

CdS nanocrystals for laser scanning microscopy

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In this paper we report the synthesis and characterization of CdS semiconductor quantum dots for potential use in bioimaging. Nanocrystals structure and their mean size were determined by means of XRD. Photoluminescence characteristics of the nanocrystals were investigated using TPE laser scanning microscopy.

(Received February 21, 2010; accepted April 11, 2010)

Keywords: CLSM, TPE, XRD, CdS nanocrystal

1. Introduction

Semiconductor nanocrystals (NCs) with optical properties set by the quantum confinement effect are currently being studied in both physics and chemistry. Because these new nanostructures are not limited by the rules of chemical binding, when they are included in artificial molecules or solids they offer intriguing possibilities that are not possible in natural molecules and solids. II-VI semiconductor NCs are of great technological interest as thin film electroluminescence devices [1] and for optical amplifiers in telecommunication networks, due to their strong bandgap luminescence. The NCs large optical nonlinearities with fast response times can be suitable for ultrafast all-optical switching elements too [2].

Nanoscale materials become more and more the object of intense investigation with the intention of further use in cancer diagnosis and therapy [3]. Each synthetic route for NCs fabrication has its own advantages and limitations, and both synthesis and preservation are important from the application point of view. But, with a proper setup of the processing parameters, the chemical methods can provide inexpensive and reliable means for the fabrication of such NCs reaching an efficiency level not far behind the MBE. Prime motivation for this choice includes simplicity, large-scale production, low-cost infrastructure, and the possibility of doping, coating and reducing the local surface-trap states. Almost all the II-VI semiconductors got a direct-bandgap and allow the manipulation of properties by stoichiometry control. Thus, nanocrystalline semiconductor materials such as CdS have attracted great interest mainly because of their theoretical importance and significant potential applications [4]. Few reports describe their preparation as photo-luminescent probes [5].

Modern bioimaging techniques like PET and CT have an image resolution restricted by a large investigation volume that should be scanned in a limited amount of time. The confocal laser scanning microscopy (CLSM) and TPE, parts of the optical microscopy family can

provide an improvement in bio-image resolution [6] that cannot be neglected, especially for research purposes.

The optical microscopy for cell study relies heavily on the fluorescence techniques, as a method to obtain contrast with a high degree of sensitivity coupled with the ability to specifically target structural components and/or dynamic processes in chemically fixed as well as in living cells or tissues. The important contribution of CLSM, when is used in biology and medical investigations is that one can perform "optical slicing" with access to the sample's volume [3], similar to tomography but on a smaller scale.

Unfortunately, one of the major drawbacks of CLSM used for investigations in fluorescence regime is that during the excitation of the fluorescent dyes, the whole thickness of the specimen is affected by every scan and out-of-focus fluorescence is generated, although not detected in the final image [6]. This will generate a large amount of photobleaching and phototoxicity that is not truly necessary and can affect the quality of the biological observation. In this context, the use of a two-photon excitation (TPE) allows alleviating these problems and opens new possibilities. In the field of biological and medical imaging, at the optical-resolution level, among the most promising and very fast growing areas are the two-photon excitation microscopy and other nonlinear techniques [3,6]. The TPE belongs to a class of imaging techniques that allow the observation of biological specimens in conditions that are similar to their natural environment and therefore the possibility of understanding the complex relationship between the biological structure and its function is increasing [7]. Only after the advent of new mode-locked femtosecond lasers these new bioimaging possibilities are finally available.

In general, two-photon microscopy has a radial resolution comparable to that of one-photon conventional microscopes. The most interesting aspect is that in TPE microscopy, the point spread function or psf is axially confined (on z, the optic axis). This aspect implies that those regions away from the focal volume of the objective lens do not suffer photobleaching and do not contribute to

the signal detected when a TPE scheme is used [3]. Because they are not involved in the excitation process, a confocal-like effect is obtained, without the necessity of a spatial filtering technique, implemented by means of a confocal pinhole in CLSM.

For bioimaging purposes, the main materials requirements are water dispersibility, biocompatibility, chemical stability and robust optical properties. When a multilabeling protocol is used in the study, different fluorescent markers will light up different cellular structures with a separate detection implemented for each one of them. The emission spectrum of a molecular fluorescent marker is usually quite extended and the presence of several markers in a single observation will require a compromise in the detection scheme. This potential problem can be solved with markers that have a narrow emission spectrum, like the semiconductor NCs. These semiconductor engineered markers exhibit size dependent properties, such as a blue shift of absorption onset, a change of electrochemical potential band edge, and an enhancement of photocatalytic activities, with decreasing crystallite size [8]. Nanoparticles (NP) like CdS, with properties that can be precisely tailored by chemical methods and engineered to replace the molecular probes will be an important new workhorse in biotechnology.

The cytotoxicity of CdS quantum dots (QD) is considered to be caused by the presence of free cadmium (NP core degradation), free radical formation, or interaction of QDs with intracellular components, leading to loss function. *In vitro* studies suggested that the cytotoxicity was more pronounced with smaller positively charged QDs (2.2 ± 0.1 nm) than with larger equally charged QDs (5.2 ± 0.1 nm) at equal concentrations [9].

The markers luminescence is crucial in the field of optical probing. Due to the large surface-to-volume ratio of these NCs, the most common reason for poor luminescence efficiency is non-radiative recombination of light-generated charge carriers at surface traps. Elimination of these traps can be achieved either by proper chemical modification of the particle surface or by growing an inorganic passivation shell around the semiconductor core [10]. This can be used to implement the NP's specific binding, too.

In this paper we report the synthesis of CdS NCs through a chemical method. These NCs were investigated for crystal size and structure by XRD. The optical response was recorded with a broadband multiphoton laser scanning microscopy system. Results are discussed from the bio-imaging point of view.

2. Experimental

Reactants used in the synthesis were cadmium nitrate tetra hydrate $\text{Cd}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ from Aldrich and sodium sulphide nona hydrate ($\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$) from Fluka with analytical grade purity. The water used in the experiments was Millipore filtered.

Cadmium ions in the solution were obtained from the solubilization in purified water of salt as cadmium nitrate, and were mixed with a solution of reducing agent such as sulphide ions (Na_2S). Colloidal cadmium sulphide particles were prepared by mixing quickly (at 400 rpm) the two solutions. The system was purged with nitrogen gas for 30 minutes to prevent the photocorrosion and the formation of the colloidal sulfur. The aqueous solutions containing $\text{Cd}(\text{NO}_3)_2$ and Na_2S were freshly prepared and were used over a short period of time from their preparation.

After the formation of CdS particles, the water present in the system was evaporated under vacuum. Adding ethanol, the impurities were solubilized and the CdS NPs were separated by centrifugation. The CdS NPs were repeatedly washed with ethanol and then kept under vacuum in order to obtain CdS in powder form.

The structural characterization of the NPs was carried out by analysis on the diffraction pattern recorded in a Bragg-Brentano configuration, with a high-resolution powder diffractometer HRD 3000 from ItalStructures (GMR). The parallel incident X-ray beam was monochromatic ($\lambda = 1.541 \text{ \AA}$, GM) obtained from a Philips long fine focus X-ray tube.

Optical properties were studied on a Leica TCS SP2 microscopy platform that allowed us to record the PL emission spectra with a 4 nm resolution. PL Fluotar Leica objective 40X NA 0.75 was used.

For the TPE effect, a mode-locked Ti:sapphire Tsunami femtosecond laser pumped by a 5W Millennia Vs diode-pumped cw laser from Spectra Physics was used. This laser system is tunable over the 700 nm – 900 nm wavelength range with a pulse repetition rate of 80 MHz and 80 fs pulse width.

3. Results and discussion

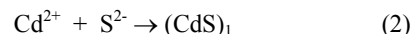
The obtaining of CdS particles results from the reaction between the metal ions (Cd^{+2}) and sulphide ions (S^{2-}) as follows:



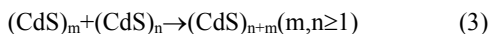
It has been experimentally documented that the precipitated uniform particles formation involves two distinct dynamical stages [11]. In the first process nanosize crystalline precursors, primary particles are nucleated in supersaturated solution, while in the second process, these primary particles aggregate into larger colloids, secondary particles [12].

The formation mechanism of these particles is similar with the one described by Towey and coworkers [13]. The reaction of CdS NPs involves three steps: the chemical reaction step, the nucleation step and the particle growth step.

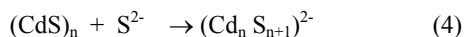
Formation of the CdS species:



Formation of aggregates with higher agglomeration numbers from reactions of small particles:



Growing of particles by addition of Cd^{2+} and S^{2-} ions:



The XRD pattern of the CdS NCs is presented in fig. 1. Peaks are broadened indicating that the crystals are very small in size. Data were associated to ICDD Card 2-549, corresponding to the Greenockite mineral.

NCs mean size is reported using the recorded diffractogram and the Debye-Scherrer formula:

$$d = \frac{0.9\lambda}{w \cos \theta} \quad (6)$$

where: d - crystallite mean size, λ - wavelength of the monochromatic X-rays beam ($CuK\alpha = 1.541 \text{ \AA}$), w - FWHM of the prominent diffraction peak, θ - diffraction angle for the (110) peak.

The average particle size of the CdS QDs was found to be: $66.7 \text{ \AA}$. This size is suggesting that the confinement regime is not very strong and that these NCs will have a better cytotoxicity behavior than the smaller ones, very well for optical probing.

The identified spatial group of CdS QDs in the international Hermann-Mauguin notation was P63mc. Within the hexagonal (wurtzite) crystallographic system, the Rietvelt calculated unit cell parameters were: $a=b=4.122 \text{ \AA}$ and $c = 6.743 \text{ \AA}$. The instrumental broadening is taken into account automatically.

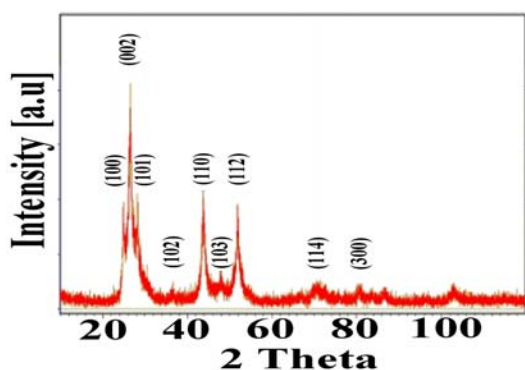


Fig. 1. XRD pattern of CdS NCs at room temperature, in atmospheric pressure.

For small CdS QDs, after their XRD investigation, Banerjee et al [14] have reported a cubic (zinc blend) structure. In this case when crystal size increases, the structure transform from cubic to hexagonal with the appearance of a weak peak {102} close to $2\theta=37^\circ$ and a {103} peak between the {110} and {112} peaks (Fig.1).

The idea of a wurtzite structure with a cubic core is supported by the low intensity of the {103} peak which in a pure wurtzite crystal is higher than the intensity of the neighbor {110} and {112} peaks. A sketch of this behavior is presented in Fig. 2 for a presumable spherical NC. The core boundaries and the mechanism that triggers the phase shift are not fully understood.

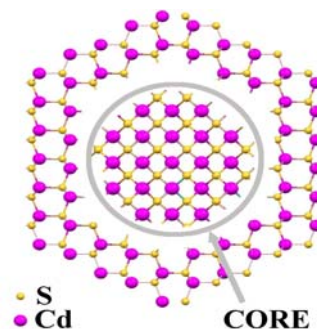


Fig. 2. Schematics of a CdS sectioned NC.

In CdS QDs, quantum size effect occurs as the crystallite diameter is comparable or below the exciton diameter of 5-7 nm [15] when both electrons in the conduction band and holes in the valence band are confined spatially. As a result of the confinement regime, the lowest energy of optical transitions from the valence band to the conduction band increases or in other words there is an increase in the effective band gap. In order to make these transitions possible a bigger or blue shifted energy will be required. The amount of maximum and necessary extra-energy was approximated using the framework of the Effective Mass Approximation [16]:

$$\Delta E = E_{gNANO} - E_{gBULK} = \frac{h^2}{8MR^2} \quad (7)$$

where: h is Planck's constant, R is the mean radius of the particle and M is the reduced effective mass of the system ($M=1.92 \times 10^{-31} \text{ Kg}$ - wurtzite CdS [14]). We have used the previously recorded XRD investigation and the determined mean particle size in order to choose the proper excitation wavelength in our laser scanning microscopy (LSM) study. Taking into account the results of the XRD investigation, the amount of energy shift can be approximated at $\sim 0.16 \text{ eV}$. In the case of bulk CdS, the effective band gap is $\sim 2.42 \text{ eV}$ [15], with the absorption edge-threshold located close to 514 nm. With the NC confinement regime and the increased effective band gap, the absorption edge will be blue-shifted at $\sim 480 \text{ nm}$ in one photon excitation regime or an equivalent 960 nm in two-photon excitation regime. Keeping this in mind, the Tsunami femtosecond laser system was set up at 730 nm. This setup of the laser system can provide sufficient excitation for the CdS NCs. The output laser power measured in femtosecond regime at this wavelength was 630 mW. For the PL spectrum, the NCs were deposited on a microscopic glass, at room temperature in atmospheric pressure (Fig. 3).

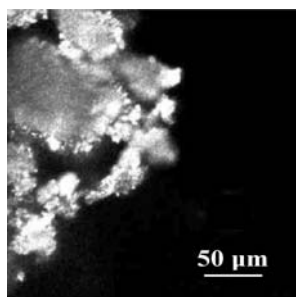


Fig. 3. CLSM image of CdS NCs prepared for TPE observation.

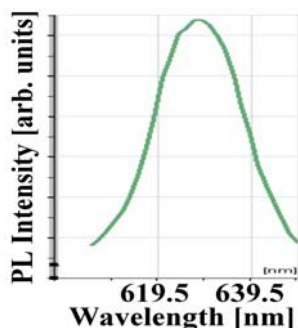


Fig. 4. PL photoluminescence emission spectrum of the CdS NCs.

The recorded PL spectra for the CdS NCs investigated with TPE microscopy technique, have a FWHM of 25 nm with a symmetric distribution around the central maximum located at 625 nm (fig.4). The spectral profile is not skewed towards the longer wavelengths, typical signature for QD structures. This emission spectrum was recorded in a point by point scanning fashion with the excited volume for each point that is producing signal, estimated at ~ 0.1 femtoliter. In the recorded optical spectrum, the presence of crystal impurities or NCs blinking was not detected.

A large bandgap of the CdS QDs can be an inconvenient in biological labeling because this might require UV excitation. To solve this, TPE uses NIR wavelengths and obtains the same equivalent UV excitation through non-linear absorption. Furthermore, in LSM this will allow a deeper penetration than in the conventional UV case and this is very convenient for biological tissue studies. Also, the future biological markers will inherit the strong optical properties of the CdS semiconductor. The excitation conditions in LSM are several orders of magnitude more severe than in the case of common optical microscopy techniques. Only QDs with a high quantum yield (PL-QY), like CdS can be useful for LSM bioimaging. The possibility of stimulating multiple QDs in the same specimen with a single excitation wavelength, obtaining PL signals with a good signal to noise ratio is the reason to consider these artificial structures as excellent candidates for the LSM multiple labeling experiments.

4. Conclusions

We obtained CdS semiconductor NCs through a simple and efficient method. The NCs size and crystal structure was determined by means of X-Ray diffraction. A mean size of 6.7 nm for the CdS NCs implies a reduced cytotoxicity when comparing with very small QDs. The determined crystal structure is wurtzite with a nanocubic core. Photoluminescence characteristics were investigated with a Leica TCS SP2 and a modern TPE optical microscopy technique. The optical PL response is strong and has a symmetric distribution with a maximum at 625 nm, 25 nm FWHM and not skewed towards longer wavelengths. No blinking or crystal impurities presence was observed in the optical recorded spectrum. All these facts are a good premise for the future use of the synthesized semiconductor NCs in LSM bioimage studies.

Acknowledgements

The present work has been carried out with financial support from National Program of Research PN-II-IDEI-PCE, Grant 1566/2009, UEFISCU CNCIS.

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