

CDTE Quantum Dots – Preparation, Physico-chemical Properties and Application as Fluorescent Markers

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Abstract:

A set of water-soluble CdTe quantum dots (QDs) was prepared by the chemical reaction between cadmium chloride and sodium hydrogen telluride in the presence of 3-mercaptopropionic acid (MPA). QDs were characterized using fluorescence spectroscopy, lifetime measurements, slab-gel and capillary electrophoresis. The particle sizes, determining the wavelength of the fluorescence emission, were evaluated to be from 2.5 nm (500 nm) to 5.2 nm (750 nm). The fluorescence lifetimes determined by the time-resolved fluorescence spectrometry, increases from 3.05 to 20.5 ns with the increasing size of particles. The uptake of non-conjugated QDs into the living cells, which has a potential for single cell analyses, was apparent for human lymphocytes and yeast cells (*Saccharomyces cerevisiae*) after 30 minutes and 3 hours, respectively. The cell manipulation, lysis and monitoring was performed in microfluidic devices with laser-induced fluorescence detection. The conjugation of QDs with functional biomolecules allows the selective labeling and localization of specific molecules. Several coupling agents such as N-hydroxysulfosuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) or carbonyldiimidazole (CDI) were used. The concentrations of reaction products were checked by capillary electrophoresis with laser-induced fluorescence detection.

INTRODUCTION

Semiconductor quantum dots (QDs) are bright fluorescent nanocrystals in the size range from 1 to 10 nm. QDs consist of semiconductor central core stabilized by shell, mainly from inorganic salts (e.g. CdS, ZnS) [1]. Water-solubility is provided by charged compounds covalently attached to the surface of QDs via thiol groups [2]. The molecules attached to the outer shell serve as mediator for the conjugation with functional ligands or biomolecules [3, 4]. Due to their unique optical properties, such as sharp and symmetric emission spectra, high quantum yield of fluorescence, good chemical stability, practically no photobleaching [5-8] and emission wavelength dependent on the nanoparticle size [2], QDs are becoming attractive as alternative fluorescent labels in analytical chemistry, cell biology and medicine. Various methods are used for characterization of QDs size and structure, e.g. transmission electron microscopy [9-11], atomic force microscopy [12], analytical centrifugation [13], X-ray photoelectron spectroscopy or powder diffraction [9, 10, 14], dynamic light scattering [15] and fluorescence correlation spectroscopy [16] and electrophoretic methods including capillary electrophoresis (CE).

The QDs conjugates with functional biomolecules (e.g. proteins, DNA, antibodies or enzymes) are used in immunoassays [17, 18], cellular labeling [19, 20], single-molecule/cell tracking [21, 22], deep-tissue imaging [4, 23] and as a fluorescence resonance energy transfer donors [4, 24, 25]. There are several possibilities for QDs connection with functional

biomolecules. It should be electrostatic or hydrophobic attractions [26-28], avidin-biotin interactions [27, 29], direct interactions with QD surface [25, 30, 31], and the most frequently used, covalent bonding [6, 15, 17, 19]. Zero cross-linkers such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) or carbonyldiimidazole (CDI) and two step procedure combining EDC with sulfosuccinyl imide (NHS), are used for bonding QDs with biomolecule via peptide bond [32].

This work is focused on the survey of methods for the preparation, physico-chemical characterization and conjugation of CdTe QDs and their application in cell analyses. The different sizes CdTe nanoparticles stabilized by 3-mercaptopropionic acid (MPA) were synthesized. Their excitation and emission spectra and fluorescence lifetimes were measured. The uptake time of non-conjugated QDs by human lymphocytes and *Saccharomyces cerevisiae* was followed using epifluorescence microscope. Consequently, the QDs were conjugated with antibody and the possibility of selective labeling of cells and the time of the uptake were tested.

EXPERIMENTAL

Chemicals and reagents

The following chemicals were used for the preparation of QDs: NaBH₄ (98.5 %), tellurium powder (99.8 %), CdCl₂ (99.99 %) 3-mercaptopropionic acid (99 %), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS) (98.5 %) from Fluka. The monoclonal Anti-CD3 antibody

was purchased from Sigma-Aldrich (St. Luis, MO, USA).

Preparation and conjugation of CdTe quantum dots

CdTe quantum dots were prepared using reaction between cadmium chloride, sodium hydrogen telluride at the presence of 3-mercaptopropionic acid (MPA) with the molar ratio $\text{CdCl}_2 : \text{NaHTe} : \text{HS-CH}_2\text{-CH}_2\text{-COOH} = 2 : 1 : 4.8$ according to procedure described by Li et al. [14]. Briefly, the sodium hydrogen telluride was prepared by the reaction of 84.85 mg sodium borohydride with 126.97 mg tellurium powder and 2 ml of deionized water. The reaction mixture was set to the ice cubes and stirred for approximately 4 hours. After this time, 2 ml of the dark violet solution of NaHTe was transferred to boiling flask with 80 ml deionized water, degassed with N_2 for 30 minutes, 370 mg CdCl_2 and 420 μl MPA. The pH of the created red solution was adjusted to 7.0 using 0.1 mol/l NaOH or MPA. The reaction mass was heated under the reflux condenser. MPA coated QDs were conjugated with antibodies containing primary amino groups by reaction with NHS and EDC coupling agents which mediate the formation of peptide bond between carboxylic group and amino group [32-35]. The main reason of utilization of EDC was its water solubility and chemical stability. The purpose of adding sulfo-NHS to EDC is to increase the stability of the active intermediate at pH range from 5 to 7.3 [32]. In fact, a mixture of 10 μl QDs (10⁻⁴ M), 2 μl EDC solution (60 mg/ml) and 10 μl NHS (0.15 mg/ml) reacted in an Eppendorf microtube for 30 minutes. Finally, 20 μl of the antibody solution were added and the mixture was left at a room temperature for 2 hours.

Fluorescence and lifetime measurements

Fluorescence spectra were measured on Aminco-Bowman AB-2 (Thermo Spectronic, NY, USA) with continuous 150 W xenon lamp. Life-time measurements were done on a home made instrument. Sample was excited by 6 mW 266 nm Nd:YAG laser (LCS-DTL-382QT, Laser – compact Co. Ltd., Moscow, Russia). Fluorescence was collected by reflective objective 25-0522-190 (Ealing Catalog Inc., Rocklin, CA, USA) with magnification 36x, scattered laser light was removed by long pass filters 269 nm and 295 nm (Omega Optical, Inc., Brattleboro, VT, USA). Fluorescence was detected by photomultiplier tube H5783-03 (Hamamatsu Photonics K.K., Iwata City, Japan). The output signal was collected by digital oscilloscope WaveRunner 6050 (LeCroy, New York, NY, USA) with software version 5.0.0.2 and XStream Browser version 1.0.3.

RESULTS AND DISCUSSIONS

Preparation and characterization of CdTe QDs

MPA coated CdTe QDs were synthesized according to method described by Li et al [14]. The size and emission maximum of nanoparticles should be easily controlled and different sizes of QDs can be prepared during one synthesis. The refluxing time of last reaction stage determine the size and emission maximum of rising nanoparticles [14]. Several samples were taken during refluxing in different times. The fluorescence spectra of these samples were measured and emission maxima and bandwidths were determined. The excitation spectra of different samples were practically the same with a breadth of 300 – 550 nm. The refluxing time was in the range from 10 minutes for the smallest 2.5 nm nanoparticles with the emission maximum of 500 nm to 44 hours for 5.2 nm particles with the emission maximum of 750 nm (Fig. 1). The emission bandwidth of the prepared nanoparticles increased from 45 nm to 100 nm with increasing the refluxing time and size.

The fluorescence lifetime, characteristic of fluorophores frequently used in analytical chemistry, was determined using time-resolved fluorescence spectroscopy. It was found out that fluorescence lifetimes of QDs vary from 3.05 to 20.5 ns with their increasing size. The shortest lifetime, which belongs to 2.0 nm particles, is similar to low molecular mass fluorophores, e.g. fluorescein (3.8 ns).

The narrow emission together with the broad excitation spectra are advantageous for parallel analyses of several analytes labeled by different QDs sizes. Thus, it is not necessary to use several different light sources for the excitation of particular fluorescent probe but only a single light source is satisfactory. The resistance against photobleaching allows for repetitive observations and long collection time of signals.

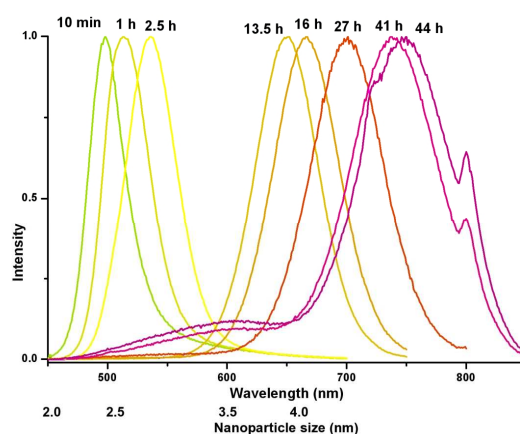


Fig. 1: Fluorescence emission spectra of QDs prepared for different refluxing time (normalized spectra)

Non-selective labeling of cells

The uptake of QDs by different types of cells was studied. Whenever QDs conjugated with biomolecules will be used for the selective and specific labeling of analytes in the cell, it is necessary

to know the uptake of non-conjugated QDs. Human lymphocytes and *Saccharomyces cerevisiae* cells were selected as model cells. Human lymphocytes and *Saccharomyces cerevisiae* represent cells sensitive to changes in their environment. Cells were mixed with QDs and its uptake was monitored under epifluorescence microscope. The uptake was apparent after 3 hours and 30 minutes for yeasts and lymphocytes, respectively (Fig. 2).

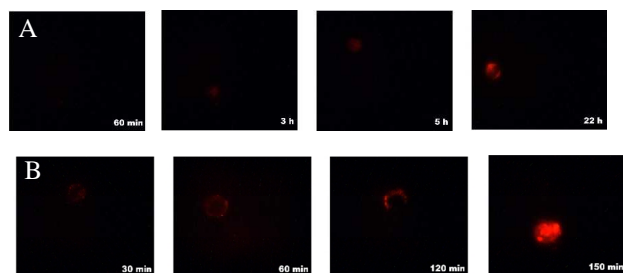


Fig. 2: Uptake of QDs into *Saccharomyces cerevisiae* cells (A) and human lymphocytes (B)

Selective labeling of cells

The QDs were conjugated via EDC and NHS coupling agents with CD3 antibody. This is the antibody against CD3 protein specific for T-lymphocytes while B-lymphocytes do not contain this membrane protein. CD3 antibody was conjugated with QDs and fluorescein. These conjugates were added to a mixture of T- and B-lymphocytes. Sufficient labeling was apparent immediately after mixing in the case of T-lymphocytes (Fig. 3 A and B). In the case of B-lymphocytes no labeling was apparent (Fig.3 C).

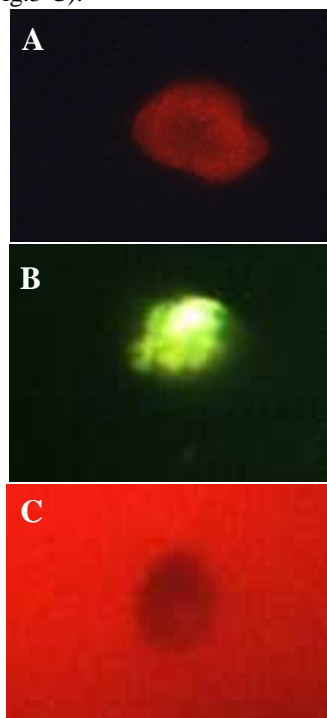


Fig. 3: Human T-lymphocytes labeled by QD-antiCD3 conjugate (A), T-lymphocyte labeled by fluorescein-antiCD3 conjugate (B), no interaction of B-lymphocyte with QD-antiCD3 conjugate (C).

CONCLUSIONS

The set of different sizes of CdTe nanoparticles coated with MPA was synthesized and characterized by fluorescence spectra and lifetimes. The size range of prepared nanoparticles was from 2.5 nm with emission maximum 500 nm to 5.2 nm with emission maximum 750 nm. The lifetimes of these QDs vary from 3.05 ns to 20.5 ns with increasing size of nanoparticles. Nanoparticles with emission maximum 610 nm were conjugated with CD3 antibody to selectively label T-lymphocytes. The selectivity of labeling was confirmed via adding QD-antiCD3 conjugate to the mixture of T- and B- lymphocytes where only T-lymphocytes were labeled immediately. Non-selective labeling of human lymphocytes and *Saccharomyces cerevisiae* with MPA coated QDs was tested. It was found out that the uptake of non-conjugated QDs is 30 min and 3 h for human lymphocytes and *Saccharomyces cerevisiae*, respectively.

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REFERENCES

- [1] J. van Embden; J. Jasieniak; D. E. G lmez; P. Mulvaney; M. Giersig, "Review of the Synthetic Chemistry Involved in the Production of Core/Shell Semiconductor Nanocrystals," Australian Journal of Chemistry, vol. 60, pp. 457-471, 2007.
- [2] A. Eychmuller; A. L. Rogach, "Chemistry and photophysics of thiol-stabilized II-VI semiconductor nanocrystals," Pure and Applied Chemistry, vol. 72, pp. 179-188, 2000.
- [3] C. Burda; X. B. Chen; R. Narayanan; M. A. El-Sayed, "Chemistry and properties of nanocrystals of different shapes," Chem. Rev., vol. 105, pp. 1025-1102, 2005.
- [4] J. M. Klostranec; W. C. W. Chan, "Quantum dots in biological and biomedical research: Recent progress and present challenges," Advanced Materials, vol. 18, pp. 1953-1964, 2006.
- [5] J. Ma; J. Y. Chen; J. Guo; C. C. Wang; W. L. Yang; N. H. Cheung; P. N. Wang, "Improvement of the photostability of thiol-capped CdTe quantum dots in aqueous solutions and in living cells by surface treatment," Nanotechnology, vol. 17, pp. 5875-5881, 2006.
- [6] J. H. Wang; H. Q. Wang; H. L. Zhang; X. Q. Li; X. F. Hua; Y. C. Cao; Z. L. Huang; Y. D. Zhao, "Purification of denatured bovine serum albumin coated CdTe quantum dots for sensitive

- detection of silver(I) ions," *Analytical and Bioanalytical Chemistry*, vol. 388, pp. 969-974, 2007.
- [7] J. Ma; J. Y. Chen; J. Guo; C. C. Wang; W. L. Yang; L. Xu; P. N. Wang, "Photostability of thiol-capped CdTe quantum dots in living cells: the effect of photo-oxidation," *Nanotechnology*, vol. 17, pp. 2083-2089, 2006.
- [8] J. Ma; J. Y. Chen; Y. Zhang; P. N. Wang; J. Guo; W. L. Yang; C. C. Wang, "Photochemical instability of thiol-capped CdTe quantum dots in aqueous solution and living cells: Process and mechanism," *Journal of Physical Chemistry B*, vol. 111, pp. 12012-12016, 2007.
- [9] . Rajh; O. I. Micic; A. J. Nozik, "Synthesis and Characterization of Surface-Modified Colloidal Cdte Quantum Dots," *Journal of Physical Chemistry*, vol. 97, pp. 11999-12003, 1993.
- [10] C. B. Murray; D. J. Norris; M. G. Bawendi, "Synthesis and Characterization of Nearly Monodisperse Cde (E = S, Se, Te) Semiconductor Nanocrystallites," *Journal of the American Chemical Society*, vol. 115, pp. 8706-8715, 1993.
- [11] G. N. Guo; W. Liu; J. G. Liang; H. B. Xu; Z. K. He; X. L. Yang, "Preparation and characterization of novel CdSe quantum dots modified with poly (D,L-lactide) nanoparticles," *Materials Letters*, vol. 60, pp. 2565-2568, 2006.
- [12] D. Gerion; F. Pinaud; S. C. Williams; W. J. Parak; D. Zanchet; S. Weiss; A. P. Alivisatos, "Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots," *Journal of Physical Chemistry B*, vol. 105, pp. 8861-8871, 2001.
- [13] E. E. Lees; M. J. Gunzburg; T.-L. Nguyen; G. J. Howlett; J. Rothacker; E. C. Nice; A. H. A. Clayton; P. Mulvaney, "Experimental Determination of Quantum Dot Size Distributions, Ligand Packing Densities, and Bioconjugation Using Analytical Ultracentrifugation," *Nano Lett.*, 2008.
- [14] L. Li; H. F. Qian; N. H. Fang; H. C. Ren, "Significant enhancement of the quantum yield of CdTe nanocrystals synthesized in aqueous phase by controlling the pH and concentrations of precursor solutions," *Journal of Luminescence*, vol. 116, pp. 59-66, 2006.
- [15] B. I. Ipe; A. Shukla; H. C. Lu; B. Zou; H. Rehage; C. M. Niemeyer, "Dynamic light-scattering analysis of the electrostatic interaction of hexahistidine-tagged cytochrome P450 enzyme with semiconductor quantum dots," *Chemphyschem*, vol. 7, pp. 1112-1118, 2006.
- [16] S. Ito; N. Toitani; L. Pan; N. Tamai; H. Miyasaka, "Fluorescence correlation spectroscopic study on water-soluble cadmium telluride nanocrystals: fast blinking dynamics in the mu s-ms region," *Journal of Physics-Condensed Matter*, vol. 19, pp. -, 2007.
- [17] H. T. Feng; W. S. Law; L. Yu; S. F. Y. Li, "Immunoassay by capillary electrophoresis with quantum dots," *Journal of Chromatography A*, vol. 1156, pp. 75-79, 2007.
- [18] J. Shen; F. Xu; H. Jiang; Z. Wang; J. Tong; P. Guo; S. Ding, "Characterization and application of quantum dot nanocrystal-monoclonal antibody conjugates for the determination of sulfamethazine in milk by fluoroimmunoassay," *Analytical and Bioanalytical Chemistry*, vol. 389, pp. 2243-2250, 2007.
- [19] S. Dwarakanath; J. G. Bruno; A. Shastry; T. Phillips; A. John; A. Kumar; L. D. Stephenson, "Quantum dot-antibody and aptamer conjugates shift fluorescence upon binding bacteria," *Biochemical and Biophysical Research Communications*, vol. 325, pp. 739-743, 2004.
- [20] Y. M. Shan; L. P. Wang; Y. H. Shi; H. Zhang; H. M. Li; H. Z. Liu; B. Yang; T. Y. Li; X. X. Fang; W. Li, "NHS-mediated QDs-peptide/protein conjugation and its application for cell labeling," *Talanta*, vol. 75, pp. 1008-1014, 2008.
- [21] W. J. Parak; R. Boudreau; M. Le Gros; D. Gerion; D. Zanchet; C. M. Micheel; S. C. Williams; A. P. Alivisatos; C. Larabell, "Cell motility and metastatic potential studies based on quantum dot imaging of phagokinetic tracks," *Advanced Materials*, vol. 14, pp. 882-885, 2002.
- [22] S. Pathak; S.-K. Choi; N. Arnheim; M. E. Thompson, "Hydroxylated Quantum Dots as Luminescent Probes for in Situ Hybridization," *Journal of the American Chemical Society*, vol. 123, pp. 4103-4104, 2001.
- [23] A. H. Fu; W. W. Gu; C. Larabell; A. P. Alivisatos, "Semiconductor nanocrystals for biological imaging," *Curr. Opin. Neurobiol.*, vol. 15, pp. 568-575, 2005.
- [24] I. L. Medintz; H. T. Uyeda; E. R. Goldman; H. Mattoussi, "Quantum dot bioconjugates for imaging, labelling and sensing," *Nature Materials*, vol. 4, pp. 435-446, 2005.
- [25] K. E. Sapsford; T. Pons; I. L. Medintz; H. Mattoussi, "Biosensing with luminescent semiconductor quantum dots," *Sensors*, vol. 6, pp. 925-953, 2006.
- [26] E. R. Goldman; G. P. Anderson; P. T. Tran; H. Mattoussi; P. T. Charles; J. M. Mauro, "Conjugation of luminescent quantum dots with antibodies using an engineered adaptor protein to provide new reagents for fluoroimmunoassays," *Analytical Chemistry*, vol. 74, pp. 841-847, 2002.
- [27] E. R. Goldman; E. D. Balighian; M. K. Kuno; S. Labrenz; P. T. Tran; G. P. Anderson; J. M. Mauro; H. Mattoussi, "Luminescent quantum dot-adaptor protein-antibody conjugates for use

- in fluorimmunoassays," *Physica Status Solidi B-Basic Research*, vol. 229, pp. 407-414, 2002.
- [28] X. J. Ji; J. Y. Zheng; J. M. Xu; V. K. Rastogi; T. C. Cheng; J. J. DeFrank; R. M. Leblanc, "(CdSe)ZnS quantum dots and organophosphorus hydrolase bioconjugate as biosensors for detection of paraoxon," *Journal of Physical Chemistry B*, vol. 109, pp. 3793-3799, 2005.
- [29] J. Lee; A. O. Govorov; J. Dulka; N. A. Kotov, "Bioconjugates of CdTe nanowires and Au nanoparticles: Plasmon-exciton interactions, luminescence enhancement, and collective effects," *Nano Letters*, vol. 4, pp. 2323-2330, 2004.
- [30] S. Y. Ding; G. Rumbles; M. Jones; M. P. Tucker; J. Nedeljkovic; M. N. Simon; J. S. Wall; M. E. Himmel, "Bioconjugation of (CdSe)ZnS quantum dots using a genetically engineered multiple polyhistidine tagged cohesin/dockerin protein polymer," *Macromolecular Materials and Engineering*, vol. 289, pp. 622-628, 2004.
- [31] F. Pinaud; D. King; H. P. Moore; S. Weiss, "Bioactivation and Cell Targeting of Semiconductor CdSe/ZnS Nanocrystals with Phytochelatin-Related Peptides," *J. Am. Chem. Soc.*, vol. 126, pp. 6115-6123, 2004.
- [32] G. Hermanson *Bioconjugate techniques*; Academic Press: San Diego, 1995.
- [33] X. Y. Huang; J. F. Weng; F. M. Sang; X. T. Song; C. X. Cao; J. C. Ren, "Characterization of quantum dot bioconjugates by capillary electrophoresis with laser-induced fluorescent detection," *Journal of Chromatography A*, vol. 1113, pp. 251-254, 2006.
- [34] Y. C. Kuo; Q. Wang; C. Ruengruglikit; H. L. Yu; Q. R. Huang, "Antibody-conjugated CdTe quantum dots for Escherichia coli detection," *Journal of Physical Chemistry C*, vol. 112, pp. 4818-4824, 2008.
- [35] Z. Grabarek; J. Gergely, "ZERO-LENGTH CROSSLINKING PROCEDURE WITH THE USE OF ACTIVE ESTERS," *Anal. Biochem.*, vol. 185, pp. 131-135, 1990.