BIOPHYSICAL STUDY OF CdTe QUANTUM DOTS INTERACTIONS WITH ALBUMIN AND ANTIBODY AS THE BASE OF PHOTODYNAMIC THERAPY

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Abstract

Photodynamic therapy is a relatively new type of cancer treatment, or eventually it is used at the elimination of undesirable pathogenic microorganisms. Quantum dots (QDs) are semiconducting nanocrystals with a size of 2-20 nm and can be used in photodynamic therapy. Two types of CdTe QDs were prepared by microwave synthesis (500 W), typical absorption spectra had maxima λ_{green} = 554 nm, λ_{yellow} = 580 nm. The determined size of the generated nanoparticles ranged between 5 - 10 nm. CdTe QDs were further studied by fluorescence analysis at excitation wavelength λ = 250 nm to obtain emission maxima (λ_{em552}) for green and (λ_{em582}) for yellow nanoparticles. Interaction study of CdTe QDs with bovine serum albumin (BSA) and with polyclonal chicken antibodies against sarcosine (AntiSar) was performed. BSA (100, 50, 25, 12.5, 6.3, 3.1, 1.6 and 0 μM) and AntiSar (20, 10, 5, 2.5, 1.3, 0.7, 0.3, and 0 g/L) were monitored with 1:1 addition of 50 μM QDs_{green} or QDs_{yellow}. The decrease in intensity of the normalized fluorescence signal in the presence of BSA or AntiSar was observed by 70-90%. The observed dependencies showed a linear trend (R^2 = 0.9) with relative error of 9-12% calculated from 5 independent repetitions. In addition, it was possible to monitor the signal shift to shorter wavelengths at the highest applied BSA concentration by 4-16 nm. The obtained data suggest that the size of individual QDs will affect intensity of an interaction with biomolecule. Nanoconstructs should therefore be targeted according to these experimental data for their intended use. In further experiments, CdTe QDs modified with AntiSar will be used for targeted prostate cancer therapy using photodynamic effect.

Keywords: Reactive oxygen species, prostate cancer, photodynamic therapy, CdTe quantum dots, nanomedicine

1. INTRODUCTION

Photodynamic therapy is a relatively new type of treatment in tumor diseases or it may be used by the elimination of undesirable pathogenic microorganisms [1,2]. The basic principle of photodynamic therapy is photoactivation of photosensitive substance / drugs. The process involves the transfer of electrons, and energy to form reactive oxygen intermediaries or reactive oxygen species. The molecules formed by this way react with cellular structures and lead to cell death. It is known that many factors affect these biologically significant processes (concentration, interaction time, wavelength, energy, and others) [3]. In addition, defensive mechanisms of cells (biomolecules containing thiol groups such as cysteine, reduced glutathione, and metallothionein) [4-6] should be considered in this complex system. Quantum dots (QDs) represent a possible

alternative to traditionally used photosensitive substances [7]. To increase the predicted therapeutic efficacy, the surface of QDs may be further modified (porphyrins) [7].

From physico-chemical point of view QDs are photoluminescent semiconductor nanocrystals constructed from elements of Group II (Zn, Cd, Hg)-VI (Se, S and Te), III-V and IV-VI of the periodic table. Until the last decade, most studies focused on II-VI QDs (CdSe or CdTe) [8-10]. The typical diameter of QDs is in the 2-20 nm range and they can contain from 100 to 100,000 atoms per nanoparticle [11]. Some of the most attractive properties of QDs are high quantum yield, high molar extinction coefficients, broad absorption spectra, narrow and symmetric emission bands (30-50 nm), large effective Stokes shifts and high resistance to photobleaching and chemical degradation [12]. Current applications of QDs are widespread, their use as fluorescence labels in bioassays being one of the most promising [13]. These nanoparticles are usually conjugated to highly specific biomolecules like proteins, antibodies, oligonucleotides, enzymes or aptamers to improve assay selectivity [11,14].

For the therapeutic use of such molecules it is necessary to know their pharmacokinetics in the living organism. Due to the considerable complexity of ongoing phenomena, the necessary simplification is needed at the beginning of the study to understand these mechanisms. Therefore albumin, main protein of blood plasma, is intensively monitored in connection with its interactions to nanoparticles. For QDs bioconjugation bovine serum albumin (BSA) is one of the most extensively studied proteins, mainly due to its structural homology with human serum albumin [9,15,16]. BSA is often used as a coat protein for the surface treatment of nanoparticles due to its strong affinity for a variety of nanoparticles such as gold nanoparticles, nanoparticles of silica and QDs [17-19]. In our previous experimental work we performed several pilot experiments aimed at monitoring the interaction of metallothionein with QDs [5,6]. The aim of this study was to investigate the interaction of QDs with various emissions with BSA and polyclonal chicken antibodies against sarcosine (AntiSar) by fluorimetry for their potential use in photodynamic therapy (see in **Figure 1**).

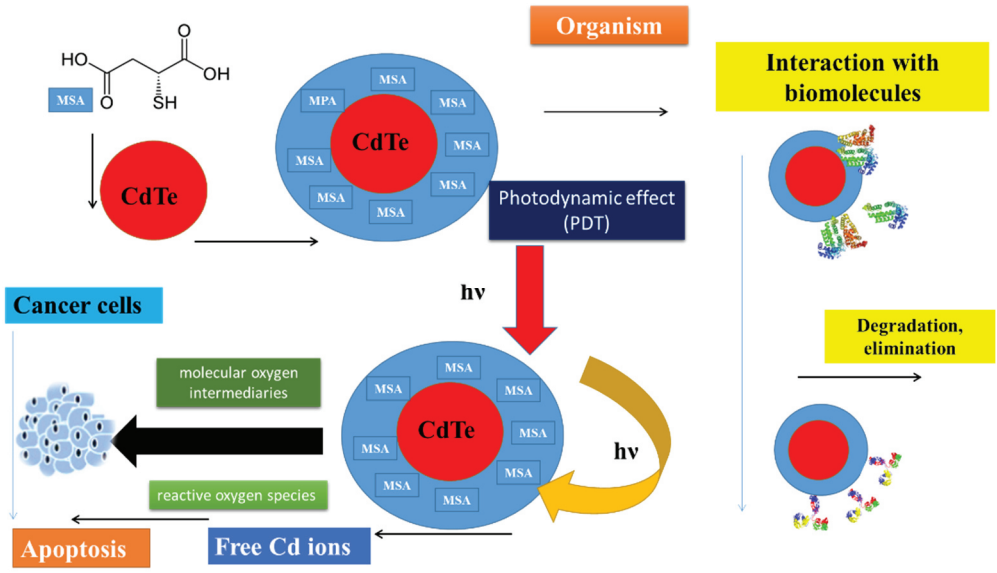

Figure 1 Schematic representation of the hypothesis of QDs function for nanomedicine application. Typical CdTe QDs stabilized with mercaptosuccinic acid (MSA) after exposure to specific radiation at a given wavelength and energy leads to excitation of a system that is capable of generating a whole group of molecular oxygen intermediaries and relative oxygen species. If such a particle is targeted toward the tumor cell (by various nanomedical approaches), an increased level of oxygen radicals can trigger apoptosis processes, and the tumor cell is destroyed. In addition, due to the photodynamic effect of CdTe QDs, free cadmium ions are released. They can also be very effectively involved in the initiation of apoptosis, or may be used as additional guidance particles for targeting other nanotransporters

2. MATERIAL AND METHODS

Chemicals: All chemicals used in this study (Cd $(CH_3COO)_2 \cdot 2H_2O$, Na_2TeO_3, mercaptosuccinic acid, bovine serum albumin, Trizma base, HCl) were purchased from Sigma-Aldrich (St. Louis, MO, USA), in ACS purity. $NaBH_4$ was purchased from Merck (Darmstadt, Germany), 25% aqueous NH_4OH was purchased from Lach-Ner s.r.o. (Neratovice, Czech Republic). Chicken polyclonal antibodies against sarcosine (AntiSar) were obtained from the Hena (Prague, Czech Republic). Deionized water was prepared using an Aqual 25 reverse osmosis device (Brno, Czech Republic). The deionized water was further purified using a MiliQ Direct QUV device equipped with a UV lamp. Resistance was 18 MΩ. The pH was measured using a WTW inoLab pH meter (Weilheim, Germany).

Preparation of CdTe quantum dots (QDs): 10 ml of solution $Cd(CH_3COO)_2 \cdot 2H_2O$ (0.266 g / 50ml) + 76 ml of H_2O + 1 ml of mercaptosuccinic acid (MSA) solution (3g / 50ml), 5 ml of Na_2TeO_3 (0.2215 g / 50 ml) + 40 mg of $NaBH_4$ was stirred on a magnetic stirrer (VMS-C4, VWR International Ltd., Darmstadt, Germany) for at least 2 hours until bubbling stopped. Subsequently, the volume was adjusted to 100 ml. 2 ml of the prepared solution was pipetted into the glass vials (Sigma Aldrich, St. Louis, MO, USA), with a white cap (Anton Paar, Graz, Austria) and a teflon cap (Anton Paar, Graz, Austria). The thus prepared vial was placed in a microwave which was set to a power of 300 W and the heating took place for 2 minutes with green QDs and 4 minutes for yellow QDs. After preparation, QDs were purified with isopropanol. After 500 µl of isopropanol was added to 500 µl of QDs, centrifugation was carried out at 14,000 g for 5 minutes. After pipetting the supernatant, pellet QDs was dissolved in 500 µl of 10 mM Tris-Cl buffer, pH 8. The final concentration of QDs was 2 mM. These purified QDs were used for further analysis.

Preparation of dilution series of BSA and AntiSar: From a stock solution of BSA (500 µM) in 10 mM Tris-Cl buffer, pH 8 a dilution series were prepared in a concentration range of 100, 50, 25 , 12.5, 6.3, 3.1, 1.7 and 0 µM. Similarly from a stock solution of chicken antibodies (20 g/L) a dilution series were prepared in a concentration range of 20, 10, 5, 2.5, 1.3, 0.7, 0.3 and 0 g /L.

Fluorescence spectra: Fluorescence spectra were obtained with the Tecan Infinite 200 PRO Multifunctional Reader (TECAN, Switzerland). For absorption spectra, the wavelength was in the range 230 - 800 nm. For fluorescence spectra, it was used as an excitation wavelength of 250 nm and an emission wavelength in the range 280 nm to 800 nm.

3. RESULTS

The detailed physico-chemical study of QDs has been published in a number of papers [20] including the study of their interactions with biomolecules. QDs nanoparticle is made up of an inorganic core and bound molecules on its surface. It is likely that the solvent and surface-bound molecules affect the physicochemical properties of QDs (hydrodynamic particle size). In our recent study, we studied the basic spectroscopic and electrochemical properties of CdTe QDs [21]. In the **Figure 1** are summarized the main experimental objectives of this work. We have decided to use CdTe QDs stabilized with mercaptosuccinic acid (MSA) in microwave synthesis. Two types of CdTe QDs were prepared by microwave synthesis (500 W), and the determined size of the generated nanoparticles ranged between 5 - 10 nm. CdTe QDs were studied by fluorescence analysis at λ = 250 nm to obtain excitation maxima ($\lambda_{em}552$) for green and ($\lambda_{em}582$) for yellow nanoparticles. In further experiments, we focused on the basic interaction study of CdTe QDs with bovine serum albumin (BSA) and polyclonal chicken antibody (AntiSar). Fluorescence spectra of BSA (100, 50, 25, 12.5, 6.3, 3.1, and 0 µM) were monitored in 1:1 addition to 50 µM of QDs_{green}. BSA at the studied concentrations and the observation interval (500-700 nm) did not provide any fluorescence signal that is observable at shorter wavelengths. In case we added QDs_{green} to the given BSA concentrations, a decrease in the intensity of the normalized fluorescence signal was observed by 76%, the dependence showed a linear trend y = -508.57x + 32064 (R^2 = 0.9355), with 5 independent repeats and a relative error of determination was about 10%. In

addition, it was possible to monitor the signal shift to shorter wavelengths at the highest applied BSA concentration by more than 15 nm (see in **Figure 2**).

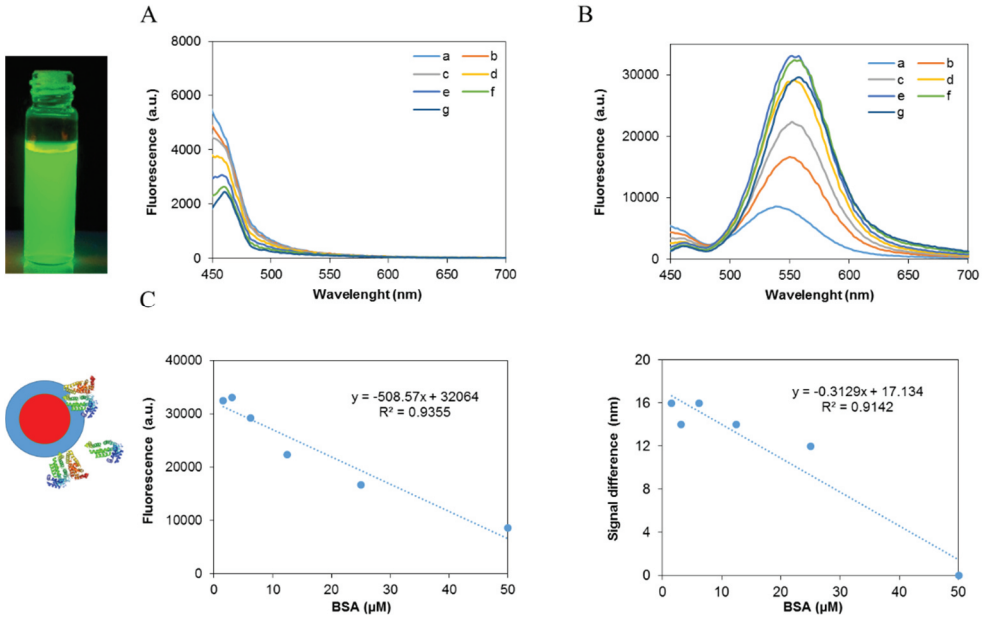

Figure 2 Emission fluorescence spectrum of bovine serum albumin (BSA) (A) and conjugate 1: 1 BSA with green QDs (50 μM) (B) in an environment of Tris-Cl buffer pH 8 at different concentrations (a) 50, (b) 25, (c) 12.5, (d) 6.3, (e) 3.1, (f) 1.6 a (g) 0 μM; (C) Dependence of fluorescence peak values of QDs obtained from chart B in the wavelength range 538 - 554 nm to BSA concentration; (D) The shift of fluorescence intensity maximum QDs value from chart B in dependence on BSA concentration

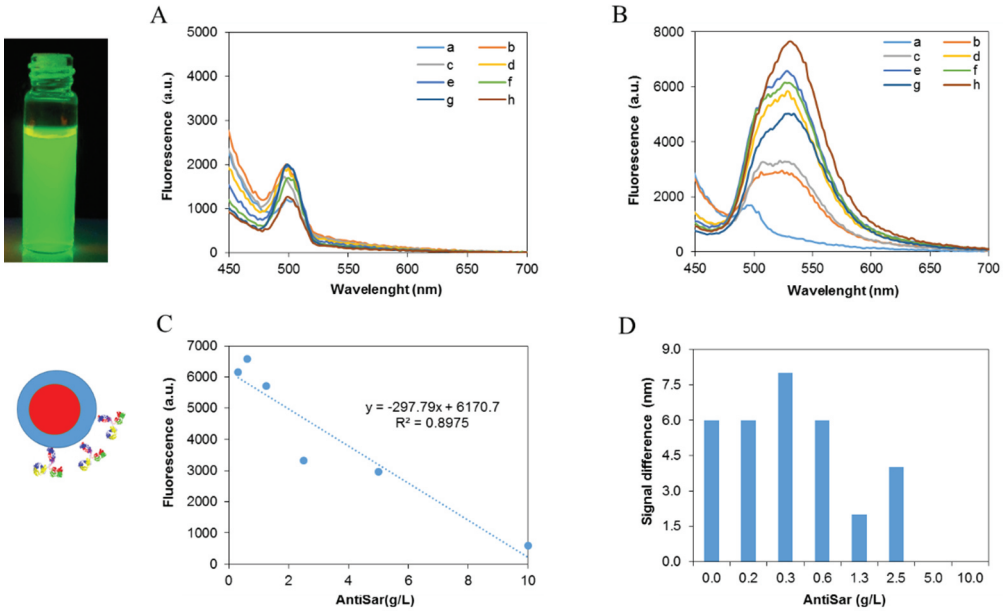

Figure 3 Emission fluorescence spectrum of chicken antibodies against sarcosine (AntiSar) (A) and antibody conjugate (1: 1) with green QDs (50 μM) (B) in Tris-Cl buffer, pH 8 at different concentrations (a) 20, (b) 10,(c) 5, (d) 2.5, (e) 1.3, (f) 0.7, (g) 0.3 and (h) 0 g / L. (C) Dependence of the fluorescence maxima of QDs obtained from Chart B in the wavelength range 522-530 nm on antibody concentration; (D) Shift of the maximum fluorescence intensity QDs from Chart B, depending on the AntiSar concentration

When we added QDs_{yellow} to the given BSA concentrations, we observed a decrease in the intensity of the normalized fluorescence signal by 68% similar to QDs_{green}, but the acquired signal dependence exhibited a polynomial trend $y = 6.6751x^2 - 602.71x + 21900$ ($R^2 = 0.8506$), with 5 independent repetitions and a relative error was 12%. For these nanoparticles it was possible to monitor the signal shift to shorter wavelengths at the highest applied BSA concentration by more than 10 nm (results not shown). In the following experiment, we studied the interaction of QDs_{green} or QDs_{yellow} with AntiSar.

Figure 3 shows typical fluorescence records of the antibodies themselves at different concentrations. In the studied range (450-700 nm), an observable signal at 500 nm is probably corresponding to some protein structures in the antibody. In case we added QDs_{green} to the given BSA concentrations, a decrease in the intensity of the normalized fluorescence signal was observed by 87%, the acquired dependence of the decrease in the signal showed a linear trend and the slope $y = -297.79x + 6170.7$ ($R^2 = 0.9$), in 5 independent repetitions and relative error of around 9%. With these nanoparticles it was possible to monitor the signal shift to shorter wavelengths at the highest applied concentration of AntiSar by 8 nm (see **Figure 3**).

In the case of QDs_{yellow}, the changes were less dramatic, the decrease in the fluorescence signal was by 99% and the dependence of the decrease also had a linear character $y = -837.97x + 24509$ ($R^2 = 0.90$), with 5 independent repetitions and a relative error of around 11%. For these nanoparticles it was possible to monitor the signal shift to shorter wavelengths at the highest applied BSA concentration by 4 nm (results not shown). The obtained data suggest that the size of individual QDs of nanoparticles will affect interaction with biomolecules. Nanoconstructors should therefore be targeted for their intended use according to these experimental data.

4. CONCLUSION

In further experiments, QDs modified with AntiSar will be used for targeted prostate cancer therapy. We assume that exposure to specific radiation at a given wavelength and energy will result in the excitation of a system capable of generating of a whole group of reactive oxygen species. If such a particle is targeted toward the tumor cell (by various nanomedical approaches), an increased level of oxygen radicals can trigger apoptosis processes, and the tumor cell would be destroyed.

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REFERENCES

[1] YAGHINI, E., SEIFALIAN, A. M.,MACROBERT, A. J. Quantum dots and their potential biomedical applications in photosensitization for photodynamic therapy. *Nanomedicine,* 2009, vol. 4, No. 3, pp. 353-363.

[2] CHOI, M., CHOI, J., KIM, S., NIZAMOGLU, S., HAHN, S. K.,YUN, S. H. Light-guiding hydrogels for cell-based sensing and optogenetic synthesis in vivo. *Nature Photonics,* 2013, vol. 7, No. 12, pp. 987-994.

[3] RUTTKAY-NEDECKY, B., NEJDL, L., GUMULEC, J., ZITKA, O., MASARIK, M., ECKSCHLAGER, T., STIBOROVA, M., ADAM, V.,KIZEK, R. The Role of Metallothionein in Oxidative Stress. *International Journal of Molecular Sciences,* 2013, vol. 14, No. 3, pp. 6044-6066.

[4] HEGER, Z., RODRIGO, M. A. M., KRIZKOVA, S., RUTTKAY-NEDECKY, B., ZALEWSKA, M., DEL POZO, E. M. P., PELFRENE, A., POURRUT, B., STIBOROVA, M., ECKSCHLAGER, T., EMRI, G., KIZEK, R.,ADAM, V. Metallothionein as a Scavenger of Free Radicals - New Cardioprotective Therapeutic Agent or Initiator of Tumor Chemoresistance? *Current Drug Targets,* 2016, vol. 17, No. 12, pp. 1438-1451.

[5] GUSZPIT, E., KRIZKOVA, S., KEPINSKA, M., RODRIGO, M. A. M., MILNEROWICZ, H., KOPEL, P.,KIZEK, R. Fluorescence-tagged metallothionein with CdTe quantum dots analyzed by the chip-CE technique. *Journal of Nanoparticle Research,* 2015, vol. 17, No. 11, pp.

[6] TMEJOVA, K., HYNEK, D., KOPEL, P., KRIZKOVA, S., BLAZKOVA, I., TRNKOVA, L., ADAM, V.,KIZEK, R. Study of metallothionein-quantum dots interactions. *Colloids and Surfaces B-Biointerfaces,* 2014, vol. 117, No. pp. 534-537.

[7] VIANA, O., RIBEIRO, M., RODAS, A., REBOUÇAS, J., FONTES, A.,SANTOS, B. Comparative Study on the Efficiency of the Photodynamic Inactivation of Candida albicans Using CdTe Quantum Dots, Zn(II) Porphyrin and Their Conjugates as Photosensitizers. *Molecules,* 2015, vol. 20, No. 5, pp. 8893.

[8] BILAN, R., NABIEV, I.,SUKHANOVA, A. Quantum Dot-Based Nanotools for Bioimaging, Diagnostics, and Drug Delivery. *Chembiochem,* 2016, vol. 17, No. 22, pp. 2103-2114.

[9] FOUBERT, A., BELOGLAZOVA, N. V., RAJKOVIC, A., SAS, B., MADDER, A., GORYACHEVA, I. Y.,DE SAEGER, S. Bioconjugation of quantum dots: Review & impact on future application. *Trac-Trends in Analytical Chemistry,* 2016, vol. 83, No. pp. 31-48.

[10] MOULICK, A., BLAZKOVA, I., MILOSAVLJEVIC, V., FOHLEROVA, Z., HUBALEK, J., KOPEL, P., VACULOVICOVA, M., ADAM, V.,KIZEK, R. Application of CdTe/ZnSe Quantum Dots in In Vitro Imaging of Chicken Tissue and Embryo. *Photochemistry and Photobiology,* 2015, vol. 91, no. 2, pp. 417-423.

[11] ESTEVE-TURRILLAS, F. A.,ABAD-FUENTES, A. Applications of quantum dots as probes in immunosensing of small-sized analytes. *Biosensors & Bioelectronics,* 2013, vol. 41, No. pp. 12-29.

[12] ALGAR, W. R., TAVARES, A. J.,KRULL, U. J. Beyond labels: A review of the application of quantum dots as integrated components of assays, bioprobes, and biosensors utilizing optical transduction. *Analytica Chimica Acta,* 2010, vol. 673, No. 1, pp. 1-25.

[13] STANISAVLJEVIC, M., KRIZKOVA, S., VACULOVICOVA, M., KIZEK, R.,ADAM, V. Quantum dots-fluorescence resonance energy transfer-based nanosensors and their application. *Biosensors & Bioelectronics,* 2015, vol. 74, no. pp. 562-574.

[14] KRIZKOVA, S., HEGER, Z., ZALEWSKA, M., MOULICK, A., ADAM, V.,KIZEK, R. Nanotechnologies in protein microarrays. *Nanomedicine,* 2015, vol. 10, No. 17, pp. 2743-2755.

[15] YAMASAKI, K., MARUYAMA, T., KRAGHHANSEN, U.,OTAGIRI, M. Characterization of site I on human serum albumin: Concept about the structure of a drug binding site. *Biochimica Et Biophysica Acta-Protein Structure and Molecular Enzymology,* 1996, vol. 1295, No. 2, pp. 147-157.

[16] KOTRESH, M. G., INAMDAR, L. S., SHIVKUMAR, M. A., ADARSH, K. S., JAGATAP, B. N., MULIMANI, B. G., ADVIRAO, G. M.,INAMDAR, S. R. Interaction and energy transfer studies between bovine serum albumin and CdTe quantum dots conjugates: CdTe QDs as energy acceptor probes. *Luminescence,* 2017, vol. 32, no. 4, pp. 631-639.

[17] GAO, X. H., CHAN, W. C. W.,NIE, S. M. Quantum-dot nanocrystals for ultrasensitive biological labeling and multicolor optical encoding. *Journal of Biomedical Optics,* 2002, vol. 7, No. 4, pp. 532-537.

[18] LUNDQVIST, M., SETHSON, I.,JONSSON, B. H. Protein adsorption onto silica nanoparticles: Conformational changes depend on the particles' curvature and the protein stability. *Langmuir,* 2004, vol. 20, No. 24, pp. 10639-10647.

[19] BREWER, S. H., GLOMM, W. R., JOHNSON, M. C., KNAG, M. K.,FRANZEN, S. Probing BSA binding to citrate-coated gold nanoparticles and surfaces. *Langmuir,* 2005, vol. 21, No. 20, pp. 9303-9307.

[20] PONS, T., UYEDA, H. T., MEDINTZ, I. L.,MATTOUSSI, H. Hydrodynamic Dimensions, Electrophoretic Mobility, and Stability of Hydrophilic Quantum Dots. *The Journal of Physical Chemistry B,* 2006, vol. 110, No. 41, pp. 20308-20316.

[21] NEJDL, L., RICHTERA, L., XHAXHIU, K., KENSOVA, R., KUDR, J., RUTTKAY-NEDECKY, B., KYNICKY, J., WAWRZAK, D., ADAM, V., KIZEK, R.,KOPEL, P. UV Tuning of Cadmium Telluride Quantum Dots (CdTe QDs) - Assessed by Spectroscopy and Electrochemistry. *International Journal of Electrochemical Science,* 2016, vol. 11, No. 1, pp. 175-188.