Lithuanian Journal of Physics, Vol. **51**, No. 2, pp. 163–171 (2011)

SPECTROSCOPIC INVESTIGATIONS OF CdTe QUANTUM DOT STABILITY IN DIFFERENT AQUEOUS MEDIA

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Received 5 May 2011; revised 7 June 2011; accepted 21 June 2011

For the successful use of quantum dots (QDs) in biomedicine their chemical and optical stability is of great importance. In this study the changes of photoluminescence parameters of CdTe QDs coated with mercaptopropionic acid (MPA) dependently on time and environment are presented. The presence of salt ions in the QD water solution decreases photoluminescence band intensity and induces red shift. The pH value of the solution also influences spectroscopic properties of QDs. In the pH range from 2.5 to 9 a decrease of photoluminescence intensity is observed. The fastest one, leading to the complete luminescence bleaching, occurs in the most acidic medium. Changes of QD spectral properties in cell growth media were studied as well. The results imply that spectroscopic changes of CdTe–MPA QDs are caused by the interactions between the ions present in the solution and ligand coating of QDs. The model of possible processes is proposed.

Keywords: quantum dots, photoluminescence, ions, pH

PACS: 87.64.kv, 87.85.Rs

1. Introduction

Due to quantum confinement effects quantum dots (QDs) exhibit unique physicochemical properties distinctly different from their corresponding individual molecules or bulk materials. QDs are exclusive fluorophores because of their broad excitation and narrow emission spectra, high photostability, and high quantum yield of luminescence. The extraordinary feature of QDs is that the particle size determines their photoluminescence (PL) properties, most importantly the wavelength of fluorescence. Due to the unique PL properties QDs are becoming widely used in many different applications. One of the most promising areas is biomedical labelling for *in vivo* imaging. However, the application of nanoparticles in this area is highly limited by their stability.

Usually QDs refer to the nanocrystal–ligand complex comprising inorganic core and organic ligand coating. The stability of the complex and consequently the constancy of QD spectral properties are determined by the strength of the bond between the ligand molecules and core surface atoms. Since the water soluble thiols contribute greatly to the stability of the nanoparticles, QDs used in the biomedical-related studies are often coated with covalently bound thiols. It was proven that the thiol-end of mercaptoacetic acid is the anchor of the nanocrystal, while the carbonic acid acts as a secondary coordinator [1]. Nevertheless, still there is a lack of comprehensive knowledge about the nature and properties of the binding between the core of QDs and the ligands.

For QDs covered with a capping layer of ligands it is often assumed that the core is protected from the action of outside environment. This assumption leads to the expectation that optical properties of QDs should not be affected by external factors. However, thiol-capped QDs are highly sensitive to pH, concentration of ions, and other parameters of living systems. Changes in physicochemical properties following a decrease in size of QDs could be responsible for a number of interactions leading to toxicological effects [2], therefore QDs are possible toxicants to living organisms. A slow release of toxic Cd^{2+} and Te^{2-} ions into the solution was observed [3–5]. Fitzpatrick et al. [6] reported the detection of the traces of QDs in mice tissue up to 2 years after the injection. Blue shift of QD spectral band indicated chemical instability of nanoparticles and possible release of heavy metal ions into organism. Therefore more information is still required for better understanding of the processes of QD instability and degradation that could cause deleterious effects on living systems.

 ISSN 1648-8504

It has been noticed that photophysical properties of QDs depend on temperature, pH value, presence and concentration of salts [1, 3, 4, 7]. The decrease of PL intensity, red or blue shifts of PL bands indicating aggregation and degradation of QDs were reported [3, 8, 9]. The decrease of PL intensity with time is thought to be related to the oxidation and surface ligand desorption [8, 10].

PL properties of QDs depend on the solution pH: in acidic solutions emission intensity decreases much faster than in alkali solutions. In highly acidic solutions QDs lose about 80% of their luminescence [11]. Boldt et al. [8] examined the stability of CdTe nanocrystals in various biochemical buffers and observed profound red and blue shifts of the emission maximum. Blue shift is thought to be the result of a partial destruction of QDs when ions are removed from the nanocrystal core. Red shift, which is usually observed in acidic solutions [1], might occur because of the aggregation – a process when QDs lose their stabilizing ligands and bind each other [8]. On the basis of spectroscopic studies it was stated that the pH-dependent changes of PL of the CdTe QDs are caused by structural changes on the surface rather than the size of the nanocrystals [12].

Recently a few reports that suggest exploring the unique surface-sensitive properties of QDs in the development of sensors for various analytes, especially in application as pH probes, appeared [13–15]. They state that the surfaces of nanoparticles are very important, as the optical properties of QDs are very sensitive to the interactions between the surrounding medium and QDs surfaces.

In this study we have investigated the changes of spectroscopic properties of CdTe–MPA QDs in saline of different pH and in the cell growth media and made an attempt to explain reasons and to provide the model of processes observed.

2. Materials and methods

2.1. Reagents

Cadmium telluride QDs stabilized with mercaptopropionic acid (MPA) emitting at 705±5 nm were purchased from PlasmaChem GmbH. Sodium chloride (purity >99.5%) was from Fluka. The Dulbecco's modified Eagle's medium (DMEM) and Roswell Park Memorial Institute (RPMI) medium were purchased from Sigma–Aldrich.

2.2. Preparation of CdTe solutions

The stock solution of QDs ($c = 10^{-8}$ M) was prepared by solving crude powder in distilled water. For further dilution up to 10^{-9} M distilled water, saline, or cell growth media was used. The pH of saline solutions was adjusted to the value of interest by the addition of HCl or KOH. The specimens were prepared at various pH values ranging from 2.5 to 12.

2.3. Spectroscopic measurements

The PL spectra of QD solutions were measured at different time intervals starting from the preparation up to few days. PL spectra were recorded on a *Cary Eclipse* spectrometer (Varian, USA). Excitation wavelength was 400 nm. All optical measurements were performed at room temperature under ambient conditions and in the dark.

To evaluate the spectral changes of CdTe–MPA QDs in aqueous solutions and in cell growth media induced by time and environmental parameters, the intensity of PL at the wavelength of the emission maximum, and shifts of the PL band maximum position were observed. The parameters of PL were measured immediately after the solution preparation, then followed every few minutes up to 1 h and later were controlled over a time span of few days until the intensity of PL of QDs solutions became less than 10% of the initial level.

3. Results

3.1. Spectral changes of QDs in distilled water and in saline

Figure 1 presents temporal changes of the emission intensity of QDs dissolved in (a) distilled water and (b) in saline. The PL intensity of QDs in pure water decreased slightly during all the period of measurements and lost about 7% of the initial level during the first 2 h and about 12% during the following 7 days. A negligible red shift of maximum position from 721 to 726 nm was detected during the whole period of observation (Fig. 1(a)). It seems that QDs in pure water are rather stable.

Temporal changes of the PL intensity of QDs dissolved in saline are presented in Fig. 1(b). In the presence of ionic species (Na^+ and Cl^-) a drop down of emission intensity was faster: about 45% of the initial intensity was lost during 2 h. PL intensity decrease was followed by a notable red shift of the band maximum

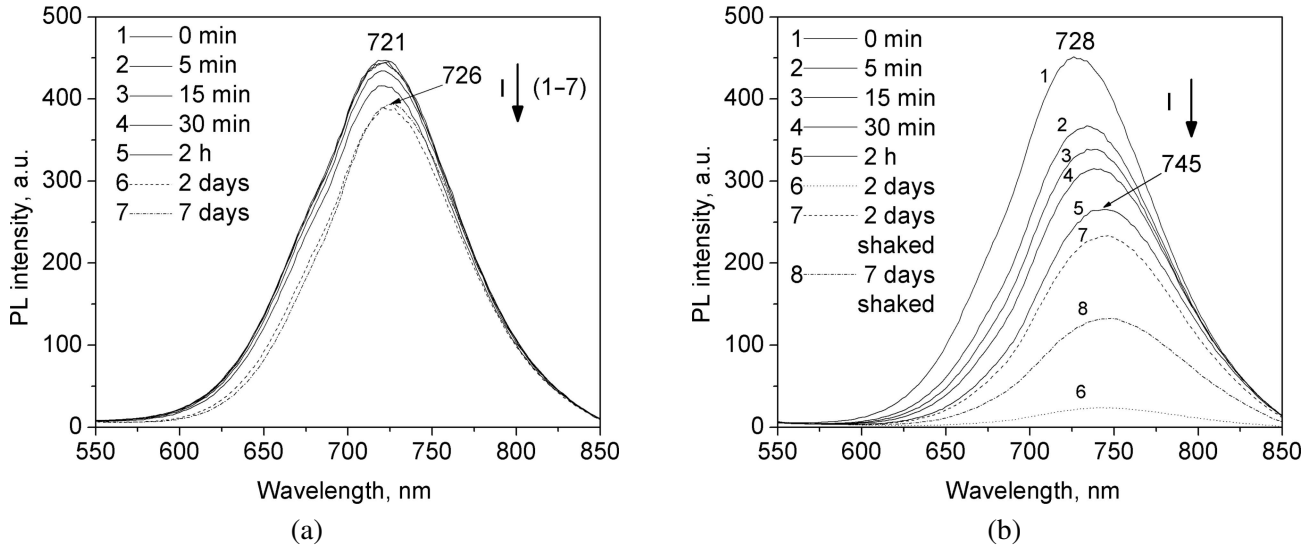

Fig. 1. Changes of PL spectra of CdTe–MPA QDs dissolved in (a) distilled water (pH 6.3) and (b) saline (pH 6.0). Concentration of QDs $\approx 5 \cdot 10^{-9}$ M, $\lambda_{ex} = 450$ nm.

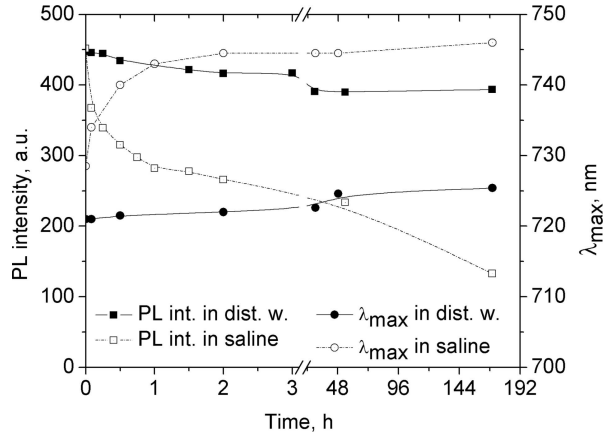

Fig. 2. Temporal changes of the PL intensity and maximum position of QDs in pure water and in saline.

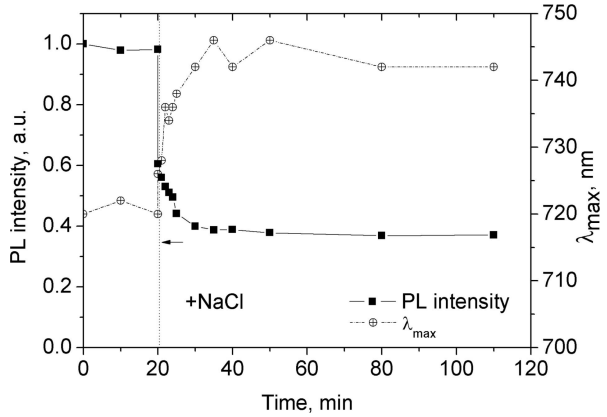

Fig. 3. Effect of the salt ions on the PL properties of QDs. PL intensity is presented as ratio I/I_0, where I_0 is the intensity at $t = 0$, and normalized to 1.

(from 728 nm in freshly prepared solution to 745 nm after 2 h). So the presence of salt ions in the solution induced faster and more significant spectral changes of QDs in comparison with the changes in pure water (Fig. 2), namely, a notable decrease of PL intensity and band red shift for about 20 nm. Influence of ionic species on PL intensity of QDs was known earlier [14]. It was noticed that some of cations quench PL of QDs significantly [1]. Our results show that the presence of cations and/or anions in the solution effects the stability of PL properties of QDs.

The addition of solid NaCl (to reach the concentration of saline) to the pure water solution of QDs 20 min after the solution preparation induced spectral changes similar to those obtained immediately after the dissolution in saline (Fig. 2). The loss of PL intensity for about 60% of the initial value and a shift of the band maximum position for about 10 nm was observed immediately after the addition of the salt (Fig. 3). During the following 10 min PL intensity dropped down to about 40% of the initial value and then became stable. Simultaneously the band maximum position was shifted from 721 to about 745 nm. These data confirm the presumption that salt ions induce some processes in the QD water solution that result in the changes of spectroscopic characteristics.

It should be noted that the precipitation of nanocrystals in saline was observed on the second day of the solution preparation. If the solution was not shaken, almost no PL was registered after two days. Slight restoration of PL was detected after the shake of solution (Fig. 1(b) curve *7*). In contrast, no precipitation of QDs was observed in the pure water.

The concentration of QDs in solutions also influences temporal changes of PL intensity and band

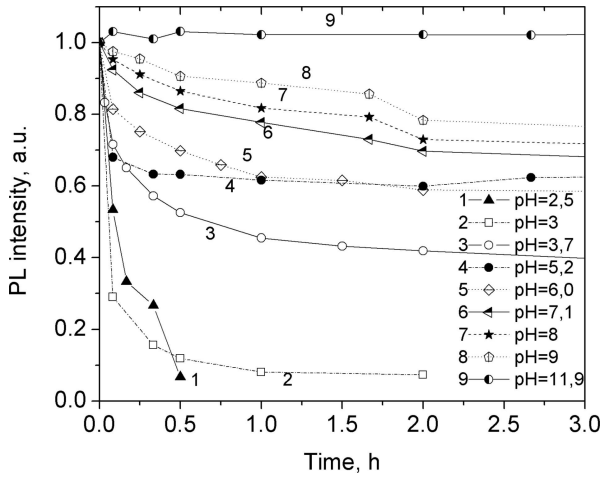

Fig. 4. Temporal changes of QD PL intensity in the saline of different pH. PL intensity is presented as ratio I/I_0, where I_0 is the intensity at $t = 0$, and normalized to 1.

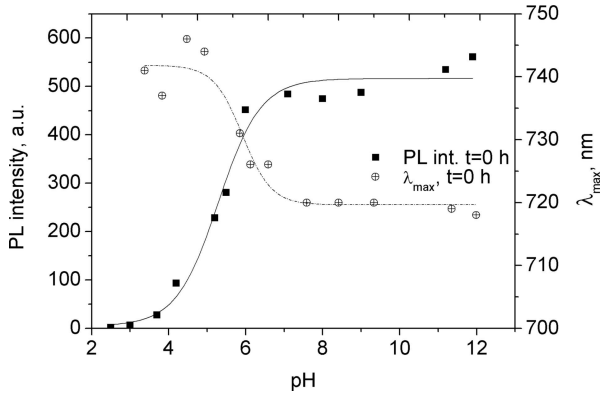

Fig. 5. Dependence of the initial PL intensity and $\lambda_{\max}$ of QDs on pH .

maximum position shifts. In the distilled water solutions as well as in saline of higher concentrations (10^{-7} M) of QDs the intensity of PL is stable during the observation time of 6 days (data not presented).

3.2. Influence of pH on the spectral characteristics of QDs

Investigations of the spectral changes of CdTe–MPA QDs were performed in saline of different pH: from acidic to strongly alkaline. Figure 4 shows temporal changes of QD PL intensity dependently on pH. As is seen, the considerable intensity changes occur during the first thirty minutes. In the pH range from 2.5 to 9 PL decay was observed, the fastest, which led to the complete PL bleaching, being in the most acidic medium (at pH 2.5 and 3). In strongly alkaline solution (pH $>$ 11) PL intensity of QDs remained almost stable.

The value of the initial PL intensity was consistent with the solution pH as well (Fig. 5). In acidic solutions (pH $<$ 4) the emission of QDs was very low. At a higher pH the initial emission of QDs grew up and, when pH was $>$6, reached the highest value and became almost stable.

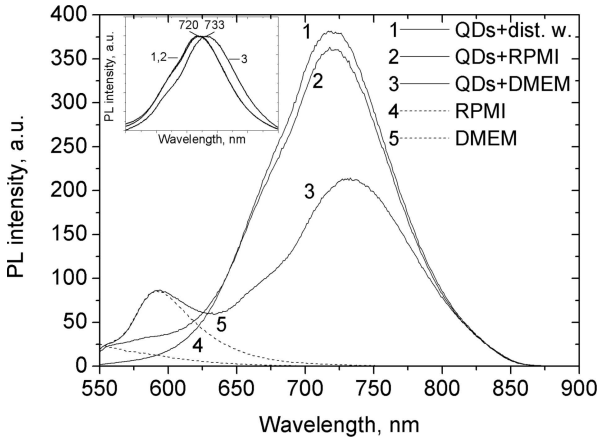

Fig. 6. PL spectra of freshly prepared CdTe–MPA solutions in distilled water, RPMI medium, and DMEM ($t = 0$). The inset shows normalized spectra after subtracting the autofluorescence of the media.

Together with the PL variations, pH changes were followed by the shifts of the emission bands indicating the interplay of complicated processes occurring on the surface of nanoparticles. Red shift is characteristic of QD solutions of almost all pH values $<$7. If one compares the position of PL band maximum in pure saline (pH 6) and in saline with added HCl (pH between 4 and 6), a red shift for about 20 nm is observed immediately after the solution preparation ($t = 0$), indicating that this shift is caused by acid. In alkaline solutions immediately after the preparation PL band maximum is located at about 720 nm (Fig. 5) and no shifts are observed with time.

3.3. Spectral changes of QDs in cell growth media

The *in vitro* experiments with cell colonies are mandatory for the most of QD biomedical applications. Therefore comprehensive knowledge about the spectroscopic properties and stability of QDs in the cell culture environment is required. The DMEM and RPMI are the most commonly used media for cell cultivation. Therefore the spectroscopic properties of CdTe–MPA QDs solved in DMEM and RPMI were investigated and compared with those obtained in distilled water. The differences of QD spectroscopic characteristics in two different cell growth media can be observed immediately after the solution preparation (Fig. 6). The PL intensity of QDs in RPMI medium is lower by about 5% as compared with that in distilled water. The position of spectral band peak is stable. The initial PL intensity

of QDs in DMEM constitutes only ∼55% of PL intensity in distilled water. Moreover, a significant (about 13 nm) red shift of the band up to 733 nm is observed.

The subsequent temporal changes of QD PL properties in RPMI and DMEM are different as well. PL intensity in RPMI is continuously decreasing with time and after 2 h corresponds to ∼70% of the initial intensity of PL (Fig. 7). A slow shift of the band peak position from 720 to 729 nm also occurs during 24 h.

More significant changes of the spectral properties of QDs take place in DMEM. The intensity of PL is decreasing faster and after 24 h PL cannot be distinguished from the medium autofluorescence background (Fig. 7(b)). PL decay is followed by the red shift of band peak maximum up to 745 nm. It is important to note that the precipitate was formed in DMEM, while it was not observed in other samples. After shaking and resuspending the solution, intensity of PL can be temporarily restored (Fig. 7(b)), but precipitate forms back over the course of minutes. Meanwhile, the stirring of solution makes no effect in RPMI medium implying no precipitate formation. Conversely, the aggregates of QDs in DMEM are observed under microscope already in the freshly prepared solution (Fig. 7(b), inset). Such aggregates were not observed in other investigated solutions.

4. Discussion

QDs used in this study of spectral characteristics dependently on environmental properties, represent a kind of core-coating system with a sulphur-capped surface created by mercapto-group covalently attached to the surface cadmium atoms. The results of spectroscopic measurements imply that pH, salt ion, and cell growth media ingredient dependent properties of CdTe–MPA nanocrystal PL are caused by the structural changes on the surface of QDs. The spectral characteristics of QDs dissolved in pure water were rather constant during a longer time of observation: intensity of PL decreased insignificantly and the red shift of the band was negligible (Figs. 1(a) and 2). However, when other ionic species (namely, ions of salt) were present in the solution of QDs, the following spectral changes were observed: a rather fast PL intensity drop down, red shift of the band maximum (Fig. 1(b)), and finally the appearance of precipitation. Yu et al. [14] and Boldt et al. [8] also reported a significant decrease of the intensity of PL in the presence of buffers compared with the intensity in pure water. They also noted a significant red shift of QD fluorescence band in DMEM, which is a mixture of so many substances that the reason for the instability of the particles cannot be easily determined [8].

On the basis of our spectroscopic measurements and taking into account the results of relevant reports, we suggest the following model (Fig. 8), which summarizes the possible processes of the degradation of QD coating and formation of precipitate. We suppose that these processes induce changes in the spectral characteristics of QDs, such as decrease of the intensity of PL and shifts of band maximum position.

In the pure water solution the core of QDs is surrounded by a rather stable coat of MPA molecules with terminal negative carboxyl groups exposed to the surrounding environment (Fig. 8(a)). Since the $\mathrm{pK_{COOH}}$ for MPA is 4.32 [1], it is reasonable to presume that in the solution at pH 6.3 more than half of carboxylic acid molecules are converted into carboxylates. Nevertheless it seems that at such conditions (no salt ions in the environment) the core–ligand bonds are stable enough and no processes influencing spectral properties of QDs take place. Coulomb repulsion between QDs with surface charge of the same polarity prevents aggregation and no significant PL decay and/or shift of PL band is observed (Fig. 1(a)).

The sensitivity of QD spectral properties to pH most probably is a function of surface modifications occurring with the changes of $\mathrm{H^+}$ concentration. As shown in our experiments (Fig. 5) and also stated by others [8, 14], the most significant decrease of QD PL intensity takes place in the acidic environment. The fundamental mechanisms of these changes are still unknown, but it is clear that PL properties of QDs conjugated with polar molecules containing thiol groups are sensitive to $\mathrm{H^+}$ concentration. The process might be described by the equilibrium proposed by Aldana et al. [16]:

$$(\mathrm{CdTe})_n\text{–}\mathrm{L}_m + m\mathrm{H}^+ \Leftrightarrow [(\mathrm{CdTe})_n]^{m+} + m\mathrm{HL}\,, \quad (1)$$

where $(\mathrm{CdTe})_n\text{–}\mathrm{L}_m$ correspond to QDs coated with ligands (L) and $[(\mathrm{CdTe})_n]^{m+}$ to bare nanocrystals as illustrated by the model presented in Fig. 8(b–e). High concentration of $\mathrm{H^+}$ in acidic solution neutralizes negative carboxyl groups of MPA (Fig. 8(b)) and due to this small protons can freely penetrate through the ligand coating (Fig. 8(c)) and induce MPA reduction according to the above equation. Therefore the reason of the detachment of the ligands is protonation of the thiolate with hydrogen ions in the acidic solution (Fig. 8(d)). Free molecules of MPA dissociate from the surface of QDs and ions may induce disturbances on the vulnerable unstabilized nanoparticle surface (Fig. 8(e)). The

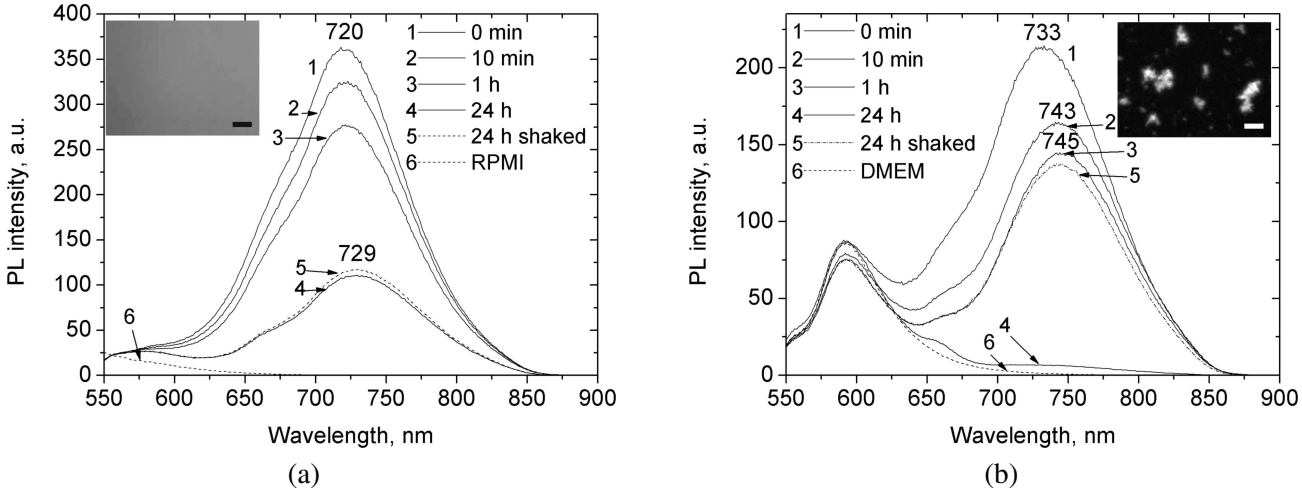

Fig. 7. Temporal changes of QD spectral properties in (a) RPMI medium and (b) DMEM. Shaking after 24 h made no effect on RPMI medium solution ((a) curve *5*), but restored PL intensity in DMEM ((b) curve *5*). Insets: fluorescence microscopy images of freshly prepared solutions showing (a) monodispersed QDs in RPMI medium and (b) formation of aggregates in DMEM. Inset scale bar is 50 μm.

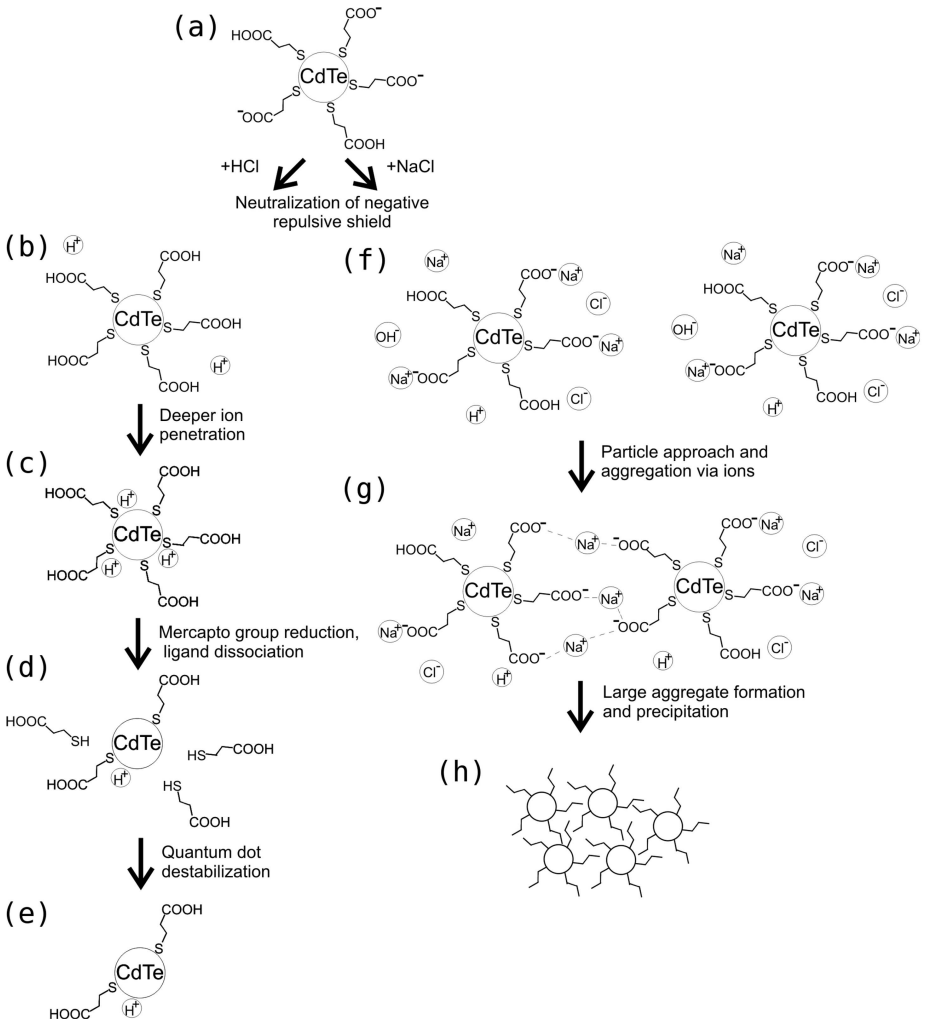

Fig. 8. Proposed model of processes occurring in QD solutions in the presence of protons and salt ions.

result of this process is loss of PL intensity in the acidic solution. However, no blue shift of PL band was observed in these conditions which means that no destruction leading to the reduction of QD core size took place.

Similarly, in the saline solutions of QDs at neutral pH values positive ions of sodium are attracted by MPA terminal $-COO^-$ groups and negative shields of MPA are neutralized (Fig. 8(f)). Due to this process, analogously as in the acidic solution, the way is open for deeper penetration of positive salt ions through the ligand layer which leads to ligand–ion interactions and as a result to the formation of MPA molecules and their detachment from the QD core surface. As more free MPA molecules are released into the solution, the surface coverage of QDs deteriorates. These environmental changes most probably could be one of the reasons of PL intensity decrease. Since together with the PL decay a red shift of emission band peak and appearance of precipitation was observed after the addition of NaCl, the following explanation of salt action might be proposed. In the presence of salt the negative surface charges of QDs are partly shielded by the Na^+ and this allows QDs to approach each other closely enough to form aggregates. It seems that after some time the electrostatic repulsion of surface charges is no longer sufficient to keep the nanoparticles apart and they begin to aggregate most probably via the positive ions of sodium (Fig. 8(g)). The first signs of this process were manifested few hours after the solution preparation as a red shift of the PL band (Fig. 1(b)). Later (1–2 days after) the appearance of precipitation was observed which meant that the strength of electrostatic repulsion decreased and due to the attraction via Na^+ QDs stuck together and settled out of the solution (Fig. 8(h)). Moreover, if the solution was shaken, a restoration of PL was detected (Fig. 1(b) curve *7*). Since the position of the PL band maximum after shaking did not change, the presumption could be made that aggregates were not disrupted by mechanical action.

As it was stated, the almost unchanged spectroscopic properties of QDs in distilled water indicate that CdTe–MPA nanoparticles remain chemically stable in the absence of ions. To use QDs for labelling in a living organism, the knowledge of nanoparticle PL dependence on the interaction with biological environment is essential. The investigation of spectral properties of QDs in a cell growth media, used as a simplified model of biological environment, has indicated that nanoparticles behave differently in the media of different composition.

The decrease of the intensity of PL of QDs in RPMI medium (Fig. 7(a)) might be caused by several processes occurring on the surface of particles: the reorganization and structural changes of surface ligands, quenching of PL by ions and organic molecules present in the medium, desorption of ligand molecules, or others. It is difficult to determine which of the medium components play the major role because of the complicated chemical composition of RPMI. However, QDs dissolved in DMEM undergo different and more significant changes than those in RPMI medium (Fig. 7(b)). Microscopic observations and registered spectral changes, particularly the decrease of PL intensity and red shift of the band, indicate that QDs in DMEM aggregate immediately after dissolution.

The processes observed in both investigated media might be determined by their different chemical composition. According to manufacturer's specifications, media contain different concentrations of salts, amino acids, glutathione, pyruvic acid, phenol red, and other components. Therefore it is difficult to determine which compound or its concentration predestines the stability of QDs. Besides, since QDs are not stable in saline (0.9% NaCl), and cell growth media contain similar or higher amounts of salt ions (0.6% NaCl, 0.4% $NaHCO_3$, and others), it means that aggregation of QDs in DMEM might be supported by the salt ions. However, the aggregation is not observed in RPMI medium (Fig. 7(a), inset), suggesting that QDs are stabilized and protected from ligand desorption and further clotting. DMEM lacks some amino acids which are more abundant in RPMI. It is possible that the interaction of CdTe QDs with protein components significantly enhances their stability and prevents them from aggregation as shown by Poderys et al. [17].

Finally, it should be noted that the results discussed above were obtained in the dark. It has been noticed that light induces additional changes in the spectral properties of QDs which will be a subject of our further experiments.

5. Conclusions

Our results demonstrate that water-soluble CdTe–MPA QDs are stable only in the distilled water solution. The presence of salt ions, components of cell growth media, and pH value variation induce changes in the spectroscopic properties of QDs. The most significant changes are decrease of the intensity of PL and the red shift of band maximum position. Careful analysis of spectroscopic data imply that changes of the spectral

characteristics of CdTe–MPA QDs in different media are caused by the interactions between the components of environment and ligand coating of QDs. The model of possible processes is proposed.

It is reasonable to stress that before using QDs for biolabelling, it is essential to have knowledge on how the photoluminescence properties of nanoparticles depend on environmental conditions.

Acknowledgement

This work was supported by the project "Multifunctional nanoparticles for specific noninvasive early diagnostics and treatment of cancer" (No. 2004-LT0036-IP-1NOR).

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CdTe KVANTINIŲ TAŠKŲ STABILUMO VANDENINĖSE TERPĖSE SPEKTROSKOPINIAI TYRIMAI

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Santrauka

Kvantiniai taškai (KT) yra puslaidininkinės nanodalelės, pasižyminčios išskirtinėmis optinėmis savybėmis. Pastaraisiais metais sparčiai plečiasi jų taikymas biologijoje ir medicinoje. Siekiant sėkmingai taikyti KT įvairiose biomedicinos srityse, būtina nuodugniai ištirti jų cheminių ir spektrinių savybių stabilumą įvairiose vandeninėse terpėse. Šiame darbe spektroskopiniais metodais buvo tirti merkaptopropiono rūgštimi dengtų CdTe KT fotoliuminescencijos savybių pokyčiai einant laikui ir keičiantis terpės sudėčiai. Nustatyta, kad tirpale esantys druskų jonai mažina KT fotoliuminescencijos intensyvumą ir sukelia ilgabangį emisijos juostos postūmį. Terpės rūgštingumas taip pat veikia KT spektrines savybes. Kai tirpalo pH < 9, KT fotoliuminescencijos intensyvumas mažėja. Šis kitimas ypač išryškėja didėjant vandenilio jonų koncentracijai tirpale. Taip pat buvo tirti KT spektrinių savybių pokyčiai ląstelių auginimo terpėse. Gauti rezultatai rodo, kad KT spektrinių savybių pokyčius lemia tirpaluose esančių jonų sąveika su KT paviršių dengiančiais ligandais. Pateikiamas galimas šių procesų modelis.