

Synthesis of AS1411-Aptamer-Conjugated CdTe Quantum Dots with High Fluorescence Strength for Probe Labeling Tumor Cells

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Abstract In this paper, we report microwave-assisted, one-stage synthesis of high-quality functionalized water-soluble cadmium telluride (CdTe) quantum dots (QDs). By selecting sodium tellurite as the Te source, cadmium chloride as the Cd source, mercaptosuccinic acid (MSA) as the capping agent, and a borate-acetic acid buffer solution with a pH range of 5–8, CdTe nanocrystals with four colors (blue to orange) were conveniently prepared at 100 °C under microwave irradiation in less than one hour (reaction time: 10–60 min). The influence of parameters such as the pH, Cd:Te molar ratio, and reaction time on the emission range and quantum yield percentage (QY%) was investigated. The structures and compositions of the prepared CdTe QDs were characterized by transmission electron microscopy, energy-dispersive X-ray spectroscopy, selective area electron diffraction, and X-ray powder diffraction experiments. The formation mechanism of the QDs is discussed in this paper. Furthermore, AS1141-aptamer-conjugated CdTe QDs in the U87MG glioblastoma cell line were assessed with a fluorescence microscope. The obtained results showed that the best conditions for obtaining a high QY of approximately 87 % are a pH of 6, a Cd:Te molar

ratio of 5:1, and a 30-min reaction time at 100 °C under microwave irradiation. The results showed that AS1141-aptamer-conjugated CdTe QDs could enter tumor cells efficiently. It could be concluded that a facile high-fluorescence-strength QD conjugated with a DNA aptamer, AS1411, which can recognize the extracellular matrix protein nucleolin, can specifically target U87MG human glioblastoma cells. The qualified AS1411-aptamer-conjugated QDs prepared in this study showed excellent capabilities as nanoprobe for cancer targeting and molecular imaging.

Keywords CdTe · Microwave-assisted synthesis · Bioimaging · AS1411 · Aptamer targeting · U87MG

Introduction

Quantum dots (QDs) are semiconductor nanocrystals. QDs systems with small dimensions exhibit quantum mechanical phenomena such as the emission of excitons that are confined in all three spatial dimensions [1–3]. The electronic characteristics of these materials are intermediate between those of bulk semiconductors and discrete molecules [4–6]. Owing to their photostability and narrow emission bandwidth, QD nanocrystals show the promise of having very interesting applications such as sensing, detection, and bioimaging [7–11].

Traditionally, QDs are synthesized in organic media and the resultant QDs has no intrinsic aqueous solubility. During the growing process, they are coated and protected with hydrophobic capping agents such as trioctyl phosphine oxide (TOPO). Then, a cap exchange procedure is required to solubilize QDs in water. However, the cap exchange procedure can be adapted to prepare functionalized QDs with reduced colloidal stability and quantum yields (QYs) [12–14]. Moreover, water-soluble QDs can be produced through a simple

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single-stage synthesis process by using thiols [15–18] and phosphates [19] that act as stabilizing agents in aqueous media. The stabilizing agents used in this method serve as biocompatible ligands on the surface of QDs, and therefore, the cap exchange procedure is not required. In this regard, Gaponik et al. [20] analyzed the effects of thiol-capping of soluble cadmium telluride (CdTe) QDs and demonstrated that each ligand has a different effect on the characteristics and quantum yield percentage (QY%) of synthesized QDs.

The large exciton Bohr radius (7.3 nm) and narrow bulk band gap (1.475 eV) of CdTe QDs render them a versatile semiconductor material for various applications. Over the past two decades, great efforts have been focused on CdTe QDs to use them as fluorescence probes and sensors [21–24]. However, it is worth noting that some of their photoluminescence deficiencies may hinder their applications. Owing to a prolonged reaction process to produce QDs in the aqueous phase (ranging from a number of hours to several days), numerous surface defects are often generated that result in photoluminescence deficiencies. To shorten the synthesis of high-quality CdTe QDs, hydrothermal or microwave-assisted methods can replace traditional reflux methods [25–29]. In microwave-assisted methods, microwaves as a heat source are utilized for the rapid synthesis of QDs, resulting in uniform heating of the reaction solution and providing size- and shape-controlled synthesis with a higher QY than conventional heating techniques. NaHTe has usually been employed as the tellurium source in the synthesis of CdTe QDs or Te nanostructures. However NaHTe is unstable owing to its spontaneous oxidation in the presence of oxygen [25–30]. For this reason, CdTe QDs have to be synthesized in an inert atmosphere. On the other hand, both absorption and emission spectra of these QDs can shift to longer wavelengths by increasing the reaction temperature or prolonging the reaction time, as expected on the basis of “quantum-confined size effects.”

In this paper, we report the synthesis of CdTe QDs by using sodium tellurite (Na_2TeO_3) as the Te source and

mercaptosuccinic acid (MSA) as the stabilizer; the synthesis occurs via a facile one-pot microwave irradiation reduction route. We used a precursor, namely, borate–acetic acid buffer solution, with a pH range of 5–8 as the reaction medium. By increasing the pH of the precursor buffer, the absorbance and emission of the prepared QDs were shifted to longer wavelengths in less than one hour without increasing the temperature. Since Na_2TeO_3 is air-stable, the synthesis does not require an inert atmosphere or complicated vacuum manipulations. With microwave dielectric heating, the growth rate of the CdTe QDs can be greatly accelerated, and CdTe QDs with a high QY can be obtained through this simple reaction route within a short period of time [31, 32].

There has been growing interest in the use of target-functionalized QDs for biosensing and bioimaging applications [33–35]. As fluorophores, QDs may be used as a passive label. In the current study, the synthesized high-quality carboxylic-acid-functionalized CdTe QDs were attached to an amine- C_6 AS1411 aptamer that can recognize the extracellular matrix protein nucleolin (Fig. 1). Finally, the capabilities of the AS1411-aptamer-conjugated QDs were determined for targeted bioimaging of the U87 glioblastoma cell line in vitro.

Methods

General

All reagents and solvents were commercially available from suppliers (Merck AG and Sigma Aldrich) and used without further purification. Roswell Park Memorial Institute (RPMI) 1640 medium, foetal bovine serum (FBS) and trypsin were obtained from GIBCO (Germany), while DNase RNase free water was procured from Invitrogen (Germany). Deionized water was used in the preparation of buffers and aqueous solutions.

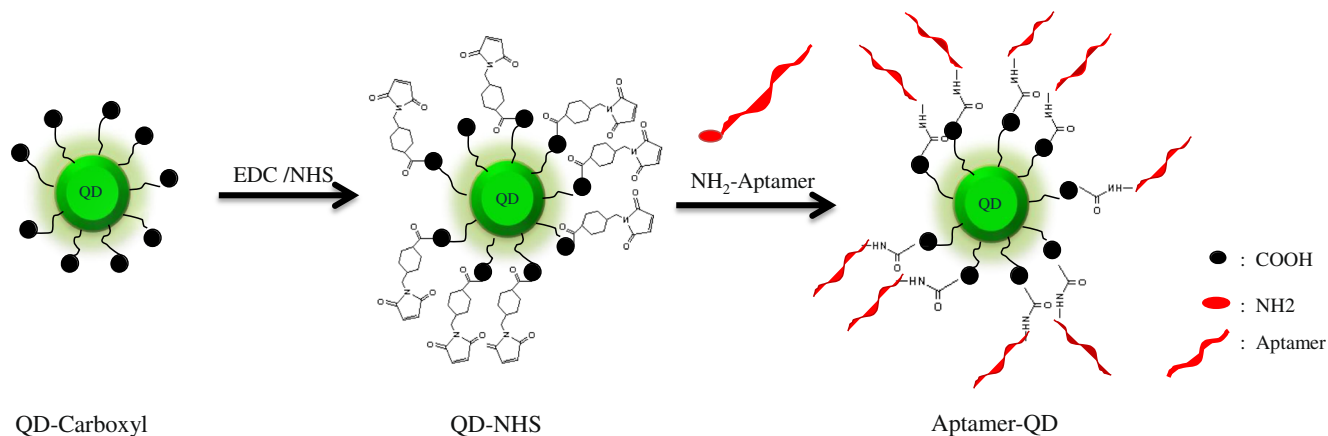


Fig. 1 Schematic represents the conjugate of QDs with AS1411- aptamer

Synthesis of CdTe QDs

CdTe QDs were synthesized through a simple and reliable method by using microwave irradiation. All the reactions occurred in 100 mL borate–acetic acid buffer solution containing 100 μ L glacial acetic acid (17 mM) and 342 mg $\text{Na}_2\text{B}_4\text{O}_7$ (17 mM), and were adjusted to different pH values with 1 M HCl or 1 M NaOH [36].

The precursor solution was prepared by mixing a solution of 20 mg CdCl_2 (1 mM), 4.4 mg Na_2TeO_3 (0.2 mM), and 45 mg MSA (3 mM) in 100 mL of the aforementioned borate buffer solution (pH 5–8) in a round bottom flask at room temperature. The Cd:Te:MSA molar ratio was 5:1:15.

After vigorously stirring for 10 min, 46 mg of NaBH_4 powder was added rapidly to the precursor solution and the reactant mixture was stirred vigorously for another 10 min. The CdTe precursor was then put into a Teflon vessel and microwave-irradiated (400 W) at 100 °C for time periods ranging from 10 to 60 min. After microwave irradiation, the mixture was allowed to cool to temperatures lower than 50 °C, and the CdTe QDs sample was diluted with 100 mL of absolute ethanol and centrifuged at 6,000 rpm for 15 min. By controlling the pH (5–8) of the precursor buffer and the reaction time (10–60 min), CdTe QDs with desired fluorescence emission spectra were obtained.

Aptamer Synthesis

An anti-nucleolin ssDNA aptamer reported by Mongelard and Bouvet was employed as the targeting ligand for bioimaging tumor cells [37]. Nucleic acids were synthesized on a Polygene DNA synthesizer (Polygene, Germany) by using reagents from Sigma (Germany). The synthesis was performed according to standard phosphoramidite chemistry. The synthesized DNA was ethanol-precipitated three times, and the concentration was determined from absorbance at 260 nm. The sequence of the modified aptamer was 5' L GGTGGTGG TGGTTGGTGGTGGTGGTGG where L represents a 5' amine C_6 linker ($\text{NH}_2(\text{CH}_2)_6\text{PO}_4$).

Characterization of CdTe QDs

The ultraviolet–visible (UV/Vis) absorption and fluorescence measurements spectra of the QDs were recorded using a Varian CARY 100 UV/Vis spectrophotometer (California, USA) and a Jasco FP-6200 spectrofluorometer (Tokyo, Japan), respectively. For the fluorescence QY estimation at room temperature, QDs in deionized water at different concentrations and rhodamine 6G in absolute ethanol with an absorbance of <0.1 at 400 nm were prepared, and the QY% was calculated according to the protocol reported by Debas and Crosby [38].

High-resolution electron microscopy, selective area electron diffraction (SAED) patterns, and energy-dispersive X-ray spectroscopy were performed using a high-resolution transmission electron microscope (HR-TEM; JEOL-2100) equipped with an energy-dispersive X-ray spectrometer (EDS, INCA) operated at 200 kV with a Gatan Orius SC600 CCD camera. The sample for TEM observation was prepared as follows: The aqueous solution of the QDs was sonicated for 30 min, subsequently dropped onto copper grids coated with an amorphous carbon film, and dried thoroughly in an electronic drying cabinet at a temperature of 25 °C and a relative humidity of 45 %. The powder X-ray diffraction (XRD) patterns were recorded using a Philips PW3040/60 powder X-ray diffractometer (Netherlands) equipped with graphite monochromatized high-intensity $\text{Cu K}\alpha$ radiation ($\lambda = 1.5438367 \text{ \AA}$). The test was conducted at 2θ angles in the range of 10–80° on 500 mg of QDs.

The Fourier transform infrared (FT-IR) spectra of MSA-capped CdTe QDs in the form of KBr pellets were recorded using a Paragon 1000 (Perkin Elmer, USA) FT-IR spectrophotometer.

Coupling of Aptamer and QDs

The carboxylic groups on the surface of the QDs were coupled to the amine-modified AS1141 aptamer according to ethyl(dimethylaminopropyl) carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) chemistry [39]. A solution containing 13.8 mg of EDC and 8.2 mg of NHS was added to 400 μ L of a 100 mM phosphate-buffered saline (PBS) buffer (100 mM NaCl, pH 6). Subsequently, 30 μ L of 8 μ M (3.5 mg/mL stock solution) MSA-capped QDs (emitting at 525 nm) and an additional 470 μ L of the PBS buffer were added, thereby increasing the total volume to 1,300 μ L. The mixture was sonicated for 5 min to prevent the aggregation of the QDs and to prepare a homogeneous solution. The mixture was incubated in the dark at room temperature for 1 h to activate the carboxyl groups on the surface of the QDs with EDC and NHS. The reaction mixture was then transferred to a 2 mL DNase/RNase-free tube and centrifuged at 13,000 rpm for 30 min. The supernatant was gently discarded and checked in a UV cabinet to confirm that there was no green fluorescence. The pellet was then washed twice with 400 μ L of 50 % methanol. The pellet was dispersed in 200 μ L of a 50 mM NaHCO_3 buffer (pH 9). Then, 350 μ L of 50 % methanol and 60 μ L of 800 μ M AS1411-amine-terminated DNA were added to the resultant solution. The QD:DNA ratio in this process was 1:200. The mixture was sonicated for 5 min to form a homogenous fluorescent orange solution. This solution was then stirred using a Teflon-coated bar magnet (length = 0.7 cm, $d = 0.1$ cm) at 4 °C, 200 rpm, for 18 h in the dark to allow the coupling of the amine-terminated aptamer to the activated carboxylic groups on the surface of the QDs. The

reactant was then centrifuged for 30 min at 13,000 rpm to produce a light orange pellet (green when illuminated in the UV cabinet). The supernatant was carefully removed and checked again to ensure that there was no green fluorescence. The pellet was washed twice with 400 μL of 50 % methanol and dried in air for 10 min. In the final stage, the pellet was resuspended in 30 μL of deionized H_2O (sonicated for 5 min). The UV absorbance at 260 nm was obtained from the combined supernatants to assess the amount of DNA not conjugated to the surface of the QDs, from which the amount of DNA conjugated to the QDs was estimated. The pellet was stored at 4 $^\circ\text{C}$ in the dark until use, and the sample was used within 3 days.

Gel Electrophoresis

Agarose (2.5 %) gel electrophoresis was performed to verify the amide coupling chemistry of the DNA aptamer to the surface of the QDs. The DNA aptamer and QD-aptamer complex were loaded on the gel (containing 0.2 $\mu\text{g}/\text{mL}$ ethidium bromide), and the gel was run in a tris-borate-EDTA (TBE) buffer at 80 V for 40 min. Then, the DNA fragments were visualized using a standard transilluminator.

Cell Imaging

The U87MG glioblastoma cell line was obtained from the National Cell Bank of Iran, Pasteur Institute of Iran. The cells were routinely cultured in a RPMI medium supplemented with 10 % (v/v) heat-inactivated foetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 $^\circ\text{C}$ in a humidified atmosphere (95 %) containing 5 % CO_2 for three days. The cells were collected and seeded at a density of 2×10^5 cells/well in a six-well tissue culture plate and allowed to adhere overnight. The cells were washed three times with a cold PBS containing 1 mM CaCl_2 and 1 mM MgCl_2 , and were then incubated in 1,000 μL of a cell-binding buffer containing 0.6 μM complexes of Apt-QDs or free QD, 10 % FBS, 1 mg/mL bovine serum albumin, 1 mM CaCl_2 , and 1 mM MgCl_2 at 4 $^\circ\text{C}$. After incubating for 1 h, the cells were washed three times with a cold PBS containing 1 mM CaCl_2 and 1 mM MgCl_2 and were then subjected to imaging under a Juli Smart Cell Analyzer (Baker and Baker Ruskin, USA).

Results and Discussion

Synthesis of MSA-Stabilized CdTe QDs

In preparing the precursor solution by adding NaBH_4 , a large amount of bubbles was released from the mixture and the clear solution of reactant turned pale green in less than 2 min,

depending on the pH of the precursor buffer and the Cd:Te molar ratio. The crude precursor solution was pale green, and owing to the small volume of QDs in the crude solution, no luminescence was observed. After heating for several minutes, the color of the solution became green (pH 5), light orange (pH 6), orange (pH 7), and light red (pH 8), and radiant luminescence was detected. High-quality CdTe QDs with different sizes and controllable emission spectra could be prepared under microwave irradiation (400 W) by adjusting the pH of the precursor solution and the irradiation time at 100 $^\circ\text{C}$. Four samples were prepared under different conditions. By increasing the irradiation time and pH of the precursor solution in the ranges 10–60 min and 5–8, respectively, the size of the CdTe QDs increased and their absorption/photoluminescence emission spectra shifted to longer wavelengths owing to quantum confinement. Hence, the size of the CdTe QDs could be tuned by the pH of the precursor solution and was detected by absorption and photoluminescence emission spectra.

Figures 2 and 3 demonstrate the absorption and photoluminescence spectra of the MSA-stabilized CdTe QDs prepared in an aqueous solution under different conditions. Also presented are the images of the MSA-stabilized CdTe QDs with different sizes under room light conditions (Fig. 4a) and those irradiated under an ultraviolet lamp ($\lambda_{\text{excitation}} = 365$ nm) (Fig. 4b).

In the current study, the effect of the pH of the precursor buffer on the optical properties of the CdTe QDs was accurately evaluated. The obtained results proved that the pH of the precursor buffer significantly affected the optical properties of the CdTe QDs. It should be considered that the use of a reaction medium with a suitable buffer capacity is pivotal for the synthesis of CdTe QDs with high fluorescence strength. CdTe QDs can properly form at a pH of 5–8.

Our results showed that decreasing or increasing the pH below 4 and above 8 significantly decreased the reaction rate and consequently the QY% of QDs.

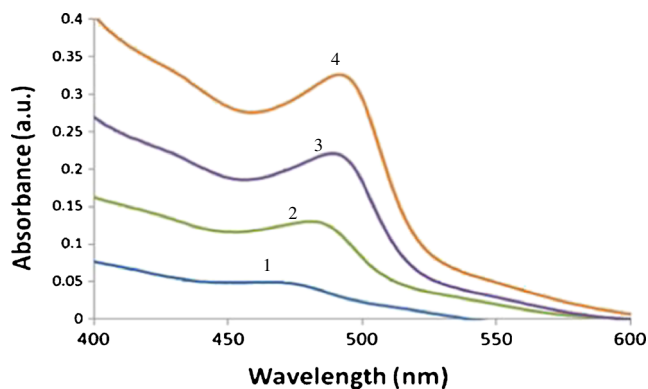
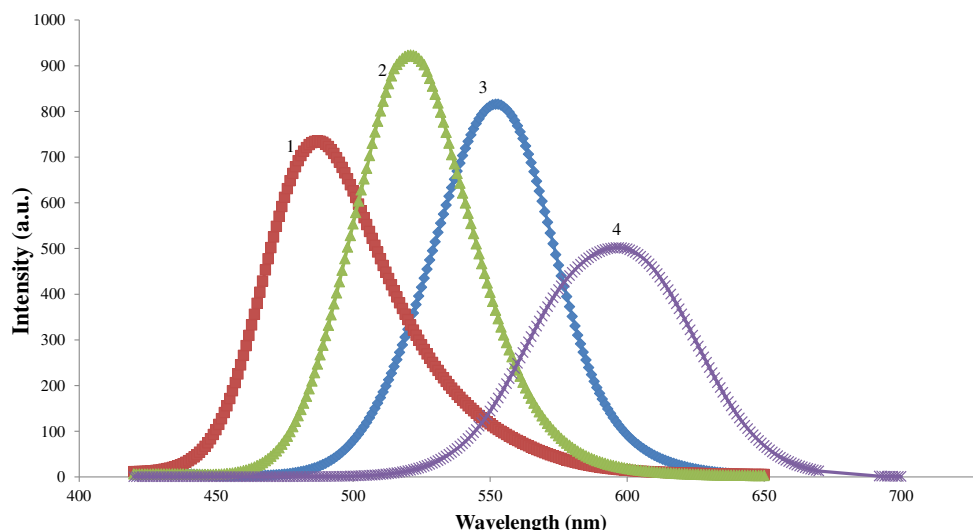


Fig. 2 Absorbance spectra of CdTe quantum dots at different pH values and different irradiation times at 100 $^\circ\text{C}$ (1) pH=5, t =10 min; (2) pH=6, t =30 min; (3) pH=7, t =45 min; and (4) pH=8, t =60 min

Fig. 3 Fluorescence emission of CdTe quantum dots at different pH values and different microwave irradiation times at 100 °C and $\lambda_{\text{exc}}=360$ nm. The maximum spectral emission was observed at (1) 480 nm, pH=5, $t=10$ min; (2) 523 nm, pH=6, $t=30$ min (3) 565 nm, pH=7, $t=45$ min; and (4) 595 nm, pH=8, $t=60$ min



The low pH values (below 5) will lead to the protonation of mercaptosuccinic acid (MSA) and weaken their bonding with Cd^{2+} , which is critical for the configuration of a defect-free surface and significantly decrease the QY% of QDs.

On the other hand, strong basic pH values (above 8) can decrease reduction rate of TeO_3^{2-} to Te^{2-} due to the decrease of the reducing capability of NaBH_4 .

In our study, during irradiation for about 10 to 60 min, the emission peaks of the CdTe QDs shifted to a longer wavelength. The excitonic peak positions in the absorption spectra ranged from 475 nm (1) to 525 nm (4), and the photoluminescence (PL) emission peaks of the CdTe QDs

ranged from 480 nm (1) to 595 nm (4). The red shifts of the absorption edge and the maximum PL emission wavelength indicated the growth of the CdTe QDs during the heat treatment, which was achieved by increasing the pH of the precursor solution. The sizes of the QDs could be estimated from the UV-vis first absorption maximum [40] by using the following equation:

$$D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0064)\lambda - (194.84) \quad (1)$$

Where D is the diameter of the QDs and λ is the first maximum absorption of the QD nanocrystals.

The diameters of the QDs were found to be in the range 1.48–2.89 nm, suggesting that the size of the QDs could be easily tuned by varying the pH of the precursor solution and irradiation time. The fluorescent color under UV irradiation changed from blue to green to yellow and orange with increasing pH and heating time.

The red-shift of the exciton absorption and fluorescent peaks with the increase of the irradiation time (10 to 60 min) and pH value (5 to 8) indicated the increase of QDs size.

Briefly, the pH increasing allows the gradually dissolve and reprecipitate CdTe to form larger crystals, probably via an Ostwald ripening mechanism [41].

The relative QY% of the four different CdTe samples was estimated to be 20, 87, 74, and 52 %, respectively, by using rhodamine 6G as a reference.

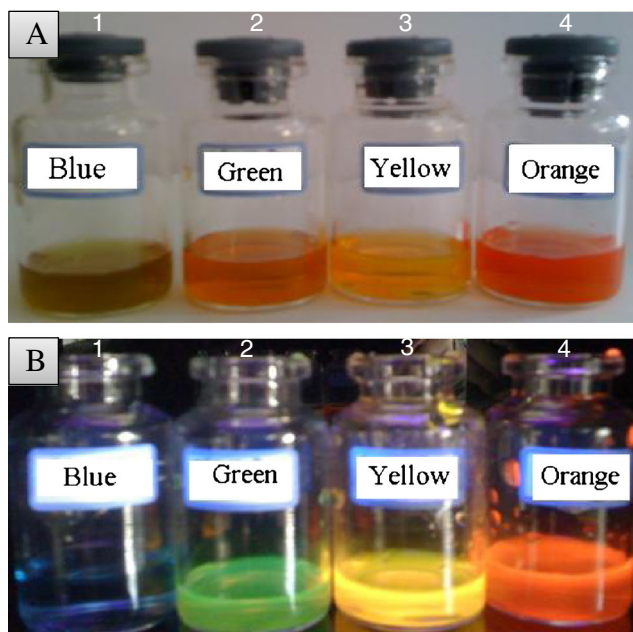
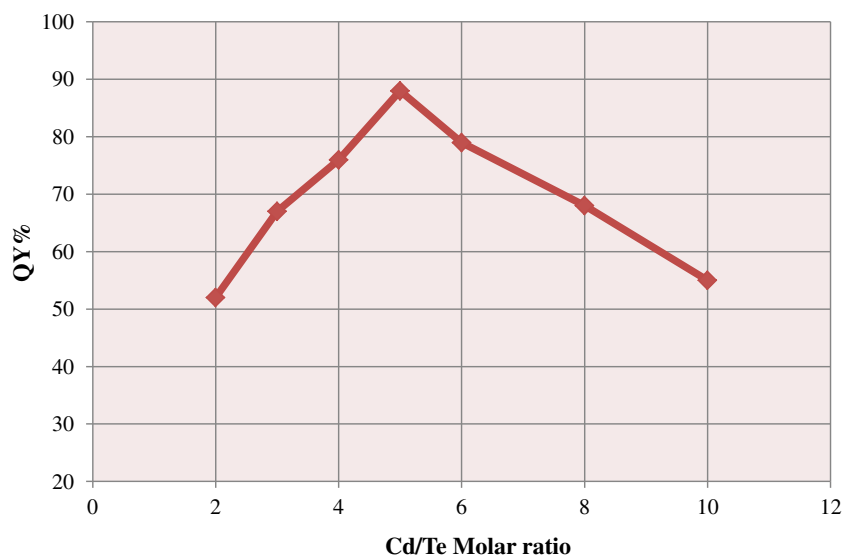


Fig. 4 **a** Image of MSA-capped CdTe QDs under ambient conditions. **b** Image of MSA-CdTe QDs under ultraviolet irradiation at 365 nm. (1) pH=5, $t=10$ min at 100 °C; (2) pH=6, $t=30$ min at 100 °C; (3) pH=7, $t=45$ min at 100 °C; and (4) pH=8, $t=60$ min at 100 °C

Influence of Molar Ratio and Reaction Time

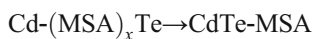
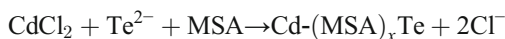
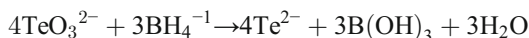
The CdTe QDs were obtained by a reaction between Te and Cd in the presence of MSA. The influence of the various synthesis parameters, including the reaction time and Cd:Te molar ratio on the QY of the QDs, were investigated. Figure 5

Fig. 5 QY% of CdTe QDs prepared at different Cd:Te molar ratios: 2, 3, 4, 5, 6, 8, and 10 at pH=6 under 30 min microwave irradiation at 100 °C



shows the influence of the Cd:Te molar ratio on the QY% of the QDs synthesized in a precursor buffer with a pH of 6. During the synthesis, the Te:Cd molar ratio was adjusted from 1:2 to 1:10 while the amounts of other reagents were fixed. The CdTe QDs became tightly capped by the Cd²⁺-MSA complexes at a Te:Cd molar ratio of 1:5, which passivated the QD surface and decreased the number of surface defects.

The following chemical reactions occurred during the synthesis of the CdTe QDs via the reduction route under microwave irradiation:



In the current study, the dissolved oxygen in water was not eliminated and owing to the high sensitivity of Te to oxygen, NaTeO₃ was used instead of NaHTe or HTe as the source of Te [42, 43]. During the synthesis, TeO₃²⁻ was reduced to Te²⁻ by using NaBH₄, which is an excellent reductant.

Since the reduced Te²⁻ could be reoxidized to a higher valence of Te by the oxygen dissolved in water, an adequate amount of NaBH₄ was added to water in order to reduce the reoxidized Te²⁻ and also to remove the dissolved oxygen in water. In this regard, NaBH₄ was utilized as a strong reductant for the reduction of TeO₃²⁻ ions and also to create an oxygen-exhausted atmosphere to prevent the reoxidation of Te²⁻. An excess amount of NaBH₄ with a NaBH₄:TeO₃²⁻ molar ratio of 12:1 was used in the synthesis to reduce all TeO₃²⁻ to Te²⁻. The NaBH₄ residue was oxidized by the oxygen diffused from air.

During the synthesis of the CdTe QDs, owing to the introduction of excess NaBH₄, large amounts of B(OH)₃ and H₂O

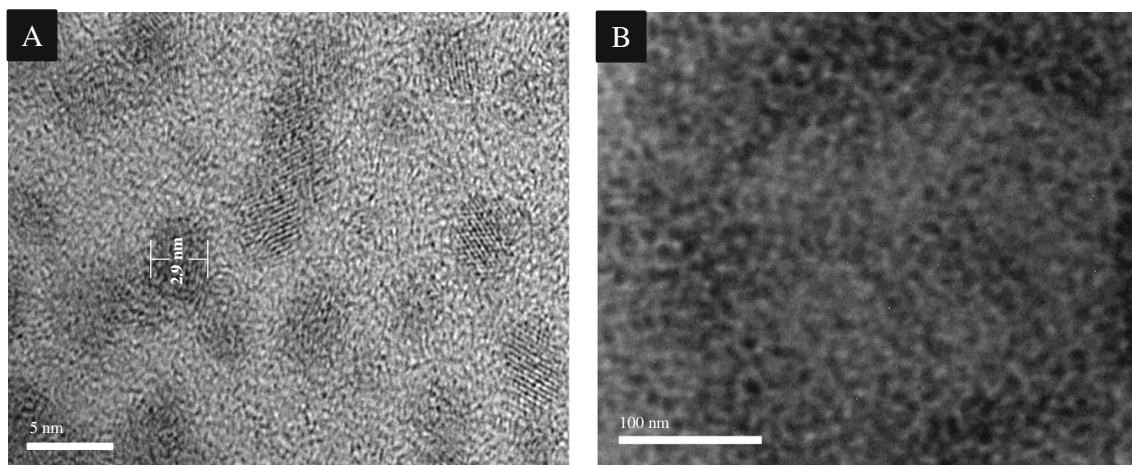
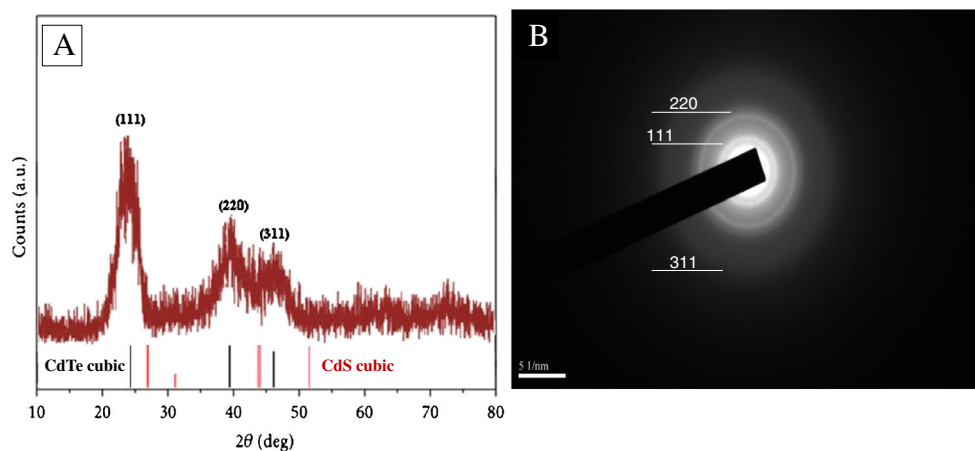


Fig. 6 High-resolution TEM image of MSA-capped CdTe QDs: **a** scale bar, 5 nm and **b** scale bar, 100 nm

Fig. 7 **a** XRD pattern of CdTe QDs and **b** SAED pattern of MSA-capped CdTe and assigned Miller indices



were produced. The buffer capacity of the precursor solution was thus crucial for controlling the pH of the reaction mixture [36]. In fact, by using an aqueous solution without buffer capacity, the QY of the synthesized MSA-CdTe was severely reduced (QY% < 7 %, data not shown). Therefore, it is essential that MSA capped-CdTe QDs are synthesized in a precursor buffer with enough buffer capacity to attain proper growth conditions of QDs during microwave irradiation. Under our experimental conditions, the borate–acetic acid buffer had enough buffer capacity to resist pH changes upon the addition of NaBH_4 .

In this study, the growth rate of CdTe QDs was accelerated by increasing the pH of the reaction medium from 5 to 8 without increasing the temperature or prolonging the reaction time over hours. As expected, the longer exposure to temperature increased the QD growth rate and larger QDs were obtained. According to previous studies, the synthesis of QDs at low temperatures results in the generation of various surface defects in the QDs and a reduction in QY. Although higher temperatures lead to an increase in the crystallinity of QDs and an enhancement of QY, an extended heating time can

cause decomposition of the stabilizer and a reduction in QY [25, 26].

To obtain high-quality QDs, the reaction time has to be optimized to realize equilibrium of the attachment/detachment rate of the cadmium-thiol complexes around the QD surfaces [44]. In our experiment, a 10–60 min reaction time at a temperature of 100 °C favored the formation of high-quality QDs with different colors (blue, green, yellow, and orange) under microwave irradiation of a precursor solution with pH 5–8. As discussed above, CdTe QDs with a high QY were obtained by optimizing the reaction parameters. CdTe QDs with a maximum QY of 87 % were prepared by reaction at 100 °C for 30 min, with a $\text{Te}:\text{Cd}^{2+}$ molar ratio of 5:1 and at pH 6.0.

Characterization of CdTe QDs

The synthesized CdTe QDs were characterized by TEM, EDS, XRD, and FT-IR. High-resolution electron microscopy analyses (Fig. 6a, b) revealed that the nanocrystal structures had a diameter of 2.9 nm with homogenous size distribution.

Fig. 8 Energy-dispersive X-ray spectroscopy analysis results of CdTe QDs

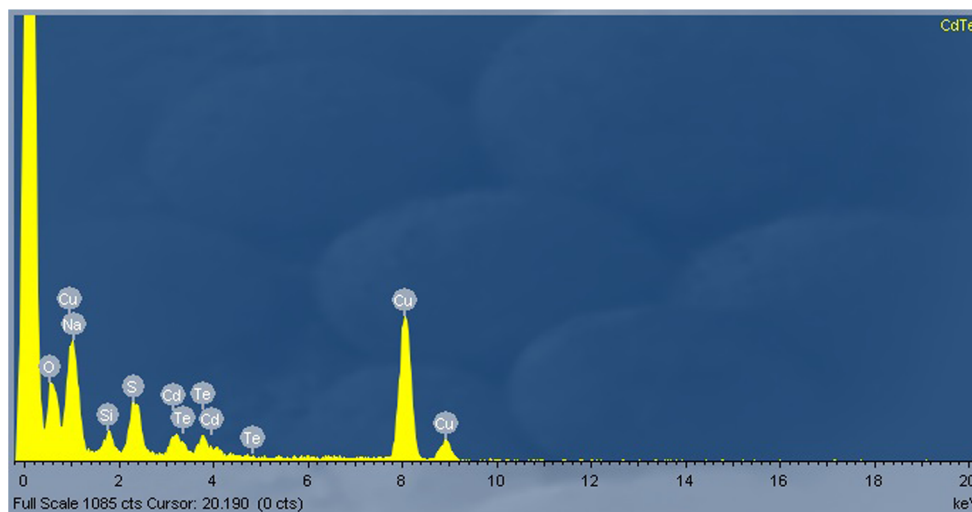
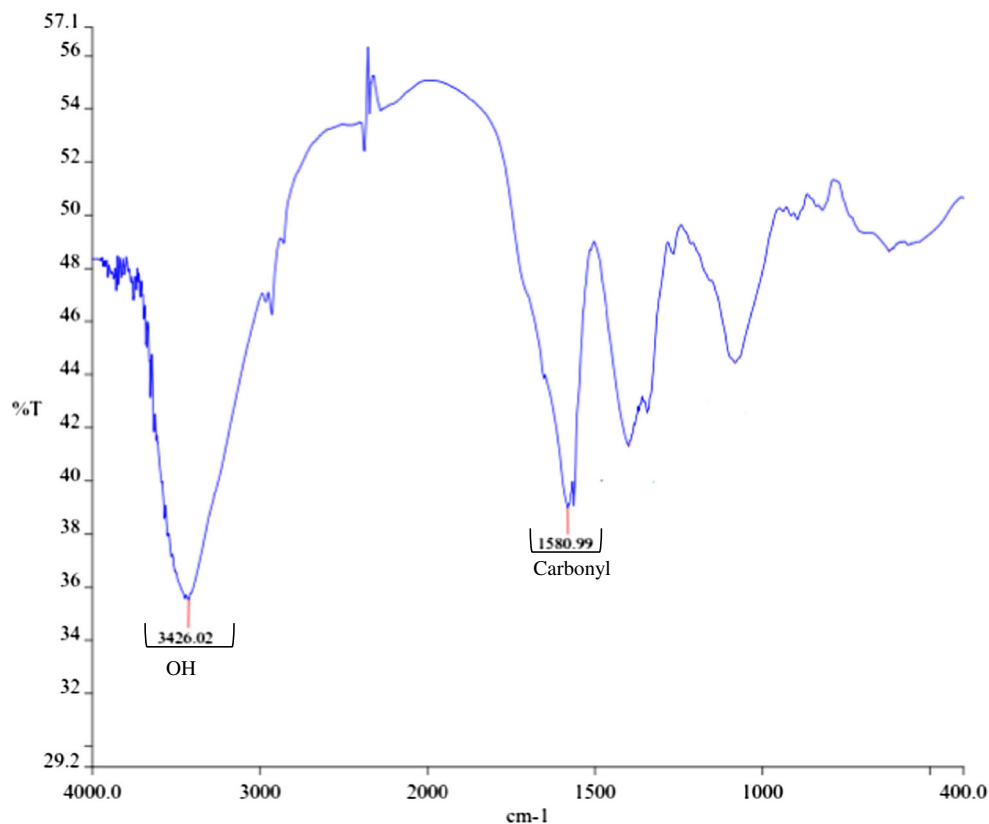


Fig. 9 FT-IR spectra of synthesized MSA-capped CdTe QDs



SAED patterns illustrate diffuse rings consistent with the cubic phase of CdTe nanocrystals without any impurities such as cadmium oxide or tellurium (Fig. 7b).

The XRD pattern of the CdTe QDs, illustrated in Fig. 7a, demonstrates three reflections at $d=24.5^\circ$, 40.6° , and 48° , which is characteristic of a typical cubic zinc-blend structure corresponding to (111), (220), and (311) planes [45]. The positions of XRD peaks matched quite well with the reported peaks of cubic CdTe (JCPDS card no. 15–0770).

The cubic CdTe reflections (black) and cubic CdS reflections (red) are shown as solid lines below the scanned diffraction pattern. Noticeably, all the three peaks are close to the peaks of cubic CdS (JCPDS 89–0440). This result indicated the formation of CdS on the surface of CdTe, which has been reported previously [46].

In addition, the crystallite size of CdTe QDs as calculated by Scherrer's formula [47] on the basis of XRD spectra was about 3.2 nm, which is consistent with the TEM result.

The EDS pattern of the CdTe QDs further confirmed that the major elements of the nanocrystals were Cd, Te, and S, which demonstrated the formation of mixed CdTe (S) (Fig. 8).

The FT-IR spectra of the MSA-capped CdTe QDs are shown in Fig. 9. In the spectrum of CdTe-MSA QDs, peaks at 3,426 and 1,580 cm^{-1} represent the O–H vibration and carbonyl-stretching vibration of MSA, respectively, on the surface of the QDs.

Aptamer Conjugation

To verify the conjugation of the AS1411 aptamers to the MSA-capped CdTe QDs, agarose (2.5 %) gel electrophoresis was used to separate the components in the coupling reaction.

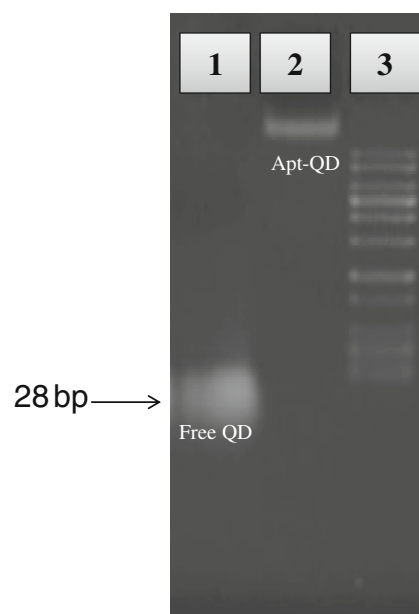
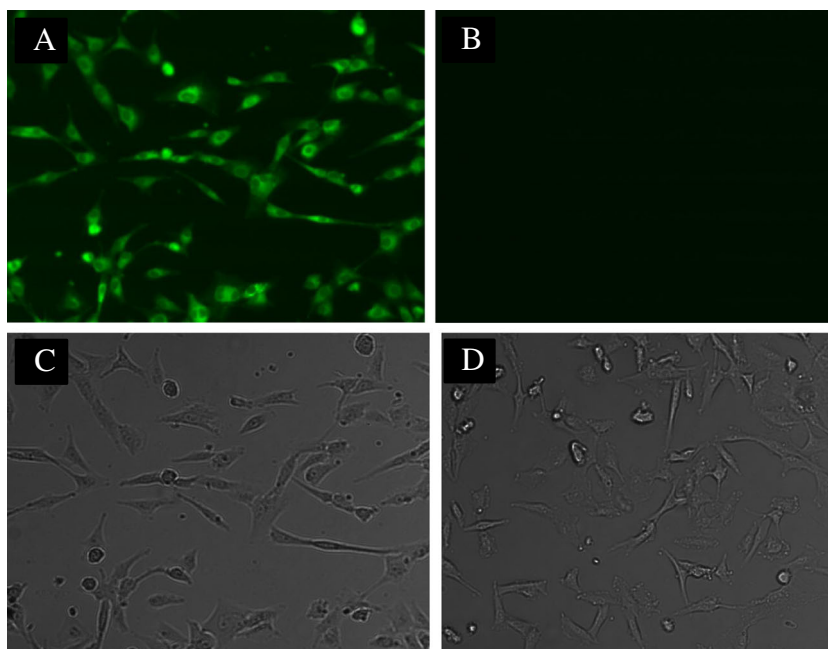


Fig. 10 2.5 % gel agarose electrophoresis results. Lane 1: free AS1411 aptamer; Lane 2: AS1411-aptamer-conjugated CdTe QDs; Lane 3: DNA marker

Fig. 11 In vitro staining of human glioblastoma U87MG cells using AS1411-aptamer-conjugated QDs nanoprobe. **a** and **c** Microscopy images of cells with Apt-QDs under fluorescent and bright field, respectively. **b** and **d** Microscopy images of cells with free QDs under fluorescent and bright field, respectively



The gel electrophoresis data in Fig. 10 demonstrate that Lane 2 (Apt-QDs nanoprobe) was unable to run on the gel, indicating the formation of a QD-Apt complex. Moreover, other bands were not observed in the lane of Apt-QDs probes, suggesting that free aptamers had been removed.

Cell Imaging

To implement QDs as an efficient nanoscale-targeted imaging agent for in vitro or in vivo bioimaging, they need to be conjugated with specific biorecognition molecules (e.g., nucleic acids, proteins, and antibodies) [48–50]. We chose the ssDNA aptamer AS1411 over nucleolin in order to develop QD bioconjugates. The high-level expression of nucleolin is correlated with the proliferative status of cells, and nucleolin levels are higher in tumors and actively dividing cells. AS-1411 is the first ssDNA aptamer to reach phase I and II clinical trials for the targeting and treatment of cancers such as gliomas, myelomas, and lymphomas. AS-1411 can bind to nucleolin specifically and is subsequently internalized into the tumor cell [51, 52].

In the current study, we investigated the potential of the Apt-QDs complex for targeting and bioimaging cancer cells. For this reason, the binding ability of the Apt-QDs to U87MG glioblastoma cells was first evaluated. As shown in Fig. 11a, the U87MG glioblastoma cells incubated with Apt-QDs exhibited a strong green color that could not be washed away. This suggests that the Apt-QDs were able to bind to the U87MG glioblastoma cells with high binding affinity. These data are in agreement with previous reports [53]. Conversely, free QDs cannot bind with U87MG glioblastoma cells (Fig. 11b, d), indicating that the cell surface of nucleolin

interacts with U87MG but not with free QDs at 4 °C. The obtained results confirmed that the Apt-QDs complex can target U87MG glioblastoma cells with high affinity and specificity.

Conclusions

We successfully synthesized high-quality MSA-capped CdTe QDs through a simple and reliable microwave-assisted reduction route that uses air-stable sodium tellurite as the Te source and cadmium chloride as the Cd source. The application of a buffer with good buffering capacity over a broad pH range as a reaction medium is crucial to synthesize CdTe QDs with high fluorescence strength. By varying the pH of the precursor buffer between 5 and 8 and the reaction time between 10 and 60 min at 100 °C under microwave irradiation, CdTe QDs with four colors were prepared in less than one hour without prolonging the reaction time over hours or increasing the reaction temperature.

In the current study, by selecting MSA, MSA-capped CdTe QDs were synthesized at a low pH (5–8) and with a high QY of up to 87 %. In contrast to other thiol-capping agents, including MPA, TGA, cystamine, or GSH, which preserve the ability at a basic pH of >8, MSA can functionalize the surface of CdTe QDs at lower pH values (<8). Since the reducing capability of NaBH₄ enhances at low pH values (5–8), the reduction rate of TeO₃²⁻ to Te²⁻ increases, ensuring facile formation of CdTe QDs. Thus, this method provides an easy and reliable route for the synthesis of high-fluorescence MSA-capped CdTe QDs of a very small size without the use

of harsh experimental conditions generally employed in previously reported methods. Moreover, the synthesized CdTe QDs exhibited excellent stability as their fluorescence intensity was maintained stable for two months when stored in the dark at 4 °C. Finally, we synthesized AS1411-aptamer-conjugated QDs and successfully developed a class of Apt-conjugated nanoprobe that could bind with human glioblastoma U87MG cells and exhibited in vitro molecular imaging with high fluorescence strength and specificity.

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Competing Interests The authors declare that they have no competing interests.

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