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Polystyrene-diphenyloxazole-chlorin e_6 nanosystem for PDT: Energy transfer study

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ABSTRACT

As the step to design nanosystems for X-ray excited sensitizing of singlet oxygen, nanoparticles of polystyrene and polystyrene with encapsulated 2,5-diphenyloxazole were synthesized. Binding of the photosensitizer chlorin e_6 molecules to both kinds of nanoparticles was shown. Excitation energy transfer from styrene chromophores of polystyrene to the bound chlorin e_6 molecules occurs in the case of both kinds of nanoparticles. Efficiency of the energy transfer in 2,5-diphenyloxazole-mediated case is higher (0.17) as compared to the non-mediated one (0.12).

KEYWORDS

styrene nanoparticles; encapsulation; electronic excitation energy transfer; photosensitizer; chlorin e_6; photodynamic therapy

Introduction

Photodynamic therapy is the method for the cancer treatment, where the photosensitizer is excited by the light causing the generation of singlet molecular oxygen that is toxic for the tumor tissue [1]. Together with several advantages, this method has its main drawback i.e. the small depth of the exciting light penetration into the tissue [2]. Thus the concept of X-ray excited sensitizers composed of scintillating and photosensitizing parts with the electronic excitation energy transfer (EEET) from the first to the last one was proposed [3]. Last years, several nanosystems based on this idea were described with various materials used as scintillators, various photosensitizers and different ways of binding them to a system [2, 4–7]. Using similar concept, the X-ray stimulated luminescence of the porphyrin sensitizer was demonstrated for the nanoparticles (NP) containing polystyrene (PS, Fig. 1), vinylpyridine, 2,5-diphenyloxazole (PPO, Fig. 1) and hematoporphyrin; scintillation was provided by styrene due to its high concentration [8]. The electronic excitation energy transfer (EEET) was supposed to occur in that system, but this process was not studied. Earlier, keeping in mind further designing of more complex and efficient nanosystems for X-ray excited sensitizing of singlet oxygen, we synthesised PS-PPO NP; EEET from styrene matrix to encapsulated PPO that took place in these NP was studied, efficiency of such transfer was roughly estimated to be about 0.37 [9]. Here we investigated binding of PS-PPO NP with the photosensitizer chlorin e_6 (Fig. 1) and EEET in the formed PS-PPO NP - chlorin e_6 nanosystem; PS NP were used for the comparison and for determining of the PPO role.

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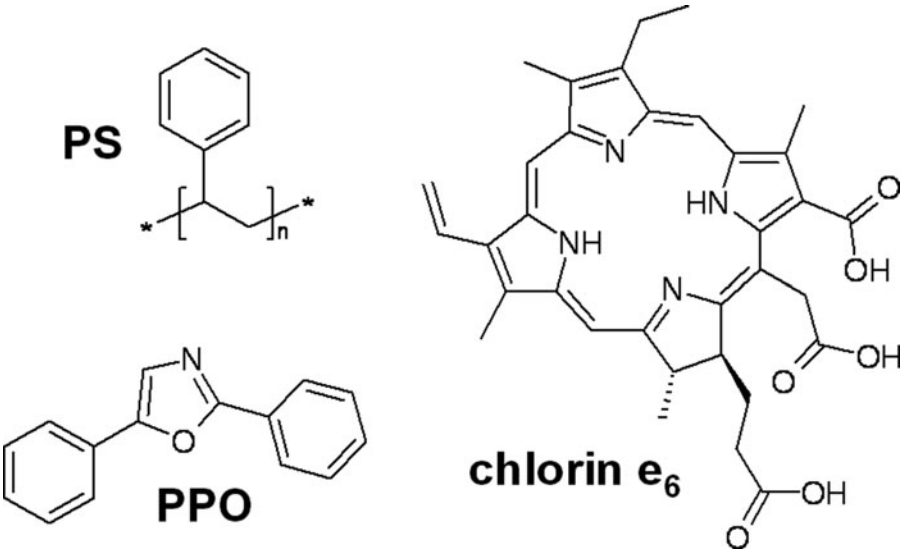

Figure 1. Structures of the studied compounds: polystyrene (PS), 2,5-diphenyloxazole (PPO) and chlorin e_6.

Materials and methods

Materials

Styrene of p.a. quality was purified *via* standard method directly before polymerization. Chlorin e_6 (Frontier Scientific Inc.) was a generous gift of T.Y. Ohulchanskyy (Institute for Lasers, Photonics and Biophotonics at the State University of New York at Buffalo). Other chemicals were purchased from Sigma-Aldrich Inc. and used without further purification.

Synthesis of nanoparticles

Microemulsion polymerization was used for the synthesis of PS NP as well as for incorporation of PPO into PS nanoparticles [8–11]. Latex beads containing organic compound were synthesized directly by the polymerization of a mixture of styrene and PPO with potassium persulphate (KPS) as initiator in a micellar aqueous solution of sodium dodecylsulphate (SDS). Namely, synthesis of PS NP and PS-PPO NP was performed as follows.

PS NP. Styrene was polimerized in a micellar aqueous solution of SDS with KPS as initiator: 2g styrene was added slowly over a period of 1.5 hours to a vigorously stirred solution of 0.01 g NaH_2PO_4, 0.2 g SDS and 0.01 g KPS in 10 ml of water at 70°C in argon atmosphere. The mixture was stirred for additional 3 hours at 70 °C and for 1 h at 90 °C. The mixture was cooled to room temperature and dialyzed during 48 hours using cellulose membrane with MWCO 3 500Da.

PS-PPO NP. Styrene was polymerized in a micellar aqueous solution of SDS with KPS as initiator: a mixture of 2 g styrene and 0.09 g 2,5-diphenyloxazole (PPO) was added slowly over a period of 1.5 hours to a vigorously stirred solution of 0.01 g NaH_2PO_4, 0.2 g SDS and 0.01 g KPS in 10 ml of water at 70 °C in argon atmosphere. The mixture was stirred for additional 3 hours at 70 °C and for 1 h at 90 °C. The mixture was cooled to room temperature and dialyzed during 48 hours using cellulose membrane with MWCO 3 500Da.

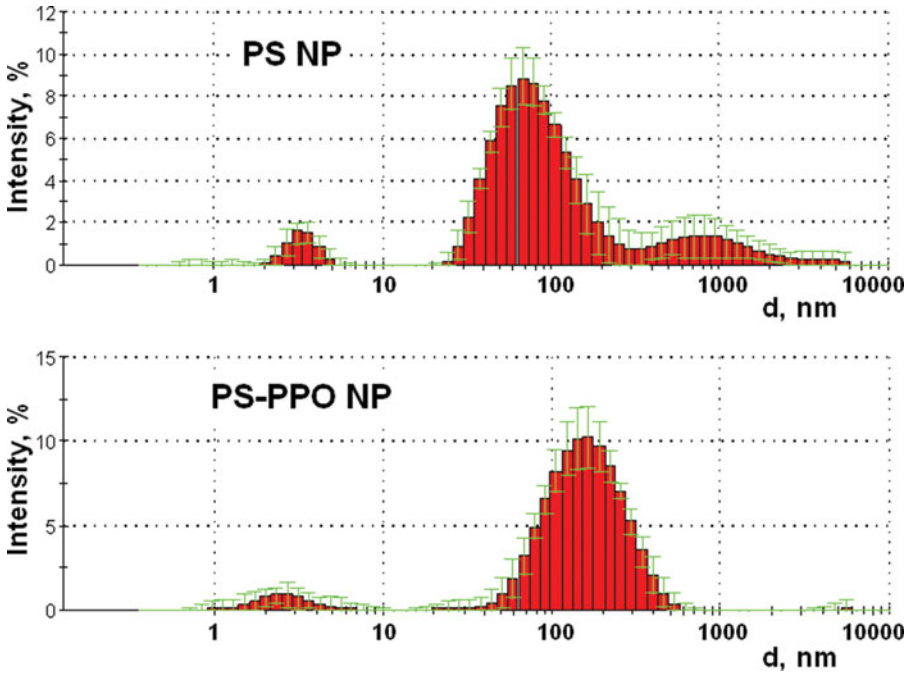

Figure 2. Distribution of DLS intensity for PS (top) and PS-PPO (bottom) nanoparticles, averaged for 9 and 11 measurements respectively.

Characterization of nanoparticles

Particle size distribution was studied by the dynamic light scattering (DLS) technique using Zetasizer Nano ZS (Malvern Instruments) apparatus (Fig. 2). For the obtained SDS-coated PS NP, intensity distribution of the hydrodynamic diameter gave main maxima at 80 ± 40 nm (about 77% of intensity), 3.3 ± 0.6 nm (about 6%), and 1000 ± 500 nm (about 17% of intensity), while for the PS-PPO NP main maxima were at 170 ± 90 nm (about 94% of intensity) and 2.6 ± 0.6 nm (about 5%). Since larger particles are known to make higher contribution to the DLS intensity, real average diameter values should be lower. Zeta potential of the synthesized NP was determined as (−90 ± 11) mV for PS NP and (−84 ± 13) mV for PS-PPO NP; such high negative potential could be due to the SDS coating of the NP.

Spectral measurements and samples preparation

Absorption spectra were measured using Specord M40 spectrophotometer (Carl Zeiss, Germany). Fluorescence excitation and emission spectra were registered with the help of Cary Eclipse fluorescent spectrophotometer (Varian, Australia). Absorption and fluorescence measurements were performed in 1 cm × 1 cm quartz cell at room temperature. 50 mM TRIS-HCl buffer, pH 7.2 was used as a solvent (this pH value was used since it is the intracellular pH of the living cell). For spectral measurements, solutions of nanoparticles were dissolved in 1000 times in buffer. Solution of sodium dodecylsulphate (SDS) in buffer was used in 5 mg/mL concentration that is higher than the critical micelle concentration for this surfactant; we thus believe SDS to form micelles in solution. Stock solution of chlorin e_6 at the concentration 2 mM was prepared in DMF. Small aliquot of chlorin e_6 stock solution was then added to either

NP or SDS solution, the final concentration of chlorin was 2 μM; DMF admixture was thus 0.1%.

Estimation of EEET efficiency

To clarify the pathway of the excitation transfer in PS NP - chlorin e_6 and PS-PPO NP - chlorin e_6 nanosystems, quantitative estimation of PS-to-PPO, PS-to-chlorin e_6 and PPO-to-chlorin e_6 EEET efficiency was performed. Namely, efficiency of excitation energy transfer from donor (D) to acceptor (A), i.e. the ratio of the number of quanta transferred to A to this absorbed by D was roughly estimated as it was made for PS-PPO NP in [9]. For this, the EEET efficiency could be determined as

$$E_{EEET} = \frac{N^{A}_{\text{abs}}}{N^{D}_{\text{abs}}} \times \frac{N^{\text{exD}}_{\text{emA}}}{N^{\text{exA}}_{\text{emA}}}$$

where N^{A}_{abs} and N^{D}_{abs} are the numbers of quanta absorbed by A and D respectively, while N^{exD}_{emA} and N^{exA}_{emA} are the numbers of quanta emitted by A upon excitation of D and A respectively. Further, D and A excitation wavelengths were selected so that optical density of D and A are roughly equal; in this case we could consider $N^{A}_{abs} = N^{\text{D}}_{abs}$ and hence:

$$E_{EEET} = \frac{N^{\text{exD}}_{\text{emA}}}{N^{\text{exA}}_{\text{emA}}} = \frac{I^{\text{exD}}_{\text{emA}}}{I^{\text{exA}}_{\text{emA}}}$$

where $I^{\text{exD}}_{\text{emA}}$ and $I^{\text{exA}}_{\text{emA}}$ are fluorescence intensities of A emission upon excitation of D and A respectively; from the $I^{\text{exD}}_{\text{emA}}$ value the contribution corresponding to direct excitation of A at the absorption wavelength of D was subtracted.

Results and discussion

Absorption and fluorescence spectra of chlorin e_6 in the presence of PS NP and PS-PPO NP

Absorption spectra of chlorin e_6 in buffer, in the presence of PS and PS-PPO nanoparticles, as well as in the presence of SDS micelles (5 mg/mL SDS) are presented at the Fig. 3.

It is seen from the Fig. 3 that the most long-wavelength band of chlorin e_6 in buffer has its maximum at 655 nm, while in the presence of both PS NP and PS-PPO NP it shifts to 662 nm; Soret band maximum also shifts from 403 to 406 nm. Thus, chlorin e_6 binds to both PS NP and PS-PPO NP in a similar way (since its spectra in the presence of both kinds of NP are close). The mechanism of chlorin e_6 binding to NP could be the most possibly its intercalation into the SDS shell of the NP; but the spectrum of chlorin e_6 in the presence of SDS micelles measured for the comparison revealed more complicated behavior of chlorin e_6 spectra. Unlike in the presence of NP, in the presence of SDS micelles the main maximum of the most long-wavelength Q-band (657 nm) is only slightly shifted as compared to that of the free chlorin e_6; besides, short-wavelength shoulder near 640 nm (connected perhaps with the form of chlorin e_6 that is dominant at pH $< \sim$6.1 [12]) as well as slight long-wavelength shoulder (about 670 nm) are manifested. Thus the presence of both kinds of studied organic NPs at the used concentrations results in binding of the majority of chlorin e_6 molecules to NPs. We suppose this binding to occur via chlorin e_6 intercalation into the SDS shell of the NP; it is interesting that such interaction results in only one form of bound chlorin e_6 molecule, unlike

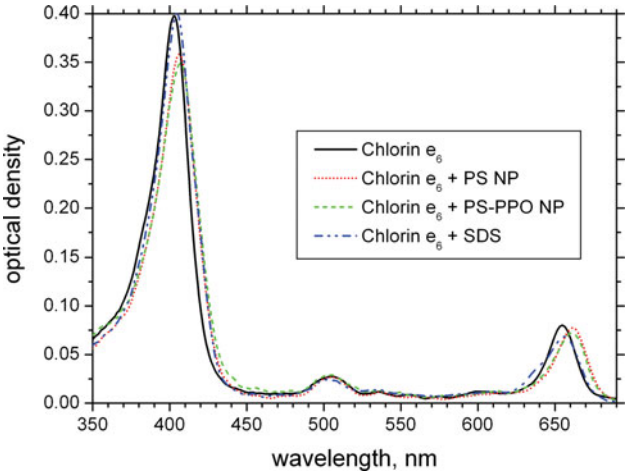

Figure 3. Absorption spectra of chlorin e_6 (2 mkM) in buffer, in the presence of SDS micelles (5 mg/mL SDS), PS NP and PS-PPO NP.

the case of SDS micelles; possibly SDS shell on the NP surface is more ordered as compared to SDS molecules in the micelle.

Fluorescence emission spectra (excitation at 400 nm) of chlorin e_6 in buffer, in the presence of SDS micelles, PS NP and PS-PPO NP (Fig. 4) support the above made conclusions about the binding of chlorin e_6 molecules to PS and PS-PPO NPs as well as about several forms of chlorin e_6 formed upon binding to SDS micelles. At the same time, in the fluorescence excitation spectra of chlorin e_6 in the presence of PS and PS-PPO NP (Fig. 5) besides of the Soret and Q-bands bands of chlorin e_6 itself, the bands attributed to PS and PPO excitation are strongly manifested. Namely, the presence of PS NP leads to the appearance of the 263-nm band in the chlorin e_6 excitation spectrum. This band corresponds to the PS excitation [9] leading to chlorin e_6 emission and points thus on the EEET from the PS NP directly to the bound chlorin e_6 molecule. Besides, existence of such transfer is one more proof of the PS NP - chlorin e_6 complex formation. Thus, vinylpyridine used in [8] for the hematoporphyrin binding to the NP turned out not to be necessary here.

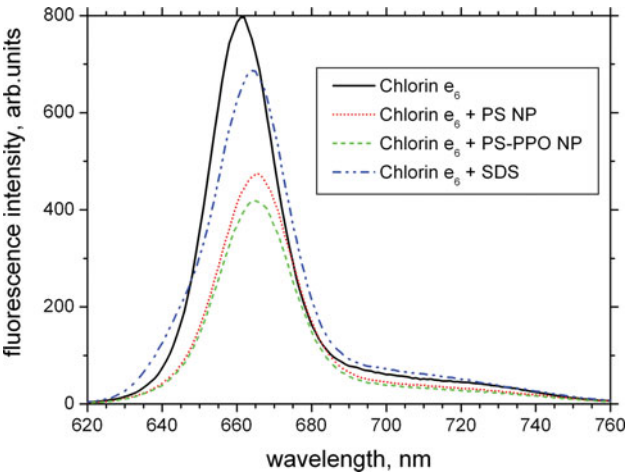

Figure 4. Fluorescence spectra (excitation at 400 nm) of chlorin e_6 (2 mkM) in buffer, in the presence of SDS micelles (5 mg/mL SDS), PS NP and PS-PPO NP.

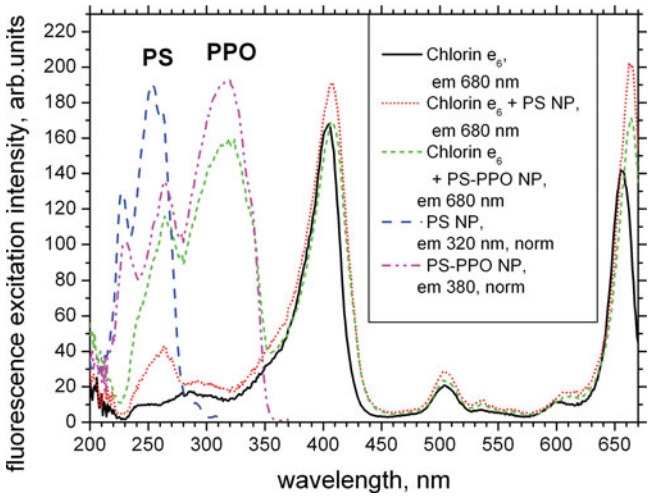

Figure 5. Fluorescence excitation spectra (emission at 680 nm) of chlorin e_6 (2 mkM) in buffer, in the presence of PS NP and PS-PPO NP. Normalized excitation spectra of PS NP (emission at 320 nm) and PS-PPO NP (emission at 380 nm) are provided for the comparison.

As for the PS-PPO NP, their presence leads to the manifestation of the 265-nm and 318-nm bands in the chlorin e_6 excitation spectrum. These bands correspond to the PS and PPO excitation respectively [9] leading to chlorin e_6 emission and point thus on the EEET from PS and PPO to the bound chlorin e_6. EEET from PS NP and PS-PPO NP to the bound chlorin e_6 molecules is also revealed by the quenching of PS and PPO fluorescence and by appearing of chlorin e_6 emission upon PS excitation (Fig. 6).

Excitation energy transfer in PS NP - chlorin e_6 and PS-PPO NP - chlorin e_6 nanosystems

Earlier we have demonstrated EEET in the PS-PPO NP from PS matrix to encapsulated PPO and have estimated the efficiency of this transfer to be 0.37 [9]. Here we have shown the existence of PS-to-chlorin e_6 EEET in PS NP - chlorin e_6 nanosystem, as well as PS-to-chlorin e_6

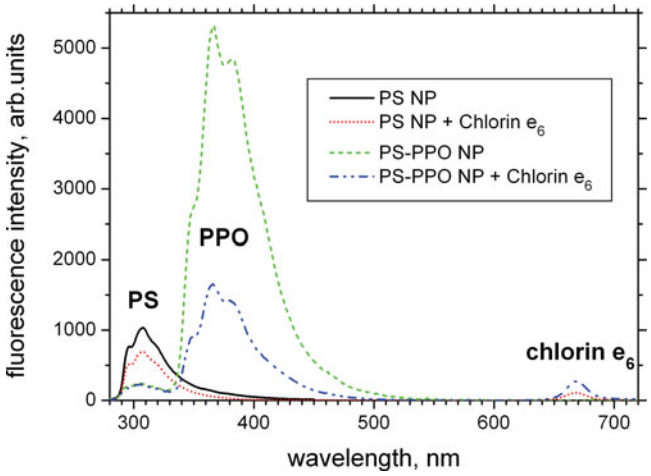

Figure 6. Fluorescence spectra (excitation at 260 nm) of PS NP and PS-PPO NP in the absence and in the presence of chlorin e_6 (2 mkM).

and PPO-to-chlorin e_6 EEET in PS-PPO NP - chlorin e_6 nanosystem. In the case of the latter nanosystem, while PPO-to-chlorin e_6 EEET is obviously direct, PS-to-chlorin e_6 transfer, though could be direct as well, the most possibly occurs *via* PPO, since (1) both PS-to-PPO and PPO-to-chlorin e_6 EEET processes take place, and (2) overlapping of PS emission and chlorin e_6 absorption spectra is rather poor, while corresponding spectra of the PS-PPO and PPO-chlorin e_6 pairs do overlap much stronger.

To clarify the pathway of the excitation transfer in PS NP - chlorin e_6 and PS-PPO NP - chlorin e_6 nanosystems, we have estimated PS-to-PPO, PS-to-chlorin e_6 and PPO-to-chlorin e_6 EEET efficiency (E_{EEET}), i.e. the ratio of the number of quanta transferred to acceptor to this absorbed by donor. Thus, for the case of PS NP - chlorin e_6 nanosystem, efficiency of PS-to-chlorin e_6 energy transfer was found to be $E_{EEET}{}^{PS\text{-}Ce6}$ [PS NP] = 0.12; in other words, 12% of the quanta absorbed by styrene chromophores in PS NP are transferred to the bound chlorin e_6 molecules (Fig. 7a). Taking into account rather poor overlapping of the PS emission and chlorin e_6 absorption spectra, the obtained value is unexpectedly high and could be explained by the close proximity of the bound chlorin e_6 molecules to styrene chromophores. Further, let's regard the model where chlorin e_6 molecules completely cover the PS NP surface and collect all the excitations of styrene chromophores situated in the external layer of certain width. Thus the value $E_{EEET}{}^{PS\text{-}Ce6}$ [PS NP] = 0.12 and simple geometry reasoning will yield that this width is about 4% of the NP radius; if we accept the DLS value of PS NP diameter equal to 80 nm, the mentioned layer would be 16Å thick. In other words, in the frames of the regarded model the bound chlorin e_6 molecules are the traps for the excitations of styrene chromophores situated not deeper than 16Å from the NP surface. In general, 16Å is the realistic value of the Förster radius. But taking into account (i) poor overlapping of the PS emission and chlorin e_6 absorption spectra, (ii) the fact that the probability of the energy transfer is not unity, especially for deep styrene chromophores, and (iii) possibility that the NP surface is not completely covered by chlorin e_6 molecules, we cannot reject the possibility that chlorin e_6 could also catch excitations from styrene chromophores situated much deeper than 16Å from the NP surface, possibly due to the excitation migration between styrene chromophores from the NP center to its periphery.

As for the PS-PPO NP - chlorin e_6 nanosystem, there could be three possible ways of energy migration: (i) from PS to PPO, (ii) from PPO to chlorin e_6, and (iii) directly from PS to chlorin e_6. Estimation of the efficiency of PS-to-PPO EEET gives $E_{EEET}{}^{PS\text{-}PPO}$ [PS-PPO NP] = 0.41 that is near to the previously obtained result [9], while the efficiency of the PPO-to-chlorin e_6 energy transfer equals to $E_{EEET}{}^{PPO\text{-}Ce6}$ [PS-PPO NP] = 0.45 (Fig. 7b). These two values are rather high that reflects good overlapping between corresponding absorption and

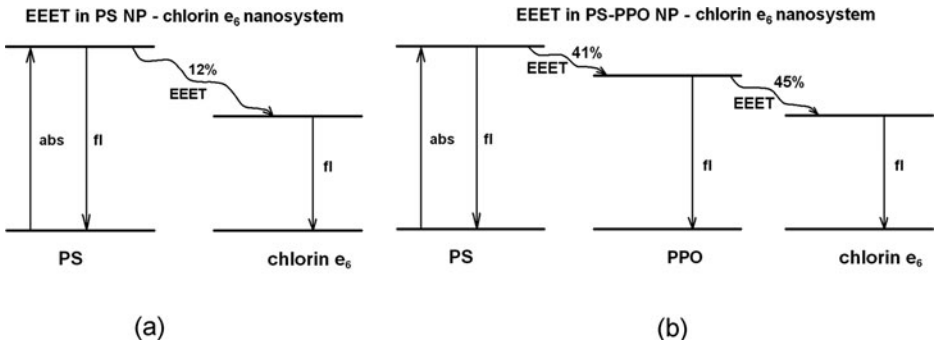

Fig. 7. EEET scheme in PS NP - chlorin e_6 (a) and PS-PPO NP - chlorin e_6 (b) nanosystems.

emission spectra; the product of these two values $E_{EEET}^{PS\text{-}PPO}$ [PS-PPO NP] × $E_{EEET}^{PPO\text{-}Ce6}$ [PS-PPO NP] = 0.18 is the efficiency of the PS-to-chlorin e_6 EEET that takes place using PPO as a mediator. But we can also estimate the total efficiency of the PS-to-chlorin e_6 EEET that includes both direct and PPO-mediated transfer; this value is $E_{EEET}^{PS\text{-}Ce6}$ [PS-PPO NP] = 0.17. In other words, total efficiency of the PS-to-chlorin e_6 EEET is about the same as PPO-mediated efficiency of this transfer. That means that in the presence of PPO mediator, direct PS-to-chlorin e_6 EEET does not occur. Though the direct PS-to-chlorin e_6 transfer is possible in the absence of PPO, the presence of the latter obviously redirects the transfer of PS excitations. It should be mentioned that the efficiency of the PS-to-chlorin e_6 EEET in the case of PS-PPO NP (0.17; indirect transfer trough PPO) is higher as compared to PS NP (0.12; direct transfer); thus encapsulation of PPO into PS NP really increases the PS-to-chlorin e_6 energy transfer efficiency, though this increase is not very high. At the same time, using the above regarded model and accepting the DLS value of PS-PPO NP diameter equal to 170 nm, we obtain that the bound chlorin e_6 molecules are the traps for the excitations of styrene chromophores situated not deeper than 50Å from the NP surface (that would be three times deeper as compared to the case of PS NP).

Conclusions

1. Synthesized polystyrene nanoparticles with and without encapsulated PPO are able to bind chlorin e_6 molecules.
2. Excitation energy transfer from styrene matrix to the bound chlorin e_6 molecules occurs in the case of both PS NP - chlorin e_6 and PS-PPO NP - chlorin e_6 nanosystems.
3. Efficiency of the energy transfer in PPO-mediated case is higher (0.17) as compared to the non-mediated case (0.12).

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