Journal of Luminescence 174 (2016) 17–21

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Full Length Article

A pH dependence study of CdTe quantum dots fluorescence quantum yields using eclipsing thermal lens spectroscopy

C. Estupiñán-López [a], C. Tolentino Dominguez [a,d], P.E. Cabral Filho [a,b], B.S. Santos [a,c], A. Fontes [a,b,*], R.E. de Araujo [a,**]

[a] Laboratory of Biomedical Optics and Imaging, Federal University of Pernambuco, Recife, PE, Brazil
[b] Biophysics and Radiobiology Department, Federal University of Pernambuco, Recife, PE, Brazil
[c] Pharmaceutical Sciences Department, Federal University of Pernambuco, Recife, PE, Brazil
[d] Centre for Telecommunication Studies, Pontifical Catholic University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article history:
Received 13 June 2015
Received in revised form
29 December 2015
Accepted 30 December 2015
Available online 29 January 2016

Keywords:
Quantum dots
Thermal lens
Quantum efficiency
Emission spectroscopy

ABSTRACT

In this study we evaluated the absolute fluorescence quantum yield (Φ) of hydrophilic CdTe QDs in function of different pHs, modified from the alkaline to acid, by using two different chemicals compounds, the mercaptosuccinic acid (MSA-the stabilizing agent of the QDs synthesis) or hydrochloric acid (HCl). The pH control of QDs suspensions is essential for the use of fluorescent nanoparticles in biological systems. We used the eclipsing thermal lens spectroscopy technique to determine the absolute fluorescence quantum yield values. The results showed variations on the Φ values as a function of the pH, which allowed a better understanding of QDs emission characteristics, establishing parameters for their use in biomedical applications such as optical images of biological systems, immunoassays, flow cytometry, biosensors and others.

1. Introduction

In life sciences, the development and characterization of new materials are of great importance, specifically those that enable their innovation and improvement for purposes related to biological process comprehension, diagnosis and therapies. In this context, fluorescence based techniques such as microscopy for imaging living cells, tissues and small animals; flow cytometry; fluoroimmunoassay and optical biosensors [1–4], which explore fluorescent probes, have been shown to be important tools to understand biological mechanisms [5]. Fluorescent probes allow the development of fluorescence based assays with high sensibility, which can provide the identification and quantification of biomolecules with high specificity. Among the fluorescent probes, the Quantum Dots (QDs) present valuable advantages, which have allowed us to enjoy the full potential of fluorescence. QDs are fluorescent colloidal nanocrystals composed by semiconductor materials, with dimensions ranging from 2 to 10 nm, which present unique optical properties, as: (1) broad absorption bands, allowing the excitation of multiple QDs' colors by a single light source. This advantage is also interesting for experiments with QDs as FRET (Forster Resonance Energy Transfer) donors for minimizing the possibility of direct excitation of the acceptor [6]; (2) high resistance to photobleaching, approximately 100 times higher than organic fluorophores, allowing to follow in time several long and lasting biological processes, and (3) narrow emission spectra, permitting multiplex analysis [7–9]. Due to these special properties, QDs have been applied as fluorescent probes for molecular, cellular and *in vivo* imaging; cytometry and fluoroimmunoassays; for biosensors and also, more recently, as photosensitizers in photodynamic therapy (PDT) [10].

QDs have a complex structure where the core, responsible for the fluorescence emission, is coated with a passivation layer that offers the photostability and provides the quality of the emission of these nanocrystals. Besides, hydrophilic QDs present a functionalization layer conferred by the stabilizing agents that contains chemical functional groups, which also allow QDs conjugation with biomolecules. The bioconjugation provides specificity to the optical fluorescence techniques, leading to particular interactions between QDs and biological systems. The conjugation can be performed by adsorption or by covalent bound. In order to apply bare QDs or conjugated QDs, it is always required adjustments of physical and chemical conditions, as temperature and pH, in order to have nanocrystals suspensions appropriated for biological applications [11]. It is known that these adjustments, especially for

* Correspondence to: Departamento de Biofísica e Radiobiologia, CCB, UFPE, Av. Prof. Moraes Rego, s/n, 50670-901 Recife, PE, Brazil.
** Correspondence to: Departamento de Eletrônica e Sistemas, Centro de Tecnologia, UFPE, R. Acadêmico Hélio Ramos, s/n, 50740-530 Recife, PE, Brazil.
E-mail addresses: adriana.fontes.biofisica@gmail.com (A. Fontes), renato.earaujo@ufpe.br (R.E. de Araujo).

http://dx.doi.org/10.1016/j.jlumin.2015.12.052
0022-2313/

conjugation, can modify the fluorescence efficiency of these nanocrystals [12]. These changes can be quantified by absolute fluorescence quantum yield (Φ) measurements, which evaluate the efficiency of fluorophores to emit radiation [13].

Fluorescence quantum yield is defined as the ratio of the number of emitted photons by the number of absorbed photons by a fluorophore. There are still few works that evaluate changes in absolute quantum yields and they are usually related to hydrophobic QDs [14,15]. Moreover, different techniques have been explored to measure fluorescence quantum yield. These techniques can be classified into absolute and relative method [15,16]. Briefly, relative techniques, that compare fluorescence emission spectra, use reference materials with known quantum yield values to quantify the fluorescence efficiency of a new sample. The need for reference materials limits the use of relative methods. Besides, absolute methods, exploring integrated sphere or thermal lens spectroscopy (TLS), can directly provide quantum yield quantification without the use of standard samples. In particular, TLS emerges as a technique which presents more accurate results for Φ values when compared with methods that directly explore relative and absolute fluorescence analyses [16,17].

This study aimed to evaluate the absolute fluorescence quantum yield of Cadmium Telluride (CdTe) QDs, synthesized in aqueous medium, according to different pHs adjusted by the addition of mercaptosuccinic acid (MSA) or hydrochloric acid (HCl), which are two chemical compounds usually explored on the pH control of QDs suspensions [18,19]. For example, the pH of hydrophilic QDs soon after the synthesis is usually around 10.0, the pH of biological samples is around 7.0 and the pH to perform covalent conjugation by using the most applied coupling agents, EDC and Sulfo – NHS (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysulfosuccinimide sodium salt), is around 6.0 [20]. CdTe QDs are the most used hydrophilic nanocrystals for biological applications.

In order to calculate the Φ values for the samples, we used the thermal lens (TL) spectroscopy in single-beam configuration and eclipsing mode detection (S-ETL), which joins the higher sensitivity of the eclipsing mode (when compared to conventional TL modes) with the more accurate results of Φ provided by TL techniques (when compared to other methods that explore relative and absolute fluorescence analysis) [21,22]. These results can provide a better understanding of processes that modify the QDs' emission characteristics, when the pH values of the suspension is changed, helping to establish parameters and specific limits for improving their applications in life sciences.

2. Experimental procedures

2.1. Samples preparation

CdTe QDs were synthesized in aqueous medium with adaptations of the method described by Andrade and co-authors [19]. Briefly, QDs were prepared by adding reduced tellurium, Te^{2-}, in a solution containing $Cd(ClO_4)_2$ (cadmium perchlorate) at pH > 10 in the presence of MSA, which acts as stabilizing and functionalizing agent, in a molar ratio of 5:1:6.0 Cd:Te:MSA. The reaction proceeded for 5 h at 80 °C, in inert atmosphere and under constant stirring. All reagents were obtained from Sigma Aldrich. The Te^{2-} aqueous solution was prepared by reducing metallic tellurium with $NaBH_4$ at a high pH (using 100 μL of NaOH, 2 mol L^{-1}), also under heating and in inert nitrogen atmosphere. Finally, the suspension was diluted in a proportion of 1:6 (QDs:Water – v/v), resulting in samples with a final pH = 10.3 and absorbance less than 0.1 at the wavelength of 532 nm.

Two groups of samples were prepared. A first group with the pHs adjusted by the addition of the stabilizing agent, MSA (4.9%), obtaining samples with pHs: 9.8; 9.1; 8.4; 7.3; 6.2 and 5.3. A second group of samples with pHs: 9.8; 9.1; 8.4 and 8.0, with values adjusted using HCl (1 mol L^{-1}). All samples were prepared to reach equal molar concentrations, and therefore the samples absorbance remains the same for all pH values. All samples were prepared and analyzed in quintuplicate, for each pH suspension.

2.2. Thermal lens spectroscopy

When a laser beam travels through an absorbing sample, the illuminate region suffers a local increase of its temperature, which is generated by energy absorption followed by a non-radiative energy decay in the material. The last process produces a sample heating that in turn induces localized changes in its refractive index (n). The localized changes in n result on an optical lens behavior of the sample, known as thermal lens (TL) effect [13,23].

In order to create the TL effect, the light from a Gaussian beam laser is focused on an absorbing sample and the temporal intensity dynamics of the transmitted beam is evaluated. The most acceptable theoretical model that explains the TL effect in a single-beam configuration is based on a Gaussian beam propagation theory and Fresnel diffraction. The model was developed by Sheldon and collaborators [24]. The analytical expression for the temporal behavior of the intensity of transmitted beam is:

$$I = I_0\left[1-\frac{\theta}{2}\tan^{-1}\left(\frac{2V}{\left[9+V^2\right]\left(\frac{t_c}{2t}\right)+3+V^2}\right)\right]^2 \quad (1)$$

where I_0 is the beam intensity at $t=0$, θ is the thermal phase shift and V is given by

$$V = \frac{z}{z_R}+\left(\frac{z_R}{z_2}\right)\left[1+\left(\frac{z}{z_R}\right)^2\right] \quad (2)$$

Considering the position of the beam waist as the origin of the z coordinate, z is the position of the sample and z_2 is the distance of the sample to the photodetector; z_R is the Rayleigh length; t_c is the characteristic heat diffusion time, given by $t_c=\omega_{ex}/4D$, being $D=\kappa/\rho C_P$ the thermal diffusivity, ρ is the volumetric density and C_P the specific heat of the sample and ω_{ex} the excitation beam waist.

The amplitude of θ, in Eq. (1), is determined from Eq. 3:

$$\theta = -\frac{\varphi P_{abs}}{k\lambda_{ex}}\frac{dn}{dT} \quad (3)$$

being φ the fraction of absorbed energy converted in thermal energy, also called absolute nonradiative quantum yield; λ_{ex} is the excitation wavelength; κ is the thermal conductivity of solvent; dn/dT the thermo-optic coefficient and P_{abs} is the laser power absorbed by the sample, which is obtained from $P_{abs}=P_{ex}\,(1-e^{-\alpha L})$, α is the linear absorption coefficient at the excitation wavelength (λ_{ex}), P_{ex} the incident excitation power and L is the optical path length of the sample.

The nonradiative quantum yield, φ, for fluorescent materials is given by.

$$\varphi = 1-\Phi\frac{\lambda_{ex}}{\langle\lambda_{em}\rangle} \quad (4)$$

Thus, the Φ of the sample can be obtained analyzing the temporal dynamics of the laser beam that passes through the sample, determining the power absorbed (P_{abs}) and by using Eqs. (1), (3) and (4).