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A highly efficient capillary electrophoresis-based method for determination of water-soluble CdTe, CdTe/ZnS, and multilayer CdTeSe/CdS/CdZnS/ZnS quantum dots

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ABSTRACT Various applications and synthesis methods of quantum dots require reliable analytical methods to determine composition, colloidal stability, monodispersity, as well as to identify quantum dots. Therefore, their analysis is of great interest. As a rule, water-dispersible nanoparticles have a surface charge, which makes electrophoretic methods of analysis promising for characterising quantum dots (QDs). Hydrophilic CdTe, CdTe/ZnS, and multilayer CdTeSe/CdS/CdZnS/ZnS QDs were studied using capillary zone electrophoresis (CZE). A method for analyzing and characterizing colloidal QDs by CZE was developed, the influence of factors of the electrophoretic process on the parameters of QDs migration was studied and the conditions for QDs analysis were selected. Optimal conditions have been established for determining quantum dots with a minimum analysis duration and using a borate buffer containing surfactant as a background electrolyte. Since the synthesis of multilayer quantum dots is multi-stage, the developed analysis method can be used for express analysis and characterization of hydrophilic QDs obtained at each synthesis step. In this study, it was shown that by the type of electropherogram and the width of the peak corresponding to QDs, conclusions can be made about the heterogeneity of the synthesized samples in size, the efficiency of each stage of QDs synthesis and purification, and the processes of their degradation during storage.

KEYWORDS quantum dots, fluorescent labels for proteins, capillary electrophoresis, capillary zone electrophoresis, semiconductor nanoparticles

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1. Introduction

Most modern biomedical research methods and diagnostics are based on using of fluorescent labels of biomolecules. Application of colloidal QDs as fluorophores is an innovative direction [1–5]. Compared to organic dyes, QDs has a number of advantages: the ability to control the luminescence wavelength, a high extinction coefficient, solubility in a wide range of solvents, a narrow and symmetrical luminescence peak, high photostability, and the dependence of the wavelength of the emitted light on the structural and dimensional characteristics of the nanocrystal.

QDs can be conjugated to biomolecules due to covalent binding between groups of ligands covering QDs and functional groups of biomolecules. Currently, conjugates of semiconductor nanoparticles with various protein molecules, such as antibodies, DNA, hormones, and many others, have been obtained [6–8]. In this form, they can be used as fluorescent tags in a wide variety of bioanalysis applications: from immunochemical test methods to imaging of affected tissues and drugs tracking in the body. For today, using of QDs in bioanalysis is one of the most perspective trend. QD's conjugates are suitable fluorophores for multiplex analysis and multicomponent detection of various components in cells using multi-color probes with a single excitation, which allows one to get a complete picture of cell damage [9, 10].

The variety of QD's synthesis methods and the expansion of their practical application potential make it necessary to search for reliable and effective analysis methods that allow identifying QDs, determining their quality, and tracking degradation processes during storage and operation. Various physical and chemical analysis methods, such as photoluminescence, X-ray diffraction, transmission electron microscopy (TEM) and scanning electron microscopy (SEM), and dynamic light scattering (DLS), are used to evaluate the quality of synthesized QDs and characterize them [11–14]. Each of these methods has advantages and disadvantages over the others.

The capillary electrophoresis (CE) method allows separating the charged components of a mixture in a capillary under an applied electric field (voltage up to 30 kV) based on differences in their electrophoretic mobility. The advantages of the CE method include high separation efficiency (hundreds of thousands of theoretical plates), short analysis time and relatively simple instrumentation needed to perform analysis. In this regard, CE is an alternative to existing methods of QDs analysis and characterization [15, 16].

Various variants of capillary electrophoresis, differing in the principles and mechanism of separation, were used in the analysis: capillary zone electrophoresis (CZE) [8, 9], micellar electrokinetic capillary chromatography (MEKCC) [17], capillary gel electrophoresis (CGE). Capillary gel electrophoresis (CGE) is usually used to determine the size of QDs [18, 19]. The CZE method has also been used to separate various nanoparticles, including particles of colloidal metal oxides, SiO_2, silver and gold nanoparticles, where separation occurs due to differences in the electrophoretic mobility of the components of the analyzed mixture [20, 21].

Thus, the CE method is a good alternative to the existing QD assay methods. This method allows one to separate QDs of different compositions and structures, as well as characterize the processes of their degradation and conjugation with biological molecules. In this regard, the purpose of the work was to study the possibility of characterizing chalcogenide QDs of different sizes and compositions and to develop a method for their analysis by capillary electrophoresis. To identify the possibilities of the CE method in determining QD, it is necessary to study the effect of various process parameters (pH, lead electrolyte concentration, applied voltage, temperature) on the electrophoretic mobility of analytes and separation efficiency under CE conditions.

For using QDs as fluorescent labels of biomolecules, they should be highly soluble in aqueous matrices. At different pH colloidal QDs are in the form of charged particles, which is due to the dissociation of groups of stabilizer covering the QDs. The object of the present study was quantum dots different in size, composition, and stabilising coating. Hydrophilic chalcogenide quantum dots CdTe-TGA, CdTe/ZnS-TGA, CdTe-L-cys, CdTe-MEA, CdTeSe/CdS/CdZnS/ZnS-PTVP (PTVP – poly (vinylpyrrolidone-co-maleic anhydride-co-ethylene glycol dimethacrylate) were investigated in the work, where L-cysteine (L-cys), 2-mercaptoethanolamine (MEA) and thioglycolic acid (TGA) were used as ligands.

2. Experimental

2.1. Materials and reagents

Tubes with filters Amicon Ultra, 0.5 ml, 50 kDa, Merck (Millipore, USA), syringe Filters "Phenex" (pore size 0.20 microns), cellulose filters with a pore size of 0.45 microns, quantum dots CdTe-TGA (3.50 ± 0.18 nm), CdTe/ZnS-TGA, CdTe-L-cis (4.28 ± 0.21 nm), CdTe-MEA (3.38 ± 0.17 nm), water-alkaline solution (Dubna State University), quantum dots CdTeSe/CdS/CdZnS/ZnS-PTVP (PTVP – poly (vinylpyrrolidone-co-maleic anhydride-co-ethylene glycol dimethacrylate) with end thiol groups), $d = 10.5 \pm 0.5$ nm, solvent: water (Research Institute of Applied Acoustics), sodium hydroxide, chemically pure ("Vekton", Russia), hydrochloric acid, 37 % chemically pure ("AppliChem", Germany), sodium tetraborate, $Na_2B_4O_7 \cdot 10H_2O$, fixanal, TU 6-09-2540-72 (Russia), cetyltrimethylammonium bromide (CTAB), (Fluka, USA), isopropyl alcohol, extra-pure grade ("Laverne", Russia), acetone, GOST 2768-84, ("Khimprodukt-Balakhna", Russia), deionized water. The scheme of the studied QDs is shown in Fig. 1.

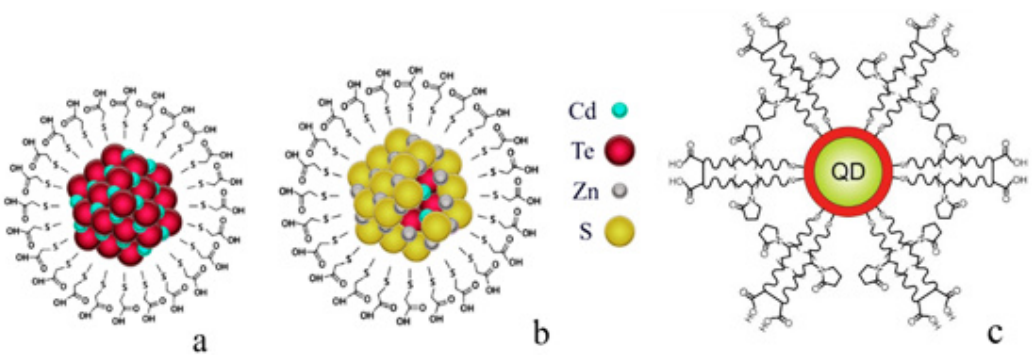

FIG. 1. Structures of hydrophilic CdTe-TGA QD (a), CdTe/ZnS-TGA QD (b) and CdTeSe/CdS/CdZnS/ZnS-PTVP QD (c)

2.2. Instrumentation

System of capillary electrophoresis CE 7100 (Agilent, USA) with a Diod Array Detector and a quartz capillary with a polyimide coating with a diameter of 50 microns and $L_{\text{tot}}/L_{\text{eff}} = 64.5/56$ cm, spectrophotometer UNICO-2100 (UNITED PRODUCTS & INSTRUMENTS, USA), Zetasizer Nano Z (Malvern Instruments Ltd, UK), Zetasizer Nano S (Malvern Instruments Ltd, UK), microcentrifuge "MiniSpin plus" (Eppendorf, Germany), centrifuge (Thermo Scientific Sorvall ST 16R, USA), water purification system "Elix Advantage 5" with E-POD unit (Millipore, USA), analytical balance AW-220 (Shimadzu, Japan), ultrasonic cleaner "UZV-4.0 TTTs" (Sapphire, Russia).

2.3. Procedure of capillary electrophoresis

QDs was purified from low-molecular-weight impurities by ultrafiltration using centrifuge filters with a molecular weight cut-off of 30 kDa at 8000 rpm for 15 minutes. Additional QDs purification was performed by depositing twice volume of acetone in relation to the initial QDs solution during centrifugation at 5000 rpm for 7 minutes. Then the deposited QDs was dried at 70 °C and dissolved in deionized water.

The hydrodynamic size of the studied nanoparticles was determined by the DLS method (Table 1). It should be noted that the hydrodynamic radius of polymer-coated QDs is larger than the core size and can exceed it by more than 20 nm [22, 23].

TABLE 1. QD's with different composition sizes

QDs	Hydrodynamic diameter of QDs, nm (by DRS)
CdTe-TGA	7.71 ± 0.68
CdTe/ZnS-TGA	27.00 ± 1.84
CdTe-L-cys	6.81 ± 0.81
CdTe- MEA	12.23 ± 2.63
CdTeSe/CdS/CdZnS/ZnS-PTVP	71.65 ± 20.78

The process of electrophoretic separation is affected by the charge of the particles in the solution of the leading electrolyte. The choice of a particular stabilizer affects the charge of the nanoparticle surface. The surface of QDs stabilized with mercaptoacids is negatively charged at neutral pH, whereas stabilization with cystamine (2-mercaptoethanolamine, MEA) makes it positively charged [24]. Cysteine (L-cys) is also actively used as a ligand [25], since it forms a zwitterion at physiological pH, making the surface of the nanocrystal electrostatically neutral. Therefore, a study of the dependence of the ζ-potential on the pH of the solution of the studied QDs was carried out, which allows us to characterize the structure of the double electric layer (DEL) of colloidal nanoparticles and determine the pH range favorable for the analysis by the CE method. The ζ-potential for CdTe QDs stabilized by TGA and L-cys, and for CdTeSe/CdS/CdZnS/ZnS QDs coated with a layer of PTVP was obtained using a Zetasizer Nano Z analyzer (Malvern Instruments Ltd, UK). The obtained dependences of ζ-potential value on pH are shown in Fig. 2. In the case of CdTe QDs stabilized by TGA the surface charge is caused by the presence of deprotonated carboxyl groups (COO^-) as potential determining ions. As a result, we observe a negative value of the ζ-potential in the entire pH range under study.

In CdTe QDs stabilized by L-cys on the surface in addition to COO^- groups, there may be NH_3^+ groups that shift the position of the isoelectric point and determine the positive value of the ζ-potential at pH < 4. CdTeSe/CdS/CdZnS/ZnS QDs coated with PTVP also have carboxyl groups on the surface which causes a negative value of the ζ-potential in the entire pH range under study. Thus, for CdTe-TGA QDs, CdTe-L-cys QDs, as well as for CdTeSe/CdS/CdZnS/ZnS-PTVP QDs, it is recommended to use buffer solutions with a high pH value. However, the studied QDs at pH ≥ 10 are unstable and tend to coagulate. In this state, QDs are not only not subject to research by CE method, but can also lead to clogging of capillary. Therefore, a buffer with pH in the range from 7 to 9.5 was selected as the background electrolyte for the QDs study using the CE. For the CE analysis, QDs samples were dissolved in deionized water, the resulting solutions were filtered through a syringe filter with a pore size of 0.20 um and centrifuged at 4000 rpm for 5 minutes. Degassing of solutions used was carried out on a centrifuge at 5000 rpm for 5 minutes.

CE analyses were carried out on the Capillary Electrophoresis System CE 7100 with a DAD detector (Agilent, USA) with quartz capillary with a diameter of 50 micron and length ($L_{\text{tot}}/L_{\text{eff}}$) was 64.5/56 cm. Prior to first use, the capillary was pretreated by flushing sequentially with 0.1 M NaOH, water, and background electrolyte (10 min each). Between CE runs, the capillary was conditioned with 0.1 M NaOH (3 min) and background electrolyte (BGE) (5 min). CE separations were carried out at voltages up to 20 kV. Samples and segments were injected hydrodynamically at 50 mbar 2 sec. All CE experiments were performed at constant temperature of 25 °C.

3. Results and discussion

Varying parameters such as pH, buffer system composition, ionic strength of the solution and temperature allows minimizing the influence of factors that lead to changes in the initial distribution of QDs forms, which is the key to the reliability of the analysis results. Thus, the CE meets one of the main requirements of the analysis, which is associated with preserving the chemical forms of the object components unchanged during the separation and determination process [26].

The choice of a suitable buffer as a background electrolyte (BGE) is most important for the separation of QDs in CE. The migration time and shape of QDs peak are affected by the pH of background electrolyte, size of the nanoparticles and charge.

Since at a pH greater than 7, the surface of the studied QDs is negatively charged, a tetraborate buffer with pH = 9.2 was chosen as the leading electrolyte. The resulting electrophoregram is shown in Fig. 3.

As can be seen from the electrophoregram, analysis under these conditions takes quite a long time. Adding substances to the buffer that can modify the quartz capillary walls, reducing their interaction with QDs and preventing his agglomeration, is one of the solutions to this problem. Dynamic decontamination of the inner surface of the capillary walls was provided by adding a cationic surfactant, cetyltrimethylammonium bromide (CTAB) to the background electrolyte. Analysis was performed at negative polarity.

The mechanism of separation of sample components is determined by hydrophobic interactions or is ion exchange in nature [27]

$$A^{x-} + IS - B^{y-} \leftrightharpoons B^{y-} + IS - A^{x-},$$

where A^{x-} – analyte anion (QDs), B^{y-} – buffer anion, IS – inner surface of the capillary.

Isopropyl alcohol was also added to the buffer, since the organic additive (from a few fractions of a percent to 30 % by volume) helps to reduce critical micelle concentration (CMC) and hydrophobic interactions between the analyzed component and the surfactant. The electrophoregram for CdTe-TGA QDs is shown in Fig. 4.

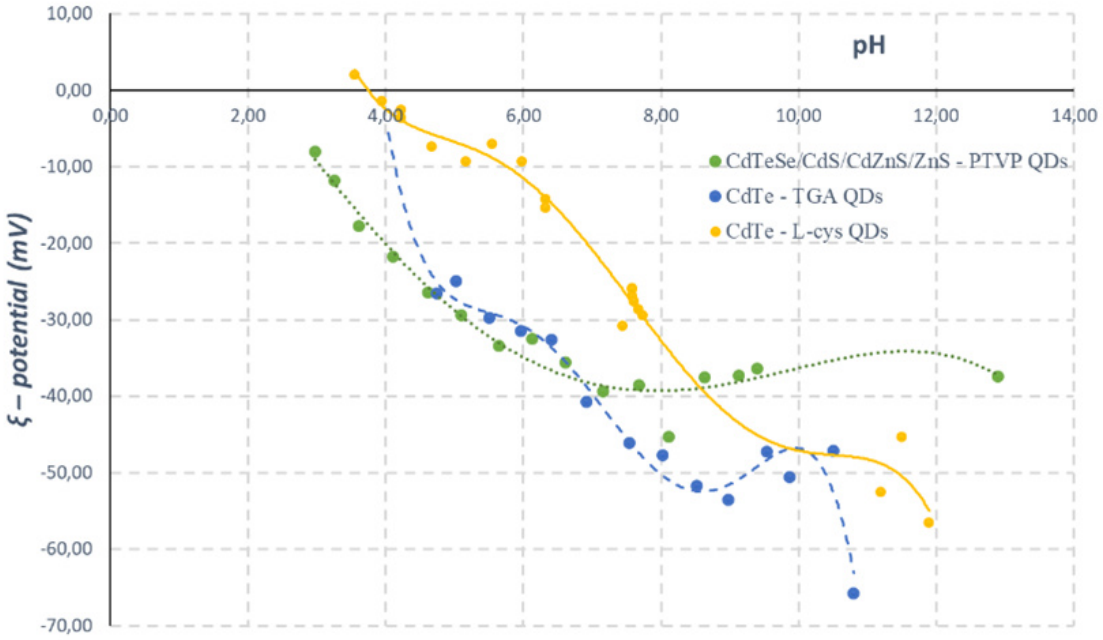

FIG. 2. Dependence of the ζ-potential on pH for CdTe-TGA QD, CdTe-L-cis QD and CdTeSe/CdS/CdZnS/ZnS-PTVP QD

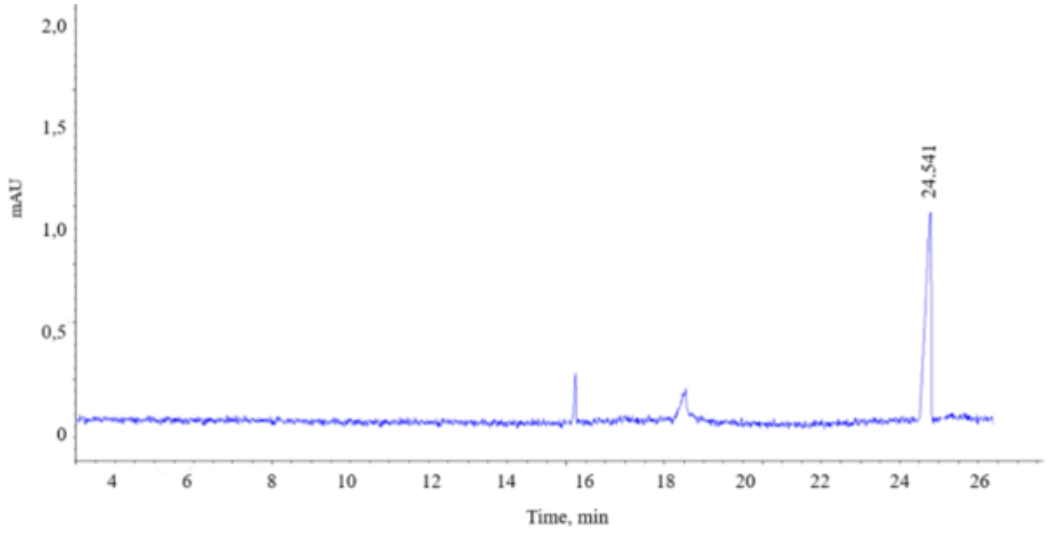

FIG. 3. Electropherograms of hydrophilic CdTe-TGA QD. CZE analysis conditions: BGE 25 mmol/L sodium tetraborate, pH = 9.2; −20 kV; detection at 220 nm

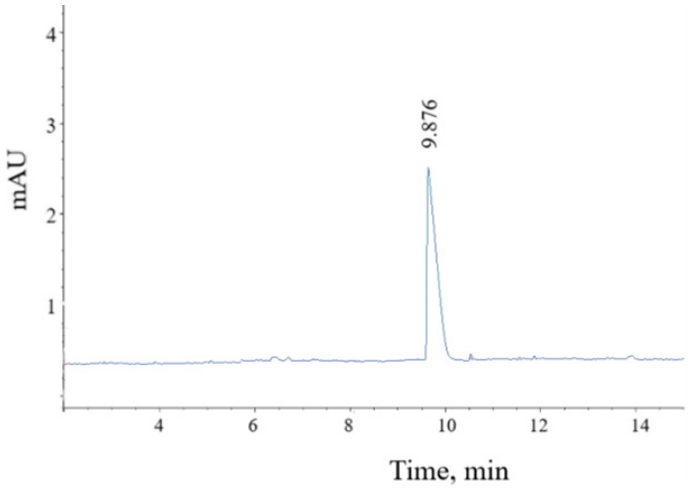

FIG. 4. Electropherogram of hydrophilic CdTe-TGA QD. CZE analysis conditions: BGE 25 mM sodium tetraborate, 0.1 mM CTAB, pH = 9.2; voltage −20 kV

It can be seen that under the conditions shown in Fig. 4, QDs migration time significantly decreased. However, QDs peak has expanded, which indicates that the electrophoretic system used is not sufficiently efficient. To increase efficiency, influence of various process parameters (concentration of the leading electrolyte, volume fraction of surfactants, applied voltage) on the electrophoretic analytes mobility and analysis efficiency was studied.

The dependence of QDs migration time on the amount of surfactants in lead electrolyte was studied for CdTe-TGA QDs. 25 mM solutions of borate buffer containing CTAB in the amount of 0.1 and 2 mM were used as background electrolytes. The resulting electrophoregrams are hown in Fig. 5.

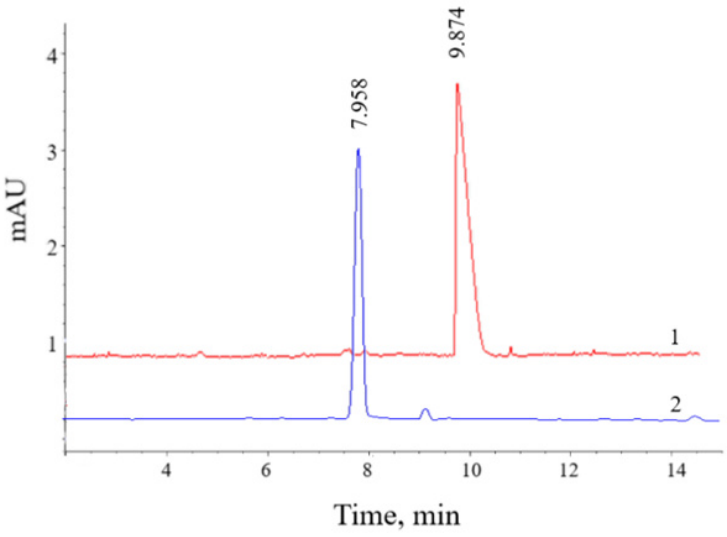

FIG. 5. Electropherograms of hydrophilic CdTe-TGA QDs with different volume fractions of CTAB. CZE analysis conditions: voltage −20 kV, detection at 220 nm; BGE No. 1: 25 mM sodium tetraborate, 0.1 mM CTAB, 5 % vol. isopropyl alcohol; BGE No. 2: 25 mM sodium tetraborate, 2 mM CTAB, 5 % vol. isopropyl alcohol

With an increase in CTAB volume fraction in borate buffer solution, an increase in the total electrophoretic mobility of QDs is observed. Insufficient efficiency may be due to too high concentration of the lead electrolyte for this system. Variation of ionic strength of the lead electrolyte in CZE is simplest and most effective way to influence characteristics of the electrophoretic process (Fig. 6).

Electrophoregrams for CdTe-TGA QDs (Fig. 6) obtained at concentrations of borate buffer 25 and 30 mM show a decrease in the efficiency of electrophoretic system and a decrease electroosmotic flow's speed with an increase in the electrolyte concentration. The broadening of the QDs migration zone occurs due to longitudinal diffusion and temperature effects typical for systems with relatively high ionic strength of solutions. At concentration of sodium tetraborate 10 mM,

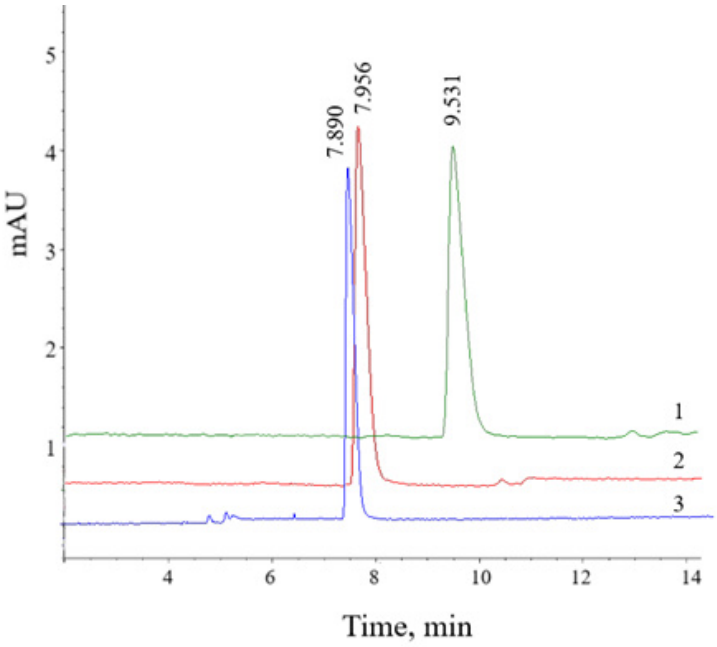

FIG. 6. Electropherograms of hydrophilic CdTe-TGA QD. CZE analysis conditions: voltage −20 kV, detection at 220 nm, PV: BGE No. 1: 30 mM sodium tetraborate, 2 mM CTAB, 5 % vol. isopropyl alcohol; BGE No. 2: 25 mM sodium tetraborate, 2 mM CTAB, 5 % vol. isopropyl alcohol; BGE No. 3: 10 mM sodium tetraborate, 2 mM CTAB, 5 % vol. isopropyl alcohol

this dependence is not observed, which may be due to an increase in the value of the critical concentration of micelle formation for solutions with low ionic strength and a change in the mechanism of the electrophoretic separation process.

At a sodium tetraborate content of 10 mM, no such dependence is observed, which may be due to an increase in the critical micellar structure for solutions with low ionic strength and a change in the mechanism of the electrophoretic separation process.

The effect of voltage on the rate of QDs migration in the CZE conditions was studied for CdTe-L-cys QDs with the composition of background electrolyte, for which the highest efficiency value was observed in CdTe-TGA QDs analysis (Fig. 7).

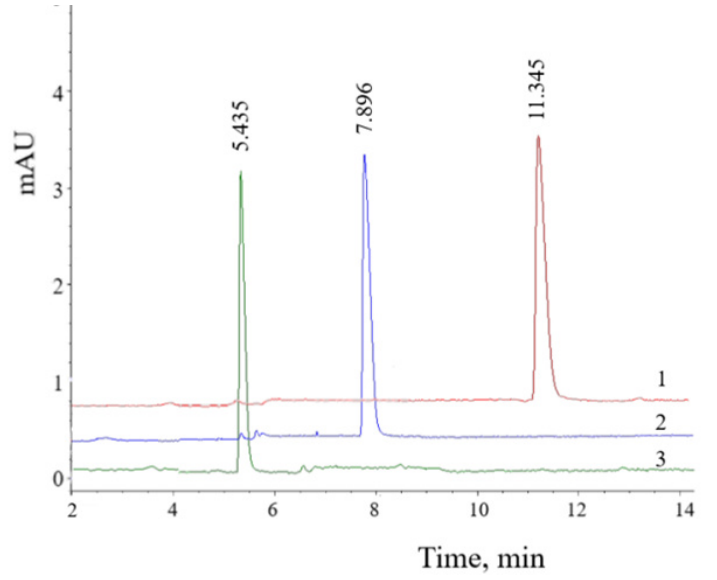

FIG. 7. Electropherograms of hydrophilic CdTe-TGA QD at voltage. CZE analysis conditions: detection at 220 nm, BGE: 10 mM sodium tetraborate, 2 mM CTAB, 5 % vol. isopropyl alcohol, No. 1 – voltage −15 kV, No. 2 – voltage −20 kV, No. 3 – voltage −25 kV

As can be seen from Fig. 7, the electrophoretic mobility of CdTe/ZnS-TGA QDs increases with increasing voltage, which leads to a reduction in duration of analysis and an increase in efficiency of the process.

Thus, based on the established regularities of the electrophoretic behavior of studied QDs, the conditions for their determination with high efficiency and short duration of analysis in the medium of a borate buffer solution with addition of cationic surfactant were selected. Table 2 shows the optimal conditions for separation of hydrophilic QDs by CE method.

Hydrophilic QDs with different stabilizing coatings were analyzed under the selected conditions (Fig. 8).

TABLE 2. Recommended QDs analysis conditions for "Kapel-105 CE system"

Parameter	Value
Capillary	$L_{\text{eff}}/L_{\text{tot}} = 50/60$ cm, ID $= 75$ mkm
Temperature	20 °C
Wavelength	220 nm
Entering a sample	50 mbar, 2 sec
Voltage	-25 kV
Analysis time	10 – 20 min
Composition of background electrolyte	10 mM of sodium tetraborate, 2.0 mM CTAB, 5 vol %. isopropyl alcohol

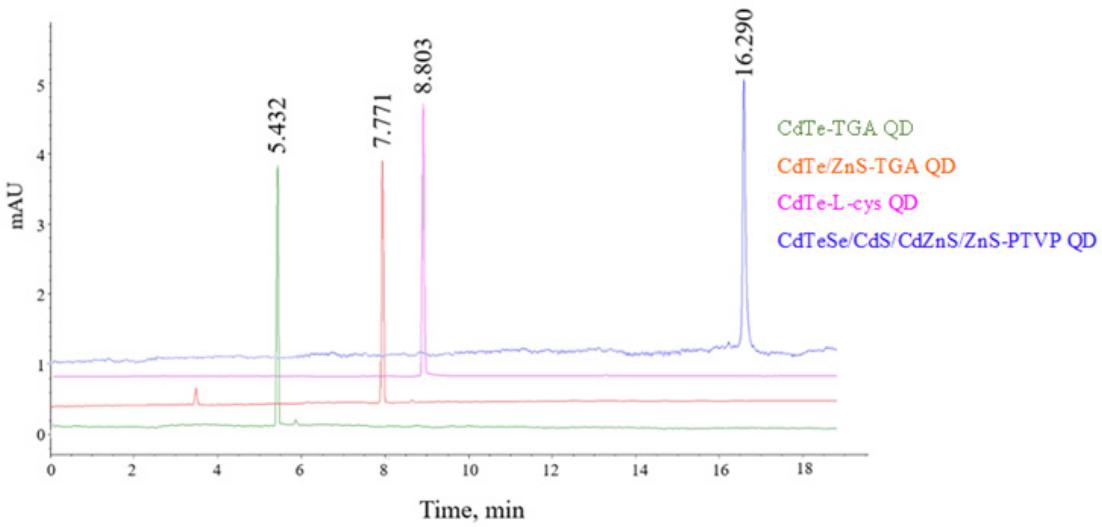

FIG. 8. Electropherograms of hydrophilic QD. CZE analysis conditions: voltage -25 kV, detection at 220 nm, BGE: 10 mM sodium tetraborate, 2 mM CTAB, 5 % vol. isopropyl alcohol

The sequence of migration of the studied QDs (Fig. 8) is consistent with data of DLS and ζ-potential measurement. Thus, among CdTe-TGA QD and CdTe-L-cys QD that are close in size, CdTe-TGA QD are the first to migrate, which have a large negative charge. Larger CdTe/ZnS-TGA QD have lower electrophoretic mobility. The last to come out are multi-layer CdTeSe/CdS/CdZnS/ZnS-PTVP QD. Positively charged CdTe-MEA QD were not determined under the conditions of this electrophoretic system.

Values of the parameters of various QDs electrophoretic determination are shown in Table 3.

4. Conclusions

A method for analyzing and characterizing colloidal QDs by CZE using a borate buffer containing surfactants as a background electrolyte has been developed. During the study of influence of various factors of electrophoretic process on QDs migration conditions were selected for effective electrophoretic separation and analysis of hydrophilic CdTe QDs, CdTe/ZnS QDs, CdTe-L-cys, and multilayer CdTeSe/CdS/CdZnS/ZnS QDs. It is shown that the CZE method is a relevant alternative to existing methods of QDs characterization and analysis, where separation occurs due to differences in the

TABLE 3. Parameters of QDs electrophoretic determination

QDs	t_M, min	μ, $\mathrm{cm^2V^{-1}c^{-1}}$	N, pcs.	H, mm	ζ-potential, mV
CdTe-TGA	5.43	$3.686 \cdot 10^{-4}$	10151	2.46	-49.55
CdTe/ZnS-TGA	7.77	$2.576 \cdot 10^{-4}$	23235	1.08	
CdTe-L-cys	8.80	$2.227 \cdot 10^{-4}$	22903	1.09	-41.52
CdTeSe/CdS/CdZnS/ZnS-PTVP	16.29	$1.227 \cdot 10^{-4}$	26461	0.94	-47.7

electrophoretic mobility of the components of the analyzed mixture. The synthesis of multilayer QDs is generally multi-stage and is carried out in several steps. The developed method can be used for rapid analysis and characterization of hydrophilic QDs obtained at each stage of synthesis. It was also shown that the type of electrophoregrams can be used to conclude sample size heterogeneity, purification efficiency of QDs, and processes of their degradation in various aqueous solutions.

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