BIOSENSORS

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CHARACTERIZATION OF THE INTERACTION OF CdTe QUANTUM DOTS WITH HUMAN SERUM ALBUMIN BY OPTICAL SPECTROSCOPIC TECHNIQUES

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Abstract. The interaction between CdTe quantum dots (QDs) and human serum albumin (HSA) was studied by absorption and photoluminescence spectroscopy. Three aqueous-compatible samples of colloidal CdTe nanoparticles with average size of 2.8, 2.9 and 3.1 nm were tested. In the absorption spectra of the colloidal CdTe QDs exciton band was found to be shifted to higher photon energy as compared with that for bulk crystals due to the quantum confinement effect. It was shown that addition of HSA to colloidal CdTe nanoparticles leds to a gradual decrease of absorption and broadening of exciton structure. The photoluminescence quenching results indicated that the quenching effect of QDs on HSA fluorescence depend on the size and temperature and the nature of quenching is static, resulting in forming QDs–HSA complexes. Stern – Volmer plots were made and quenching constants were thus obtained. The results suggested the quenching constants increasing with increasing of the sizes QDs and decrease with increasing temperatures of QDs-HSA solutions.

Keywords: CdTe, quantum dot, nanoparticles, nanocrystal, optical density, photoluminescence, fluorescence quenching, human serum albumin

ХАРАКТЕРИСТИКА ВЗАЄМОДІЇ КВАНТОВИХ ТОЧОК CdTe ІЗ СИРОВАТКОВИМ АЛЬБУМІНОМ ЛЮДИНИ МЕТОДАМИ ОПТИЧНОЇ СПЕКТРОСКОПІЇ

І. Д. Столярчук, А. Й. Савчук, Р. Войнаровська, Я. Політ

Анотація. Дослідження спектрів поглинання та фотолюмінесценції застосовувались для вивчення взаємодії між квантовими точками (КТ) CdTe і сироватковим альбуміном людини (HSA). Дослідження проводились для колоїдних розчинів наночастинок CdTe з середніми розмірами 2.8 нм, 2.9 нм та 3.1 нм. Екситонна структура в спектрах поглинання колоїдних розчинів наночастинок CdTe виявилась зміщеною в область високих значень енергій у порівнянні з об'ємними кристалами, що зумовлено проявом квантово розмірного ефекту. Додавання сироваткового альбуміну людини до колоїдного розчину КТ CdTe призводить до поступового зменшення поглинання та розмиття екситонної структури спектру. Дослідження гасіння фотолюмінесценції квантових точок CdTe при додаванні сироваткового альбуміну крові людини дозволили встановити статичний характер гасіння та формування комплексів нанокристал - сироватковий альбумін людини. Використовуючи рівняння Штерна - Фольмера було встановлено зростання константи гасіння зі збільшенням розмірів КТ та її зменшення із підвищенням температури розчинів КТ-HSA.

Ключові слова: CdTe, квантова точка, наночастинка, нанокристал, оптична густина, фотолюмінесценція, гасіння флуоресценції, сироватковий альбумін людини

ХАРАКТЕРИСТИКА ВЗАИМОДЕЙСТВИЯ КВАНТОВЫХ ТОЧЕК CdTe С СЫВОРОТОЧНЫМ АЛЬБУМИНОМ ЧЕЛОВЕКА МЕТОДАМИ ОПТИЧЕСКОЙ СПЕКТРОСКОПИИ

И. Д. Столярчук, А. И. Савчук, Р. Войнаровская, Я. Полит

Аннотация. Оптическое поглощение и фотолюминесценция использовались для характеристики взаимодействия квантовых точек (КТ) CdTe с сывороточным альбумином человека (HSA). Исследования проводились для коллоидных растворов наночастиц CdTe со средними размерами 2.8 нм, 2.9 нм и 3.1 нм. Экситонная структура в спектрах поглощения коллоидных растворов КТ CdTe оказалась смещенной в область высоких значений энергий по сравнению с объемными кристаллами, что обусловлено проявлением квантово-размерного эффекта. Добавление сывороточного альбумина человека к коллоидному раствору КТ CdTe приводит к уменьшению поглощения и размытию экситонной структуры спектра. Исследование тушения фотолюминесценции квантовых точек CdTe при добавлении сывороточного альбумина человека позволило установить статический характер тушения и формирование комплексов нанокристалл - сывороточный альбумин человека. Используя уравнения Штерна - Фольмера было установлено рост константы тушения с увеличением размеров КТ и ее уменьшение с повышением температуры растворов КТ - HSA.

Ключевые слова: CdTe, квантовая точка, полупроводниковая наночастица, оптическая плотность, фотолюминесценция, тушение флуоресценции, сывороточный альбумин человека

1. Introduction

In the past decade, a variety of nanoscale structures have been used for a range of biological and biomedical applications. Major classes of biologically relevant nanostructures include semiconductor nanoparticles, magnetic nanoparticles, carbon-based nanostructures and metallic nanoparticles [1]. Research on semiconductor nanocrystals (NCs), also known as quantum dots (QDs), has increased rapidly in the past few decades [2]. QDs are useful as a novel probe in biosensor and bioimaging due to their unique size dependent optical and electrical properties. Moreover, semiconductor QDs are also becoming valuable analytical tools for biological and biomedical applications as they offer the opportunity to design luminescent probes for labeling, imaging, and sensing with unprecedented performance [3].

Due to the tremendous focus on applying the nanoparticles to biological and biomedical applications, there has been increasing interest in estimating the toxicity of II-VI undoped and doped semiconductor based nanoparticles. It is well known that the human serum albumin (HSA) is the most abundant protein in blood plasma and involved in the transport of a variety of endogenous and exogenous ligands. Transportation, distribution, physiological and toxicological actions of the ligands in vivo are closely related to their binding with proteins. So, it is very significant to investigate the interaction between the nanoparticles and the major carrier protein like HSA. Several reports have been devoted to study such kind of interaction between II-VI semiconductor based nanoparticles and bovin serum albumin (BSA) and HSA. Shao et al. speculated that the interaction of CdTe QDs with BSA was mainly attributed to electrostatic attraction [4]. Xiao et al. proved that binding of colloidal CdSe/ZnS QDs and HSA is a result of the formation of QDs–HSA complex and electrostatic interactions play major role in stabilizing the complex [5]. This group also studied [6] the conformation changes of HSA induced by CdTe quantum dots with different sizes and the obtained results indicated that the biological activity of HSA is weaker for quantum dots with bigger sizes. Wu et al. [7] reported on the interaction between BSA and ZnS quantum dots by spectroscopic techniques and showed strong quenching of fluorescence. Recently, Hemmateenejad and Yousefinejad [8] have revealed the presence of static type of quenching mechanism in the binding of ZnS nanoparticles to HSA. Bhogale et al. [9] studied the interaction of ZnO nanoparticles with HSA and discuseed the quenching of fluorescence of fluorophores in HSA, which was attributed to formation of HSA-ZnO complex in the solution.

In the present work, we report on investigation of the influence of CdTe quantum dots size and temperature on the interaction with human serum albumin. Main attention is paid to conventional UV-Vis absorption and fluorescence spectroscopic methods.

2. Experimental

2.1. Sample preparation

Aqueous synthesis of QDs offer many benefits for biological studies. The basic principle of chemical synthesis of nanostructured materials (the so-called bottom-up method) is to initiate chemical reactions and control the nucleation and growth of the reaction products. This can be achieved by conducting the reactions within a confined environment or controlling the reaction process via dynamic binding of surface ligands. In colloidal solution synthesis, controlling the size or shape is done by adjusting the ratio of the chemicals concentrations, selecting capping material, value of pH, and temperature. Nanoparticles of CdTe were prepared in aqueous solution at room temperature using procedure similar to described in [10]. Briefly, Cd precursor solutions were prepared by mixing 3 mmol of $CdCl_2$ with 225 ml of ultrapure water followed by 7.7 mmol of thioglycolic acid (TGA) under magnetic stirring. The pH value of the mixed solution was adjusted to 10.0 by dropwise addition of 1M NaOH solution. Then, gas mixture of Ar and H_2Te was passed through the solution. The reaction time was varied to achieve different molar ratio of Cd^{2+}:Te^{2-}:TGA.

Transmission electron microscopy (TEM) was used in order to confirm the nanoparticles of the

grown samples, estimate shape and determine the average size of nanocrystals. A TEM instrument Tecnai Osiris X-FEG TEM microscopy that provides maximum resolution of 0.136 nm has been used.

HSA was purchased from PJSC Biofarma (Ukraine) at the concentration of 1,5 x 10^{-6} mol L^{-1}. Solutions of CdTe nanocrystals with HSA were prepared by adding the set amount of quantum dots (from 0,1 x 10^{-6} mol L^{-1} to 1,9 10^{-6} mol L^{-1}) to fixed volume of HAS (1 mL) and stirred for 2 min. The experiments were started in 10 min after the sample was inserted in the instrument to allow the temperature to equilibrate.

2.2. Measurements

The absorption and photoluminescence spectra were recorded using UV-Vis spectrometer on the base of diffraction monochromator MDR-23 (LOMO). Quartz cells (1cm path length) and a thermostatic bath were used for all measurements. The excitation of photoluminescence was carried out by a He-Cd laser operating at wavelength of 325 nm and power of 10 mW.

3. Results and Discussion

The HR TEM image of typical colloidal CdTe nanoparticles is shown in Fig. 1. For this kind of microscopic analysis a drop of colloidal suspension is placed on special carbon-coated copper grid. As can be seen the shape of the nanoparticles is close to spherical and the average diameter of the nanoparticles is found to be approximately from 2.5 to 5 nm.

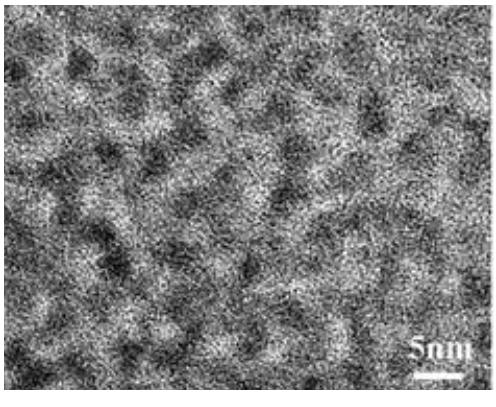

Fig. 1. HR TEM images of the colloidal CdTe quantum dots.

To study optical spectra three colloidal solutions were chosen with an average diameter of CdTe quantum dots about 2.8 nm, 2.9 nm and 3.1 nm. Fig. 2 shows optical density as a function of photon energy for four solution samples contained in the same quartz container with inner thickness of 10 mm which correspond to different materials. Curve 1 corresponds to the sample of CdTe nanoparticles with an average diameter of 2.9 nm and curve 2 corresponds to HSA solution. Curves 3 and 4 correspond to mixed solutions of CdTe quantum dots and HSA. In optical absorption spectrum of CdTe nanocrystals one can see clear exciton band with maximum at 2.32 eV. Its maximum is blue shifted as compared with bulk CdTe crystals. A blue shift with respect to the absorption peak of bulk crystals is due to the confinement effect. The correlation between the bandgap and the radius of semiconductor nanocrystals was given by Brus [11,12] in the following equation:

$$E(r) = E_g^{bulk} + \frac{\pi^2 h^2}{2er^2}\left(\frac{1}{m_e^*} + \frac{1}{m_h^*}\right) - \frac{1{,}8e}{4\pi\varepsilon\varepsilon_0 r} + P, \quad (1)$$

where E (r) corresponds to the nanoparticles energy bandgap, E_g^{bulk} is the bulk semiconductor energy bandgap, r is the crystallite radius, m_e^* is the electron effective mass, m_h^* is the hole effective mass, e and P is the dielectric constant and polarization term, respectively. By neglecting small polarization term and using values of m_e^*, m_h^*, ε for bulk CdTe, the experimental value of E (r) - E_g^{bulk} = 0.83 eV an average radius of the studied CdTe nanoparticles was estimated as r ≈1.4 nm.

As shown in Fig. 2 (curves 3 and 4), an addition of HSA to colloidal CdTe nanoparticles leads to a gradual decrease of optical density and broadening of exciton structure. However, energy position of the exciton band in this case remains not shifted. The obtained results indicate that the binding process between quantum dots and protein molecules may change the conformation of HSA.

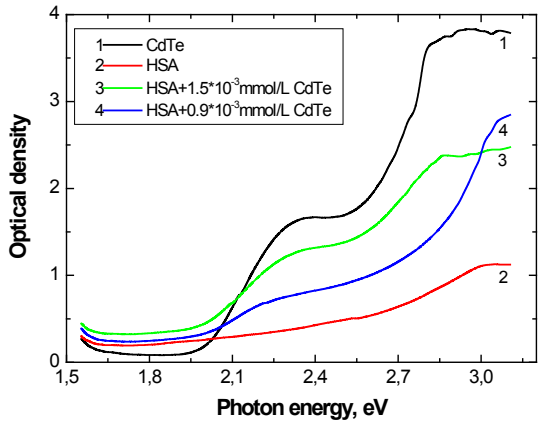

Fig. 2. Optical density as function of photon energy for solution of colloidal QDs CdTe with average size of 2,9 nm and HSA (curve 1 corresponds to CdTe QDs only, 2 corresponds to HSA solution only, 3 – corresponds to HSA + 1.5 x 10^{-3} mmol L^{-1} QDs, 4 – corresponds to HSA + 0.9 x 10^{-3} mmol L^{-1} QDs).

Photoluminescence is the process of photon emission as a result of the return of an electron in a higher energy orbital back to a lower orbital. A variety of the molecular interactions can result in quenching, including excited-state reactions, energy transfer, ground-state complex formation, and collisional quenching [11]. The mechanisms of quenching are usually classified as either dynamic quenching or static quenching. These can be distinguished by their varying dependence on temperature or by luminescence lifetime measurements. The dynamic quenching depends on diffusion, since higher temperature results in larger diffusion coefficients. For this reason, the quenching constants are expected to increase with increasing temperature. In contrast, increased temperature is likely to result in decreased stability of complexes, and, therefore, lower values of the static quenching constants [13]. Fig. 3 shows photoluminescence spectra of colloidal CdTe nanoparticles (curve 1), HAS (curve 5) and their solutions (curve 2-4). Main finding from these experiments is so-colled quenching effect. The photoluminescence intensity of HSA progressively decreases with the increasing concentration of CdTe quantum dots. The fluorescence quenching mechanism can be analyzed quantitatively at different temperatures (293, 303 and 309 K) with the Stern–Volmer equation [14]:

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q] \qquad (2)$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, Kq 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.

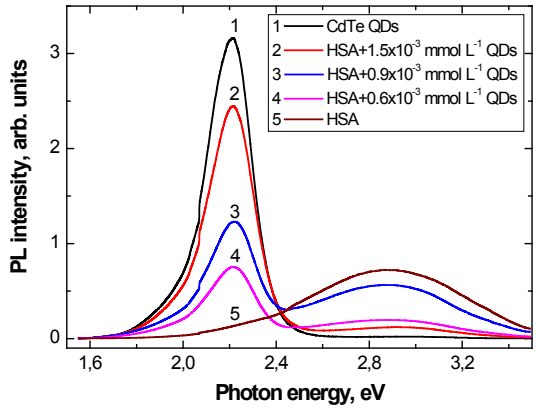

Fig. 3. Photoluminescence spectra of CdTe QDs (2,9 nm) in HSA (curve 1 corresponds to CdTe QDs only, 2 corresponds to HSA + 1.5 x 10^{-3} mmol L^{-1} QDs, 3 – corresponds to HSA + 0.9 x 10^{-3} mmol L^{-1} QDs, 4 – corresponds to HSA + 0.6 x 10^{-3} mmol L^{-1} QDs, 5 - corresponds to HSA solution only).

Fig. 4 shows the Stern–Volmer plots of F_0/F versus [Q] at three different temperatures. The quenching constants decrease with increasing temperatures, which indicates that the quenching mechanism mainly arises from static quenching [15]. As such, a ground state complex is formed between HSA and CdTe QDs that leads to fluorescence quenching.

The quenching ratio (F/F0) of the HSA photoluminescence with different average radius of CdTe nanoparticles is shown in Fig. 5. For equal concentrations of nanoparticles in solutions with HSA the intensities of photoluminescence slowly decrease with an increase of size of quantum dots. These results indicated that the quenching effect of HSA on CdTe quantum dots photoluminescence depended on their sizes.

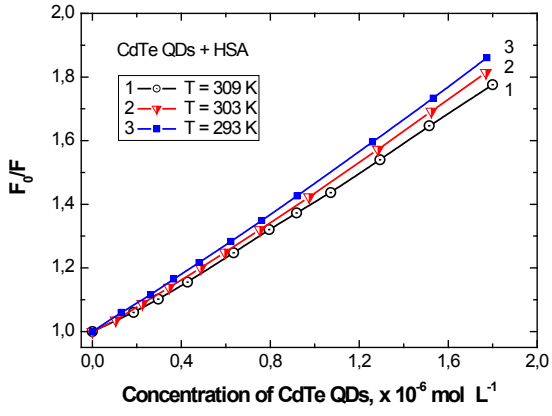

Fig.4. The Stern–Volmer plots for HSA photoluminescence quenching by CdTe QDs for different temperatures.

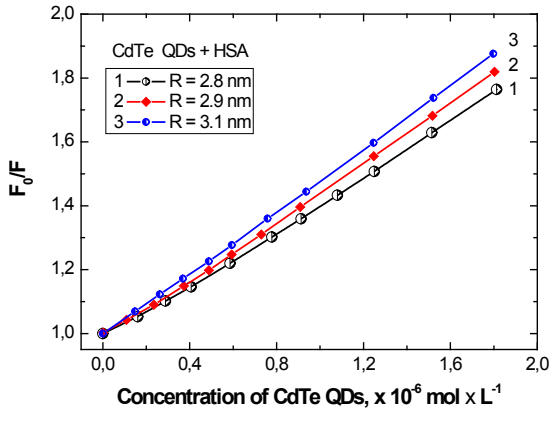

Fig. 5. The Stern–Volmer plots for HSA photoluminescence quenching by different average size of CdTe QDs at 293 K.

The quenching constants K_q of nanoparticles CdTe with average radius 2.8nm, 2.9 nm and 3.1 nm for HSA were calculated to be 2.12×10^{13} , $2.14\times10^{13}$and 2.28×10^{13} L mol^{-1} s^{-1}, respectively. According to the literature [16,17], for dynamic quenching, the maximum scatter collision quenching constant of various quenchers with the biopolymer is 2.0×10^{10} L mol^{-1} s^{-1}. Considering that in our experiment the rate constants of the HSA quenching procedure initiated by nanoparticles were much greater than 2.0×10^{10} L mol^{-1} s^{-1}, it can be concluded that the nature of quenching is not dynamic but probably static, resulting in forming QDs–HSA complexes.

4. Conclusions

In summary, three different CdTe QDs with average radiuses of 2.8 nm, 2.9 nm and 3.1 nm were obtained and their interactions with HSA were investigated. The addition of HSA to colloidal CdTe QDs leads to a gradual decrease of optical density and broadening of exciton structure. Photoluminescence spectroscopy provides qualitative and quantitative information about the interaction between QDs and HSA. Our results showed that the intrinsic fluorescence of HSA was quenched through static quenching mechanism. The quenching constants and the number of binding sites increase with increasing of average size of QDs.

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