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Delivery and reveal of localization of upconversion luminescent microparticles and quantum dots in the skin in vivo by fractional laser microablation, multimodal imaging, and optical clearing

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Abstract. Delivery and spatial localization of upconversion luminescent microparticles [Y2O3:Yb, Er] (mean size \( \sim 1.6 \) \( \mu \)m) and quantum dots (QDs) (CuInS2/ZnS nanoparticles coated with polyethylene glycol-based amphiphilic polymer, mean size \( \sim 20 \) nm) inside rat skin was studied in vivo using a multimodal optical imaging approach. The particles were embedded into the skin dermis to the depth from 300 to 500 \( \mu \)m through micro-channels performed by fractional laser microablation. Low-frequency ultrasound was applied to enhance penetration of the particles into the skin. Visualization of the particles was revealed using a combination of luminescent spectroscopy, optical coherence tomography, confocal microscopy, and histochemical analysis. Optical clearing was used to enhance the image contrast of the luminescent signal from the particles. It was demonstrated that the penetration depth of particles depends on their size, resulting in a different detection time interval (days) of the luminescent signal from microparticles and QDs inside the rat skin in vivo. We show that luminescent signal from the upconversion microparticles and QDs was detected after the particle delivery into the rat skin in vivo during eighth and fourth days, respectively. We hypothesize that the upconversion microparticles have created a long-time depot localized in the laser-created channels, as the QDs spread over the surrounding tissues. © 2018 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.23.2.026001]

Keywords: upconversion microparticles; quantum dots; skin; fractional laser microablation; optical clearing; luminescence spectroscopy; optical coherence tomography; confocal microscopy; histochemical analysis.

1 Introduction

Transcutaneous delivery of micro- and nanocarriers directly to the targeted sites inside skin allows for excluding the interaction of drugs with the surrounding tissue during the diffusion and creating a long-time drug depot inside the skin. Passive diffusion of the micro- and nanoparticles into deeper layers of skin is extremely hampered due to protective properties of the skin barrier.1 Both size and formulation of the particles govern their penetration into skin.2 For example, less than 0.03% of the applied ZnO nanoparticles with an average size \( \leq 100 \) nm included in a sunscreen formulation penetrate to the human epidermis.3 As it has been shown by many authors, at skin topical application, the main site of nanoparticle localization in the skin is hair follicles.2,4 In particular, in Ref. 4, the authors have studied cutaneous localization of nonbiodegradable polystyrene nanoparticles labeled by fluorescein 5-isothiocyanate using confocal laser scanning microscopy (CLSM). It follows from the CLSM images that accumulation of 20- and 200-nm polystyrene particles in follicular openings depends on both size of the particles and time elapsed, and there are no evidences of permeation of the nanoparticles in the nonfollicular region. The authors of Ref. 5 also demonstrate accumulation of ZnO and TiO2 nanoparticles in hair follicles. To implement targeted particle delivery deep into the skin, massage,6 low-frequency sonophoresis,7,8 microporation,9 and fractional laser microablation (FLMA)10,11 are typically used. FLMA in combination with ultrasonic treatment is a promising technique for this purpose.12

Creating artificial microchannels in the upper skin layers by FLMA promotes deeper and more efficient delivery of micro- and nanoparticles. The sufficiently fast restoration of the skin...

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Ultrasonic treatment facilitates administration of micro- and nanoparticles into the skin and their targeted subsurface localization within the tissue. One of the most rapidly developing applications of luminescent particles is their use in molecular and cellular biology, medical diagnostics and therapy. For example, in order to monitor encapsulated drug delivery pathways, luminescent particles are introduced into microcapsules at the stage of synthesis. 

Currently, quantum dots (QDs) and upconversion particles (UCPs), along with the traditionally used luminescent labels, are widely used for biological research. The use of luminescent spectroscopy allows for detection of optical signal from micro- and nanoparticles in real time during several days. In Ref. 22, the authors have studied the transdermal delivery capacity of water-soluble CdSeS QDs through mouse skin and their deposition in the body. Experimental results indicate that QDs can penetrate into the dermal layer through hair follicles and then get into blood circulation. QDs were found in liver and kidney. Dye-containing polymeric 320-nm nanoparticles accumulated in the hair follicles can stay there for up to 10 days, while a nonparticulate form can be detected only up to 4 days. Subcutaneous injection of ethylenediaminetetra (methylenephosphonic acid)-coated NaYF4: Er/Yb nanoparticles in mice has demonstrated that the particles of both types exhibit bright green luminescence upon laser excitation of the skin for up to three days after injection.

In order to visualize spatial localization of the luminescent nanoprobe in tissues, deep penetration of excitation radiation into the tissue is required. In addition, the emitted optical radiation has to be in the so-called “diagnostic” spectral window. For this, the upconversion luminescence excited in the near-infrared (NIR) spectral range can be effectively used. Indeed, high scattering of the visible and NIR radiation in tissues as well as optical detection method significantly limits the spatial resolution and the probing depth. This problem can be solved by application of hyperosmotic immersion liquids known as optical clearing agents (OCAs).

The combined use of several optical imaging techniques, so called “multimodal imaging,” can provide more advanced opportunities to visualize spatial distribution of micro- and nanoparticles within tissues.

In this paper, multimodal optical imaging, i.e., luminescent spectroscopy, optical coherence tomography and confocal microscopy, and immersion optical clearing technique are used for spatial localization and monitoring of upconversion luminescent microparticles and QDs during days within rat skin microchannels created by FLMA.

2 Materials and Methods

2.1 Animals

In vivo studies were carried out on six outbred albino rats 1 year of age and the body mass of ~400 g. During all experimental studies, animals were kept in accordance with the European Convention for the Protection of animals used for experimental and other scientific purposes. Prior to all treatments, the rats were anesthetized with Zoletil 50 (Virbac, France) in a dose of 0.05 mg/kg. On the sites under study, hair was thoroughly removed using a cream for depilation.

2.2 Particles

Commercially available lanthanide-doped UCPs [Y2O3: Yb, Er] P Tir660-UF (Phosphor Technology, United Kingdom) with an average size of ~1.6 μm and in-house synthesized CuInS2/ZnS QDs with an average size ~20-nm coated with polyethylene glycol (PEG)-based amphiphilic polymer (see Appendix) were used in the experiments.

The UCPs have a luminescent band near the 650-nm wavelength, upon 980-nm laser excitation [Fig. 1(a)]. The UCPs [Y2O3: Yb, Er] are a promising new generation of agents for bioimaging. Upconversion as a phenomenon utilizes
a sequential absorption of multiple photons via long lifetime (metastable) and real ladder-like energy levels of trivalent lanthanide ions embedded in an appropriate inorganic host lattice to produce higher energy anti-Stokes luminescence.\textsuperscript{31-33} It thereby converts two or more low-energy excitation photons of NIR light into shorter wavelength emission in NIR and visible ranges. High photostability, large blue shift of the upconversion luminescence relative to the wavelength of the exciting radiation, and absorption in the range of maximal transparency of tissues (600 to 1000 nm) make it possible to track single particles and perform their long-term detection.\textsuperscript{34}

The synthesized QDs have an irregular shape with the crystal size less than 10 nm and a hydrodynamic diameter around 20 nm.\textsuperscript{35} The luminescent peak of QDs is at the 680-nm wavelength [Fig. 1(b)] with the quantum yield of $\sim$45\% measured at 488-nm excitation with Rhodamine 6G as a reference dye.

Aqueous suspensions of the particles were prepared immediately before their use. Concentrations of UCPs and QDs were 0.625 $\mu$g/$\mu$L and 0.6 $\times$ 10$^{-6}$ mol/L, respectively. To prevent aggregation of the particles, UCP and QD suspensions were sonicated in an ultrasonic bath before their topical application to the skin surface.

\subsection{2.3 Experimental Methods}

\subsubsection{2.3.1 Particle delivery protocol}

All six rats were subjected to the procedure of FLMA. For carrying out the FLMA procedure, a commercially available Palomar Lux 2940 pulse erbium laser (Palomar Medical Technologies Inc., USA) operating at the 2940-nm wavelength was used. Laser pulse energy was 3 J with a duration of 20 ms; the temporal profile of the pulse was modulated by three subpulses with duration of each about 5 ms. The laser beam was split into 64 microbeams using a microlens array. Thus, 64 vertical microchannels were created over the skin area.

The animals were divided into two groups. In the first one (two animals), the efficiency of administration of UCP and QD suspensions was studied by using ultrasound or massage.

Five sites were marked as shown in Fig. 2(a): the site 0 corresponded to intact skin without any treatment, the sites 1 and 3 were treated by the UCP suspension, and the sites 2 and 4 by the QD suspension. The volume of both suspensions was equal to 23 $\mu$L. The UCPs and the QDs were delivered into microchannels by using sonophoresis (sites 3 and 4) or mechanical massage (sites 1 and 2) for 5 min each.

A commercially available device Dynatron 125 (Dynatronics, USA), equipped with a 2.2-cm diameter US transducer was applied for sonophoresis in the continuous-wave mode with the frequency of 1 MHz and the power density of 1.5 W/cm$^2$. Remains of the suspensions were removed from the skin surface using distilled water.

In the second group (four animals), six sites were marked as shown in Fig. 2(b): the site 0 corresponded to intact skin without any treatment, the sites 1 and 4 were treated by UCP suspension, and the sites 2 and 5 by the QD suspension. The volume of the applied suspensions was the same as for the first group. The site 3 remained untreated by any particle suspension. This site (control) was used for the monitoring of the changes of skin state after FLMA. The UCPs and the QDs were delivered into the microchannels by using sonophoresis (the sites 1, 2, 4, and 5) for 5 min each.

\subsubsection{2.3.2 Particle imaging}

Imaging of the particles was performed using different optical modalities, such as optical coherence tomography, luminescent spectroscopy, and confocal microscopy, as well as conventional optical microscopy (histological analysis).

The microchannels were visualized just after FLMA and after filling them by the particle suspensions in the first and the second groups. The imaging was performed with a commercially available spectral-domain optical coherence tomography (OCT) system OCP930SR (Thorlabs, USA), operating at the central wavelength of 930 $\pm$ 5 nm with 100 $\pm$ 5 nm full-width-at-half-maximum spectrum and having the following characteristics: numerical aperture of 0.22, optical power of 2 mW; the scanning length along the skin surface of 6 mm with the axial and the lateral resolutions on the air of 6.2 and 9.6 $\mu$m, respectively.

To study the influence of ultrasound and massage on intensity of luminescent signal from particles inside the skin, we carried out the spectral measurement immediately after FLMA and particle delivery in the first group of animals.

For monitoring UCP and QD localization in the rat skin in vivo (the second group), luminescent spectroscopy was applied every day during the entire period of in vivo studies (nine days). Figure 3 schematically shows the experimental system for detection of UCP and QD luminescent spectra by...
a spectrometer QE6500 (Ocean Optics, USA). The excitation of UCPs was performed at 980-nm wavelength and that of QDs at 405-nm using a semiconductor laser module DMH980-200 (Laser Systems LAS, Russia, 20 mW) and a violet laser pointer Pen Style (HangZhou NaKu Technology Co. Ltd., China, 300 mW), respectively. The fiber-optic probe was fixed over the skin surface at the distance of 25 mm. Luminescent spectra were measured with a 100-ms acquisition time.

The withdrawal of the animals from the experiment and sampling of tissues for the luminescent and morphological study was performed on the first day (two rats from the first and the second groups) and on the last day of the experiment (two rats from the second group).

Unstained sections were studies with luminescent microscopy. Since luminescence of UCPs and QDs is excited by different wavelengths, we used different equipment for the observation. Luminescent images of skin histological sections with QDs were obtained at room temperature (∼20°C) using a standard confocal luminescent microscope (Leica TCS SP8, Germany) with 10× and 100× magnification under 405-nm laser excitation. Images of UCPs were taken with an optical microscope OGME-PZ (Sapphire, Russia) equipped with a CCD camera (Videocan 415/P-USB, Videocan, Russia) under 980-nm laser excitation.

Then the sections were stained with hematoxylin–eosin according to the standard procedure. Morphometric studies of histological preparations were done using a digital image analysis system μVizo-103 medical microvisor (LOMO, Russia).

2.3.3 Optical clearing agent
To enhance the luminescent signal from the particles inside skin dermis, the skin sites 1, 2, and 3 were exposed during 20 min to OCA Omnipaque™ (GE Healthcare, Ireland) before each spectroscopic measurement.

Omnipaque™ (iohexol; GE Healthcare, Ireland) was used as an OCA to decrease skin scattering. Omnipaque™ is a nonionic, water-soluble x-ray contrast agent with iodine concentration of 300 mg/mL commonly used for in vivo angiography and imaging of internal organs (arteriography, phlebography, etc.). Iohexol has a low osmolarity comparable with blood plasma and cerebral spinal fluid. It is approved by the U.S. Food and Drug Administration (FDA) for in vivo topical use. Omnipaque™ (300) has molecular weight of 821, neutral pH (6.8 to 7.6), refractive index 1.439 at the wavelength 589 nm, and low toxicity.

Figure 4 shows the series of OCT images of skin sites from the first group of rats immediately after FLMA alone (a), FLMA followed by QD delivery with massage (b), FLMA followed by UCP delivery with massage (c), FLMA followed by QD delivery with sonophoresis (d), and FLMA followed by UCP delivery with sonophoresis (e). In the images, two layers—epidermis (E) and dermis (D)—are clearly distinguishable. Microchannels (MC) look like cones. Figure 4(a) allows for evaluation of the separation between the centers of the laser-perforated channels as ∼1 mm with the diameter of their openings at skin surface as 150 to 250 μm and the depth as 450 to 500 μm.

As follows from OCT images (c) and (e), after both massage and sonophoresis, the largest part of the microchannel is filled up by the UCP suspension. It is well seen that QDs cannot be visualized with high-contrast on the background of the skin scattering [see images (b) and (d)]. UCPs provide high contrast of OCT visualization of the microchannels because of their much lower scattering than QDs.

3 Results and Discussion
3.1 Optical Coherence Tomography Imaging
Figure 4 shows the series of OCT images of skin sites from the first group of rats immediately after FLMA alone (a), FLMA followed by QD delivery with massage (b), FLMA followed by UCP delivery with massage (c), FLMA followed by QD delivery with sonophoresis (d), and FLMA followed by UCP delivery with sonophoresis (e). In the images, two layers—epidermis (E) and dermis (D)—are clearly distinguishable. Microchannels (MC) look like cones. Figure 4(a) allows for evaluation of the separation between the centers of the laser-perforated channels as ∼1 mm with the diameter of their openings at skin surface as 150 to 250 μm and the depth as 450 to 500 μm.

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larger size than QDs (1.6 μm versus 20 nm). Comparison of data presented in Figs. 4(c) and 4(e) shows that in contrast to the massage, the ultrasound treatment allows for deeper filling of the ablated channels in the skin. It is in a good agreement with the results of Ref. 12, where the increase of skin permeability for both in vitro and in vivo studies using ultrasound irradiation was shown.

3.2 Luminescent Analysis

The spatial localization of the particles in the microchannels immediately after FLMA and the particles delivery can be observed on luminescent images presented in Fig. 5. Luminescence of UCPs was detected by optical microscope under 980-nm laser excitation [see Figs. 5(a) and 5(b)] and luminescent of QDs was detected by confocal microscope under 405-nm laser excitation [see Figs. 5(c) and 5(d)].

It is well seen that near the skin surface, the particles were localized along the channel walls; the main volume of the suspensions, apparently, was removed from the channels during the washing-off. However, as we can see in Fig. 4, the suspension remained at the depth of 400 to 500 μm. After healing of the tissue around the channels and as a result of their closing, the particles are accumulated within a localized space in tissue. QDs and UCPs were not detected in the histological sections with a confocal luminescent microscope after nine days of particle delivery.

Figure 6 shows the luminescent spectra of the UCPs [see Fig. 6(a)] and QDs delivered into skin by massage and sonophoresis [see Fig. 6(b)]. It can be seen that sonophoresis results in the increased luminescent signal from the particles in comparison with the massage, apparently, due to the increase of quantity of the particles penetrated into the channels.

Measured kinetics of luminescent intensity (Figs. 7 and 8) and histological analysis (Figs. 9 and 10) confirm that the depot of the particles can be created inside the microchannels.

Figure 7 shows the luminescent intensity produced by UCPs (a) and QDs (b) embedded into the rat skin in vivo.

Under excitation by 980-nm laser, skin autofluorescence in the red spectral region is negligible. By contrast, 405-nm radiation excites fluorescence from endogenous skin fluorophores: three bands of porphyrins in the 630 to 710-nm region and NADH near 480 nm.37

The luminescent peak of QDs shifted in the skin from 680 to 670 nm overlaps with the fluorescent bands of porphyrins [see Fig. 7(b)]. The QD and UCP luminescence was observed for four days [see Fig. 7(b) (3, 4)] and eight days [see Fig. 7(a) (3, 4)], respectively, with a gradual decrease in the intensity with time.

![Fig. 5 Luminescent images of histological section of the perforated skin obtained using: (a, b) an optical microscope equipped with CCD camera under 980-nm laser excitation of the UCPs and (c, d) a confocal microscope under 405-nm laser excitation of the QDs. The fluorescing UCPs are marked with the red circle. The images were obtained from the depth of (c) 0 μm and (d) 40 μm immediately after FLMA and particle delivery.](https://remotesensing.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)
Healing and regeneration of skin epidermis (about 4 to 5 days) can prevent penetration of 405-nm light to the QD depot in the skin. At the same time, 980-nm light penetrates significantly deeper and can excite luminescence of UCPs for more prolonged time during skin healing and regeneration and afterward.

The decrease of luminescent signal can also be connected with the diffusion of the particles from the observation zone to surrounding tissues. In Ref. 38, it was shown that contrast of OCT-images of human skin with embedded particles also decreased during a few days after their delivery. After the full regeneration of the skin integrity, quantity of the particles was so little that they did not register in the OCT images. Difference between time interval (days) during which luminescence from UCPs and QDs is detectable can be related to particle size and their diffusion from the initial delivery volume: larger UCPs remained localized inside the skin channels for a long time, whereas much smaller QDs diffuse from the channels and distributed in the surrounding tissues.

It is well seen that the maximal luminescent signal is observed after the skin optical clearing. Figure 8 shows kinetics of the maximal intensity of the luminescent bands of the UCPs
Fig. 8 The time dependence of the luminescent maximum of the (a) UCPs ($\lambda_{\text{max}} = 658$ nm) and (b) QDs ($\lambda_{\text{max}} = 680$ nm) embedded into the rat skin by sonophoresis and with (black squares) and without (red circles) optical clearing by topical application of Omnipaque™.

($\lambda_{\text{max}} = 658$ nm) (a) and QDs ($\lambda_{\text{max}} = 680$ nm) (b) embedded into the rat skin by sonophoresis and with and without optical clearing by topical application of Omnipaque™.

Figures 7 and 8 show that the topical application of the immersion agent Omnipaque™ allows for enhancement of the luminescent signal from the particles located inside skin dermis due to reduction of light scattering of tissue layers, where excitation and fluorescent signals propagate. This is a so-called optical clearing technique application that increases probing depth and contrast of optical imaging methods.

It was demonstrated, in particular, for fluorescent spectroscopy and microscopy. Omnipaque™ has refractive index (1.439) close to refractive index of main skin scatterers—collagen fibers (1.43). Thus, a partial replacement of the interstitial fluid by Omnipaque™ causes the matching of the refractive indices of the tissue components, its better homogeneity and reduction of scattering.

In Fig. 8(a), we can see that skin treatment by Omnipaque™ does not affect the intensity of the luminescent signal after four days post-FLMA and particle delivery. We suppose that after full regeneration of skin barrier function, Omnipaque™ does not penetrate effectively into skin dermis, and therefore, the 20-min agent application is not enough for signal enhancement.

3.3 Histological Analysis

Microphotographs of histological sections of the studied skin areas from the first group measured immediately after UCPs delivery are shown in Fig. 9. The control sample [intact skin without any treatment; see Fig. 9(a)] represents skin with normal multilayer epithelium (epidermis). The skin appendages are visible in the dermis. QDs could not be detected in the histological sections with conventional optical microscopy due to their small size (20 nm).

For the samples taken from the FLMA-zone [ablation only; see Fig. 9(b)], crater-like defects crossing epidermis and dermis, regularly occurring on average every 600 μm with the development of coagulation necrosis in tissue around them, are observed. In Fig. 9(b), one of the craters covers an area of width $\times$ depth equal to $153 \times 170$ μm$^2$ as the maximal depth of necrosis edge is $363$ μm. Coagulation necrosis of the thickness about $10$ μm is formed around the perforated channel. In addition, full-blooded vessels around the zone of influence are observed.

Figure 9(c) shows UCPs on crater walls delivered by massage; this crater is an internal part of the microchannel. Outside, the channel is surrounded by a necrotic zone with a radius approximately equal to the crater depth. The size of the crater is $155 \times 58$ μm$^2$.

It is important to note that sonophoresis increases the channel size. The form of the channel changes from conical to spherical. We anticipate that the necrotic tissue is detached and pushed out from the channel by sonophoresis. This hypothesis is confirmed by a thinner zone of coagulation necrosis observed in Fig. 9(d) than in Fig. 9(c).

Figure 10 presents a fragment of skin with a microchannel filled by UCPs of the rat from the second group. A depth of localization is $323$ μm. The size of the defect is $21 \times 63$ μm$^2$.

Analysis of this histological section confirms our hypothesis that particles with the microscale dimension localized inside the channels remain there after the channel healing and do not penetrate in surrounding tissue. Thus, a depot of particles can be created for prolonged drug release.

QDs, on the contrary, can diffuse through the surrounding tissue and leave the channel zone. Therefore, we cannot see accumulation of the particles in the histological sections and luminescent signal to the ninth day post-treatment. It was shown earlier that the use of FLMA allowed for delivery of microparticles into dermis and creation of a particle depot. For example, biocompatible CaCO$_3$ microcontainers (4.0 ± 0.8 μm) containing Fe$_3$O$_4$ nanoparticles were delivered to the depth about 300 μm in dermis. On the seventh
day, the dissolving of the microcontainers and release of the content into dermis was observed. In Refs. 38 and 39, it was shown that particles with diameter about 100 nm and more can remain in skin in vivo during 30 days.

4 Conclusions
It is shown that UCPs are visualized in skin more efficiently in comparison with QDs. The UCPs have a stable narrow-band emission at excitation in the NIR spectral range. Under excitation by 980-nm laser, the skin autofluorescence in the spectral region of the microparticle luminescence is absent. The luminescent images of the UCPs distribution indicate the advantage of the particle delivery into the skin by FLMA with sonophoresis. The UCPs are observed in the channels by optical coherence tomography, conventional microscopy, and luminescent imaging during eight days after the skin treatment. The QDs luminescence is detected for four days with a gradual decrease of the intensity. It is shown that the use of the immersion agent Omnipoque™ results in the enhancement of the luminescent signal from the particles inside skin dermis. In the process of the channel healing, QDs were displaced from the channels to the surface of the skin. This fact explains the absence of the QD luminescence signal on the fifth day of the experiment. The UCPs were located deeper in the tissue and remained there after the healing of the channels. This explains the presence of the luminescent signal of the UCPs for eight days. The difference in the depth of the particles localization can be explained by the fact that ultrasound more effectively influences heavier and larger UCPs than QDs.

Thus, the multimodal imaging approach provides capabilities for a more advanced investigation of spatial localization of
UCPs and QDs within the skin, which is of interest for different biomedical applications.

Appendix: CuInS$_2$/ZnS Core/Shell Synthesis

A Zn precursor was prepared by mixing 1.6-g zinc stearate Zn(St)$_2$ (2.53 mmol), 4.3-mL oleic acid, 14-mL octadecene in a flask and flushing the mixture for 30 min with nitrogen, followed by heating up to 190°C until a colorless solution was obtained. Then the flask was cooled down to 90°C, 0.84 mL dodecanethiol was added and the mixture was left under nitrogen at 75°C for further use. For the core/shell synthesis, the flask with CuInS$_2$ cores grown at 220°C was cooled down to 80°C. At this temperature, 2.7 mL of the Zn precursor (Zn:In = 1:1) was added and the flask was immediately heated up to 215°C and was kept at this temperature for 30 min. Afterward, the flask was cooled down again to 80°C, 2.7 mL of the Zn precursor (2.7 ml) was added followed by heating up the flask again up to 215°C. The same procedure was repeated five times. Finally, the solution was cooled down, diluted with chloroform [V(CHCl$_3$): V(solution) = 1:5] and centrifuged at 3000g for 10 min. The formed precipitate was discarded, and ethanol was added to the supernatant until opalescence. The mixture was centrifuged at 3000g for 10 min and the supernatant was decanted. The precipitate was dispersed in toluene. This precipitation/dissolution procedure was repeated three times and in such a way obtained QDs were stored in toluene at 4°C.

Disclosures

All authors have no financial interest.

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