Speaker – Prof. Khodonov A.A.

Study of synthetic approaches for producing modified CdTe quantum dots in aqueous solutions with programmable optical properties

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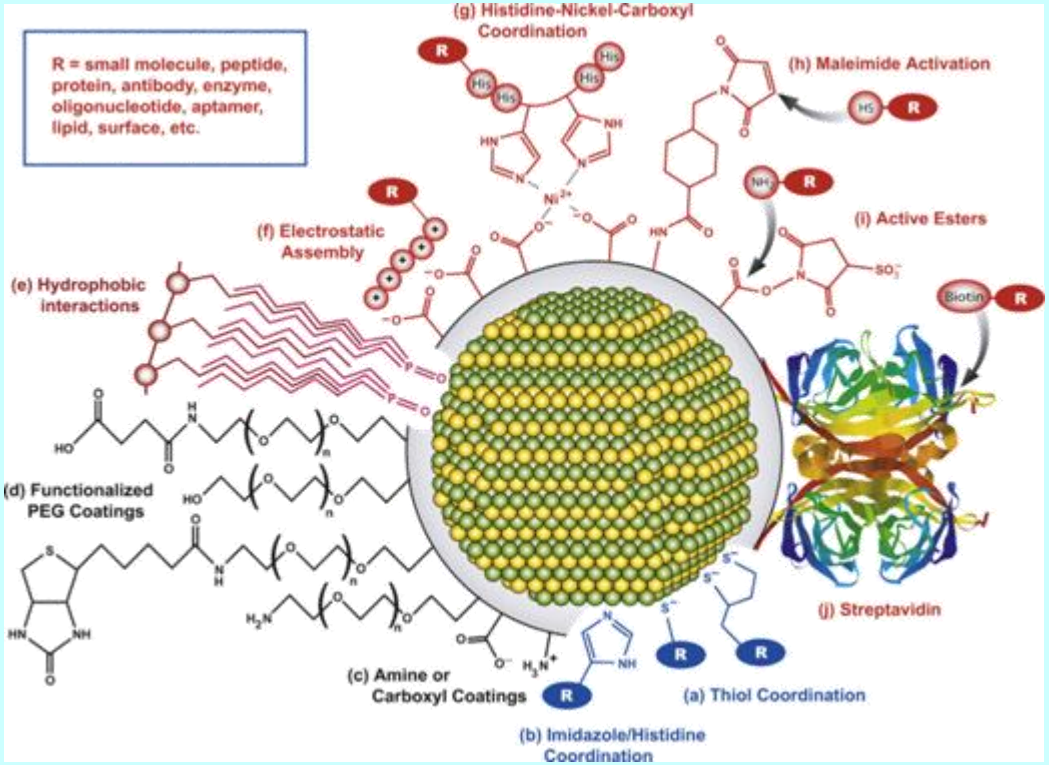
Introduction



Among the currently known families of fluorophores, semiconductor nanocrystals (quantum dots, QDs) are the most promising for use in various fields.

They have unique optical and chemical characteristics that distinguish them favorably from organic and biological fluorophores. The unique fluorescent properties of QDs have a number of advantages over organic fluorophores commonly used in biology and medicine:

1)narrow and symmetric fluorescence bands (FWHM 20–35 nm);

2) the possibility of creating on the basis of QDs samples of the same composition, but differing in nanoparticle sizes, a wide spectral range of fluorescent probes and labels from the UV region to the near IR range;

3) higher photostability;

4) comparable values of the quantum yield and higher values of the molar extinction coefficient, which made it possible to obtain systems for visualizing single molecules with good sensitivity and image brightness;

5) the possibility of implementing methods of surface modification using additional coatings or shells (ZnS, etc.) or immobilisation methods of various reactive groups, molecular addresses or bionanosized objects on it, allows the use of these systems in nanomedicine in diagnostics both *in vitro* and *in vivo* options, and in the long term - for the treatment of patients.

QDs of different sizes and shapes absorb light in a wide range of the optical spectrum from UV to near IR, while their emission spectrum is extremely narrow (FWHM) and is 20-25 nm, ideally symmetric, and the position of the maximum fluorescence emission of QDs is determined by their diameter.

Thus, QDs with diameters from 3 nm to 6 nm can be excited by a light source of the same wavelength, while emitting fluorescence in the range from blue to near infrared, depending on their size. In addition, QDs are more photostable (by many orders of magnitude) than the best organic fluorophores, providing the ability to multiply both the excitation power and long-term (days to months) tracking of the label's behavior in real time.

Preparation methods

Over the past 30 years after the discovery of QDs, several methods for their preparation have been developed:

1) high-temperature colloidal synthesis (HTCS);
2) hydrothermal synthesis in aqueous solutions;
3) methods for replacing the type of coating on the surface of QDs;
4) mixed variants of surface modification and combinations of those mentioned in paragraphs 1-3 methods.

High temperature colloidal synthesis (HTKS).

This method has found wide application for obtaining various hydrophobic QDs of the composition CdTe, CdSe, InP, InAs, CdSe / ZnS, etc. The method consists in the fact that a cold solution of selenium (Te or other organo-metallic precursors) in trioctylphosphine (20 C) is introduced into a hot (270-300 C) solution of cadmium oxide (or other sources of Cd) in a mixture of trioctylphosphine oxide (TOPO) and a hydrophobic stabilizer, for example, hexadecylamine, and the growth of QDs occurs with a controlled change in the process temperature. The surface of hydrophobic QDs is covered with fragments of TOPO and hydrophobic stabilizer. They are soluble only in low-polarity organic solvents (chloroform, toluene, heptane, hexane). QDs have a perfect crystal structure, small scatter in sizes (<5%), but the quantum yield of fluorescence remained relatively low (~ 10%). A significant increase in the quantum yield (up to 70%) was achieved by the formation of an additional ZnS or CdS shell around the main QDs core.

The solution of the main task of this study - the realization of the possibility of using QDs as labels and probes in biological systems requires a directed change in the surface properties of QDs, its modification with the formation of a sufficient number of **hydrophilic polar groups on the particle surface**, some of which could be an anchor group for attaching to them biological target molecules.

There are 2 main approaches:

(1) the use of bifunctional modifiers capable of replacing TOPO molecules and containing terminal hydrophilic anchor groups, which, after attachment to QDs, are exposed to an aqueous solution, and thus provide solubility in water;

(2) incorporation of QDs into polymer matrices, sol-gels, dendrimers. Although surface hydrophobic ligands (trioctylphosphine oxide and stabilizer) can be replaced by target hydrophilic ligands by exchange in a weakly polar solvent, this approach is not optimal: as a rule, such a ligand replacement leads to a significant decrease in the quantum yield of fluorescence and the stability of QDs samples.

Selection of QDs composition and nature

After analyzing the information on the availability, cost of the initial precursors in the domestic and foreign markets, the potential hazard in handling these reagents and their stability to the effects of various factors during the synthesis, comparing the data on two possible candidates (1) hydrophobic CdSe QDs and (2) MPA-modified hydrophilic QDs CdTe, we found that the synthesis of CdSe QDs was almost 8 times more expensive. Thus, we opted for a hydrophilic candidate (2), for which we developed a technology for hydrothermal synthesis of target CdTe QDs in aqueous solutions**.**

To develop protocols for selective labeling of various recombinant target proteins containing a hexahistidine tag, we needed to develop a technology for preparation high-quality CdTe QDs samples modified with various ligands in aqueous solutions.

A

$$4TeO_3^{2-} + 3BH_4^- \rightarrow 4Te^{2-} + 3BO_2^- + 6H_2O$$

$$CdCl_2 + Te^{2-} + MPA \rightarrow Cd{-}(MPA)_xTe_y + 2Cl^-$$

$$Cd{-}(MPA)_xTe_y \xrightarrow{\Delta} CdTe$$

B

Fig. 1. A - Basic chemical reactions in the technology of obtaining CdTe QDs stabilized with 3-mercaptopropionic acid (MPA), B - Basic technological stages of the synthesis of CdTe QDs.

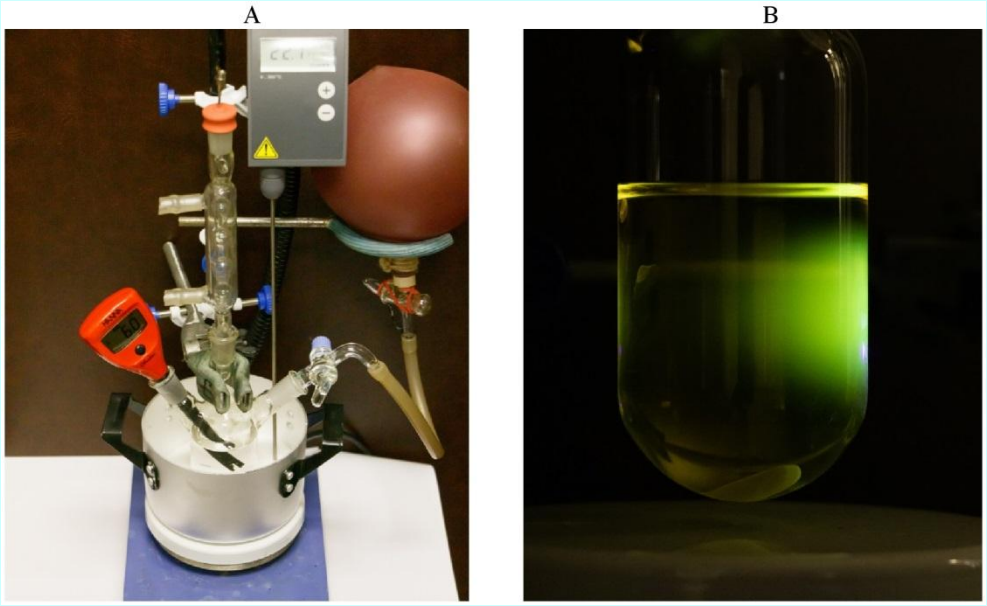


Fig. 2. A - General view of the installation for producing CdTe QDs stabilized with 3-mercaptopropionic acid, B - "Light beam in the dark kingdom" - fluorescence of the CdTe QDs reaction mix after reflux before the stage of separation $\lambda_{max\ fl}$ 520 nm.

CdTe QDs synthesis

Among the variants of the hydrothermal synthesis of CdTe QDs preparations known in the literature:

a) generation of the precursor (Te^{2-}) - hydrogen telluride solution (H_2Te) in situ from the Al_2Te_3 / H_2SO_4 system,

b) generation of the precursor (Te^{2-}) - NaHTe solution in situ from the Te / system $NaBH_4$,

c) generation of the precursor (Te^{2-}) in situ from Me_2TeO_3 by reduction of $NaBH_4$,

We have chosen and significantly modified the technology for preparations of water-soluble CdTe quantum dots stabilized with sulfur-containing ligands by the method (c) of hydrothermal synthesis.

After analyzing the results of early publications, we opted for the following modified technology for the synthesis of the colloidal system CdTe QDs, shown in Fig. 1-2. We selected the most accessible Cd and Te salts as the initial precursors. The pilot experiments carried out made it possible to select and optimize the parameters of the process: the order of mixing the reagents, their ratio, the temperature of the main reaction, the pH of the main reaction (values of the pH range from 9 to 12). A number of different sulfur-containing ligands with a terminal polar carboxyl group were tested (among them the most optimal ligand was 3-mercaptopropionic acid), the type of reducing agent, temperature parameters and duration of reflux of the reaction mixture, solvent for the size-selective sedimentation procedure, speed and duration of centrifugation.

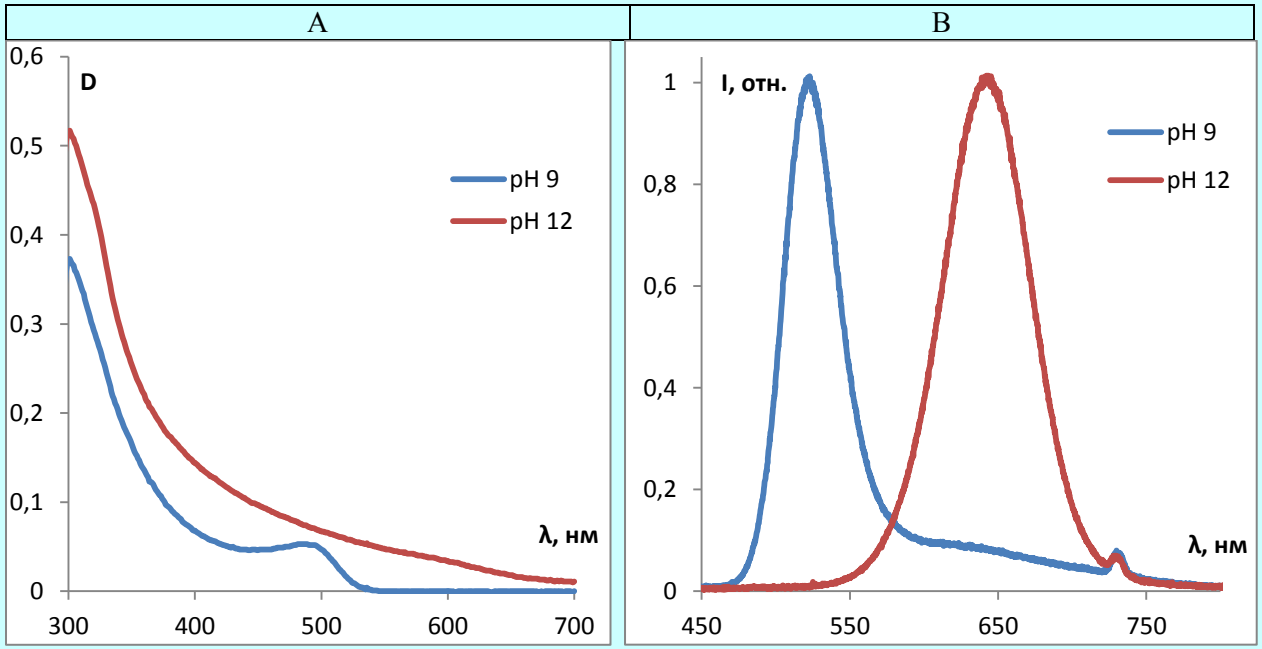
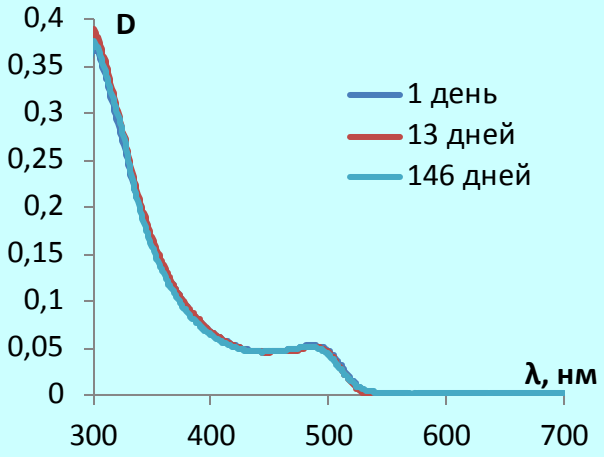


Fig. 3. Electronic spectra of CdTe QDs preparations (A) and their fluorescence spectra (B).

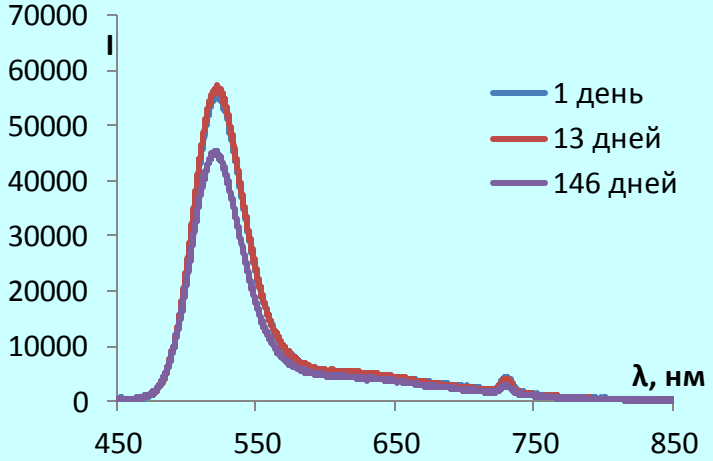
Table 1. Physical-chemical properties of CdTe QDs.

	pH 9	pH 12
Wavelength of main fluorescence band maximum, nm	520	642
Full width at half maximum (FWHM) of main fluorescence band, nm	45	70
First exciton absorption maximum, nm	488 (±3)	610 (±10)
Particle diameter, nm	1,9 (±0,2)	3,7 (±0,2)
Molar extinction coefficient, ε ($l \cdot mol^{-1} \cdot cm^{-1}$)	$4{,}3(\pm 0{,}9) \cdot 10^4$	$1{,}6(\pm 0{,}2) \cdot 10^5$

Stability of QD CdTe-MPA (pH 9) at 4 C (in reaction mix)



Absorption spectra in water



Fluorescence spectra in water, excitation with UV filtered by UFS-2

The synthesized CdTe QDs preparations during storage at 4 C in solution or in the solid state practically did not change their spectral parameters and characteristics for a long time (till half of year).

Research results

Thus, we have developed a technology for obtaining a serie of samples of the CdTe QDs colloidal system with programmed optical fluorescence parameters from 520 to 642 nm.

In the future, these intermediate CdTe QDs, after the introduction of a molecular address - an NTA fragment, will be used to develop protocols for the selective labeling of various recombinant target proteins.

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