Magneto-luminescent nanostructures for biomedicine

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Abstract. Magneto-luminescent nanosystems can be widely applied in biomedicine, namely for disease theranostics, early tumor imaging, and as drug delivery and hyperthermia agents. The creation of such systems using superparamagnetic iron oxide nanoparticles (SPIONs) and semiconductor quantum dots (QDs) approach is popular, since SPIONs are characterized by excellent magnetic properties and zero coercive force, and QDs have advantages over the majority of organic phosphors. In this work, the alloyed (CdₓZn₁₋ₓSe₁₋ₚS₁₋ₚ)/ZnS quantum dots are used to reduce the probability of Förster Resonance Energy Transfer or electron transfer, that quench QD photoluminescence, due to a thick gradient semiconductor shell. Moreover, Magnetic Circular Dichroism (MCD) spectroscopy has been successfully applied as a method for monitoring both magnetic properties and colloidal stability of magnetic nanoparticles in a solution, which is crucial for their biomedical applications. The simplicity of SPION/QDs nanostructure formation due to the sequential addition of a SPION solution to a QD solution is a major advantage of this approach. It was demonstrated that these SPION/QDs nanostructures are stable, can be efficiently absorbed by the HeLa cells, and do not show high cytotoxicity at low concentrations (up to 25 nM).

1. Introduction

There are no materials in nature that are simultaneously characterized by a high quantum yield of luminescence and a high degree of magnetization. However, such systems could be beneficial in biomedicine, namely, for disease theranostics, early tumor imaging, and as drug delivery and hyperthermia agents [1,2]. For this reason, active research is aimed at creating magneto-luminescent structures. The approach to such systems creation using superparamagnetic iron oxide nanoparticles (SPIONs) and semiconductor quantum dots (QDs) is widespread, since SPIONs are characterized by excellent magnetic properties and zero coercive force [3], and QDs have several advantages over organic phosphors [4].

Förster Resonance Energy Transfer (FRET) and electron transfer (ET) from QDs to SPIONs complicate the production of bright luminescent nanostructures, since photoluminescence (PL) of QDs, efficient energy and electron donors, is quenched [5]. In this work, the alloyed (CdₓZn₁₋ₓSe₁₋ₚ)/ZnS quantum dots are used to reduce the probability of FRET or ET due to a thick gradient semiconductor shell. Moreover, a method of Magnetic Circular Dichroism (MCD) spectroscopy has been successfully applied to monitor both magnetic properties and colloidal stability of magnetic nanoparticles in a solution, which is crucial for their biomedical applications.
2. Results and discussion

The SPION/QD nanostructures were formed by sequentially adding a solution of magnetic nanoparticles to a solution of quantum dots in dimethyl sulfoxide (DMSO) in the ratios of 0.2, 0.5, and 1. The formation of complexes in the aprotic bipolar solvent DMSO was ensured by coordination of the carboxyl group of the stabilizing QD molecules (L-cysteine) on the SPION surface iron atoms.

2.1. Spectral-luminescent properties

All samples were characterized by a maximum in the long-wavelength region in the absorption spectra at a wavelength of 552 nm (Figure 1). The PL spectra of the samples (Figure 1) were analyzed considering the internal filter effect due to high optical density of SPIONs. A sequential increase in SPIONs concentration in samples was accompanied by effective quenching of QD luminescence. Based on this, the formation of complexes in these samples is assumed. However, this does not directly confirm the presence of complexes, since a decrease in the PL quantum yield of quantum dots occurs in cases of their aggregation, which is often observed when their nearest environment changes. For this reason, the kinetics of QD luminescence at various SPIONs concentrations was studied (Figure 2).

![Figure 1. Absorption and PL spectra of a QD solution and solutions with complexes; 1 – free QDs, 2-4 – mixture with n = 0.2; 0.5; 1.](image1)

![Figure 2. Luminescence decay of samples and dependencies of characteristic decay times on the recording wavelength. 1 – free QD, 2-4 – mixture with n = 0.2; 0.5; 1.](image2)

In accordance with Figure 2, the PL decay times do not depend on the recording wavelength within the error limit. Thus, there is no energy transfer between quantum dots, which means the absence of their aggregation. The analysis of PL kinetics showed that the PL decay of QDs is approximated by three exponential dependencies, their characteristic times and amplitudes are shown in Table 1.

| Table 1. PL lifetimes and amplitudes. |
|--------------------------------------|
| Concentration ratio (n) | <τ> n | τ₁, ns | A₁, % | τ₂, ns | A₂, % | τ₃, ns | A₃, % |
|-------------------------|--------|--------|--------|--------|--------|--------|--------|
| 0                       | 21.2±1.1 | 3.2±0.2 | 41.1±2.1 | 13.9±0.7 | 51.4±2.6 | 43.9±2.2 | 7.6±0.4 |
| 0.2                     | 17.9±0.9 | 2.4±0.1 | 49.4±2.5 | 11.7±0.6 | 41.8±2.1 | 34.2±1.7 | 8.8±0.4 |
| 0.5                     | 16.7±0.8 | 2.0±0.1 | 51.1±2.6 | 10.6±0.5 | 39.6±2.0 | 31.0±1.6 | 9.3±0.5 |
| 1                       | 11.7±0.6 | 1.4±0.1 | 61.0±3.1 | 7.0±0.4 | 32.5±1.6 | 24.2±1.2 | 6.4±0.3 |

The data in Table 1 demonstrate that an increase in SPIONs concentration is accompanied by a symbate reduction in all characteristic decay times of luminescence. It confirms the FRET or ET from QDs to SPIONs, thus indicating the SPION/QDs structure formation, rather than a spontaneous
aggregation of QDs, which is usually accompanied by a sharp increase in the percentage of QD fraction with the shortest characteristic PL decay time.

The newly recorded luminescence kinetics after 10 days showed that the average decay time of QD luminescence in the complexes decreased by 0.59 ns during storage, which indicates the stability of QDs and complexes.

2.2. MCD as a method for studying magnetic properties and stability of complexes

The dependencies of the normalized intensity of the bands in MCD spectra (G-factor) on the magnitude and the sign of the external magnetic field repeat the sample magnetization curve [6]. This means that the MCD spectra intensity reflects the data on sample magnetization, which makes MCD spectroscopy an effective method to assess magnetic properties of samples.

The MCD spectra of SPIONs and complexes show the same bands at 415 nm, 350 nm, and 310 nm wavelengths, which correspond to the $^6A_1\rightarrow^4E$, $^4A_1(^4G)$, $^6A_1\rightarrow^4T_2(^4D)$, and $^6A_1\rightarrow^4T_1(^4P)$ transitions [7]. Figure 3 shows that the G-factor of the complex decreases with a decrease in SPION SPIONs concentration. This is consistent with the theory, since magnetic material is diluted with a non-magnetic one.

**Figure 3.** Dependencies of the $^6A_1\rightarrow^4T_1(^4P)$ SPION transition G-factor in samples with various relative SPION/QD concentrations.

**Figure 4.** Dependence of the $^6A_1\rightarrow^4E$, $^4A_1(^4G)$ SPION transition G-factor on the size of SPION aggregates under the influence of a 0.5 T magnetic field.

It is known that the aggregation of magnetic nanoparticles prevents their excretion from the body [8]. Therefore, their colloidal stability is a key parameter to apply SPIONs in biomedical applications. A traditional method for characterizing SPION magnetic properties is to measure the magnetization curve of the dried powder. This method does not reflect information on whether SPIONs are aggregated in a solution. Therefore, it was relevant to investigate magneto-optical properties of not only monodisperse magnetic nanoparticles but also specially formed aggregates.

It has been established that the G-factor in the MCD band of the $^6A_1\rightarrow^4E$, $^4A_1(^4G)$ SPION transition is sensitive to changes in the aggregate state of SPIONs in samples. Thus, the dependence shown in Figure 4 demonstrates that the aggregation of the SPIONs is accompanied by a noticeable decrease in the G-factor in this band. Based on this method, it was proved that SPIONs in the obtained complexes are in a monodisperse state, and not in aggregates.

2.3. Complexes in cells

To determine whether the complexes remain bound after their introduction into cells, the samples were investigated by confocal microscopy using an indicator dye for iron and by QD luminescence. To obtain transmission images by confocal microscopy (Figure 5), HeLa cells with SPION/QD complexes were stained with dye to detect iron ions using the Pearl’s reaction typical for histology.
The concentration of the complexes added was calculated by the concentration of QDs. Figure 6 shows an image of cells with complexes by luminescent confocal microscopy.

Since complexes are detected in cells by confocal microscopy using both QD luminescence and the cytological method of staining for iron compounds, this proves that these complexes interact with cells and do not disintegrate.

The analysis of cell viability after cultivation with complexes for 24 hours at various concentrations (10, 25, 64, 140 nM) showed that the complexes do not exhibit high cytotoxicity at low concentrations (up to 25 nM).

3. Conclusion
The simplicity of SPION/QDs nanostructure formation due to the sequential addition of a SPION solution to a QD solution is a major advantage of this approach. The formation of complexes in the samples was confirmed by studying the decay kinetics of QD luminescence. The research shows that these SPION/QDs nanostructures are stable, can be efficiently absorbed by the HeLa cells, and do not show high cytotoxicity at low concentrations (up to 25 nM). These results clearly demonstrate that the alloyed (Cd$_{x}$Zn$_{1-x}$Se$_{y}$S$_{1-y}$)/ZnS QDs can be successfully combined with SPIONs to reach new hybrid nanostructures. Furthermore, it has been proven that the circular dichroism method can be effectively applied to control and study the conditions of colloidal stability of complexes based on SPIONs.

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