

Research Article

Spectroscopic Investigation of Chlorin-Based Photosensitizers in Polymer Matrix

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Chlorin e6 and its derivatives are the basis of a number of drugs used in medicine in the treatment of various diseases, including cancer, by photodynamic therapy. Nonpolar derivatives of Chlorin e6—dimethyl ether of Chlorin e6 (DME Ce6) and trimethyl ether of Chlorin e6 (TME Ce6)—are actively studied for application during photodynamic therapy. In this work, based on the electron optical absorption spectra, the interaction of photosensitizer molecules with branched star-like copolymer dextran-graft-polyacrylamide in anionic form was investigated and the possibility of using the latter as a carrier for drug delivery to tumor cells was suggested.

1. Introduction

Photodynamic therapy (PDT) is an advanced method of treating cancer and various benign diseases, including infections [1]. The main components for photochemical reactions are light-activable molecule-photosensitizer (PS), light, and oxygen [2]. The principle of the method of PDT is that PS under irradiation with light of a specific wavelength is able to initiate the generation of singlet oxygen and other reactive oxygen species that either kill tumor cells directly or damage tumor vasculature [3, 4]. PDT has a number of advantages over conventional methods of cancer treatment. Among the PSs of natural or synthetic origin, the most promising is Chlorin e6 (Ce6), boasting high efficacy and very low toxicity [5, 6]. There are a number of drugs based on Ce6 or its derivatives that have been already created and applied in the clinic, such as Photolon, Radachlorin, Photoditazin, Talaporfin, and Pirlitin. To date, several modified derivatives of this compound have been developed that would increase the effectiveness of PDT. The low solubility of Chlorin e6 and its derivatives in water forces them to look for special carriers for their address delivery. A modern trend in PDT is the use of multifunctional polymer nanocarriers which can enhance target-oriented PDT. The polymer nanocarriers

can be of few types: linear and branched polymers, dendrimers, micelles, and nanogels [7]. Our previous study has shown that branched star-like copolymer dextran-graft-polyacrylamide in anionic form could be efficient nanocarriers for drug delivery to tumor cells [7]) and can be efficient matrices for in situ synthesis of gold nanoparticles [8]. The theoretical [9–11] and experimental [12, 13] studies of star-like polymers proved that branched macromolecules have a higher local concentration of functional groups capable of binding drugs or other substances causing its incorporation into polymer nanocarriers.

The characteristics of the nanocomposite depend on the properties of PS and polymer matrices. The aim of this work was the comparative investigation of the absorption and fluorescence spectra of Chlorin e6, dimethyl ether of Chlorin e6, and trimethyl ether of Chlorin e6 (Ce6, DME Ce6, and TME Ce6, respectively) both in aqueous solutions and in water solution of branched polymer dextran-graft-polyacrylamide.

2. Materials and Methods

In this work, Chlorin e6 (Frontier Scientific, USA) was used. The Chlorin e6 (Ce6) derivatives dimethyl ester (DME Ce6) and trimethyl ester (TME Ce6) of Chlorin e6 were obtained

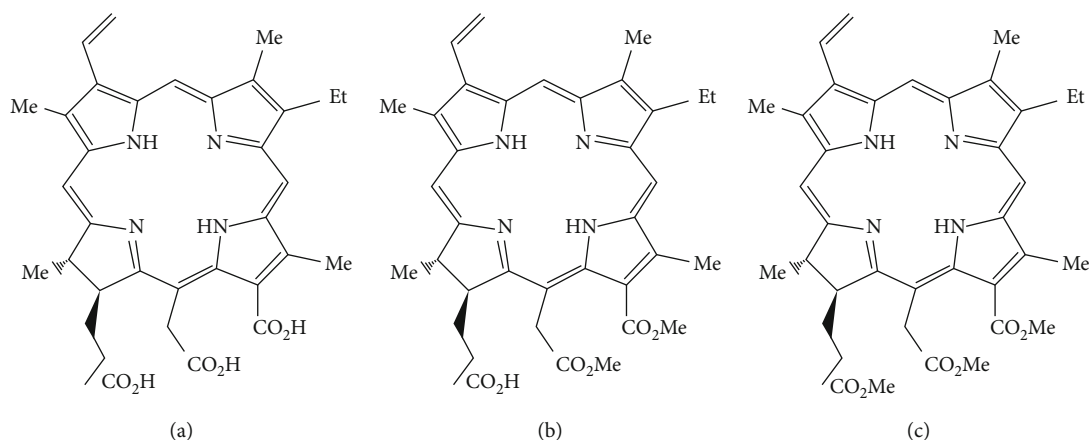


FIGURE 1: The structural formulas of the studied PSs: Ce6 (a), DME Ce6 (b), and TME Ce6 (c).

from the Laboratory of Biophysics and Biotechnology (Belarusian State University, Belarus). The structural formulas of the studied PSs are presented in Figure 1. As can be seen, the DME Ce6 and TME Ce6 molecules differ from the Ce6 molecule by the presence of two or three ether groups instead of carboxyl ones, which leads to their greater hydrophobicity and, consequently, the probability of aggregation of these molecules in aqueous solutions increases

Stock (concentrated) solutions of the Chlorin-type photosensitizers were prepared by dissolving the PS powder in ethanol. The weight was determined using analytical balance Vibra (Japan); the weighing accuracy is 0.05 mg. The solutions were prepared as follows: first, a stock solution in ethanol (2×10^{-3} M) was prepared; then, it was diluted in water to a concentration of 10^{-5} M.

As a nanocarrier, a branched copolymer obtained by grafting polyacrylamide (PAA) chains onto dextran ($M_w = 7 \times 10^4$ g/mol⁻¹) backbone was used. The details of the synthesis, identification, and analysis of the internal polymer structure were described in [14, 15]. The theoretical number of grafted polyacrylamide chains to the polysaccharide backbone for the sample we used as a polymer nanocarrier in the present work was equal to 5, and the related dextran-graft-polyacrylamide copolymer was referred to as D70-g-PAA. Molecular parameters of the polymer sample are $M_w = 2.15 \times 10^{-6}$ g/mol, $R_g = 85$ nm, and $M_w/M_n = 1.72$. The choice of this copolymer among the series of the branched samples synthesized was based on our previous research.

The D70-g-PAA copolymer was saponified by alkaline hydrolysis, by using NaOH to obtain branched polyelectrolyte dextran-graft-(polyacrylamide-co-polyacrylic acid) referred to as D70-g-PAA (PE) throughout [16]. The degree of saponification of carbamide groups to carboxylate ones in PAA-grafted chains determined by the potentiometric titration was equal to 43% [16].

The absorption spectra were registered with a spectrophotometer Cary 60 (Agilent) in the range of 200–800 nm. The spectral resolution was 1 nm. Fluorescence spectra were registered with a Cary Eclipse (Varian, Australia) fluorescent spectrophotometer in the range of 200–800 nm. The spectral width of slits for fluorescence measurements was of 5 nm. The

recording of all the spectra were carried out in standard quartz cuvettes $1 \times 1 \times 4$ cm (transmission range 170–1000 nm).

3. Results and Discussion

3.1. The Manifestation of Chemical Structure of Photosensitizers (Ce6, DME Ce6, and TME Ce6) in Absorption Spectra. Due to the highly coupled pi-electron system, porphyrins have a very characteristic absorption spectrum: one, the so-called Soret band, a very intense band in the region of 380–420 nm, and several less intense bands in the region of 500–750 nm (Q-band). Figure 2 shows absorption spectra of aqueous solutions of Ce6, DME Ce6, and TME Ce6.

In the absorption spectrum of the molecules under study in the UV and visible spectral regions, the distribution and intensity of absorption maxima characteristic of Chlorins are observed. The most intense bands appear at the boundary of the visible and UV regions of the spectrum at 399 nm (the Soret band) and at the boundary of the visible and IR regions of the spectrum at 662 nm. The optical density of Soret bands is approximately the same for all three compounds. The broadening of the bands and their redshift indicate an increase in aggregation processes with increasing hydrophobicity of the system. At the same time, the intensity of bands in the vicinity of Q-band at ~670 nm increases in the row: Ce6 → DME Ce6 → TME Ce6, i.e., demonstration increasing of ratio D_{670}/D_{405} and enhancement of PDT activity of molecules. These results are in good agreement with published data [17, 18].

3.2. The Influence of Polymer Matrix on Absorption Spectra of Chlorin-Based Photosensitizer. The absorption spectra of Ce6 solutions in water and in anionic polymer D70-g-PAA (PE) are shown in Figure 3. The most intense Soret band (maximum absorption 405 nm) is located in the close UV range, while the Q-band (504 and 648 nm) is in the red part of the spectrum. The general appearance of the experimentally recorded spectrum coincides with the literature data [17, 18]. The following spectra show that the shape of the band in the absorption spectrum in the visible range of the photosensitizer Ce6 dissolved in water and in the presence of polymer D70-g-PAA (PE) practically does not change the optical

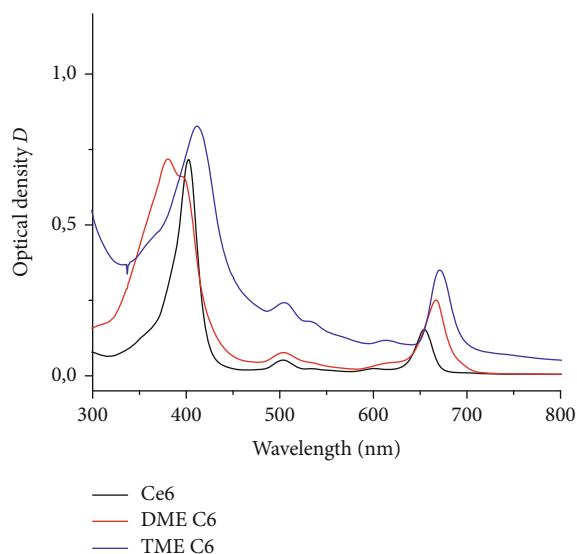


FIGURE 2: Absorption spectra of aqueous solutions of Ce6 (black line), DME Ce6 (red), and TME Ce6 (blue line).

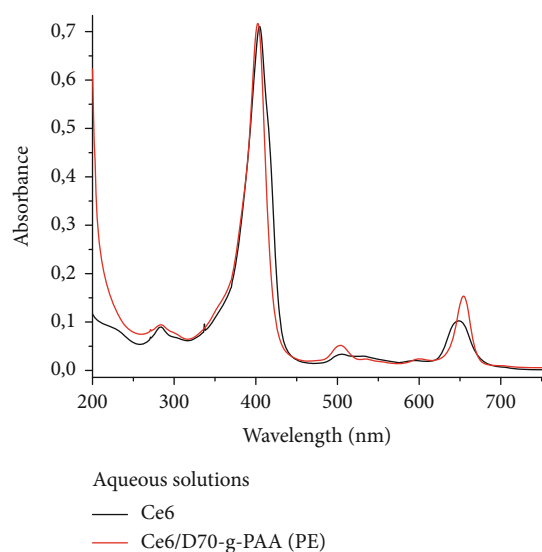


FIGURE 3: The absorption spectrum Ce6 solutions in water and with the presence of anionic polymer.

density, but the ratio changes of Soret and Q-band intensities are clearly visible. It has also been found that, depending on the polymer, the spectral position and optical absorption density of the Soret band and the Q-band with a maximum of 504 nm remain unchanged, while for the Q-band located around 650 nm, there is a slight redshift for the ionic polymer $648 \rightarrow 654$ nm. Additionally, it can be seen that the bands become slightly narrower, which indicates that the presence of the polymer interferes with the aggregation processes inherent in the aqueous solutions of Ce6.

Figure 4 displays the more detailed absorption spectra of Ce6, DME Ce6, and TME Ce6 solutions in water and in

anionic polymer matrix D70-g-PAA (PE) in the vicinity of the Soret band. If we compare the shape of the Soret band lines for Ce6 and its modified forms, we will see the following features: for Ce6, it becomes narrower and symmetrical; the barely noticeable redshift of the shoulder, indicating the presence of aggregation in aqueous solution, disappears; for TME Ce6, there is a small low-frequency shift; for DME Ce6, a doublet structure is clearly observed. Such changes indicate that all three photosensitizers are embedded in the polymer matrix, but differently. This is especially true of Ce6 dimethyl ether, the absorption spectrum of which (the doublet nature of the Soret band) can demonstrate the aggregation of photosensitizer molecules that may not integrate into the polymer structure.

Regarding Q-bands in the spectral region at 450–620 nm (Figures 3 and 5), one can see that Ce6 shows the greater sensitivity to the polymer matrix, while for DME Ce6 and TME Ce6, they remain virtually unchanged.

Of particular interest is the band Q4, because greater is the PDT effect achieved for more intensity of this band. Figure 5 shows that in the presence of polymer, the shape of this band becomes more symmetrical and sharp, and its intensity increases in the row: Ce6 \rightarrow DME Ce6 \rightarrow TME Ce6. Such changes in the spectrum once again confirm the fact of preventing the aggregation of photosensitizer molecules by the polymer matrix. Note that there is a redshift of the bands, which is an important factor for the effectiveness of PDT.

A generalized analysis of the spectral position of the bands is given in Table 1.

3.3. The Manifestation of Chemical Structure of Photosensitizers (Ce6, DME Ce6, and TME Ce6) in Absorption Spectra. The interaction of the studied photosensitizers with the polymer matrix was also confirmed by the fluorescence spectra, which are presented in Figure 6.

Ce6 in the presence of a polymer gives the PL spectrum already described in the literature [17, 18]. The PL excitation spectrum coincides with the absorption spectrum, which indicates that the same centers are involved in the absorption and emission processes.

The fluorescence spectrum of Ce6 in the polymer when excited by radiation with $\lambda = 405$ nm (Figure 6(a), red line) shows an intense band with a maximum of 661 nm and a low-intensity broad shoulder of ~ 715 nm. In the same figure, the black line shows the absorption spectrum, which completely coincides with the excitation spectrum (dashed red line) of fluorescence, which indicates participation in the radiation process of the same centers.

For DME C6 (Figure 6(b)), the fluorescent band is redshifted by 2 nm. The shape of this line remains unchanged. But a comparison of the fluorescence excitation spectrum with the absorption spectrum indicates that the Soret band is a doublet, but intensities are “pumped,” and the main contribution to the emission is made by the transition represented by the 399 nm band, which is less intense in the absorption spectrum, and the more intense absorption band (380 nm) in the PL excitation spectrum is presented only in the form of a low-intensity short-wavelength “shoulder.”

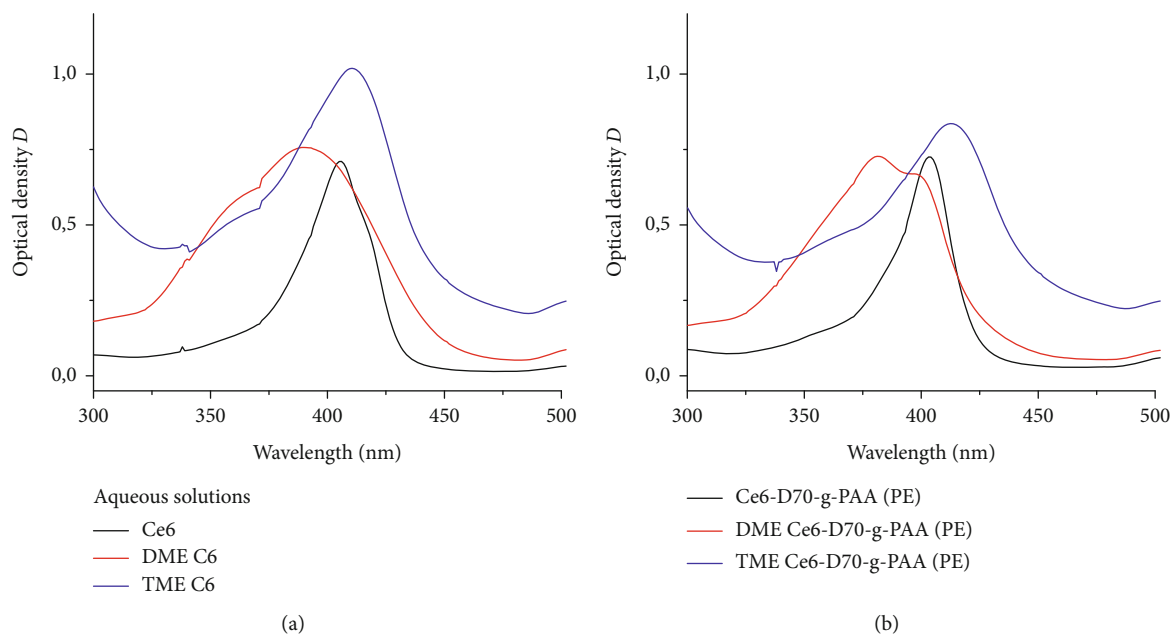


FIGURE 4: Absorption spectra of aqueous (a) and polymer (b) solutions of photosensitizers Ce6 (black line), DME Ce6 (red line), and TME Ce6 (blue line) in the region of Soret band.

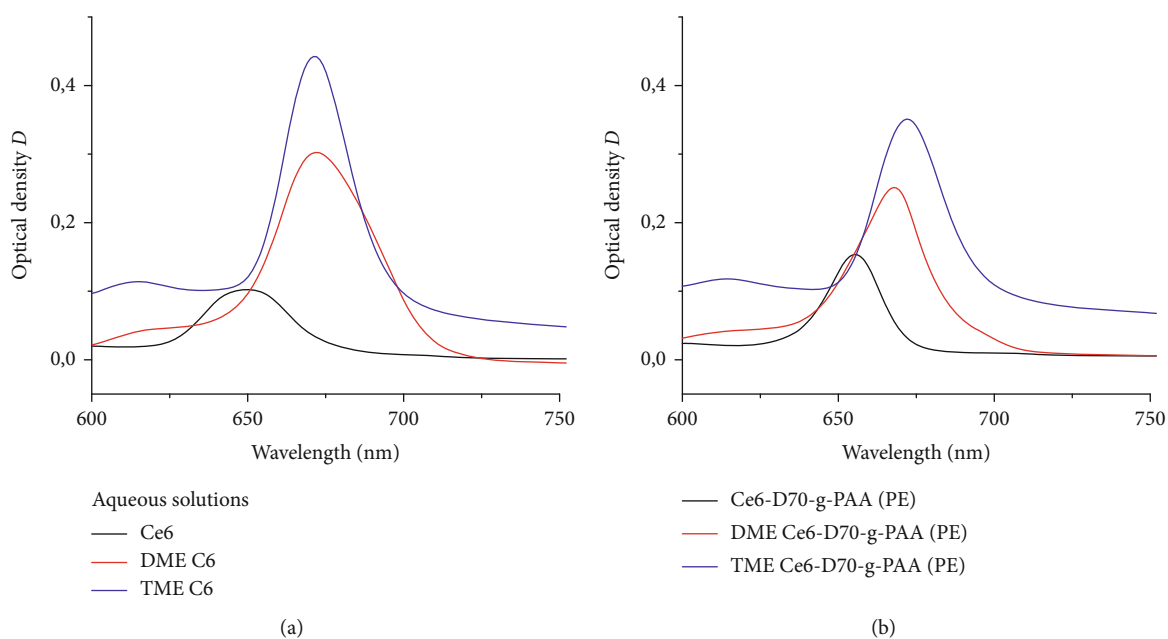


FIGURE 5: Absorption spectra of aqueous (a) and polymer (b) solutions of photosensitizers Ce6 (black line), DME Ce6 (red line), and TME Ce6 (blue line) in the region of Q4 band.

The TME Ce6 spectrum (Figure 6(c)) differs significantly from the two previous ones: first, an unexpected high-intensity band ~ 420 nm appeared, and second, in the region of the Q4 band, we see two bands with maxima of 678 and 752 nm. The spectral positions of these bands correspond to the Q4 band in the previous spectra (an intense band at 661 and 663 cm^{-1} in Ce6 and DME Ce6, respectively, and a broad low-intensity band at about 750 nm) at 678 and 752 nm. However, in this case, a redistribution of

intensities occurred, and the second band (represented in the spectra of Ce6 and DME Ce6 by a wide low-intensity band) is narrow and clearly pronounced, and its intensity exceeds the intensity of the main Q4 band. That is, we see a significant increase in the probability of transition. The PL excitation spectrum indicates that the emission center is the same as for Ce6 and DME C6. For correct interpretation, it is necessary to carry out quantum mechanical calculations.

TABLE 1: The position of the bands in the absorption spectra of Ce6, DME Ce6, and TME Ce6 and composites D-g-PAA/Ce6, D-g-PAA/DME Ce6, and D-g-PAA/TME Ce6.

PS/solution	Spectral position of absorption bands (nm), FWHM (nm), and optical density					
	λ	Soret FWHM	D	λ	Q4 FWHM	D
Ce6	405	34	0.91	648	33	0.1
Ce6/D70-g-PAA (PE)	402	28	0.72	654	22	0.15
DME Ce6	389	82	0.76	671	37	0.3
DME Ce6/D70-g-PAA (PE)	380	62	0.72	667	29	0.25
	399		0.65			
TME Ce6	410	65	1.02	670	26	0.44
TME Ce6/D70-g-PAA (PE)	412	54	0.83	671	24	0.35

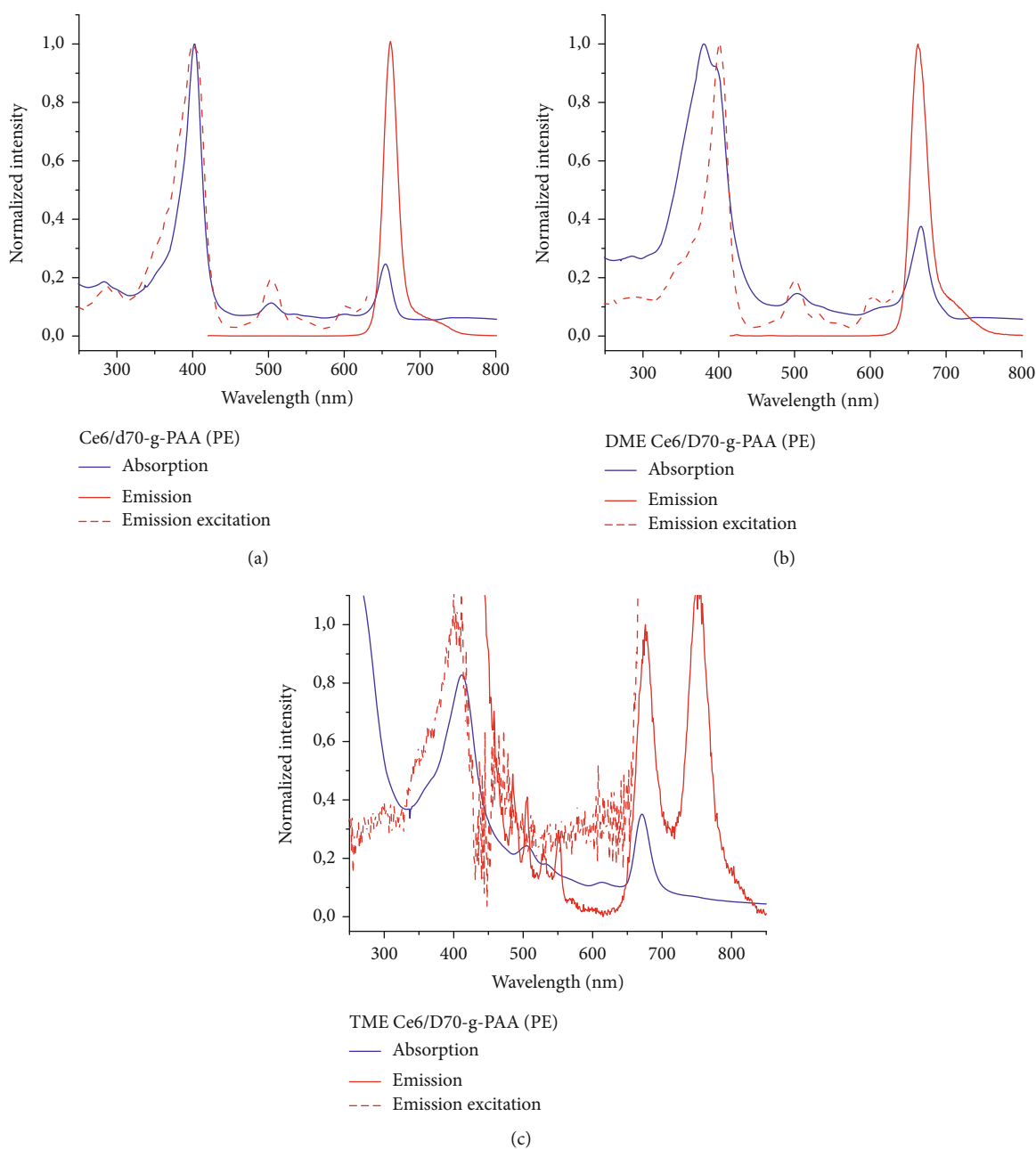


FIGURE 6: Fluorescence (red line) and fluorescence excitation (dotted red line) spectra of PS/D70-g-PAA (PE) systems, where PS = Ce6 (a), DME Ce6 (b), and TME Ce6 (c). The absorption spectra for comparison are given by a blue line.

4. Conclusions

The spectral characteristics of nanosystem polymer/photosensitizers of the Chlorin series have been studied. Comparison of the absorption and fluorescence spectra of solutions of Ce6 in water and in the presence of polymers dextran-polyacrylamide in anionic form showed a visible effect of the anionic matrix on the photophysical properties of studied photosensitizers. It was shown that in the absorption spectra of aqueous Ce6 and its modified forms—dimethyl (DME) and trimethyl (TME) esters—in the UV range and the visible range of the spectrum, there is a characteristic position of absorption maxima: Soret band 389-410 nm and Q-band 506, 536, and 590-616 nm; however, upon transition from Ce6 to DME and TME, changes in the intensities and band shapes of these spectra are observed. The broadening of the bands and their redshift indicate an increase in aggregation processes in aqueous solutions of three PSs with increasing hydrophobicity of the system. It was shown that the polymer matrix effectively prevents the aggregation processes of photosensitizers. A comparison of the absorption spectra of PS/D70-g-PAA (PE) systems with the corresponding aqueous solutions showed that in the presence of a polymer, the FWHM of the characteristic bands decrease, which indicates an increase in the PS monomer content in the solutions. The shifts of the maxima of the position of the Soret band (blue for DME and red for TME) and Q4 band indicate the incorporation of PS into the polymer matrix, i.e., complex formation. This is also confirmed by fluorescence spectra. In addition, the redshift of the Q4 band in the row Ce6-DME Ce6-TME Ce6 and appearance of the band at 752 nm (for TME Ce6) when these PSs are introduced into the polymer matrix makes them somewhat more promising for PDT in comparison with aqueous solutions. In addition, the redshift of the Q4 band in the Ce6-DME-TME series and the appearance of a band at 752 nm (for TME), when these PSs are introduced into the polymer matrix, make them more promising for PDT compared to aqueous solutions.

Data Availability

Data is available on request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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