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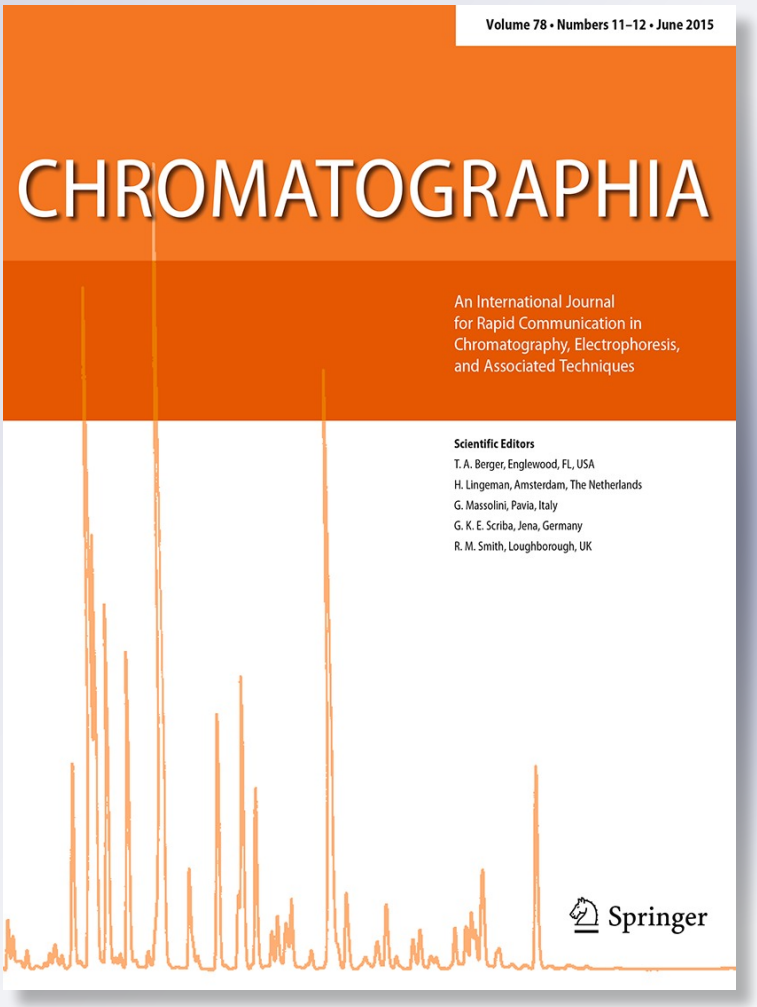

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ORIGINAL

SDS-PAGE as a Tool for Hydrodynamic Diameter-Dependent Separation of Quantum Dots

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Abstract The purpose of this study was to test quantum dots (QDs) separation by native and Tris–Glycine SDS-PAGE according to the protocols commonly used for protein analyses. To study the electrophoretic behaviour of quantum dots, ten samples of previously synthesized CdTe QDs stabilized with mercaptosuccinic acid (MSA) were used. Prior to electrophoresis the hydrodynamic diameters of QDs and zeta potentials were determined, as well as the fluorescence properties and stability of QDs in the running buffers. After verification of QDs stability and separation in native polyacrylamide gel, SDS-PAGE in gradient 4–20 % polyacrylamide gel was performed. Under UV irradiation a colour-dependent separation of QDs was observed, which was consistent with their hydrodynamic diameter distribution. The electrophoretic conditions were further optimized with respect to achieving the optimal colour separation, fluorescence stability and to minimize the time of analysis. Based on the results obtained, for further work 15 % polyacrylamide gels with SDS were used and the times (30–60 min) and voltage (100–150 V) used for separation were optimized. Under the optimal separation conditions (30 min, 100 V) the addition of MSA in the concentration range 0–4 mM was used to improve visualization of QDs with diameters in the range from 7 ± 2 to 4 ± 2 nm.

Keywords Quantum dots · SDS-PAGE · Electrophoresis · Separation · Hydrodynamic diameter-dependent

Abbreviations

QDs	Quantum dots
DLS	Dynamic light scattering
AGE	Agarose gel electrophoresis
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
PAGE	Polyacrylamide gel electrophoresis
DNA	Deoxyribonucleic acid
SDS	Sodium-dodecyl sulphate
SDS-PAGE	Polyacrylamide gel electrophoresis in presence of sodium-dodecyl sulphate
MPA	Mercaptopropionic acid
MSA	Mercaptosuccinic acid
T-G	Tris-glycine
EDTA	Ethylenediaminetetraacetic acid

Introduction

Quantum dots (QDs) are semiconductor nanocrystals with diameters in the range of 1–10 nm. They have unique spectral properties featured mainly by the hydrodynamic diameter and/or composition-tuneable emissions due to quantum size effects [1–4]. The range of their use varies from chemical to physical, computing and biological sciences [5]. In biological applications, QDs are employed in imaging, labelling and sensing. The unique optical properties enable to use them as in vivo and in vitro fluorophores, as well as tools for the labelling of cells and tissues; they are also

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efficient donors in fluorescence resonance energy transfer [6–8]. QDs can form bioconjugates with proteins, small molecules and oligonucleotides by a direct link to defined binding sites. These bioconjugates have a wide field of applications, from immunoassays to cells and organelles imaging or mutation detection. Other important applications of QDs appear to be traceable drug delivery, intracellular sensors [9], vectors for gene therapy [10], quantum computers, [11] and photodetectors [12].

Several analytical techniques, namely laser dynamic light scattering (DLS), laser Doppler electrophoresis, capillary electrophoresis and gel electrophoresis are suitable to characterize the nanocolloids [13–17]. Optical characterization of QDs is usually provided by UV–VIS and photoluminescence spectroscopy, which offers fast, non-destructive and contactless option. The hydrodynamic diameter of QDs is generally calculated using conventional techniques such as scanning electron microscopy and/or transmission electron microscopy. In addition, a number of different techniques have been employed to characterize QDs surface chemistry: particularly X-ray photoelectron spectroscopy, nuclear magnetic resonance spectroscopy, and Rutherford backscattering may be utilized [1]. To use size-dependent nanoparticles properties, such as quantum confinement, it is critical to have nanoparticles with the lowest diameter dispersion possible. Therefore, the hydrodynamic diameter-selective separation of metal nanoparticles has become increasingly important for both fundamental studies and biological applications [18–20]. Gel electrophoresis is a widely applied technique used for biomolecules analysis. As well as for biomolecules, gel electrophoresis has been used to analyse nanoparticles. Agarose gel electrophoresis (AGE) using Tris–Acetate–EDTA (TAE) or Tris–Borate–EDTA (TBE) buffer, commonly used for nucleic acids analysis is most frequently employed. Main purposes are to purify QDs conjugates from unconjugated ligands [21, 22], to check the interaction of QDs with the ligand [23, 24] and/or to prove the stability of the conjugates and coatings [25]. AGE has proven to be an excellent means for the hydrodynamic diameter-selective separation of nanoparticles. Hydrodynamic diameter and ζ-potential can be evaluated by variation of the agarose content and application of a Ferguson plot [26].

Polyacrylamide electrophoresis (PAGE) generally allows more effective separation of both proteins and nucleic acids. Unlike in native DNA, where all molecules have similar charge and shape and the only resolution factor is the molecular weight of the molecule, proteins have different molecular weight, charge, shape, and surface modifications [27]. As well as proteins, QDs generally may differ in all above mentioned parameters [28, 29]. Separation of crystalline dendrimer-stabilized gold nanoparticles by native PAGE with Coomassie-blue staining has been reported by Shi et al. [30].

Tris–Glycine SDS-PAGE (Laemmli system) has been commonly used for protein analysis for more than four decades. This method enables separation of proteins mixture according to their molecular weight. This is achieved by reducing of proteins charge and shape influence by SDS. Effectiveness of proteins separation is increased by use of stacking and resolving gels, which differ in polyacrylamide concentration, ionic strength and pH. This system allows effective separation of proteins in the range approximately from 300 to 10 kDa.

The aim of this study was to compare QDs separation by AGE and SDS-PAGE. To study electrophoretic behaviour of quantum dots, CdTe QDs stabilized with mercaptosuccinic acid (MSA) were used.

Experimental Section

Chemicals

Unless otherwise indicated, all chemicals were purchased from Sigma Aldrich (USA) in ACS purity. Deionized water underwent demineralization by reverse osmosis using the instruments AquaOsmotic 02 (AquaOsmotic, Tisnov, Czech Republic) and then it was subsequently purified using Millipore RG (MilliporeCorp., USA, 18 MΩ)-MilliQwater.

Preparation of Quantum Dots

10 mL of cadmium acetate (5.32 mg/mL) and 1 mL mercaptosuccinic acid (60 mg/mL) were mixed properly with 76 mL of deionized water using a magnetic stirrer. 1.8 mL of ammonia (1 M) solution and 1.5 mL of sodium tellurite (4.43 mg/mL) were also added and mixed very well. 40 mg of sodium borohydride was added slowly later to the solution. Then the solution was stirred for around 2 h until the bubble formation stopped and finally the volume of the solution was made up to 100 mL with deionized water. 2 mL of this solution was taken in small glass vessels and heated at different temperatures (50, 60, 70, 80, 90, 95, 100, 110 and 120 °C) and 300 W for 10 min (ramping time 10 min) under microwave irradiation (Multiwave3000, Anton-Paar GmbH, Graz, Austria) to prepare QDs of different colours. The prepared CdTe QDs were stored in dark at 4 °C.

Determination of QDs Hydrodynamic Parameter and Zeta Potential

The average particle hydrodynamic parameters and diameters distribution were determined by quasielastic laser light

scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, UK). Nanoparticle distilled water solution of 1.5 mL (1 mg/mL) was put into a polystyrene latex cell and measured at a detector angle of 173°, a wavelength of 633 nm, a refractive index of 0.30, a real refractive index of 1.59, and a temperature of 25 °C.

Spectrophotometric and Fluorimetric Measurements

Spectrometric measurements were performed using a multifunctional microplate reader Tecan Infinite 200 PRO (Tecan group Ltd., Männedorf, Switzerland). The absorbance scans were recorded in the range of 230–1000 nm each 2 nm. Emission wavelengths were measured at excitation wavelengths of 380 and 312 nm each 2 nm.

The stability of QDs fluorescence in the presence of single gel components was tested by mixing QDs in ratio of 1:1 (V/V) letting to interact for 20 min at 25 °C in total volume of 100 μL.

Determination of Quantum Yields

The quantum yields were determined by the emission maxima analysis with the excitation at 380 nm. The absorbance was analysed using the same excitation wavelength. The quantum yields were calculated on the basis of integration absorption and emission value. Absolute values were calculated using the standard samples, which have a fixed and known fluorescence quantum yield value, according to the following equation:

$$\phi_X = \phi_{ST}\left(\frac{\mathrm{Grad}_X}{\mathrm{Grad}_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right) \quad (1)$$

where the subscripts ST and X denote standard and test, respectively, ϕ is the fluorescence quantum yield, Grad the gradient from the plot of integrated fluorescence intensity vs. absorbance, and η the refractive index of the solvent. The determination error was calculated to about 6 %.

Tris–Glycine SDS-PAGE

Tris–Glycine SDS-PAGE was performed according to Laemmli et al. [31]. For electrophoresis Mini Protean Tetra apparatus with gel dimension of 8.3 × 7.3 cm (Bio-Rad, USA) was used. The Mini Protean TGX gels with gradient polyacrylamide concentration of 4–20 % were purchased from Bio-Rad, USA. For hand-casted gels, first 15 % (m/V) running, and then 5 % (m/V) stacking gel was poured. The gels were prepared from 30 % (m/V) acrylamide stock solution with 1 % (m/V) bisacrylamide. Composition of running gel was as follows: 15 % (m/V) acrylamide, 0.5 % (m/V) bisacrylamide, 0.1 % SDS (m/V), 0.083 % TEMED (V/V), 0.05 % APS (m/V), 0.376 M Tris/HCl, pH 8.8. Composition of stacking gel is as follows: 4.5 % acrylamide (m/V), 0.15 % bisacrylamide (m/V), 0.1 % SDS (m/V), 0.1 % TEMED (V/V), 0.05 % APS (m/V), 0.125 M Tris/HCl, pH 6.8. For preparation of native gels SDS was omitted both in running and stacking gels. The polymerization of the running or stacking gels was carried out at room temperature for 45 min. To minimize free polymer subunits and catalysers concentrations, the gels were casted in advance and stored overnight in 4 °C. The samples were loaded in 5 μL Sample Buffer (200 mmol L^{-1} Tris, pH 6.8; 2 % SDS, 40 % glycerol, 0.04 % Coomassie brilliant blue G-250; Bio-Rad, USA) in 1:1. For monitoring of the separation, mobility and fluorescence normalization the prestained fluorescently labelled protein ladder "Precision plus protein standards" from Bio-Rad was used. The electrophoresis was run at 150 V for 1 h at 20 °C in Tris–Glycine running buffer (0.025 M Trizma-base, 0.19 mol L^{-1} glycine and 3.5 mmol L^{-1} SDS, pH 8.3). For native electrophoresis the same running buffer was used with omitting of SDS. The separation conditions of the electrophoretic separation (time and voltage) were optimized. To test the effect of MSA on QDs separation, gels with MSA in concentrations of 0, 1, 2 and 4 mM were used.

Agarose Electrophoresis

The gels were prepared from 1.5 % agarose in 0.5 × TAE buffer (20 mmol L^{-1} Tris, 10 mmol L^{-1} acetic acid, 0.5 mmol L^{-1} EDTA, pH 8). The samples were loaded 5:1 in sample buffer (200 mmol L^{-1} Tris, pH 6.8; 2 % SDS, 40 % glycerol, 0.04 % Coomassie brilliant blue G-250; Bio-Rad, USA). The electrophoresis was run at 100 V for 50 min at room temperature (Biometra, Germany) in 0.5 × TAE buffer. The fluorescence of QDs was monitored in 5-min intervals. To test the effect of MSA, 2 mM MSA was added to gel.

Gels Documentation and Processing

Separated QDs in gels were visualized using common UV transilluminator at wavelength of 312 nm and photographed.

Function molecular weight (MW-RF/OD) of Biolight software (Vilber Lourmat, France) was used for calculation of relative mobilities of the bands by contour recognition. The image analysis was performed by image processing software ImageJ using the Gels function.

Results and Discussion

Characterization of QDs

CdTe QDs were prepared from stock solution of cadmium acetate, mercaptosuccinic acid and sodium tellurite

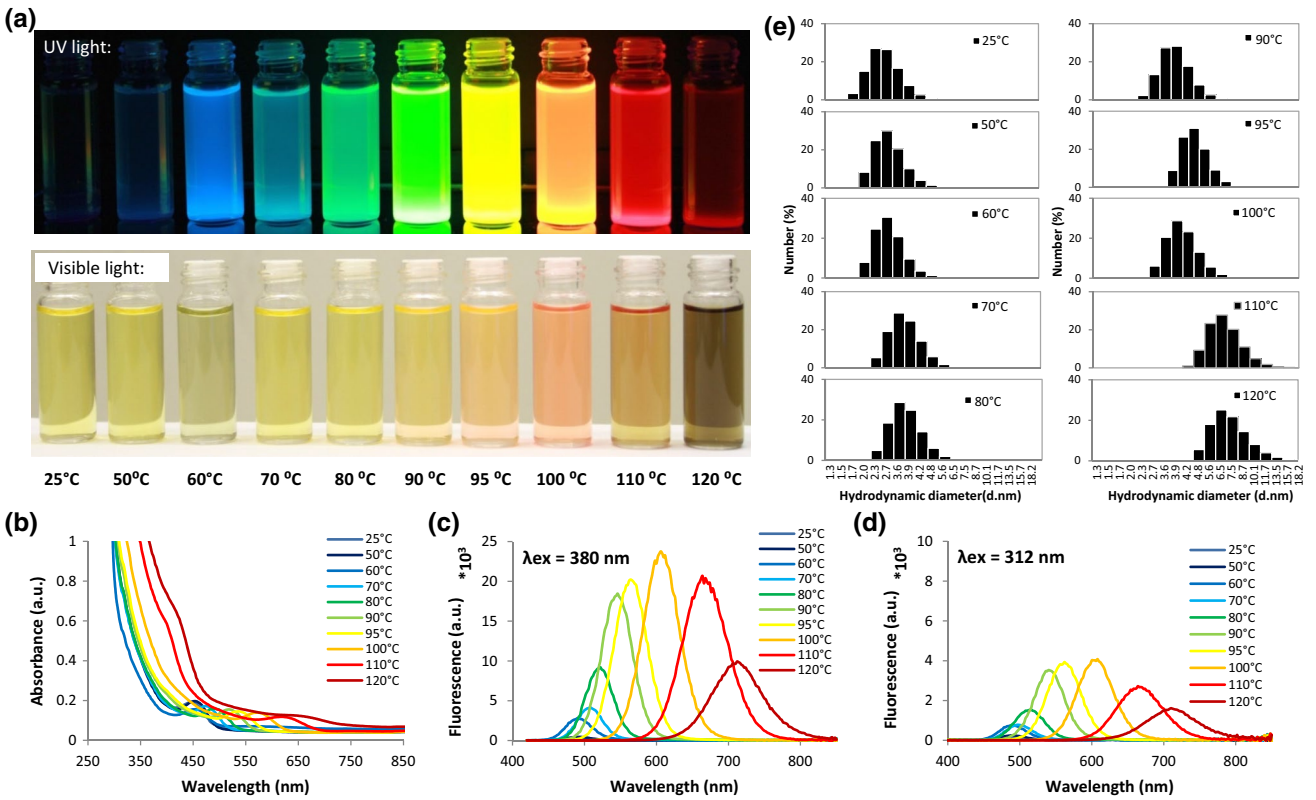

Fig. 1 Characteristics of the CdTe/MSA QDs synthesized under different temperatures. **a** Photography of the QDs under UV irradiation (*upper*) and under visible light (*lower*), **b** absorption spectra, **c** emission spectra at excitation wavelength of $\lambda = 380$ nm, **d** emission spectra at excitation wavelength of $\lambda = 312$ nm, **e** size profiles of QDs (diameters)

by microwave heating. This process was optimized and according to the temperature different colour QDs can be prepared (for details see "Experimental Section"). The colour of the QDs was checked under UV transilluminator (Fig. 1a). Further, the absorbance and the emission spectra of CdTe QDs were measured (Fig. 1b–d). At excitation wavelength of 380 nm the highest intensities of fluorescence were obtained, while at excitation wavelength of the UV transilluminator ($\lambda = 312$ nm) approximately 10 % of original fluorescence intensity was achieved (Fig. 1c, d). The absorbance and fluorescence characteristics of CdTe QDs are summarized in Table 1.

Quantum yields for single QDs were in the range from 0.3 to 42 %, for summary see Table 1. The smallest QDs (25 °C) exhibited very low quantum yields, only 0.3 %. The quantum yields increased with the increasing hydrodynamic diameters of the QDs up to nanoparticles with the medium diameters [39 % for green QDs (90 °C) and 42 % for yellow QDs (95 °C)]. In samples with the higher excitation maximum, the quantum yields were again decreased with 7 % for the largest QDs (120 °C).

The hydrodynamic diameters of the QDs were measured using Zetasizer by DLS method. The hydrodynamic diameter of blue CdTe QDs was found to be 2 ± 1 or 3 ± 2 nm, for green QDs it was found to be 3 ± 2 nm, for yellow QDs 4 ± 2 nm, for orange QDs was 5 ± 2 nm and for red QDs it was found to be 7 ± 2 nm, respectively, for summary see Table 1. From QDs hydrodynamic diameter distribution graphs (Fig. 1e), it follows that the resulting fluorescent spectra are resultant from QDs in more hydrodynamic diameters that are present in the sample.

The zeta potentials were for all QDs in range from −26 to −48 mV indicating their negative charge (Table 1).

Electrophoretic Separation of QDs

First, the stability of QDs fluorescence in TAE buffer of different concentrations was tested (Fig. 2a). After 30-min interaction with the buffer, at largest QDs (120–80 °C), the differences in fluorescence were in range of standard deviation. At smaller QDs (70–25 °C), approximately 20 % quenching of QDs fluorescence was observed, indicating the stability of QDs in TAE buffer.

After separation of QDs by agarose gel electrophoresis in 1.5 % agarose gel in 0.5× TAE buffer [32], QDs migrated as one monochromatic band. Without stabilizer (2 mM MSA), the fluorescence of QDs was unstable. Even after 5-min separation, only five samples of QDs with

Table 1 Fluorescent properties, quantum yields, major hydrodynamic diameters and zeta potentials of analysed CdTe/MSA QDs

Sample no.	λ_{em} (nm)	λ_{abs} (nm)	$\lambda_{em\ 312}$ (nm)	Quantum yield (%)	Major hydrodynamic diameter (nm)	ζ-potential (mV)
1	486	448	492	0.3	2 ± 1	−39.2
2	494	454	488	1	3 ± 2	−35.3
3	490	454	490	10	3 ± 2	−34.9
4	508	474	498	10	3 ± 2	−42.9
5	522	490	516	23	4 ± 2	−38.5
6	546	518	540	39	4 ± 2	−41.2
7	564	536	562	42	4 ± 2	−32.0
8	606	572	602	35	5 ± 2	−25.5
9	664	620	664	18	7 ± 2	−47.9
10	704	662	710	7	7 ± 2	−39.8

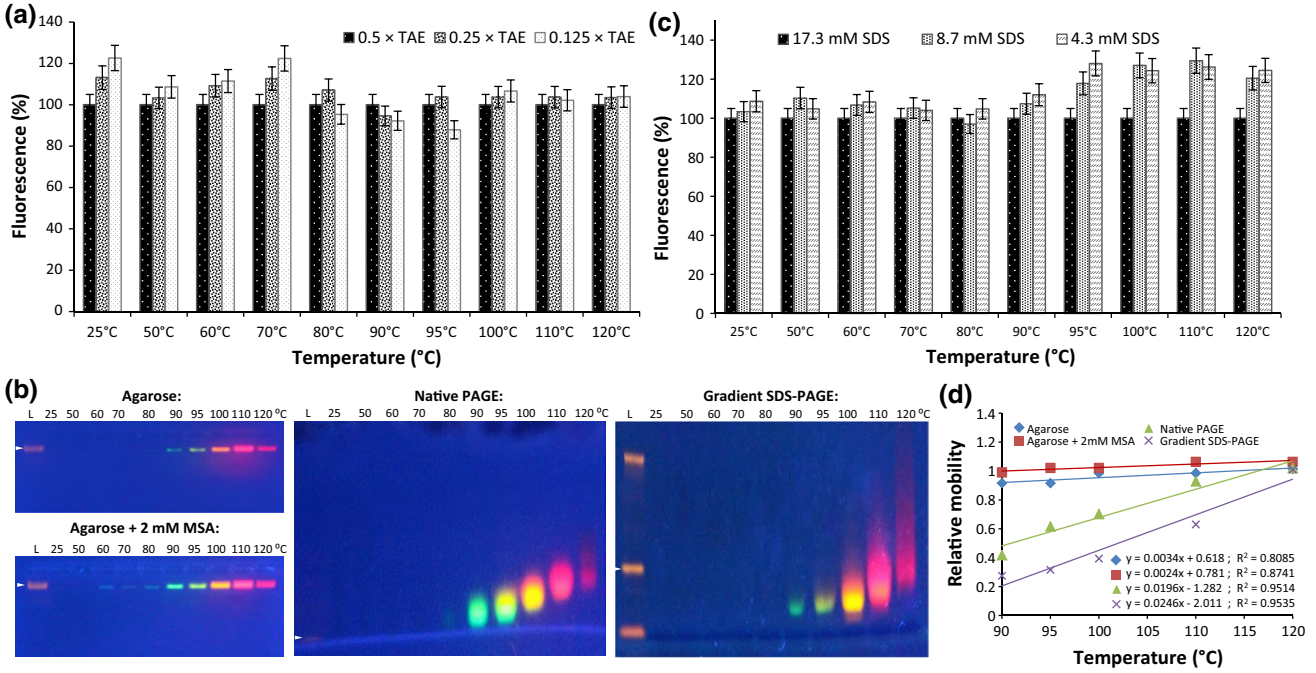

Fig. 2 **a** Stability of CdTe/MSA QDs fluorescence in TAE buffer, **b** comparison of QDs separation by agarose electrophoresis in 0.5× TAE buffer with or without addition of 2 mM MSA to gel and by native PAGE and gradient SDS-PAGE. *Arrow* indicates the band of the standard used for relative mobility estimation, **c** stability of CdTe/MSA QDs fluorescence in the presence of SDS, **d** dependence of the QDs relative mobilities after separation in agarose gel with or without 2 mM MSA and in native and gradient PAGE

highest hydrodynamic diameters were visible. To improve the stability of QDs during the separation, 2 mM MSA was added to the gel. After 5-min separation all QDs except for two smallest ones (25 and 50 °C) were detectable after UV irradiation (Fig. 2b). After 10-min separation without MSA addition, the fluorescence was detectable only at red QDs, in the presence of MSA, the fluorescence was detectable only at QDs with the highest size (red to green). After 15-min separation, the fluorescence was detectable only at red QDs (110 and 120 °C). Compared to AGE without MSA addition the fluorescence of red QDs was visible even after 50-min separation, but no differences in samples mobilities were observed. For complete fotodocumentation see Supplementary Materials.

PAGE has generally higher separation efficiency compared to AGE. In protein electrophoresis, SDS is added to sample to improve protein separation. Prior the electrophoresis in polyacrylamide gels the fluorescence properties and stability of QDs in Tris–Glycine running buffer with addition of SDS were studied (Fig. 2c).

Fluorescence intensities and maxima were not influenced by buffer presence. The addition of SDS to

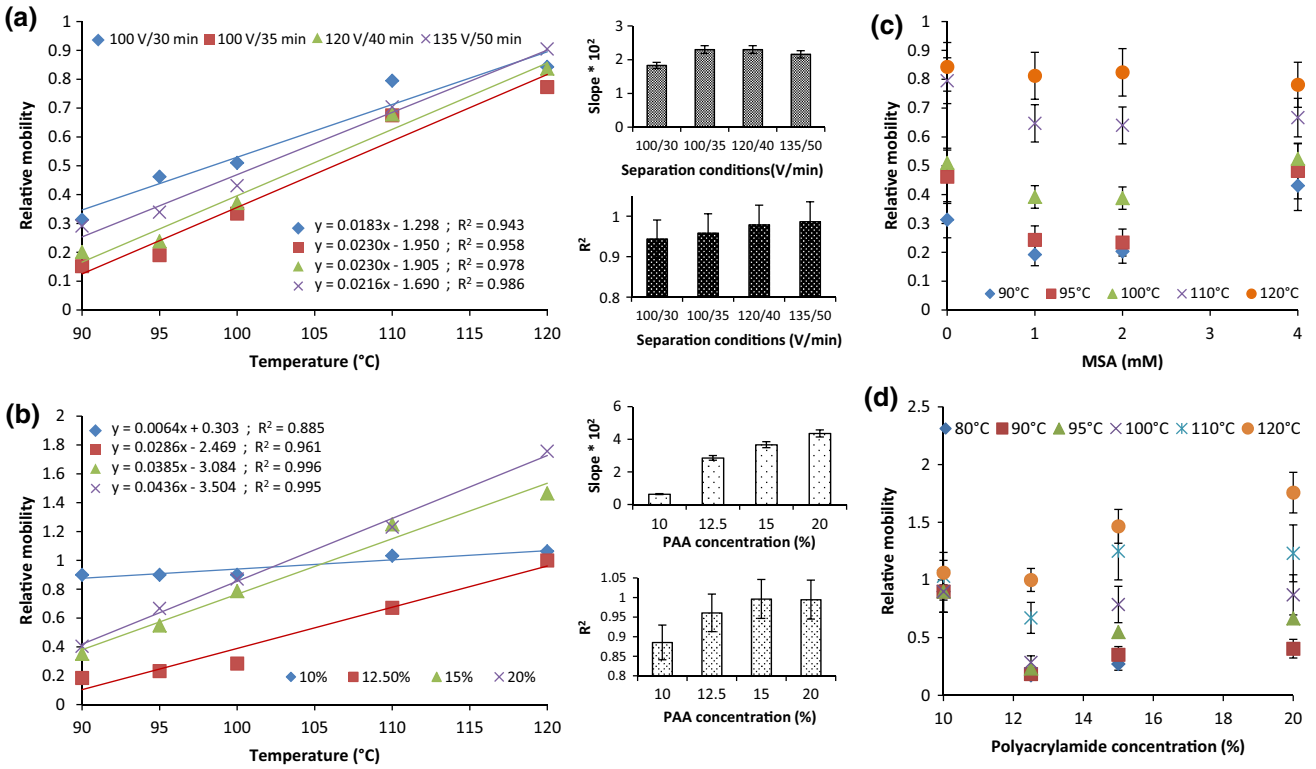

Fig. 3 **a** Dependence of the QDs relative mobilities after separation by SDS-PAGE under different voltages and times, **b** dependence of the QDs relative mobilities after separation by SDS-PAGE in gels with different polyacrylamide concentrations and with addition of 4 mM MSA, in the inset: slopes and reliability coefficients of the dependences, **c** relative mobilities of various-sized QDs in dependence on MSA concentration, **d** relative mobilities of various-sized QDs in dependence on polyacrylamide concentration

samples after 20-min interaction caused approximately 30 % quenching of the fluorescence of QDs with the highest diameters (120–90 °C). At the smaller QDs, the addition of SDS did not influence the signal height. In the native system, a shift of QDs mobility in dependence on their hydrodynamic diameter was observed at five largest QDs. Moreover, it was observed, that the bands are colour-separated from green to yellow and red (Fig. 2b). The mobility shift and colour separation was improved in SDS-system with gradient polyacrylamide concentration in range 4–20 % (Fig. 2b).

To quantify the separation of QDs, relative mobilities of the bands to the fluorescent marker were determined. The dependence of the relative mobilities for single QDs on their preparation temperature, i.e., hydrodynamic diameters, are shown in Fig. 2d. While in the agarose gels all samples migrated with practically uniform mobilities, in polyacrylamide gels the relative mobility of the QDs increased linearly in dependence on their preparation temperature, which exhibited as two-order increase of the slope value and increase of the determination coefficient above 0.95.

The electrophoretic conditions were further optimized with respect to achieving the optimal colour separation, fluorescence stability and minimizing the time of analysis. For further work 15 % polyacrylamide gels with SDS were used, due to their better resolving in QDs migration area, as estimated from Fig. 2b gradient gel. In this system, different voltages and electrophoretic times were tested (Fig. 3a). With increasing voltage and separation time, both slopes and determination coefficients increased slightly. The highest slope together with determination coefficient was obtained after 40-min separation at 120 V. Even after 50-min separation the fluorescence of QDs was sufficient for imaging, but no improvement in separation efficiency was achieved. After 60 min, the QDs fluorescence was slightly quenched, especially at smaller QDs (for fotodocumentation see Supplementary Materials).

Despite all effort, the smallest blue and green QDs were still not detected in SDS-PAGE system without stabilizers. The addition of MSA to the gels increased QDs fluorescence in the gels and further improved the separation (Fig. 4a). In the presence of 1 and 2 mM MSA the range of relative mobilities for single QDs broadened, i.e., the slope values increased with determination coefficients above 0.98. In the presence of 4 mM MSA the range of relative mobilities narrowed, i.e., the slope value decreased, but the determination coefficient of the separation was above 0.99 (Fig. 4b). Moreover, addition of 4 mM MSA allowed us to

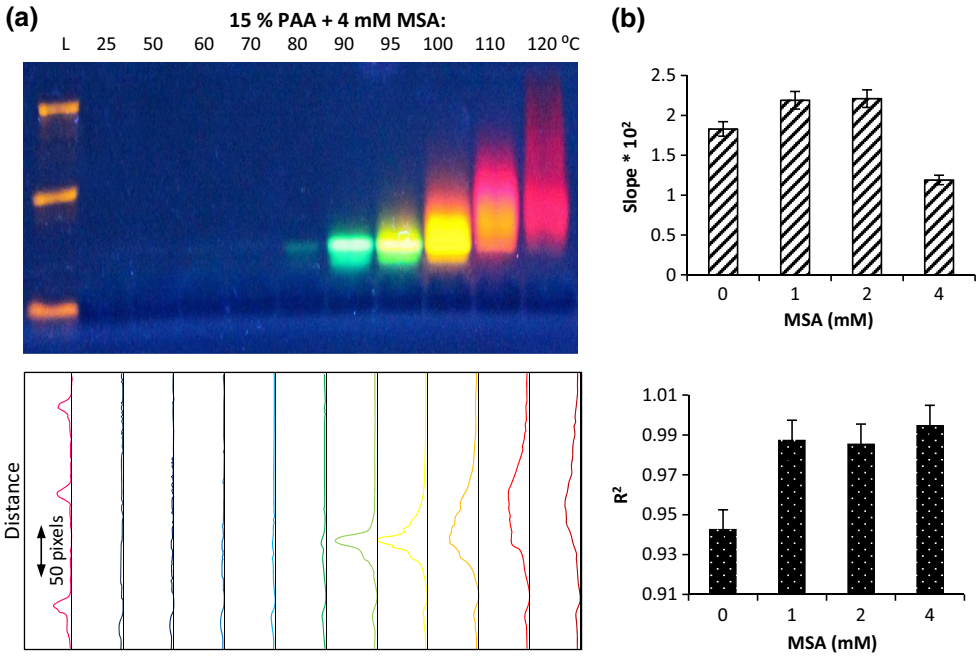

Fig. 4 **a** Photograph of gel electrophoresis analysis of QDs in 15 % polyacrylamide gel (*top*) and software transformation of the gel image to densitometric curves (*bottom*), **b** dependence of the QDs relative mobilities after separation by SDS-PAGE with MSA addition and dependence of the slopes and reliability coefficients of the mobility dependences after separation of QDs by SDS-PAGE with MSA addition

visualize the smaller green QDs prepared at temperature of 70 °C (Fig. 4a).

After separation in polyacrylamide gels, a band broadening at red QDs was observed (Fig. 4a; Supplementary Materials). This indicated, that QDs with higher hydrodynamic diameter are either adsorbed to gel matrix or retained due to small pores diameters in the gel. The mean hydrodynamic diameter of red QDs was determined as 7 ± 2 and 5 ± 2 nm or lower for other QDs (Table 1). According to hydrodynamic diameters distribution, also QDs in size above 8 nm are present in the red QDs, which are not present at smaller QDs (Fig. 1e). The mean pore diameters and its distribution in polyacrylamide gels are dependent on total gel concentration (%T), crosslinker concentration (%C), TEMED and APS concentration and polymerization temperature. For SDS-PAGE with gels of equivalent crosslinker concentration the mean pores diameter of 7.5 % gel was approximately 5–10 nm [18], Lo et al. [33] predicted pores size in 9 and 12 % polyacrylamide gel as 5 and 4 nm, respectively, or 2.5 nm in 14 % polyacrylamide gel according to [34]. Based on mean QDs hydrodynamic diameters and distribution in the samples, the gels with different polyacrylamide concentration (10, 12.5, 15 and 20 %) were tested at 120 V for 40 min. From graph in Fig. 3b, c it is obvious, that QDs were not separated in 10 % gels; the range of relative mobilities was narrow and the dependence slope value was comparable with agarose. At higher polyacrylamide concentrations QDs were separated, the range of relative mobilities broadened and the dependence slope value increased with increasing polyacrylamide concentration. The determination coefficient of the dependences was above 0.99 for 15 and 20 % gels. However, at 20 % gels the QDs prepared at 80 °C were not detectable. Nevertheless, the blue QDs remained still undetectable using a common transilluminator. One reason may be their very low fluorescence at excitation wavelength used for gels imaging (Fig. 1d), or poor visibility of blue light in UV irradiation, which could be improved after using a more suitable imaging system. Other reason may be their lower stability compared QDs with higher hydrodynamic diameter [35]. It was reported, that fluorescence of QDs was quenched due to the residues of polymerization agents ammonium persulfate [36], tetramethylethylenediamine [37] or acrylamide [38]. However, after polymerization of the gels, the free polymer subunits (acrylamide and bisacrylamide) and polymerization catalysers are present in trace concentration and their concentration is lowered by overnight storage of the gels [39].

After comparison of the QDs diameters with fluorescence spectra, bands colours and intensities, it was observed that the band colour was consistent with QDs diameters. Similar results were observed also for CdTe, CdSe and CdS QDs stabilized either with MPA or glutathione (not shown). It is known that optical properties of quantum dots depend on their hydrodynamic diameter [40]. QDs with diameter of approximately 5 nm emit red light, QDs with diameter of approximately 1.5 nm emit blue light [41]. Determination of QDs hydrodynamic diameter by fluorescence was previously performed by Mutavdzic et al. [42]. However, except of used buffer the emission maximum can be shifted with stabilizer and its concentration [43]. Moreover, the fluorescence lifetime of QDs

is dependent on their hydrodynamic diameter, thus larger QDs are more stable than smaller QDs [42].

Our results showed that SDS-PAGE was the most effective for hydrodynamic diameter-dependent CdTe/MSA QDs separation with direct UV imaging. Agarose gel electrophoresis using TAE or TBE buffer is commonly used for nucleic acids analysis, especially due to its simplicity. Main purposes are checking of QDs conjugation with ligands, such as antibodies, streptavidin or DNA. The resolving capacity of agarose gel is limited by its thickness, pore diameters and buffer composition (especially pH and ionic strength) [26].

PAGE generally allows more effective separation of both proteins and nucleic acids. Polyacrylamide is capable to resolve QDs more effectively, as shown in study of Hlavacek and Skladal [44], who in polyacrylamide Tris–Glycine system replaced SDS with 2 mM thioglycolic acid (QDs stabilizing agent). Separation of crystalline dendrimer-stabilized gold nanoparticles by native PAGE with Coomassie-blue staining has been reported by Shi et al. [30], who also observed diameter-dependent shift in nanoparticles mobility. In our work, the separation of QDs in PAGE was improved by addition of SDS and MSA compared to native system.

Conclusion

To our knowledge, SDS-PAGE in common arrangement has not been used for quantum dots separation and characterization yet. Generally, QDs are stabilized and hydrophilized by surface modification either with organic acid, such as thioglycolic acid, MSA, MPA, aminoacid, peptide, and protein, or with chemical group enabling further conjugation with target ligand. In general, quantum dots are stable at slightly basic pH and vary in their hydrodynamic diameter and charge. SDS was previously used as QDs stabilizer [45], therefore it can be hypothesized that SDS is able to replace other QDs stabilizers and in QDs separation it plays a similar role as in proteins electrophoresis, for example, charge and shape unification.

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Conflict of interest The authors have declared no conflict of interest.

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