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Review

Colloidal quantum dots conjugated with human serum albumin – interactions and bioimaging properties☆

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ABSTRACT

Quantum dots, due to their unique optical properties, constitute significant materials for many areas of nanotechnology and bionanotechnology. This work presents a review of researches dedicated to the interaction between quantum dots (QDs) with human serum albumin (HSA) and human cell culture as important for nanomedicine applications. The optical properties of bio-nanocomplexes formed by nanoparticles including colloidal QDs (e.g., CdTe, CdS, CdCoS) and albumin are displayed. The absorption spectra show that adding HSA to colloidal QDs leads to a gradual decrease of absorption and broadening of the exciton structure. The photoluminescence quenching results indicate that the quenching effect of QDs on HSA fluorescence depends on the size and temperature. The nature of quenching is rather static, resulting in forming QD-HSA complexes. The CdTe QD-HSA complexes show chemical stability in a PBS buffer. Furthermore, it is stable in cytoplasm and suitable for cell labeling, tracking, and other bioimaging applications.

Contents

1. Introduction

Semiconductor nanocrystals (NCs), also known as quantum dots (QDs) because of their unique optical and electronic properties, are promising materials for a number of applications including optical [1], electronic [2], biological [3], and medical [4]. In the recent decades, the employing of II–IV QDs in nanomedicine has rapidly

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Table 1
Photophysical properties of quantum dots and chemical dyes [6–8].

Properties	QDs	Organic fluorophores
Photoluminescence (PL) phenomenon	Present	Present
Photostability	Superior	Low
Photoluminescence lifetime	Longer (>10 ns)	Shorter (<10 ns)
Quantum yield	High (10–90%)	Lower
Molar extinction coefficients	High	Lower (~10–100× lower than QDs)
Excitation–emission range	Wide (UV–vis–IR)	VIS
Excitation band	Broad band	Narrow band
Emission band	Narrow band (FWHM ~25–40 nm)	Broad band
Photobleaching threshold	High resistance to photobleaching, as well as photo and chemical degradation	Poor resistance
Additional advantages	Size-dependent emission spectra	Fixed emission wavelength
	Excitation of multicolor light	Excitation of monochromatic light
	Large Stokes shifts	–
	Efficient fluorescence resonance energy transfer (FRET) donors	–
	Easy surface modification and change in optical and utility properties	–
	Easily observable by conventional microscopic and spectroscopic techniques	Easily observable by conventional microscopic and spectroscopic techniques

increased [5]. Quantum dots, which are characterized by photoluminescence phenomenon, superior photostability and longer photoluminescence (PL) lifetime [1] can be successfully used for bioimaging. The main photophysical properties of quantum dots in comparison to chemical dyes are provided in Table 1.

The QDs, due to their unique optical properties, are used as fluorescent probes for cancer cells' detection [9]. The use of QDs for a bioimaging application has many advantages. One of the most valuable is the size-dependent emission spectra [10]. This allows e.g. for a multi-color detection. The different sizes of QDs may be excited with a single wavelength of light, while many emission colours may be detected simultaneously. Additionally, in comparison with conventional chemical fluorophores, the QDs have a narrow, tunable, and symmetric emission spectrum. High photochemical stability makes the nanocrystallites stand out. They are more resistant to a photo and chemical degradation, as a result of their endurance to bleaching, and a fluorescence signal, as well as microscopic images can be analyzed longer and more precisely [6,7]. Moreover, the semiconductor QDs allow for a fluorescence resonance energy transfer process (FRET) and, as a result, can be commonly used as biological molecular probes [11]. FRET is a nonradiative transfer of energy from a donor molecule to an acceptor molecule through a near-field dipole–dipole interaction. The phenomenon is very sensitive and utilitarian in the development of biosensors and detection assays [7]. The QDs optical properties could be easily modified in composition with other chemicals or materials, e.g., the surface of QDs can be changed and bio-functionalized by organic molecules, nucleic acids, proteins or antibodies [12,13]. These changes can improve usability of QDs and greatly expand the possible usage, e.g., improve the biocompatibility, precision targeting of nanoparticles and specificity of molecules' detection [6]. Thus, such nanocrystal probes are complementary to biosensors and in some cases may be superior to existing fluorophores.

All presented advantages result in QDs having an important role in fluorescence imaging techniques, which can provide high sensitivity and high spatial resolution. According to publications [4,10,14], fluorescent nanoparticles can be useful for *in vitro*, as well as for *in vivo* imaging in medical diagnostics, to image different kinds of cells and tissue in the biological research. Medintz et al. [6] presented a detailed list of cellular components and proteins that have been labeled with QDs: nucleus, mitochondria, microtubules, actin filaments, endocytic compartments, mortalin, cytokeratin, serotonin transport proteins, prostate-specific membrane antigen, glycine receptors and p-glycoprotein [6]. QDs have been also used for a real-time tracking of single molecule dynamics for *in vitro* and targeting tumor sites of mice cancer [8].

Despite above mentioned superiority, the pure QDs have some disadvantages. The main problem is their toxicity, resulting from the elemental composition of nanocrystals and their nanometric size [7,15]. It is known, that Cd, Se, Te, Hg, Pb, which are often used in QDs, are toxic. Additionally, some reports describe the QDs DNA damage [6]. There are not enough results of the QDs researches to have clear information about their long-term toxic effects. The additional problem, that researchers have to overcome, is the reproducibility improvement. One of them, is the inorganic passivation which could cause the undesirable changing of the QDs properties.

The biocompatibility and bioavailability of QDs constitute very important issues. QD cytotoxicity is the induced effect, which depends not only on the elemental composition, but also it is related to the concentration of nanocrystals. The lack of influence on cell growth and development of concentration below 1×10^{-7} mol/l is observed in the case of CdSe/ZnS core–shell QDs [8]. For other types and sizes of nanocrystals a toxicity threshold is different. However, several works have demonstrated the remarkably reduced toxicity of *in vitro* cells after coating of the QDs with albumin [16]. Apart from protein binding, there are two other ways to target more biocompatible QDs: connection with antibodies or with small molecules [17]. Furthermore, the binding of additional molecules (multi-functionalized QDs), which specifically affects cellular receptors or other cell molecules, may improve the cellular uptake of QDs [16].

As mentioned in the previous paragraph, the toxicity of QDs restricts their use in the human body. This leads to the creation of such QDs which will preserve their unique optical properties, biological sensitivity and primarily will be safe for using in nanomedicine. Toxicity of QDs can be minimized in different ways *e.g.* appropriate composition of nanoparticles or combining them with some bionanoparticles [15]. The information on the optical properties of some important QDs types: CdTe, CdS and CdCoS QDs is presented in this work. The main part of the research is dedicated to the interaction of the QDs with human serum albumin (HSA) and bioimaging properties of this bio-nanocomplex – QD-HSA. The different methods are used for the optical properties of the QD-HSA complex characterization: the UV–vis absorption, fluorescence spectroscopy and fluorescence microscopy. The results of the excitation and absorption edge as well as the photoluminescence spectra of QDs, are presented. The excitation–emission studies are complemented by infrared spectroscopy used for chemical func-

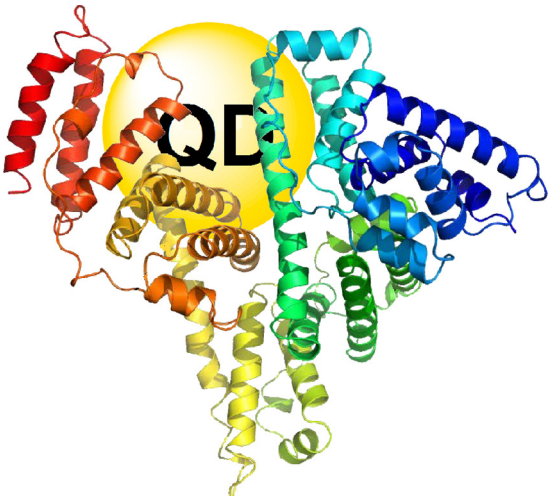

Fig. 1. Illustration of HSA protein interacting with QDs surface for QD-HSA complex formation.

tional groups identification, and detection of some changes in HSA protein secondary structure, through a change in the recorded oscillation spectra.

The paper consists of six sections including introduction and conclusion. The role of HSA in human organism as well as in the QDs usage is presented in Section 2. In Section 3 the preparation of the QD-HSA complexes is described, while the optical properties – in Section 4 as well as in Section 5 – the bioimaging properties of QD-HSA complex and examples of their use as a fluorescence probes are shown.

2. Role of human serum albumin

The HSA is the most abundant and one of the most important proteins in blood plasma. It was firstly described by Denis in 1840 and it is one of the earliest known proteins in the human body. This protein occurs in plasma, cerebrospinal fluid and lymph. HSA is produced by liver and it provides more than 50% of the total proteins in plasma. Albumin has many different and very important physiological functions: connecting and handling to the cells a variety of endogenous and exogenous ligands (including hormones, metabolites, ions or fatty acids), drug transportation, toxins binding, and maintaining of the oncotic pressure (through the water-binding capacity) [17–20]. It can be also a diagnostic marker for determining some diseases like inflammatory states [21]. From the biochemical point of view, albumin is a protein composed of three α-helical domain (I–III), which are divided into two sub-domains: A and B. By the presence of the elastic loop it is possible to move the relative domains toward each other, which makes the structure of protein easily adaptable to binding a large number of ligands [22].

As mentioned previously, the protein plays an important role in the transportation of many ligands and during this property it is used to create new diagnostic methods, contrast agents or drugs [23]. Albumin with the capability of binding metal ions can be used to create a biosensor chip that detects and quantifies such ions: Cd^{2+}, Ni^{2+}, Zn^{2+} [24].

Besides the optical and functional properties of the QDs, the interactions between QDs and HSA and their mechanisms, have been investigated in many works. Several reports have been devoted to the study of such kinds of interactions of the II–VI based nanoparticles with HSA as well as with bovine serum albumin (BSA) [25–31]. However, the processes of the interactions of these molecules between each other and with other molecules in human body are highly complex and complicated and still are not well known. Therefore, it is very significant for nanomedicine purpose to investigate the processes occurring between the nanoparticles and the major carrier protein like HSA.

Furthermore, covering of QDs by the natural protein seems to be a promising solution for reducing their toxicity. Therefore, forming the QD-HSA complexes is important in order to remove toxicity from the QDs. The toxic effect of QDs is mainly related to the processes occurring on the surface of nanoparticles. The most significant is the release of metal ions, particularly heavy metals and the oxidation processes connected with the reactive oxygen species forming. This effect can be minimized by combining the QDs with some materials *e.g.* with other metals, polymers or biomolecules which cover the QD surface. However, such modifications alter their properties and functional features. As shown below, the HSA covering is neutral and slightly change the properties of the CdTe QDs.

3. Preparation of QD-HSA complexes

The synthesis of quantum dots with the precisely defined sizes requires the use of appropriate technologies. The chemical colloidal methods of QDs synthesis fulfill those requirements. They involve the creation of colloidal quantum dots by precipitation of nanosemiconductor crystal in the solution [32] with the size of few nanometers and usually spherical symmetric shape. Two types of quantum dots can be obtained by such methods: pure and homogeneous QDs or core–shell QDs with semiconductor core (with a smaller band gap) surrounded by a layer of a second semiconductor material (with a larger band gap).

The role of the QDs has increased rapidly due to their optical properties [25,33]. The CdTe and CdS QDs are one of the most widely examined II–VI semiconductor nanocrystals. An energy band gap of the CdS QDs is located at 2.42 eV, at 300 K. However, co-doped II–VI semiconductor QDs are even more promising because of their chemical stability and strong fluorescence in visible region. Fediv et al. [34] described the properties of the CdS QDs modified by adding magnetic manganese ions. The optical and magnetic properties of these nanocomposites have been changed. Additionally, nanoparticles behave as a typical paramagnet. Properties of the CdS quantum dots doped by cobalt ferromagnetic chemical element have also been studied [35,36].

Nanoparticles of CdTe can be prepared in aqueous solution at high pressure and high temperature [37] but also it is possible to synthesize them at room temperature [33,38,39]. As it is described in publication by Savchuk et al. [33] $CdCl_2$ solution can be used as a Cd precursor and mixed with thioglycolic acid (TGA), which is commonly used for the QDs modification and stabilization [40,41]. The pH value is adjusted to 10.0 by a drop wise addition of the NaOH solution. Then the gas mixture of Ar and H_2Te is passed through the solution. The reaction time is fixed to obtain certain molar ratio of Cd^{2+}:Te^{2-}:TGA. In other publication [41] the sodium tetraborate NaHTe (formed by a suspension of the tellurium in the sodium borohydride solution) and the $CdCl_2$ solution in the presence of the TGA were used as a substrate for the CdTe QDs preparation. The solution was mixed and it was heated to 100 °C at the N_2 atmosphere.

The pure CdS QDs and doped $Cd_{1-x}Me_xS$ (Me: Mg, Mn, Co, Zn) can be prepared by a co-precipitation method. In order to vary the nanoparticle compositions the $[CdCl_2]$:$[Na_2S]$, or the $[CdCl_2]$:$[CoCl_2]$ molar ratios can be changed. The CdS QDs can be also synthesized by using wet chemistry method, where the cadmium acetate solution is mixed with the thioglycerol capping agent, and the sodium sulfide solution is added drop by drop. The colloidal CdS QDs starts to precipitate rapidly [31].

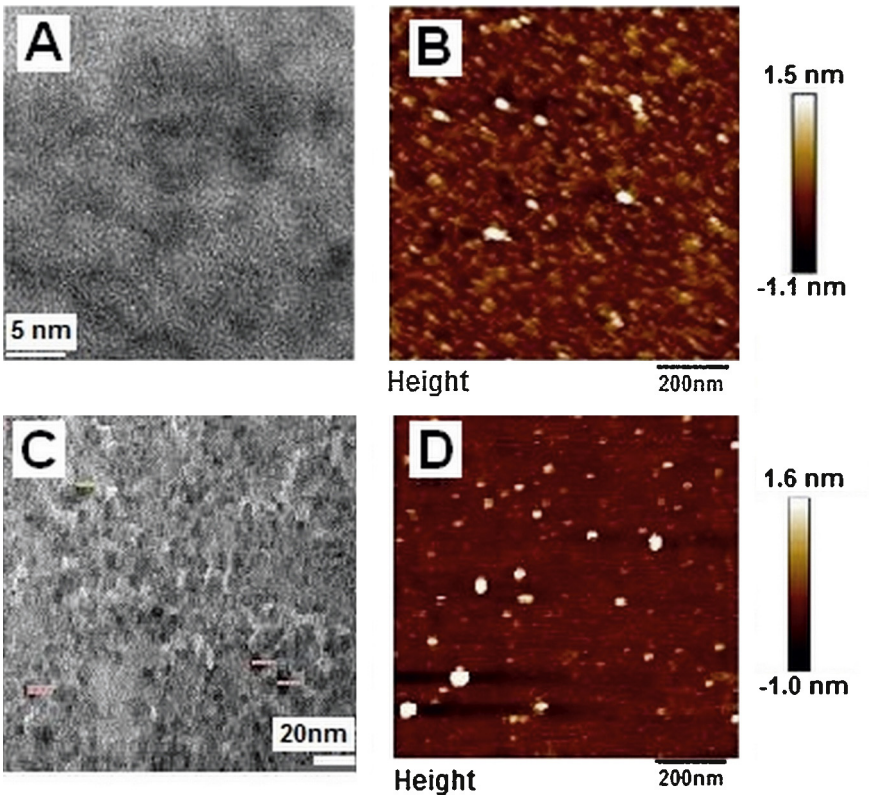

Fig. 2. TEM and AFM images of the colloidal CdCoS QDs (A – TEM; B – AFM) and CdTe QDs (C – TEM; D – AFM).

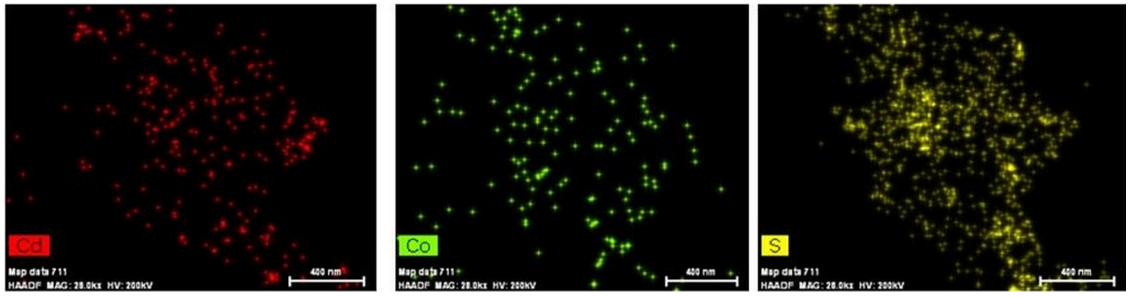

Fig. 3. EDX scheme of presence of chemical elements: Cd, Co, and S in the CdCoS QDs structure.

Quantum dots must be more biocompatible for bio-imaging and other biological applications. It can be achieved by linking QDs with biomolecules. However, it is necessary to preserve the biological activity of the conjugated molecules. A lot of successful conjugation methods have been described in the literature. They can be divided into different types of a binding mechanism: covalent, non-covalent attachment and specific conjugation [42]. Albumin, as well as other proteins can be bonded with QDs using all of the above mentioned methods. The QD-HSA bio-nanocomplex can be easily prepared by mixing the set amount of albumin with colloidal solution of QDs to obtain a certain concentration of reagents. The mixture is usually stirred and incubated for at least 15 minutes to several hours at room temperature to form connections between molecules [8,30,43]. The schematic picture of the QD-HSA complex formed by a non-covalent (electrostatic) interaction between nanocrystal and protein is presented in Fig. 1. The specific chemicals named as linkers are used for covalent connections of the QDs with proteins. 2-mercaptoethanol [44], *N*-acetyl-L-cysteine, glutathione reduced, cysteamine chydrochloride [45], and EDC-NHS linker (1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; N-hydroxysuccinimide) [8] are often employed for this purpose. In this case, there is no direct bonding between the QD and the protein.

4. Characterization: microstructure and optical properties

4.1. Microscopy analysis

Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM) are often used in order to confirm the structure of nanoparticles and bio-nanocomplexes. The estimate shape and average size of nanocrystals, as well as the composition and crystal structure are analyzed by the above mentioned methods. A drop of colloidal suspension is placed on a carbon-coated copper grid and dried for the TEM analysis. Samples are usually placed on the highest grade mica or graphite surface before an AFM measurement. The composition SEM microscope with an energy dispersive X-ray spectroscopy (EDS) can be used for the chemical analysis of QDs.

The TEM and the AFM images of the colloidal CdCoS and CdTe nanoparticles are shown in Fig. 2. As can be seen, the shape of the nanoparticles is close to spherical and the average diameter of the

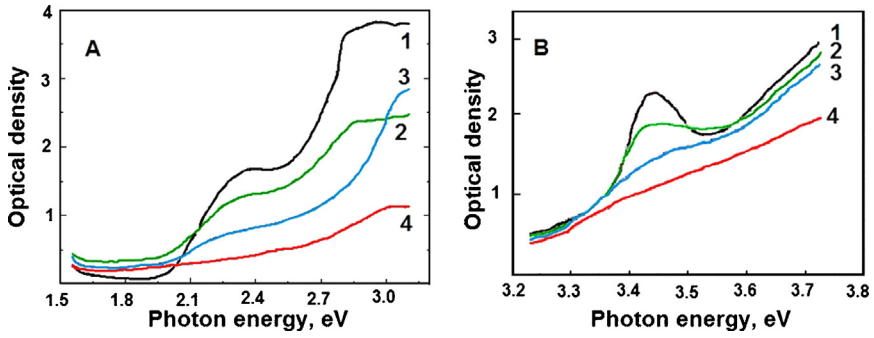

Fig. 4. Optical density as a function of photon energy for solution of colloidal CdTe (A) and CdCoS (B) QDs with average size of 3 nm and 5 nm, respectively; curve 1 – corresponds to QDs only, 2 – corresponds to HSA + QDs (1.5×10^{-3} mmol/l for CdTe and 0.9×10^{-3} mmol/l for CdCoS), 3 – corresponds to HSA + QDs (0.9×10^{-3} mmol/l for CdTe and 0.6×10^{-3} mmol/l for CdCoS), 4 – corresponds to HSA solution only.

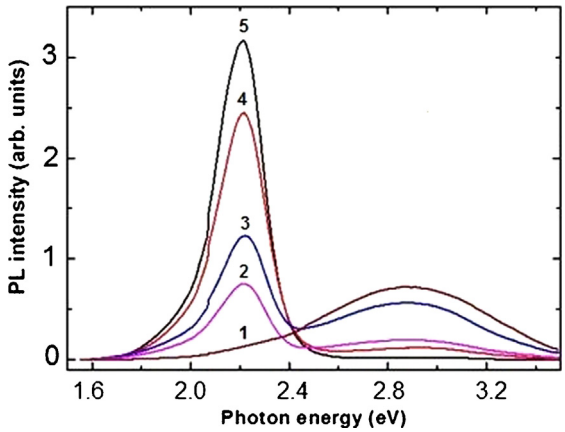

Fig. 5. Photoluminescence (PL) spectra of an HSA solution, CdTe QDs (3.0 nm) solution, and CdTe QD-HSA complexes (curve 1 – corresponds to pure HSA solution, 2 – corresponds to HSA + 0.6×10^{-3} mmol/l QDs, 3 – corresponds to HSA + 0.9×10^{-3} mmol/l QDs, 4 – corresponds to HSA + 1.5×10^{-3} mmol/l QDs, 5 – corresponds to pure CdTe QDs).

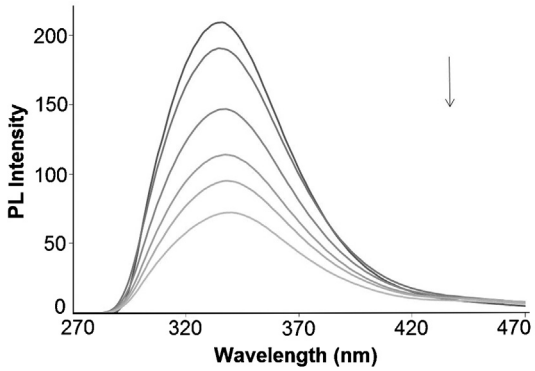

Fig. 6. The photoluminescence spectra of 10 mg/ml HSA under interaction with different concentration of CdCoS QDs (a – 0; b – 0.1; c – 0.2; d – 0.3; e – 0.4; f – 0.5 mg/ml).

nanocrystals is found to be approximately from 2.5 to 5 nm for CdTe QDs (Fig. 2C, D) and 4 to 8 nm for CdCoS QDs (Fig. 2A, B). In the AFM image some larger structures can be also observed. This aggregate can be created by sample drying in air atmosphere.

Fig. 3 shows EDX results on the content of chemical elements for CdCoS QDs. All of the compounds are clearly visible in the structure of the material.

4.2. Optical spectra

The absorption spectra are recorded and analyzed for the evaluation of optical properties of pure QDs, pure HSA and QD-HSA complex. The region of the fundamental absorption edge of the CdTe QDs and the CdCoS QDs are studied. Fig. 4A shows optical density as a function of photon energy for four samples. Curve 1 corresponds to the sample of the CdTe QDs, curve 2 was obtained for the CdTe QDs + HSA with a higher concentration of QDs of 1.5×10^{-3} mmol/l, curve 3 for the CdTe QDs + HSA with a lower concentration of QDs (0.9×10^{-3} mmol/l), and curve 4 corresponds to pure HSA solution. Curves 1 and 2 in Fig. 4A demonstrate the exciton states of QD [46] – pronounced plateau at photon energies 2.3–2.5 eV as well as at 2.8–3.2 eV. An addition of HSA to colloidal CdTe nanoparticles leads to a gradual decrease of optical density (curve 2) and broadening of exciton structure (curve 3). However, energy position of the exciton bands is not shifted. The obtained results indicate that the binding process between the QDs and the protein molecules do not change the electron states in the QDs.

As shown in Fig. 4B, the energy position of the excitation band for CdCoS QDs is at around 3.4–3.45 eV. An addition of HSA to colloidal CdCoS QDs does not change the position of the band, however, it leads to a gradual decrease of the optical density and broadening of an exciton structure [47], similar to the previously described CdTe QDs.

4.3. Photo-luminescent assay

The photoluminescence of QDs is characterized by practically a 100% efficiency. The increase in a photoluminescence quantum efficiency results from a surface passivation effect of the core, where the number of non-radiative recombination sites, such as holes and gap states are reduced, enhancing the charge transfer [42,48]. A variety of the molecular interactions can result in quenching, including excited-state reactions, energy transfer, ground-state complex formation, and collisional quenching [47].

Photoluminescence spectra of HSA under interaction with QDs have been recorded on spectrofluorymeter upon the excitation wavelength optimal for the investigated QDs. The optical properties are dependent on composition, size and shape of QDs, resulting in a certain position of absorption bands. For the CdS QDs and the CdCoS QDs the excitation band is approximately at 250 nm and 325 nm for CdTe QDs, respectively. The measurements are usually performed at room temperature. The changing of photoluminescence properties of HSA by the presence of QDs is described in several scientific reports [24–31]. The fluorescence of HSA mainly comes from tryptophan, tyrosine and phenylalanine amino acids. When small molecules bound to HSA, the changes of the protein luminescence are mainly due to the tryptophan side chains [49,50]. The phenomenon of fluorescence quenching is commonly regis-

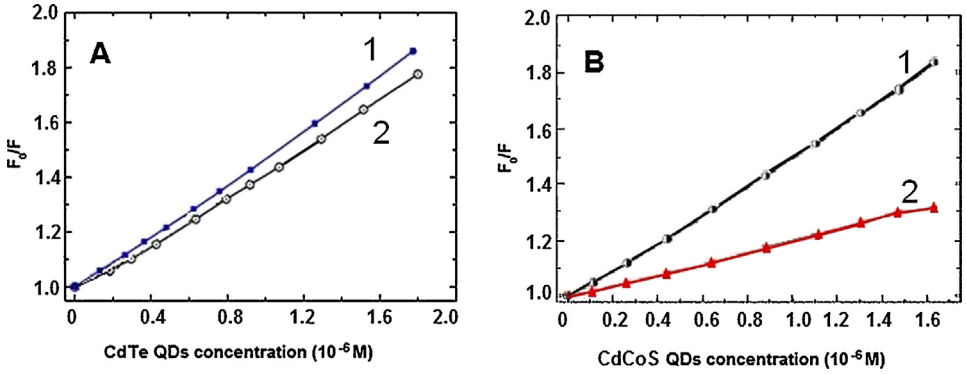

Fig. 7. Stern–Volmer plots for CdTe QDs (A) at different temperatures 1–293 K and 2–309 K, and CdCoS QDs (B) with different concentration of Co element: 1 – $Cd_{0.9}Co_{0.1}S$ and 2 – $Cd_{0.95}Co_{0.05}S$, under interaction with HSA.

Table 2
Identification of the lines observed in the FTIR spectra (Fig. 8); s – strong, m – medium, w – weak.

Chemical bonds	Peak position (cm^{-1}) – HSA	Peak position (cm^{-1}) – HSA + CdTe	Peak position (cm^{-1}) – HSA + CdCoS
Amide I (**C=O** stretch, CN stretch, CCN deformation, NH bend)	1651 (s)	1652 (s)	1650 (s)
Amide II (**NH** bend, **CN** stretch, CO in plane, CC, NC stretch)	1546 (s)	1548 (s)	1541 (s)
C—H bend	1446 (w)	1446 (w)	1452 (w)
COO— stretch and C—H bend	1402 (m)	1402 (m)	1390; 1410 (m)
Amide III (**CN** stretch, **NH** bend, CO in plane, CC stretch)	1236 (w)	1236 (w)	1238 (w)

tered, however, sometimes the strengthening of the light emission is noticed. The modification of the QDs photoluminescence by the HSA presence is not so thoroughly described.

Fluorescence quenching is a powerful tool for determining the type of interaction between protein and quenchers dispersed in a solution. The photoluminescence quenching mechanism can be calculated by the Stern–Volmer equation [51]:

$$\frac{F_0}{F} \equiv 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, K_q is the quenching rate constant of the bimolecular, τ_0 is the average lifetime of the fluorophore without quencher, K_{SV} and [Q] are the Stern–Volmer dynamic quenching constant and the concentration of the quencher, respectively.

The mechanisms of quenching are usually classified as the dynamic quenching or the static quenching [51,52]. These can be distinguished by their varying dependence on temperature or by luminescence lifetime measurements. Since the dynamic quenching depends on diffusion [53] the quenching constants are expected to increase with increasing temperature [54]. In contrast, increased temperature result in decreased stability of the complexes, and therefore, lower values of the static quenching constants [48].

As mentioned in previous part, interactions between some molecules including nanoparticles, ions or other ligands can affect the changes in the protein photoluminescence properties. Fig. 5 shows photoluminescence spectra of an HSA solution (curve 1), colloidal CdTe QDs (curve 5) and their combinations (curve 2–4). The photoluminescence intensity of the protein is decreased with the increasing concentration of CdTe QDs. The quenching effect occurs.

Fig. 6 shows photoluminescence spectra in the range between 270 and 470 nm, which is typical for the HSA photoluminescence emission spectrum. HSA has a strong fluorescence emission band at 336 nm, with the excitation wavelength at 250 nm. When different amounts of CdCoS QDs are added to the HSA solution, the decrease in the fluorescence intensity of protein is observed. These suggest, that the quenching effect occurs in the CdCoS QD-HSA complex and may lead to changes in the protein conformation [30]. Xiao et al. studied the relationship between sizes of CdSe/ZnS QDs (nanocrystals size from 2 nm to 4.8 nm) and occurrence of changes in the BSA molecular structure. Conducted research confirmed stronger reduces of the protein biological activity by QDs with bigger diameter [27].

The photoluminescence intensity of the HSA progressively decreases with the increase of the CdTe QDs, as well as the CdCoS QDs concentration. Similar experiments were carried out also for other types of QDs: ZnS [28,29], ZnO [30], CdSe/CdS [55], and CdSeS/ZnS [56], *e.g.*, the obtained results have been similar, and they have shown the occurrence of quenching effect. However, different intensity of the process was noticed.

In Fig. 7 the Stern–Volmer plots for CdTe QDs (A), and CdCoS QDs (B) in the interaction with HSA are shown. Both studied QDs have static mechanism of quenching. However, the Co concentration in the CdCoS QDs plays an important role in this mechanism. The static mechanism occurs when the interaction between the quencher and the fluorophore takes place in the ground state of molecules. This type of interaction is also demonstrated by Hemmateenejad and Yousefinejad [30]. They described the presence of static mechanism of fluorescence quenching in the interaction between the HSA and the ZnS nanoparticles.

The quenching constant K_q of CdTe QDs with the average radius of 3.0 nm for HSA was calculated to be approximately of 2.2×10^{13} $lmol^{-1}$ s^{-1}. It occurred, that the quenching constants decrease with the increase of temperatures, which indicates that the quenching mechanism mainly arises from static quenching as mentioned before [57]. According to Refs. [57,58], the maximum scatter collision quenching constant of various quenchers with the biopolymer is of 2.0×10^{10} $lmol^{-1}$ s^{-1} for dynamic quenching. The rate constants of the HSA quenching procedure initiated by nanoparticles were calculated much greater than 2.0×10^{10} $lmol^{-1}$ s^{-1}, it can be concluded and additionally confirmed that the nature of quenching is not dynamic, but probably static, resulting in forming QD-HSA complexes. Simultaneously the interaction between QDs and HSA does not affect the electron structure of QDs.

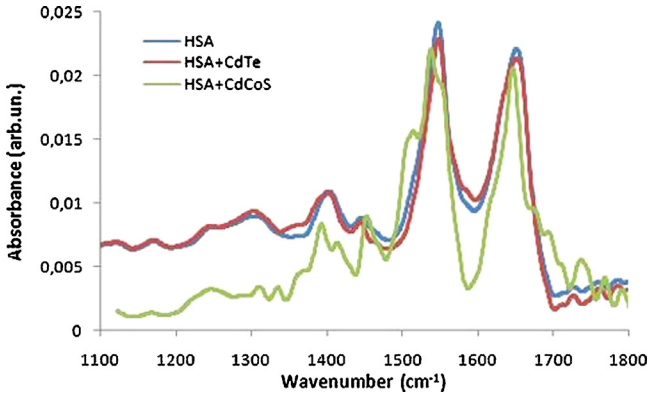

Fig. 8. FTIR spectra of the HSA (blue), HSA after interaction with: CdTe QDs (red) and CdCoS QDs (green).

4.4. Oscillation spectra

The infrared spectroscopy and Raman spectroscopy are commonly used for the chemical structure determination, conformation changes identification, and the study of the interactions between molecules [41,59]. The results of oscillation spectra can give important information about the processes occurring during a bio-nanocomplex formation. In Fig. 8 the spectra of pure HSA, and HSA after interaction with the CdTe QDs and the CdCoS QDs are shown. Table 2 contains the most important oscillation lines of the investigated protein, and their shifting after the HSA connection with different types of QDs.

Changes of molecules, chemical structure (including changes in the secondary structure of proteins) can be observed through the infrared spectroscopy method [60]. The two main peaks at 1651 cm^{-1} and 1546 cm^{-1} are observed in all IR spectra and they correspond to the amide I and the amide II bands, respectively [61–63]. Amide I is mainly associated with C=O stretch vibration, amide II is described by C–N stretch coupled with N–H bending vibrations. Both of them indicate the secondary structure of protein [49,64]. Apart from these two main lines, weaker line was observed at 1236 cm^{-1} and it corresponds to the amide III band. It can be assigned mainly to CN stretching, and NH bending vibrations [62,65] (Table 2).

The changes of the peaks position and shape are small for the HSA in the interaction with the CdTe QDs. The position of the peak maximum is shifted, from 1651 cm^{-1} to 1652 cm^{-1} for the amide I and from 1546 cm^{-1} to 1548 cm^{-1} for the amide II. The peaks' shapes and peaks intensity slightly changed also. However, for HSA + CdCoS QDs sample the secondary structure of the protein is modified more strongly. The amide II band is shifted from 1546 cm^{-1} to 1541 cm^{-1} for HSA in the interaction with CdCoS, while the amide I peak at 1651 cm^{-1} is shifted only at about 1 cm^{-1} (Table 2). However, the peaks' shapes changed more significantly, which indicates the secondary structure modification [60]. The shape of amide II line of the pure HSA is mainly characteristic for the α-helical protein structure [41]. After interaction with the CdCoS QDs some HSA signals at around 1626 cm^{-1} and 1682 cm^{-1} which are associated with a β-sheets' structure [66,67] are noticed. An additional maximum at 1514 cm^{-1} in amide II bond was also observed. It can be associated with tyrosine amino acid vibrations or changes in the vibrations of C—N or N—H chemical bonds. The results indicate a slight reduction of α-helical folding protein type. The HSA conformation is not much changed after the interaction with CdTe QDs, and the protein structure is similar to the natural one. However, the presence of the CdCoS QDs modified the structure of albumin more strongly than CdTe QDs.

Table 3
Advantages and disadvantages of QD-HSA complex in biomedical applications [6,16,72,73].

Advantages	Disadvantages
Better biocompatibility	Quenching effect between QDs and protein
Reduction of toxicity	Less photoluminescence intensity than pure QDs
Easy to conduct	Non-specific binding
Water soluble	Bigger size (around 10–15 nm)
Good stability (short term)	
Maintaining resistance to photobleaching, photo and chemical degradation	
Flexible modifications (multifunctional probes)	
Insignificant quantum yield decrease	

5. Bioimaging properties

The unique optical properties of QDs allow them to be widely used in medicine for diagnostic purposes, including detection of cancer cells, as well as their labeling and tracking, especially with using a fluoresce microscopy technique [9,68–71] and other bioimaging methods [72,73]. Many researches focus on an early cancer diagnosis such as breast cancer, cervical carcinoma, prostate cancer, and hepatocellular carcinoma [8]. Functionalized quantum dots seem to be the most promising material, which can be combined with selected biological or chemical ligands like proteins or DNA sequences. They can detect the cancer cells or other molecules as a magnificent fluorescent probe [68]. Specific detection of the cancer cells or other tumor markers is possible in case of using quantum dots connected with antibodies. This solution gives better visibility of the tumor during the diagnosis and the treatment process [69].

Developed quantum dots are also used for modern nanosensors creations. A biosensors which are based on the nanocrystals often employ fluorescence resonance energy transfer phenomenon (FRET) [11]. The mechanism of FRET consists of the energy transfer between donor and acceptor. The excited donor transfers energy to the acceptor, which emits it in the form of fluorescence. Generally, the use of quantum dots involves the formation of new systems, that will be delivered to the body and the monitoring of their distribution in the body will simultaneously be possible.

As mentioned, the surface of QDs can be easily modified. It gives several advantages, *e.g.*, improving endocytosis process, selective molecules recognizing, fluorescence properties developing and finally toxic effect reduction [70,71]. In the literature we can find some reports which indicate that the attachment of the protein to the QDs surface can have benefits. Researchers describe better biocompatibility, the reduction of toxicity, as well as the improvement of other functional properties of the material. Mansur et al. [72] present the results concerning the formation of the CdS QDs + BSA complex leading to the stabilization of quantum dots' fluorescence in the dispersion systems. Nithyaja et al. [25] demonstrate that the effective control of the CdS QDs band gap may be possible by varying the concentration of the protein or nucleic acid. Other published results show that the protein improves the stability and the luminescence quantum yield of CdTe quantum dots [73]. The main advantages and disadvantages of QD-HSA complex in biomedical applications are presented in Table 3.

The side-effects of the pure QDs and the QD-HSA complexes using in living organisms could be different. The nanoparticles can induce oxidative stress in living cells, that may lead to cell death and/or stress adaptation. Stress-induced premature senescence (SIPS) should be also taken in consideration [74]. The nanoparticles are usually taken up by the phagocyte cells of the immune system (*e.g.*, macrophages) that may promote undesirable interactions between nanoparticles and the immune system such as

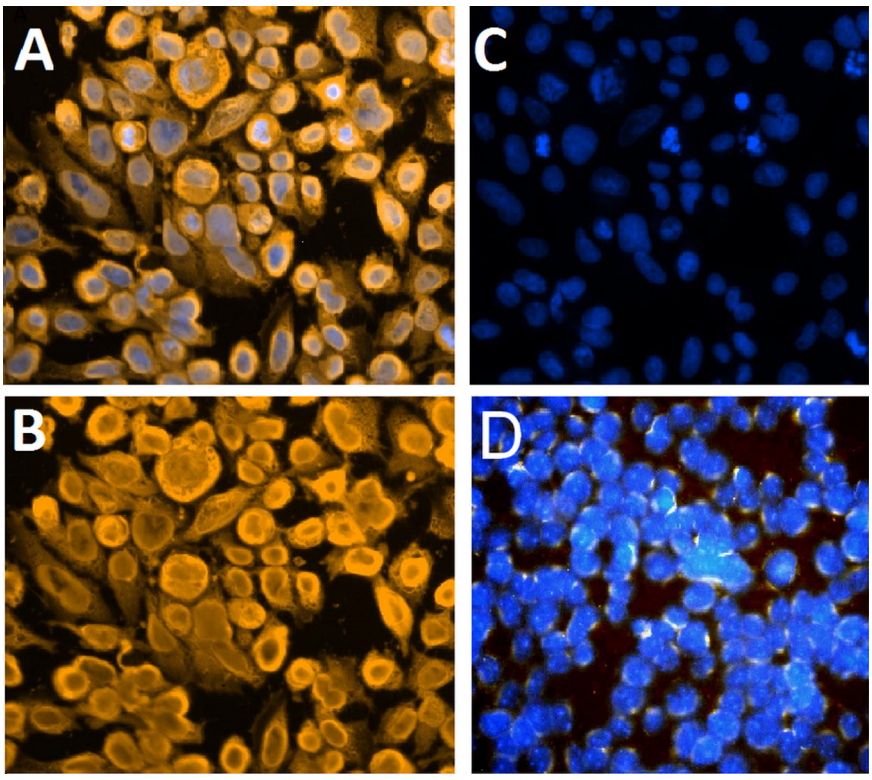

Fig. 9. Osteosarcoma cells incubated with CdTe QDs (A–C) and CdCoS QDs (D); orange colour – CdTe QDs (A, B) and CdCoS QDs (C), blue colour – Hoechst 33342 fluorescent dye (A, C, D).

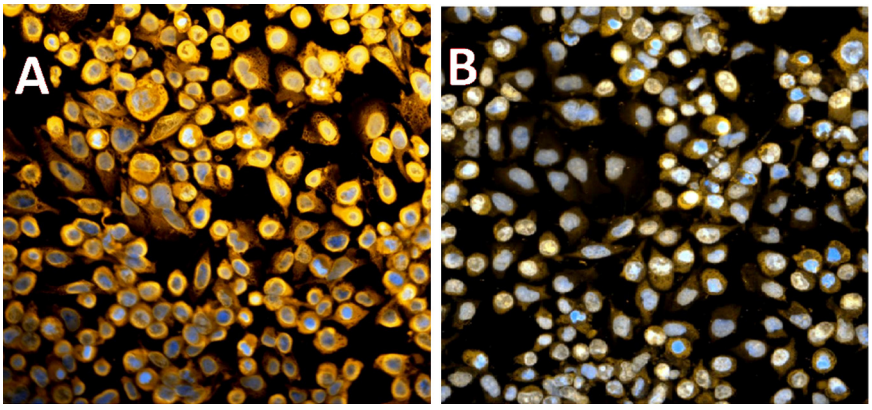

Fig. 10. Osteosarcoma cells treated by pure CdTe QDs (A) and CdTe QD-HSA bio-nanocomplex (B).

immunostimulation or immunosuppression. The latter ones could stimulate inflammatory or autoimmune disorders, as well as could increase host susceptibility to infections and cancer [75]. The main function of the immune system is to protect the host from foreign substances. However, improper recognition of nanoparticles as foreign agents by the immune cells may result in a multi-level immune response against the nanoparticles and eventually lead to toxicity in the host and/or lack of therapeutic efficacy [75]. Therefore, covering the QDs with specific molecules can complicate its interaction with the immune system while covering the surface of the nanoparticles with human protein may cause greater biocompatibility of the material. The QDs may become to be invisible to the immune system and in this way to deceive them if their surface possesses the specific HSA antigen.

A current imaging techniques like X-ray, computed tomography or ultrasound are very important for the cancer diagnosis, but they have two major limitations. They do not have sufficient sensitivity (especially in case of small numbers of malignant cells), and sufficient specificity. Quantum dots imaging probes, provide the potential to fulfill the requirements for cancer cells imaging [47] and searching for nanoparticles with optimal properties for bioimaging and diagnostic applications is still widely pursued [76–79].

A semiconductor QDs such as: CdTe, CdS, CdSe, PbTe, PbS, PbSe and InP QDs [76] were tested for their utility in the study of cancer cells. Rodriguez-Torres et al. [77] prepared TGA-capped CdSe QDs with varying content of selenium (10 and 15 mM) as a bioimaging agents to efficiently detect colo-205 human cancer cells. Prepared QDs do not have toxic effect in small concentrations. They are cytotoxic above the concentration of 2.0 mM. Investigated TGA-CdSe QDs were distributed within the cytosolic side of cell membranes and can be potentially employed for colo-205 cancer cells marking with strong fluorescence emission efficiency after 48 h of incubation with the cells.

CdSe QDs were also investigated by Vibin et al. [78] for the human cervical cancer cell line bioimaging. In this study the CdSe QDs were covered by silica and used for *in vivo*, as well as *in vitro* study. In both cases the small toxic effect was observed. It is so low that it does not preclude using them. As we can find in Jankiewicz et al. [79] the surface of silica particles or core/shell particles with silica layer can be easily modified by a number of chemical compounds having various functional groups and molecular structure. Through these modifications, some physical and chemical properties of nanoparticles can be altered and improved.

In the presented review, the investigation of using of CdTe, CdCoS QDs and QD-HSA complexes for 134b osteosarcoma cells bioimaging is described more precisely as one of the examples of using this type of nanomaterials for cancer cell bioimaging. The human osteosarcoma is the primary malignant bone tumor in children and adult. Early detection is particularly important, because the tumor is prone to metastasize (especially in the lung) and further mutation and genetic changes often occur [80].

Both types of pure CdTe and CdCoS QDs do not penetrate into the living osteosrcoma cells. The fluorescence of QDs is not detected inside the cells. The emission of light is noticed only on the surface of cells which suggest that the QDs interact with cells and covered them. Only damage or dead cells have a relatively higher uptake of the QDs, and fluorescence inside these cells is noticed. To improve the diffusion through the cell membrane without cells modification, the surface of QDs should be modified by connecting some chemicals, e.g. mercaptopropionic acid, PEG – polyethyleneglicols, Pluronic F127 [81], methotrexate [82] or chitosan-peptide conjugate [83], which interact with some cell's receptors.

The uptake of CdTe and CdCoS QDs to the cytoplasm space of permeabilized osteosarcoma cells is demonstrated in Fig. 9. The fluorescence intensity is higher for CdTe QDs than CdCoS QDs which suggests better optical properties and usability as a bioimaging probe. The QDs fluorescence is recorded in the whole cell except for the nucleus region, which is marked by a standard fluorescence dye – Hoechst. However, the strongest signal is noticed around the nuclear membrane. It suggests, that most of QDs are located on the surface of nuclear membrane, but cannot penetrate it. Similar conclusions are also described by Kuo et al. [84]. The fluorescence intensity of intra cellular QDs was noticed even after very short incubation (10 min) and stability during 24 h. Additionally, cells treated with QDs could be imaged even after 30 days. The images are still clear and informative. The CdTe QDs are recognized as strong cells' fluorescent indicator (Fig. 9A, B), which can be used for marking, labeling and tracking of the cells.

The CdTe QD-HSA bio-nanocomplex is recognized as a more biocompatible fluorescence material, which can be used for fluorescence analytical methods as a measuring probe. Less fluorescence intensity is observed after cells' treatment by the CdTe QDs connected with albumin than CdTe without the protein (Fig. 10). It is caused by the quenching effect described in previous section, but also it can be associated with less penetration of the bio-nanocomplexes into the cells. However, the fluorescence efficiency is still satisfactory and it provides opportunities for bioimaging applications of this material.

More complicated system than the described above is presented by Meng et al. [16]. CdTe/ZnS quantum dots were conjugated with BSA and additionally, with folic acid (FA) for FA-BSA-QDs complex forming. According to the authors this system can be used for monitoring cancer cells with overexpression of FA receptors such as human nasopharyngeal carcinoma cells. The presented characteristics suggest that FA-BSA-QDs have better efficiency of luminescence for FA-positive cells. As a result, they can also be the potential candidates for cancer diagnosis. It is also possible to include selective antibodies to the surface of QDs and to use immunofluorescence methods to achieve even greater specificity than the receptor–ligand interaction. However, the problem of non-specific binding, as well as improving other properties such as solubility or stability in aqueous solutions requires more research.

6. Conclusion

As shown, searching for new tools and methods for a more accurate and earlier diagnosis of cancer is still valid and it is the main problem of nanomedicine. Currently, numerous studies of the production, modification and practical use of such nano-materials as the semiconductor QDs are conducted. They are becoming common fluorescence markers for *in vitro* imaging rather than *in vivo* analysis, not to mention the possible toxicity of some components.

Covering of QDs by natural protein seems to be a promising solution for the reduction of their toxicity. Therefore, forming the QD-HSA complexes is important in order to remove toxicity from the QDs.

Two different types of QDs: CdTe and CdCoS are the interesting examples of bioimaging properties of biofunctionalized of QDs protein. Their interactions with HSA, as well as fluorescence properties after QD-HSA complex forming seem to be particularly promising.

In most cases of a QD–protein interaction the fluorescence is modified through static quenching mechanism but quantum yield decreases insignificantly. Therefore, CdTe QD-HSA bio-nanocomplex could be a useful material for cancer cell marking, labeling and tracking, as a more biocompatible than pure QDs.

Traditional fluorescent probes are still used more often than modern fluorescent nanoparticles. However, we can expect that because of their favorable properties like a high fluorescence efficiency, increased ability to multiplex of emitted light wavelength, long fluorescence life time and resistance to photo-bleaching they will be increasingly used.

References

[1] S.F. Wuister, I. Swart, F. van Driel, S.G. Hickey, C. de Mello Donega, Highly luminescent water-soluble CdTe quantum dots, Nano Lett. 3 (4) (2003) 503–507.
[2] R. Kaur, A.K. Paul, A. Deep, Conjugation of chlorinated carbon nanotubes with quantum dots for electronic applications, Mater. Lett. 117 (2014) 165–167.
[3] T. Jamieson, R. Bakhshi, D. Petrova, R. Pocock, M. Imani, A.M. Seifalian, Biological applications of quantum dots, Biomaterials 28 (2007) 4717–4732.
[4] X. Michalet, F.F. Pinaud, L.A. Bentolila, J.M. Tsay, S. Doose, J.J. Li, G. Sundaresan, A.M. Wu, S.S. Gambhir, S. Weiss, Quantum dots for live cells, in vivo imaging, and diagnostics, Science 307 (5709) (2005) 538–544.
[5] M. Wiśniewski, P. Rossochacka, K. Werengowska-Ciećwierz, Medical aspects of nanostructural carbonaceous materials, Inż. Ochr. Środow. 16 (2) (2013) 255–261.
[6] I.L. Medintz, H.T. Uyeda, E.R. Goldman, H. Mattoussi, Quantum dot bioconjugates for imaging, labelling and sensing, Nat. Mater. 4 (6) (2005) 435–446.
[7] T.M. Samir, M.M.H. Mansour, S.C. Kazmierczak, H.M.E. Azzazy, Quantum dots: heralding a brighter future for clinical diagnostics, Nanomedicine 7 (11) (2012) 1755–1769.
[8] D. Chen, J. Liu, X.-F. Yu, M. He, X.-F. Pei, Z.-Y. Tang, Q.-Q. Wang, D.-W. Pang, Y. Li, The biocompatibility of quantum dot probes used for the targeted imaging of hepatocellular carcinoma metastasis, Biomaterials 29 (2008) 4170–4176.
[9] W.C. Chan, D.J. Maxwell, X. Gao, Luminescent quantum dots for multiplexed biological detection and imaging, Curr. Opin. Biotechnol. 13 (1) (2002) 40–46.
[10] K.D. Wegner, N. Hildebrandt, Quantum dots: bright and versatile in vitro and in vivo fluorescence imaging biosensors, Chem. Soc. Rev. 44 (2015) 4792–4834.
[11] M. Li, F. Li, Z. He, J. Zhang, J. Han, P. Lu, Two-photon-excited fluorescence resonance energy transfer in an aqueous system of CdTe quantum dots and Rhodamine B, J. Appl. Phys. 116 (2014) 233106-1–233106-6.
[12] M. Yao, X. Zhang, L. Ma, W. Chen, A.G. Joly, J. Huang, Q. Wang, Luminescence enhancement of CdTe nanostructures in LaF_3:Ce/CdTe nanocomposites, J. Appl. Phys. 108 (2010) 103104-1–103104-7.
[13] M. Gao, C. Lesser, S. Kirstein, H. Möhwald, A.L. Rogach, H. Weller, Electroluminescence of different colors from polycation/CdTe nanocrystal self-assembled films, J. Appl. Phys. 87 (2000) 2297–2302.
[14] M. Fang, C.-W. Peng, D.-W. Pang, Y. Li, Quantum dots for cancer research: current status, remaining issues, and future perspectives, Cancer Biol. Med. 9 (2012) 151–163.

[15] Handbook of Nanophysics: Nanomedicine and Nanorobotics, in: J. Sattler, D. Klaus (Eds.), CRC Press, 2011, ISBN 978-1-4200-7546-5.
[16] H. Meng, J.Y. Chen, L. Mi, P.N. Wang, M.Y. Ge, Y. Yue, N. Dai, Conjugates of folic acids with BSA-coated quantum dots for cancer cell targeting and imaging by single-photon and two-photon excitation, J. Biol. Inorg. Chem. 16 (1) (2011) 117–123.
[17] S.J. Rosenthal, J.C. Chang, O. Kovtun, J.R. McBride, I.D. Tomlinson, Biocompatible quantum dots for biological applications, Chem. Biol. 18 (2011) 10–24.
[18] J. Gburek, K. Gołąb, K. Juszczyńska, Renal catabolism of albumin – current views and controversies, Postępy Hig. Med. Dośw. 65 (2011) 668–677.
[19] A. Miller, W.W. Jędrzejczak, Albumina – funkcje biologiczne i znaczenie kliniczne, Postępy Hig. Med. Dośw. 55 (2001) 17–36.
[20] C.H. Park, T. Maack, Albumin absorption and catabolism by isolated perfused proximal convoluted tubules of the rabbit, J. Clin. Invest. 73 (1984) 767–777.
[21] G.J. Quinlan, G.S. Martin, T.W. Evans, Albumin: biochemical properties and therapeutic potential, Hepatology 41 (6) (2005) 1211–1219.
[22] D.C. Carter, X.M. He, S.H. Munson, P.D. Twigg, K.M. Gernert, B. Broom, T.Y. Miller, Three-dimensional structure of human serum albumin, Science 244 (1989) 1195–1198.
[23] L. Trynda-Lemiesz, K. Wiglusz, I. Mucha, The role of albumin in diagnostics. Binding of ions and metal complexes, Wiad. Chem. 64 (2010) 84–101.
[24] C.M. Wu, L.Y. Lin, Utilization of albumin-based sensor chips for the detection of metal content and characterization of metal–protein interaction by surface plasmon resonance, Sens. Actuators B: Chem. 110 (2005) 231–238.
[25] B. Nithyaja, K. Vishnu, S. Mathew, P. Radhakrishnan, V.P.N. Nampoori, Studies on CdS nanoparticles prepared in DNA and bovine serum albumin based biotemplates, J. Appl. Phys. 112 (2012) 064704-1–064704-6.
[26] L.W. Shao, C.Q. Dong, F.M. Sang, H.F. Qian, J.C. Ren, Studies on interaction of CdTe quantum dots with bovine serum albumin using fluorescence correlation spectroscopy, J. Fluoresc. 19 (2009) 151–157.
[27] Q. Xiao, S. Huang, W. Su, P. Li, J. Ma, F. Luo, Y. Liu, Systematically investigations of conformation and thermodynamics of HSA adsorbed to different sizes of CdTe quantum dots, Colloids Surf. B: Biointerfaces 102 (2013) 76–81.
[28] D. Wu, Z. Chen, X. Liu, Study of the interaction between bovine serum albumin and ZnS quantum dots with spectroscopic techniques, Spectrochim. Acta A Mol. Biomol. Spectrosc. 84 (2011) 178–183.
[29] J. Hemmateenejad, S. Yousefinejad, Interaction study of human serum albumin and ZnS nanoparticles using fluorescence spectrometry, J. Mol. Struct. 1037 (2013) 317–322.
[30] E.A. Bhogale, N. Patel, J. Mariam, P.M. Dongre, A. Miotello, D.C. Kothari, Systematic investigation on the interaction of bovine serum albumin with ZnO nanoparticles using fluorescence spectroscopy, Colloids Surf. B: Biointerfaces 102 (2013) 257–264.
[31] M. Ghali, Static quenching of bovine serum albumin conjugated with small size CdS nanocrystalline quantum dots, J. Lumin. 130 (2010) 1254–1257.
[32] C.B. Murray, C.R. Kagan, M.G. Bawendi, Self-organization of CdSe nanocrystallites into three-dimensional quantum dot superlattices, Science 270 (1995) 1335–1338.
[33] A.I. Savchuk, I.D. Stolyarchuk, P.M. Grygoryshyn, O.P. Antonyuk, T.A. Savchuk, Interaction of human serum albumin with CdTe quantum dots probed by optical spectroscopy methods, Proc. SPIE 9066 (2013) 906618.
[34] V.I. Fediv, A.I. Savchuk, V.M. Frasunyak, V.V. Makoviy, O.A. Savchuk, Magnetic and magneto-optical properties of CdS:Mn quantum dots in PVA matrix, J. Phys.: Conf. Ser. 245 (2010), 012084 1–4.
[35] L. Sharma, M. Inpasalini, S. Mukherjee, Defect induced ferromagnetism in luminescent and doped CdS quantum dots, J. Mater. Sci. Mater. Electron. 26 (10) (2015) 7621–7628.
[36] I. Rashid, K. Imad, A.H.A. Rahnamaye, A. Zahid, A. Iftikhar, Density functional studies of magneto-optic properties of CdCoS, J. Magn. Magn. Mater. 351 (2014) 60–64.
[37] H. Zhang, L. Wang, H. Xiong, L. Hu, B. Yang, W. Li, Hydrothermal synthesis for high-quality CdTe nanocrystals, Adv. Mater. 15 (20) (2003) 1712–1715.
[38] C. Wang, Q. Ma, W. Dou, S. Kanwal, G. Wang, P. Yuan, X. Su, Synthesis of aqueous CdTe quantum dots embedded silica nanoparticles and their applications as fluorescence probes, Talanta 77 (4) (2009) 1358–1364.
[39] Y.J. Hu, Y. Liu, L.X. Zhang, R.M. Zhao, S.S. Qu, Studies of interaction between colchicine and bovine serum albumin by fluorescence quenching method, J. Mol. Struct. 750 (2005) 174–178.
[40] L. Zhao, X. Zhao, B. Yang, C. Gao, X. Hao, Y. Wu, New strategy for the evaluation of CdTe quantum dot toxicity targeted to bovine serum albumin, Sci. Total Environ. 407 (2009) 5019–5023.
[41] J. Liang, Y. Cheng, H. Han, Study on the interaction between bovine serum albumin and CdTe quantum dots with spectroscopic techniques, J. Mol. Struct. 892 (2008) 116–120.
[42] M.A. Walling, J.A. Novakl, J.R.E. Shepard, Quantum dots for live cell and in vivo imaging, Int. J. Mol. Sci. 10 (2009) 441–491.
[43] G.R. Bardajee, Z. Hooshyar, Interaction of a novel starch-capped CdS quantum dots with human serum albumin and bovine serum albumin, Starch 68 (2016) 329–338.
[44] Q.-F. Chen, W.-X. Wang, Y.-X. Ge, M.-Y. Li, S.-K. Xu, X.-J. Zhang, Direct aqueous synthesis of cysteamine-stabilized CdTe Quantum dots and its deoxyribonucleic acid bioconjugates, Chin. J. Anal. Chem. 35 (1) (2007) 135–138.
[45] L. Lai, C. Lin, Z.-Q. Xu, X.-L. Han, F.-F. Tian, P. Mei, Spectroscopic studies on the interactions between CdTe quantum dots coated with different ligands and human serum albumin, Spectrochim. Acta Mol. Biomol. Spectrosc. 97 (2012) 366–376.
[46] Y. Masumoto, T. Takagahara, Semiconductor Quantum Dots Physics, Spectroscopy and Applications, Springer Science & Business Media, 2002, pp. 96–107.
[47] H. Zhang, D. Yee, C. Wang, Quantum dots for cancer diagnosis and therapy: biological and clinical perspectives, Nanomedicine 3 (1) (2008) 83–91.
[48] B.O. Dabbousi, J. Rodriguez-Viejo, F.V. Mikulec, J.R. Heine, H. Mattoussi, R. Ober, K.F. Jensen, M.G. Bawendi, (CdSe)ZnS core–shell quantum dots: synthesis and characterization of a size series of highly luminescent nanocrystallites, J. Phys. Chem. B 101 (1997) 9463–9475.
[49] A. Sulkowska, Interaction of drugs with bovine and human serum albumin, J. Mol. Struct. 614 (2002) 227–232.
[50] P.N. Naik, S.T. Nandibewoor, S.A. Chimatadar, Non-covalent binding analysis of sulfamethoxazole to human serum albumin: fluorescence spectroscopy, UV–vis, FT-IR, voltammetric and molecular modeling, J. Pharm. Anal. 5 (3) (2015) 143–152.
[51] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd ed., Springer Science Business Media, 2006, pp. 278–280.
[52] E.R. Carraway, J.N. Demas, B.A. DeGraff, Luminescence quenching mechanism for microheterogeneous systems, Anal. Chem. 63 (4) (1991) 332–336.
[53] H. Ditlbacher, J.R. Krenn, N. Felidj, B. Lamprecht, G. Schider, M. Salerno, A. Leitner, F.R. Aussenegg, Fluorescence imaging of surface plasmon fields, Appl. Phys. Lett. 80 (2002) 404–406.
[54] C. Röcker, M. Pötzl, F. Zhang, W.J. Parak, G.U. Nienhaus, A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles, Nat. Nanotechnol. 4 (2009) 577–580.
[55] Q. Wang, F. Ye, P. Liu, X. Min, X. Li, Conjugation and fluorescence quenching between bovine serum albumin and L-cysteine capped CdSe/CdS quantum dots, Protein Pept. Lett. 18 (4) (2011) 410–414.
[56] K.S. Adarsh, M.K. Singh, M.G. Kotresh, L.S. Inamdar, M.A. Shivkumar, B.N. Jagatap, B.G. Mulimani, S.R. Inamdar, Spectroscopic investigation of water-soluble alloyed QDs with bovine serum albumin, Luminescence 32 (1) (2017) 35–42.
[57] H. Cao, D.H. Wu, H.X. Wang, M. Xu, Effect of the glycosylation of flavonoids on interaction with protein, Spectrochim. Acta Mol. Biomol. Spectrosc. 73 (5) (2009) 972–975.
[58] M.G. Bawendi, M.I. Steigerwald, L.E. Brus, The quantum mechanics of larger semiconductor clusters ("quantum dots"), Annu. Rev. Phys. Chem. 41 (1990) 477–496.
[59] J.N. Tian, J.Q. Liu, W.Y. He, Z.D. Hu, X.J. Yao, X.G. Chen, Probing the binding of scutellarin to human serum albumin by circular dichroism, fluorescence spectroscopy, FTIR, and molecular modeling method, Biomacromolecules 5 (5) (2004) 1956–1961.
[60] Q. Wang, P. Liu, X. Zhou, X. Zhang, T. Fang, P. Liu, X. Min, X. Li, Thermodynamic and conformational investigation of the influence of CdTe QDs size on the toxic interaction with BSA, J. Photochem. Photobiol. 230 (2012) 23–30.
[61] C.M. Orphanou, The detection and discrimination of human body fluids using ATR FT-IR spectroscopy, Forensic Sci. Int. 252 (2015) 10–16.
[62] A. Barth, Infrared spectroscopy of proteins, Biochim. Biophys. Acta 1767 (9) (2007) 1073–1101.
[63] K. Rahmelow, W. Hübner, Secondary structure determination of proteins in aqueous solution by infrared spectroscopy: a comparison of multivariate data analysis, Methods Anal. Biochem. 241 (1996) 5–13.
[64] S. Tabassum, W.M. Al-Asbahy, M. Afzal, F. Arjmand, Synthesis, characterization and interaction studies of copper based drug with Human Serum Albumin (HSA): spectroscopic and molecular docking investigations, J. Photochem. Photobiol. B: Biol. 114 (2012) 132–139.
[65] A.I. Ivanov, R.G. Zhbankov, E.A. Korolenko, E.V. Korolik, L.A. Meleshchenko, M. Marchewka, H. Ratajczak, Infrared and Raman spectroscopic studies of the structure of human serum albumin under various ligands loads, J. Appl. Spectrosc. 60 (1994) 305–309.
[66] J. Kong, S. Yu, Fourier transform infrared spectroscopic analysis of protein secondary structures, Acta Biochim. Biophys. Sin. 39 (8) (2007) 549–559.
[67] S. Saha, T. Kamilya, R. Bhattacharya, A.K. Bhunia, Unfolding of blood plasma albumin protein in interaction with CdS nanoparticles, Sci. Adv. Mater. 6 (2014) 1–7.
[68] E.B. Voura, J.K. Jaiswal, H. Mattoussi, S.M. Simon, Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy, Nat. Med. 10 (9) (2004) 993–998.
[69] M.J. Kim, J.Y. Lee, U. Nehrbass, Detection of melanoma using antibody-conjugated quantum dots in a coculture model for high-throughput screening system, Analyst 137 (6) (2012) 1440–1445.
[70] L.-W. Liu, S.-Y. Hu, Y. Pan, J.-Q. Zhang, Y.-S. Feng, X.-H. Zhang, Optimizing the synthesis of CdS/ZnS core/shell semiconductor nanocrystals for bioimaging applications, Beilstein J. Nanotechnol. 5 (2014) 919–926.
[71] S. Zheng, J.-Y. Chen, J.-Y. Wang, L.-W. Zhou, Q. Peng, Effects of cell cycle on the uptake of water soluble quantum dots by cells, J. Appl. Phys. 110 (2011) 124701-1–124701-6.
[72] H. Mansur, A. Mansur, J. Gonzales, Protein-semiconductor quantum dot hybrids for biomedical applications, Phys. Status Solidi 96 (2012) 1435–1438.
[73] V. Poderys, M. Matulionyte, A. Selskis, R. Rotomskis, Interaction of water-soluble CdTe quantum dots with bovine serum albumin, Nanoscale Res. Lett. 6 (9) (2011) 1–6.
[74] J. Mytych, K. Pacyk, M. Pepek, J. Zebrowski, A. Lewinska, M. Wnuk, Nanoparticle-mediated decrease of lamin B1 pools promotes a TRF

protein-based adaptive response in cultured cells, Biomaterials 53 (2015) 107–116.
[75] Q. Jiao, L. Li, Q. Mu, Q. Zhang, Immunomodulation of nanoparticles in nanomedicine applications, BioMed Res. Int. 2014 (2014) 1–19.
[76] O. Mashinchian, M. Johari-Ahar, B. Ghaemi, M. Rashidi, J. Barar, Y. Omidi, Impacts of quantum dots in molecular detection and bioimaging of cancer, Bioimpacts 4 (3) (2014) 149–166.
[77] M.R. Rodriguez-Torres, C. Velez, B. Zayas, O. Rivera, Z. Arslan, M.N. Gonzalez-Vega, D. Diaz-Diestra, J. Beltran-Huarac, G. Morell, O.M. Primera-Pedrozo, Cyto-compatibility of direct water synthesized cadmium selenide quantum dots in colo-205 cells, J. Nanopart. Res. 17 (266) (2015) 1–11.
[78] M. Vibin, R. Vinayakan, A. John, V. Raji, N.S. Rejiya, A. Abraham, Cytotoxicity and fluorescence studies of silica-coated CdSe quantum dots for bioimaging applications, J. Nanopart. Res. 13 (6) (2011) 2587–2596.
[79] B.J. Jankiewicz, D. Jamiola, J. Choma, M. Jaroniec, Silica–metal core–shell nanostructures, Adv. Colloid Interface Sci. 170 (2012) 28–47.
[80] H.H. Luu, Q. Kang, J.K. Park, W. Si, Q. Luo, W. Jiang, H. Yin, A.G. Montag, M.A. Simon, T.D. Peabody, R.C. Haydon, C.W. Rinker-Schaeffer, T.-C. He, An orthotopic model of human osteosarcoma growth and spontaneous pulmonary metastasis, Clin. Exp. Metastasis 22 (2005) 319–329.
[81] J. Liu, R. Hu, J. Liu, B. Zhang, Y. Wang, X. Liu, W.C. Law, L. Liu, L. Ye, K.T. Yong, Cytotoxicity assessment of functionalized CdSe, CdTe and InP quantum dots in two human cancer cell models, Mater. Sci. Eng. C Mater. Biol. Appl. 57 (2015) 222–231.
[82] M. Johari-Ahar, J. Barar, A.M. Alizadeh, S. Davaran, Y. Omidi, M.R. Rashidi, Methotrexate-conjugated quantum dots: synthesis, characterisation and cytotoxicity in drug resistant cancer cells, J. Drug Target. 15 (2015) 1–14.
[83] A.A. Mansur, S.M. de Carvalho, H.S. Mansur, Bioengineered quantum dot/chitosan-tripeptide nanoconjugates for targeting the receptors of cancer cells, Int. J. Biol. Macromol. 82 (2016) 780–789.
[84] K.W. Kuo, T.H. Chen, W.T. Kuo, H.Y. Huang, H.Y. Lo, Y.Y. Huang, Cell uptake and intracellular visualization using quantum dots or nuclear localization signal-modified quantum dots with gold nanoparticles as quenchers, J. Nanosci. Nanotechnol. 10 (7) (2010) 4173–4177.