Bio-functionalized water-soluble ZnS quantum dots using carboxymethylchitosan

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Abstract. The major goal of this study was to develop an innovative green route for synthesizing biocompatible water-soluble luminescent QDs using chemically modified chitosan as the ligand in aqueous media. The preparation of ZnS QDs bio-functionalized by carboxymethylchitosan (CMC) was performed using a single-step aqueous colloidal process at room temperature. The results showed that water-dispersible ZnS nanocrystals capped by CMC were produced within the quantum-size confinement regime. Moreover, the luminescent properties of ZnS QDs were significantly affected by the pH during the synthesis due to the size distribution of the nanoparticles and their density of surface states.

1. Introduction

Recent advances in nanotechnology and nanoscience for biological and medical applications have been the major driving force in the creation of new nanohybrids and nanoconjugates. Essentially, to be considered as nanosized, the systems must present one or more components with at least one dimension ranging from 1 to 100 nm. Besides the low dimensions, nanohybrids and nanoconjugates may bring together the intrinsic functionalities of inorganic nanoparticles combined with the biocompatible surfaces and interfaces offered by biomolecules and biopolymers, such as carbohydrates, proteins, and enzymes [1-2]. Among several alternatives of inorganic nanoparticles, semiconductor nanocrystals (or quantum dots, QDs) have emerged as an attractive nanomaterial owing to their unique physico-chemical, electronic, and optical properties. However, for potential applications of QDs in nanomedicine and biology their cytotoxicity must be eliminated because they are predominantly made of highly toxic heavy metal cores [2]. Presently, the most commonly used QDs contain divalent cadmium (Cd\(^{2+}\)), widely known as a toxin, due to its accumulation in living tissues and organs [3-4]. In that sense, “cadmium-free” nanomaterials are very promising possibilities, such as zinc divalent cations (Zn\(^{2+}\)), due to their natural environmental abundance and they are commonly found in several living organisms as crucial metabolic species. Another interesting approach has been used for producing QDs with biocompatible surfaces and interfaces based on capping ligands such as biomolecules and biopolymers rendering them biocompatibility for biomedical applications [3-5]. In view of the large number of available options of biocompatible
ligands to produce QD nanoconjugates for bio-applications, such as carbohydrates, proteins, and enzymes, chitosan and its derivatives have been often chosen, due to their biocompatibility, physico-chemical and mechanical properties, and relative chemical solubility and stability in aqueous medium [3-5]. Chitosan (poly-β(1→4)-2-amino-2-deoxy-D-glucose) is one of the most abundant polysaccharides (semi-processed) from natural sources, second only to cellulose [3-5] but it has very low solubility at physiological environment (pH~7.0). Consequently, chitosan derivatives such as carboxymethylated chitosans (O-carboxymethyl chitosan, O-CMC and N-carboxymethyl chitosan, N-CMC) may be good candidates as ligands for preparing biocompatible colloid semiconductor nanoparticles because they are water-soluble in acidic, alkaline and physiologic media [5,6]. These combined features of chitosan and its derivatives offer limitless possibilities for developing water-dispersible QD conjugates for biological, pharmaceutical [2,5] and environmental purposes [7].

Hence, this research focused on the synthesis of ZnS quantum dots directly capped by carboxymethyl chitosan (CMC) using a green, facile, reproducible, and single-step aqueous process at room temperature.

2. Experimental

2.1. Materials

All reagents and precursors, ZnCl₂ (Sigma-Aldrich, USA, ≥ 98%), Na₂S·9H₂O (Synth, Brazil, >98%), NaOH (Merck, USA, ≥ 99%), CH₃COOH (Synth, Brazil, ≥ 99.7%), HCl (Sigma-Aldrich, USA, 36.5-38.0%), and the sulfur source solution (Sigma-Aldrich, USA, 99.9%) were used as-received. Chitosan powder (Aldrich, USA, MM = 310,000 to >375,000 g/mol, DD ≥ 75.0%, and viscosity 800-2000 cPoise, at 1 % in 1 % acetic acid) was used as the reference ligand. Deionized water (Millipore Simplicity™) with resistivity of 18 MΩ·cm was used in the preparation of all solutions and the syntheses were performed at room temperature (R.T. = 23±2 °C) unless specified.

2.2. Synthesis of carboxymethylchitosan (CMC) and ZnS quantum dots (QD_CMC and QD_CHI)

The method used to prepare carboxymethylchitosan at R.T. was based on the process previously reported by our group [2,4]. In brief, the chitosan (CHI) was chemically modified using the reaction with monochloroacetic acid in highly alkaline (NaOH) medium of isopropanol for approximately 4 h. In the sequence, the suspension was neutralized (HCl), filtered and the solid filtrate rinsed in ethanol to neutral, and then vacuum dried, leading to the formation of CMC flakes. Finally, the CMC solution (1.0% w/v) was prepared by dissolving 0.5 g of CMC flakes in 50 mL of deionized water under moderate magnetic stirring until complete solubilization occurred. ZnS nanoparticles were synthesized via an aqueous route at R.T. using CMC or CHI as the ligand, the zinc precursor solution (ZnCl₂, 8x10⁻³ mol.L⁻¹) and the sulfur source solution (Na₂S·9H₂O, 1.0x10⁻² mol.L⁻¹) which were added to the reaction flask (S:Zn = 1:2) and stirred for 1 h. ZnS QDs colloidal suspensions were referred to as QD_CMC-pH4, QD_CMC-pH10 and QD_CHI-pH4 as a function of the pH and ligand of the QDs synthesis. Sampling aliquots were collected at different time intervals for Ultraviolet-Visible spectroscopy (UV-vis) measurements to evaluate the colloidal nucleation and stability.

2.3. Characterization of CMC and QD_CMC and QD_CHI colloidal quantum dots

The characteristic functional groups of chitosan, CMC and conjugates were evaluated by Fourier transform infrared spectroscopy (ATR-FTIR, in the range of 650 to 4000 cm⁻¹, Thermo Fischer, Nicolet 6700, 16 scans, 2 cm⁻¹ resolution, with a background subtraction). The water solubility of CMC as a function of the pH was evaluated by the turbidity method using UV-vis in the range 190 nm ≤ λ ≤ 800 nm based on the literature [8], which considered the sample insoluble when the transmittance was ≤ 85% (λ = 450 nm). The nucleation, growth and stabilization of colloidal ZnS QDs in CMC were monitored by UV-vis, and their physicochemical and optical properties were assessed using photoluminescent spectroscopy (PL). ZnS conjugates fluorescence emissions were measured at
R.T. using Nanodrop 3300 fluorospectrometer (Thermo Scientific, UV LED, $\lambda_{\text{excitation}}=365\pm10$ nm). Nanostructural characterizations of the QD nanoconjugates were based on the images and selected area electron diffraction patterns (SAED) obtained using a Tecnai G2-20-FEI transmission electron microscope at an accelerating voltage of 200 kV. Energy-dispersive X-ray spectra (EDX) were collected using the TEM for element chemical analysis. In all of the TEM analyses, the samples were prepared by dropping the colloidal dispersion onto a porous carbon grid. The QD size and size-distribution data were obtained based on the TEM images by measuring at least 100 randomly selected nanoparticles using an image processing program (ImageJ, version 1.44, public domain, National Institutes of Health). All of the experiments were conducted in triplicates (n=3) unless otherwise noted. The data are presented as the mean±standard deviation.

3. Results and Discussion

The FTIR spectra of chitosan (CHI) and carboxymethylchitosan (CMC) are showed in figure 1(a) and figure 1(b), respectively. Unlike the CHI spectrum, CMC spectrum presented a characteristic band at approximately 1731 cm$^{-1}$, which is assigned to the carboxyl (–COOH) groups. The protonation of the amine group in CMC causes the appearance of two new bands, the first band at 1640-1620 cm$^{-1}$, associated with the antisymmetric stretching of -NH$_3^+$ overlapping the amide I band, and the second band at 1550-1500 cm$^{-1}$ is related to the -NH$_3^+$ symmetric deformation, indicating the acid-form of CMC (H-CMC) [4,8].

The solubility tests based on UV-vis turbidity test indicated that CMC solutions were soluble under acidic and alkaline conditions, and only insoluble in small range of pH 6.0 < pH < 7.5. That is one of the major advantages of CMC derivative compared to chitosan because it is soluble in both acid and alkaline aqueous media and chitosan is insoluble at pH higher than 6.5.

The UV-vis absorption spectra of the colloidal ZnS nanoparticles produced using CHI and CMC as the stabilizing ligand are showed in figure 2. In general, the curves exhibit a broad absorption band in the range from 250 to 350 nm, associated with the first excitonic transition indicating nucleation/growth of ZnS nanoparticles within the “quantum confinement regime”. It was observed the energy “blue-shift” for the all the ZnS-conjugates curves as compared with the “bulk” value (figure 2, arrow at $\lambda=343$ nm) [9]. Therefore, the optical band gap energy of ZnS QD ($E_{QD}$) is larger than that of the original bulk material ($E_g$). The optical band gap (absorbance onset) and the “blue-shift” values were determined from the absorption coefficient data as a function of wavelength using the “Tauc relation” [10] extracted from the UV-vis spectra. The results support the formation of ZnS QDs in the CHI and CMC media because the band gap energy values of QDs ($E_{QD}$) equal to 3.74±0.02 eV for QD_CHI-pH4 and 3.83±0.02 eV for QD_CMC-pH4 and QD_CMC-pH10, respectively) were significantly greater than the ZnS bulk (i.e., 3.61 eV) [4]. The average ZnS nanoparticle sizes (2R) were estimated from the optical band gap value ($E_{QD}$). The ZnS QDs were produced and stabilized by CMC with estimated diameters (2R) of 4.2±0.1 nm and 3.2±0.1 nm at pH 4.0 and 10.0, respectively, compared to the larger size 4.7±0.1 nm found for ZnS-CHI conjugate (at pH 4.0).

Figure 3 shows the photoluminescence spectra of the QD_CHI and QD_CMC colloidal nanoparticles collected at R.T. with their relative intensities. Based on the absorbance curves and the band gap energies evaluated under excitation, QD_CHI and QD_CMC nanoconjugates were expected to emit light in the UV range ($E_g \geq 3.6$ eV). However, band edge recombination was not detected, and other bands in the violet-blue range were observed (inset, figure 3). These results are in agreement with the reported emission spectra of ZnS nanoparticles, which typically exhibit radiative recombination in the 400-550 nm wavelength range arising from trap states associated with point and surface defects [4,12]. The emission band at approximately 416 nm is due to transitions involving Zn$^{2+}$ interstitial states (I$_{2a}$), while the emission band at 435 nm is assigned to sulfur vacancy states (V$_{S}$), compatible with the procedure used in this work using a stoichiometric molar ratio of Zn$^{2+}$:S$^{2-} = 2:1$. The band at approximately 470 nm, observed for all systems (independent to the pH of the synthesis), may be assigned to surface defects [4,13].
Figure 1. The FTIR spectra of chitosan (a) and CMC (b).

Figure 2. UV-Vis spectra for (a) QD_CMC-pH4, (b) QD_CMC-pH10, and (c) QD_CHI-pH4.

Figure 3. PL spectra of ZnS conjugates. Inset: schematic drawing of excitonic recombination pathway (not to scale) and blue luminescence image of ZnS conjugates.

4. Conclusions
In the present work, ZnS QDs directly bio-functionalized by carboxymethyl chitosan were synthesized using an eco-friendly one-step colloidal process in aqueous medium at room temperature. The results indicated that by varying the pH within a relatively broad range, from acid (pH=4.0) to alkaline...
(pH=10.0) CMC solutions, the average size of ZnS QDs were significantly affected, leading to important changes in the photoluminescent properties of the ZnS nanoconjugates.

Figure 4. TEM images of QD_CMC-pH4 (A), QD_CMC-pH10 (B), and QD_CHI-pH4 (C) with their respective histograms of size distributions of ZnS nanoparticles (bottom).

Moreover, the results indicated that the stabilization mechanism of the QD_CMC nanoconjugates was determined by the interactions of the carboxylate functional groups of CMC with Zn$^{2+}$ species at the surface of the QDs as compared to chitosan (QD_CHI) that was predominately by amine groups.

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