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Colloidal Synthesis and Characterization of Hydrophilic CdTe Quantum Dots for Medical Diagnostics

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THE PRESENT article deals with colloidal quantum dots (QDs) synthesized in an aqueous medium using thioglycolic acid (TGA), L-cysteine (L-cys) and mercaptoethylamine (MEA) as stabilizers. In contrast to high-temperature synthesis in an organic medium, this method of synthesis enables us to skip an additional time-consuming stage of hydrophilization of QDs. The resulting CdTe QDs were studied by spectroscopic methods of analysis. In the absorption spectra of the QDs there is an exciton peak which corresponds to the minimum energy required to form excitons in the QDs. The average size of the QDs, which is about 3-4 nm, was calculated based on the position of the exciton peak. The zeta potential of the QDs was measured and the results were compared with literature data.

Keywords: Quantum dots, Colloidal quantum dots, Semiconductor nanocrystals, Nanoparticles,CdTe QDs.

Introduction

Recently, technologies based on semiconductor nanoparticles have been developing successfully in different areas: medicine [1], printing [2], photovoltaics [3], electronics [4], textile industry [5], etc. Some products still exist at the level of prototypes, somewhere the technology is partially implemented, and some are already used in practice.

Colloidal QDs are semiconductor nanocrystals ranging from 2 to 10 nm in sizeand having from 10^3 to 10^5 atoms in their structure. A decrease in theparticle size of a substance to a size smaller than the exciton Bohr radius leads to the fact that such properties as the band gap and the extinction coefficient come to be determined not as much by the chemical composition of nanoparticles as by their size and shape. Such fluorescent semiconductor nanocrystals are a relatively new class of fluorophores, which possesses a number of physicochemical features which are not typical of other fluorescent dyes[6, 7].

The main advantages of QDs over traditional fluorophores are a wide absorption spectrum, a narrow symmetric peak of luminescence, high photostability and a high quantum yield (QY) of fluorescence. These properties make them particularly attractive for alternative use in medical diagnostics [8-10].

QDs can be conjugated with biomolecules. Their conjugates are used in a variety of bioassay applications−from immuno chemical test methods to visualization of affected tissues and tracking of drugs in the body, which require the registration of several parameters simultaneously [11, 12]. Using biolabels based on QDs allows multicomponent detection, i.e. creating multicolor probes, with which one can get a complete picture of the affected cells with a single excitation [13]. In general, a conjugate is a complex which

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consists of covalently "crosslinked" QDs and biomolecules, which is more preferable for further use in bioassay. To obtain conjugates with biomolecules, water-soluble surface-modified QDs are usually used. At present, conjugates of semiconductor nanoparticles with various protein molecules, including antibodies, DNA, hormones, and many others, have been obtained [14].

Classical QDs, which are fluorescentin the near-IR region, are systems based on inorganic semiconductor materials (CdSe, InP, Si, etc.). The development of new, more advanced methods for synthesizing QDs, which are fluorescent in the near-IR range, is one of the promising areas of colloidal synthesis of nanomaterials with unique optical properties [8].

Nowadays, there are two main directions of synthesizing colloidal QDs (Fig. 1) which are described in [15].

The first directionis organometallic colloidal synthesis (OCS, Fig.1a) at high temperature (HT). In this type of synthesis, high-boiling organic solvents such as tri-n-octylphosphine oxide (TOPO), trioctylphosphine(TOP), or hexadecyl amine (HAD) are used as coordinating solventsand a surfactant mixture is used as a ligand. To connect the coordinating solvent (TOPO or HAD) with metal precursors (cadmium stearate, TOPO-Cd), chalcogenide (TOP-Se) and to start the nucleation process in the OCS, high temperatures (≈ 300 °C) and an inert medium are used. During the synthesis, ligands attach terminal functional groups (phosphines, phosphine oxides and amines) to the surface of the QDs, leaving alkyl chains directed from the surface.

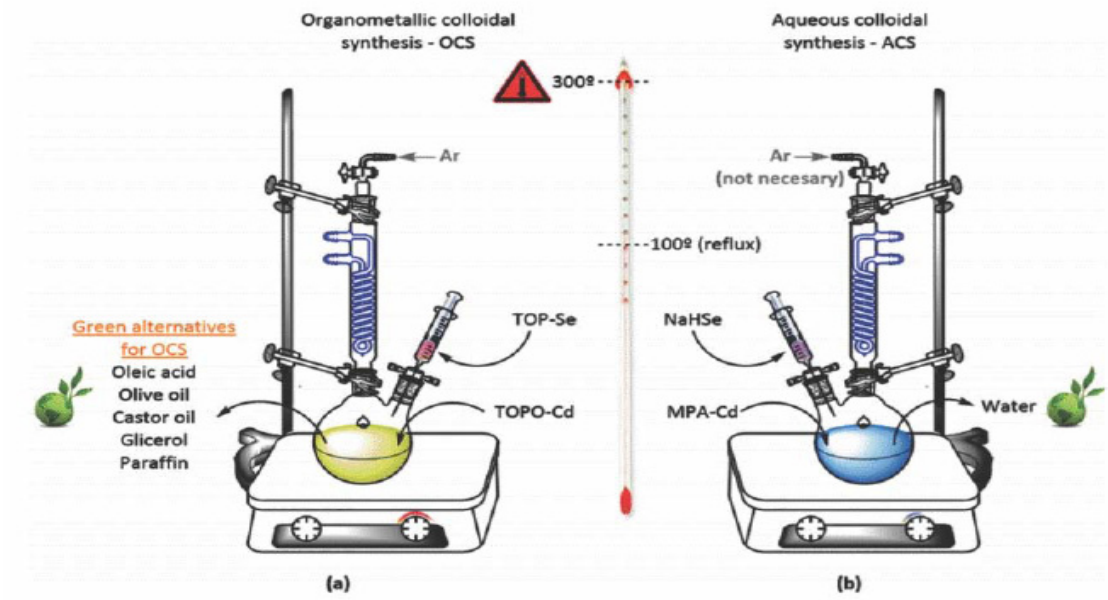

Fig. 1. Schematic representation of two main QDs synthesis methods, OCS (a) and ACS (b) in CdSe QD spreparation [15].

The second directionis ACS (Fig. 1b). It includes direct water synthesis of QDs using a less toxic and the most biocompatible solvent, i.e. water. In ACS, heavy metals precursors (for example, $CdCl_2$, $Cd(NO_3)_2$ and $Cd(CH_3COO)_2$) readily dissolve in water and are coordinated by hydrophilic agents such as TGA [16, 17], mercaptopropionic acid (MPA) [18], L-cys, [19], glutathione (GSH) [20], MEA [21], mercaptosuccinic acid (MSA) [22]. Chalcogen precursors can be NaHE (E = Se, Te) which are freshly prepared in an aqueous medium and obtained by chemical reduction of selenium (Se) or tellurium (Te) in the presence of boron sodium hydride ($NaBH_4$). Due to the possibility of oxidation of NaHE (E = Se, Te) with air oxygen, the synthesis is carried out in an inert atmosphere. To accelerate the growth of the QDs, after adding one of the precursors to the hot solution of the other, the mixture is refluxed. During the synthesis, the coordinating agents attach their functional group (-SH) to the surface of the QDs, leaving hydrophilic groups available for subsequent bioconjugation processes [14, 23].

When using OCS, the QDs are obtained with a narrow luminescence peak and a high quantum yield. However, it is essential to use high-boiling organic solvents and high temperatures in this synthesis. Furthermore, the QDs obtained by this method are hydrophobic and require an additional modification for further use in bioanalysis, during which the QY is considerably reduced.

The QDs obtained by ACS have a wider peak of luminescence, a smaller quantum yield, and lower stability. Meanwhile, toxic organic solvents are not required in ACS and the resulting QDs do not require further hydrophilization. Taking into account the advantages and disadvantages, ACS was chosen as a method for synthesizing QDs in this paper.

The main purposes of the paper areto obtain hydrophilic colloidal CdTe QDs for bioanalysis by the method of low-temperature colloidal synthesis in polar media and to studytheir optical properties.

At present, there are a lot of papers on ACS devoted to studying the effect of various stabilizers and their ratios on the growth rate, homogeneity, and optical properties of QDs. Thiol-containing ligands are often used as stabilizers [24-27]. It is noted in the paper [28] that with a decrease in the amount of a stabilizer, which is TGA, QDs are insufficiently covered, which results in a decrease in QY and stability. In the following paper [29], L-cys is used as a stabilizing ligand.

The nature of a stabilizer affects the characteristics of synthesized QDs. For example, using L-cys as a stabilizer shifts the fluorescence peak to a longer wavelength region [29]. This is due to the fact that L-cys,which is present on the surface of the QDs, forms hydrogen bonds between adsorbed thiols, which lead to a shift of luminescence to a longer wavelength region, which is caused by the transfer of the exciton energy between the QDs rather than by an increase in the size of the nanoparticle itself.

In addition, the nature of a stabilizer is characterized by the length of the alkyl chain [30]. Using the short-chain mercaptocarboxylic acids as stabilizers (for example, TGA, MPA) shifts the absorption and fluorescence peaks to a longer wavelength region, whereas using long-chain ligands such as mercaptohexanoic acid (MHA), mercaptoundecanoic acid (MUDA), and bulk ligands MSA slow down the growth of particles.

The pH of a medium has a major effect on the optical properties of QDs [21]. The QDs coated with ligands with carboxyl functional groups are stable in the pH range of 10-5. A decrease in the pH of the medium leads to a sharp decrease in the intensity of fluorescence, which is caused by a decrease in the negative surface charge and the formation of aggregated microparticles [21, 31].

In this paper, CdTe QDs weresynthesized by the method of ACS using TGA, L-cys and MEA as stabilizers. Their characteristics were studied depending on the reaction time, the amount and the nature of a stabilizer.

Methods for Analyzing QDs

The most universal and common methods for studying theproperties of QDs are optical methods. They enable us to characterize and to control the quality of QDs: to determine their chemical composition and size, interface quality, and reveal defects.

If the energy of QDs is greater than the width of the forbidden zone, they absorb photons. As a result, electrons move from the valence band to the conduction band, forming a hole. Theelectron-holepairiscalled an exciton. In the absorption spectra of qualitative QDs, there is always an exciton peak which corresponds to the minimum energy required to form excitons in the QDs. In addition, the position of the exciton peak can be used to estimate the average size of the QDs using the formula [32].

$$D = (1{,}6122 \cdot 10^{-9}) \cdot \lambda^4 - (2{,}6575 \cdot 10^{-6}) \cdot \lambda^3 + (1{,}6242 \cdot 10^{-3}) \cdot \lambda^2 - 0{,}4277 \cdot \lambda + 41{,}57 \quad (1)$$

where λ is the wavelength of the first exciton peak in the absorption spectra.

Based on the photoluminescence spectra of QDs, the width of the spectral line showing the dispersion of particles by size can be determinedat half height from the radiation maximum. The average particle size in a sample of a colloidal solution of QDs can be also estimated based on thethe position of the radiation maximum in the fluorescence spectra [33].

Dynamic light scattering (DLS) is often used to characterize QDs and determine their size. The

method is based on determining the diffusion coefficient of dispersed particles in a liquid by analyzing the correlation function of fluctuations in the intensity of scattered light. Then, the program calculates the radius of nanoparticles from the diffusion coefficient.

The charge or zeta potential acquired by a particle or a molecule in a solution characterizes the surface charge of the particles dispersed in the liquid phase. The value of the zeta potential carries important information about the stability of colloidal QDs in various media.

Experimental

Materials and reagents

Anhydrous cadmium chloride ($CdCl_2$, ≥ 99% Aldrich), sodium borohydride ($NaBH_4$, ~ 98% Aldrich), tellurium powder (Te, 30 mesh, 99,997% Aldrich), thioglycolic acid (TGA, ≥99% Aldrich), L-cysteine (L-cys, ≥ 98% Aldrich), mercaptoethylamine (MEA, ≥ 98% Aldrich), 20 mM borate buffer, deionized water, argon.

Spectrophotometer UNICO-2100 (UNITED PRODUCTS & INSTRUMENTS, USA), spectrofluorometer FluoroLog 3 model FL3-21 (Horiba JobinYvon SAS, France), Zetasizer Nano Z (Malvern Instruments Ltd, UK).

Synthesis of CdTe-QDs

Synthesis of CdTe-QDs was carried out in three stages. The first stage of the synthesis is to obtain NaHTe. For this purpose, a hitch of Te (0.0494 g) and a portion of sodium borohydride $NaBH_4$ (0.0640 g) are placed in a flask with a volume of 25 cm^3 and mixed. Then 5 cm^3 of degassed distilled water is added and the reaction mass is stirred at room temperature for 1 hour until the powder has dissolved completely and a light pink color has appeared. The synthesis is carried out in an argon atmosphere.

The second stage of the synthesis is to obtain the precursor of cadmium Cd^{2+}. For this purpose, a portion of cadmium chloride $CdCl_2$ is put in a 100 cm^3 round bottom flask and is dissolved in degassed distilled water. Then, a stabilizer is added to the resulting solution. TGA, L-cys, MEA are used as stabilizers (Fig. 4). To obtain the salt of TGA and L-cys, the pH of the resulting solutions is adjusted to a value of 10 by a sodium hydroxide solution with a concentration of 0.5 M. If MEA is used as a stabilizer, the pH of the medium is adjusted with a 1M acetic acid solution.

The third stage is to synthesize CdTe-QDs. For this purpose, the cadmium precursor solution with a stabilizer is heated to 90 °C and a freshly prepared NaHTe solution (tellurium precursor) is quickly added with constant stirring, the (Cd^{2+}):Teratio is 1:0.3. Complying with these conditions helps to accelerate the growth of QDs. The conditions of synthesizing QDs with various stabilizers are shown in Table 1.

TABLE 1.Conditions of syntheses.

The ratio of Cd^{2+}: stabilizer (molar)	Stabilizer	$CdCl_2$ weight, g	Stabilizer weight, g	pH
1:6	TGA	0.0183	0,0276	10
1:3	TGA	0.0183	0,0552	10
1:6	L-cys	0.0183	0.0727	10
1:6	MEA	0.0183	0.0463	5

The appearance of red coloration when injecting NaHTe into the Cd precursor solution signals the onset of the formation of QDs. To study the process of QDs formation, aliquots are selected every 15 minutes after the start of the synthesis.

Results and Discussion

OCS was chosen as a method for synthesizing hydrophilic CdTe-QDs, since it is the cheapest andthe least toxic one, and also gives good values of the quantum yield and a high degree of monodispersity of the resulting nanostructures. The given paperpresents the selection of conditions for synthesizing CdTe-QDs,which was made in order to obtain stable biocompatible QDs. The scheme of the three-stage synthesis of CdTe-QDs using various stabilizers is shown in Fig. 2.

At the first stage, a tellurium precursor, NaHTe sodium tellurium hydride, was synthesized. The scheme of synthesis of NaHTe can be shownby the following equation:

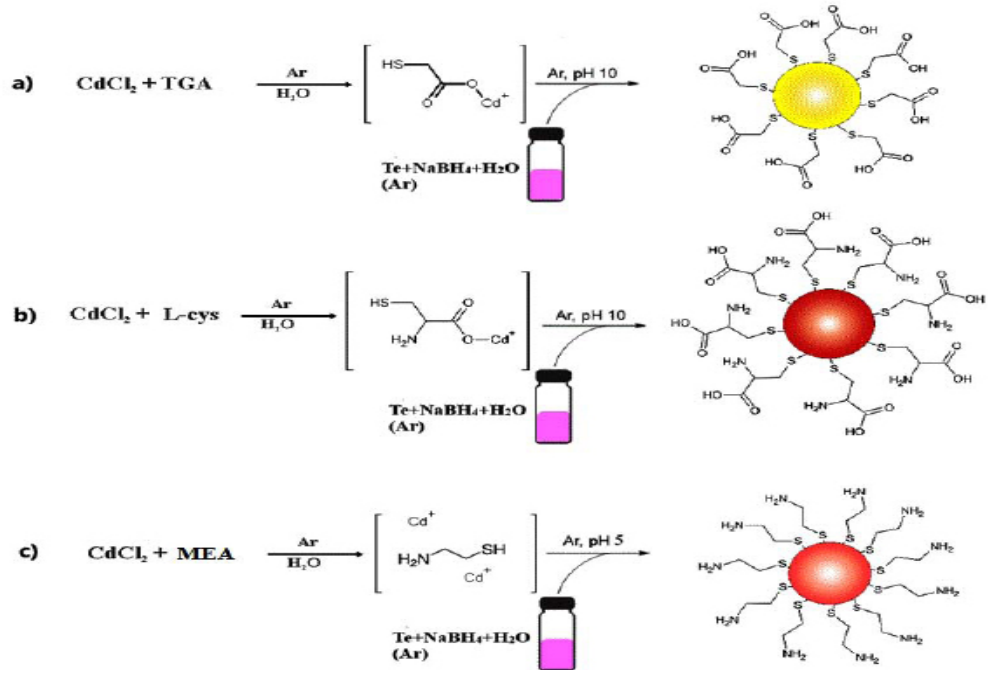

Fig. 2. Scheme of CdTe QDs. a) CdTe QDs stabilized TGA, b) CdTe QDs stabilized by L-cys, c) CdTe QDs stabilized by MEA.

The samples of the QDs, which were selected after mixing reagents,were unstable even after 15 minutes of synthesizing and quickly aggregated and precipitated. More stable CdTe QDs are formed with an increase in the reaction time to 30-40 minutes (Fig.3).

The main parameters on which the characteristics of the resulting QDs depend are as follows: temperature, the concentration of

$$4NaBH_4 + 2Te + 7H_2O \longrightarrow 2NaHTe + Na_2B_4O_7 + 14H_2. \quad (2)$$

precursors, the nature of a stabilizer and the reaction time. The rate of particle formation increases with increasing temperature, and the size of a precursor increases with increasing its concentration, and vice versa. Thus, by varying the concentration of the components, the rate at which they are fed into the reaction mixture, and the reaction time, nanocrystals of various dimensions and chemical composition can be grown. The amount of an added stabilizer will affect the stability of the QDs and their size.

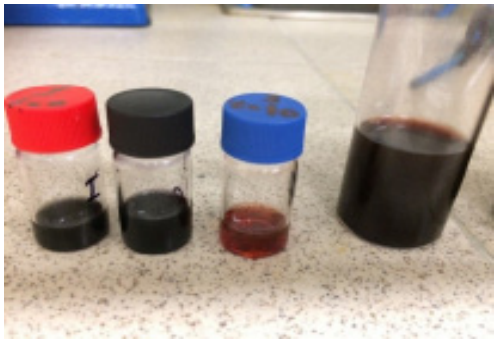

Fig. 3. CdTe QDs synthesized for 0, 5, 15, 40 min.

The paper studies the effect that the concentration of a stabilizer has on the properties of the resulting QDs. More stable CdTe-QDs obtained were analyzed by spectroscopic methods.

As the reaction time increases, the maximum of the exciton peak (Fig.4, left) and the fluorescence peak (Fig. 4, right) shift to a longer wavelength region, indicating an increase in the size of the QDs with increasing the reaction time.

The QDs obtained at different ratios of cadmium salt and a stabilizer led to the following result (Fig. 5).

The exciton peak is at 555 nm at Cd: TGA

ratio of 1 : 3, and is at 540 nm at Cd : TGA ratio of 1 : 6. This can be explained by the fact that the excess stabilizer prevents the QDs from growing by reducing the diffusion of cadmium and tellurium ions.

Additional syntheses were performed using L-cys and MEA to select the optimal stabilizer. The resulting QDs were also studied by spectral methods of analysis. The spectra for CdTe-QDs covered with L-cysare shown in Fig. 6.

The exciton peak in the absorption spectra of the QDs stabilized by L-cys is weakly expressed, which may be due to wide size dispersion or defects in the structure of the QDs. In addition,

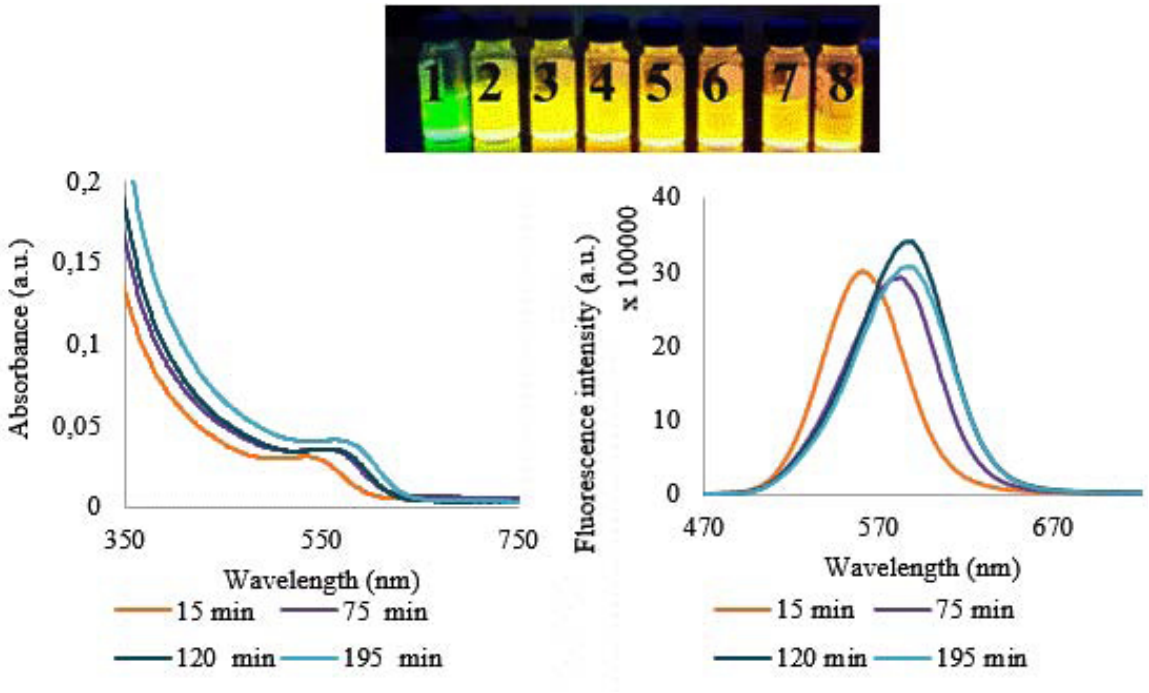

Fig. 4. UV-visible absorption and photoluminescence spectra of CdTe QDs stabilized by TGA depending on the reaction time. 1–15 min, 2–45 min, 3–75 min, 4–105 min, 5–120 min, 6–150 min, 7–180 min, 8–195 min.

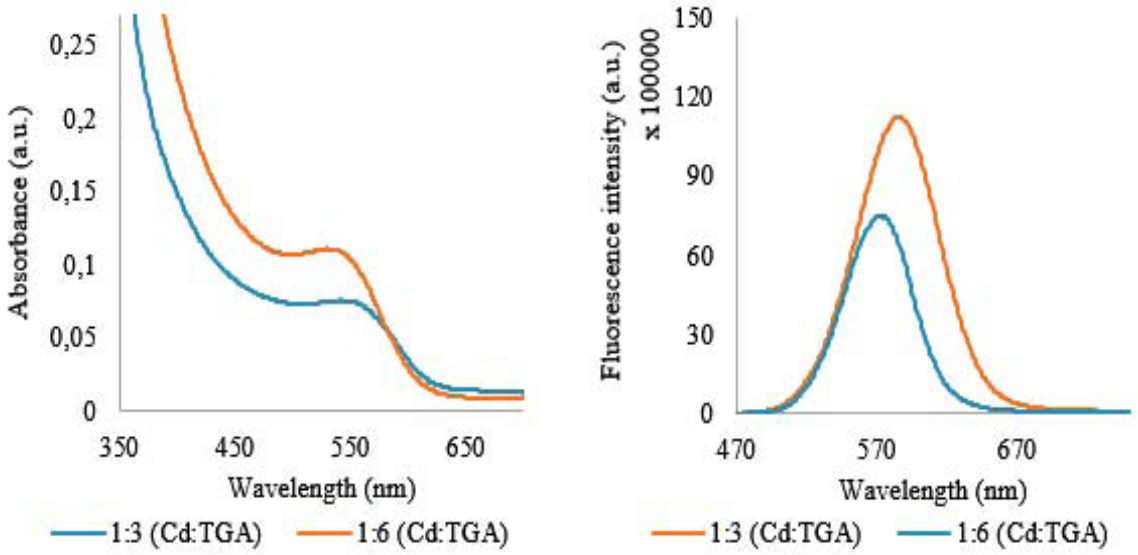

Fig. 5. Absorption and luminescence spectra of QDs depending on Cd: TGA ratios (reaction time is 45 min).

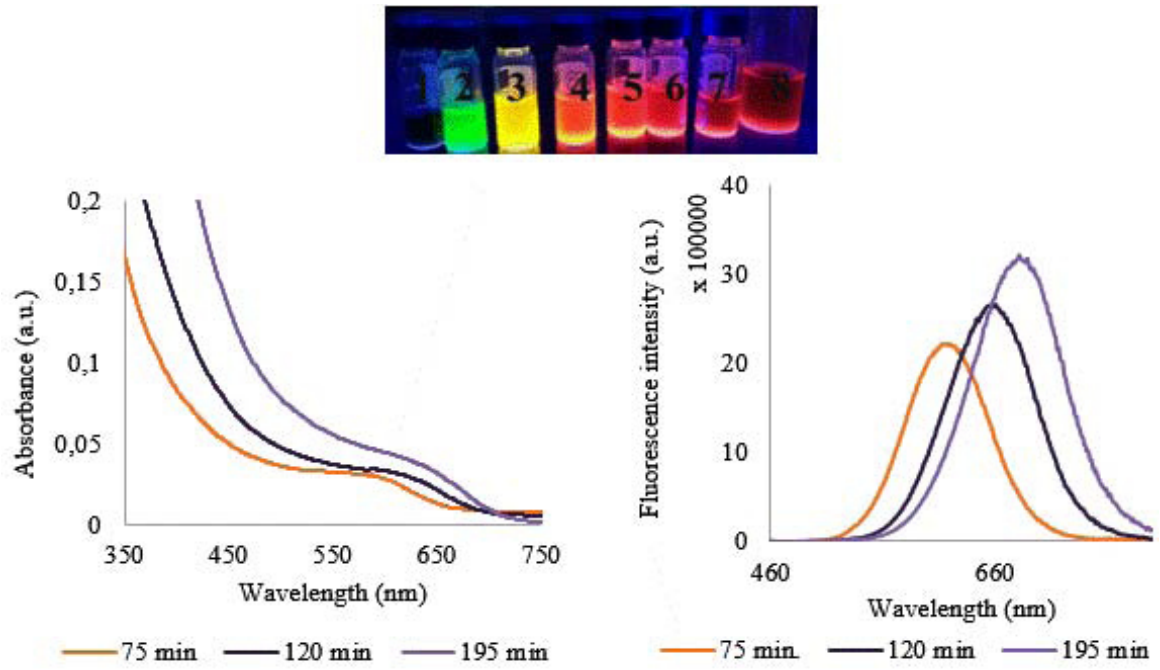

Fig. 6. UV-visible absorption and photoluminescence spectra of CdTeQDs stabilized by L-cys depending on the reaction time. 1–15 min, 2–45 min, 3–75 min, 4–105 min, 5–120 min, 6–150 min, 7–180 min, 8–195 min.

the QDs have a wide peak of luminescence, which confirms the wide size dispersionof particles.

The result of synthesizing the QDs by MEA is shown in Fig. 7

The absorption and luminescence spectra of the QDs with different stabilizers under the same conditions and reaction time are shown in Fig. 8.

A comparison of the spectral data of the QDs stabilized by TGA, L-cys, MEA shows that when TGA or MEA is used as a stabilizer, the maximum of the exciton peak is about 554 nm, and the maximum of the luminescence peak is about 590 nm. If L-cys is used, the exciton peak is located at 645 nm, and the luminescence maximum is at 681 nm, that is, for the QDs stabilized by L-cys it can be observed that the exciton peak and the maximum of luminescence shift to a longer wavelength region. From this it follows that if TGA or MEA are used as stabilizers, the QDs have an approximately equal growth rate but the growth rate is higher when L-cys is used. This is related to the high solvating ability of L-cys.

The average size of the QDs can be estimated by using the position of the exciton peak and the formula (1). The calculated QDs size with different stabilizers and depending on the reaction time are presented in Table 2.

The resulting data correspond to the spectral studies and show that the size of the QDs depends on the reaction time, the nature of a stabilizer, and the Cd^{2+} : stabilizer ratio.

The zeta potential was measured to determine the stability of the resulting QDs in the solutions stabilized by TGA, L-cys, MEA (Fig. 9).

An analysis of the dependence of zeta potential on pH shows that the surface of CdTe QDs stabilized by TGA has a significant negative charge in the entire pH range, which indicates their colloidal stability. A study of the dependence of the zeta potential on the pH of CdTe in the QDs stabilized by L-cys shows that the surface is negatively charged in the pH range 4 – 12. Near the isoelectric point at pH = 4, the QDs coagulated and at pH < 4 L-cys becomes cationic and the surface acquires a positive charge. For this reason, it can be concluded that the QDs coated with L-cys are stable in a wide range of pH except the area of the isoelectric point. The study of the dependence of the zeta potential on pH for CdTe-MEA QDs shows a significant positive charge in the pH range 7-11, which is caused by the presence of protonated amino groups on the surface of the QDs. The isoelectric point is reached at pH = 12.

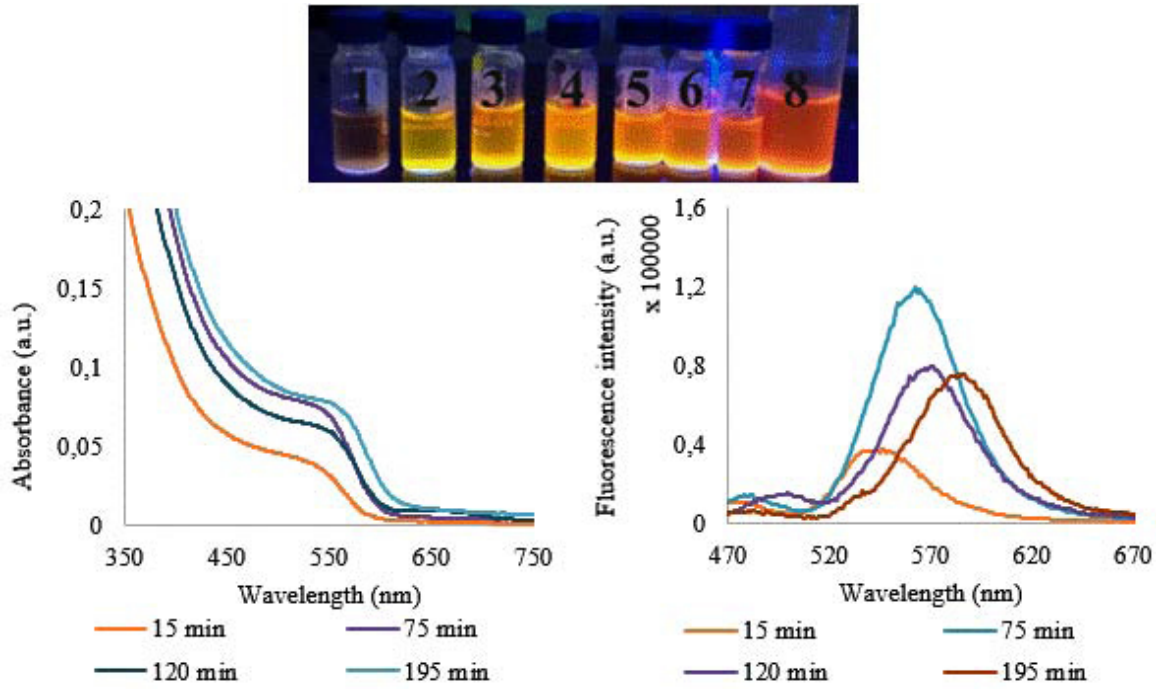

Fig.7. UV-visible absorption and photoluminescence spectra of CdTe QDs stabilized by MEA depending on the reaction time. 1–15 min, 2–45 min, 3–75 min, 4–105 min, 5–120 min, 6–150 min, 7–180 min, 8–195 min.

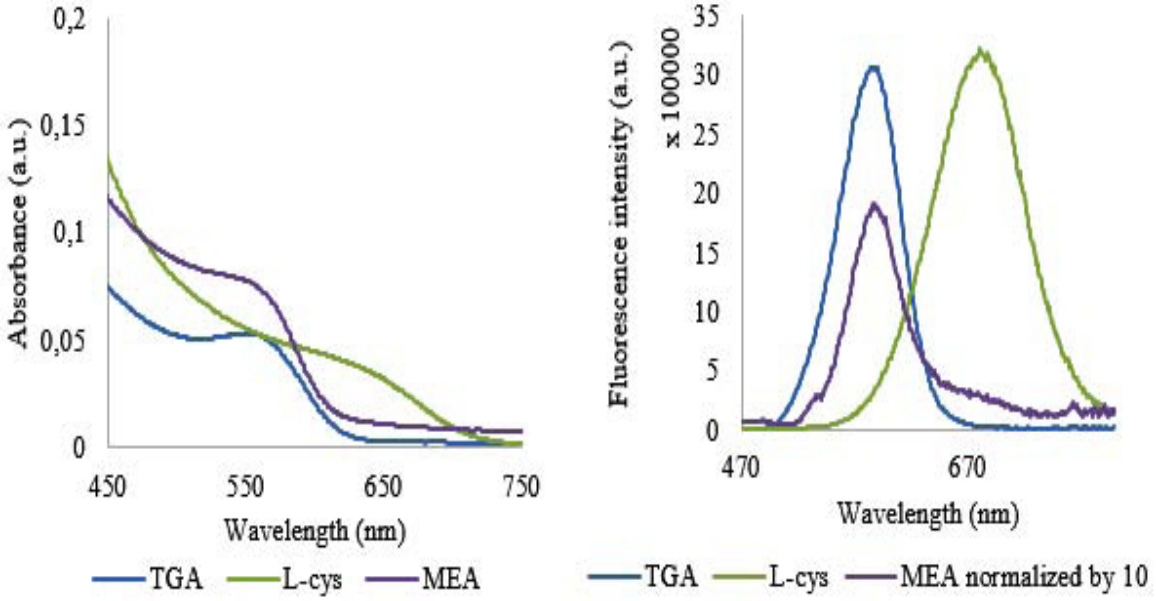

Fig. 8. Absorption and luminescence spectra of CdTe-QDs stabilized by TGA, L-cys, MEA (reaction time is 195 min).

TABLE 2. Dependence of QDs size on reaction time and stabilizer.

QDs	Dependence of QDs size on reaction time			
	15 min	**75 min**	**120 min**	**195 min**
CdTe - TGA	3,20 nm	3,40 nm	3,43 nm	3,50 nm
CdTe - L-cys	-	3,57 nm	4,02 nm	4,28 nm
CdTe - MEA	3,17 nm	3,26 nm	3,32 nm	3,38 nm

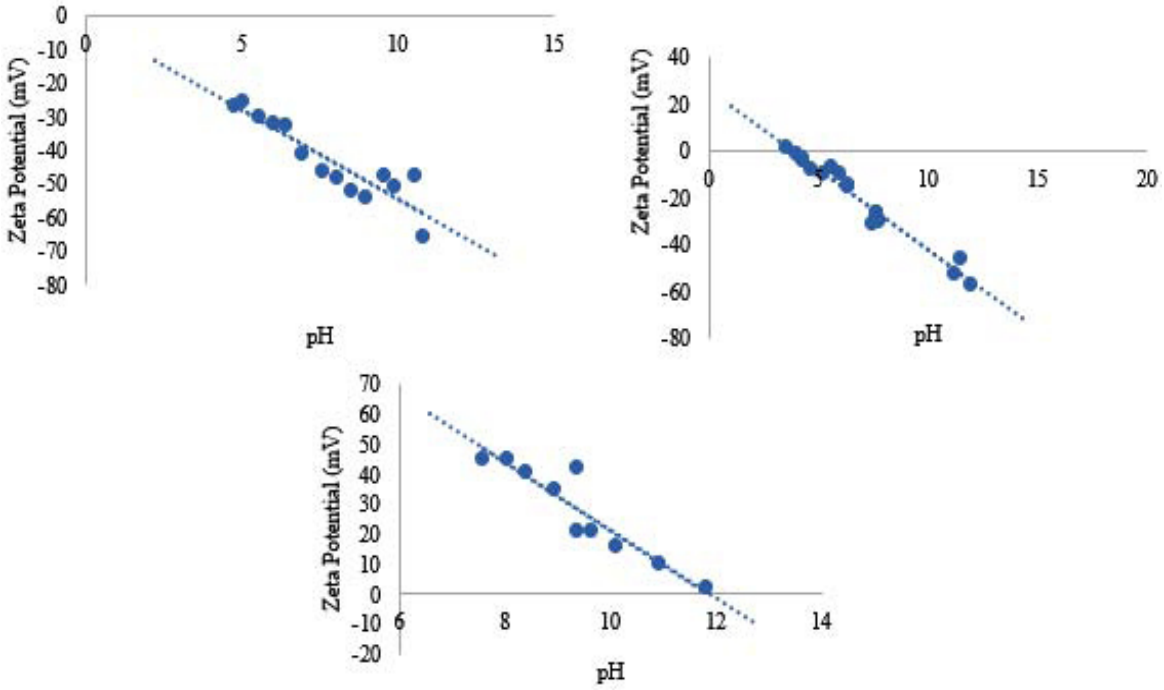

Fig. 9. Dependence of zeta potential on pH CdTe-TGA QDs (left), CdTe-L-cys QDs (right) and CdTe-MEA QDs (bottom).

Upon further increasing pH, the deprotonation of amino groups occurs on the surface of the QDs, and the group NH_3^+ changes into NH_2, which reduces the surface charge and the aggregation of particles occurs.

The value of zeta potential of the QDs obtained in the paper is twice as much as that described in the literature [21, 34] although the nature of the dependence is the same. The difference in charges may be due to the fact that there are more functional groups of a stabilizer on the surface of the QDs.

Conclusion

Recently, the method of colloidal synthesis of nanoparticles in aqueous media has been widely used to obtain QDs. The advantages of this method are as follows: low cost, environmental friendliness and the ability to obtain nanocrystals with a hydrophilic surface without having to transfer particles from a non-polar environment to an aqueous one,in contrast to high-temperature synthesis, which is the key point for using QDs in bioanalysis.

Cadmium chalcogenides are the most popular and well-studied group of materials which are luminescent in the visible and near IR ranges. QDs based on cadmium chalcogenides are the most available ones and are used in bioanalysis as fluorescent probes. The high QY of the QDs based on CdTe, CdSe is obtained by covering the nuclei with passivating materials with a larger band gap such as, ZnS or CdS.

In this paper, CdTe-QDs were obtained by the method of ACS using TGA, L-cys and MEA as stabilizers. The characteristics of the QDs were studied depending on the amount and nature of a stabilizer, and the reaction time.

The resulting CdTe QDs were studied by spectroscopic methods of analysis. In the absorption spectra of the QDs, there is an exciton peak which corresponds to the minimum energy required to form excitons in the QDs. The average size of the QDs was calculated based on the position of the exciton peak.

The zeta potential of the QDs was measured for CdTe-TGA and CdTe-L-cys. It shows a large negative surface charge in a wide pH range due to the presence of carboxyl groups. CdTe-MEA QDs are characterized by a positive surface charge due to the presence of functional amino groups.

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تحضير وتوصيف نقاط كمية من معلق تليريد الكادميوم للتطبيقات الطبية

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تتناول الدراسه الحالية طريقة لتحضير النقاط الكمية الغروية (QDs) التي يتم تحضيرها في وسط مائي باستخدام حمض الثيوجليكوليك (TGA) ، والحمض الاميني السيستين L-cysteine (L-cys) وكذلك ميركابتو ايثيل امين mercaptoethylamine (MEA) كمثبتات. علي الجانب الاخر فان ما يتم في الطرق الاخري للتحضير يكون باستخدام درجات حرارة عالية وفي اوساط عضوية، بينما الطريقة الحالية المستخدمه في هذا البحث تتم دون استغراق وقتًا طويلاً عن طريق اختصار الخطوات. وتعتبر هذه الطريقة التي تتم في هذا البحث من الطرق الرخيصة و التي يمكن من خلالها استخدام النقاط الكمية في التطبيقات الحيوية الطبية. وفي هذا البحث يتم دراسة النقاط الكمية الغروية لتليريد الكادميوم الناتجة (CdTe QDs) بواسطة طرق التحليل الطيفية. وقد اظهرت اطياف الاشعه فوق البنفسجية وجود منحني يمثل قمه أكسيتون في أطياف الامتصاص الخاصة بالنقاط الكمية الغروية والتي تتوافق مع الحد الأدنى من الطاقة اللازمة لتشكيل الأكسيتون في النقاط الكمية الغروية. كما تم حساب متوسط حجم النقاط الكمية الغروية ، ووجد انه مابين 3 الي 4 نانومتر، بناءً على موضع قمة الاكسيتون. كما تم حساب قيمة زيتا المحتملة (potential zeta) للنقاط الكمية الغروية و تمت مقارنة النتائج التي تم التوصل اليها مع تلك التي تمت في الدراسات السابقة. وقد اظهرت نتائج زيتا المحتمله لكل من CdTe-TGA و CdTe-L-cys وجود سطح كبير بشحنات سالبه لوجود مجموعات الكروبكسيل بينما اظهرت نتائج زيتا المحتملة CdTe-MEA QDs وجود سطح بشحنات موجبة نتيجة وجود مجموعات الاميد.