Polymer 52 (2011) 1045–1054

Contents lists available at ScienceDirect

Polymer

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

Synthesis and characterization of CdS quantum dots with carboxylic-functionalized poly (vinyl alcohol) for bioconjugation

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ARTICLE INFO

Article history:
Received 14 October 2010
Received in revised form
22 December 2010
Accepted 1 January 2011
Available online 11 January 2011

Keywords:
Poly(vinyl alcohol)
Nanoparticles
UV–vis spectroscopy

ABSTRACT

In the present research it is reported the synthesis and characterization of CdS nanoparticles (NPs) prepared using carboxylic-functionalized poly (vinyl alcohol) (PVA) as the ligand via aqueous route at room temperature and ambient pressure. Different molar concentrations of carboxylic-PVA and PVA were investigated aiming at producing stable colloidal systems. Carboxylic-PVA was conjugated with BSA (bovine serum albumin) and used as capping ligand in the preparation of CdS nanocrystals. UV–visible spectroscopy, photoluminescence spectroscopy, and transmission electron microscopy were used to characterize the kinetics and the relative stability of polymer-capped CdS nanocrystals. The results have clearly indicated that the carboxylic-functionalized PVA was much more effective on nucleating and stabilizing colloidal CdS nanoparticles in aqueous suspensions compared to PVA. In addition, the CdS nanocrystals were obtained in the so-called "quantum-size confinement regime", with the calculated average size below 4.0 nm and fluorescent activity. Thus, a novel simple route was successfully developed for synthesizing nanohybrids based on quantum dots and water-soluble chemically functionalized polymers with incorporated carboxylic moiety with the possibility of direct bioconjugation.

1. Introduction

Since the early studies pioneered by Brus and co-workers [1] quantum confinements of semiconductors have drawn the attention of the research community. Physical, chemical, and electronic properties of these zero-dimensional entities change dramatically because of quantum effects. The semiconductor nanocrystals, often referred as "quantum dots" (QDs), are one of the most popular terms in nano-science of the 21st century, essentially because of the alterations in the material as a result of the direct influence of the ultra-small length scale on the energy band distribution in the material [2]. These are promising nanomaterials concerning to the exploitation of their properties in all areas of science, mainly those associated with biomedical applications such as nanobiotechnology and nanomedicine [2,3]. The synthesis of QDs was first described in 1982 by Efros [4] and Ekimov [5] who grew nanocrystals of semiconductors in glass matrices. Since then, several publications have reported the preparation of semiconductor nanoparticles in all sorts of systems, LB films [6,7], SAM [8], zeolites [9], organic [10], biomolecules [11,12] and conductive polymers [13]. Nevertheless, despite the relative success on developing QDs, most of the methods are in hydrophobic medium using organic solvents at high temperatures. Usually, these nanocrystals have surfaces that readily oxidize when exposed to water. Moreover, they have to be synthesized in organic solvents under an inert atmosphere and then transferred into aqueous media through a capping ligand exchange. Despite reasonably effective, ligand exchange typically produces less than ideal surface stabilization and product luminescence yield may be limited due to particle agglomeration and growth during the exchange process [3]. More recently, few studies of water-soluble nanoparticles have been published, but frequently with complex routes and/or using expensive precursors [14]. In addition, to be used in biological environments they must exhibit compatibility to the physiological medium where water is abundant and with the large number of molecules such as peptides, enzymes, proteins, among others. Regardless the route one may choose, the final system size should be maintained within a very restricted range as the "hydrodynamic diameter" will determine its behavior in most biological applications [3,10]. As a consequence, the development of novel methods for producing water soluble QDs, with long-time stability, narrow size distributions and biocompatibility, associated with the least possible "hydrodynamic diameter" is still a challenge

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doi:10.1016/j.polymer.2011.01.004

to be overcome. Amongst several choices, polymeric materials come as an interesting possibility of synthesizing semiconductor nanocrystals, as they share some of the major requirements for that purpose. Polymers are commercially available, many are water soluble, biocompatible, and have been widely used in the chemistry of colloids as dispersants, chelates, surfactants, food industry, ligands and so forth. In the realm of water compatible polymers reported in literature, poly (vinyl alcohol) (PVA) and its related products appear as one very interesting choice for preparing colloidal suspensions, due to their biocompatibility and biodegradability aiming at medicine, biology and pharmaceutics applications [15,16]. PVA is a hydrophilic semi-crystalline polymer produced by polymerization of vinyl acetate to poly (vinyl acetate) (PVAc), and successive hydrolysis to PVA. This reaction is incomplete resulting in polymer with different degrees of hydrolysis (DH) [17]. So, it is a copolymer of poly(vinyl alcohol) and poly(vinyl acetate) referred as poly(vinyl alcohol-co-vinyl acetate). PVA is commercially available in highly hydrolyzed grades (DH > 98.5%) and partially hydrolyzed ones (DH from 80.0 to 98.5%) [18]. The degree of hydrolysis or the molar content of acetate groups in PVA affects its physical–chemical properties, such as solubility, hydrophilic/hydrophobic interactions, pH-sensitivity and viscosity [19]. The pH-sensitive systems have experienced increasing interest as novel polymeric materials [18]. Stimuli-responsive structures based on PVA may be achieved during the synthesis (monomers and precursors) or by posterior chemical grafting, for instance, with carboxylic groups [20]. Despite of being a very dynamic area of research, the use of chemically modified PVA as stabilizer for the production of quantum dots colloidal suspensions is relatively unexplored. In fact, several studies have been published using PVA or poly (vinylpyrrolidone) (PVP) as stabilizers for semiconductor nanoparticles, but none of them have actually reached biological application [21–23]. Actually, the relatively low reactivity of alcohol groups has significantly narrowed its direct bioconjugation with biomolecules such as proteins, antibodies and carbohydrates. The usual alternative has been the incorporation of amine and carboxylic groups to enhance such possibility of molecule coupling but at the cost of increasing the "hydrodynamic size" of the entire bioconjugated system [14]. So, direct aqueous-phase synthesis of semiconductor nanocrystals in a single step with suitable quantum yield would provide a convenient and plausible alternative to the labor-intensive method of organic-phase synthesis followed by ligand exchange concerning to biomedical applications.

Thus, in the present study, it is reported the synthesis and characterization of CdS quantum dots obtained in PVA and carboxylic-PVA aqueous solutions using colloidal chemistry. To the best of our knowledge, this is the first report to investigate CdS nanoparticles successfully produced in the quantum size range using carboxylic-functionalized PVA as "capping" ligand. Also, these results were compared with conventional PVA, regarding to the polymer concentration and colloidal stability. Moreover, CdS quantum dots were successfully produced and stabilized using PVA-carboxylic bioconjugated to bovine serum albumin. Hence, the synthesis of such nanohybrid system with incorporated carboxylic moiety in the polymer chain has brought the possibility of direct bioconjugation.

2. Materials and methods

All reagents and precursors, thioacetamide (Sigma–Aldrich, Cat#163678, >99%), cadmium perchlorate hydrate (Aldrich, Cat#401374, $CdClO_4.6.H_2O$), sodium hydroxide (Merck, Cat# 1.06498.1000, ≥99%) were used as-received, without any further purification. Poly(vinyl alcohol) with degree of hydrolysis >99.3% and average molecular weight (M_w) = 85 000–124 000 g/mol was supplied by Aldrich (Cat#363146). PVA chemically functionalized (PVA–COOH) containing 1.0 mol% carboxylic acid units was kindly donated by Kuraray Corporation (Poval KM-118, Viscosity 26.0–34.0 [mPa.s], Mw = 85 000–124 000 g/mol, degree of hydrolysis = 95.5–98.5%). Strictly, the polymer can be referred as a terpolymer Poly(vinyl alcohol-vinyl acetate-itaconic acid). Bovine serum albumin (BSA) lyophilized powder was supplied by Sigma (Cat#A9418). EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride, Sigma–Aldrich) in the presence N-Hydroxysulfosuccinimide sodium salt (NHS-sulfo, Sigma–Aldrich) was used as cross linker. De-ionized water (DI-water, Millipore Simplicity™) with resistivity of 18 MΩ cm was used in the preparation all solutions.

2.1. Preparation methods of precursor solutions

2.1.1. Preparation of a solution of thioacetamide 8.0×10^{-3} mol l^{-1} (8.0 mM)

Approximately 0.0601 g CH_3CSNH_2 was added to 75 ml of DI-water in a 100 ml flask and homogenized under moderate manual stirring for 10–15 min. Then, the volume was completed to 100 ml with DI-water. This sulfur precursor stock solution was referred as "SOL-A".

2.1.2. Preparation of a cadmium perchlorate solution 1.0×10^{-2} mol l^{-1} (10 mM)

Approximately 0.4193 g of $Cd(ClO_4)_2.6H_2O$ was added to 75 ml of DI-water in a 100 ml flask and homogenized under moderate manual stirring for 10–15 min. Then, the volume was completed to 100 ml with DI-water. This cadmium precursor stock solution was referred as "SOL-B".

2.1.3. Preparation of poly(vinyl alcohol) solution: 1.0 mol l^{-1} (1.0 M)

Approximately 4.4 g of PVA (PVA molar unity of 44.0 g/mol) was added to 80 ml of DI-water in a 100 ml flask. Then, the mixture was heated to (85 ± 5)° under vigorous magnetic stirring and kept for 4 h when a clear solution was reached. This PVA stock solution was referred as "SOL-C". This PVA solution was diluted by 10, 100 and 1000 for preparing the 1.0×10^{-1} mol l^{-1}, 1.0×10^{-2} mol l^{-1} and 1.0×10^{-3} mol l^{-1} solutions, respectively. For clarity, Poly(vinyl alcohol) will be referred as PVA–OH.

2.1.4. Preparation of PVA-carboxylic (PVA-COOH) solution: 1.0 mol l^{-1} (1.0 M)

Approximately 4.4 g of Poly(vinyl alcohol-vinyl acetate-itaconic acid) (molar unity of ~44.6 g/mol) was added to 80 ml of DI-water in a 100 ml flask. Then, the mixture was heated to (85 ± 5)° under vigorous magnetic stirring and kept for 4 h when a clear solution was reached. This PVA-COOH stock solution was referred as "SOL-D". This PVA-COOH solution was diluted by 10, 100 and 1000 for preparing the 1.0×10^{-1} mol l^{-1}, 1.0×10^{-2} mol l^{-1} and 1.0×10^{-3} mol l^{-1} solutions, respectively.

In Fig. 1 it is shown the schematic representation of the experimental procedure performed for the synthesis of CdS-PVA-OH and CdS-PVA-COOH capped systems.

2.1.5. Bioconjugation of BSA to PVA-COOH

PVA-COOH solution (1.0 mol l^{-1}) was prepared according to previously described procedure in Section 2.1.4 ("SOL-D"). EDC (10 mg, 1.0 wt%), NHS-sulfo (23 mg, 2.3 wt%) and BSA (5 mg, 0.5 wt %) were dissolved in phosphate saline buffer pH 7.4 (1.0 ml). Bioconjugation process was carried out as follows: 500 μl of 1.0 wt% EDC solution and 500 μl of 2.3 wt% NHS-sulfo solution were dissolved in 10 ml of PVA-COOH aqueous solution (1.0 mol l^{-1}) and magnetically stirred for 15 min at (23 ± 2)C°. Under stirring, 200 μl of 0.5 wt% of BSA solution was added. After 10 min of mixing, the system was incubated at (23 ± 2)°C for 2–3 h. The reaction has led

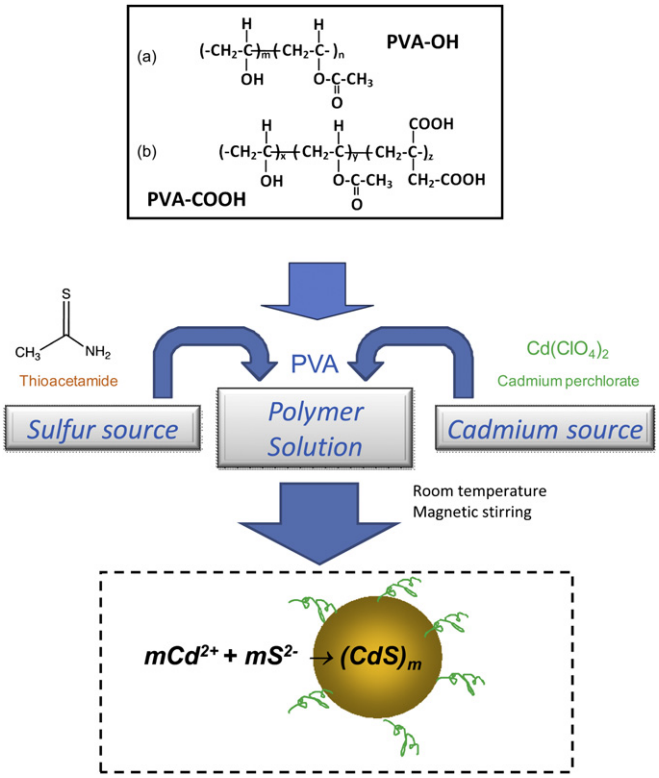

Fig. 1. Representation of the designed experimental procedure for the CdS-PVA colloidal system; Molecular structures of (a) poly(vinyl alcohol) PVA-OH, and (b) PVA-COOH.

to the formation of amide bonds between carboxylic groups from PVA-COOH and amines from BSA (protein-NH_2). This bioconjugated complex was referred as "PVA-C(O)NH-BSA" ("SOL-E"). The bioconjugated system "PVA-C(O)NH-BSA" was purified and concentrated using ultra-centrifugal device with 100 000 M_W cutoff cellulose membrane (Amicon filter, Millipore). Centrifugation was conducted for 30 min (6 cycles × 5 min per cycle, @ 12 000 rpm, 4 °C) using a Hettich Mikro 200R centrifuge. After the first cycle, it was washed 5 times with 300 μl DI-water. Centrifugal force removes the excess of reagents (EDC Mw = 191.7 g/mol; NHS-sulfo = 217.1 g/mol) or unreacted BSA (~67 kDa) and PVA-COOH through the membrane into the filtrate vial. The resulting conjugate (PVA-C(O)NH-BSA) was resuspended in 500 μl of DI-water and stored at 4 °C until further use.

2.2. Synthesis of CdS nanoparticles in PVA solutions

CdS nanoparticles were synthesized via aqueous route in a reaction flask by using the stock solutions as detailed in the previous section, Cd^{2+} and sulfur precursors, and PVA-OH, PVA-COOH or PVA-C(O)NH-BSA as capping ligands. The simplified reaction is represented in Equation (1):

$$Cd^{2+} + CH_3C(S)NH_2 + H_2O \rightarrow CdS + CH_3C(O)NH_2 + 2H^+ \qquad (1)$$

A typical synthesis was carried out as follows: 2 ml of PVA solution (SOL-C for PVA-OH, SOL-D for PVA-COOH or SOL-E for PVA-C(O)NH-BSA) and 45 ml of DI-water were added to the flask reacting vessel. Under moderate magnetic stirring, the pH was adjusted to (11.5 ± 0.5) with NaOH (1.0 mol l^{-1}) slowly added in droplets for approximately 2–5 min. Then, 4.0 ml of cadmium precursor ($Cd(ClO_4)_2$, SOL-B) and 2.5 ml of sulfur source solution (CH_3CSNH_2, SOL-A) were added to the flask (S:Cd molar ratio was kept at 1:2). The solution turned yellowish and sampling aliquots of 1.0 ml were collected at different time intervals (3 h, 6 h, 8 h, 24 h, 48 h and 9 days) for UV–vis spectroscopy measurements, which were used for kinetics analysis and colloidal stability evaluation. The actual polymer concentration during the synthesis of the nanoparticles was diluted by approximately 25 times the original stock solutions (~2 ml:50 ml).

2.3. UV–visible spectroscopy characterization

UV–Vis spectroscopy measurements were conducted using Perkin–Elmer equipment (Lambda EZ-210), wavelength from 600 nm to 190 nm, in transmission mode, using quartz cuvette. The absorption spectra were used to monitor the reaction for the formation of CdS QDs and their relative colloidal stability in the medium. Moreover, based on the "absorbance onset" of the curve it was possible to calculate the average nanoparticles sizes and their optical properties. All experiments were conducted in triplicates ($n = 3$) unless specifically noted. Statistical analysis was performed assuming the mean and the standard deviation where needed.

2.4. TEM-EDS spectroscopy characterization

The nanostructural characterizations of the CdS-polymer samples based on the images and the electron diffraction patterns (ED) were conducted using a Tecnai G2-20-FEI transmission electron microscope (TEM) at an accelerating voltage of 200 kV. The Energy-dispersive X-ray spectra (EDS) were collected in the TEM for chemical analysis.

2.5. Photoluminescence spectroscopy characterization

The emission spectra of the CdS-Polymer nanohybrids were acquired by using an Ocean Optics USB4000 VIS–NIR spectrophotometer and a Helium–Cadmium (HeCd) laser at $\lambda = 442$ nm (violet–blue, 15 mW of power) as the excitation source. All the photoluminescence (PL) spectra were collected at room temperature.

3. Results and discussion

3.1. UV–Vis spectroscopy of PVA-capped CdS quantum dots

3.1.1. Effect of PVA concentration on CdS formation and stability

In order to investigate the effectiveness of PVA working as a stabilizer for preparing CdS nanocrystals in aqueous medium, three different concentrations of PVA solutions were tested. It is broadly known that semiconductor nanoparticles made from II–VI elements exhibit a very pronounced change in their optical absorption properties when their sizes were reduced below a certain diameter. Hence, in this study CdS nanoparticles colloids were extensively characterized based on the UV–visible spectroscopy results. In Fig. 2, it is shown the UV–vis spectra of the system immediately after the thioacetamide precursor was added to the cadmium perchlorate and PVA solutions. It can be observed in Fig. 2 that, at relatively high PVA concentrations, i.e. 1.0 and 10^{-1} mol l^{-1}, represented by curves (a) and (b), respectively, there were significant changes in the absorption spectra at about 500 nm. That aspect can be attributed to the formation of CdS nanoparticles shifting the relative position of the absorption onset. On the other hand, at a lower PVA content [10^{-2} mol l^{-1}, Fig. 2(c)], the spectrum did not present noticeable changes on the absorption curve in that same wavelength range. Thus, at this polymer concentration, the "as-prepared" colloidal suspensions of CdS nanoparticles were not stable. It should be mentioned that the peak at approximately

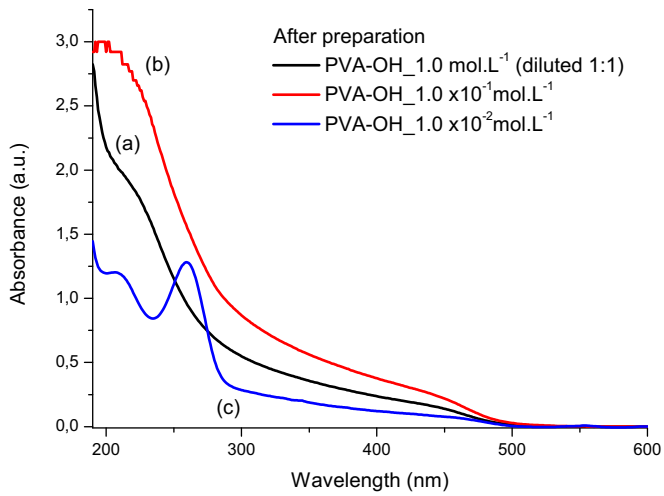

Fig. 2. UV–Vis spectra of PVA-OH solutions with different concentrations (at initial stage): (a) 1.0 mol l^{-1}, (b) 1.0 × 10^{-1} mol l^{-1}, and (c) 1.0 × 10^{-2} mol l^{-1}.

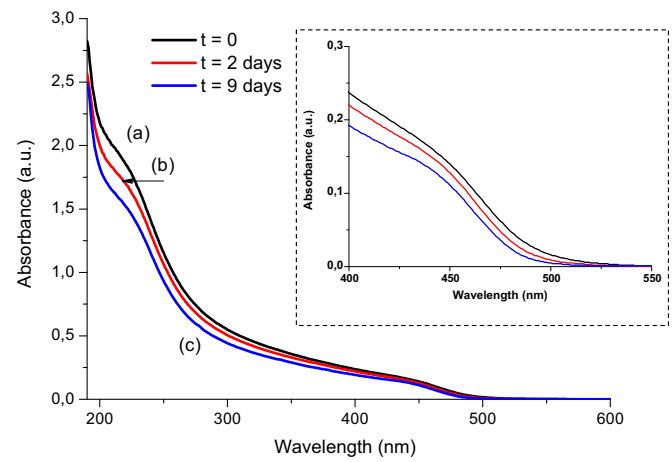

Fig. 4. UV–Vis spectra of PVA-OH at 1.0 mol l^{-1} concentration regarding to the stability of the quantum dots after the preparation (a); 2 days (b); 9 days (c). Insert: detail of the "absorbance onset" region.

~280 nm was associated with the thioacetamide precursor. At alkaline pH, the thioacetamide was practically all consumed based on the reaction (Eq. (1)), as the kinetics of thioacetamide decomposition is favored by the increase in pH. The stabilization effect was even more evident after 24 h from the initial reaction of the CdS nanocrystals formation, as it can be verified in the spectra shown in Fig. 3. The "absorbance onset" values were not apparent in both curves, Fig. 3b and Fig. 3c, at the PVA concentrations of 10^{-1} mol l^{-1} and 10^{-2} mol l^{-1}, respectively. Thus, it could be assumed that the CdS-PVA colloidal systems were not stable at these concentrations. It means the CdS nanocrystals may have flocculated or they perhaps have grown to bulk-size. In both cases, the spectra would be consistent with the curves presented in Fig. 3b and Fig. 3c. Nevertheless, the system was stable for 24 h at the PVA concentration of 1.0 mol l^{-1} [Fig. 3a]. In fact, it can be observed in Fig. 4 that the semiconductor nanocrystals of CdS formed in the PVA aqueous solution (1.0 mol l^{-1}) did not present major changes based on their visible absorption curves from the initial stage up to 9 days.

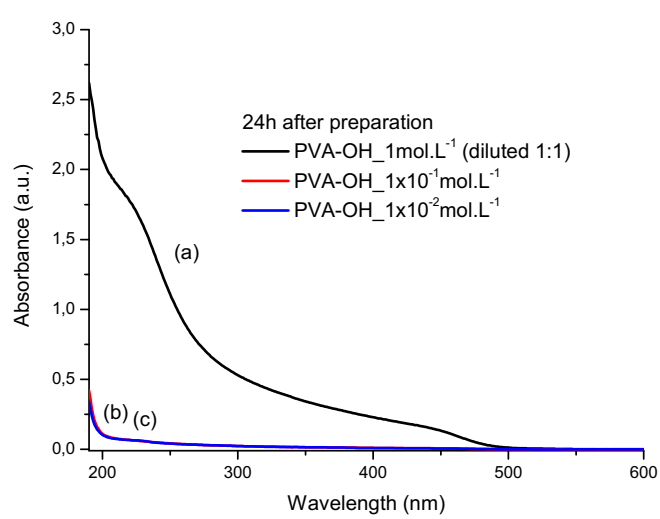

Fig. 3. UV–Vis spectra of PVA-OH solutions with different concentrations (at 24 h): (a) 1.0 mol l^{-1}, (b) 1.0 × 10^{-1} mol l^{-1}, and (c) 1.0 × 10^{-2} mol l^{-1}.

3.1.2. CdS nanoparticle size determination in colloidal media

At this point, it is assumed that a short background on physics and chemistry of semiconductor nanocrystals would be recommended. This subject is intrinsically multidisciplinary and some fundamental considerations are required to properly understand and explore the number of possibilities on conjugating QDs with polymers. Nevertheless, an in-depth analysis of this topic based on quantum theory is beyond the scope of this paper. So, optical excitation of electrons from the valence band to the conduction band produces an abrupt increase in the absorbance at the wavelength associated with the band gap energy. Due to their extreme small size, semiconductor nanoparticles with the dimensions below the so-called "bohr-radius" will present a quantum-confinement effect, related to the strong interaction between the pair "hole-electron" generated by exciting photon [1]. It means that after reaching a specific threshold in particle size (R = radius), the band gap is larger than that of the original bulk material [3]. The reported bulk value for CdS band gap is approximately 2.4 eV or wavelength $\lambda = 514$ nm. For CdS, the quantum size effect occurs as the cluster diameter becomes comparable to or smaller than the exciton diameter of about 6.0 nm (about 3000–4000 atoms) [24]. As a consequence, through UV–vis spectra data, the "absorbance onset" on the curve will be directly related to the altered band gap caused by the quantum-confinement effect.

Essentially, in this study, the average nanoparticle size was determined from Henglein's empirical model [25] which relates the CdS nanoparticle diameter ($2R$) to the optical "absorption onset" from UV–vis spectra according to Eq. (2):

$$2R_{cds}(\mathrm{nm}) = 0.1/(0.1338 - 0.0002345\lambda_{\mathrm{QD}}) \quad (2)$$

Where: λ_{QD} is the wavelength from the spectral "absorption onset". The parameter λ_{QD} can be estimated directly from the intersection of the sharply decreasing region of the UV–vis spectrum with the baseline. However, the assessment of the optical band gap has been accepted as a more accurate method for obtaining the wavelength value (λ_{QD}) associated with the "absorbance onset".

Therefore, in the present research, the optical band gap was estimated from absorption coefficient data as a function of wavelength using the "Tauc relation" [26,27], as shown in Eq. (3):

$$\alpha h\nu = B\left(h\nu - E_{qd}\right)^n \quad (3)$$

Where: α is the absorption coefficient, $h\nu$ is the photon energy, B is the band form parameter, E_{qd} is the optical band gap of the

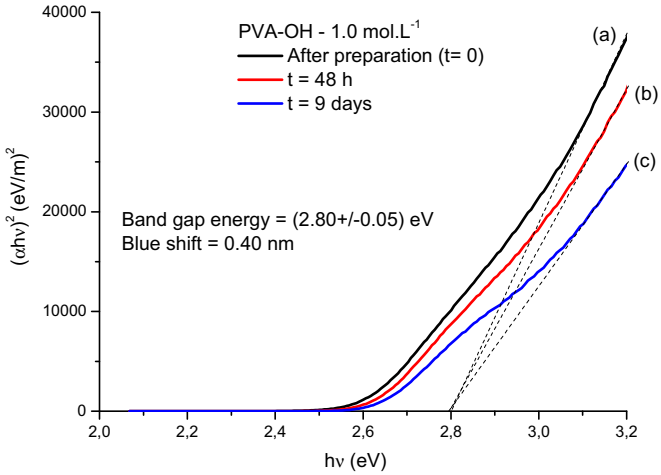

Fig. 5. Optical absorption spectra of CdS nanoparticles in PVA-OH solution at the concentration of 1.0 mol l^{-1} after preparation (a); 48 h (b); 9 days (c).

nanoparticles, and $n = 1/2$ for direct band gap and 2 for indirect band gap. It this case, $n = 1/2$ as CdS is a direct band gap semiconductor. Therefore, one can estimate the direct band gap value from the plots of $(\alpha h\nu)^2$ versus $(h\nu)$ and extrapolating the straight portion of the graph to $(h\nu)$ axis, i.e. at $\alpha = 0$ (dashed lines in Fig. 5). It should be pointed out that these are relative values extracted from the method which are usually compared to bulk values. Nevertheless, it has been widely accepted as the most accurate way of estimating average particle size from the UV–vis spectroscopy technique.

Based on these theories, it was possible to characterize the formation, stability and average sizes of cadmium sulfide nanoparticles in PVA colloidal solutions though UV–vis spectroscopy. Furthermore, the bandgaps of the samples were calculated in order to determine if there was a "size quantization" effect in the synthesized materials.

In Fig. 5, it is presented the results for the stability of the system which was monitored since the very beginning when the reaction has initiated up to 9 days. It can be observed that the synthesized CdS colloidal suspensions were rather stable, with the calculated average band gap of $E_{qd} = (2.80 \pm 0.05)$ eV, which is higher than the bulk value of 2.4 eV reported for CdS. Thus, the "blue-shift" of about 0.4 eV (i.e. 2.8–2.4 eV or from $\lambda = 514$ nm–443 nm) has proven that the CdS quantum dots were successfully synthesized under these conditions. Moreover, this fact has given strong evidence that they were chemically stable as colloidal aqueous solution for a fairly long "shelf-life" period.

Hence, a similar analysis was conducted for the whole set of colloidal systems using PVA polymer as the "capping" agent and the results for band gap energy and CdS nanocrystals sizes are summarized in Table 1. These results for PVA-OH samples are presented in Fig. 6. It can be observed the correlation between the calculated band gap energies ("blue-shift") with time of colloidal preparation for each PVA-OH concentration, considering the time immediately after the addition of the sulfur precursor up to 168 h (9 days).

From the colloidal chemistry perspective, these results can be primarily analyzed and discussed by assuming that both thermodynamics and kinetics aspects associated with the formation of cadmium sulfide are very favorable. That is, the reaction of Cd^{2+} with sulfides in water medium producing CdS crystals would certainly cause a reduction in the free-energy ($\Delta G < 0$). Regarding to kinetics, the reaction rate is determined by the extremely small value of the "solubility product constant" ($K_{sp} = 8.0 \times 10^{-27}$). However, after being formed, the nucleated nanocrystals would tend to grow or agglomerate mostly driven by the decrease in surface energy, i.e. by reducing the surface area (S) to volume (V) ratio as in Eq. (4):

$$S/V = \left(\pi R^2\right) \Big/ \left((4/3)\pi R^3\right) = 3/(4R) \tag{4}$$

Thus, based on classic theories the growth of the nanoparticles may have occurred by two favored pathways [22]:

a) The growth of CdS on the nucleated seeds (Eq. (5)):

$$Cd^{2+}(aq) + S^{2-} + (CdS)_m \rightarrow (CdS)_{m+1} \tag{5}$$

b) The process of "Ostwald ripening" whereby larger seeds grew at the expense of the smaller ones (Eq. (6)):

$$(CdS)_n + (CdS)_k \rightarrow (CdS)_{k+1} \qquad (k > n) \tag{6}$$

Therefore, in order to stabilize the just formed particles in the aqueous solution, a relatively high concentration of PVA-OH capping agent has to be utilized. Apparently, the most likely mechanism acting on the system is the reduction of the surface energy by the interactions with the hydroxyl groups from PVA-OH chains, as schematically represented in Fig. 7. In order words, it could be considered that the CdS quantum dots were effectively stabilized by a polymeric-capping effect, as the diffusion of ions (Cd^{2+} or S^{-2}) was significantly restricted by the PVA surface layer limiting further particle growth. Obviously, several other interactions were present in such complex dynamic system as described in the excellent recent review from Nel and co-workers [28]. For instance, electrostatic forces, hydrophilic and hydrophobic interactions, hydrogen bonds (PVA alcohol groups), steric hindrance among polymer chains will influence the overall behavior of the colloidal system. It was not found in the literature previous reports addressing the effect of molecular weight (M_W), molecular weight distribution and degree of hydrolysis (DH) of PVA on the stability of

Table 1
Parameters of CdS nanoparticles formation in PVA-OH and PVA-COOH media at different concentrations and reaction time: band gap energy, "blue-shift", size.

PVA concentration			1.0 mol l^{-1}		1.0×10^{-1} mol l^{-1}		1.0×10^{-2} mol l^{-1}	
Time			t_o	t_∞	t_o	t_∞	t_o	t_∞
Stabilizer	PVA-OH	Band Gap (eV)	2.80	2.80	2.78	Bulk*	2.80	Bulk*
		"Blue-shift" (eV)	0.40	0.40	0.38	Bulk*	0.40	Bulk*
		Absorbance onset (λ, nm)	443	443	446	Bulk*	443	Bulk*
		Particle Size (2R,nm)	3.3	3.3	3.4	Bulk*	3.3	Bulk*
	PVA-COOH	Band Gap (eV)	2.81	2.81	2.78	2.78	2.75	Bulk*
		"Blue-shift" (eV)	0.41	0.41	0.38	0.38	0.35	Bulk*
		Absorbance onset (λ, nm)	441	441	446	446	451	Bulk*
		Particle Size (2R,nm)	3.3	3.3	3.4	3.4	3.6	Bulk*

t_∞ = after 9 days of reaction; t_o = initial time of reaction; Bulk* = bulk crystals or flocculated.

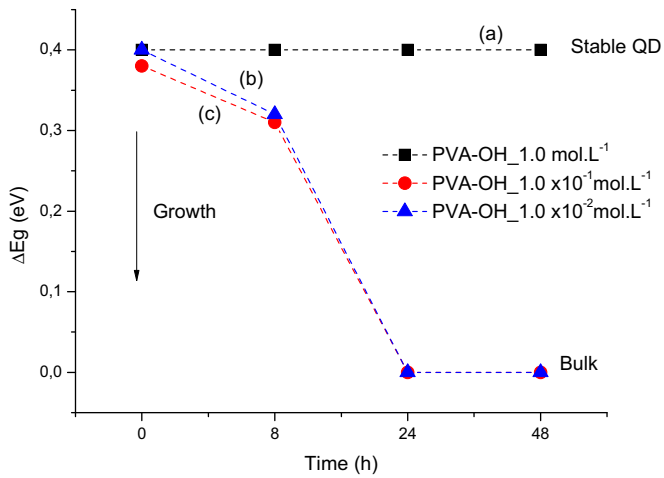

Fig. 6. Evolution of the "blue-shift" of CdS nanoparticles in PVA-OH solution at the concentration of (a) 1.0 mol l^{-1}; (b) 1.0 × 10^{-1} mol l^{-1}; (c) 1.0 × 10^{-2} mol l^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

CdS quantum dots in biological or clinical use. However, theoretically, it can be assumed that PVA with low M_W and intermediate DH would be easier to be biodegraded by the mammals' metabolic system and later excretion by liver and kidneys. On the other hand, there is a limit on reducing the polymer M_W as it should also work as capping ligand during the QDs formation. That would certainly require a more in-depth investigation and also a large number of experiments to reach the optimum balance among all properties needed to be suitable for biomedical applications.

3.1.3. *Effect of PVA-COOH concentration on CdS formation and stability*

Aiming at moving a step forward on understanding these interactions between CdS quantum dots and polymer-capping agents, PVA chemically functionalized with carboxylic groups (PVA-COOH)

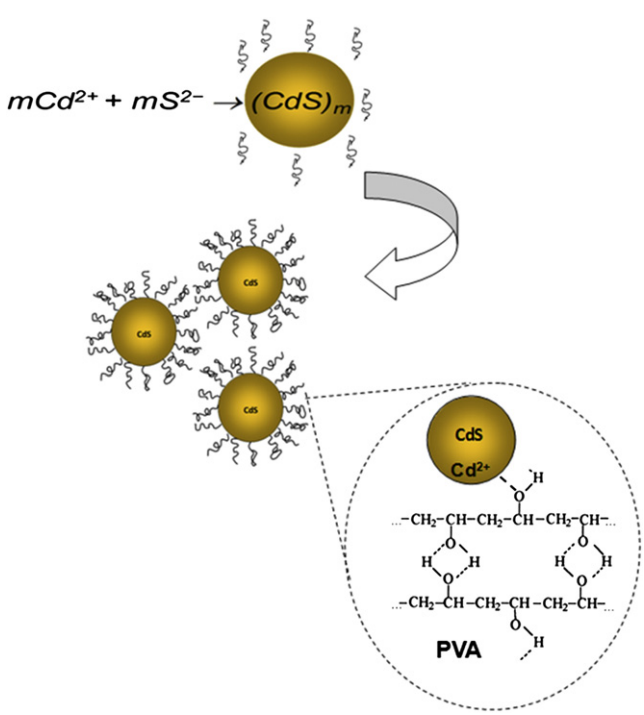

Fig. 7. Schematic representation of the mechanism acting in the system: reduction of the high surface energy by interaction with the hydroxyl groups from PVA-OH chains.

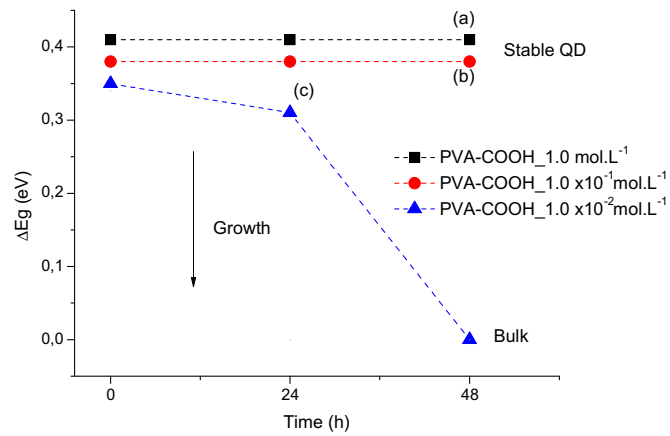

Fig. 8. Stability and band gap analysis of CdS nanoparticles in PVA-COOH solution at the concentration of (a) 1.0 mol l^{-1}; (b) 1.0 × 10^{-1} mol l^{-1}; (c) 1.0 × 10^{-2} mol l^{-1}.

was also prepared via aqueous colloidal routes using. Fig. 8 presents the results of the changes on band gap energy using PVA-COOH at 3 M concentrations, 1.0, 10^{-1}, and 10^{-2} mol l^{-1}. It can be clearly observed that for PVA-COOH with the concentrations of 1.0 mol l^{-1} and 10^{-1} mol l^{-1} (Fig. 8a and Fig. 8b, respectively) no significant alterations were detected from the initial stage of the reaction up to 48 h. On the other hand, the band gap shift was smaller at a lower PVA-COOH concentration (10^{-2} mol l^{-1}, Fig. 8c). That was directly related to the CdS nanoparticles growth and/or coalescence causing the formation of agglomerates as discussed in previous section (Eq. (5) and (6)). These results of PVA-COOH functionalized compared to those of PVA-OH without chemical modification of the polymer chain (Fig. 6) have evidenced a major contribution from the carboxylic groups on stabilizing the CdS nanoparticles in the quantum-size range. Indeed, it is worth mentioning that the capping behavior in the PVA-COOH solution was approximately 10 times more effective than the solution of PVA-OH by taking into account their respective molar concentrations, 10^{-1} mol l^{-1} and 1.0 mol l^{-1}.

Once more, by considering the mechanism of colloidal particles stabilization in solution, it may be assumed that the new carboxylic species added to the PVA-COOH polymeric chains have been attracted by the dangling bonds of Cd–S at the quantum dots surfaces. Besides that, at the used alkaline pH (pH > 11.0) the

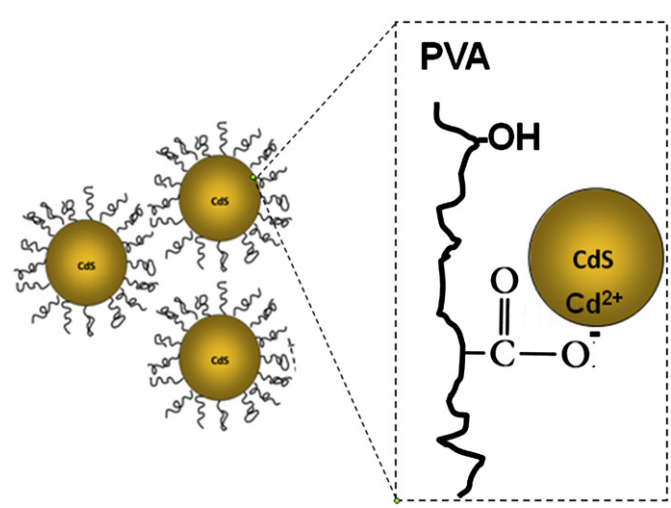

Fig. 9. Schematic representation of the mechanism acting in the system: reduction of the high surface energy by interaction with the carboxylate groups from PVA-COOH chains.

carboxylic groups from PVA-COOH should be partially ionized forming carboxylates (COO^-). As a consequence, these negatively charged regions of PVA-COOH would have driven to a much stronger attractive force with the positively charged ions (Cd^{2+}) at the nanoparticles surface. The function of the polymer in this case was to compete with sulfide (S^{2-}) for metal ion-binding sites and, presumably, to sterically hinder small nanoparticles from aggregating together and growing into larger ones. Also, Cd^{2+} at the surface would be stabilized by forming chelates with carboxylates ("electron-donor"). The schematic representation of this system is illustrated in Fig. 9. It can be assumed that, for charged polymer systems, such as PVA-COOH, mainly strong electrostatic interactions have worked and influenced the adsorption at the nanoparticles surfaces balancing their charges. On the other hand, for uncharged polymers (PVA-OH), H-bonding and solvation forces were expected to be more significant.

It should be emphasized that it is a simplified approach for the developed system. Although the adsorption behavior of PVA polymer on solid–liquid interface is well documented in the literature, the amount adsorbed and the kinetics are strongly dependent on the molecular weight and the number of acetate groups of PVA, resulting in different properties and behavior. The adsorption forces usually increase when the degree of hydrolysis of the PVA is increased mostly due to the formation of hydrogen bonds. Also, the presence of the hydrophobic acetate groups affects the conformation of polymer chains adsorbed onto the nanoparticles surfaces [29]. By adding functional groups such as carboxylic, the adsorption phenomenon is more complex as the polymer chain has to reach a 3D conformation to match the overall balance of forces involved. That effect was reported in our recent work where CdSe nanocrystals were successfully produced in carboxylic modified polymer via aqueous colloidal route [30].

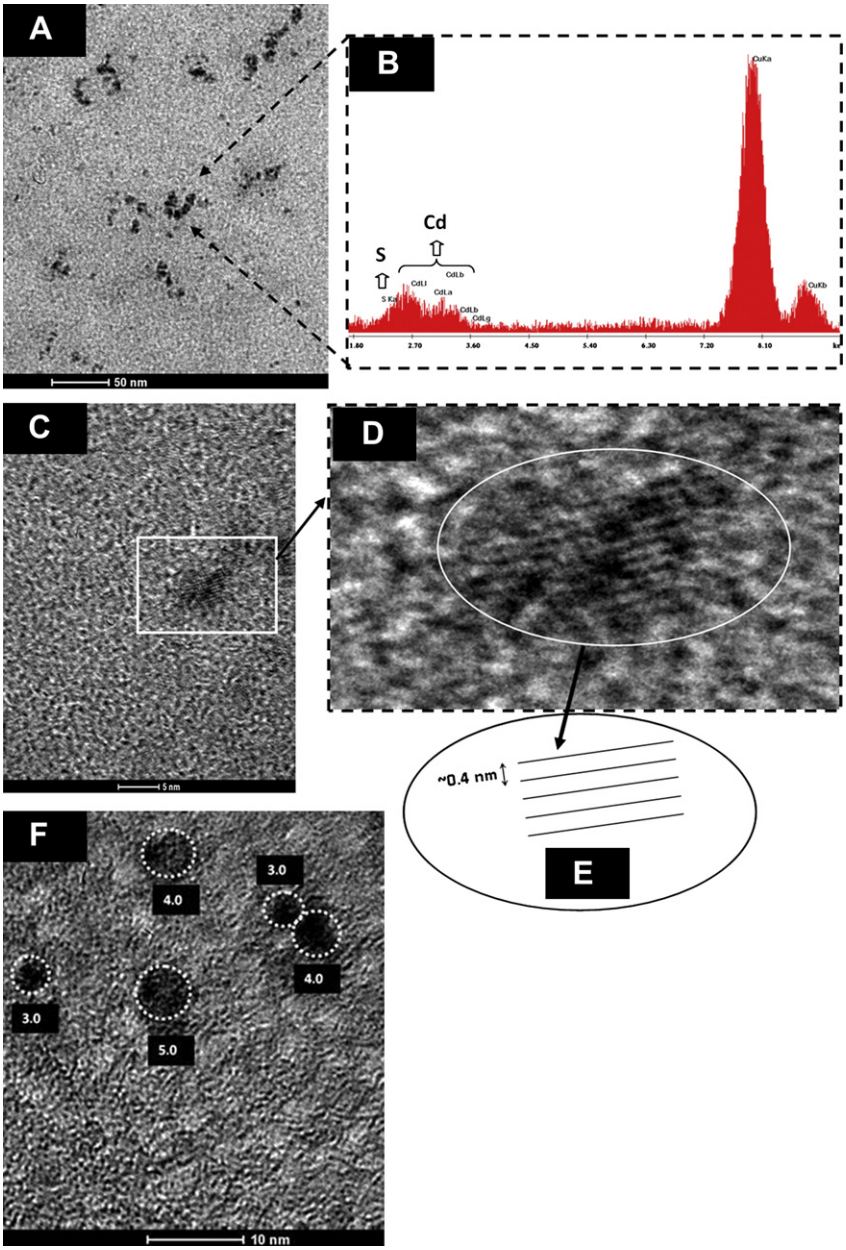

Fig. 10. (A) TEM image of produced CdS/PVA-COOH quantum dots; (B) EDS spectrum of CdS/PVA-COOH QDs; (C) Image of QDs lattice arrangements by electron diffraction; (D) detailed nanocrystal plane spacing and representative drawing (E); TEM image of CdS/PVA-COOH quantum dots with estimated nanoparticles sizes (F);

3.2. Characterization of CdS quantum dots by TEM coupled to EDS spectroscopy

UV–visible absorption spectroscopy has been widely utilized for estimating semiconductor nanoparticles sizes, as it is a straightforward technique of monitoring the nanocrystals nucleation and growth in colloidal media. Nevertheless, in this research a more in-depth and complementary evaluation of the quantum dots characterization was carried out using transmission electron microscopy (TEM). The morphological and structural analyses of the quantum dot-polymer systems were performed by electronic transmission microscopy experiments associated with the electron diffraction patterns (EDP). A representative TEM image of CdS-PVA-COOH system is shown in Fig. 10A and F. It can be observed a fairly uniform nanoparticles size distribution, typically within the range of 1–5 nm and the estimated mean size centered at 3–4 nm. Also, the Energy-dispersive X-ray spectra (EDS) have shown the chemical analysis of the nanocrystals with Cd and S as the major elements (Fig. 10B), excluding the copper and carbon peaks related to the TEM grid. These TEM results have proven that CdS quantum dots were properly stabilized by the PVA-COOH capping polymer within the quantum-confinement size range and they were also consistent with the values estimated by UV–Visible spectroscopy in the previous section. In Fig. 10C and D it can be clearly verified the electron diffraction patterns associated with the CdS quantum dots crystalline structure with plane spacing of about ~4–5 Å. These results are well-matched to the values reported in the literature for the CdS QDs with wurtzite-like crystal structures (wurtzite, a = 4.160; c = 6.756 Å) [31,32].

3.3. Photoluminescence spectroscopy of polymer-capped CdS quantum dots

Despite not being the major goal of the present study, it is known that some other properties are important to be investigated before using such colloidal system as biomarkers. For instance, the QDs photo-stability could be accessed by photoluminescence spectroscopy. In that sense, photoluminescence (PL) spectroscopy was conducted aiming at verifying the preliminary activity of the CdS nanocrystals produced via aqueous route using a relatively facile method with carboxylic-functionalized PVA as capping agent. Fig. 11A presents a typical PL emission spectrum for CdS nanocrystals synthesized with the carboxylic modified PVA as capping ligand. The PL emission peak energy was centered at approximately λ = 520 nm ("green"), for all polymer concentrations tested. A similar behavior was also observed for CdS nanocrystals stabilized by "conventional" PVA (PVA-OH, PL not shown). Also, the fluorescence images were captured by using a 12 million pixels color digital camera. Fig. 11B shows the characteristic "green" fluorescence image of CdS/PVA-COOH QDs in aqueous colloidal solution excited by laser source at λ = 442 nm (violet–blue, 15 mW of power). Fig. 11C presents the image of the synthesized CdS/PVA-COOH QDs with a bright green fluorescence when exposed to ultraviolet light radiation (compartment, light source λ = 254 nm). These results have given support for considering this novel system based on CdS-PVA-COOH to be potentially used in biolabeling applications as it has indeed shown PL behavior. Besides that, it brings an environmentally friendly alternative to the labor-intensive method of organic-phase synthesis followed by ligand exchange. The choice of PVA polymer comes as an alternative to other aqueous routes, with some advantages. PVA and PVA-COOH are biocompatible and biodegradable comparable to Polyethylene glycol (PEG). Different from PEG, which is always very hydrophilic, PVA and its derivatives can be chosen based on the polymer chain and the degree of hydrolysis (ratio of acetates/alcohol groups) which offers a unique opportunity for preparing solutions with a wide range of properties (viscosity, solubility, degradability etc.) (Fig. 12).

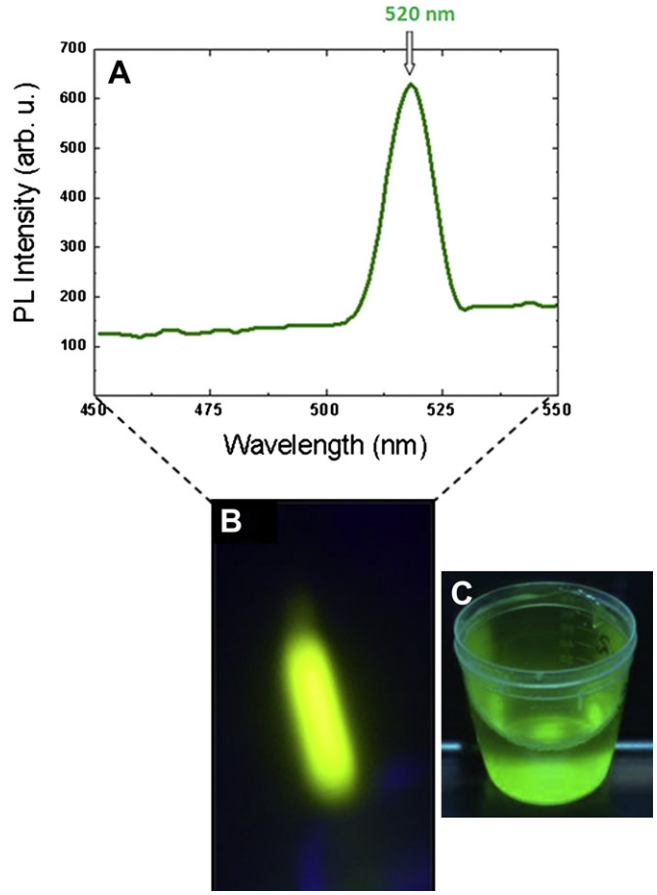

Fig. 11. (A) Fluorescence spectrum and (B) Fluorescence image of CdS/PVA-COOH QDs in aqueous colloidal solution excited by laser source at λ = 442 nm (violet–blue, 15 mW of power); (C) image of CdS/PVA-COOH QDs in ultraviolet light compartment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Therefore, it can be highlighted the following aspects when comparing to other published studies:

1) PVA-derived polymer [terpolymer, Poly(vinyl alcohol-vinyl acetate-itaconic acid)] was utilized as the novel capping agent with carboxylic pendant groups (PVA-COOH) which has decreased about 10 times the concentration of ligand needed when compared to PVA(PVA-OH);
2) The precursor [Cd:S] molar ratio used was [2:1] as compared to the large majority of previously consulted publications which have used [1:1]. That has made possible some "free" Cd^{2+} cations at the quantum dots surfaces for charge balancing with carboxylate anions ($-COO^-$). As a consequence, it has resulted in more stable colloidal systems;
3) The partial substitution of alcohol by carboxylic groups increases the potential reactivity of the quantum dots-Polymer systems with biomolecules;
4) In addition, the choice of using a carboxylic functional polymer may contribute for reducing the total "hydrodynamic diameter" of the bioconjugate QD-polymer-protein, as it can be produced in a single-step process.

Thus, in order to move further aiming at biological applications of this developed work, one may consider a designed system with

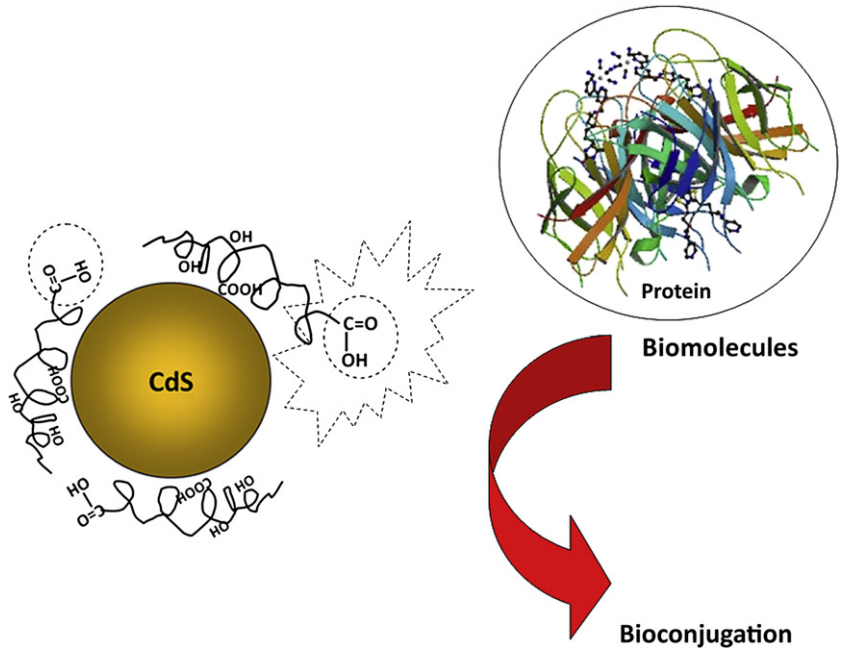

Fig. 12. Representative drawing of potential alternatives for bioconjugation with the carboxylate groups from PVA-modified chains (not in scale).

the choice of the carboxylic group as a chemical functionality in PVA to be utilized as a reactive "coupling group" for potential bioconjugation. For instance, the synthesis of quantum dot-polymer (CdS/PVA-COOH) structures conjugated with biological moieties (Fig. 12) such as proteins.

3.4. CdS quantum dots capped by PVA-C(O)NH-BSA bioconjugates

As a proof of concept, it was designed and performed a set of experiments. PVA-COOH was conjugated with BSA (bovine serum albumin) considered as a "model" biomolecule. The reaction of

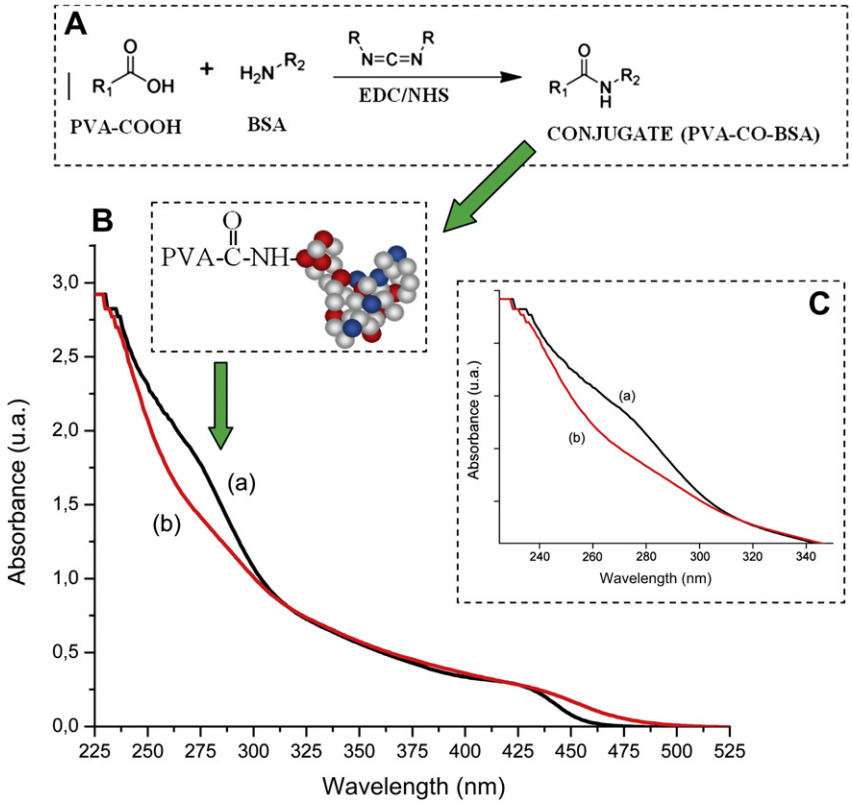

Fig. 13. (A) Schematic representation of the bioconjugation reaction between PVA-COOH and BSA with the formation of an amide using a carbodiimide linker; (B) UV–Vis spectra of CdS quantum dots with PVA-COOH (a) and with (b) PVA-C(O)NH-BSA bioconjugation; (C) Insert: detail of the UV–Vis spectra of the amide region.

the carboxyl-reactive 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was utilized as a "zero-length" cross linker in the presence of NHS-sulfo (catalyst) for covalently coupling the carboxylic group from PVA-COOH to the amines from BSA (protein-NH_2). The formation of an amide linkage is expected to occur straightforward ("PVA-C(O)NH-BSA"). Also, EDC has the advantages of not being incorporated into the structure of the protein and not being toxic in comparison with cross-linkers (glutaraldehyde). The results are summarized in Fig. 13. In Fig. 13A, it is shown the schematic representation of the bioconjugation reaction with the formation of an amide using carbodiimide (EDC/NHS-sulfo). It can be observed in the UV–Vis spectra (Fig. 13B and C) the increase of absorbance in the amide region (curve b) of the conjugated PVA-C(O)NH-BSA compared to the PVA-COOH not conjugated (curve a). It is important to be pointed out that no detectable differences were observed in the CdS quantum dots average sizes prepared using both capping ligands, PVA-COOH or PVA-C(O)NH-BSA. Therefore, the results have clearly proven that CdS quantum dots were successfully produced/stabilized using a novel approach based on PVA-COOH bioconjugated to BSA.

4. Conclusion

It can be summarized that in the present study an aqueous colloidal route for producing CdS quantum dots was developed using both PVA-OH and carboxylic-functionalized PVA-COOH as polymer-capping agents. Regarding to the colloidal system stability, the carboxylic groups in the polymeric chain have decreased about 10 times the amount of ligand required for obtaining water stable suspensions. They have shown relatively adequate activity and stability in PL assays, good dispersion, and the capped surfaces by alcohol and carboxylic groups. The use of carboxylic group in the functionalized PVA (PVA-COOH) potentially increases the overall interactions leading to stability of the system with other chemical species when compared to alcohol groups (PVA-OH). In fact, the designed system based on the chemically active polymer (PVA-COOH) was successfully bioconjugated to the "model protein" (BSA), via carboxylic-amine reactions, leading to the formation of amide covalent bonds (PVA-C(O)NH-BSA). The synthesized CdS/PVA-COOH and CdS/PVA-C(O)NH-BSA nanocrystals represent hybrid inorganic–organic materials that can be both fabricated and functionalized with biomolecules in a relative facile synthetic route. In that sense, this novel nanostructured system opens a wide window of possibilities in biomedical research and applications for producing water soluble inorganic fluorophores based on quantum dots.

Acknowledgments

The authors acknowledge the financial support from CAPES (Proc:5548-09-8; PNPD/2010; 433/2010), FAPEMIG (Proc:PPM-00343-09), and CNPq (Proc:305098/2007-1). Also, the authors thank the contribution from Microscopy Center/UFMG staff for TEM analysis.

References

[1] Brus LE. J Chem Phys 1984;80:4403–9.
[2] Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Nature Materials 2005;4:435–46.
[3] Mansur H. Wiley Int. Reviews: Nanomedicine and Nanobiotechnology 2010;2:113–29.
[4] Efros AL. Sov Phys Semicond 1982;16:772–5.
[5] Ekimov AI, Onushchenko AA. Sov Phys Semicond 1982;16:775–8.
[6] Mansur HS, Grieser F, Marychurch MS, Biggs S, Urquhart RS, Furlong DN. J Chem Soc, Faraday Trans 1995;91:665–72.
[7] Mandal P, Srinivasa RS, Talwar SS, Major SS. Applied Surface Science 2008;254(16):5028–33.
[8] Shin SK, Yoon HJ, Jung YJ, Park JW. Current Opinion in Chemical Biology 2006;10(5):423–9.
[9] Jeong NC, Kim HS, Yoon KB. Langmuir 2005;21(13):6038–47.
[10] Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI, et al. Nature Biotechnology 2007;25:1165–70.
[11] Ma N, Tikhomirov G, Kelley SO. Acc Chem Res 2010;43(2):173–80.
[12] Pinaud F, King D, Moore H-P, Weiss S. J Am Chem Soc 2004;126:6115–23.
[13] Mansur HS, Vasconcelos WL, Grieser F, Caruso F. Journal of Materials Science 1999;34(21):5285–91.
[14] Li H, Shih WY, Shih W-H. Ind Eng Chem Res 2007;46:2013–9.
[15] Peppas NA, Simmons REP. J Drug Del Sci Tech 2004;14(4):285–9.
[16] Costa-Júnior ES, Barbosa-Stancioli EF, Mansur AAP, Vasconcelos WL, Mansur HS. Carbohydrate Polymers 2009;76(3):472–81.
[17] Hassan CM, Peppas NA. Adv Polym Sci 2000;153:37–65.
[18] Mansur HS, Sadahira CM, Souza AN, Mansur AAP. Materials Science and Engineering: C 2008;28(4):539–48.
[19] Briscoe B, Luckham P, Zhu S. Polymer 2000;41:3851–60.
[20] Moritani T, Kajitani K. Polymer 1997;38(12):2933–45.
[21] Wang H, Chen Z, Fang P, Wang S. Materials Chemistry and Physics 2007;106:443–6.
[22] Ma X-D, Qian X-F, Yin J, Xi H-A, Zhu Z. Journal of Colloid and Interface Science 2002;252:77–81.
[23] Yang YJ, Xiang BJ. Journal of Crystal Growth 2005;284:453–8.
[24] Spanhel L, Haase M, Weller H, Henglein A. J Am Chem Soc 1987;109:5649–55.
[25] Weller H, Schmidt HM, Koch U, Fojtik A, Baral S, Henglein A, et al. Chemical Physics Letters 1986;124(6):557–60.
[26] Tauc J, Menth A. J Non-Cryst Solids 1972;8-10:569–85.
[27] Winter JO, Gomez N, Gatzert S, Schmidt CE, Korgel BA. Colloids and Surfaces, A: Physicochemical and Engineering Aspects 2005;254(1–3):147–57.
[28] Nel AE, Mädler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, et al. Nature Materials 2009;8:543–57.
[29] Chibowski S, Paszkiewicz M. J Dispers Sci Technol 2001;22:281–9.
[30] Mansur HS, Mansur AAP. Materials Chemistry and Physics 2011;125(3):709–17.
[31] Reiss P, Protiere M, Li L. Small 2009;5(2):154–68.
[32] Li Y, Zhu Y, Yang X, Li C. Cryst Growth Des 2008;8(12):4494–8.