

Maja Stanisavljevic¹
Marketa Vaculovicova^{1,2}
Rene Kizek^{1,2}
Vojtech Adam^{1,2}

¹Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic
²Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

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Review

Capillary electrophoresis of quantum dots: Minireview

It has been already three decades, since the fluorescent nanocrystals called quantum dots (QDs) appeared and attracted attention of a broad scientific community. Their excellent not only optical but also electronic properties predetermined QDs for utilization in a variety of areas. Besides lasers, solar cells, and/or computers, QDs have established themselves in the field of (bio)chemical labeling as well as medical imaging. However, due to the numerous application possibilities of QDs, there are high demands on their properties that need to be precisely controlled and characterized. CE with its versatile modes and possibilities of detection was found to be an effective tool not only for characterization of QDs size and/or surface properties but also for monitoring of their interactions with other molecules of interest. In this minireview, we are giving short insight in analysis of QDs by CE, and summarizing the advantages of this method for QDs characterization.

Keywords:

Capillary electrophoresis / Conjugation / Quantum dots / Separation
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1 Introduction

Nanotechnology is widely spread technology applied in almost every field of science today [1–6]. Its backbone is hidden in principle of creating or engineering materials in the atomic or molecular scale. The year 1959 in history of nanotechnology can be marked as turning point because of brilliant speech of Richard P. Feynman who gave the vision that science and technology can be based on nanoscale [7]. However, the beginning of nanotechnology or closely speaking of nanomaterials was given much earlier by Michael Faraday in 1857 by observing characteristics of gold nanoparticles produced in aqueous solution [8]. Nanotechnology roots are tightly connected with development of colloids and physical chemistry, thus the great names such as Albert Einstein with his Brownian motion theory and Nobel prized Jean-Baptiste Perrin should not be neglected [9].

The biggest member of the family of nanomaterials is a group of nanoparticles that covers metal nanoparticles, metal oxide nanoparticles, polymer nanoparticles, and/or silica nanoparticles. The most interesting is group

of gold nanoparticles with their well-known optical characteristics as absorption, luminescence, and stability. Iron nanoparticles also play important role due to their magnetic properties [10–12]. Beside nanoparticles, carbon-based nanomaterials such as nanotubes, fullerenes, graphene which exhibit very good optical, electrochemical, and adsorptive properties are subject of constant investigation [13]. Other nanomaterials which cannot be neglected nor considered less important are liposomes [14, 15] and dendrimers [16, 17].

In this review, we focused on finding of articles containing phrases quantum dot (QD) and electrophoresis. We were interested mainly in the field of characterization of these unique materials using electrophoresis and further in biomolecules binding.

1.1. Quantum dots

QDs belong to the family of nanoparticles and they are defined as semiconductor nanocrystals with size from 1 to 10 nm usually spherical shape, but they can also be cubic, rod-like, or tetrapod-like [18]. QDs are mostly made of elements of II–VI groups as CdSe, CdTe (Fig. 1A), CdS, and ZnSe or III–V groups as InP and InAs [19, 20] and their optical and electronic properties can be placed between those of bulk materials and isolated molecules or atoms [21]. Size-depending properties and quantum confinement give them unique characteristics such as symmetric and narrow emission (Fig. 1B and C), continuous absorption spectra, and high emission quantum yields. For biological and medical usage, their photostability and resistance to chemical degradations are

Correspondence: Dr. Vojtech Adam, Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic
E-mail: vojtech.adam@mendelu.cz
Fax: +420-5-4521-2044

Abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; FRET, fluorescence resonance energy transfer; HRP, horseradish peroxidase; PAA, polyacrylamide; QD, quantum dot; SMCC, succinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxy; TOPO, triethylphosphine oxide

Colour Online: See the article online to view Figs. 1–3 in colour.

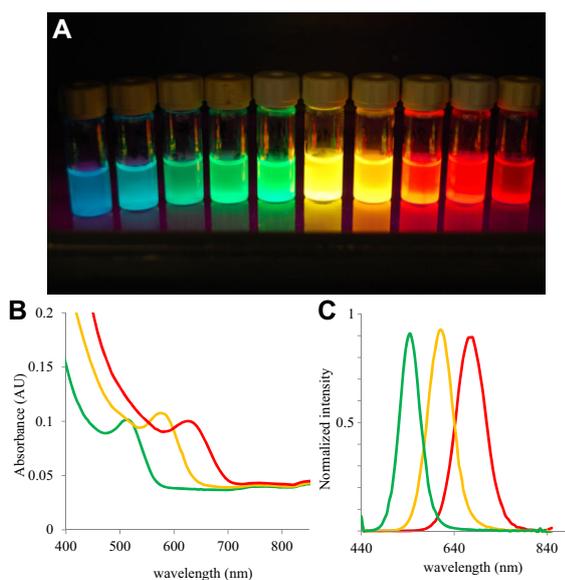


Figure 1. (A) Photography of quantum dots made from CdTe under various conditions. For more experimental details, see [84]. (B) Absorption spectra of three types of CdTe QDs. (C) Normalized emission spectra of three types of CdTe QDs (excitation wavelength: 400 nm).

valuable [22, 23]. However, the core of QDs created with inorganic elements is toxic for living systems and cells. Moreover, QDs are usually hydrophobic and this makes them unsuitable for working in aqueous environment or for any application in biological system. Therefore, synthesis methods of water-soluble QDs have been developed [24–26] and different surface modifications provide easy conjugations with biomolecules through covalent or noncovalent attachment [27, 28]. Nowadays, when concern for environment is emphasized, more environment-friendly technologies are explored and used for QDs synthesis [29–31]. The newest discovery in this field is the synthesis of QDs in earthworms using earthworm's metal detoxification pathway for it [32].

QDs exhibit one specific phenomenon called “blinking” defined as fluctuations in luminescence. Besides blinking on QDs luminescence effect environment, some molecules or reagents can cause increasing or decreasing of fluorescent signal of QDs. The blinking and the environment influence can cause limitation and issues in QDs application or analysis and are considered as drawbacks. The mechanism and explanations of drawbacks remain unknown and unclear [33]. Nevertheless, the topic which still occupies and worries science is the toxicity of QDs. On the other hand, one may mention that even large-scale production of these materials cannot cope with the well-known sources of metals, which are much greater. Even modified QDs bring doubts because nobody can tell with certainty what will happen to the materials used for modification in biological environment [34]. Other opened question is the environment itself and disposal of QDs in it [35]. Development and improving of QDs are equally followed with research about their toxicity [36–38]

and even methods for evaluation of their toxicity have been developed [39]. Based on these facts, there still remain unanswered questions concerning these materials and their well characterization is one of the keys to answer them.

1.2 Capillary electrophoresis

In the area of nanomaterials, CE is powerful tool not only for analysis of nanomaterial properties and validation of the synthesis process, but also for monitoring of nanomaterials interactions with other molecules. CE-LIF method was found as excellent method for not only characterization of QDs based on their fluorescent properties but also for analysis of biomolecules employing QDs as an efficient fluorescent label [22, 40]. Moreover, in the 90s, chip-based electrophoresis experienced a big breakthrough representing miniaturization of CE [41, 42]. Microchip electrophoresis provides very good separation with low sample and reagent consumption. Due to separation channels with even lower amount of injected analyte, extremely sensitive detection technique is required more than ever before and therefore highly fluorescent labels are essential [43].

2 Characterization of QDs with CE

Progress in technology of CE is still going further. Basic CE method was modified and developed according to different separation mechanisms and conditions. Based on these advances, CE methods have been used for characterization and separation of QDs CZE, MEKC, and CGE, as we discussed on the following paragraphs.

Characterization of QDs was done by Song et al. with CGE employing LIF detector for the first time [44]. This group used linear polyacrylamid (PAA) as sieving media as successful choice for characterization and separation of different size QDs, also providing valuable information of QDs behavior in wide pH range. Very important is a study of peak broadening in sieving media as well as percentage of used sieving media. A challenge to perform characterization of QDs with CZE was fulfilled by Pereira et al. [45]. Measurements included characterization of commercially available QDs with ultraviolet (UV) detection, LIF detection, and sodium phosphate as BGE. Two CE instruments were constructed solely for the study of QDs and ADS620 and T2-Evitag QDs were analyzed. The degree of net negative charge present on the QD surface can be assessed based on the migration times.

Very interesting insight in QDs separation was given by Pyell and co-workers on CdSe/ZnS/SiO₂ core/shell/shell nanocrystals [46]. Pyell's group was using, previously given by Ohshima group [47], formula of electrophoretic mobility μ independence of zeta potential ζ , particle radius r , and ionic strength I . The theory matched with practice and it proved that mobility and size of nanoparticles are in non-linear function. A comparison of calculated with experimentally determined distributions of the electrophoretic mobility

clearly showed that the observed broad bands in CE studies of colloidal nanoparticles are mainly due to electrophoretic heterogeneity resulting from the particle size distribution. The calculated data were in a good agreement by transmission electron microscopy method. Therefore, this procedure is well suited for the routine production control of charged nanoparticles with a ζ potential of 50–100 mV.

In addition, CZE was used for monitoring synthesis conditions which determine size of QDs with CdTe in core and with thioglycolic acid as capping agent by Clarot et al. [48]. In following work, Li et al. used CE method with added polymer additives as sieving medium to BGE for size determination of CdSe/ZnS QDs and observing the influence of different concentration of polymer to separation resolution, concentration of BGE, and pH. Novelty of this work was mathematical formula for size calculation, which relied on correlation between electrophoretic mobility and QD size. Confirmation of formula accuracy was done by transmission electron microscopy method [49].

Interesting work was done by Oswaldowski group [50], where they have used MEKC and CZE to separate mixture of CdSe QDs coated with cationic, anionic, and nonionic surfactants. The method is relied on the formation of bilayer between hydrophobic trioctylphosphineoxide (TOPO) and ionic and nonionic surfactants and gives a possibility of monitoring interactions between these layers. Oswaldowski group have also done research using preconcentration and micellar plug as a new method for analysis of QDs surface modified with amphiphilic, bidentate ligands, and biologically active molecules, which give QDs neutral or charged surface [51–53]. Besides, the role of ligands on growth rate and size distribution of CdTe QDs was monitored by micellar electrokinetic CE with LIF detector and it was confirmed that proper ligands give QDs bigger size, but narrower size distribution [54].

An interesting analysis of BSA-coated QDs was done by Bucking et al. [55]. A preparation method for BSA-coated CdSe/ZnS, ZnS:Mn²⁺/ZnS and InP/ZnS core/shell QDs was described and their electrophoretic properties were compared. Characterization was done in combination of agarose gel electrophoresis, dynamic light scattering, laser Doppler electrophoresis, and isotachopheresis methods. It was assumed that a negative charge observed in CdSe/ZnS and ZnS:Mn²⁺/ZnS QDs originated from the crystals due to defects in the crystal's lattice. This behavior was not observed in InP/ZnS QDs, probably due to a more covalently assembled lattice with fewer defects. Finally, InP/ZnS QDs showed almost the same behavior as the CdSe/ZnS QDs, both particles could be water-soluble without affecting their colloidal stability, confirming the successful conjugation to BSA, which attracted attention as possible coating because of solubility in concentrated salt solutions, low cost, and variety of functional groups capable of interaction.

However, the best resolution for QDs separation, whose surface was modified with trioctylphosphine oxide/trioctylphosphine (TOPO/TOP) and SDS, was done by Carrillo-Carrion et al. using MEKC. Authors separated QDs

with 0.5 nm difference in diameter and 19 nm difference in luminescence emission maximum [56]. The summary of CE conditions employed for QD analysis is given in Table 1.

3 CE of bioconjugated QDs

As it was aforementioned, after preparation of quantum dots, their surface has to be capped, functionalized, and/or bioconjugated. Applying surface modification and bioconjugation on QDs certainly affects their characteristics important for CE analysis, such as size, charge, and therefore electrophoretic mobility. Selected bioconjugation strategies are schematically demonstrated in Fig. 2.

Pioneers in this work were Huang et al., who observed QDs capped with mercaptopropionic acid as ligand and coupled with BSA and horseradish peroxidase (HRP). The authors efficiently separated bioconjugated QDs and free QDs adjusting buffer's pH foreseeing that CE-LIF will be used in further investigations of bioconjugated QDs [57]. QDs were coupled to BSA via electrostatic attraction and to HRP via covalent conjugation using EDC reagent. The E% of QD–BSA conjugation was about 54.9% and the splitting peak profiles for BSA–CdTe QDs were caused by BSA isoforms. In the case of HRP–QD conjugates, it was observed that with increased buffer pH, the negative charges of the QD bioconjugate increased. With the decrease in buffer pH, the negative charges of the QD bioconjugate were reduced, and the QD bioconjugates migrated slower than free QDs. The E% was calculated to be 91.7%, and this result illustrated that HRP was successfully labeled with QDs using EDC.

Application of QDs as fluorescent label in immunoassay was reported for the first time by Feng et al. [58]. QDs were conjugated with antibody and subsequently tested by electrophoretic separation of free antibody and antibody–antigen complex. Satisfactory separation of complex from free antibody could be achieved with 20 mM sodium tetraborate as separation buffer, at pH 9.8. On three differently bioconjugated QDs — with streptavidin, biotin, and IgG — Vicente and Colon showed using CE-LIF that electrophoretic mobility is dependent on biomolecule attached on QDs, and using polymeric additives can improve the resolution of bioconjugates. The group was also observing separation of bioconjugated QDs with different emission maxima using one excitation source. Besides, this was first reported separation of three differently bioconjugated QDs, because, only separation of QDs and bioconjugated QDs has been performed since than [59].

After this successful bioconjugation QDs with antibodies, more research have followed from other authors showing that QDs have a bright future as fluorescent label in immunoassay probes. Wang et al. reported how QDs enhanced immunoassay for the detection of antibenzo(a)pyrene diol epoxide-DNA adducts in lung cancer. Authors prepared QD-antibody-DNA complex and using CE-LIF method they showed how the formed complex can be not only successfully separated but also focused by the method [60]. Liskova

Table 1. Summary of CE methods and separation parameters applied for analysis of QDs and their bioconjugates

QDs type	CE method	BGE	Capillary parameters $l_{\text{eff}}/l_{\text{tot}}; \text{ID}$	Capillary coating	Detection	References
CdTe-MPA	CGE	2xTB (178 mM Tris and 178 mM boric acid (pH 8.8), 5% PAA)	30/40 cm; 75 μm	Linear polyacrylamide (PAA)	PDA; LIF	[44]
CdTe/CdS-MPS (ADS620), CdSe/ZnS carboxylic acid (T2-Evitag)	CZE	Sodium phosphate (5–25 mM, pH 7.5–11)	42/49.5 cm; 51 μm	Uncoated fused-silica	UV; LIF	[45]
CdSe/ZnS/SiO ₂	CZE	17.5, 8.74, 4.37 mM Britton Robinson buffer pH 9.0	20/27 cm; 18.7/25.7 cm; 17.8/24.4 cm; 75 μm	Uncoated fused-silica	UV	[46]
CdTe/ZnS with thioglycolic acid	CZE	25 mM borate pH 8.5	30/37 cm; 75 μm	Uncoated fused-silica	UV, PDA	[48]
CdSe/ZnS	CGE	25 mM tetraborate (pH 9.2) water-soluble polymeric additive (PEG, PVP, PVA)	35/60 cm; 75 μm	Uncoated fused-silica	LIF	[49]
CdSe/TBP/TOPO//SDS, CdSe/TBP/TOPO//N101, CdSe/TBP/TOPO//CTAB	MEKC/CZE	20–100 mM SDS or CTAB, 10 mM sodium tetraborate/20 mM sodium tetraborate buffer (pH 9.2)	60/70 cm; 75 μm	Uncoated fused-silica	PDA	[50]
CdSe NCs with dihydroliipoic acid, 1,10-phenanthroline derivative, DNA oligomer	MEKC/CZE	50 mM SDS with 10 mM sodium tetraborate/10 mM sodium tetraborate pH 9.4; 5 or 10 mM sodium tetraborate, pH 9.4 by adding methanol or ACN in the range up to 40% v/v	60/70 cm; 75 μm	Uncoated fused-silica	PDA	[51, 53]
CdSe/ZnS with dihydroliipoic acid and/or α -diimine derivatives	MEKC/CZE	50 mM SDS with 10 mM sodium tetraborate; 75 mM SDS with 10 mM sodium phosphate/20 mM potassium chloride pH 6.0	60/70 cm; 75 μm	Uncoated fused-silica	PDA	[52]
CdTe/ZnS with mercaptoacetic acid, l-cysteine (Cys) and reduced glutathione	MEKC	25 mM borate with 50 mM SDS pH 8.5	50/57 cm, 75 μm	Uncoated fused-silica	LIF	[54]
CdSe/ZnS/BSA, InP/ZnS/BSA	Agarose GE/ITP	Tris–glycine buffer, gel (running buffer and sample buffer ((5 stock solution, 50% glycerine in Tris–glycine buffer))/leading electrolyte: 60 mM Tris pH 9.1; terminating electrolyte: 25 mM glycine	N/A	N/A	Conductivity and UV	[55]

l_{eff} : effective length of the capillary; l_{tot} : total length of the capillary; MPA: mercaptopropionic acid; MPS: sodium mercaptopropionate; PDA: photodiode array detectors; TB: Tris-boric acid buffer; TBP: tributylphosphine.

et al. pointed out the importance of the binding way of QDs and proteins, emphasizing antibodies, in the immunoluminescence probes. In this paper, the authors observed and optimized conjugation of QDs and antibodies via two different zero-length cross-linkers, long-chain linkers, and highly specific linkers, concluding that the best choice are highly specific linkers, which do not compromise effective usage of antibodies what can happen with nonselective linkers [61]. The same group of authors prepared QDs–ovalbumin complex and observed creating antigen–antibody complex and its separation from free conjugates of QDs in the following paper [62]. The obtained CE-LIF electropherograms clearly

showed the successful QDs and antiovalbumin interactions (Fig. 3A).

Biomolecules with QDs are usually bonded through strong covalent bonds, but Shi et al. gave a new way of bonding them by simple adsorption of antibodies on the QD's surface. Characterization was done by CE-LIF, fluorescence spectrometry, and fluorescence correlation spectroscopy and these QDs had high luminescence, small radii, and good stability in aqueous environment [63].

The simple adsorption of antibodies on the QDs surface as well as commonly used bioconjugation via organic linkers such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

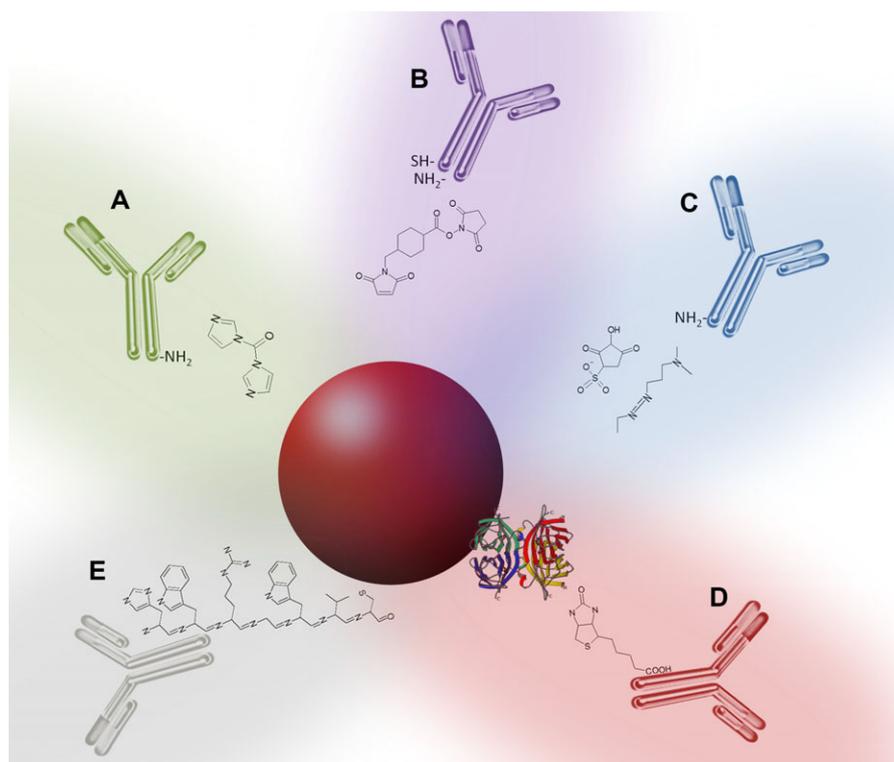


Figure 2. Summary of QD–antibody conjugation strategies. The conjugation serves as surface modification of dots to further bind antibodies. The following substances are used for this purpose: (A) carbonyldiimidazole, (B) succinimidyl 4-[*N*-maleimidomethyl]-cyclohexane-1-carboxylate, (C) EDC/sulfo-NHS, (D) biotin-streptavidin, and (E) heptapeptide HWRGWVC. All of these strategies are based on the affinity of antibodies with the mentioned compounds. Sulfo-NHS: *N*-hydroxysulfosuccinimide.

(EDC) or two-step procedure combining EDC with *N*-hydroxysulfosuccinimide may lead to the inactivation of the antibody due to the sterically inappropriate binding. This was addressed in the work of Janu et al. [64], where an artificial peptide was used as a linker with specificity to the Fc fragment of the antibody and therefore active site remained active (Fig. 3B). Moreover, the important aspect investigated by Wang et al. was the influence of *pI* of biomolecule as well as pH of the used buffer and it was showed that efficiency of biomolecule–QDs conjugation is highly dependent of this values and CE-LIF and fluorescence correlation spectroscopy as suitable methods for observation [65]. Later, the bioconjugation of QDs with a short oligonucleotide sequence via streptavidin–biotin linkage was demonstrated by Stanisavljevic et al. employing the CE-LIF and CE-UV for the evaluation of the interaction process [66]. Bioconjugation possibilities are summarized in Table 2.

4 Detection modification using QDs

QDs with their great fluorescent properties can enhance CE detection of the samples in many different scientific fields, what will be subject of the following chapter. Relying on successful application of QDs in immunoassay, possible usage of QDs in fluorescence resonance energy transfer (FRET) analysis was next challenge. In the literature in FRET as energy-transfer processes, QDs are playing role of energy donors. Li's group reported FRET analysis based on QDs as donor–acceptor system. Two different QDs were attached

to antibody and antigen whose strong affinity brings QDs close enough to make FRET. CE-LIF was successfully applied in this work [67]. A combination of in-cuvette fluorescent analysis and CE with fluorescent detection (CE-FL), Wang and Xia observed binding process between Cy5-labeled polyhistidine peptide dendrimer and glutathione-capped CdSe QDs and proofed CE-FL as a powerful analytical method [68]. In the following paper, the same authors observed amide bond cleavage by a specific protease based on FRET and monitored it by CE-FL [69]. Inspired with variety of biomolecules conjugated with QDs, Wang et al. brought a new idea of detecting residues of glucose. The authors conjugated QDs with concanavalin (con A), lectinprotein, which react with specific sugar residues. CE-LIF was used for validation of “one-step” and “two-step” conjugation procedure between con A and QDs mediated by glutaraldehyde [70].

Li et al. work combine QD and CE in DNA mutations analysis improving conventional molecular beacon methods. QD molecular beacon probes measured by CE are more sensitive, accurate, have low sample consumption, and provide simultaneous multiplex detection [71]. Metal-enhanced fluorescent effect is very promising, particularly Ag and Au nanoparticles enhancing strength of the electromagnetic field increase the fluorescence of nearby fluorophores. This phenomenon was used by the same authors enhancing QDs fluorescent with Au nanoparticles. Needed distance was provided by modifying particles with the wanted base numbers of two complementary DNA oligonucleotides. This method was successfully monitored by CE-LIF [72]. Zhao et al. used characteristics of QDs to enhance CE-chemiluminescence

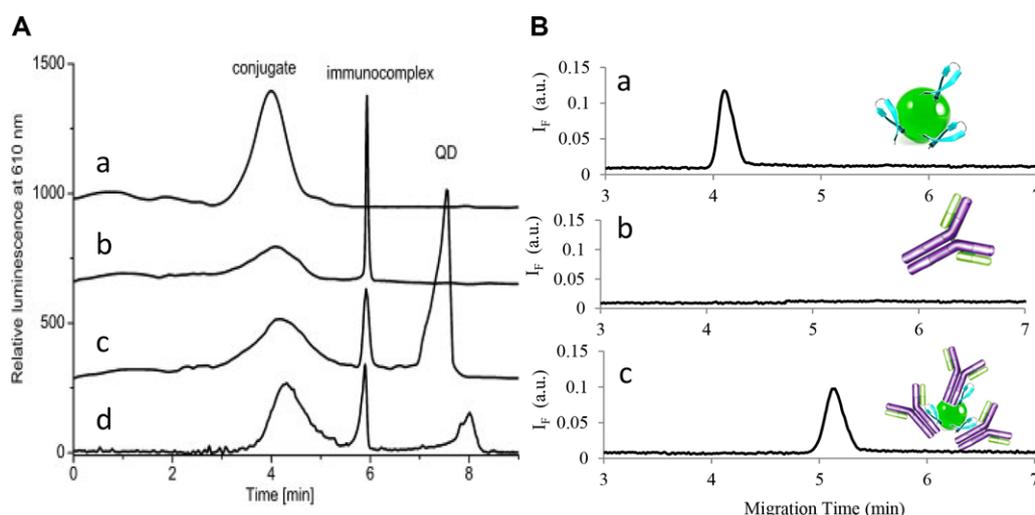


Figure 3. (A) CE-LIF of (a) HWRGWVC-QDs, (b) human immunoglobulins, and (c) their bioconjugate. Experimental conditions—excitation: 488 nm, emission: 520 nm, capillary: 75 μ m id, 47.5 cm/40 cm, BGE: 20 mM sodium borate, pH 9.2, voltage: +20 kV, injection: 3.4 kPa, 20 s. (B) CE-LIF electropherograms of crude reaction mixture after conjugation between QDs and antiovalbumin, the same mixture after the addition of ovalbumin and free QDs. (a) Conjugate of 3.5 nm CdTe QDs (emission maximum 610 nm) with antiovalbumin. (b) Formation of immunocomplex with equimolar amount of ovalbumin. (c) Standard addition of free QDs at concentration 1.2×10^{-5} M. (d) Conjugate of QDs with ovalbumin. Formation of immunocomplex with equimolar amount of antiovalbumin (conditions are the same as in b and c) and addition of free QDs. Separation conditions: BGE 0.1 M Tris/TAPS at pH 8.3. Bare silica-fused capillary: id 75 mm, effective/total length 15/25 cm. Excitation at 488 nm (Ar-ion laser), detection at 610 nm. Separation and injection voltage: 6 kV. Reprinted with permission from [62].

Table 2. Summary of biomolecules conjugated to QDs and interactions used for bioconjugation

Molecule	Bonding via	CE mode	References
BSE	Electrostatic attraction	CE-LIF	[57]
Horseradish peroxidase	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, EDC	CE-LIF	[57]
Human IgM	Succinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate (SMCC) and DTT	CE-LIF	[58]
Streptavidin	An active ester coupling reaction	CE-LIF	[59]
Biotin	Carbodiimide-mediated coupling reaction	CE-LIF	[59]
IgG	SMCC and DTT	CE-LIF	[59]
Goat F(ab') ₂ anti-mouse IgG secondary antibody	EDC/sulfo-NHS	CE-LIF	[60]
Antiovalbumin	EDC/sulfo-NHS	CE-LIF	[61,62]
Antiovalbumin	Carbonyldiimidazole	CE-LIF	[61]
Antiovalbumin	Sulfo-SMCC	CE-LIF	[61]
Antiovalbumin	Oxidized antibody glycans	CE-LIF	[61]
Epidermal growth factor receptor antibody	Adsorption affinity	CE-LIF	[63]
Human IgG	Short artificial peptide—HWRGWVC	CE-LIF, CE-UV	[64]
Proteins (AFP1A6, AFP2A5, streptavidin, Erbitux, and peroxidase)	EDC/sulfo-NHS	CE-LIF	[65]
Cancer sequence BCL-2	Streptavidin-biotin linkage	CE-LIF, CE-UV	[66]
Viral hepatitis B virus	Streptavidin-biotin linkage	CE-LIF, CE-UV	[66]

AFP1A6: mouse anti-human alpha-fetoprotein antibody; AFP2A5: mouse anti-human alpha-fetoprotein antibody; BCL-2: B-cell lymphoma 2; Erbitux: commercial name of the epidermal growth factor receptor (EGFR) antibody; Sulfo-NHS: N-hydroxysulfosuccinimide.

detection of neurotransmitters such as dopamine and epinephrine.

Obtained results indicate that chemiluminescence detection signal increased with the increasing of QDs size. This improved and simplify detection of these neurotransmitters

is important as indicators of mental diseases such as multiple sclerosis, and/or Parkinson's disease [73].

Today, one of the wide-spread concerns of human population is usage of chemical treatments in agriculture and food industry. This is why rapid and efficient way of their

detection is asked. QDs and CE-LIF with their characteristics are promising methods for analysis of these substances. Chen and Fung used CE and QDs for detection of organophosphorus pesticides in tomato. Procedure involve immobilization of QDs onto the capillary wall and making mercaptopropyltrimethoxysilane network, this method is called MEKC-QD/LIF method. The method gave very good results by detecting ten times lower concentration than maximum residue levels allowed by Codex Alimentarius Commission [74]. The principle of this method is taking the advantage of the enhancement of the QD fluorescence by interaction with organophosphorus pesticides and when they are passing through the detection window. Moreover, the resolution of the separation was increased by using SDS as a buffer additive and therefore MEKC principle was introduced.

Recently, it has been proven that QDs may be utilized also as a fluorescent agent in the indirect LIF detection [75]. By this method, nicotiny pesticide residues in vegetables were determined in carrot, cucumber, and tomato. Also in this case, the SDS was utilized as a buffer modifier.

Another food industry application is for detection of acrylamide or 2-propenamide contaminants occurring in products prepared by high temperature, in this case potato crisps. The issue of detection of contaminants was its weak UV absorption. QDs as fluorescent labeling material in combination with LIF as detector solved the lack of UV absorption. QD-acrylamide complex was analyzed by MEKC-QD/LIF method as in the previous case [76].

Beside pesticides and contaminants, propylparaben, sodium dehydroacetate, sorbic acid, benzoic acid, and sodium propionate as food preservatives were determined by CE and QDs as background substance [77] as well as analysis plant peptide hormone from tomato called systemin [78].

In aforementioned description of QD's characteristics, one of the concerning facts is their influence on environment. Celiz et al. described how CE-LIF can be powerful method for studying of the interaction between QDs and humic substances (humic and flavic acids) in waters. To follow quantitative information of free zinc or cadmium ICP-MS in combination with CE-LIF was used. Data obtained from research showed that there is no degradation of QDs in the environment, but they do not exclude influence of sunlight and other environment parameters which have not been considered here [79].

5 Conclusion

QDs have already found wide applications in different scientific fields. Following rapid growth of technology they are one of the most promising nanoparticles. Taking into consideration the great size-dependent characteristics and high luminescence of QDs, CE as analytical method is very suitable for their analysis, especially in combination with LIF detector. High separation efficiency of CE and its relative methods is enabling detail investigations on the interaction mechanisms between QDs and biomolecules and it is also beneficial for

characterization of the nanoparticles during their routine production.

Moreover, the employment of semiconductors light sources such as high-power LEDs and/or laser diodes covering nearly the whole range of wavelengths is advancing significantly the miniaturization process. Chip-based CE with LED (laser diodes)-induced fluorescence detection is pushing the progress toward portable instruments and therefore the application of fluorescent tags is inevitable. Such miniaturization may decrease the size of analytical instruments to the hand-held appliances which enables the realization of the point-of-care concept in practice. Furthermore, the broad excitation band of QDs is extremely beneficial for multicolor labeling because only single-wavelength excitation source is needed. The fact that the excitation wavelength of the most QDs is close to 400 nm is moreover beneficial due to the fact that the emission wavelengths of currently commercially available high-power LEDs are currently approaching 400 nm.

Improvements in QDs synthesis and its application are followed with research about their toxicity and deleterious effect on living systems. Cadmium accumulates in the liver, bones, and kidneys. Exposure of QDs cadmium core to UV light or air will cause surface oxidation and release free cadmium ions that have harmful effect. Nevertheless, in the existing research it is also proven that ZnS layer and coating can provide safe longtime measurements in vitro [80]. When we are talking about harmful effect of QDs, cadmium every day consumption data should be taken into consideration. Cadmium is a common pollutant in environment caused by nature (volcano activity, erosions, etc.) and mainly human (smoking, mining, fossil fuel combustion, phosphate fertilizers, electronic and municipal waste, etc.) activity. In 2010, World Health Organization and Food and Agriculture Organization established a provisional tolerable monthly intake for cadmium of 25 $\mu\text{g}/\text{kg}$ body weight. Concentration of cadmium in a cigarette smoke is not negligible and it is within the range from 1000 to 3000 $\mu\text{g}/\text{kg}$ [81, 82]. From one pack of cigarettes, which contains 20 cigarettes, 2–4 μg of Cd deposits into the smokers lungs and some of it is released into the air [83]. A moral dilemma in science is: could we use QDs knowing risks for human health? Hitherto research with QDs benefits has advantage over their toxicity. Neither possibility of releasing free cadmium ions should be overlooked nor QDs numerous advantages.

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