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Characterization of CdSe quantum dots with bidentate ligands by capillary electrophoresis

Research Article

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Abstract: The CdSe quantum dots (QDs) with bidentate ligands: α-diimine (NN) and dihydrolipoic acid (DHLA) were synthesized and characterized by UV-Vis, particle size and capillary electrophoretic techniques. Two systems were analyzed: CdSe with one ligand (CdSe/ligand) and CdSe with two different ligands (CdSe//ligand1/ligand2), where ligand = α-diimine or DHLA. Hydrodynamic features of functionalized QDs were characterized by zone capillary electrophoretic (CZE), and particle size techniques and these methods were consistent. It was established that CZE, micellar (MEKC) and microemulsion (MEEKC) modes were suitable for separating charged CdSe QDs and that no peaks were obtained for QDs passivated with electrically neutral ligands. For CdSe QDs with neutral (NN) ligands, a preconcentration method with the use of a micellar plug was introduced for visualizing these QDs. A sharp peak representing neutral QDs was obtained within the zone of micellar plug of a non-ionic surfactant, Here, a ligand character used for CdSe modification and the type of the electrophoretic method applied were the determining factors for the QDs peak visualization. Moreover, examples of visualization of charged and neutral QDs on the same run were presented, and for this purpose, dual mechanism (separation/preconcentration) was proposed.

Keywords: *Nanocrystals CdSe • Capillary electrophoresis • Ligand exchange • Dihydrolipoic acid • Surfactants*

1. Introduction

The unique photophysical properties of quantum dot (QD) nanocrystals have drawn considerable attention for sensing, optoelectronic, and energy storage applications. However, incorporating QDs in various milieus requires precise control of the QD surface chemistry for proper dispersion and adaptation to different environments.

As generally prepared, QDs consist of an inorganic nanoparticle surrounded ("passivated") by a layer of organic ligand, *e.g.* trioctylphosphine (TOP). The hydrophobic surface does not allow QDs to disperse in aqueous solutions. Thus, it is often necessary to exchange a surface ligand in order to provide surface reactivity and/or to disperse QDs in different solutions [1-3]. This is often accomplished by displacing the original surface ligand with thiol-terminated ligands of specific functionality for subsequent surface reactions. Several examples of ligand-exchange for QD surface modification have been reported, where sophisticated interfacial architecture have been proposed [1-5]. However, the exact nature of the QD surface chemistry is still relatively unknown, in terms of how the nanoparticle parameters, such as surface charge density and hydrodynamic radius affect colloidal stability, monodispersity, and surface reactivity, or electrostatic interactions between QDs and other entities in solution. This lack of characterization is due, in part, to lack of suitable analytical methods for making such determinations. To date, most QD characterizations are accomplished by transmission electron microscopy (TEM), UV-Vis, and photoluminescence where determinations are primarily relegated to QD size.

Among these, the electrophoretic techniques, planar gel and capillary, were frequently applied to the characterization of nanocrystals. The planar gel

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electrophoresis is probably the most frequently used in the laboratory practice among electrophoretic methods, which was recently examined towards determination of QDs hydrodynamic size and ζ-potential [6], as well as towards the number of biological ligands attached to a particular nanocrystal [7,8].

The capillary electrophoretic methods for the separation and characterization of particles or nanoparticles were recently reviewed in the context of theory and the method development [9-12]. Numerous reports have shown that CE methods could be employed for the separation of various-type nanoparticles. The earliest reports focused on the separation of colloidal Au, Ag, or Ag/Au (core/shell) nanoparticles [13-16]. It was shown, for example, that the separation of water-soluble Ag/Au nanoparticles could be achieved in the micellar mode (MEKC) of CE [13,14]. More recently, it was shown that CE could also be applied to the separation and characterization of semiconducting QDs after various surface modifications [17-23]. Capillary zone electrophoresis (CZE) has also been demonstrated as a useful tool for monitoring the efficiency of exchange for surface ligands of QD and QDs surface functionality towards biomolecules (*e.g.* streptavidin/biotin) [20].

Despite considerable effort to establish convenient capillary electrophoretic methods for the routine practice, there is only a slight progress in the method development. This is due to intrinsic feature of (charged) particles, where the following effects play the main role in their mobility: (i) the electrostatic force exerted by external electric field, (ii) the Stokes friction, (iii) the electrophoretic retardation, and (iv) the relaxation effects [9,16,22]. In order to overcome limitations enforced by those effects, some non-conventional modes of the capillary electrophoresis were recently tested. As a result, a successful CE separation of CdTe nanocrystals according to size with the size span 2.6 nm was recently proposed, with a polymer as sieving medium [17]. The reversed electrode polarity stacking method of CE with the use of an anionic surfactant was examined for the enrichment of Au nanoparticles allowing an increase in detection, up to 260 folds [24,25]. The method of determining effective QDs surface charge using fluorescence correlation spectroscopy/microchip electrophoresis was recently reported [23]. Strong interaction between QDs and micellar aggregates, using QDs coated with surfactants, was also recently reported. This effect allows focus QDs using CZE or MEKC mode of separation [26].

The present work has two aims. First, QDs modified by bidentate ligands: dihydrolipoic acid DHLA and/or α-diimine (NN) derivatives of various polarities as shown in Supplementary Fig. 1A, were obtained. Bidentate ligands provide the superior stability over monodentate ones [27]. The former assure the QDs highest stability, preventing them from aggregation and ligand dissociation, which is a key factor in obtaining data repeatability. Additionally, such approach with well characterized (NN) ligands [28] used for QDs covering, allows for obtaining QDs differing in key parameters (*e.g.* charge, hydrodynamic size), but in a controllable manner. This allows establish a system, where thus prepared and well characterized QDs can be used as a tool for the characterization of other techniques or procedures.

The second aim was to examine capillary electrophoretic techniques, zone CZE, micellar MEKC and microemulsion MEEKC for the separation and characterization of QDs.

Although, the capillary electrophoretic method development for the separation of nanocrystals was the main aim of the present work, the experimental data presented here were supported by additional techniques. Thus, particle size analysis was applied to determine the QDs hydrodynamic parameters, used as the reference to the CE results. A tool – molecular modeling at the *ab initio* DFT level - was introduced to support the approach applied to ligands exchange, where TOP (trioctylphosphine) surface ligands were replaced by bidentate ligands *via* a pyridine intermediate, as proposed in [29]. Both these issues were included to the Support Electronic Section.

The present work provides direction in the area, where α-diimine (NN) ligands play the crucial role during metal ↔ ligand charge transfer, and it is important for up-to-date projects concerning energy transformation such as light → electricity. On the other hand, due to its (S,S) binding atoms, DHLA ligand is widely used as covering a ligand (commercially available QDs) for CdSe or CdSe/ZnS QDs. Ligand also enables further bioconjugation (*e.g.* [1-4]).

2. Experimental Procedure

2.1 Instrumentation

A P/ACE MDQ CE system (Beckman Coulter, Fullerton, CA) with photo-diode array (PDA) detection was used. An IBM PC and Beckman 32 Karat 5.0 software was utilized to control the CE instrument and acquire the data. Fused-silica capillaries with 75 μm inner diameter were purchased from (Polymicro Tech., Phoenix, AZ). The capillary length (window/total length) was 60/70 cm. The injection was typically performed at a pressure of 0.5 psi for 5 s. Prior to the daily use, the silica capillary

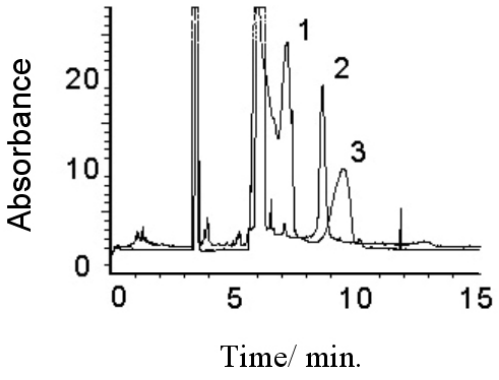

Figure 1. Comparison of separation of CdSe/DHLA and CdSe/$(PhSO_3)_2$phen QDs by means of MEKC method. Peaks: 1, ligands (DHLA, $(PhSO_3)_2$phen); 2, CdSe/DHLA; 3, CdSe/$(PhSO_3)_2$phen. Both QDs have the same size, 2.6 nm. MEKC conditions: background buffer: 50 mM SDS and 10 mM sodium tetraborate, voltage +20 kV.

was pretreated by flushing sequentially for 15 min with 0.1 M NaOH, 5 min with water, and 5 min with background buffer. The capillary was also rinsed with organic solvents (chloroform + methanol) to remove the QDs adsorbed to the wall of the capillary (4 min), water (3 min), 0.1 M NaOH (5 min), water (3 min) and buffer (5 min) between each run. The separation capillary was temperature controlled at 25°C by liquid cooling of the P/ACE MDQ instrument.

Particle size analyzer, Zetatrac (Microtrac, Mantgomeryville, PA, USA) was used for the measurement of hydrodynamic parameters (size, ζ-potential) of nanocrystals analyzed in the present work.

The HP8453 UV-VIS spectrophotometer with HP ChemStation software was used (Hewlett-Packard, Palo Alto, CA), and spectra were collected with the use of a 1 cm optical length cuvette. Amicon Ultra 15 centrifugal filter tubes (50,000 NMWL), (Millipore, Billerica USA) were used for purification of water or water/MeOH soluble QDs.

2.2 Chemicals and reagents

All chemicals and reagents used herein were of analytical grade. For the synthesis of the TOP coated CdSe nanocrystals, the cadmium oxide (~1 micron, 99.5%), selenium powder (100 mesh, 99,999%), trioctylphosphine (TOP, 90%) were used. Ligands: α−lipoic acid was used for preparation of DHLA; α-diimine (NN): bpy (2,2`-bipyridyl), phen (1,10-phenanthroline), $(Ph)_2$phen (4,7-Diphenyl – 1,10-phenanthroline), $(PhSO_3)_2$phen (4,7-Diphenyl-1,10-phenanthroline-disulfonic acid disodium salt). The ligand concentration was in the range of $(2 \pm 0.2) \times 10^{-4}$ mol L^{-1}. Surfactants: SDS (sodium dodecyl sulfate) and Tritons: X-100 (polyethylene(10) isoctylphenyl ether) and N-101 (polyoxyethylene nonylcyclohexyl ether); Poly(ethylene oxide) M_v 8,000,000 and 600,000, were obtained from Sigma-Aldrich (St. Louis, MO) and 1-octadecene (ODE) from Acros Organics (Morris Plains, NJ). Aqueous ionic surfactant solutions in the range 10–700 mM and non-ionic Triton (N-101 and TX-100, 5% and 10%, respectively) were prepared. Salts: sodium tetraborate, potassium chloride, sodium acetate and sodium phosphate (Na_2HPO_4) (Sigma, St. Louis, USA) were utilized. A background buffer of potassium chloride 20 mM was used for zone CE separation and the pH was fixed by diluted HCl and NaOH solutions. The MEKC buffer was based on SDS and the 50 mM SDS with 10 mM sodium tetraborate or on 75 mM SDS with 10 mM sodium phosphate (Na_2HPO_4). The background buffer for MEEKC (stable macroemulsion (weight%): 91.26%, 10 mM sodium phosphate (Na_2HPO_4), 0.82% n-heptane, 1.44% SDS, 6.48% 1-butanol) was prepared. Dimethyl sulfoxide (DMSO) and 4-dodecylaniline (DA) (Sigma-Aldrich) were used as markers for the migration window in MEKC and MEEKC techniques.

2.3 Procedures

2.3.1 Synthesis and purification of CdSe/TOP QDs

The CdSe/TOP QDs in the range of 2.2 - 3.7 nm were synthesized with the use of octadecene and oleic acid [30]. The CdSe/TOP QDs were purified by precipitation method with the use of excess of methanol and acetone. It was experimentally observed that the purification is convenient for larger QDs (> 3 nm). In this case the spontaneous precipitation of such QDs from the solution was observed. For smaller QDs (especially < 2.5 nm) no spontaneous fallout of QDs was observed. In such a case, the modified procedure was applied by heating the mixture containing post reaction solution and excess of methanol and acetone (50 – 60°C, 10 – 15 min) followed by centrifugation (3000 rpm, 10 min). The obtained pellet of CdSe/TOP QDs was washed with 1 mL of MeOH.

2.3.2 Preparation of DHLA ligand

The DHLA ligand was prepared according to the procedure proposed in [4]. Shortly, the commercial α−lipoic acid was reduced by means of sodium borohydride ($NaBH_4$) and the reduced form of dihydrolipoic acid (DHLA) was extracted from the reaction medium with toluene. The excess of water was removed from the toluene phase using magnesium sulphate. The toluene DHLA solution was used for preparation of CdSe/DHLA QDs [4]. During the synthesis of the CdSe with mixed ligands containing DHLA, the DHLA ligand was prepared by removing toluene under vacuum and dissolution of the solid DHLA in ans appropriate solvent (MeOH or mixture of MeOH/water depending on QDs to be prepared).

2.3.3 Ligand exchange

For the synthesis of CdSe/DHLA QDs, procedures proposed in [4,29] were applied. Both syntheses, direct (CDSe/TOP with toluene solution of DHLA) [4] and indirect (CdSe/TOP → CdSe/py → CdSe/DHLA) [29] are recommended here.

The CdSe/pyridine (CdSe/py) QDs were an intermediate product of the synthesis producing variety of final CdSe QDs with various ligands (Supplementary Fig. 1A, supplementary materials). For the preparation of CdSe/py, QDs were added to the 10 - 15 mg of CdSe/TOP QDs, 5 mL of pyridine. It was observed that CdSe/TOP QDs were easily dissolved in pyridine and under conditions (stirring, 60 – 70°C, 1 h), the ligand exchange (TOP → py) was taking place. Next, the obtained CdSe/py QDs were precipitated by the addition of excess hexane. Also in this case, the larger CdSe/py QDs were fallen out spontaneously, however for the smaller one, the modified procedure (heating: 50 – 60°C, 10 – 15 min and centrifugation: 3000 rpm, 10 min) was applied. For the quantitative ligand exchange, the exchange (TOP → py) and purification procedures were repeated three times.

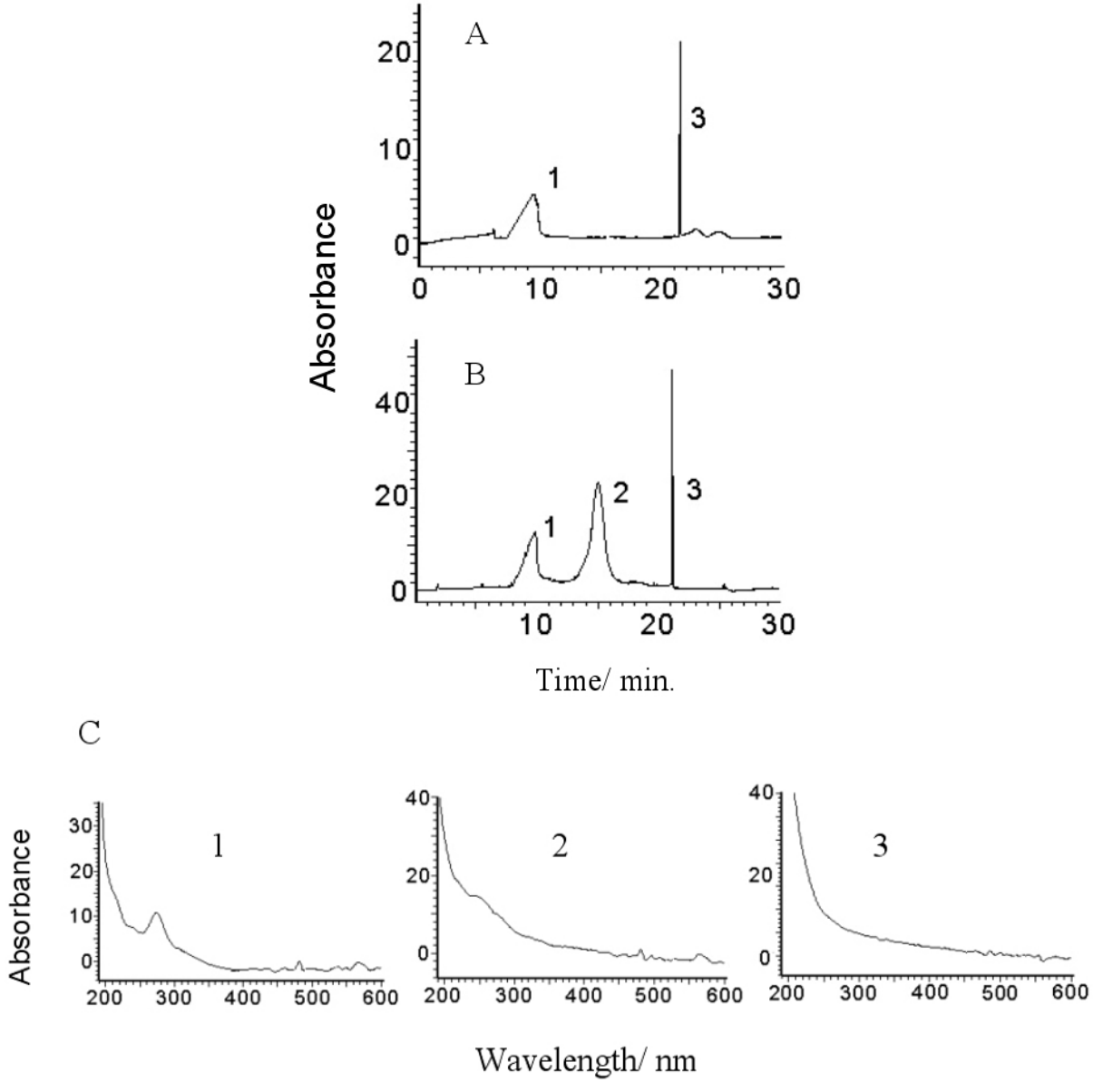

Figure 2. CZE separation of charged QDs: CdSe/ligand and CdSe//ligand$_1$/ligand$_2$. Frames: A, 1 CdSe/DHLA, 3 CdSe/(PhSO$_3$)$_2$phen; B, peaks 1, 3 the same as in frame A; 2, CdSe//(PhSO$_3$)$_2$phen/DHLA; C, spectra of peaks of CdSe QDs. Conditions: background buffer, 20 mM potassium chloride; pH 6.0; voltage 20 kV (→ 44 μA). All QDs were synthesized form CdSe/TOP, size 3.2 nm.

In final synthesis, 300 – 500 µL of ligand solution (NN, DHLA) was added, to the pellet of CdSe/py, in order to obtain CdSe QDs modified by a pair of ligands (CdSe/(NN, DHLA)). The mixture was dispersed under ultrasonification for 1min, followed by stirring at room temperature for 2 hours. Depending on the current conditions (size of CdSe, type of ligand exchange) the transfer of solid CdSe/py into a soluble form (CdSe/ligand) was observed as a sign of the ligand exchange process. However, for a ligand with high polarity ($(PhSO_3)_2$phen), an incomplete dissolution of CdSe/py QDs was observed and in this case an additional heating (60^0C, 30 min) was advised. The obtained CdSe/(NN, DHLA) QDs were purified and concentrated using Amicon Ultra-15 centrifugal filter tubes, which allows removal of an excess ligand and concentrating the obtained QDs. For this, tubes with 50,000 MWCO cut off were applied (3000 rpm, 10 - 15 min) and the progress in purification was controlled using CE separation (MEKC, MEEKC).

2.3.4. Preparation of QDs samples with non-ionic surfactants

20 µL of non-ionic surfactant was added to the methanolic solution of CdSe passivated with neutral ligands. After the sample was left for 2 h for methanol evaporation , it was suitable for CE separation.

2.3.5. Separation of functionalized CdSe nanocrystals by capillary electrophoresis

In order to separate particular QDs or mixtures of QDs, the samples were prepared according to Sections 2.3.3 or 2.3.4 and injected at the anode. Concentrations of QDs were in the range of 10^{-5} (smaller QDs) - 10^{-6} (larger QDs), due to significant difference in their extinction coefficient. The sample injection was done under pressure, typically of 0.5 psi and for 5 seconds. For the separation under CZE mode, 20 mM potassium chloride in the pH range 6 – 8.5 and + 20 kV voltage was applied. In separation of QDs with the use of micellar background buffer (MEKC, MEEKC) the sample injection and voltage, were the same as for CZE mode. Current electrophoretic conditions are presented in a caption of an appropriate figure, which illustrates an appropriate separation. Peaks of QDs were verified according to characteristic diode – array spectra for QDs and times of the QDs have been determined based on this accurate migration. Electropherograms presented in figures were collected at 214 nm, unless stated otherwise, which assured the highest ratio (QDs peak (height, area)/background noise).

2.3.6. Measurement of hydrodynamic parameters for CdSe NCs using the particle size analyzer

The hydrodynamic parameters (size and ζ-potential) for CdSe NCs were obtained from particle size measurement using Zetatrac instrument. For this, a diluted solution of nanoparticles in appropriate solvents (CdSe/TOP in chloroform, CdSe/pyridine and CdSe/(NN) in methanol (apart from CdSe/$(PhSO_3)_2$phen → in water), and CdSe modified by both DHLA and (NN) ligands, in water) were measured using standard procedure: set zero 90 s, run time 100 s, number of runs 2. The particle distribution was measured according to the intensity option. Before measurement the instrument was checked using reference material (Microtrac reference material, silica suspension, part # 400196-100), which allowed testing both parameters (size and ζ-potential).

3. Results and Discussion

3.1 Synthesis and characterization of CdSe/TOP QDs

Synthesis of CdSe/TOP QDs was accomplished by adapting a procedure reported elsewhere [30] The obtained QDs were purified according to a standard procedure [31]. Basic characterization of the synthesized QD core size and concentration was accomplished by UV-VIS spectrophotometry, according to a previously described method [31,32]. By measuring the position of the first exciton peak in the UV-Vis range, it was found that the synthesis procedure resulted in QDs in a relatively narrow size range of 2.2 – 3.7 nm. These QDs, in terms of size, were characterized by TEM technique [26]. A similar procedure for QDs characterization has been previously reported for CdTe nanocrystals prior to their CE separation [17].

3.2 Ligand exchange at the CdSe crystal surface

Synthesis of the ligand exchange products used in these CE studies is illustrated in Supplementary Fig. 2 (Electronic Support Section). In agreement with Kloepfer *et al*. [29] we found that optimal synthesis was achieved when a pyridine (py) ligand intermediate was used, *e.g*. CdSe/TOP → CdSe/py → CdSe/product-ligand. It is believed that this indirect synthesis method improves ligand transfer efficiency due to a better solvent compatibility of pyridine as opposed to hydrophobic TOP. As shown in Supplementary Fig. 2, synthesis products contained either monodentate or bidentate ligands with S or

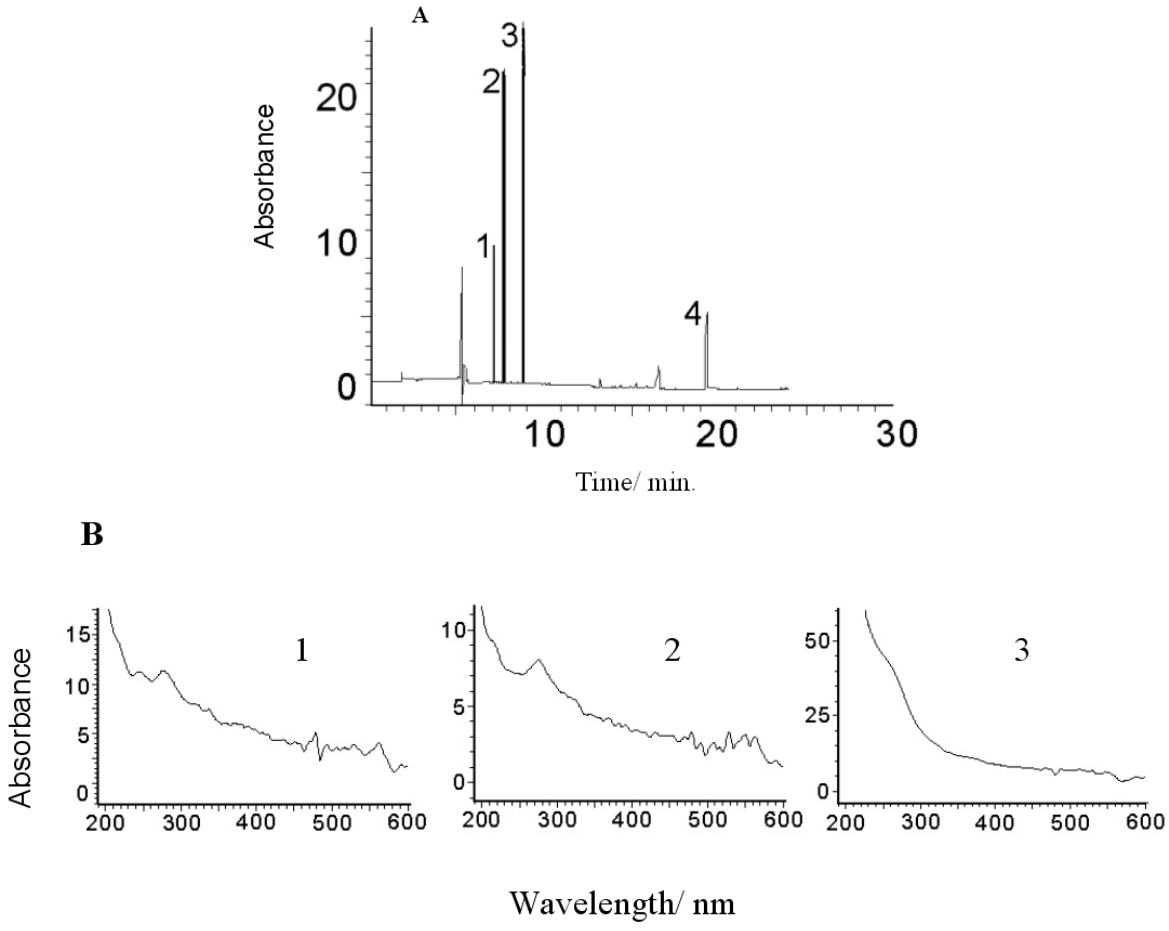

Figure 3. CZE separation of CdSe QDs coated with the mixed ligands, as CdSe//(NN)/DHLA. Frames: A, 1, CdSe//$(Ph)_2$phen/DHLA, 2, CdSe//phen/DHLA, 3, CdSe//bpy/DHLA; 4, CdSe/DHLA; B, spectra of peaks for CdSe QDs from frame A. Spectrum of peak 4 (CdSe//DHLA) is presented in Fig. 2. Conditions: background buffer, 20 mM potassium chloride; pH 8.0; voltage 20 kV (→45 μA). All QDs have the same core size dimension, 3.5 nm.

N functionality for coordination of the nanoparticle surface. To demonstrate versatility, bidentate ligands were chosen with a distribution of polarities (Supplementary Fig. 1A). Purification through 50 kDa MWCO centrifuge filters was applied to remove excess of ligands and to concentrate the final product. To monitor purification, microemulsion electrokinetic chromatography (MEEKC) CE was used to observe both components, ligand and CdSe/ligand QDs, simultaneously. Supplementary Fig. 3 (Electronic Support Section) shows an example used for CdSe/DHLA QDs.

The surface modification of CdSe QDs, due to passivation with two different ligands (NN, DHLA), simultaneously was an interesting continuation of this scheme. Supplementary Fig. 2 (Electronic Support Section) presents a graphical interpretation of this approach. These QDs were synthesized in the way described in the Section 2.3.3, using for the final synthesis (CdSe/py → CdSe//$ligand_1$/$ligand_2$) a mixture, 1:1 = $ligand_1$:$ligand_2$ (mol:mol), which lead to the formation of QDs passivated with two ligands. Although the synthesis with three or more ligands would be interesting, this was not considered in the present work. Also, only the ratio of ligands: 1:1 mol:mol, used for QDs preparation, was considered here. The presence of a (NN) ligand at CdSe surface was confirmed by the UV-Vis spectrophotometry, due to characteristic band from (NN) ligand in UV range; this was in agreement with reported CdSe/ZnS//(NN) QDs system [33]. The same was observed due to: (i) the DAD spectra for CdSe//(NN) peak (Figs. 2-4,6) or (ii) UV-Vis spectra of CdSe//(NN) solutions after ligands exchange and QDs purification (Supplementary Fig. 4). In cases of dual surface ligands, where the ligand polarities are substantially different, a combination of miscible solvents of differing polarity was necessary to maintain a stable nanoparticle suspension. For example, transfer solution of CdSe/py with methanol soluble DHLA and water soluble $(PhSO_3)_2$phen requires a solvent of compromised polarity, *e.g.* methanol/water.

Both, UV-Vis and particle size measurements (estimation of a core and a hydrodynamic size, respectively) were applied to QDs characterization, with respect to ligands exchange. Based on UV-Vis spectra (Supplementary Figs. 4 and 5, Electronic Support Section), it was observed that during the process, a slight decrease in a core size, (CdSe/TOP→CdSe/py): takes place and no further change in a core size, because of ligands exchange (*e.g.* CdSe/py→CdSe/(NN)), was seen. On the other hand, the QDs hydrodynamic size obtained from particle size measurement is a net value due to superposition of two effects: (i) change in a core size, and (ii) effect of a surface ligand volume. This is clearly seen through analysis of Supplementary Fig. 6, where hydrodynamic sizes from CdSe/TOP starting material, through CdSe/py intermediate, to final product CdSe/(NN) QDs were compared. It can be stated that for the same CdSe core size, the hydrodynamic sizes are different for NN and NN/DHLA derivatives, however within each group NN vs. NN/DHLA sizes are similar (Supplementary Fig. 7, Electronic Support Section).

To support the choice regarding surface modification, as well as nature of ligands binding to CdSe surface, short theoretical considerations based on the molecular modeling were included in the Support Electronic Section. This is a valuable extension of the experimental results presented here, from atomic perspective. Obtained results from modeling confirm the binding scheme applied in the present work (binding energy; bidentate > monodentate) and show that binding of ligands with S,S atoms can be different from the one with N,N atoms.

3.3 CE separation of CdSe QDs passivated with (NN) or DHLA ligands

Preliminary CE characterization was done with the use of either bare silica capillary or capillary coated with *N,N*-dimethylacrylamide. The latter has demonstrated greater stability at larger pH values and with various organic solvents applied [34].

The coated capillary was tested for the separation of charged ($(PhSO_3)_2$phen, DHLA) and uncharged (bpy, phen, $(Ph)_2$phen) derivatives of CdSe QDs with the use of either CZE mode (20 mM KCl) or MEKC mode (50 mM SDS, 10 mM acetate). However, no peaks of QDs were observed in this case. The broad peaks of charged QDs were seen when the CZE buffer modified by MeOH was applied (20 mM KCl, 30% MeOH). In this case the migration time of charged CdSe/ligand QDs was long (30 – 40 min) due to suppressed EOF, which led to significant broadening of the peaks. Taking this into account, only an uncoated (bare) silica capillary was used. With this capillary, both modes (zone, micellar CE) were examined for the visualization of charged and uncharged QDs coated with (NN) or DHLA ligands. First, the uncharged CdSe/(NN) (NN = bpy, phen, Ph_2phen) were examined with the use of CZE or NACE (non-aqueous capillary electrophoresis) mode, based on aqueous or methanol background buffers, as both these CE modes cover a wide range of solute hydrophobicity. However, despite the diversity of volumes and polarities of (NN) ligands ($\log P_{o/w}$: bpy (1.6) < phen (2.1) << Ph_2phen (5.9) [28]), applied to the modification (CdSe/(NN) QDs), no peaks of uncharged QDs were seen in this experiment. The same can be stated using micellar (MEKC, MEEKC) mode of separation.

Next, the charged CdSe/ligand (ligand = $(PhSO_3)_2$phen or DHLA) QDs were examined and peaks for these QDs were obtained with the use of both CZE and MEKC modes. First, the MEKC mode (50 mM SDS, 10 mM sodium tetraborate) was examined in detail (Fig. 1). Results, due to applying this mode of separation can be concluded as follows: (i) the general migration order is: ligand < CdSe/ligand, (ii) for the QDs covered with the same ligand (CdSe/$(PhSO_3)_2$phen or CdSe/DHLA), no separation according to core size was observed, and (iii) separation of CdSe/ligand QDs was observed only when CdSe QDs were covered with different ligands. Features (ii and iii) indicate that parameters related to the size of QDs have minor importance, whereas interfacial properties related to QDs surface architecture play the major role in electrophoretic separation of QDs.

The next conclusion is, that by application of the micellar background buffer, peaks of QDs are broad and close to each other for CdSe passivated with one ligand (CdSe/ligand), as well as with two ligands (CdSe//$ligand_1$/$ligand_2$), thus the applicability of this separation mode for the characterization of these QDs is deeply limited. This may be due to a mixed separation mechanism involving additional coating of QDs, with surfactant molecules, during a run [14]. On the other hand, (MEKC, MEEKC) modes were useful for practical purposes *e.g.* for controlling QDs purification (Fig. S3) and due to the possibility of visualizing all sample components on the same electropherogram.

The separation mode (CZE) was the next examined method. In this mode the migration of charged QDs is a direct function of hydrodynamic properties of QDs; thus, some features for these QDs can be estimated.

3.4 CZE separation of QDs system: CdSe// $ligand_1$/$ligand_2$

The first example considered here was the system where CdSe QDs were passivated with either DHLA or $(PhSO_3)_2$phen ligand. Both QDs were negatively charged and the Fig. 2 shows that a separate peak was

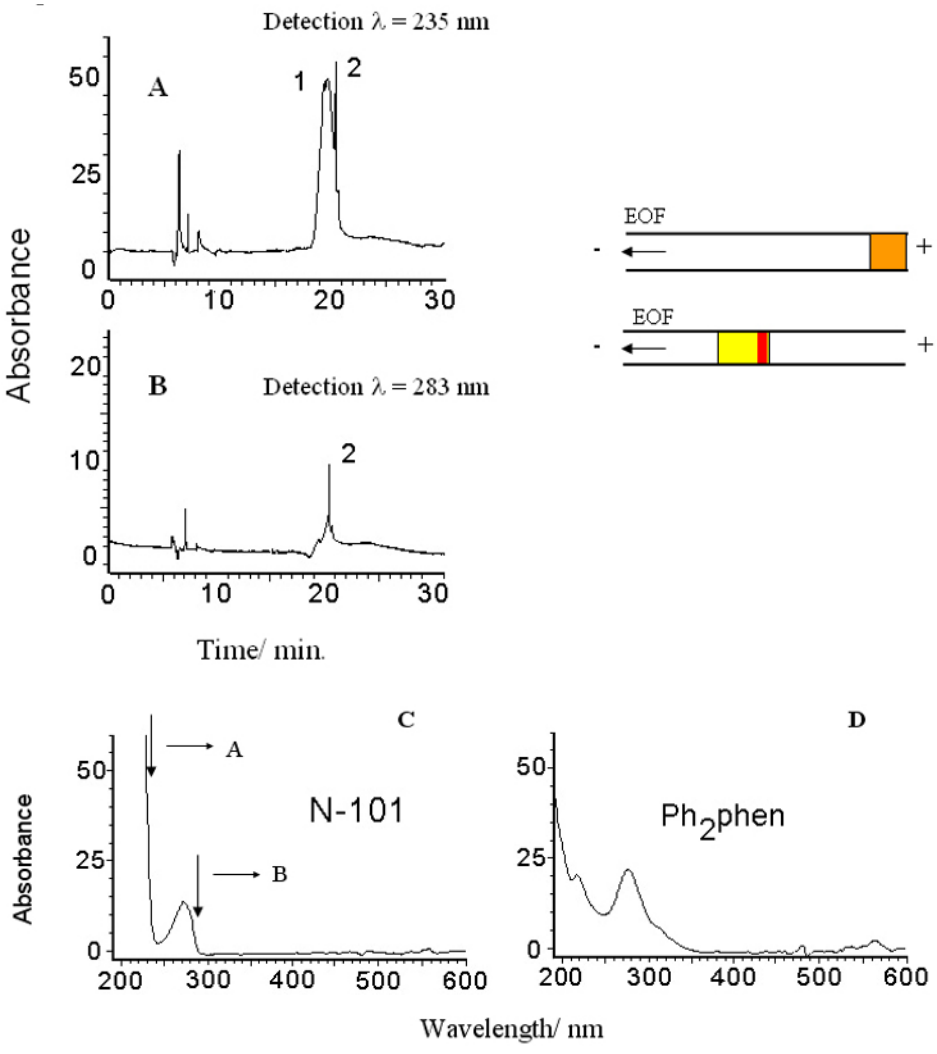

Figure 4. Visualization of uncharged CdSe/(NN) QDs due to MEEKC electrophoresis of a micellar plug of a non-ionic surfactant. Sample: CdSe/$(Ph)_2$phen QDs in the presence of 10% solution of Triton N-101. Frames: A, electropherogram recorded at 235 nm shows both peaks: (1) Triton N-101, and (2) CdSe/Ph_2phen; B, electropherogram recorded at 283 nm shows only peak of CdSe/Ph_2phen; C, spectrum of N-101. Arrows at frame (C) show wavelengths used to record A and B electropherograms; D, spectrum of peak 2, typical for CdSe/(NN) QDs. Conditions: MEEKC background buffer, 20 kV (→ 70 μA). CdSe/$(Ph)_2$phen QDs, core size 3.5 nm. As an inset is the graphical interpretation of the focusing effect. Upper - starting point, orange layer denotes injected plug – a sample of mixture CdSe/$(Ph)_2$phen QDs and Triton N-101. Below, the situation at detection window, yellow layer represents the surfactant plug inside capillary, narrow red layer denotes zone of CdSe/$(Ph)_2$phen QDs. Arrow shows the electroosmotic flow.

obtained for CdSe/DHLA or CdSe/$(PhSO_3)_2$phen QDs, respectively, under the same run. For both these QDs, as an example, the dependence of migration of QDs, on pH of the background electrolyte was examined (Electronic Support Section, Supplementary Fig. 8). It was concluded that, constant electrophoretic mobility was observed in the pH range of 6 – 8.5 and the range was applied to all separations, applied in the present work. The second aspect, an impact of an electrolyte pH on the separation selectivity was also considered, as CdSe/DHLA contains, able to ionize carboxylic groups, whereas CdSe/$(PhSO_3)_2$phen, containing ($-SO_3^-$) groups, should not be sensitive to pH in this range. This statement is supported by [23], where for CdTe/MPA (MPA = mercaptopropionic acid) QD, its surface charge, estimated from electrophoresis data, was a function of electrolyte pH, and used to confirm ligands exchange at the QDs surface. However, data presented in Supplementary Fig. 8 show that a switch in the migration selectivity can be at pH < 4, which is difficult to obtain, due to a significant decrease in EOF, in this pH range, observed through the position of the acetone peak (marker) (inset, Supplementary Fig. 8).

The second issue was the separation of CdSe QDs coated with a pair of ligands (CdSe//$ligand_1$/$ligand_2$ QDs), which were prepared according to Section 2.3.3.

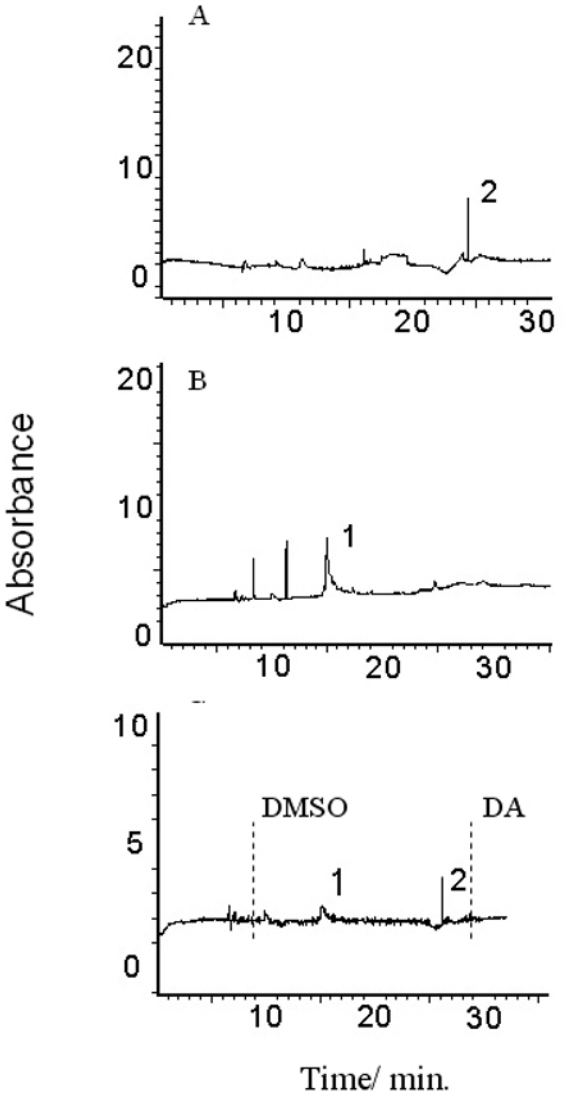

Figure 5. Separation of charged CdSe/$(PhSO_3)_2$phen and uncharged CdSe/Ph_2phen QDs with the use of Triton N-101 as a micellar plug, by means of MEEKC method. A, B, electropherograms of single QDs, C, mixture of both. Peaks: 1, CdSe/$(PhSO_3)_2$phen, 2, CdSe/Ph_2phen. Conditions – MEEKC background buffer, 20 kV (→ 70 mA). The dashed vertical lines denote the separation window detected by DMSO and DA, respectively. Both QDs have the same core size, 3.5 nm.

Referring to the literature, an example of QDs coated with a pair of ligands (DHLA, DHLA-PEG600) was recently reported for CdSe/ZnS QDs [6]. It was stated that, ζ-potential for QDs coated with a pair of ligands was lower than for QDs coated with only one ligand (DHLA) [6].

The first example of the CdSe//ligand$_1$/ligand$_2$ QDs system is CdSe//$(PhSO_3)_2$phen/DHLA QDs and the CZE electropherogram of the sample of QDs after ligand exchange (Section 2.3.3) shows the presence of a peak, which migrates within the window formed by both CdSe/$(PhSO_3)_2$phen and CdSe/DHLA QDs (Fig. 2B). The origin of the peak (apart from migration time) was additionally confirmed by comparing spectra obtained for each peak, from DAD detector (Fig. 2C). Thus, the absence of a shoulder in the spectrum for CdSe/DHLA was observed, whereas the DAD spectra of both peaks for CdSe/$(PhSO_3)_2$phen and CdSe//$(PhSO_3)_2$phen/DHLA QDs contain a shoulder in UV range, characteristic for QDs/(NN) system [33]. Thus, both these QDs are surely derivatives of (NN) ligands.

The similar separation procedure was applied to CdSe QDs coated with a pair of ligands, namely, neutral ligand (bpy, phen and $(Ph)_2$phen) and charged ligand (DHLA). It has been pointed out (Section 3.3), that no peak for CdSe QDs, coated with only neutral ligand (bpy, phen and Ph_2phen) was observed using either CZE or micellar (MEKC, MEEKC) mode. Thus, applying a mixed (neutral/charged) ligands system for passivating CdSe QDs, allows introduce the charge to these QDs. This was confirmed by measuring ζ − potential, using particle size analyzer (Supplementary Fig. 7) and due to an appearance of a peak with characteristic band from (NN), in QD/(NN) spectrum (Fig. 3B). Fig. 3 shows an example of the CZE separation of CdSe QDs coated with the following pairs of ligands: bpy/DHLA, phen/DHLA, $(Ph)_2$phen/DHLA and finally CdSe/DHLA, for comparison. The migration order was: CdSe//$(Ph)_2$phen/DHLA < CdSe//phen/DHLA << CdSe//bpy/DHLA << CdSe/DHLA. This issue will be discussed in the next Section 3.5.

3.5 Electrophoretic behavior of functionalized CdSe QDs – a comparison

Generally, the mobility, μ, of a rigid nonconducting spherical particle undergoing electrophoretic migration in a medium of viscosity η has been related to:

$$\mu = \frac{2\varepsilon\zeta}{3\eta \bullet f(\kappa R)} \quad (1)$$

where, ε is the dielectric permittivity of the medium, ζ the particle's zeta potential [9,22]. The function f(κR) ranges from 1 (Hückel limit) to 1.5 (Smoluchowski limit) [9,16,22]. Under the present experimental conditions, applying the same background buffer (20 mM KCl) means that both ε and η are constant. Using CdSe QDs with the same core size, thus with similar hydrodynamic size, as observed from either UV-Vis or particle size measurement, along with the same ionic strength of the background buffer, the constancy of f(κR) function can be assumed. Thus, the only hydrodynamic variable in this system is the zeta (ζ) potential.

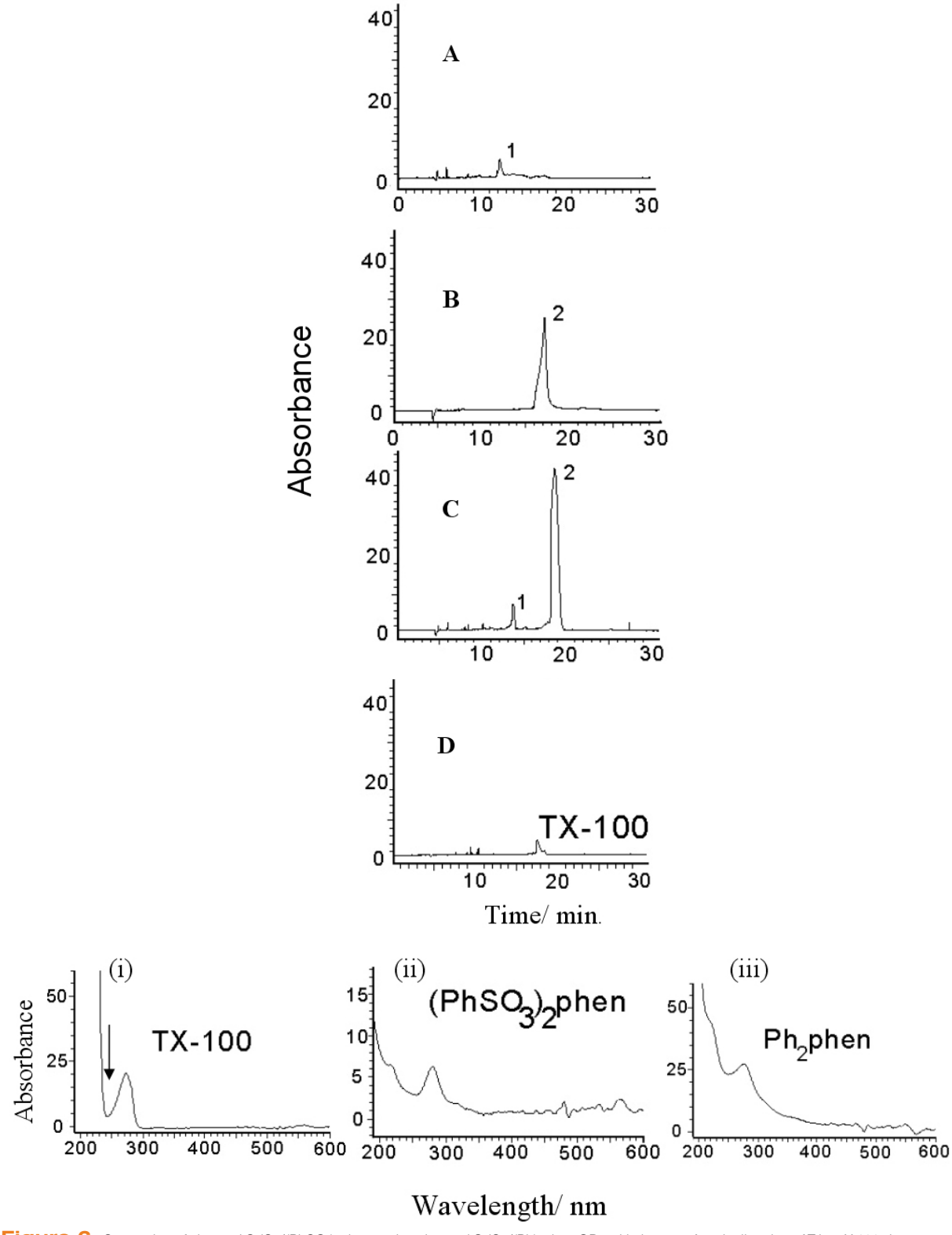

Figure 6. Separation of charged CdSe/$(PhSO_3)_2$phen and uncharged CdSe/$(Ph)_2$phen QDs with the use of a micellar plug of Triton X-100, by means of MEKC method. Conditions: background buffer 75 mM SDS, 10 mM Na_2HPO_4, voltage 20 kV (→ 58 μA). A, B, electropherograms of single QDs; C, mixture of both; D, only TX-100 at the same concentration as in A - C. Peaks: 1, CdSe/$(PhSO_3)_2$phen, 2, CdSe/$(Ph)_2$phen. Below are spectra recorded during this separation: (i) TX-100 with arrow pointing the wavelength at which electropherograms (A – D) were gathered, (ii) CdSe/$(PhSO_3)_2$phen, (iii) CdSe/$(Ph)_2$phen. Both QDs have the same core size, 3.5 nm.

The first conclusion can be drawn from Fig. 3. In this case, assuming that both, hydrodynamic radius for CdSe//(NN)/DHLA QDs and the ratio of surface ligands (NN)/DHLA, are the same for all those QDs, the ζ potential should be a function of CdSe surface architecture. In this case, the only major variable is the (NN) ligand. Indeed, for CdSe//(NN)/DHLA system, the slowest migration was observed for NN = ((Ph)$_2$phen), thus for ligand with the highest log P_{ow} (and largest molecular volume) and the fastest migration for the ligand (bpy) with opposite properties [28].

The above remarks, along with Eq. 1 allow estimate the ζ potentials for CdSe//(NN)/DHLA QDs, provided the basis for calculation as a ζ potential for DHLA coated QDs (-25 mV, [6]) and there is a linear dependence between QD charge density and its migration (an issue discussed in [9]). Thus, in this case the ζ order for CdSe QDs functionalized with a pair of ligands, as: (Ph)$_2$phen/DHLA (-8 mV) < phen/DHLA (-10 mV) < bpy/DHLA (-12 mV) < DHLA (-25 mV) can be estimated. The order is in agreement with the ζ-potential order for these QDs, obtained from particle size measurement (Supplementary Fig. 7, Electronic Support Section). To summarize, results presented here show that by applying a mixed ligands system to the functionalized QDs, the modification of a surface architecture of QDs in controllable manner is attainable and that the parameter, *e.g.* the charge density of QDs can be under control.

3.6 Visualization of electrically neutral CdSe/(NN) QDs using a micellar plug

In the present work, it has been stated that none of the CE methods (CZE, MEKC and MEEKC) were able to visualize the peak of an electrically neutral CdSe/(NN) QDs (NN = bpy, phen, (Ph)$_2$phen). Also, the literature confirmed this remark, as reported CE methods were proposed toward the separation of charged QDs ([9-12]). To solve this, a method for visualizing electrically neutral QDs, was recently implemented [26]. The method relies on injection of a micellar plug, containing a solution of a non-ionic surfactant and QDs, either uncharged or charged, followed by the plug electrophoresis. The mode can be discussed in the broader context, due to recently introduced new direction in the separation science, based on a micellar plug, being a tool for a solute preconcentration and separation [35-40]. The phenomena discussed in this work can be explained under terms, discussed in [35-40] (especially works [35,40]) with this difference that a voluminous QD represents a solute.

The schematic of the method is presented in Fig. 4. In this instance, the sample injected into the capillary, prepared according to Section 2.3.4, containing uncharged QDs and non-ionic surfactant (CdSe/(Ph)$_2$phen and Triton N-101, respectively), was run using MEEKC mode. It was observed that a sharp peak of CdSe/Ph$_2$phen QDs appeared within a zone of a plug of a non-ionic surfactant. This is the focusing phenomenon and the inset, Fig. 4, shows graphical interpretation for this. The presence of focused peak for QDs, within a micellar plug, is confirmed by DAD spectrum for the peak (Fig. 4), which shows the characteristic spectrum for CdSe/(NN) QDs, containing the band in UV range, from (NN) ligand. Despite long migration time, the calculated separation efficacy, in terms of number of theoretical plates (N) for the focused peak, was in the range of 10^7. Both factors (migration, N) denote that the enrichment of QDs, within a micellar plug, takes place in conditions applied.

Two remarks are important because of the interpretation given above. First, the method relies on preconcentration phenomenon, thus the term – sensitivity enhancement factor (SEF_{heigh}) should be applied, instead of number of theoretical plates (N). However, the absence of the QDs peak due to "conventional" separation preclude its use. Secondly, sharp peaks for particles called "spikes" have been reported in the literature [9] and these are small aggregates with not reproducible migration. The focused, in the present work, QDs were not in the form of spikes, as the migration of QDs was reproducible and DAD spectra for peaks show spectra characteristic for QDs being dispersed in solution; both confirm the status of dispersion of QDs within a plug. Also, additional experiments (sample centrifugation or ultrasound dispersion, before CE run) preclude the presence of aggregates in these samples.

Some features of the method were checked additionally. First factor, a kind of a non-ionic surfactant applied to a micellar plug was checked and both N-101 and TX-100 (structures Supplementary Fig. 1B) gave the similar results. Within this factor, POE (poly(ethylene oxide)), instead of non-ionic surfactant, was checked, as there are reports [10] of CE preconcentration of bacteria using POE, with ultrahigh efficiency (N ~ 10^9), due to the focusing mechanism. However, no peak of QDs was visualized in this case. Thus, it can be stated that only a micellar plug, is able to focus neutral QDs and the cause for this is the formation of a mixed pseudomicellar system, where the modified QDs serve as a pseudomicelle. The system can be stable as concluded from results of the previous work [26].

The second factor, a kind of a ligand used for CdSe QDs modification (NN = bpy, phen, (Ph)$_2$phen) did show that among these ligands, only the CdSe/(NN) QDs coated with the most hydrophobic ligand, as CdSe/(Ph)$_2$phen, can be visualized here. This additionally

confirms, that the hydrophobic interaction between QDs and plug can be one of main factors allowing for the incorporation of QDs to a micellar plug, which allows uncharged QDs to be visualized. Finally, the third factor, a kind of separation mode was checked and micellar techniques (MEEKC vs. MEKC) were compared. It was observed that for the system analyzed here (CdSe/$(Ph)_2$phen//non-ionic surfactant) a sharp peak was observed using MEEKC mode (Figs. 4,5), whereas using MEKC mode (Fig. 6) the peak is broaden.

Finally, both (MEKC, MEEKC) methods were tested in order to separate two types of QDs, namely, negatively charged (CdSe/$(PhSO_3)_2$phen) and uncharged (CdSe/Ph_2phen) one (Figs. 4,6). It was found that both methods were suitable for this task and two peaks were visualized here. However, in contrast to uncharged QDs, the charged QDs were found outside the micellar plug. There are two meanings of this fact: (i) modified QDs (charged) did not form the mixed pseudomicellar system or the system is weak that electric field can split a plug into zone of charged QDs and micellar zone, during a run, (ii) double separation mechanism on the same run was available, as charged QDs were migrated according to micellar CE, whereas electrically neutral QDs were focused within a micellar plug (enrichment). This is in agreement with [36], where double separation mechanism was reported, when a micellar plug was used as a separation tool.

Supplementary Figs. 1-13 are included to the Electronic Support Section.

4. Conclusion

In the present work, CdSe QDs were functionalized, in terms of coating crystal surface, using bidentate ligands containing S,S or N,N binding atoms. As a result, the stable ligands attachment to the surface of CdSe QDs was obtained, which prevented ligands exit from a CdSe QD surface. By applying this approach, QDs aggregation over time was limited, which assured repeatable results using methods based on QDs. Moreover, QD surface coating enables building a surface architecture, which allows study and control of the QD hydrodynamic properties. This issue was supported by molecular modeling, at the DFT level, in terms of binding energy and a way of ligands binding to CdSe QDs surface. These confirm the assumption about the higher than monodentate stability of the crystal/ligand system with bidentate ligands. UV-Vis work applied in the present work, particle size and capillary electrophoretic methods allow for obtaining data concerning QDs core sizes and hydrodynamic parameters and these methods were proved to be consistent. Finally, a method for visualizing electrically neutral QDs based on the enrichment of such QDs during migration of a micellar plug containing QDs, was proposed. It was established that by using this method, a dual mechanism (separation, enrichment) was available during the same electrophoretic run.

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