# Applications of nano-particles in optical chemical and biological sensors

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## ABSTRACT

Semiconductor nano-particles, or quantum dots, with their relatively high quantum yields, narrow luminescence spectrum, outstanding photostability and the ability to tune their optical properties, are ideal for biological tagging applications and a very powerful tool for chemical sensors. In this paper an overview of this rapidly expanding area of research is presented. Additionally, some results are shown, in the framework of optical oxygen sensors, which establish quantum dots as suitable temperature and intensity references for application in luminescence based chemical sensors.

**Keywords:** Quantum-dots; luminescence; optical chemical sensors; biosensors.

## 1. INTRODUCTION

The advent of nanotechnology, introducing control over matter to the nanometer scale, has created a new class of materials with exciting properties. This provides a new set of tools in a diversity of domains. In particular biochemical applications are already benefiting from the versatility of a variety of nanoparticles[1,2]. Fluorescent semiconductor nanocrystals, or quantum dots (QDs), are especially attractive in this area[3, 4]. Fluorescence is the base for a large number of bioassays and chemical sensing techniques[5]. The unique optical properties of nanometer scale QDs are highly favorable when compared to traditional fluorophores. The ability to tune their luminescence characteristics, by simple control of their size, combined with the high versatility of their chemical properties, allow the implementation of traditional techniques with superior performance and make possible an entirely new set of applications.

## 2. SEMICONDUCTOR NANOCRYSTALS

Quantum Dots are extremely small particles of semiconductor material, consisting of a few hundreds to a few thousands of atoms. Their small size is mainly responsible for their unique optical, electrical and chemical properties. This occurs due to quantum confinement. QDs are smaller than the Bohr radii of the material, the natural preferred distance between the positive and negative charges in the excited state. This happens because electrical charges cannot be separated by distances larger than the size of the dot and causes a readjustment of the electrons energy distribution according to the physical size. Thus quantum dots have larger bandgaps than the corresponding bulk semiconductor

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material and exhibit a mix between solid-state and atomic properties that can be tuned according to their size. Important properties like absorption and photoluminescent emission are size dependent and thus controllable[4, 6]. This makes them attractive for a wide range of applications.

These types of materials can be fabricated by different methods: lithography, epitaxy and colloidal chemistry. The last type is the most important in the context of optical sensors, since the resulting dots are more easily manipulated and suitable for use in solutions, or for immobilization in solgel and polymer matrices. QDs produced in this way are also known as semiconductor nanocrystals. Through a series of engineered reactions the semiconductor material is made to precipitate into a solution. The process is controlled, with the presence of stabilizing agents, in such a way that aggregation of the particles is arrested before they reach a certain critical size, while still within the quantum confinement regime. The final result is a colloidal dispersion, in which very small fluorescent semiconductor solid particles are suspended in the liquid solvent. However, these nanocrystaline QDs are very reactive and become easily polluted by solvent molecules or impurities. Impurities and surface defects provide quenching mechanisms that compromise the photoluminescence efficiency of the nanocrystals. In order to obtain chemical stability and high quantum yields, some stabilization strategies have to be followed. The most successful one consists in coating the surface of the semiconductor core with a protective shell of unreactive, transparent and structurally related material. Cadmium-Selenide (CdSe) QDs are usually coated with some layers of zinc sulfide (ZnS)[7, 8]. When provided with this passivating shell, semiconductor nanocrystals have a very bright and stable emission, and their photobleaching, which is a major problem with traditional dyes, is extremely small or non-existing. Further capping layers are usually added on top of this inert shell that provide increased stability, and can define the nanoparticle functionality. This also controls its solubility and sets its chemo or bio sensitive. Usually large inorganic molecules are used as a further passivating layer and as an interface with biomolecules or outside chemistry.

Currently, nanocrystal QDs are available in a wide variety of emission wavelengths[9-11]. Depending on the particles size, CdSe quantum dots emission can be continuously tuned from 450 nm to 655 nm, corresponding to a size range of 2 nm to 6 nm respectively. This means that any color in this range can be achieved, and because only the nanocrystals size changes, all of these particles are still CdSe, i.e. each can be prepared with the same quantum yield, surface chemistry and set of environmental sensitivities. When using traditional dyes, different emission wavelengths are provided by completely different chemical species that sometimes presents dramatically different quantum yields. There are practical limits to the size of the particles that limits the wavelength range achieved with a certain material. In order to cover a large range of wavelength we should use a variety of materials. For example, Cadmium-Tellurium (CdTe) QDs emit further into the red (600nm-725nm) while bluer emission is possible with Cadmium-Sulfur (CdS) (350nm-470nm). Infrared emission has also been reported (800nm-2000nm) using Led Selenide (PbSe) nanoparticles.

In contrast to traditional dyes, which have broad emission spectrums with a characteristic long red tail, nanocrystal QDs present a very symmetrical (Gaussian) and relatively narrow emission spectrum. Although natural broadening and even Doppler broadening can occur, the major source of emission broadening in QDs comes from their size distribution. In a colloidal dispersion the solid particles have approximately, but not exactly the same size. Because the emission of a QD is related to its size, the slight differences in size result in slight differences in the emission wavelength. The result of this is that emission spectrum of a certain sample will be much broader than the individual QD spectrum. Presently, size distributions with less than 5% variation are possible. This provides a FWHM of approximately 30 nm, which is quite narrow in comparison to traditional dyes. Additionally, with the development of more sophisticated fabrication techniques it will be possible to obtain FWHM of 20-25 nm. As the size changes, the lifetime of the excited state will change. This can be a useful feature in frequency domain techniques. For example, CdSe nanocrystal QDs have a lifetime in the 15-20 ns range.

Like bulk semiconductor materials, nanocrystal QDs will absorb any wavelength to the blue of the emission peak, i.e. any photon with energy higher than that of the bandgap will be absorbed. Moreover, the probability of absorption grows with increasing photon energy. This is because more and more valence-band/conduction-band transitions become possible. This provides QDs with a very broad absorption spectrum. This broad spectrum is a major feature when compared with organic dyes whose absorption spectra are relatively narrow, and in the vicinity of their emission (small stokes shift). This means that QDs can be excited by any optical source with higher energy than its emission peak. The excitation wavelength impacts the intensity of the resulting fluorescence, but not in the emission wavelength. Thus QDs will greatly benefit from broadband excitation, and unlike dyes, several QDs can be excited by the same optical source. This feature can contribute to reducing cost and complexity of multiwavelength systems.

All these properties make QD nanocrystals an attractive tool in a growing range of applications, from telecommunications[12], to sensors and even quantum mechanics[13]. In the field of optical sensors[4], the ability to tune quantum dots' unique optical properties and tailor their chemical and biological properties for biochemical applications

are particularly appealing[3, 14]. These would not only serve as alternatives to traditional dyes, but also as new tools with novel functionalities. The applications of nanocrystaline QDs to the field of optical sensors will be addressed in the following sections.

## 3. PHYSICAL SENSORS

Nanocrystaline QDs are most widely used in chemical and biological sensors, however, the behavior of their optical properties with temperature, make them excellent temperature probes with a wide range of applications.

CdSe QDs coated with a passivating layer of ZnS and entrapped in a polymer matrix (poly(lauryl methacrylate)-PLMA) were reported to have attractive properties for optical thermometry applications[15]. The 600 nm emitting nanocrystals, entrapped in a PLMA 5 mm thick disk, were subjected to temperature changes in a range of 200 °C, while excited with a 488 nm laser line. The monitoring of the resulting photoluminescence revealed that the wavelength maximum ($\lambda_{max}$), width, and intensity of this emission were all strongly temperature dependent. A blue shift in $\lambda_{max}$ of 20 nm was observed, while temperature decreased from 42 °C to -173 °C. In the same range, the FWHM decreased from 26 nm at higher temperatures to 22 nm at lower temperatures. The strongest effect was, however, observed in the photoluminescent intensity which increased by a factor of five as temperature decreased. In particular, for the near ambient temperature range (5 °C to 40 °C), the photoluminescent intensity decrease was linear and in the order of –1.3% per °C. It was shown that, in this particular range, the wavelength shift was small (≈2 nm) and the FWHM variation was negligible (<1 nm). All the changes were reversible with temperature. Good reproducibility was observed even after 3 hours of continuous irradiation, demonstrating the good photostability of QDs. This temperature behavior was essentially identical for QDs excited at different wavelengths (460, 530, and 580 nm) and in different matrices (polymer and solgel). This data establishes the suitability of QDs as good temperature indicators for sensing applications. The authors suggest the use of CdSe-ZnS QDs as an internal temperature reference in pressure sensitive paints. This type of paint is used in stationary wind tunnel tests. The surface under study is painted with an oxygen sensitive dye that under proper illumination provides a luminescent mapping of pressure distribution. The dyes luminescence, however, is invariantly temperature dependent and some temperature reference is needed. The fact that the fundamental emission mechanism of QDs is not sensitive to collisional quenching by oxygen makes them suitable for use as a reference in this kind of applications.

For the majority of applications, however, an intensity based measurement is not an absolute measurement and is always prone to error due to optical power fluctuations of various origins. In such situations it is possible to perform temperature measurements that are independent of optical power, by using the wavelength shift information. In order to evaluate this characteristic some experiments were performed. For this purpose CdSe-ZnS QDs, purchased from Nanoco, were used to dope non-hydrolytic sol-gel solutions. The doped sol-gel solutions were used to obtain either bulk glass samples or spin coated thin films. Two samples were tested in the experimental setup of figure 1.

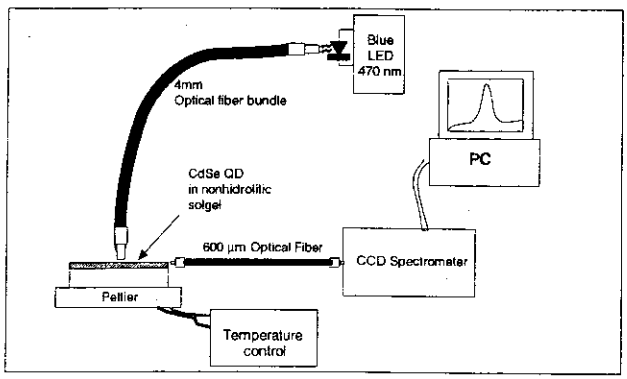


Figure 1: Experimental set-up used to test the temperature behavior of CdSe QDs.

Sample A, a 1 $cm^2$ (≈1 mm thick) bulk glass doped with a QD with $\lambda_{max}$ at 520 nm, and sample B, a thin glass film (≈5 µm thick), doped with a QD with $\lambda_{max}$ at 610 nm, deposited on a glass slide. A blue LED ($\lambda_{max}$ at 470 nm, from Nichia) was used as an excitation source. Optical power was carried from the LED to the samples through a 4mm

diameter fiber bundle. An Ocean Optics S2000 miniature spectrometer (with a 600 μm fiber cable), connected to a PC, was used for detection of the photoluminescence emission. The temperature of each sample was controlled using a peltier cooling device. The emission spectrum of each sample was recorded after thermal equilibrium was reached.

The samples emission spectrums were acquired while the temperature was changed from 11 °C to 48 °C. The spectral changes observed were reversible and independent of the direction of temperature change. Figures 2(a) and 2(b) show, respectively, the spectral response of sample A and B to such temperature changes.

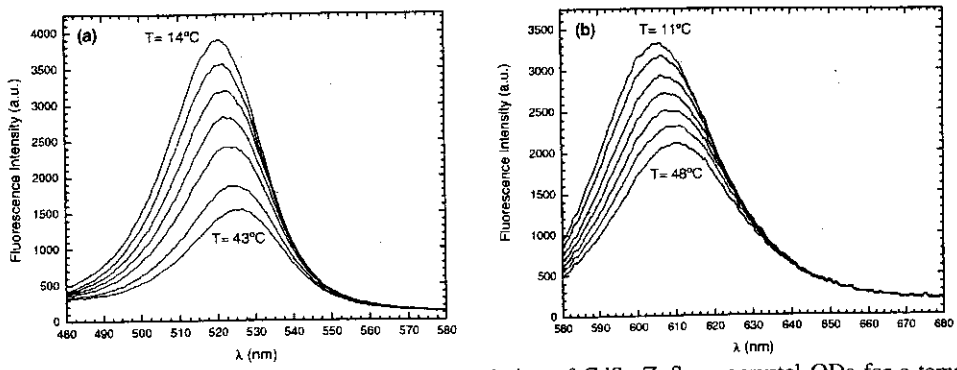


Figure 2: Temperature response of the luminescence emission of CdSe-ZnS nanocrystal QDs for a temperature range from 11 °C to 48 °C. a) Sample A (520 nm QD in solgel bulk glass); b) sample B (610 nm QD in solgel thin film).

Both samples show a behavior similar to the one reported in literature, however, it can be seen that the sensitivity of their photoluminescence intensity to temperature changes was quite different. In sample A, the rate of change in the intensity with temperature was approximately –1.6% per °C, and for sample B the rate was –0.7% per °C. On the other hand, the rate of change of the wavelength, towards shorter wavelength, as the temperature decreases was, in both cases approximately 0.2 nm per °C. This may indicate that the differences in sensitivity shown in the intensity response could be due to different immobilization environments, distinct signal-to-noise-ratios, etc. Therefore, in the face of the identical wavelength shift behavior, it is reasonable to state, that the intrinsic response of both QDs to temperature is similar. Also, deviations in linearity were observed in the intensity response of both samples, for the lower temperature range, that was due to some water condensation on the samples surface changing the coupling conditions (see figure 3(a)). This was not observed in the wavelength shift response, which was linear throughout all temperature intervals. Therefore, this mechanism is particularly well suited to perform self-referenced temperature measurements.

A simple detection scheme can be implemented in order to take self-referenced temperature measurements. If two signals, $S_1$ and $S_2$, corresponding to two narrow spectral windows on opposite sides of the spectrum are normalized according to $(S_1 - S_2)/(S_1 + S_2)$, the resulting normalized output will be proportional to temperature and independent of the optical power level in the system. This procedure was implemented using sample B. The software controlling the CCD spectrometer allowed us to obtain signals $S_1$ and $S_2$ corresponding to the spectral windows 595-600 nm and 620-625 nm respectively. Temperature measurements were performed using three different levels of the LED optical power ($P_{LED}$): 100% $P_{LED}$, 90% $P_{LED}$ and 80% $P_{LED}$. This was achieved by changing the LED drive current. Figure 3(a) shows the luminescence intensity response to temperature changes for each one of the three power levels. The corresponding normalized outputs can be seen in figure 3(b).

Comparison of figures 3(a) clearly shows that the luminescent emission response strongly depend on the change in the excitation optical power. This possible source of error can be eliminated by applying the proposed normalization scheme, Figure 3(b). The dispersion of the normalized signal was within the noise limit of the system. This shows that with this detection scheme QDs can be used as self-referenced temperature probes. Additionally, the availability of these nanoparticles in a wide range of wavelengths will allow the implementation of multiplexed temperature measurements. These features give added value to nanocrystal QDs as auxiliary tools in many applications, including optical bio and chemical sensors.

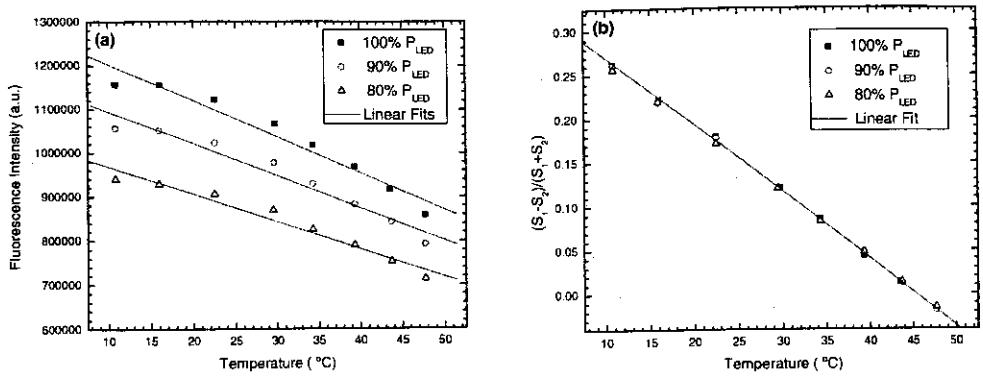


Figure 3: Calibration curves obtained for 100%, 90% and 80% of $P_{LED}$, for a temperature range from 11 °C to 48 °C. a) Luminescence intensity response); b) normalized signal response.

## 4. BIOSENSORS

The unique optical properties of QDs establish them as appealing alternatives to traditional fluorophores in the context of biotechnology, offering a potentially greater performance in fluoroimmunnoassays and bio-imaging applications[3, 16]. The introduction of QD technology in the biological domain involves its chemical stabilization, the control of its hydrophobic/hydrophilic properties and, finally, its conjugation with a biomolecule of interest which will define its functionality. All these processes will strongly impact the luminescence properties of the nanoparticle. Nevertheless, several successful strategies have been developed to covalently or non-covalently bind biomolecules to surface modified QDs, and some of these bioconjugated QDs are already commercially available[10, 11]. The size of QDs that are much bigger than single dye molecules will allow their simultaneous conjugation with more than one biomolecule. This provides them with the potential for increased sensitivity, multi-analyte detection with single QD and other new functionalities. This also raises some concerns about interference with the biomolecules mobility and functionality.

In one of the first reported applications of semiconductor nanoparticles in a bio-assay, CdSe-ZnS QDs covalently coupled to a protein presented 20 times more luminescent intensity in comparison to rhodamine. Additionally, the QDs were reported to be 100 times more resistant against photobleaching. This allowed the authors to perform ultrasensitive detection at the single-dot level. However, an on/off behavior of single dot emission was observed. This fluorescence 'blinking' was reported to affect some dye molecules, and can compromise measurements. While preserving the optical properties of the nanoparticles, they also demonstrated that the attached biomolecules were still active and able to recognize specific analytes. The first example of a QD *in-vitro* immuno-assay was the case when quantum dots were labeled with IgG antibodies, and they were recognized and agglutinated by polyclonal anti-igG. The authors also demonstrated cell labeling by transporting QD-transferrin bioconjugates into cultured HeLa cells via receptor-mediated endocytosis[17]. The potential of QDs, for multicolor assays, was first demonstrated by Bruchez et. al. when two different size CdSe-CdS nanocrystals, emitting green and red luminescence, were specifically bound to different parts of 3T3 mouse fibroblast cells, and were excited by a single optical source[18]. In this pioneer experiment some nonspecific binding was observed. Higher degrees of specificity are required in *in-vivo* applications where background biomolecules can generate false positives. Stabilization and conjugation techniques are rapidly evolving and higher levels of specificity have been achieved[19, 20]. Recently, Mattoussi et. al. prepared CdSe-Zn QDs with mixed protein adaptors to provide recognition to antibodies. The QD complexes prepared can be conjugated to a wide range of antibodies, thus providing a whole new set of highly specific probes. The obtained QD-antibody conjugates were used in several direct, sandwich and competition fluoroimmunoassays, to detect different toxins (staphylococcal enteroxin B, cholera toxin) and small molecule explosives. In the several assays performed, limits of detection were achieved that were, at least, as low as the ones obtained with dye-based assays[21].

The same authors performed the first study about fluorescence resonance-energy transfer (FRET) in QD-protein conjugates. QDs were used as energy donors to acceptor dye molecules that were attached to the conjugated proteins. This configuration allowed the exploration of the influence of parameters such as donor-acceptor spectral overlap and

donor-acceptor ratio on the FRET efficiency[22]. The resultant FRET enhancement will contribute for increased assays sensitivity.

These studies are intended to evaluate the possibility of quantifying analyte concentrations by fluorescence quenching. In sequence of the results obtained a prototype of a QD FRET sensor for sugar detection was presented. Each QD was conjugated, via His-Zn coordination, with 15 to 20 maltose binding proteins (MBP) and with further processing a QSY-9 quencher was bonded to each MBP. For two reasons the concentration dependent quenching of the QD emission was obtained; (1) the QSY-9 absorption overlapped perfectly with the emission of the 555 nm emitting QDs, and (2) its separation distance from the QD center was within the range of FRET critical radius. When maltose was added to the solution, the quencher was displaced and FRET was interrupted. An apparent binding constant of 7.0 μM was found from the titration curve with maltose. A response was obtained only with certain sugars, showing that the QD-MBP conjugate kept its specificity. These results confirmed QDs as excellent FRET donors, establishing a new tool for sensitive and specific biosensing[21].

Besides outperforming dyes in traditional bioassay applications QDs also introduced new possibilities. Their unique photostability and lack of cytotoxicity allow for long term *in vivo* imaging and monitoring of dynamic cell processes. Additionally, wavelength multiplexing for simultaneous multianalyte determination becomes possible. Recently the incorporation of different size QDs into polymeric microbeads with precisely controlled ratios has been reported. Controlling the types of QDs and their relative ratio in the bead makes it possible to create optically encoded microbeads which are highly suitable for multiplexing applications. This concept was demonstrated performing a multiplexed DNA hybridisation assay using triple-colored beads[23].

The realization of several fundamental bioassays have been demonstrated with QD-bioconjugates. Additionally, new functionalities were explored. In the face of these developments, QD-bioconjugate technology will soon transform semiconductor nanocrystals in a standard tool in biotechnology.

## 5. CHEMICAL SENSORS

### 5.1. Quantum Dots as Chemical Sensors

In contrast to the widespread use of QDs in biological applications, the use of semiconductor nanoparticles as chemical sensing probes is just starting to develop. Few reports have been made concerning the applications of semiconductor nanocrystals as luminescent indicators for detection of chemical species. However, without the passivating shell and protective capping, the luminescence of core QDs can be very sensitive to the surrounding chemical environment. This can be the path for using QDs as chemical sensors, provided some selectivity can be achieved. The desired selectivity can be controlled by chemically tailoring the outer surface of the nanoparticles.

Coating the QD surface with suitable ligands can have a strong effect on its luminescent response to specific chemical species[7]. Chen and Rosenzweig[24] were able to alter the selectivity of CdS QDs to respond either to zinc or copper ions, by changing their capping layer. They showed that, while polyphosphate-capped CdS QDs responded to almost all mono and divalent metal cations (showing no ion selectivity), thioglycerol-capped CdS QDs were sensitive only to copper and iron ions. QD luminescence was quenched by iron and copper, but was not affected by other ions occurring at similar concentrations. On the other hand, the luminescence emission of L-cysteine-capped CdS QDs was enhanced in the presence of zinc ions but was not affected by other cations like copper, calcium and magnesium. Some quenching by iron was observed, however. The quenching by iron, which interfered with the detection of copper and zinc, was attributed to an inner filter effect, and could be eliminated by adding fluoride ions to the solutions in order to form a colorless complex. Using this set of QD probes the authors established the selective detection of zinc and copper in physiological buffer samples, where several other metal ions were present. Quantitative measurements were performed where detection limits of 0.8μM and 0.1μM were achieved for zinc(II) and copper(II), respectively. This was claimed to be the first use of semiconductor nanoparticles as selective ion probes in aqueous samples. More recently, the detection of copper in physiological buffer solutions, with a detection limit of 10nM, was achieved by using CdSe-ZnS QD modified with bovine serum albumin[25].

The same principle can be applied for the detection of inorganic anions. The use of surface modified CdSe QD functionalized with *tert*-butyl-*N*-(2-mercaptoethyl)-carbamate (BMC) groups for the determination of cyanide was demonstrated by W.J. Jin et al.[26]. The luminescent emission of the modified QDs was found to be strongly quenched in

the presence of $CN^-$ ions. The presence of both static and dynamic quenching was revealed by a non-linear Stern-Volmer plot. In spite of this, an empirical fit to the quenching data allowed the determination of cyanide with a detection limit of $1.1x10^{-7}M$. The presence of other ionic species like $NO_3^-$ , $Cl^-$ , $SCN^-$ did not exhibit any significant effect on the luminescence emission of the modified QDs, demonstrating selectivity. However, some luminescence quenching was observed in the presence of the anions $I^-$ , $NO_2^-$ and $Br^-$ , but, the quenching by cyanide was much stronger and a very small concentration of these interfering ions (typical in environmental studies) should present no problem. Additionally, further surface modification can be applied in order to eliminate these cross sensitivities. Alternatively, surface modification can promote sensitivity to other ionic species. Overall, these results indicate the feasibility of using surface modified QDs as analytical probes for the determination of chemical species.

In a different approach, molecular imprinting technology is used to render the QDs photoluminescence sensitive to specific molecules[27]. If the synthesis of a polymer is made in the presence of an imprint or template molecule, cavities will be produced in the polymer, which are highly selective for the imprint. C.I. Lin et al. prepared molecular imprinted polymers (MIP) with photoluminescence property using CdSe QDs functionalized with 4-vinylpyridine. Several polymers containing the QDs were imprinted with different template molecules (Caffeine, Uric acid, L-Cysteine). The resulting solid polymers were ground to a fine powder and sieved. The template molecules were then extracted from the obtained powder. Detection of the analytes was performed by incubation of the MIPs with the corresponding template molecules in aqueous solutions. It was observed that the photoluminescence emission of the MIPs was strongly quenched in the presence of the respective templates however, no quenching occurred in the presence of other molecules. Strong quenching was observed for the caffeine imprinted polymer in the presence of Caffeine, but the presence of analogous molecular structures like theophylline and theobromine had no effect on the photoluminescence emission. Also, a control polymer, with no imprint, showed no change in the QDs photoluminescence. These results demonstrate that it is possible to couple QDs with the selective recognition capacity of MIPs, opening several possibilities of the application of semiconductor nanoparticles in optical chemical sensors.

### 5.2. Quantum Dots as auxiliary tools in chemical sensors

In the framework of optical chemical sensors QDs can be used, not only as sensors, but also as auxiliary tools for improvement of the sensing system performance.

In the case of sensors based on the detection of luminescent optical power there is, most of the times, the need for a reference scheme. Otherwise, it will be impossible to discriminate changes due to the mensurand, from the changes due to the optical power drift of the optical source, and changes in coupling conditions, etc. In the particular case of luminescence based optical sensors[5], leaching and photobleaching of the sensing dye is often the main source of optical power drift. To minimize these problems, frequency domain fluorometry is often used in optical oxygen sensors[28]. With this technique we can measure a phase shift that depends only on the excited state lifetime, and it is independent of oxygen concentration and optical power level. However, it has been reported that the photobleaching of the dye also changes the excited state lifetime and the phase response[29]. Also, sometimes an intensity-based system is often a simpler, less expensive alternative. Most common intensity reference schemes include the use of a second analyte-insensitive dye and ratiometric detection of two wavelengths. The ratiometric scheme can also be used with the detection of part of the excitation radiation. However, most dyes available suffer from photobleaching and the ratio of luminescence to the excitation radiation does not provide full reference.

Being bright and highly photostable, nanocrystal QDs can be excellent intensity references. Their wavelength can be tuned in order to avoid overlap with the emission spectrums of the sensing dye and excitation source. Control of the shell chemistry can provide insensitivity to the analyte. However, any QD whose absorption spectrum overlaps with the emission of the sensing dye cannot be used otherwise the QD emission would depend on the analyte as well. If these conditions are fulfilled, the QD luminescence intensity will only depend on the available excitation optical power. On the other hand, the fluorescence of the sensing dye is sensitive both to the analyte level and to the excitation optical power. Therefore, ratiometric processing, which can be made with a miniature CCD spectrometer or with an adequate set of filters and detectors, will output a signal which is independent of the optical power level along the system. Because the QDs can be immobilized in the same location of the sensing dye, all of the losses in the optical path to detection are compensated as well, providing a full intensity reference. This can be applied to multiple sensors in different locations as long as no spectral overlaps of any of the signals occur. Due to the different magnitudes in the

lifetimes of the QDs and the dyes, a compensation scheme in the frequency domain is also a possibility.

As already shown, in section 3, QDs are excellent temperature sensing probes. They can become temperature references to be used in luminescence based chemical sensors. Generally, such sensors rely on quenching as sensing mechanisms. Since quenching is a diffusion based process, the fluorescence characteristics of the sensing dyes, like oxygen sensing ruthenium complexes strongly change with temperature. In order to univocally determine the analyte level, knowledge of the temperature is also needed. The use of QDs with adequate spectral characteristics in combination with the sensing dye can provide a sensing system with the necessary temperature reference.

Some results regarding such applications will be given in the context of luminescence based oxygen sensors. The dynamic quenching of the fluorescence of organo-metallic Ruthenium complexes by oxygen is the most widely used sensing mechanism. Oxygen is a strong quencher of both the fluorescence intensity and of the excited state lifetime of these indicators. The absorption spectrum of these dyes overlaps well with the emission of blue LEDs. When excited with blue radiation (around 460 nm) a strong luminescent signal is emitted (centered at 620 nm). Additionally, the relatively long lifetimes of these complexes make them suitable for the implementation of frequency domain spectroscopy. Usually the sensing dye is immobilized in solgel glasses suitable for optical fiber or integrated optics configurations. Although oxygen sensors based on this technology are already commercially available there is still much room for improvement. Leaching and photo-bleaching of the indicators usually degrade the sensor performance. Also dependence on temperature and on the level of the excitation optical power should be compensated. As it was mentioned, QDs technology has the potential to solve many of these problems.

An experiment was made to demonstrate the application of QDs as an intensity reference in an optical oxygen sensor. The experimental setup is shown in figure 4.

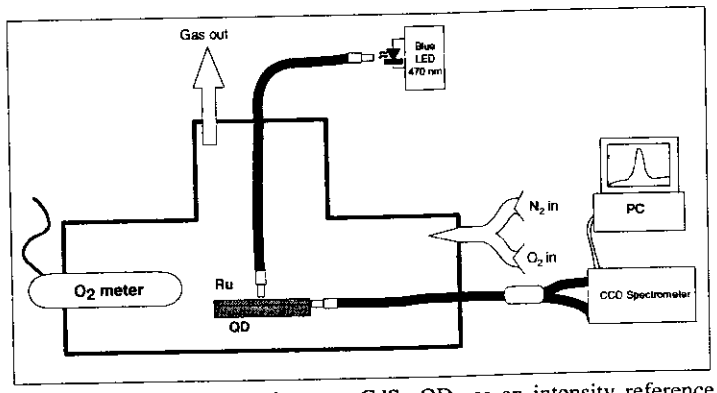


Figure 4: Experimental setup used to test CdSe QDs as an intensity reference in a luminescent oxygen sensor.

A thin film of porous sol-gel glass doped with [Ru(bpy)$_3$]-Tris (2,2'-bipyridine) ruthenium (II) chloride hexahydrate was used as the oxygen sensor. In order to produce the sensing film, a doped sol-gel solution was spin coated in a thin glass slide. The reference was a thick glass film doped with a CdSe-ZnS QD, with an emission peak at 520 nm, it was produced by placing a drop of doped non-hydrolytic sol-gel solution onto a glass slide. Both films were cured using adequate thermal treatment. The two samples were placed inside a sealed chamber. A supply of nitrogen and oxygen was released into the chamber, to allow control of the $O_2$ level inside, which was monitored using a conventional oxygen meter. Excitation was performed with a blue LED (470 nm, Nichia). The blue radiation was directed to the sample by a 4mm diameter fiber bundle. The detection was performed using a 4mm diameter Y splitter fiber bundle, which guided the sensor output into a two channel S2000 Ocean Optics miniature CCD spectrometer. Although the CCD had a built in long pass filter in one of the channels (cut-off at 550 nm), the detection fiber was oriented at 90° to the excitation fiber, in order to avoid noise from the strong excitation radiation.

In order for the reference scheme to be effective the QDs' emission must be independent of the oxygen level. For this purpose, the sol-gel was cured to obtain a non-porous glass with no permeability to oxygen. Additionally, the protective chemical shell of the QDs prevented oxidation. Finally, the spectral characteristics of the QDs are such that the radiation emitted by the ruthenium complex was not absorbed by the dot. Because of these considerations oxygen had no effect on the QDs emission, which is dependant only on the exciting optical power.

In order to verify these properties, two simple tests were performed. First, the output power of the LED ($P_{LED}$) was changed, by changing the drive current, in a constant atmosphere of 20% $O_2$. Second, the oxygen level of the chamber was changed while the drive current remained constant. The results of these tests can be observed in figures 5(a) and 5(b). As expected, when $P_{LED}$ was gradually decreased from 100%, to 82% and then to 63%, the LED, QD and the ruthenium emission decreased in the same proportion. However, when the oxygen level was increased from 0% to 20% and then 100%, only the ruthenium emission changed. The QDs emission remained constant and proportional to the LED output.

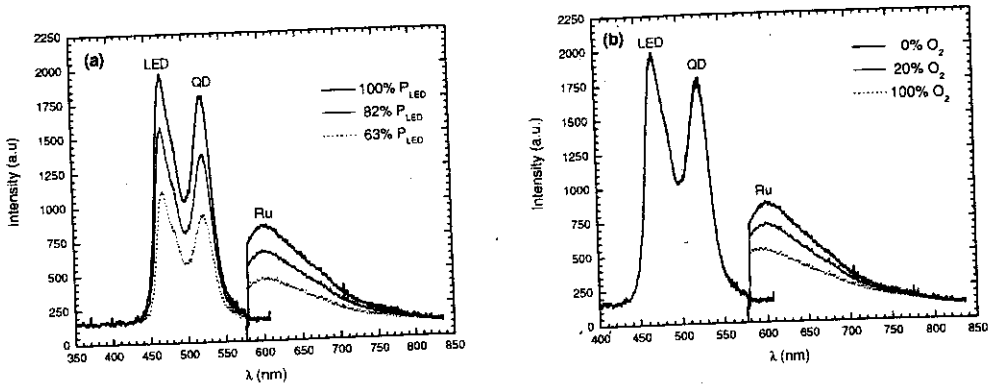


Figure 5: Behavior of excitation source, LED, reference QD and oxygen sensor (Ru) when: (a) $P_{LED}$ decreased from 100% to 82% and 63% in a 20% $O_2$ atmosphere; (b) The oxygen level changed from 0% to 20% and 100%, with constant $P_{LED}$.

The spectral distribution of the LED, QD and of the sensing dye allowed easy discrimination between the sensing signal and the reference signal. Using the CCD spectrometer and adequate software, it was straightforward to acquire each one of these signals and perform the necessary operations in order to obtain an output that was insensitive to the level of excitation optical power. The ratio between the ruthenium output ($P_{Ru}$) and the QD output ($P_{QD}$) would be independent of optical power changes, while still responding to oxygen level variations. In order to demonstrate this, some oxygen measurements were performed while $P_{LED}$ was modulated to change very slowly from 100% to approximately 70%. Figure 6 shows the response of the signals $P_{Ru}$ and $P_{Ru}/P_{QD}$ to $N_2/O_2$ saturation cycles. The variation of $P_{LED}$, in the same interval, is also shown.

It is visible that both $P_{Ru}$ and $P_{Ru}/P_{QD}$ responded to oxygen changes, while, $P_{Ru}$ was also strongly affected by the excitation power variation. The reference scheme worked effectively and the referenced signal responded only to oxygen variations being insensitive to a 30% excitation power variation.

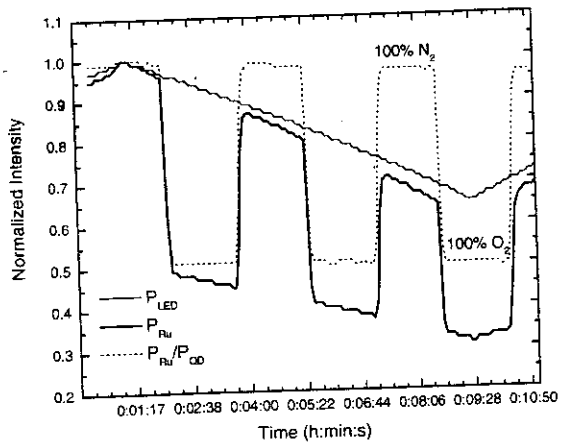


Figure 6: Sensor response ($P_{Ru}$ and $P_{Ru}/P_{QD}$) to $O_2/N_2$ saturation cycles while $P_{LED}$ changed slowly from 100% to 70%.

Further tests showed that, a decrease of 82% in the ruthenium fluorescence that was caused by a decrease of the excitation optical power changed the reference signal by only 4%. The immunity could be further improved if a mathematical operation such as $(P_{Ru}-P_{QD})/(P_{Ru}+P_{QD})$ was used rather than a simple ratio. In this case the reference signal only changed 2.7% after an 82% decrease in the LED optical power. In this particular configuration, the slight overlap between the spectrums of LED and QD prevents further improvement. When using a smaller detection fiber (400μm diameter) that is placed in a position so that no LED radiation could be collected, the highest variation observed was smaller than 1.5%, which was within the noise level of the signal. The same result can be obtained with more selective filtering or using a quantum dot with a longer wavelength and no overlap with the excitation source spectrum. These results clearly demonstrate the feasibility of using QDs as an intensity reference in luminescence based optical sensors.

In section 3, it was shown that both the peak wavelength and the luminescence intensity of QDs change in a linear way with temperature. This established QDs as suitable temperature probes for sensing applications. This feature is not incompatible with the intensity reference scheme presented. In section 3, it was demonstrated that the wavelength shift can be used to measure temperature independent of optical power level. A processing scheme can be implemented based on this technique to extract the temperature information from the QD wavelength shift. With such information, the change in QD luminescence intensity that is due to temperature could be accounted for, and any power fluctuation due to any other cause would still be detected for further compensation. With this kind of scheme it would be possible to provide simultaneous compensation for temperature and optical power drift with a single QD reference. Work is in progress to implement such scheme.

## 6. CONCLUSION

The introduction of semiconductor nanocrystals technology in the field of chemical and biological sensors is a relatively new trend. However, the applications of QDs in many fundamental bio-assay, bio-imaging and chemical sensing techniques have been successfully demonstrated. In addition to providing several advantages, when compared to traditional dyes, QDs have introduced a new set of possibilities, from increased multiplexing capabilities to long term imaging of live cells. In this paper we have explored one of these possibilities by incorporating QDs in an optical oxygen sensor. We have shown that this new oxygen sensor can simultaneously correct for the effect of intensity as well as temperature fluctuations due to the intrinsic properties of QDs. This establishes QDs as auxiliary tools with great potential to solve many problems in sensing systems.

Recent progress in the application of QDs in the field of biochemical sensors, together with a substantial worldwide research effort in the development of new QDs will surely establish semiconductor nanocrystals as high performing bioanalytical tools and provide new capabilities for the physical, biological and chemical sensors. In addition, the development of immobilization techniques of QDs in solid matrices, like sol-gels and polymers, facilitates their use in integrated optics and fiber optics sensors, which is an important step towards remote monitoring in environmental applications.

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