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Carboxymethyl chitosan functionalization of Bi_2S_3 quantum dots: Towards eco-friendly fluorescent core-shell nanoprobes

Alexandra A.P. Mansur[a], Fábio P. Ramanery[a], Luiz C. Oliveira[b], Herman S. Mansur[a,*]

[a] Center of Nanoscience, Nanotechnology and Innovation—CeNano2I, Department of Metallurgical and Materials Engineering, Federal University of Minas Gerais, Brazil
[b] Department of Chemistry, Federal University of Minas Gerais, Brazil

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ABSTRACT

Designed bioengineered nanocomposites are emerging as a novel class of hybrid materials composed of natural aminopolysaccharides and inorganic semiconductors for biomedical and environmental applications. In this study, it is reported for the first time the synthesis and characterization of water-soluble Bi_2S_3 quantum dots (QDs) functionalized with *O*-carboxymethyl chitosan (*O*-CMC) as capping ligands. UV–vis spectroscopy, transmission electron microscopy, dynamic light scattering, zeta potential, and photoluminescence spectroscopy were used to characterize these nanohybrids. The results proved the hypothesis that *O*-CMC acted as a pH-dependent multi-functional ligand by altering the mechanisms of nucleation, growth and stabilization of water-soluble colloidal Bi_2S_3 nanocrystals under acidic, physiological and alkaline conditions, using an eco-friendly aqueous process at room temperature. Moreover, the *O*-CMC capping ligand and the relative molar ratios of the precursors in solution effectively controlled the diameters of the Bi_2S_3 QDs, which ranged from 2.8 to 12.8 nm, and that exhibited luminescent properties in visible light.

1. Introduction

In the last few decades, polysaccharide-based materials and nanocomposites have received much attention for the production of biohybrids due to their wide range of applications in the pharmaceutical, nutritional, biomedical and environmental areas (Crini & Badot, 2008; El-Sherbiny & El-Baz, 2015; Jayakumar, Menon, Manzoor, Nair, & Tamura, 2010). Essentially, these nanobiohybrids may bring together the intrinsic multifunctionalities of inorganic nanomaterials combined and the versatile biocompatible interfaces offered by biomolecules, such as natural polysaccharides (Hezinger, Tessmar, & Gopferich, 2008; Mansur et al., 2012). The aminopolysaccharides are a group of polysaccharides that have a primarily animal origin and contain amino sugar units, which are most frequently D-glucosamine and D-galactoseamine. The most common natural aminopolysaccharides include chitin, chitosan, keratin sulfate, hyaluronic acid, heparin, chondroitin and dermatan sulfates. Despite their similarities regarding to the polysaccharide backbone, they significantly differ by their chemical functional groups, as chitin and chitosan contain hydroxyl, amino and acetyl groups, and the others also contain carboxyl and/or sulfate groups (Furda, 2009). Among the numerous polysaccharides, the last decade has witnessed extensive multidisciplinary research efforts focused on exploring the properties of chitosan and its derivatives, and the exoskeletons of marine crustaceans and fungi are the main sources of chitin and its semi-processed deacetylated derivative (i.e., chitosan). The main reasons for the increasing interest in chitosan and its derivatives are based on (a) their unique chemical, physicochemical, and biological properties, (b) the abundant renewable source of raw materials for their production and (c) their intrinsic properties, such as nontoxicity, biocompatibility, biodegradability and environmental friendliness (Il'ina and Varlamov, 2005; Jayakumar et al., 2010; Upadhyaya, Singh, Agarwal, & Tewari, 2013). Despite the importance of chitosan in the biomedical, pharmaceutical and nutritional fields, chitosan is only soluble under acidic conditions, which limits some of its potential scientific and industrial applications in neutral and alkaline media. Therefore, recently, carboxymethyl chitosan (CMC) has been investigated due to its better solubility in water, improved antibacterial property (Liu, Guan, Yang, Li, & Yao, 2001), enhanced biocompatibility (Chen, Wang, Liu, & Park, 2002; Zhu, Chan-Park, Dai, & Li, 2005) and safety for humans regarding to toxicity (Fu, Han, Dong, Yang, Lv, & Liu, 2011; Tokura, Nishimura, Sakairi, &

* Corresponding author at: Federal University of Minas Gerais, Av. Antônio Carlos, 6627–Escola de Engenharia, Bloco 2–Sala 2233, 31.270-901, Belo Horizonte/MG, Brazil.
E-mail addresses: hmansur@demet.ufmg.br, herman.mansur2016@gmail.com (H.S. Mansur).

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Nishi, 1996). Therefore, CMC has been broadly used in biomedical applications, such as wound healing, tissue engineering and drug delivery applications (Upadhyaya et al., 2013). In the last few years, chitosan and the CMC derivative have been employed as suitable ligands for producing novel biohybrid fluorescent materials because they can stabilize ultra-small semiconductor nanocrystals in water and simultaneously create a multifunctional organic shell for biomedical and environmental applications (Mansur, Mansur, et al., 2013; Ramanery, Mansur, Borsagli, & Mansur, 2014; Xie et al., 2005). Thus, research on semiconductor nanocrystals, which are also referred to as colloidal quantum dots (QDs), is growing at a very rapid pace due to the significant advances in nanoscience and nanotechnology focusing on optoelectronics, catalysis, biomedicine, and eco-friendly environmental applications (Lesnyak, Gaponik, & Eychmüller, 2013). In particular, interest in narrow bandgap materials, such as bismuth chalcogenides (e.g. Bi_2X_3, X = S, Se and Te) nanocrystals, has intensified in recent years, and the results indicate possibility of applications of these materials in solar cells, infrared optoelectronics (e.g. lasers, optical modulators, photodetectors and photoimaging devices) and biological imaging and biosensor systems (Aresti et al., 2014). Bismuth is a remarkably harmless element among the toxic heavy metals in the periodic Table and sparked great interest in various areas ranging from medicinal to environmental applications because bismuth compounds are even less toxic than common food salts (e.g. sodium chloride). Therefore, bismuth is unique among the chemical elements and has earned *'green element'* status (Mohan, 2010). Several studies have reported the production of Cd-based and Zn-based quantum dots with chitosan and its derivatives mostly using ligand exchange processes with organic solvents (Ma, Lin, Yang, Li, & Su, 2014) or embedded in the polymeric particles (Ghormade et al., 2015), which are more expensive, not eco-friendly, time-consuming and difficult to be performed, usually affecting the final optical properties of the conjugates.

Surprisingly, only a few studies have reported the preparation of nanomaterials based on Bi_2S_3, such as quantum dots (Aresti et al., 2014) and nanorods (Liao et al., 2012; Luo et al., 2013), which have been scarcely used in biomedical and pharmaceutical fields (Fang et al., 2013). However, no research investigating the synthesis and characterization of nanoconjugates made of Bi_2S_3 quantum dots directly biofunctionalized by carboxymethyl chitosan was found in the consulted published literature.

Thus, in this study, in order to test the hypothesis we formulated experiments aimed at the development of innovative carbohydrate-based nanoconjugates combining *O*-carboxymethyl chitosan (*O*-CMC) with luminescent Bi_2S_3 narrow bandgap semiconductor nanocrystals. For that purpose, a single-step eco-friendly aqueous process at room temperature was designed using *O*-CMC as the pH-depend capping ligand for directly synthesizing ultra-small Bi_2S_3 QDs, producing water-soluble and biocompatible colloidal core-shell nanostructures. These nanoconjugates were photoluminescent, which may offer numerous possibilities for use in a wide range of biomedical and environmentally-friendly applications.

2. Experimental procedure

2.1. Materials

All of the reagents and precursors, including bismuth chloride (Aldrich, USA, ≥98%, $BiCl_3$), sodium sulfide (Synth, Brazil, >98%, $Na_2S{\cdot}9H_2O$), sodium hydroxide (Merck, USA, ≥99%, NaOH), acetic acid (Synth, Brazil, ≥99.7%, CH_3COOH), hydrochloric acid (Sigma-Aldrich, USA, 36.5–38.0%, HCl), monochloroacetic acid (Sigma-Aldrich, USA, 99%, $ClCH_2COOH$), ethanol (Synth, Brazil, 99.8%, CH_3CH_2OH), methanol (Sigma-Aldrich, USA, 99.8%, CH_3OH), and isopropanol (Sigma-Aldrich, USA, 99.5%, $(CH_3)_2CHOH$) were used as received. Chitosan powder (Sigma-Aldrich, USA, high molecular weight of 310–375 kDa, degree of deacetylation = 83 ± 3%, viscosity 800–2000 cP, 1 wt.% in 1% acetic acid at 25 °C, Brookfield) was used as the polymer for the synthesis of carboxymethyl chitosan (CMC). Unless otherwise indicated, deionized water (DI water, Millipore Simplicity™) with a resistivity of 18 MΩ cm was used to prepare the solutions.

2.2. Methods

2.2.1. Synthesis and characterization of CMC

Carboxymethyl chitosan (CMC) was synthesized aiming at primarily reacting at hydroxyl groups of chitosan to yield *O*-CMC based on the methods reported by our group (Borsagli, Mansur, Chagas, Oliveira, & Mansur, 2015; Ramanery et al., 2014) as detailed in the sequence. Chitosan powder (3.0 g) was suspended in 70.8 mL of isopropanol and kept under magnetic stirring for 30 min. Then, 8.16 g of NaOH dissolved in 10.0 g of DI-water and 10 mL of isopropanol were added to the suspension and stirred for 1 h. Next, 14.4 g of monochloroacetic acid/isopropanol solution (1:1 m/m) was added to the suspension and the reaction proceeded for 4 h under magnetic stirring and stopped by adding 100 mL of ethanol. Then, the suspension was filtered and the solid filtrate was washed with ethanol/water mixtures of increasing ethanol content (from 70% to 90%) and dried at room temperature. The neutralization was conducted by suspending 1.0 g of dried powder in 80% ethanol/aqueous solution (100 mL), adding hydrochloric acid (1 mL, 37%), and stirring for 30 min. In the sequence, the suspension was filtered and the solid filtrate extensively washed with 70–90% ethanol to neutral, and vacuum dried, leading to the formation of the CMC derivative powder, which was stored for later use (desiccator). All of these procedures were conducted at room temperature (23 ± 2 °C).

The degree of substitution (DS) of the CMC was evaluated using the potentiometric titration test as follows: 0.10 g of CMC was dissolved in 100 mL of 0.10 mol L^{-1} HCl with moderate stirring overnight. Under moderate continuous stirring, 100 μL of 0.10 mol L^{-1} NaOH solution was added, then allowed equilibrating, and the pH was recorded. This sequence was repeated until neutralization of the HCl and carboxyl groups occurred. Degree of substitution (DS) was calculated using Eq. (1) (Abreu & Campana-Filho, 2005).

$$DS = [M * C_{NaOH} * (V_2 - V_1)]/\{m - [80 * C_{NaOH} * (V_2 - V_1)]\} \quad (1)$$

where M is the average molar mass of repetitive unit of chitosan (g mol^{-1}), C_{NaOH} is the concentration of NaOH (mol·L^{-1}), V_1 is the volume of NaOH solution consumed to neutralize excessive hydrochloric acid (L), V_2 is the volume of NaOH solution added to the titration of terminal −COOH and m is the mass of CMC used in 100 mL.

Fourier transform infrared (FTIR) spectroscopy was performed in the 650–4000 cm^{-1} range (Thermo Fischer, Nicolet 6700) using transmission mode. Chitosan derivative (CMC) films (0.025 g of polymer dissolved in 10 mL of water or 1% (v/v) acetic acid for CMC and CHI, respectively) were placed into a sample holder and immediately scanned (16 scans) at a resolution of 2 cm^{-1} with background subtraction.

The ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 30 °C in D_2O/HCl using a BRUKER–200 MHz Varian spectrometer (90 pulses and 16 scans).

The water solubility of CMC as a function of the pH was determined using the turbidity method and UV–vis spectroscopy (Abreu & Campana-Filho, 2009). The absorbance of the solution was recorded on a Lambda EZ-2100 spectrophotometer (Perkin Elmer) using a quartz cell (optical path length = 10 mm) in the 190 nm ≤ λ ≤ 800 nm range. The sample was considered insoluble