QDs of CdTe coated with albumin were used to identify C. elegans [19]. Other quantum dots, were synthesized with heavy metal for the identification and determination of other microorganisms such as bacteria, parasites, specific biomolecules or even the presence of heavy metals. For example, different techniques have been developed with biofunctionalized QDs to detect the presence of parasites such as *Giardia lamblia* and *Cryptosporidium parvum* [20], *Salmonella sp.*, Shigellas p and *Vibrio cholera* [21].

During the period of 2005-2010, the application of QDs in the biomedical field increased significantly and after 2010 decreased again due to the toxicity and non-bio-degradability and non-biocompatibility presented by the first QDs based on heavy metals without functionalized [22] (Figure 2). The first challenge for the application of QDs in medical areas was the non-use of heavy metals in the manufacture of QDs. The reduction of the toxicity of QDs has been one of the biggest obstacles in the study for its application to biomedicine since the first materials used in the synthesis were CdSe, CdS, CdTe, InP, InAs and PbSe [23, 24]. Nowadays there are other materials with low toxicity or zero toxicity such as graphene, coal, nano diamonds, silicon, gold, silver and silver and gold sulfide for the production of QDs [22]. On the other hand, the functionalization of QDs helps to decrease or eliminate toxicity and increase the biocompatibility of the material with a biological system.

*Fig. 2. Elsevier publications of QDs for biomedical applications **A)** In vivo analyses; **B)** image applications, **C)** diseases diagnostic.*

More recently, heavy metal-free QDs have been used as tests to monitor synaptic receptors in neural circuits [25] and they have been applied in luminescence tests for in situ DNA hybridization [26]. Graphene QDs have been used in *in vivo* assays of fluorescent tests [27], in hepatocytes from living mice successfully and with improved results due to its multicolor fluorescence [22]. Another application is the development of biological tests is glucose detection using surface-functionalized CdTe QDs with concavalin A (ConA) and β-cyclodextrin-modified gold nanoparticles; in the absence of glucose, the ConA binds to the β-cyclodextrin and increases the proximity between the QDs and the gold nanoparticles causing fluorescence [28].

The application of bioconjugated quantum dots for the detection of different types of cancer is reported since 2007. QDs with biomolecules were synthesized for the identification of cytokines TNF-α, IL-8, IL-6, MIP-1β, IL- 13 and IL-1β at picomolar concentrations in for the timely cancer detection [29]. In 2008 some studies were reported on QDs specially for the detection of several types of cancer such as liver [30], breast [31], pancreas [32] and hepatitis C [33,34].

In recent years quantum dots have been studied with great interest, the performance of QDs in different biomedical applications has been published for example in the development of cellular markers for detection of diseases such as hepatitis C [33]. Cytotoxic studies have shown that QDs-Ag_2S have no toxicity in terms of cell proliferation, apoptosis, necrosis, and genetic

damage as they have been good candidates for the development of QDs in biological applications. In a toxicological study, it was reported that there are no cytotoxic or genotoxic effects observed in cells exposed to Ag_2S nanoparticles [35].

It has been shown that the QDs-Ag_2S has great potential for *in vivo* imaging, disease detection and cancer diagnosis [36, 37]. Recently, studies on QDs-Ag_2S in near-infrared spectrometry tests have been performed as a molecular imaging test of living cells. The results of this research have shown that Ag_2S quantum dots in infrared spectrometry are attractive for medical studies with high efficiency and biocompatibility [37].

In the last 5 years many articles have been published related with manufacturing of sulfur quantum dots for the detection of biomolecules present in cancer cells. An interesting study was the synthesis of hybrid QDs of Ag_2S-Fe_2O_4 for the development of therapeutic and detection of luminescent properties with Ag_2S and magnetic properties of Fe_2O_4. Also, these particles has good cytohemocompatibility, showing that these nanoparticles can act as biosensors or optical and magnetic devices [38]. It should be noted that the previous study is not the only one that has developed QDs-Ag_2S with magnetic properties. Recently QDs-Ag_2S doped with gadolinium ions (Ga^{+3}) was produced. These particles show null toxicity in *in vivo* and *in vitro* studies, This study also considered the study of malignant tumours [39].

3. Quantum dots properties

The quantum dots were discovered in 1980 by Louis Brus, but it was until 1990 when they gained more importance in the field of research; Quantum Dots are semiconductor nanocrystalline structures confined in 3 dimensions and having a size range of 2 to 10 nm [4]. These nanocrystals are characterized by good optoelectric properties and high catalytic activity [40, 35]. Its unique properties are closely linked to the quantum confinement effect intrinsic to the physical magnitude of its dimensions [40]. The basic concept of quantum confinement has been known since 1970 and it is presented when the wave function of an exciton is comparable to the physical size of any of the three dimensions of a particle [41]. The study of confinement on a scale comparable to atomic size concerns to the field of quantum mechanics [42]. The QDs can be considered very similar to confined atoms because they also involve the confinement of an electron in a mixture of potential with different properties in the short and long range. In this context, the Hamiltonian is used to represent the properties of the quantum dots that are almost identical to the properties of these confined atoms [42]. The optical properties of the quantum dots includes a great absorption of photons, photoluminescence, tunnel effect, a high quantum field potential and long periods of photoluminescence drop [40]. Due to its photoluminescent properties and to its physicochemical characteristics, QDs can be used in a great variety of applications.

The QDs are zero-dimensional confined nanoparticles (0D) since they show the quantum confinement phenomenon [7, 43]. In order to explain the physical definition of the quantum point, let us say that a bulk material, also found in the literature as bulk, is classified as a three-dimensional (3D) or unconfined material since all its dimensions measure more than 10 nm; in this definition a grain of salt is a bundled, 3D or unconfined material because the physical size of its dimensions, although very small, is much larger than 10 nm in any of its three dimensions [44]. On the other hand, a confined material is one in which at least one of its three dimensions (x, y, or z) is physically less than 10 nm (Figure 3). The QDs, called universally quantum dots, are then zero dimensional materials (0D). Also, there are 2D and 1D materials, quantum wires and quantum wells, respectively. All the confined materials present a quantum confinement effect, this is a mechanical phenomenon intrinsic to its confinement and causes a profound variation in the electronic energy by modifying its optoelectronic properties as compared to the same material in bulk [5]. Due to this phenomenon, these materials present unique properties such as a strong increase in the strengthening of the hollow-electron interactions, called excitons, close to the Broglie wavelength, fluorescence, tunnel effect and laser-like optical properties. Excitons are a type of quasi-particles with excellent optoelectronic properties, due to this interaction, fluorescence is typical in confined materials [5,6].

QDs are semiconductor crystalline materials of 2 to 10 nm; They are typically synthesized with combinations of elements from II-VI, III-V and IV-VI groups of the periodic table [7]. From a physical and conceptual perspective, QDs are defined as particles with physical dimensions smaller than the radius of Bohr's exciton (aB); This is understood as the distance between a gap and an electron in the conduction band when an exciton is formed. The QD structure consists of a core formed by the semiconductor material itself and may or may not have a coating either by a polymer, organic, metallic or other functional groups [35]. The coating of QDs helps to improve the optoelectronic properties of the material, reduce its toxicity, improve its solubility, optimize the biocompatibility or other properties.

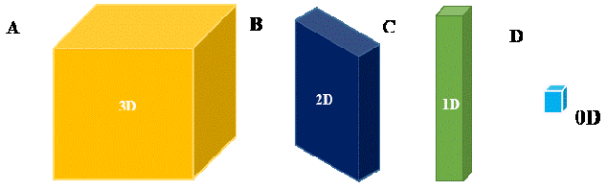

Fig. 3. A) 3D-bulk material, no confined; B) 2D-quantum well. confined; C) 1D-quantum wire-confined; D) 0D-quantum dot-confined.

QDs absorb white light and emit energy at a specific wavelength with a specific color within a few nanoseconds, depending on the band gap of the material [4]. The fluorescence characteristic of QDs has greater intensity, photostability and chemical stability compared to organic fluorophores [45]. Depending on the composition of the QD the properties can also vary, silver sulfide QDs (QDs-Ag_2S) are distinguished for their promissory properties. The α-monoclinic QDs-Ag_2S are n-type semiconductor materials, not water soluble, they have good photostability and high fluorescence intensity; They have an energetic gap of 1.1 eV in their bulk for, high absorption coefficient and non-toxicity [28, 45, 46]. They exhibit good catalytic properties, optoelectronics and high electrical conduction that together with their null toxicity make them good candidates in biomedical applications. In addition, these materials can be functionalized to increase their biocompatibility, be bioconjugated and used in the construction of biosensors [28, 47]. Due to these characteristics, the QDs have important applications such as solar energy, biomedical materials, superconductor technology, fabrications of optoelectronic devices such as fluorescent sensors, biosensors, photodetectors, optical amplifiers among other applications [47].

4. Quantum Confinement

Since 1970 it is known basic knowledge about quantum confinement, this phenomenon is showed when the wave function of exciton, in a low dimension(s) nanostructures in their lowest excited state, is comparable to the physical size of the particle [41].

This effect allows to understand remarkable changes that occur in the properties of the nano-materials compared the equivalent structures in bulk. It is well known that the properties of a material change in the nanoscale due to they are function of the size, shape structure, composition and phase. As discussed above, it is known as a quantum confinement effect, the decay of the physical size of a material of 2 to 10 nm in any of its three dimensions; that is a smaller dimension than the Bohr exciton [48]. When the material reduces its size to dimensions below 100 nm the properties of this one begin to change even when in essence it is the same chemical composition or of the same atoms. A bulk material is known as three-dimensional (3D) because all its dimensions are greater than 10 nm, quantum wells are 2D materials, and quantum wires and quantum dots are materials 1D and 0D respectively. Finite quantum systems are those in which the "quantum" term implies the use of quantum theory for its definition and understanding its behavior, the finite adjective makes a distinction between the macroscopic or bulk structures of quantum systems: atoms, quantum dots, metallic grains, nuclei of atoms or clusters [44].

One of the most important properties of QDs is their massive change in optical properties as a function of size; When the physical size of the material is reduced to its low dimensions, the electronic excitation changes to a high energy level with strong oscillations within energy transitions [45]. Quantized QDs t are smaller than Bohr's exciton radius energy levels. When the material absorbs a phonon with energy close to the band gap energy of the semiconductor constituent of the QD occurs the formation of an exciton, This allows radioactive recombination and allows the emission of phonons in a narrow band of symmetric energy [49]. The changes in properties in bulk, nano-materials, and confined materials can be explained in greater depth through quantum chemistry and the physics of ultra-small domains [42, 48].

In quantum dots the electrons are confined is a very small physical space. In other words, their motion is limited by the small space they occupy, and when the radius of the semiconductor is equal or smaller than the radius of Bohr excitation occurs a quantization of energy levels. This explains the unique spectral characteristics and the properties of the QDs between the properties of the same atoms and the materials in bulk [42, 50]. In a bulk material the electrons are free to move throughout the physical space. That is, the electrons are delocalized but in a confined material (0D, 1D and 2D). The electrons are limited to their small physical space, occupied by atoms in the atomic network. In this situation, it is not possible to delocalize the electrons because they are restricted [42]. The number of electrons for a QD is well known and estimated at n <30 and the free path in the fermi energy is approximately 100 nm with a QD diameter of 10 to 100 nm [44].

5. Synthesis of quantum dots

The synthesis of the material is fundamental for the development of different technological applications; there are several routes for the production of quantum dots since the synthesis of quantum dots can be really difficult. There are two approaches to this goal: by a route called ″from bottom to top″ universally known as *bottom-up* and a route ″from to top″ or *top-down*, Figure 4 [51].

The bottom-up route is to begin the synthesis with the precursor materials to the desired size of the nanostructure, an analogy to better understand this concept would be to assume that the precursor materials or atoms are the bricks needed to build a wall or a larger structure: dots, wires or quantum wells. This path uses chemical methods: colloidal synthesis, sol-gel, biomimetics, vapor phase deposition among others. On the other hand, the top-down synthesis consists in decreasing the size of a material in bulk (3D) until obtaining the desired size, following the previous analogy, this time the wall would represent the structure in bulk and to obtain the desired size we would have to remove the bricks from the wall or atoms of 3D material to form a 2D, 1D or 0D. This approach is carried out by physical methods such as nanolithography [51]; speaking specifically of the manufacture of quantum dots, the approaches used must reach dimensions smaller than 10 nm which can be very complicated.

There are two routes to synthesize quantum dots the botom up and top-down route as previously mentioned. Techniques such as molecular beam epitaxy (MBE), ion implantation, electronic bombardment lithography, and x-ray lithography are examples of top-down approaches. On the other hand, bottom up approaches involve chemical processes such as colloidal quantum dots [4]. The manufacture of top-down type is about the manufacture of quantum dots by the attack of a material in bulk to obtain structures of smaller size [52], this means going from larger structures to the nanostructure. On the other hand, bottom up approaches refer to placing small blocks or structures as atoms to form larger structures as a quantum point, is to build from the raw material atom by atom, molecule per molecule or ion by Ion to the desired size.

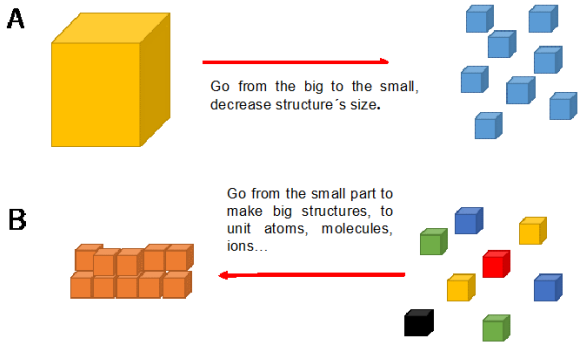

Fig. 4. A) Quantum dots synthesis by a top-down approach. B) Synthesis of quantum dots by a bottom up approach.

5.1 Top-down approach

The top-down strategies can be defined how sculpt a larger mother structure and forming it into the desired structure [53].

These types of methods are conditioned and fabricated by external parameters to create the nanostructure or the nanodevice with the desired shape and size characteristics from the bulk material. The dimensions of the bulk material are reduced to the required size: large to small [51]. The top-down strategies try to transform the technological methods to fabricate microstructures and microdevices to obtain structures in nanometric scales [54]. These methods are based on the philosophy of physical micro lithography. In conventional lithography the required materials are usually covered by a mask and the exposed material is etched by different chemical or physical pathways. The chemical etching uses acidic substances in the process and the physical engraving uses mechanical ways with different types of energy such as X-rays, ultraviolet light, electronic bombardment, among others [55]. Some characteristics of these techniques are summarized below.

5.1.1. Photolithography

The lithograph emerges in 1798 with the work of Alois Senefelder [56] his method has changed a lot over time and although it does not resemble much the initial the principle remains the same because lithography involves a large number of different techniques with the same essence: pass information from one substrate to another. As antecedent, Alois Snefelder discovered this technique when he wrote with ink made from soap, wax, and black dye on polished limestone whose edge covered with wax and on the whole surface applied acid, Alois Snefelder observed that after a few minutes with acid the patterns on which he had not written had been engraved on the stone, removing or washing the acid from the stone, discovered that the stone had been eroded with the pattern in a thickness of 1/10 as if it had been carved. With this accidental discovery Snefelder managed to pass the engraving to the paper by placing dyes on the patterns engraved in the stone and placing paper on top of it and applying a little pressure, noting that the engraving was copied on the paper Snefelder called this technique printing in stone [57]. In the scientific field this technique was first introduced in 1960 and later evolved to photolithography [55]. Several types of photolithography have been developed during the last 40 years: photolithography, soft lithography or lithography by electron bombardment among others [58]. Lithography consists of drawing a pattern on the surface of a material; in optical lithography a collimated beam of different types of energy is used: x-rays, ultraviolet, electron beam, etc., projected onto a photosensitive substrate.

The basic components for carrying out this process include a light source (for example a source of UV radiation), lenses of projection and of objective, a photo-mascara, the pattern to be recorded, a conventional optical microscope or an electron microscope depending on the size of the engraving, and a lithographic configuration system with a lighting system. In general, in photolithography, mostly used for the production of integrated circuits, the first thing that is done is to obtain the mask with the pattern or drawing; The mask is used to copy the pattern onto the substrate, then the substrate is cleaned (the latter can be a semiconductor material or another

depending on the case). The process involves two parts: the mask preparation and the transfer of the mask pattern to the substrate containing the pattern or image to be recorded.

In a typical case (Fig. 5), a wafer-shaped substrate of high chemical purity is thoroughly cleaned and coated with a resin or photosensitive substance and heated to evaporate the excess solvent; once the resin is placed on the substrate it is placed above the mask containing the pattern, the mask is placed on the substrate with resin by mechanical pressure or is retained by other means and subsequently exposed to UV or X-ray, laser or other radiation. In the exposure process the substrate-resin-mask system is directly attacked by radiation and those parts of the substrate-resin that are not covered or protected by the mask are freely exposed, etching the pattern of the mask onto the system by transmission or reflection of the radiation on the substrate. Subsequently, the substrate with the resin and the engraved pattern is heated again causing a photoacid catalysis of the photoactive compounds contained in the photosensitive resin to accelerate modification of the substrate. The resin is then dissolved in a solvent to produce a 3D replicate of the mask pattern that had been etched onto the resin on the substrate; Finely, the image on the resin is etched onto the substrate [57].

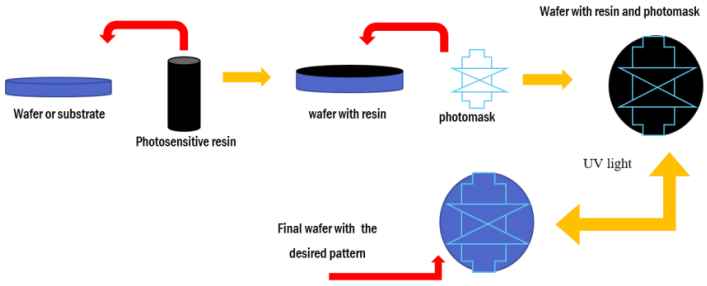

Fig. 5. Photolithography process.

5.1.2. Electron beam lithography

In recent times conventional lithography has evolved because there are applications that require special features or demand higher quality materials, processes and tools used, require more sophisticated chemical processes or lithography for semiconductors (as in the case of Quantum dots), A restriction of photolithography is that it is limited by the diffraction imposed by the wavelength of visible light and that it is surpassed by photons or electrons of shorter wavelength, since it has been found that any particle with smaller wavelength than a micrometric size has the potential to generate radiation [57] which will affect the resolution of the pattern. This premise has been used to develop newer lithography techniques, it has been reported that particles such as near ultraviolet photons, deep ultraviolet photons, electrons, soft X-rays or ions can be used in lithography techniques [57]. These types of particles used in lithography techniques have given rise to more novel lithographic processes such as far ultraviolet photon lithography, soft x-ray lithography, ion beam lithography and electron beam lithography.

Electron beam lithography (EBL) belongs to the group of techniques of lithography in which the source that realizes the pattern is not of photons but that is mediated by particles with load like electrons or ions. In this lithography, the beam that makes the pattern or image in the semiconductor substrate covered with the resin is composed of electrons. The LHE system consists of an electron source that passes through a system of obturator plates, then through the primary capacitor lenses, followed by the secondary ones and finally the beam is impacted on the mask, which contains the pattern or drawing, which is placed on the Substrate covered with resin. The resins used are divided into 2 groups according to their working principle or according to chemical resistance. The first [59].

Since the great boom of lithography in the eighties the EBL has been the most used for the manufacture of mesoscopic structures or different systems with unique advantages such as: high resolution, manufacture of sizes smaller than photolithography, high repeatability of the process, flexibility to perform pattern replications, reliable alignment and position versus the pattern [59],

The electron beam lithography technique can generate smaller patterns (less than 10 nm) than those made by photolithography [57] and can form reliefs because the electronic source generates a great depth in the substrate and is therefore used in nanotechnology[60]. Exhibiting the electrons a wavelength of 0.012-0.024 nm corresponding to 50 to 100 KeV of energy, energy three times shorter than that used by UV radiation in photolithography whereby its resolution is not affected by diffraction.

EBL has been widely used for the design of nanostructures in 2D, also used in bioscience and biomedical for example in the manufacture of nanofluid channels [59]. This technique has also been used to perform water-soluble quantum dots microarrays for biomedical applications. Other authors have also used this method to manufacture GaN quantum dots in synergy with other techniques, in this case with chemical vapor deposition to name a few examples [61].

5.1.3. Soft lithography

Soft lithography (SL) was developed by George Whitesides in 1990 [62]. This method allows to generate structures with sizes smaller than 100 nm in a great variety of material. It is called SL to the application, altogether, of several types of techniques called "non lithographic"; Such as microcontact printing (μCP), replica molding (REM), microtransfer molding (μTM), solvent assisted micromolding (CAMIM). In each of these techniques an elastomeric component or mold (stamping) is used as the key element because it is necessary to achieve the transfer of the pattern to the substrate. In SL molecules and / or flexible and organic materials are used instead of the inorganic hard materials commonly used in lithography techniques. Through SL, micro- or nano-patterns can be generated by the formation of self-assembled monolayers (SAM) on the substrate by contact printing or else nanomaterials and micro-materials can also be constructed by a process called stamping or forming of reliefs by replica molding.

In a general procedure (Fig. 6), first of all a drawing or pattern is obtained, it is printed on a material to form the mask and later photolithography or electron beam lithography is used to create a master from the mask that, upon replication, will form a stamper and it will transfer the pattern onto a substrate. Following the mentioned techniques of soft lithography: molding, printing or other mechanical process the final structure or pattern is obtained the desired substrate.

The SL is a simple procedure and its most important features are its ability to replicate the pattern rather than the pattern engraving the substrate, in addition being a fast process, using a variety of materials, has the ability to deform the elastomer substrate or either molding it to obtain the desired structure or pattern, can be performed under laboratory ambient conditions [62]. The stamper is placed on the final substrate and dimethylsilosan is added to the substrate-stamped and then "cured" with another polymer, polymethylsiloxane (PDMS). At the end of the curing the final pattern is obtained on the substrate and by means of some soft lithography technique the final structure is obtained.

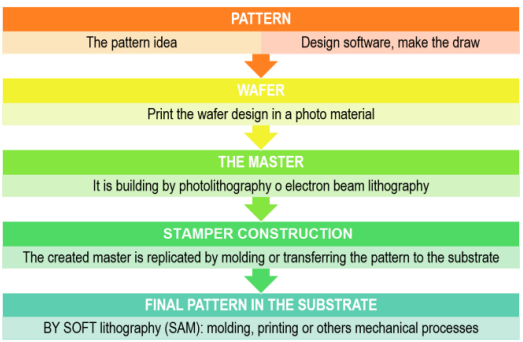

Fig. 6. Soft lithography process.

5.2. Methods of synthesis by the bottom up strategy

Bottom up approach can be considered as those techniques where structures are assembled from their subcomponents, step by step, in an additive method [53]. This process is mainly based

on chemical principles and is completely in contrast to the *top-down* approach, in this method atomic or molecular units, also named "blocks", are joined to form a complete nanometer structure [54], in simply words; this method goes from small to big structures (of course in the nanometer range). Chemical techniques, also named self-assemble techniques, provide changes of chemical binding strength and preferred orientation of bonds with a fine tuning according to the numbers of bonds atoms or atomic groups in good spatial orientation [54]. In this kind of synthesis the advantage of the physicochemical interaction and self-assembly of molecules is used to obtain the desire structures that's why the control of the precursors, which are the nanoscale building blocks, is very important factor [63]. Some examples of the different synthesis with a bottom up approach are showing a continued.

5.2.1. Colloidal synthesis

This kind of synthesis is one in which the nonmetric crystal or quantum dots are synthesis in an organic or inorganic solvent during a chemical reaction. During the process the shape and size of the QD can be controlled trough modify of the reaction parameters: temperature, reaction time, rate addition of the precursors, stoichiometric relation between the precursors, pH, concentration of the chemical reactants and the clean of the reaction medium [64].

This method is based on the fact that the system has three components: surfactants, solvents (dispersing phase) and dispersed phase also called precursor. During the reaction, the precursor is converted into monomers during the evolution of the reaction or colloidal state and when the system reaches a state of supersaturation the crystals are formed and the nucleation process begins. During this process, crystals of a particle size that is not desired may be formed due to the dissolution of the crystals of small particles and the re-deposition of particles on the surface of larger particles called Ostwald Ripening (OR). During this process the large particles gradually grow while the small particles are dispersed in the solvent. In order to obtain the desired particle size an equilibrium must be achieved by separating the spontaneous nucleation processes from the growth of nanocrystals. This route can be controlled through temperature control [65].

Usually quantum dots are synthesized by this method of wet chemistry using a temperature controlled reaction bath. Colloidal formation is induced by the rapid combination of two or more chemical reactive agents at high temperatures (commonly), if the reaction is carried out at high temperatures it is called hot injection and was first implemented by Murray who established the first methodology by wet chemistry for the synthesis of quantum dots [12]. This method consists of three stages: in an initial stage the temperature is used so that the precursor reagents are transformed into monomers and they are dispersed. At a critical temperature a second precursor is added at the set speed rate which may be fast or very slow (depending on the system) to commence nucleation. Finally, the nuclei grow into nanocrystals and the reaction cools abruptly to stop the reaction.

5.2.2. Biomimetic synthesis

The biomimetic method is defined as a method of synthesis that using biological principles for the construction of nanomaterials [66]. These procedures emerge from the desire to imitate natural biological processes of catalysis and anabolism, However although it has tried to imitate nature has not been done in great detail and perfection, through these imitation defects have emerged new tools of study as the biomimetic synthesis [67]. In this type of synthesis are biomolecules (proteins, peptides, amino acids, complexes of active biomolecules, metabolites etc.,) which lead to the growth of QDs by the formation of chelating complexes under advantageous physical conditions. A biomimetic synthesis can be carried out in living organisms such as bacteria, yeasts, fungi, plant cells or in active substances such as bovine serum albumin or the enzyme ribonuclease [14]. A biomimetic pathway allows to process nanostructures at low temperatures, in aqueous solution [68] And neutral pH which generates great advantages of production [69]. Although the synthesis of QDs and other nanostructures has been reported successfully by other physical and chemical methods and great advances have been generated in recent times, these pathways present difficulties and limitations to synthesize homologous forms and sizes as well as large scale production. In a biological system on the other hand, there are no limitations to homologate the form and size of the particle because the biosystem produces

inorganic compounds always in nanometric size and self-produce because they provide a benefit to the biosystem [69].

In a biomimetic synthesis, tools of molecular biology or the design of proteins and peptides are used that can control the growth and production of nanostructures of the desired size, even in sizes from 2 to 10 nm size desired for the QDs. Peptides are then used to carry out the control of the formation, assembly, and organization of confined nanostructures [69]. Different authors have successfully reported the synthesis of quantum dots using living organisms as bacteria: synthesis of CdS-QDs with acidophils of the genus Acidithiobacillus [70], Fungi: PbSe-QDs with Aspergillus terreus [71] or living cells of plants: synthesis of QDs-Ag2S with endoesperas of wheat [72] But also using specific proteins, one of which is bovine serum albumin and Ribonuclease-A: synthesis of RNase-A-QDs-Ag2S [73]. Biomimetic routes are usually simple procedures (in some cases) certain mechanisms can be summarized in a stage of incubation, reaction and growth after which quantum dots are obtained.

Another important aspect of this route is that when carried out at ambient temperature and using water as a solvet and microorganisms or active biomolecules as a solvent can be considered a green route [74] as long as the quantum dots or nanostructures manufactured are non-toxic, generate toxic products or their chemical composition is heavy metals since the first QDs were synthesized based on heavy metals [75, 76]. In addition the synthesis of QDs by these routes can allow to obtain quantum dots of sizes between 2 and 10 nm, functionalized in a single step, in this case protein groups are attached to the QDs by means of chelating reactions, amine and carboxyl groups of proteins besides functionalizing the surface of the QDs give them solubility in aqueous medium, diminish or eliminate its toxicity without affecting its fluorescence and quantum yield [77].

5.2.3. Atomic Layer Deposition

Atomic layer deposition (ALD) was popularized and introduced as an epitaxy of atomic layer by Suntola and Antson in 1977. It is a technique with many advantages over other methods of manufacturing quantum dots since it allows to deposit layers of a great variety of materials in vapor phase. ALD is a technique analogous to chemical vapor deposition (CVD) but unlike this ALD allows to alternate the exposure of the chemical precursors to react in such a way that the desired material is obtained and regularly it is carried out at temperatures smaller than 350 ° C [78].

The general process consists in bringing to the state of vapor the chemical precursors (A + B) which will enter the zone of reaction or reaction in alternating pulses and will react with the substrate, Figure 7. The individual reactions between each precursor and the substrate are called intermediate reactions which are only a part of the complete synthesis of the material. During each pulsation the precursor is injected into a vacuum chamber for a certain period of time, this to allow the precursor to react completely with the surface of the substrate, this is a self-limiting process which does not allow more than a single layer to form on the substrate. The chamber is then cleaned with argon or an inert gas to remove the precursor residues that did not react with the substrate, then the next precursor is injected, repeating the cycle, Figure 8.

A recent work reported the synthesis of Cu2O quantum dots, this is a material with a Bohr radius of 0.7 nm in its bulk form which makes it very difficult to obtain confined structures of this material with dimensions between 2 and 3 nm without impurities [79].

Although colloidal methods have outstanding advantages such as good particle size at low cost of equipment and small variation in particle sizes, the synthesis of quantum dots by this method also have disadvantages for example, the QDs produced by this method tend to agglomerate which increases the particle size and the loss of the quantum effect. To prevent this the quantum dots require the presence of ligands on the surface that potentiate the colloidal stability of the system although they can also affect the size of the quantum dots and therefore the confinement, for this reason colloidal methods can become very complicated [80].

On the other hand, in situ vapor deposition techniques allow to obtain good confinement sizes through the control of the barrier height energy, the effective mass of the carriers, temperature and reaction time, high purity particles are obtained and it is a simple way in practical terms [79, 80].

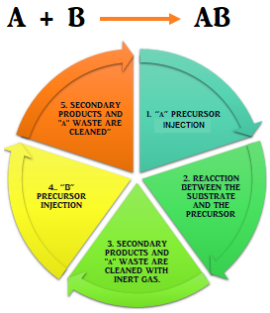

Fig. 7. Atomic layer deposition for a reaction: A + B →AB.

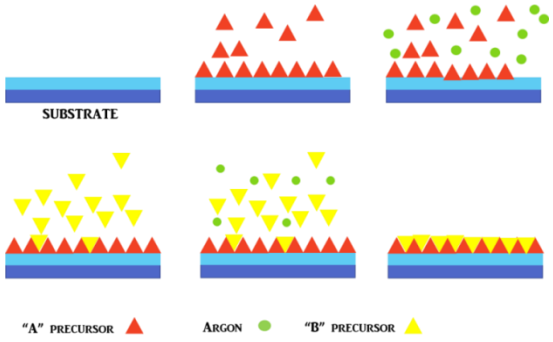

Fig. 8. Example of the atomic layer deposition process.

5.2.4. Molecular self-assembly

The molecular self-assembly method is defined as a self-directed process in which the molecular precursors or components of the system are organized autonomously into well-defined aggregates. This technique is characterized by providing benefits as stable structures being a versatile, easy, economical process and the process occurs through the state of minimal thermodynamic energy of the system [81].

In this method a self assembly process is carried out, in which the precursor molecules accommodate by themselves in well-defined three-dimensional forms directed naturally through chemical or physical processes or by biomolecules that orchestrate the selectivity, affinity and specificity of precursors in the desired nanostructures [82].

The synthesis of nanostructures by this route can be directed or accompanied by a number of different individual mechanisms or by the combination of these principles: surface forces, electrostatic forces, chemical interactions, hydrophilic and hydrophobic interactions, or by biochemical processes.

Auto assembly of molecules by electrostatic forces: this process is governed by the adsorption and desorption in equilibrium in cationic and anionic solutions that form films by a mechanism layer by layer. This route allows to obtain films and particles by the simple execution of a cycle of immersion, rinsing and drying of the precursor solutions in a substrate. The thickness of the films or particles can be controlled by the pH of the solutions used. Initially a glass substrate is deposited in an acidic solution by doping or by placing cationically charged molecules on the substrate. Then, the glass is immersed in a basic solution, by electrostatic forces a layer of negatively charged molecules is deposited, Fig. 9.

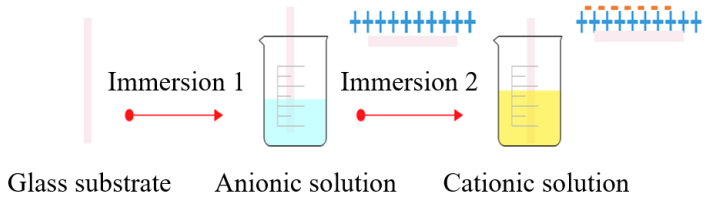

Fig. 9. Molecular autoassembly for electrostaic forces mechanism.

Surface forces: This is a simple procedure in which colloidal solutions are used that are placed in a substrate, then a controlled evaporation of the colloid dispersing phase is carried out so that the particles are deposited in the substrate in an orderly manner. The colloidal particles are assembled on the substrate either by the connective flow of the boundaries between the arrangements or patterns formed by the evaporation of the solvent or by the forces of attraction acting between the particles due to the surface tension of the surface, Figure 10 [82].

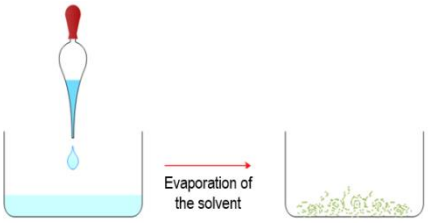

Fig. 10. Assembly due to surface forces.

Self-assembly by chemical mechanisms: This is a procedure in which nanostructures films are formed by the formation of monolayers, Figure 11. As in the case of electrostatic forces, a substrate is immersed in an indicated solution of each of the precursors until the desired composition is obtained. Unlike the other methods herein, the layers are linked by means of covalent bonds; this allows to obtain ordered arrays, controlled sequences, binding specificity, specific functional groups and permanent nanostructures.

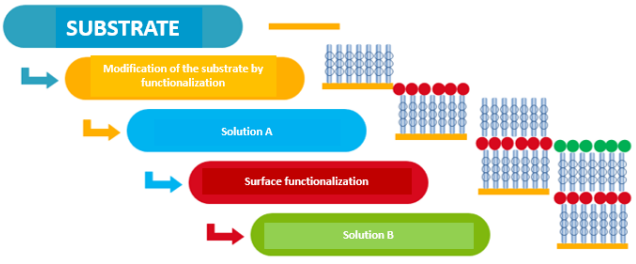

Fig. 11. Self-assembly by chemical mechanisms.

Biomolecules: This mechanism is related to the biomimetic route of synthesis described above in which the natural idea of the biochemical self-assembly is the main idea. In this mechanism biological molecules like proteins are used to act as promoters in the auto assembly, presenting non-covalent interactions such as hydrogen bonds and Van der Waals forces are presented. The biomolecules have high selectivity and affinity for certain functional groups, therefore, they direct the assembly with high specificity and autonomous way.

6. Biomedical applications of silver sulfide quantum dots

In the development of nanotechnological tools the size of the QDs is very important since their properties depend on the size of the particle, to a larger size the quantum yield of the QD is affected because the confinement is who defines the properties that they will present [83, 84]. The properties of the QDs have allowed them to be used in different biomedical applications such as bioimaging and bioimaging in vivo, controlled drug release, fluorescence optical biosensors, diagnosis of pathogens, toxins, heavy metals and / or LED technology to mention some [23, 40]. In the next section we will discuss several important applications of quantum dots and give some short examples.

6.1. Biosensors

A biosensor is a device that is used in biomedical applications and that is in contact with a biological system to detect specific information of a receptor: antigens, cellular markers, proteins, molecules, hormones, heavy metals, enzymatic activity, and ions. This device generally consists of the formation of a bioconjugate of the functionalized quantum dot bound to an active biomolecule and converts a biochemical signal into an electrical, mechanical or optical signal [48]. Once the bioconjugate is formed, it captures, binds or interacts with a specific stimulus that activates or binds to the biomolecule, this interaction is detected and a signal is emitted, the amplification of the signal allows translating a result [85]. Depending on the magnitude of the response a quantitative, semi-quantitative or qualitative analysis can be performed of the target molecule. The development of biosensors with QDs for the detection of specific biomolecules for biomedical applications is very interesting because of their small size, excellent properties and the high sensitivity they present [85].

A biosensor is composed of two parts: a recognition element and a transduction element where the elements used are constituted by biologically active molecules. The combination of these two components is used to devise a test or medical device to identify the presence and amount (if possible) of a specific biological analyte [83].

In a biosensor, molecules such as proteins, nucleic acids or anti-bodies are used to identify the analyte through specific reactions such as the antigen-antibody interaction. There are several criteria that should be taken into account for the design and construction of an ideal biosensor: sensitivity, selectivity, specificity, detection limit, detection rate, reproducibility, precision and accuracy, reusability and dynamic range. The order of importance of these factors depends on the application of the biosensor however, each of these aspects is important and bring the device on the way to the ideal biosensor. As is known the "ideal" is difficult to reach but the QDs are very close to this goal in the construction of biosensors when they are bioconjugados [83].

On the other hand, the signal transduction carried out by the specific reaction must be converted into a signal that can be interpreted by the analyzer or test executor. The most common transducer element is fluorescence [83], so the QDs are so attractive in this field of application. Diagnoses based on fluorescence are characterized by high sensitivity and very versatile. The common organic fluorophores have a narrow and weak absorption spectrum, red photoluminescence, high susceptibility to loss of fluorescence, sensitivity to pH, susceptibility to chemical degradation, short time fluorescence of only a few nanoseconds and also the organic fluorophores do not result optimal in a wide variety of applications. On the other hand, QDs have strong and wide bands of absorption, symmetrical and narrow fluorescence spectrum, resistance to fluorescence loss (photobleaching), long fluorescence time, fluorescence wavelength characteristic of QDs is determined from its size and varies between red and blue spectrum with decreasing size [83].

A recent study developed a biosensor of QDs-Ag_2S functionalized with 3-mercaptopropionic acid doped with manganese (Ag_2S-Mn) to improve its optoelectronic properties, the Ag_2S-Mn QDs were bioconjugated with bovine serum albumin (BSA) to carry out the recognition of laminin. The recognition of this protein was performed through an immunochemical reaction where the QDS Ag_2S-Mn-BSA complex is able to bind to antibodies and carry out a recognition with fluorescence production, null toxicity and high sensitivity [86].

Authors reported the development of a test for the detection of CA125 antigen (cervical cancer antigen) forming a bioconjugate with Ag_2S, 5-fluoroacyl quantum dots and aptamer of the CA 125 antigen for its detection [87].

Another study developed a photoelectrochemical biosensor with Ag_2S QDs for the detection of glucose and MCF-7 cancer cells showing good sensitivity and specificity [36].

6.2. Bioimaging

The imaging includes the realization of all kinds of diagnostic and therapeutic exams in which equipment is used to obtain images of an organism. Among the techniques used daily to obtain images are ultrasound, computerized axial tomography, nuclear magnetic resonance, radiology and microscopy for the diagnosis of diseases. Imaging techniques have been used for the observation and investigation of the shape, size and movement of cells, microorganisms or specimens related to various diseases [88]. Organic fluorophores such as fluorescent proteins allow researchers to investigate changes in subcellular structures that have led to advances in medical diagnosis, these are linked to specific proteins that are located in cell areas relevant to a study and emit green light at a certain wavelength after being exposed to a short period of shortwave excitation; In such a way that the cellular morphology can be observed with definition due to the phenomenon of fluorescence.

The data obtained through imaging studies contain great information to understand the biological processes, effects of therapeutic drugs and provide diverse indicative as the individual and heterogeneous behavior of the cells, in addition to obtaining visual and statistical evidence about a disease. Bioimaging techniques consist of multiple analyzes with different approaches and objectives: detection and segmentation, visualization, tracking or tracing to finally carry out an analysis that together with genomic and chemical data allow the design of a therapeutic mechanism against a disease or the study of the same [88].

Quantum dots as previously mentioned are fluorescent nano-structured semiconductor materials that can be used in bioimaging and diagnostic studies. The QDs exhibit outstanding and advantageous properties compared to organic fluorophores and also exhibit good electronic and optical properties for use in bioimaging studies (in vivo and in vitro) [89]. A recent study published images of live T24 cells in which cell morphology and fluorescence were clearly observed in images [90].

Near infrared quantum dots were synthesized in a study to be applied in the detection of Cu^{+2} with high sensitivity and to monitor their changes in concentration through in vivo and in vitro fluorescence imaging studies. For in vivo studies CdTe / CdS NIR QDs were used in live mice [91].

Another study showed that QDs-Ag_2S have long times of fluorescence and chemical stability in vivo bioimaging tests, the photoluminescence of QDs-Ag_2S in blood was observed in real time, which also showed no toxicity [92] this study was carried out to study the processes of angiogenesis.

A published study shows the production of bioconjugated QDs-Ag_2S with an Arg-Gly-Asp-DPhe-Lys pentapeptide with high affinity to malignant tumor integrins in vivo imaging studies, Figure 12 [93].

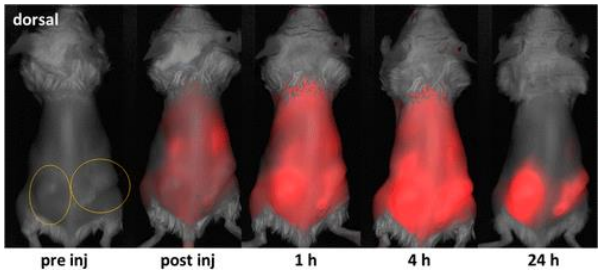

Fig. 12. Tumor location in vivo by QDs flouresence imaging (Tang et al., 2015).

The quantum dots have also been used as mapping to identify areas of tumors and the extent and magnitude of these; The quantum dots can be used to perform a tumor mapping without the need for a biopsy and to have an idea of the margin of the tumor, a test performed with silicon QDs labeled with 124I for observation by positron emission tomography, the silica-QDs were modified with cRGDY peptides for the cartographic observation of breast cancer [94].

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6.3. Controlled drug delivery

Controlled drug delivery is an emerging field focused on drug delivery directed to a specific target of the organism as a group of cells [95]. One of the most important diseases of the present time is cancer, today one in four people dies because of some type of cancer [96]; Chemotherapy is commonly used as a treatment against this disease however, its administration involves damage to both types of cells: healthy and tumor causing serious side effects. For this reason, the main objective of controlled drug delivery systems is to transport the necessary, sufficient and correct amounts of a drug to a specific site: tumor, tissue, group of cells to perform its function while minimizing side effects of the administered drug [97, 95]. Nanotechnology has gradually been directed towards the field of medicine for controlled drug delivery systems and to maximize the existing therapeutic possibilities these and reduce side effects by opening up a new field of study: nanomedicine. Nanomedicine offers possibilities and improvements in the diagnosis, monitoring, prevention and treatment of diseases using active drug carriers, diagnostic agents and drug fractions through the use of nanoparticles, polymer micelles, liposomes, dendrimers, and carbon nanoparticles [96], confined nanostructures like quantum dots.

A recent study used graphene quantum dots for the controlled and targeted release of doxorubicin a DNA intercalation drug in tumor cells. The quantum dots were covalently bound to the biotin tumor targeting module, capable of efficiently recognizing biotin receptors overexpressed in cancer cells and loaded with doxorubicin [98]. Also a study using Ag_2S-QDs bioconjuated with the cRGD peptide and linked with the drug doxorubicin were used in a study to monitor the systematic distribution of the drug by the individual in an in vivo study obtaining excellent results [99].

7. Conclusions

The quantum dots are confined nanostructures with unique properties and great advantages compared to other molecules and structures within the biomedical field. QDs of Ag2S exhibit promissory properties. They n-type semiconductor materials, not water soluble, they have good photostability and high fluorescence intensity.

They also exhibit good catalytic properties, optoelectronics and high electrical conduction that together with their null toxicity make them good candidates in biomedical applications. However, there is still much to improve and understand in their application in a field as important as health, so their development and application continue being a challenge.

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