1 Introduction

1.1 Photoluminescence

Photoluminescence occurs when chemical species are excited with photons and their return to the ground state results in emission of photons (of less energy than the ones of the excitation process) producing an emission spectrum that provides useful information for qualitative or quantitative purposes. Fluorescence is a type of photoluminescence involving transitions between energy states of the same multiplicity (singlet to singlet) and, therefore, lifetimes are relatively short, of the order of 10^{-7} to 10^{-9} s [1]. Although such nanosecond scale may seems quite short, in fact fluorescence is a process that take orders of magnitude longer than other processes like molecular vibrations (10^{-14} s) and transitions between electronic states (10^{-15} s) due to absorption of energy.

In organic substances, structures composed by a group of atoms known as chromophores may absorb photons of the UV-vis spectral region, producing electronic transitions. The energy diagram (Jablonski diagram) can be used to illustrate the various possible quantized transitions (Figure 1) involved after photon absorption. The ground state is represented by S_0 (since it is generally singlet) and further, these electronic states are divided into vibrational levels, which have energy differences beween levels are much smaller than the energy difference between subsequent electronic states. After absorption the excited population of molecules will be transferred to an excited electronic state (S_1, S_2 or S_n). By dissipating the excess energy through vibrations and collisions, the population tends to relax back to the lowest vibrational level, in general of the first excited state.

If conditions are favourable in minimizing the probability of radiationless processes such as vibrational relaxation and internal conversion, the population returns to the ground state emitting photons with energy proportional to the energy gap between the lowest vibrational level of the excited state and any vibrational level of the ground state. In terms of selection rules, such transition is

of high probability (allowed transitions). It is also possible, although less probable, the transfer of the population from the excited singlet state to the excited triplet state (intersystem crossing). Such quantum-mechanic forbidden transition may occur under special circumstances resulting in a longer lifetime emission (μs to ms time scale) with less energy than the one expected for a singlet-singlet transition [2].

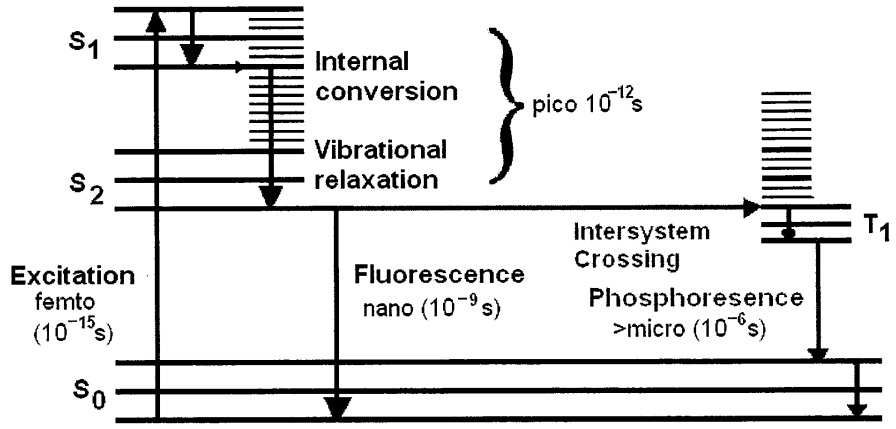


Figure 1- Modified Jablonski diagram depicting absorption and emission electronic processes.

Molecular optical sensing has been continuously dominated by the use of organic dye-based photoluminescent substances of high photoluminescence quantum yields [3]. In many cases, lanthanide complexes of these substances (ligands working as “antenna” to capture electrons) are used to enable amplification of the characteristically sharp lanthanide transition [4]. The emerging of luminescent nanomaterials in the past decade have brought new perspectives in analytical sensing due to their peculiar photo-physical properties such as intense signal, longer lifetimes and more resistance to photobleaching. Among these new materials, semiconducting nanoparticles or nanocrystals, also known as quantum dots (QDs), have been the subject of many studies involving analytical applications towards the sensitive and selective determination of analytes such as pollutants [5], drugs [6] and relevant biomolecules [7].

1.2 Semiconducting nanocrystals or quantum dots

1.2.1 Background

Semicondunting nanocrystals (quantum dots) were first reported in the late 1970's by Ekimov and co-workers and due to their unique characteristics these nanomaterials became of great interest to science community [8]. The potential applications of quantum dots involves many areas of pure and applied sciences such as life sciences (chemical and biological sensing) [9] biological tagging and labeling [10] and the developing of high quality light emitting devices [11].

These photoluminescent materials range in size (diameter) between 1 and 20 nm involving in its structure as few as 100 to 100.000 atoms per particle. The most common quantum dots consists of elements from the group II and VI, for instance, cadmium sulfide (CdS) [12], cadmium selenide (CdSe) [13], cadmium telluride (CdTe) [14], zinc selenide (ZnSe) [15], lead sulfide (PbS) [16] and mercury sulfide (HgS) [17]. There are also some quantum dots formed by elements of the groups III and V such as indium phosphide (InP) [18] and indium arsenide (InAs) [19]. Moreover, some quantum dots composed of single element (such as silicon) or of ternary systems, such as CdZnS, CdSSe and InNP, have also been synthesized [20].

The attractive photophysical properties of quantum dots are consequence of a phenomenon known as quantum confinement [21]. In bulk materials (macroscopic semiconductors), the energy levels are very close to each other resulting in small difference between them, thus behaving as continuous distribution of energy (non-quantized). The band gap is the region where the presence of electrons is forbidden and separates the so called valence band (the outermost electron shell of atoms in an insulator or semiconductor, in which the electrons are tightly bound to the atom to carry electric current) from the conduction band (the outermost orbitals in atoms in a conductor or semiconductor, in which the electrons are free enough to move and thereby carry electric current).

In bulk semiconductors, the valence band is almost fulfilled with electrons with a very small percentage of electrons occupying the conduction band. For the electrons in the valence band, the gain of energy is required for crossing the gap.

However, due to the fact that the bulk semiconductor has a continuous-like energy level distribution, therefore the band gap magnitude, is fixed and any optical transition presents a single wavelength.

In the case of nanoscale semiconductor structures, the behaviour is quite different as the quantum dots physical dimensions can approach the exciton-Bohr radius (the spatial separation of the electron from the hole), therefore, Bohr approximation can be used to determine this distance (r) as seen in Equation 1.1 [22].

$$r = \varepsilon h^2 / \pi\, m_r e^2 \qquad (1.1)$$

In the Equation 1.1, r is the radius of the sphere (corresponds to the separation of the electron-hole pair), ε is the dielectric constant of the particle, h is Planck's constant, m_r is the reduced mass of the electron-hole pair and *e* is the charge on the electron. The hole is a vacancy, essentially the absence of an electron that is created during electronic transition and can be considered as a particle with its own dimensions and effective mass.

In contrast, the bulk of the semiconductor is of much greater size than its exciton-Bohr radius, resulting in the extension of the natural confination of the exciton. In quantum dots, the physical dimensions are of the order of the exciton-Bohr radius and often less (Figure 2). This system is a prime example of the "particle in a box" quantum model [23], which theoretically illustrates the dependence on the size of the box in calculating the particle's energies. When the particle approaches the size of the exciton-Bohr radius they cease to behave like the bulk semiconductor in that the energy levels cannot be considered as continuous. On the contrary, they are now discrete (quantized differences between energy levels) and the optical transition observed has a wavelength dependence upon the size of the nanoparticle.

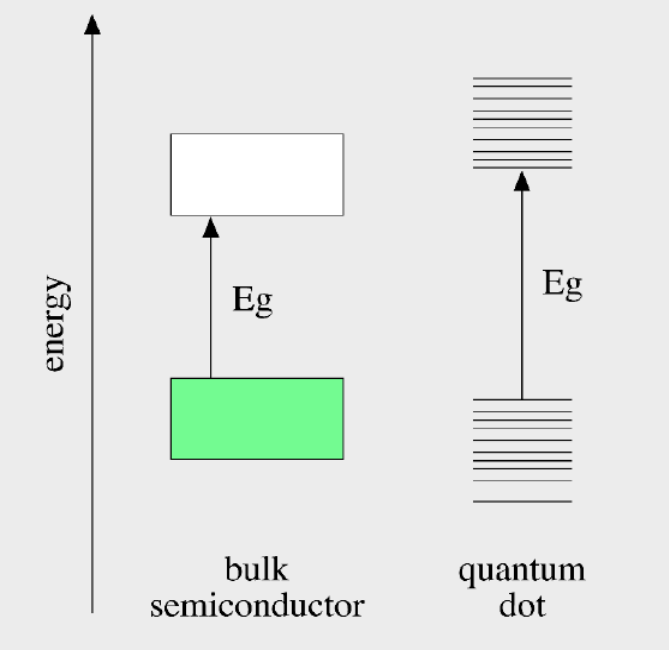


Figure 2 - Schematic describing Tunable bandgap of quantum dots compared to the fixed band gap of the bulk semiconducters.

Due to the energy band structure of semiconductor and to their nanosize scale, quantum dots exhibit distinguished photoluminescent properties, which are illustrated with the scheme in Figure 3. Quantum dots present discrete energy levels in both the valence band (VB) and the conduction band (CB) as there are a limited number of atoms in each particle. When they are excited with an energy (E_{ex}) higher than band gap energy (E_g), electrons 'jump' to the conduction band forming short-lived electron-hole pairs (the so-called excitons). Then the electrons and holes (represented by the open and closed circle) recombine quickly, and photons are emitted with a specific energy corresponding to the band gap, which is the band edge emission. As part of the energy may be radiationless released, the emited energy (E_{em0}) is usually lower than the excitation energy (Stokes shift or ΔE). When energy trap states exist in the band gap, photons with different energies (E_{em1}, E_{em2}, E_{em3}, E_{em4}) may be released. These energies are usually lower than the band gap emission energy (E_{em0}).

In conclusion, the quantum dots differ from bulk semiconductors as the emission frequencies shows dependency upon the band gap magnitude. Therefore,

frequency of the emitted light (photoluminescence) may be tuned by changing the size of the nanostructure.

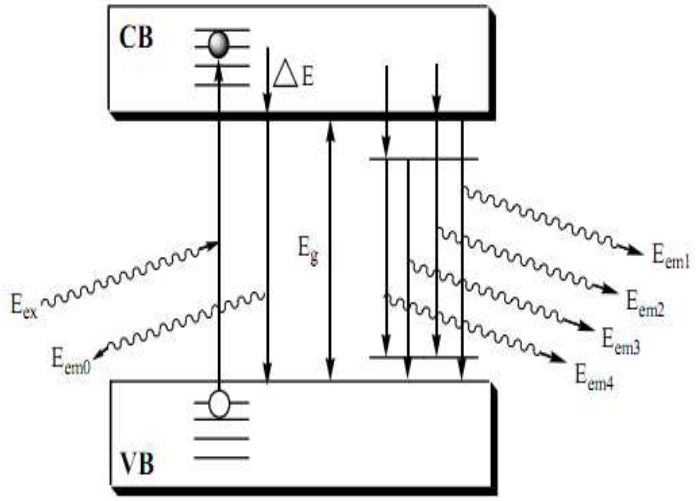


Figure 3 - Schematic of excitation and emission of quantum dots with the typical energy band structure of semiconductor. V_B is the valence band, C_B is the conduction band, ΔE is the Stokes shift, E_g is the band gap energy, E_{ex} is the excitation energy, $E_{em\ 0\text{-}4}$ are the various emission energies.

1.2.2 Synthesis of quantum dots

There are many approaches for the synthesis of quantum dots such as vapour-phase deposition, evaporation, sputtering and epitaxial growth on certain substrates (the so-called Stranski-Krastanov growth) [24]. The main limitations of these methods are the cost of fabrication and the lack of control over positioning of individual quantum dots. In the recent years, synthesis of quantum dots by the colloidal method has become the most favoured technique as the quantum dots are synthesized via chemical routes in solution allowing even the use of reverse micelles to control de size distribution of the colloidal nanocrystals [25]. The two most widely applied colloidal methods for the synthesis of quantum dots are the organometallic synthesis approach and the synthesis in aqueous phase.

1.2.2.1 The organometallic synthesis

The so called TOP/TOPO synthesis method is an approach to synthesizing CdX (X = S, Se, Te) nanoparticles from organometallic reagents. The method is based on the pyrolysis of organometallic reagents (for example, dimethylcadmium and bis-selenium) by injecting them into the hot solvents tri-n-octylphosphine (TOP) and tri-n-octylphosphine oxide (TOPO). This method is widely used due to its versatility, reproducibility and high quality of the particles produced. Traditionally, many quantum dots of the groups II-VI and groups III-V are formed in a hot mixture of TOP and TOPO that work as both solvent and stabilizing ligand. For instance, in a typical synthesis of CdSe quantum dots, a certain amount of TOPO, in a reaction flask, is purged with a flow of inert gas and heated to about 200 ^{o}C to 300 ^{o}C. Solutions of dimethylcadmium (Me_2Cd) and tri-n-octylphosphine selenide (TOPSe) are then added to the flask and mixed with vigorous stirring, where the CdSe nanocrystals promptly nucleate and grow in time [26].

In order to narrow the size distribution of the nanocrystals, the large crystals are precipitated by adding methanol and then re-dispersed in 1-butanol. This size-selective precipitation is repeated for a few times and the final product is dispersed in organic solvent. Besides practical advantages and good results achieved, this method is not environment-friedly and brings human health issues due to the high toxicity of many of the employed raw materials, especially the organometallic precursors that are also expensive, unstable and explosive/pyrophoric. Although a ‘greener’ approach was developed later using CdO as the precursor, [27,28], the hydrophobic surface of the quantum dots organic ligand coating in these systems is not suitable for biological applications, very high temperatures for reaction are still required and the complexity of the organic synthesis influences on the final cost of the commercially available quantum dots.

Further attempts to improve the organometallic synthesis have been done to produce water-soluble and biocompatible quantum dots using silica coating. For instance, 3-(mercaptopropyl) trimethoxysilane (MPS) was adsorbed on the surface of nanocrystals to replace TOPO. However, this process is tedious and

involves multiple steps to change the solvent compatibility of the nanocrystals from organic to aqueous system.

1.2.2.2 Aqueous phase synthesis

In the last two decades significant developments demonstrated the great potential to synthesize quantum dots directly in aqueous media in order to provide nanoparticles with superior properties such as strong photoluminescence, long time stability and compatibility with biological media. This approach is relatively simple, easily adjusted in scale, use water as a reaction solvent (environmental friendly and biocompatible), minimizes the requirements for the use of inert atmosphere, and allow the use of various types of capping ligands making the nanoparticle functionalization versatile.

The aqueous synthesis protocol is well established and consists in reacting a metal ion or one of its complexes with a chalcogen containing precursor, in the presence of a stabilizer. The nucleation and growth of the quantum dots is controlled by heating (temperature and time). Metal precursors in the form of water soluble salts are used (commonly perchlorates and chlorides). Sodium sulphide (Na_2S) is widely employed as the source of sulphur [29-30]. Tellurium powder is the source in synthesis of Te based quantum dots and selenium in synthesis of Se based quantum dots. For example, CdTe nanoparticles is prepared via the reaction between Cd^{2+} and NaHTe (NaHTe is produced by the reduction of Te with $NaBH_4$). They start to crystallize and show visible photoluminescence after reflux at 96 ^{o}C during a specific time [31]. CdSe has been synthesized by Se (NaHSe prepared by reduction of Se with $NaBH_4$) and solid $CdCl_2$ using thyoglicolic acid (TGA) as a capping ligand with the reaction kept at at 0 °C (ice bath).

The formation of nanoparticles is a dynamic process which is usually explained by an Ostwald ripening (see details below) mechanism mainly consisting of the growth of larger particles at the expense of the smaller ones present in the reaction vessel.

1.2.3 Growth mechanism of quantum dots

1.2.3.1 Nucleation

The growth process of colloidal quantum dots can be divided into two parts. The first stage is the nucleation when the nanoparticles are spontaneously formed through the coming together of the dispersed monomers in solution. The second stage is the actual growth process. Nucleation can be viewed as an overcoming of a barrier whereby a thermodynamically stable state is attained after the assembly of a core of monomers that do not decay back to the free atoms or ions [32]. At this point, two phases coexist, the crystalline and solution phase. As the names suggest, the crystalline phase is the one comprising atoms bound to the crystal while the solution phase refers to the freely dispersed atoms (as ions and solvated molecules) in solution. The difference in the free energy between the two phases governs the nucleation in solution at constant temperature and pressure. The driving force for this nucleation is the gain in chemical potential, in other words, the energy released by the formation of bonds in the growing crystal and the increase in total surface energy, which accounts for the incomplete saturation of surface bonds. The change in the free energy ΔG, on formation of the spherical nucleus with n atoms is governed by the Equation 1.2;

$$\Delta G = n\,(\mu_c - \mu_s) + 4\pi r^2\sigma \qquad (1.2)$$

Where μ_c is the chemical potential of the crystalline phase, μ_s is the chemical potential of the solution phase, r is the radius of the nucleus, σ is the surface tension (or, more correctly for solids, their surface energy). The given Equation 1.2 is for a simplified case where the surface tension σ remains constant for any size and morphology of the crystal. For a more detailed approach the defined crystalline surface would need to be accounted for the role of faceting (the arrangement of atoms and dangling bonds on the surface). The density of atoms will affect the total surface energy but, in order to simplify the discussion, such effect is ignored and an isotropic crystal without facets is assumed. In Equation 1.3, the variation in the free Gibbs energy (ΔG) of the nanometric system is

expressed by the radius r of the nanocrystals and the density (d_m) of the group of atoms forming the crystal:

$$\Delta G = 4\pi d_3 /3.\ r^3 (\mu_c - \mu_s) + 4\pi r^2 \sigma \qquad (1.3)$$

The minimum free energy occurs when all the atoms are freely dispersed as the chemical potential of the atoms in solution is smaller than that the ones in the

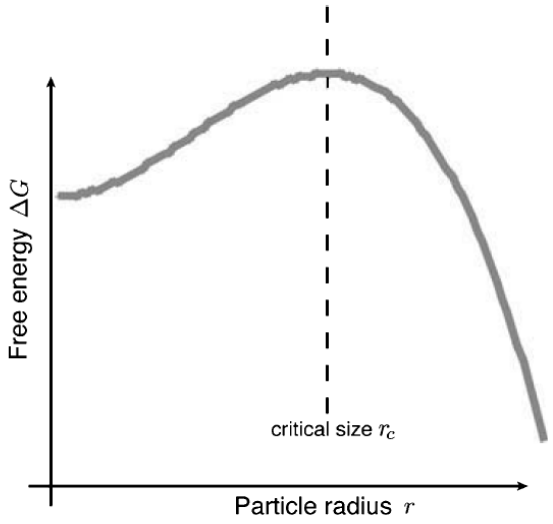


Figure 4- Free energy variation for the nucleation.

crystal. Therefore, there is no formation of stable crystals. However, the opposite case, with the chemical potential of atoms in solution being larger than that of bound atoms, is the one of interest. Thus, the first term in Equation 1.3 becomes negative and the free energy reaches a maximum for a certain radius r_c termed critical size. Only at small values of r in Equation 1.3, the term r^2 of the surface energy outcompetes the r^3 contribution of the chemical potential, so that a barrier is imposed at the critical size r_c (Figure 4). For small nuclei, the surface energy term dominates the free energy, whereas only for crystals much larger than r_c the growth is driven by the gain in chemical potential and in principle the crystals can grow to an infinite size [33].

1.2.3.2 Growth

The growth process of particles comprises of two distinct steps. The first involves the transportation of the monomers (in this case, a unity structure of the semiconductor) to the nanoparticle surface and the second step is their reaction on the surface. The growth rate is controlled by the rate of deposition of monomers on the nanocrystal. To initiate the growth process, an excess of monomers is injected into the reaction cell. Due to the high concentration of the monomer, the rate of incorporation only depends on the reaction rate for the conversion of monomers to crystal. The surface area of the nanocrystal dictates this rate. As the concentration of monomers reduces, the growth rate varies upon the rate at which the monomers reach the nanoparticle surface. In the course of a reaction, the concentration of monomers decreases and the smaller crystals enter a size focusing stage in which the critical size r_c increases and a broadening process occurs. When the radius of the crystal reaches the point where equilibrium with the monomer concentration is established, a zero growth rate is observed.

The critical size ultimately becomes greater than the radius of the smallest nanocrystals entering the Ostwald ripening stage, as shown schematically in Figure 5, which is characterized by a significant broadening of the size distribution. This is accompanied by a reduction in the concentration of the nanocrystals. The smaller crystals “melt” back into the solution, freeing the monomers and these are incorporated into the larger crystals. The nucleation stage has a major impact on the final size distribution. This stage should ideally be forced to finish before the diffusion controlled growth stage. If the nucleation event occurs over too long, then a broadening of the distribution of sizes of crystals occurs. The photoluminescence spectrum is a good indicator of the quality of the nanocrystals in terms of size distribution, measured as the full width at half maximum (FWHM), since the size of the nanoparticles is reflected in the wavelength of fluorescence. In general, a FWHM of approximately 30 nm shows good size distribution.

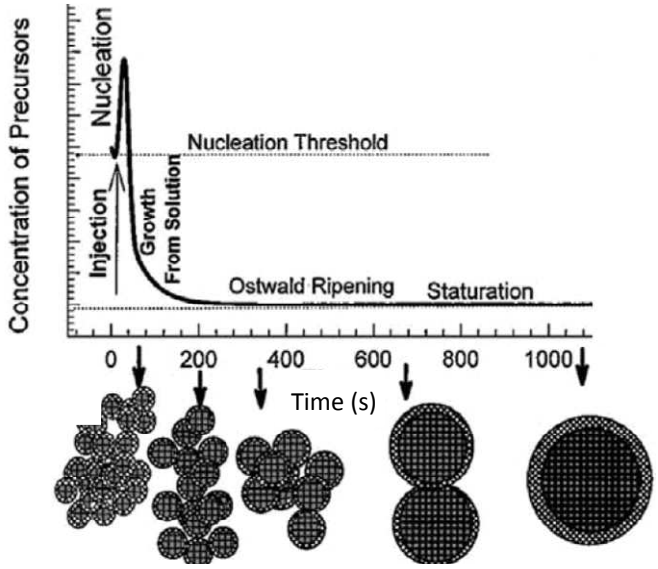


Figure 5- Model for stages of nucleation and growth of monodisperse colloidal particles [33].

In practical aspects of the synthesis, a high temperature reaction and an excess of monomers ensures a short nucleation period and reduces the chance of entering a broadening regime. Some synthetic routes cause a rapid depletion in the supply of monomers resulting in a broadening of the photoluminescence band. This can be overcomed by a fresh injection of monomers during the growth phase. Improvements in size distribution may be achieved in a somewhat laborious way by repeated precipitations of the material with an organic solvent followed by homogenization in aqueous solution.

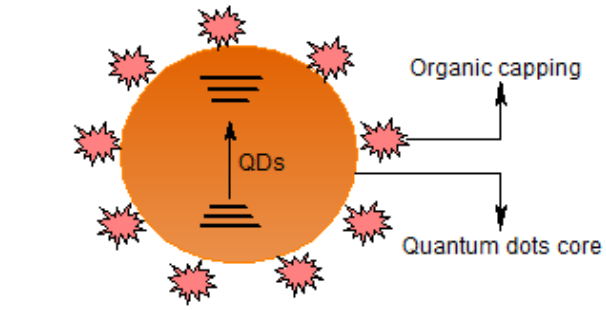
1.2.4 Surface Passivation and water solublization

The surface chemistry of quantum dots is strongly dependent on the size because the surface-to-volume ratio increases as the size decreases. A high surface-to-volume ratio means that the structural, optical, and electronic features of semiconductor quantum dots are heavily influenced by their surface properties. This ratio is large in quantum dots due to their small sizes, so they may tend to aggregate by the influence of temperature, UV light, and ionic strength of the system. The flexibility of the quantum dots in terms of surface physico-chemical interactions enable these photoluminescent inorganic probes to efficiently couple

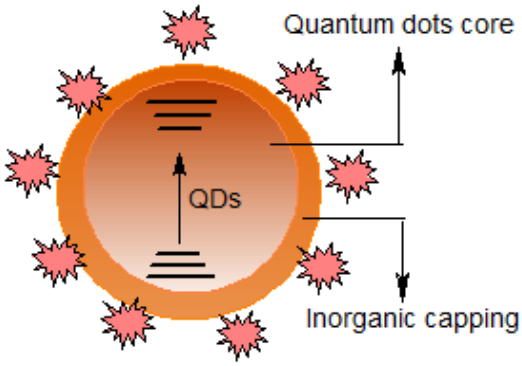
with the organic ligands that provide them with the peculiar properties attractive in many applications.

Organic stabilizers are often employed aiming the formation of a monlayer of ligand on particle surface. These stabilizers not only stabilize the quantum dots in solution but also passivate its surface decreasing the surface defects. Because surface defect on surface of particles, such as vacancies, impurities, or adsorbates cause the formation of ‘‘trap’’ states into which the photo-excited electron can fall, or the photo-excited hole can ‘‘float’, affecting the photoluminescence properties of quantum dots. Therefore these stabilizers improve the luminescence quantum yield. Among these organic stabilizers are thiol-containing molecules [34], silica shells [35] surfactants and oligomers [36], encapsulation in micelles [37], and the use of polymers are also used [38].

Due to the large structural differences between quantum dots and the organic capping molecules, a perfect passivation cannot be achieved by removing surface dangling bonds. An ideal passivation method is to use the inorganic shell of another semiconducting material (for instance ZnS) around the core quantum dots, providing dense surface protection. Such passivation of the nanocrystals surface by a thin semiconductor shell does not significantly modify the absorption and emission characteristics of the core quantum dots, but increases their luminescence quantum yield up to 70%. Examples of theses core/shell system are CdSe/ZnS, CdSe/CdS and ZnSe/ZnS [39]. As the band gap energy of the shell is higher than that of core, the shell material does not absorb the light emitted from the core material. In addition, the presence of the shell helps to eliminate the broadband emission (Figure 6).



a.



b.

Figure- 6 - (a) Representation of an organic ligand coated quantum dots (b) and a core shell quantum dots.

1.2.5. Photophysical properties

One of the unique and striking features of quantum dots is their size-dependent emission spectra [40]. By altering the size and composition of the nanoparticles their photoluminescence characteristics (emission wavelength and lifetime) alters. The photoluminescence wavelength can be tuned from the ultra-violet region of the spectrum up to the near infrared. As the particle size reduces the band gap increases resulting in emissions in shorter wavelengths (larger energies and frequencies). For instance, CdS quantum dots can be tuned to emit from 350 nm to 500 nm. For CdSe quantum dots, the tunability limits are from about 450 nm (average particle size of 1.8 nm) and 655 nm (average particle size of 7.5 nm) while for CdTe, tuning can be made from 510 nm (average particle size of 2.1 nm) to 675 nm (average particle size of 5.1 nm) [41]. Others such as the Pb-based quantum dots have been employed to extend the tunability range into the near infra-red (up to 2000 nm) [42]. The range over which the band gap and photoluminescence wavelength can be tuned depends on the materials of nanoparticles and it bulk band gap. The light emission of the quantum dots is also more narrow and symmetrical than the one of the most conventional organic dyes.

Another interesting property of the quantum dots is their broad band absorption spectra extending through the UV region, which allows versatility in the photoexcitation process as any wavelength over a large range can be selected.

The broad absorption of quantum dots corresponds to the overlapping of a series of peaks that increase in size as the wavelengths get shorter. Each peak represents an energy transition between discrete electron-hole pairs (excitons) explained by the discrete nature of their energy levels. Quantum dots will not absorb at wavelengths longer than that the one of the of the 1st exciton peak. The molar extinction coefficients for the bandgap of these quantum dots can be 10 to 50 times greater than commercially available organic dyes [41].

Surface-passivated quantum dots also show excellent photochemical and thermal stability when compared to organic dyes. The additional inorganic surface

layers completely suppressed photo-oxidation of the core for relevant time intervals.

1.3 Photoluminescent chemical sensing

The continuous monitoring of the presence of chemical species is called chemical sensing [43]. Many areas related, for instance, to chemistry, biology, clinical-biomedicine and environment requires signal sensing, which plays an important role in our day-to-day life [44].

Many features make photoluminescence one the most attractive field to report chemical recognition. A number of photoluminescence microscopy and spectroscopy techniques based on the life-time, anisotropy or intensity of the emission of photoluminescent probes have been developed over the years. The use of the right conditions to achieve high sensitivities allows even single molecule detection. In addition photoluminescence probing tends not to destroy the object of interrogation.

The most classical design of photoluminescent indicator for analyte recognition comprises two moieties, a receptor and fluorophore. There are three main approaches adapted for chemical sensing in solution. The first, results in intrinsic photoluminescent probes, which mechanism for signal transduction involves the interaction with the analyte by a chemical reaction/interaction involving, in general, groups that are part or directly bonded to the conjugated π-system of the fluorophore [45]. The second involves extrinsic photoluminescent probes, in which the receptor moiety and the fluorophore are covalently linked but are electronically independent [46]. Due to the covalent linking through a spacer, both moieties are in close proximity and the interaction of the analyte with the receptor induces a change in the fluorophore surroundings, changing its fluorescence. The third strategy is called chemosensing ensemble that is based on a competitive assay in which a receptor-fluorophore ensemble is selectively dissociated by the addition of an appropriate competitive analyte that is able to interact efficiently with the receptor resulting in a detectable effect on the fluorophore [47].

As different types of fluorescent nanoparticles have become available, they also have been explored for sensing. In the last decade, water soluble

semiconductor nanoparticles have gained great attention since their discrete electronic states are very sensitive to surfaces changes induced by presence of nearby chemical species. This fact indicates that a surface phenomenon is very important in the sensing with nanoparticles. Therefore, it can be expected that the presence of different molecules nearby the quantum dots surface may cause changes in their luminescent properties and these changes could be used for analytical purposes. Photoluminescence quenching or enhancement can be observed after during analyte-quantum dots interaction.

The discovery of these luminescent nanoparticles has opened the door to a new exciting approach for photoluminescent chemical sensing as the recent and important applications listed in Table 1.

1.4 Sensing approaches based on photoluminescence of quantum dots

The semiconductors nanoparticles have a broad range of applications that includes their use as new type of photoluminescent optical probes in analytical chemistry. Because of the strong photoluminescence emission in these nanoparticles originates from the recombination of photogenerated electron-hole pairs that takes place on the surface of the nanocrystals, the binding of samples organic molecules or ions (analytes) close the surface may affect their emission. The photoluminescence changes (responses) may be either the quenching or the enhancement of photoluminescence and these effects provide the basis to exploit their properties as chemical sensor (photoluminescent probes).

The term photoluminescence quenching refers to any event in which the observed photoluminescence is decreased. When the quenching arises from the change in optical properties of the sample, it is called trivial quenching or non-selective quenching. More selective quenching arises from the specific interaction of non-photoluminescent molecules with selected fluorophores (for instance, quantum dots). The static quenching involves the formation of ground-state fluorophore-quencher complex and, in contrast, the dynamic quenching takes place due to transient collisional interaction between an excited- state fluorophore and ground state quencher. Both static and dynamic quenching are selective, and, thus, more attractive in the analytical point of view. Dynamic quenching is mathematically described by well known Stern-Volmer Equation 1.4.

$$F_0/F = 1 + k_d \tau_f^0 C_q = 1 + K_{SV} C_q \quad (1.4)$$

Where F_0 and F are the photoluminescence emission intensity of fluorophor (in this case, the quantum dots) respectively in the absence and presence of quencher (C_q). Plotting the fluorescene ratio (F_0/F) in function of the quencher concentration leads to a linear response with the slope equal to the stern-Volmer constant (K_{SV}) and with the intercept equals to unity. The observed Stern-Volmer constant can also be expressed as the product of the second order rate constant for quenching (k_d) and the lifetime of the fluorophore in the absence of quencher (τ_f^0). Dynamic quenching is a collisional process that occurs during the excited-state lifetime of the fluorophore, therefore, changes in lifetime occurs in the presence of quencher species. Hence, measurement of photoluminescence lifetime of the fluorophore in the absence and presence of quencher molecules can be used to find out whether the quenching is static or dynamic.

To date, most of designed photoluminescence probes used for detecting a variety of analytes are based on the photoluminescence quenching and the mechanisms involved could be inner-filter effects, non-radiative recombination pathways and electron transfer. Approaches based on luminescence quenching of quantum dots are usually less selective, enabling poorer LOD values and need for corrections to compensate the inner filter effect caused by the absorbance of light produced by the analyte itself or by sample matrix components. The need for such kind of correction is often ignored in many works.

The photoluminescence enhancement from a fluorophore (in this case, quantum dots) also known as "turn on" approach is a less explored process. When compared to quenching process, the enhancement is more interesting due to its selectivity when compared to quenching process. In addition, photoluminescence enhancement is not limited by background as quenching based methods. Photoluminescence enhancement in quantum dots is attributed to the passivation of trap states or defects on the surfaces of nanoparticles. Nevertheless, the exact mechanism of photoluminescence quenching and enhancement in these nanoparticless is still not exactly known. In conclusion, by a careful choice of quantum dots and by proper optimization of experimental conditions, the

photoluminescence quenching or the photoluminescence enhancement approaches can be utilized in analytical sensing applications as indicated in Table 1.

Table 1- Recently developed quantum dots based photoluminescent sensing applications.

Analytes	Quantum dots	Limit of detection	Refs
Methimazole.	TOPO-CdSe	30 ng mL^{-1} (0.26 μmol L^{-1})	[48]
Ranitidine hydrochloride	TGA-CdS	0.38 mg L^{-1} (1.1 μmol L^{-1}) .	[49]
Roxithromycin	MPA-CdTe	4.6 mg L^{-1} (5.5 μmol L^{-1})	[50]
Uric acid	TGA-CdTe-CdSe 2-Mercaptoethylamine CdTe	0.1 μmol L^{-1}	[51]
Menadione	p-sulfonatocalix[4]arene coated ZnS	80 ng mL^{-} (0.46 μmol L^{-1})	[52]
Levodopa	Citrate-capped-Mn-modifiedCdSe/CdS	0.2 μg mL^{-1} (0.9 μmol L^{-1})	[53]
spironolactone	TOPO-CdSe QDs	0.2 mg L^{-1} (0.48 μmol L^{-1})	]54]
Sulfadiazine	TGA-CdS	8.0 μmol L^{-1}	[55]
Acetylsalicylic acid	S-βCD-MSA-CdTe	8.5 nmol L^{-1}	[56]
Methomyl	CdTe- Composite	0.08 mol L^{-1}	[57]
Deltamethrin	CdTe-MIP-SiO_2	.6 mg L^{-1} (0.3 μmol L^{-1})	[58]
Organophosphorus pesticides	CdTe-AChE-cholineoxidase	1 pmol L^{-1}	[59]
Glyphosate	Silica Spheres Coated With Calix[6]arenecapped CdTe	0.0725 nmol L^{-1}	[60]
P-nitrophenol	Cyclodextrin-QDs	7.9 nmol L^{-1}	[61]
L-cysteine	MPA-CdSe/ZnS	3.8 nmol L^{-1}	[62]
Bradykinin	CdTe	0.6 μ mol L^{-1}	[63]

Melamine	TGA-CdS	1 nmol L^{-1}	[64]
Glutathione	Rhodamine 6G conjugated and MPA-CdTe	15 nmol L^{-1}	[65]
Adenine	Hexametaphosphate- Zns	3 nmol L^{-1}	[66]
Bovine serum albumin	MPA-CdTe/CdS	54 nmol L^{-1}	[67]
Cysteine	CdS	5.0 x 10^{-5} mol L^{-1}	[68]
Rutin	2-meracpto propionic acid-CdS	1.2×10^{-6} mol L^{-1}.	[69]
Ag^{2+}	thiolactic acid-ZnS	0.5 μmol L^{-1}	[70]
Arsenic	MAA-CdS	0.07 mg L^{-1}(0.93 μmol L^{-1})	[71]
Hg^{2+}	CdTe-CdS nano composite	5.6 μmol L^{-1}	[72]
Cu^{2+}	L-cysteine-ZnS	7.1 μmol L^{-1}	[73]
Zn^{2+}	Mn-dopped ZnS	0.67 μmol L^{-1}	[74]
Cd^{2+}	l-Carnitine-CdSe/ZnS	0.15 μmol L^{-1}	[75]
Ag^{+}	Citrate-CdSe	1.7 μmol L^{-1}	[76]
F^{-}	CdSe/ZnS capped with- 1-(bis(η^5-yclopentadienyl)iron)-methyl-3-(5,7-dimercapto-heptyl)-Urea	74.0 μmol L^{-1}	[77]
Br^{-}	4-amino-2,2,6,6-tetramethylpiperidine-N-oxide-CdTe	0.6 μmol L^{-1}	[78]

- TOPO - Tri-n-octylphosphine oxide
- TGA - Thyoglicolic acid
- MMA- Mercaptoacetic acid
- MPA- Mercaptopropionic acid

1.5 The analytes of interest for sensing through optical probes

1.5.1 Captopril

Hypertension is the elevation of blood pressure of the human body that leads to overworking of both heart and blood vessels. The untreated high blood pressure may cause chest pain (angina), heart attack, stroke, kidney failure, peripheral vascular disease and eye damage (retinopathy). Antihypertensive drugs work lowering blood pressure by one or by a combination of actions : (i) opening and widening the blood vessels, (ii) preventing the blood vessels from closing and tightening, (iii) reducing the workload of the heart [79]. There are ten types of antihypertensives categorized by the mechanisms of action: diuretics, enzyme (ACE) inhibitors, angiotensin receptor blockers, alpha-blockers, beta-blockers, calcium channel blockers, angiotensin-converting, central adrenergic inhibitors, peripheral adrenergic inhibitors and blood vessel dialators. [80].

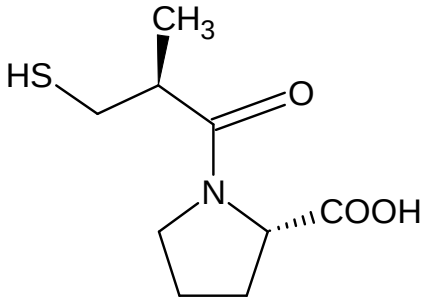


Figure -7- Chemical structure of captopril.

Captopril, 1-[(2*S*)-3-Mercapto-2-methyl-L-oxopropyl]- L-proline, (Figure 7) belongs to the group of antihypertensive drugs widely used for treatment of hypertension and congestive heart failure that blocks the angiotensin II converting ACE, which causes blood vessels to tighten. It was the first ACE inhibitor drug marketed. Although, nowadays, several other ACE inhibitors (such as enalapril, lisinopril, perindopril, ramipril) have been developed, captopril has the lowest cost and, therefore, widely indicated in prescriptions for the general population.

Captopril is a slightly yellow or white crytalline powder, easily soluble in alkalines solution, ethanol, chloroform, methanol and water. This drug can be found in medicines at various dosages and trade names.

A limited number of analytical methods are available for captopril. Indirect methods that relies on the formation and detection of chemical species formed by redox reactions in the presence of captopril enabled limit of detection (LOD) of 0.25 μg mL^{-1} (1.1 μmol L^{-1}) by absorption photometric detection of either Fe^{3+} or iodine after complexation with a chromophore reagent [81] and 0.1 μg mL^{-1} (0.46 μmol L^{-1}) through fluorimetric detection of Ce^{3+} [82].

Most of the separation methods coupled with optical detection relies on the time consuming chemical derivatization of the analyte because of captopril´s lack of strong chromophore groups. High performance liquid chromatography (HPLC) and capillary electrophoresis (CE) using absorption photometric provided LOD values for captopril down to the ng mL^{-1} level in complex samples (plasma and urine) after chemical derivatization of the analyte using different reagents such as l-benzyl-2-chloropyridinium bromide [83], 2-bromo-2'acetonaphthone [84], 2-chloro-1-methylquinolinium tetrafluorborate [85], 3-bromomethyl-propylphenazene [86], and with the Ellman's reagent [87].

A few attempts bave been made to detect captopril and its degradation products using capillary electrophoresis without chemical derivatization, however, poorly detection (LOD at the mg mL^{-1} level) were achieved. Organic substances (1-2 naphtoquinone-4-sulfonic acid and 4-methyllumbelliferone) [88,89] have been proposed as probes as they generate highly photoluminescent products in the presence of captopril. Analytical methods based on these probes allowed LOD in the ng mL^{-1} level. Recently, a voltammetric method based on the catalytic reaction captopril on a multiwall carbon nanotube (modified with a cathecol derivative) was developed, enabling LOD of 7 ng mL^{-1} (32 nmol L^{-1}) [90].

1.5.2 Histamine

Biogenic amines or biologically active amines are organic bases with aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structures that, with the exception of physiological amines, are mainly produce by microbial

decarboxylation of amino acids in several foods [91,92]. Biogenic amines may be of endogenous origin at low concentrations in non-fermented food such as fruits, vegetables, meat, milk and fish. High concentrations of biogenic amines have been found in fermented foods as a result of a contaminating microflora exhibiting amino acid decarboxylase activity [93].

The biogenic amines acts as natural substances for growth of microorganisms and plants (reserve of nitrogen). They also play an important role in several physiological functions in human and animals, participating in the regulation of gastric secretion, the contraction and relaxation of smooth muscle. These amines are also biomodulator and stimulate sensory, motor and cardiovascular neurons [94,95]. Some crucial cell activities are also dependent upon biogenic amines. For instance, spermidine and spermine are involved in the synthesis of DNA, RNA and proteins, in the stabilization of membranes and are essential for growth and replication of cells [96]. Histamine, serotonin, dopamine, adrenaline and noradrenaline are psychoactive and act as neurotransmitters in the central nervous system. The histamine-containing neurons may participate in regulating water intake, body temperature and the secretion of antidiuretic hormone, as well as the control of blood pressure and pain perception. Tyramine, phenylethylamine, isoamylamine, dopamine, adrenaline, noradrenaline and tryptamine cause an increase in blood pressure by constricting vascular system and increasing the speed and force of cardiac contraction. Typically, histamine causes dilatation of small blood vessels, resulting in redness, decreased total peripheral resistance, pressure drop systemic arterial contraction and increased heart rate and speed increased capillary permeability [97].

According to the Food and Agricultural Organization the global sea food production is expected to be about 157.3 million metric tons and the value of seafood exports was estimated to be USD 138 billion in the year 2012 [98]. A major part of seafood comprises fish, which accounts for to at least 20% of the total animal protein consumed by 2.6 billion peoples around the world. Fish has high protein content (15-20%) with significant amounts of all of the essential amino acids. They also provide nitrogenous compounds, lipids, carbohydrates, minerals and vitamins. In certain countries such as Bangladesh, Cambodia, Indonesia, Sri Lanka and Japan, fish protein contributes more than 50% of the total animal protein consumed in the diets. Fish can be harvested from aquaculture

or from the natural marine stock. Aquaculture accounts for to as much as 80% of the world fish supply [99]. Specific conditions are required to store fish and other seafood in order to maintain its integrity, avoiding the danger of seafood born chemical intoxication, which, in turn, imposes a great challenge in terms of public health and trade [100].

Among the products of animal origin, fish is subjected to rapid deterioration upon harvest because spoilage bacteria are commonly present on the skin and in the digestive tract of fish [101]. The harvested fish should be chilled in ice or frozen immediately in order to prevent and minimize the growth of bacteria and the production of toxins. Freezing or chilling of the harvested fish may not be always feasible and fish may be exposed to ambient temperature for quite some time. Thus the quality of fish is mainly dependent on the handling after each catch.

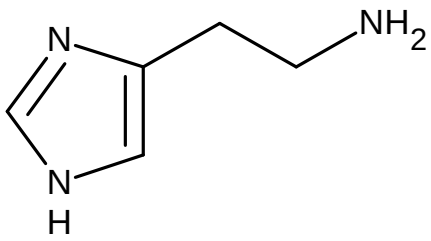


Figure-8 - Chemical structure of histmine

One of the major problems in consumption of fishes of the family *Scombroidae* (tuna, marakeral and bonito) and others from the nonscombrodae family such as sardines is the presence of histamine [102]. Histamine, (2-(4-imidazolyl) ethylamine), see Figure 8, is a biogenic amine produced from the decaroboxylication of histidine by the action of the bacterial enzyme histidine-decarboxylase. When histamine levels in fish (also valid for red wine and cheese) are larger than 500 mg kg^{-1}, it causes a food born chemical intoxication called histamine fish poisoning of HFP [103].

The major symptoms of HFP include nausea, vomit, diarrhea and allergies that last from several minutes up to 3 h after the ingestion of the spoiled food. The severity of these symptoms depends on both the amount of histamine ingested and sensitivity of the individual to histamine poisoning [104].

Non-favorable storing conditions induce the formation and accumulation of histamine as the bacterial action produces histamine–decarboxylase enzyme that turn histidine (present in the fish muscles) into histamine even at temperatures as low as 5 ^{0}C. Once histamine is produced it cannot be destroyed by heating or freezing.

The presence of histamine in foodstuffs has been used as an indicator for food quality control, especially in fish and fish products. Therefore, a relatively large number of analytical methods is available in the literature for determination of histamine in fish and fish products. The most widely used methods are based on liquid chromatography using fluorimetric determination of chemical derivatives of histamine produced by reaction with o-phthalaldehyde and dansyl chloride [105, 106] and by gas chromatography, which has been applied for determination of histamine even without derivatization [107]. Capillary electrophoresis makes use of the fluorimetric detection of histamine after chemical derivatization with O-phthalaldehyde and also direct detection without using chemical dervatization with a UV detector set at 210 nm [108-109]. Isotachophoresis has been used to improve separation of histamine from other biogenic amines exploring the differences in their pK_b. Enzymatic approaches, thin layer chromatography (with fluorimetric determination of derivatized histidine) and colorimetric methods have also been employed for quantification of histamine in fish [110-112].

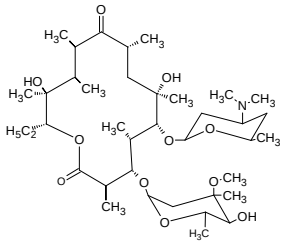
1.5.3 Aminoglycosides

Aminoglycosides are a large and diverse class of antibiotics that are characterized by one or more amino sugars linked by glycosidic bonds to an aminocyclitol component [113]. They may be classified according to the pattern of substitution of the cyclitol. The two most important sub-classes are the 4,5-disubstituted deoxystreptamine (for instance, neomycin) and 4,6-disubstituted deoxystreptamine (for instance, gentamicin, kanamycin and tobramycin). The structures of some of the important aminoglycosides are shown in Figure 9. Many actinobacteria (Actinomycetes), particulary related to the genera *Streptomyces* naturally produce aminoglyocosides. These organisms often produce a number of structurally related antibiotics simultaneously and the therapeutic product may

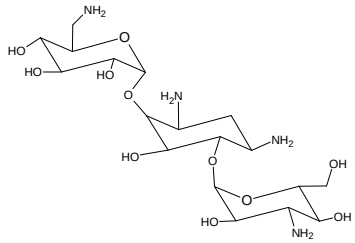
contain a mixture of active compounds [114]. The chemical synthesis of aminoglycosides is well established, but their production through fermentation is still the most economic route. The aminoglycosides are not effective against anaerobic bacteria but they are active against a broad-spectrum gram-positive and many gram-negative organisms making them useful in human and veterinary medicines [115]. However, the use of aminoglycosides presents adverse side effects like ototoxicity and nephrotoxicity.

In various types of fruits, such as apples, streptomycin (in the salt form) has been used to control bacterial infestation [116]. The use of aminoglycosides carries out the risk of their persistence presence in animal derived foods like milk and meat [117]. Their stability and high solubility in water make them potential pollutants in water bodies [118].

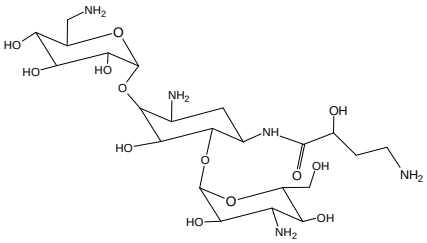
Concerns have arisen due to the adverse effects of aminoglycosides on human health, such as allergic reactions, and also to the microorganism resistance to antibiotics. For safety reasons of food for human consumption, the Council Regulation (EEC) no. 2377/90 (2000) of the European Union established the maximum residue limits (MRLs) for different aminoglycosides. For environmental waters no specific values for aminoglycosides and other antibiotic have been defined, but a general concern exist as, for instance, 1 ton of gentamycin is used in Germany for human medical purposes and little information is available about its occurrence in waste water and aqueous environment [119].



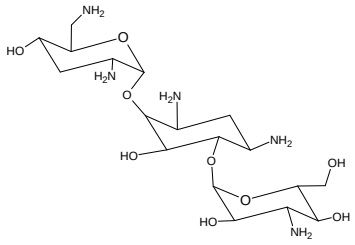
Erythromycin



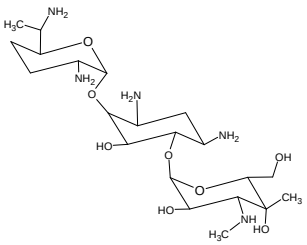
Kanamycin



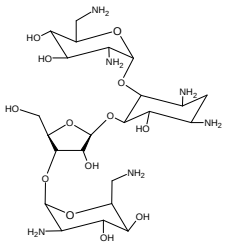
Amikacin



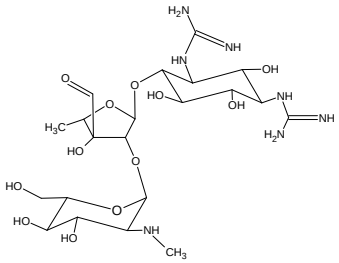
Tobramycin



Gentamycin



Neomycin



Streptomycin

Figure 9- Structures of some aminoglycosides and erythromycin antibiotics.

There are several methods described in the literature for determination of aminoglycoside antibiotics in different matrices. However, the lack of chromophore groups makes impossible the direct detection through their absorbance or through intrinsic fluorescence. Since in this work kanamycin was the aminoglycoside used as a model compound for sensing with quantum dots, the following revision focus on this aminoglycoside.

Chemical derivatization has been employed for the determination of total kanamycin as low as 60 µg mL^{-1} (1.0 x 10^{-4} mol L^{-1}) in pharmaceuticals. The reaction with acetylacetone-formaldehyde reagent in *N,N'*-dimethyl formamide enabled the formation of a derivative that strongly absorbs in the visible (maximum at 410 nm) [120]. The determination of six aminoglycoside antibiotcs, including kanamycin, was also achieved after the reaction with Eu^{3+}. The lanthanide photoluminescence at 616 nm was enhanced after binding with aminoglycosides, which not affected the photoluminescence at 592 nm. The ratio between these two photoluminescence signals corrected the response in function of the free Eu^{3+}, allowing the detection of at least 10 µg g^{-1} of kanamycin [121]. The inhibiting effect that kanamycin has on the reaction of lucigenin with hydrogen peroxide in a basic solution was also taken in advantage. Although not selective towards other aminoglycosides, such approach was employed in a flow injection system, allowing a claimed capability to detect kanamycin at concentrations as low as 1 x 10^{-13} mol L^{-1} in pharmaceuticals [122**].**

Separation of kanamicine from other aminoglycosides and even the separation of kanamycin A from their isomers (kanamycin B, C and D) in diverse samples (pharmaceuticals, varicella vaccine, raw materials and bacterial culture media) were achieved by reversed-phase high performance liquid chromatography. Either C-18 or cation-exchange resin were used as stationary phases. Detection was achieved by pre-column or post-column chemical derivatization with fluorescamine [123], 9-fluorenylmethyl chloroformate [124] and phenylisocianate [125] in order to enable photometric absorption or photoluminescence detection with absolute limit of detection (ALOD) in the tenths of ng range. Alternatively, LOD of 2.3 µmol L^{-1} (3.9 µmol L^{-1}) has been achieved by light scattering [126], amperometric detection using a gold electrode (ALOD in the low ng for 20 µL of sample) [127] and by indirect

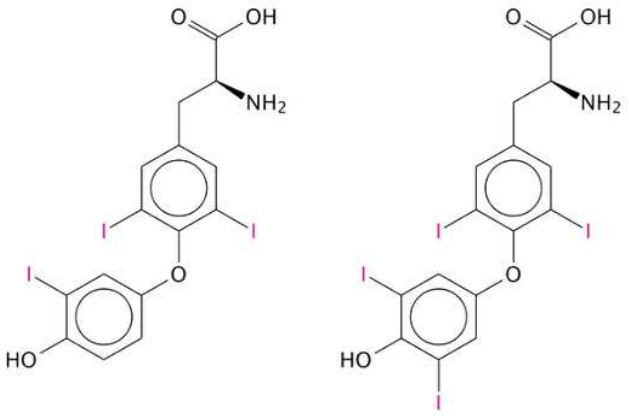
photoluminescence by the displacement of the fluorophor tryptophan (Tryp) from the non-photoluminescent $Cu(Tryp)_2$ complex (ALOD of 9 and 15 ng respectively for kanamycin A and B) [128].

Derivatization of kanamycin with *o*-phthalaldehyde and mercaptoacetic acid has been used to enable the absorption photometric detection (at 355 nm) in capillary electrophoresis (CE) analysis using a pH 10 borate buffer background electrolyte solution. Two approaches were used for derivatization. First, analyte was previously derivatized before introduced into the capillary where an on-line analyte concentration, using field amplified sampling stacking, was performed. The other approach was based on the reaction of the analyte with the derivatization reagents after they were injected into the capillary [129]. For complex samples such as serum from tuberculosis patients, a previous solid phase extraction (SPE) on a weak cation-exchange phase was performed. Limits of detection (LOD) as low as 2.0 µg mL^{-1} (3.4 x 10^{-6} mol L^{-1}) was achieved. Kanamycin was also determined by square-wave voltammetry using a mercury film electrode in injectable pharmaceuticals. Although the method enabled a LOD of 4.8 x 10^{-10} mol L^{-1} the use of mercury is nowadays discouraged [130].

Determinations of kanamycin using nanodispositives have also been accomplished. Surface plasmon absorption of gold nanoparticles are shifted to red in the presence of kanamycin due to electronic dipole–dipole interaction and coupling between plasmons of neighboring particles in the nanoparticle aggregates. This phenomenon was used to develop a method to determination of kanamycin by measuring the plasmon resonance light-scattering, which lead to a LOD of 2 x 10^{-9} mol L^{-1} in the analysis of spiked human urine [131]. The change in the absorption characteristics of gold nanoparticles aggregates has also been used to determine kanamycin and kanamycin derivatives after binding with a single-strand DNA aptamer with LOD of 2.5 x 10^{-8} mol L^{-1} [132].

1.5.4 Thyroxine

A hormone is a chemical substance produced by a specialized gland, which is carried to a target organ by blood stream or by other body fluids. The hormones can be grouped to several different chemical categories such as polypeptides or proteins, steroids, hormones derived from amino acids or fatty acids derivatives [133].



a. **b.**

Figure - 10- chemical structure of a. triiodothronine (T_3) and b. thyroxine (T_4)

The normal thyroid gland is a discrete soft body made up of a large number of vessels that produce, store, and release two key hormones: triiodothyronine, also called T3 and thyroxine or T4 (Figure 10). The numbers 3 and 4 refer to the number of iodine atoms attached to each hormone. Thyroid cells are the primary cells in the body capable of absorbing iodine, an essential nutrient. The thyroid takes in iodine, obtained through food, iodized salt, or supplements, and combines them with the amino acid tyrosine, converting them to T3 and T4. A healthy thyroid produces a proportion of about 20% of T3 and 80% of T4. T3 is the biologically active hormone that is used by the cells and it is several times stronger than T4. As needed, the body converts the inactive T4 to active T3 by removing one iodine molecule. This conversion process is called monodeiodination.

Thyroid hormones have a number of functions including: (i) enable cells to convert oxygen into energy, (ii) help the body to breathe normally, (iii) help the intestinal system to work properly, (iv) strengthen hair, nails, and skin, (v) help

the brain to function properly, (vi) help with normal bone growth, (vii) help the body process carbohydrates, (viii) aid in the proper functioning of muscles, (ix) enable proper sexual development and functioning and (x) help the heart pump properly and effectively [134].

A low secretion or even the lack of the production of the thyroid hormones (hypothyroidism) results in a number of health problems [135] and the treatment is based on the administration of thyroxine to the patient in order to achieve a regular maintenance of the hormonal deficiency. Levothyroxine (mostly prescribed drug) is an artificial thyroxine hormone most used for the therapy in thyroid dysfunction.

A limited number of analytical methods has been reported in literature for determination of thyroid hormones (T_4 and T_3) in diverse matrices. For example, the indirect detection was carried out from either the inhibition of luminol–iron (II) chemiluminescence or the enhancement of the electrochemiluminescence of Tris(2,2-bipyridyl)ruthenium(III)-NADH. Detection limits of respectively 0.1 mg L^{-1} (1.2 x 10^{-7} mol L^{-1}) and 5 x 10^{-8} mol L^{-1} were achieved [136,137]. Electrophoresis has also been utilized for simultaneous determination of thyroxine and triiodothyronine in pharmaceutical formulations. A carbon disk electrode was applied as working electrode and the 0.05 mol L^{-1} borate buffers was used as separation medium [138].

The use of high performance liquid chromatography (HPLC) for separation and detection of two thyroid hormones (T_3 and T_4) in dietary supplements enabled quantifications down to 0.002 μg mL^{-1} (2.9 x 10^{-9} mol L^{-1}) after pre-column derivatization with 4- fluoro-7- nitrobenzofuran (NBD-F) [139]. Alternatively, the indirect detection of thyroxine in urine has been achieved by spectrophotomeric detection (226 nm) of iodide after separation in an anion exchange AS4A column [140]. More recently, isotope-dilution liquid chromatography/tandem mass spectrometry method was applied for the pg mL^{-1} level determination of thyroxine in saliva [141].

The indirect photoluminescence detection of thyroxine has been achieved by measuring the quenching effect on 7-hydroxycoumarin and Eu(III)-(Pyridine-2,6-Dicarboxylate) Tris complex. LOD values of 3.4 x 10^{-8} and 2.0 x 10^{-5} mol L^{-1}, have been achieved [142-143]. Moreover, voltammetric methods based on carbon paste electrode modified with phenyl hydrazine and surfactants (SDS, CTAB,

Triton x-100) and glassy carbon electrode (modified with carbon nanotubes) were developed for determination of thyroxine with linear range from 2×10^{-4} to 1×10^{-3} mol L^{-1} [144].

1.6 Motivation and aims of the work

The main goal of the use of nanomaterials in analytical chemistry is to exploit their peculiar properties in order to improve well-established analytical methods or to develop methods for new analytes and for the analysis of different matrices. The use of nanoparticles may lead to improvements in figures of merit and might contribute with the overall reduction of the complexity of the procedures by minimizing the need for sample preparation and also contributes with the miniaturization/simplification of analytical systems. The use of nanomaterials is one important trend in analytical chemistry and may bring significant changes in this area. In this context, one of the most promising nanomaterials are the semiconductor nanoparticles (quantum dots), which have been used, for instance, as photoluminescent nanoprobes for the sensing of diverse chemical species. The Applied Spectroanalytical and Electroanalical Laboratory at PUC-Rio (LEEA-PUC-Rio) has dedicated part of their scientific efforts (human resources and funding) in such direction, since the year of 2009, in projects dealing with the use of quantum dots, gold nanoparticles and graphene. In 2012, the first scientific articles from the group reporting analytical applications of quantum dots have been accepted for publication (a total of three). The present Thesis is the first one of this research group relying exclusively on the use of nanomaterials in analytical chemistry. It describes part of the efforts made to master the synthesis and funcionalization of quantum dots (simple quantum dots at first) as the necessary commercially available quantum dots are quite expensive and their availability, especially in the case of surface modified quantum dots, is limited. Besides producing the quantum dots, the present work focused on the characterization of these nanomaterials using different approaches and techniques aiming to access their quality and to evaluate their potential as selective sensors. Analytical applications involving the use of the synthesized quantum dots as sensing probes in aqueous dispersions were proposed and their performance evaluated, however, because of the original orientation of the work and the lack of

time, it was not a goal to exhaust all of the possibilities of these methods and the procedures involved. Therefore, there is still room for improvements in these methods especially in terms of fully exploring them in the analysis of real samples.

The main goal of this work was to exploit the outstanding properties of water soluble thioglycolic acid-CdTe (TGA-CdTe), 2-mercaptopropionic acid-CdTe (2MPA-CdTe) and cystein-ZnS (cys-ZnS) nanoparticles as photoluminescent probes for sensing histamine, kanamycin, captopril and thyroxine in diverse samples. All the proposed optical probes relies on the strong photoluminescence from these materials. The aqueous dispersion of nanoarticles probes operate on the fact that under optimized experimental conditions, the photoluminescence emission intensity originating from the recombination of photogenerated electron-hole can be altered in the presence of these analytes of the interest.

The photoluminescence variations (responses) may be either quenching or enhancement in photoluminescence emission intensity. This concept provides the basis to exploit their properties as simple, inexpensive, sensitive and selective photoluminescence probes for small molecules that lacks strong chromophore groups. The proposed photoluminescence probes avoid the use of chemical derivatization procedures.

In order to achieve the main goal of the work, the following tasks were performed;

- Synthesis of carboxylic acid thiol capped CdTe and ZnS quantum dots;
- Characterization of these nanomaterials by various spectroscopic and microscopic techniques;
- Optimization of experimental parameters (concentration of quantum dots, pH of dispersion, time and temperature, etc) for probing of histamine, kanamycin, captopril and thyroxine;
- Detailed study of the interactions between quantum dots and analytes;
- Evaluation of the analytical potential of the optical probes;
- Study of selectivity in terms of possible interfering substances that may exists in samples of interest;
- Applications of proposed approaches in the analysis of diverse samples.