Co-doped CdS quantum dots and their bionanocomplex with protein: interaction and bioimaging properties

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Stolyarchuk I.D.[1], Wojnarowska-Nowak R.[2], Nowak S.[3], Romerowicz-Misielak M.[3], Kuzyk O.V.[1], Dan'kiv O.O.[1], Stolyarchuk A.I.[1]

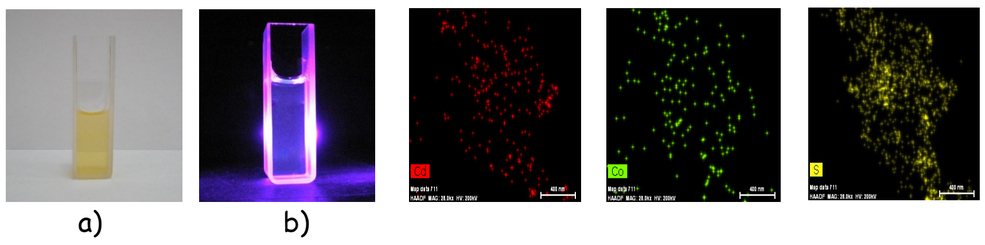
[1]Department of Physics, Drohobych Ivan Franko State Pedagogical University, 24 I.Franko str., 82100 Drohobych, Ukraine
[2] Center of Microelectronics and Nanotechnology , University of Rzeszow, 16C Rejtana Str., 35-959 Rzeszow, Poland
[3] Department of Animal Physiology and Reproduction, Institute of Applied Biotechnology and Basic Sciences, University of Rzeszow, Werynia 502, 36100 Kolbuszowa, Poland

i.stolyarchuk@dspu.edu.ua

MOTIVATION

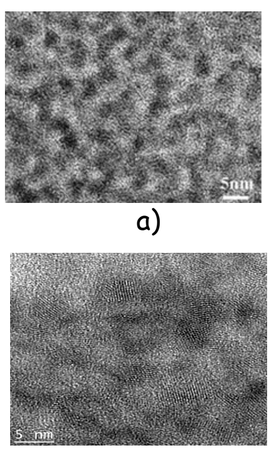
Semiconductor nanoparticles or quantum dots (QDs) constitute significant materials for many areas of nano- and biotechnology. One of the main decisive parameters for the use of QDs in bionanotechnology is the functionalization of their surface with appropriate (bio)molecules to ensure the stability of colloidal solutions in combination with effective labeling of the corresponding biological objects. It is the combination of nanoparticles with selected biological or chemical ligands, such as proteins, antibodies or DNA sequences etc., that leads to the creation of highly functional bionanomaterials (bionanocomplexes), which opens up the broadest prospects for their use in bio- and nanomedicine [1].
In the present work, we report on spectroscopy studies of interactions between colloidal CdS:Co diluted magnetic semiconductor QDs and model proteins type of human serum albumin (HSA). In addition to conventional UV-Vis absorption and fluorescence spectroscopic methods we have included Raman spectroscopy and technique of magneto-optical Faraday rotation, which is important for case of nanoparticles with magnetic impurities. The low toxicity as well as high stability of the QDs - HSA bioconjugates in the case of their using as bio-imaging probes for the 143b osteosarcoma cells has been demonstrated.

MAIN RESULT



a) b)

Fig. 1. Photo images of colloidal nanoparticles CdCoS (a) and photoluminescence of bionanocomplexes of nanoparticles $Cd_{0,95}Co_{0,05}S$ with HSA (b)



a) b)

Fig. 2. HR TEM images of CdS (a) and $Cd_{0,9}Co_{0,1}S$ (b) nanoparticles

Fig 3. EDX results showing content of CdCoS QDs

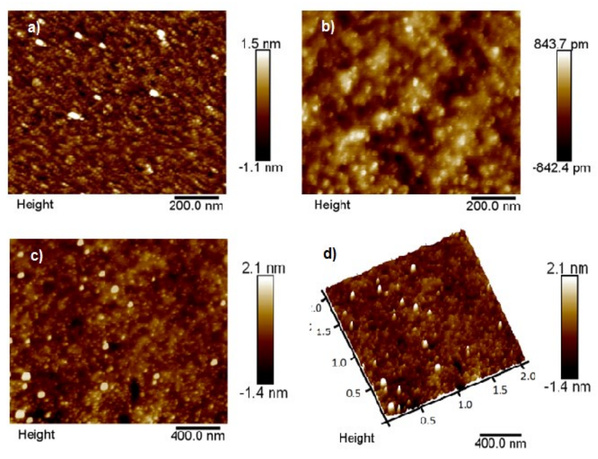


Fig. 3. AFM images of the quantum dots CdCoS(a), human serum albumin (b) and their bionanocomplex - 2D (c), 3D (d)

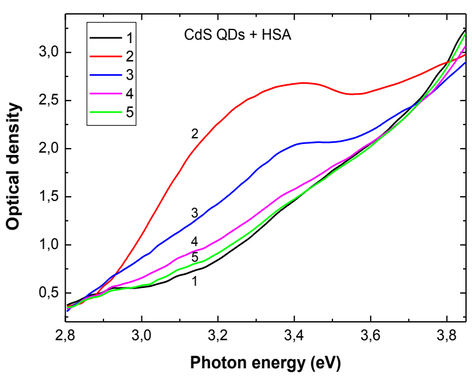


Fig. 5. Optical density of nanoparticles CdS with HSA: 1 - HSA, 2 - CdS , 3 - HSA + 0,6 x 10^{-3} mmol/L CdS, 4 - HSA + 0,9 x 10^{-3} mmol/L CdS , 5 -HSA + 1,5 x 10^{-3} mmol/L CdS

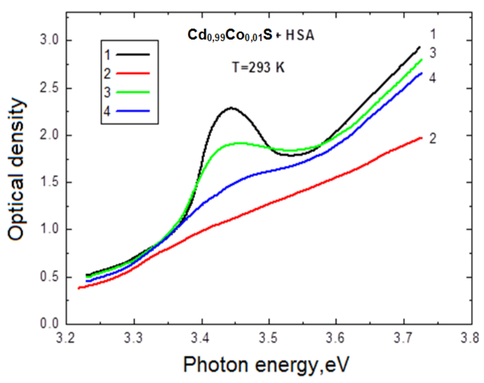


Fig. 6. Optical density of colloidal QDs $Cd_{0,99}Co_{0,01}S$ with HSA:1-QDs $Cd_{0,99}Co_{0,01}S$, 2- HSA, 3- HSA + 0,5 x 10^{-3} mmol x L^{-1} QDs, 4 - HSA + 1,2 x 10^{-3} mmol x L^{-1} QDs

SAMPLES

Synthesis of colloidal nanoparticles

Among different technological approaches for growth of CdS and CdS:Co nanoparticles we have chemical methods. In order to vary nanoparticle composition $[CdCl_2]$:$[Na_2S]$ or $[CdCl_2]$: $[CoCl_2]$ molar ratios were changed. The molar concentration of the precursors was within the range of (10^{-4}-10^{-2}) mol/l [2].

$$(1-x)CdCl_2 + xCoCl_2 + Na_2S \rightarrow CdS{:}Co + 2NaCl$$

Preparing of semiconductor nanoparticles and HSA solution

Solutions of CdS and $Cd_{1-x}Co_xS$ nanoparticles with HSA were prepared by adding a small amount of concentrated HSA solution in salin.

The human osteosarcoma 143b cell line was used for the bioimaging properties of CdS (CdS:Co) QDs and CdS:Co QDs-HSA complexes examination. The cells were maintained in Dulbecco's Modified Eagle's medium (DMEM), supplemented with fetal bovine serum (FBS, 10%), and penicillin-streptomycin (1%) antibiotic, in 5% CO_2 at 37°C. After centrifugation (7 min, 4°C, 600 g), the cells were seeded in a 96-well plate at a density of 8.0×10^4 cells per mL, and incubated during 24h. The QDs were mixed with DMEM medium in order to obtain 30×10^{-5} M, 20×10^{-5} M, and 8.0×10^{-5} M concentration of QDs. An aliquot (0.1 mL) of the prepared solution was added to each well, and incubated during 24h. In the next experiment, osteosarcoma cells were fixed by pre-cooled methanol and the cell membrane was permeated by 0.1% Triton X-100 reagent. 0.1 mL aliquot of CdS:Co QDs suspended in phosphate-buffered saline was added to the cells and incubated for 10 min. The same procedure was used for the treatment of cells by CdS:Co QDs -HSA bionanocomplexes, with 1.2×10^{-5} M of maximum concentration of protein. Cell nuclei were stained with Hoechst 33342 fluorescent dye as a standard method. The images were taken by In Cell Analyzer 2000 automated microscope (GE Healthcare, UK). The images were taken with two different excitation/emission filters: 543/605 nm for the CdS:Co QDs and the CdS:Co QDs -HSA bionanocomplexes and 350/455 nm for Hoechst 33342. The osteosarcoma cells were treatment with QDs and bionanocomplexes with the same concentrations as for photoluminescence measurements.

Characterization methods

The absorption spectra were recorded using UV-Vis spectrometr on the base of diffraction monochromator MDR-23 (LOMO). The excitation of photoluminescence was carried out by a He-Cd laser operating at wavelength of 325 nm and power of 10 mW. The fluorescence was carried out by a FL-2500 Hitachi Spectrophotometer. The size and shape of nanoparticles were studied by HRTEM and AFM technique. The images were collected in Tapping Mode with using Innova Bruker microscope. Optical properties of saples were egzaminated by Raman spectroscopy - InVia Renishaw spectrometer and IR spectrscopy – Vertex 70 Brukerwith ATR technique.

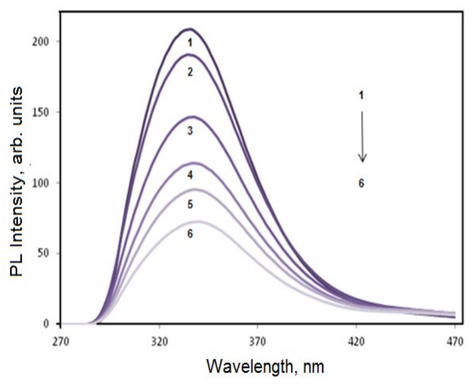


Fig. 7. The photoluminescence spectra of HSA with different concentrations of CdS:Co (1%) QDs: 1: 10 mg/ml HSA; 2: + 0,1 mg/ml QDs; 3: + 0,2 mg/ml QDs; 4: + 0,3 mg/ml QDs; 5 : + 0,4 mg/ml QDs; 6 : + 0,5 mg/ml QDs

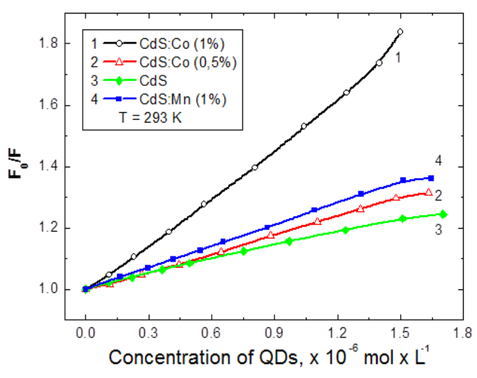


Fig. 8. The Stern–Volmer plots for HSA photoluminescence quenching

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{sv}[Q]$$

$$K_{SV} = K_q\tau_0$$

System	CdS:Co (0,5%)		CdS:Co (1%)	
T, K	293	298	293	298
K_{sv}, L mol^{-1}	7,37 10^4	6,35 10^4	9,84 10^3	8,34 10^3
K_q, L mol^{-1}c^{-1}	7,37 10^{12}	6,35 10^{12}	9,84 10^{11}	8,34 10^{11}
K_b,	1,78 10^6	1,98 10^6	2,17 10^6	2,68 10^6
n	1,03	1,06	0,93	0,98
R^2	0.992	0.988	0.997	0.991

Table 1. Quenching constants K_{sv} , bimolecular quenching constants K_q , binding constants K_b and number of binding sites - n for interaction of HSA with QDs

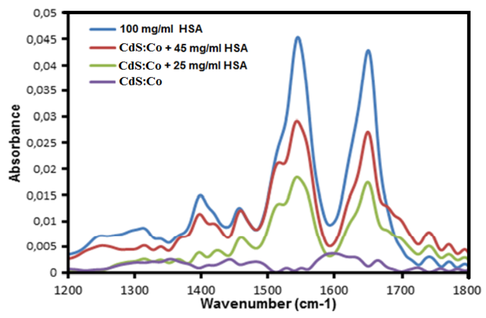
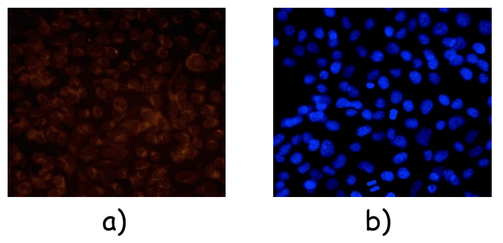


Fig. 9 The FTIR spectra of quantum dots CdS:Co, human serum albumin and their bionanocomplex

	Position of line (cm^{-1})	Identification [3,4]
1	1650 (s)	Amide I (C=O stretch, CN stretch, CCN deformation, NH bend)
2	1547 (s)	Amide II (NH bend, CN stretch, CO in plane, CC, NC stretch)
3	1446(w)	C-H bend
4	1402 (m)	COO- stretch and C-H bend
5	1319 (m)	O-H bend
6	1259 (w)	Amide III (CN stretch, NH bend, CO in plane, CC stretch)



a) b)

Fig. 10 a) – CdCoS QDs fluorescence inside the cells, b) - Hoechst 33342 fluorescent dye inside nucleus

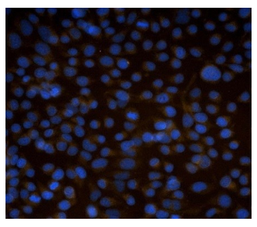


Fig. 10 143 b cells incubated CdCoS (orange) and Hoechst 33342 (blue)

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ACKNOWLEDGMENTS

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