SPECTROSCOPY
OF CONDENSED STATES

Investigation of the Spectral Properties of Noncovalent Complexes of a Polysubstituted Water-Soluble Derivative of the C$_{60}$ Fullerene and Chlorin e6 in Polar Solvents

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Abstract—Using methods of absorption and fluorescence spectroscopy, we show that noncovalent complexes based on a water-soluble fullerene derivative and chlorin e6 dissolved either in water or in ethanol are formed as a result of electrostatic interactions. The formation of these complexes causes a red shift of the absorption spectrum of chlorin e6 and efficient quenching of the intensity of its fluorescence. The Stern–Volmer quenching constants of complexes in water and ethanol are 7.3×10^5 and 2×10^5 M^{-1}, respectively. We show that, in an aqueous solution, the complex consists, on average, of one molecule of the water-soluble fullerene derivative and three dye molecules, whereas this ratio in ethanol is 1 : 1.

INTRODUCTION

At present, fullerenes and their derivatives are attracting much attention as potential photodynamic drugs. Upon their excitation by a light quantum, they pass to a triplet state with a probability close to unity and, depending on the polarity of a medium, efficiently generate either singlet oxygen $^1O_2$ or superoxide anion radical O_2^- and other active radicals [1]. However, the absorption of native fullerene in the red range of the spectrum, which is the most suitable for the photodynamic therapy (PDT), is weak. To avoid this problem, it has been proposed to create structures in which fullerene is combined with a dye that absorbs light in the given spectral range and then transfers this excitation or an electron to fullerene [2–9].

In the literature, there are a number of works devoted to the study of fullerene complexes with various dyes: phthalocyanines (with and without Zn atom), Ni(2)diporphyrin, and porphyrin [2–4]. It was shown that fullerenes form stable noncovalent complexes with dyes, in which electron transfer takes place, leading to the fluorescence quenching. However, structures based on the C$_{60}$ fullerene and porphyrin dyes that were described in these works are insolu-

ble in water, and, for this reason, they are of little use in biology and medicine. In works [5–7], complexes of water-soluble fullerene derivatives (WSFDs) with a phthalocyanine derivative, preparation Photosens, which is used in clinical practice [8], were studied.

From the point of view of PDT, the chlorin e6 dye is of particular interest. At present times, its various derivatives are widely used in clinical practice as photosensitizers [10, 11]. They have a high absorption in the red range of the spectrum, low dark toxicity, and a long lifetime of the triplet state [12, 13]. By combining chlorin and fullerene into a single structure, one can expect a significant increase in its photodynamic activity. The interaction of π orbitals of the chlorin molecule and the fullerene core leads to changes in electronic and vibrational levels of the excited dye both in the composition of the noncovalent complex and upon formation of covalent dyads; as a result of which, both the photophysical properties of chlorin e6 (its absorption spectra and fluorescence quantum yield), and the generation efficiency of active forms of oxygen change significantly [9, 14–16]. As a consequence of this interaction, it becomes possible to transfer either excitation or an electron from a photoexcited dye to fullerene, which, ultimately, can significantly enhance
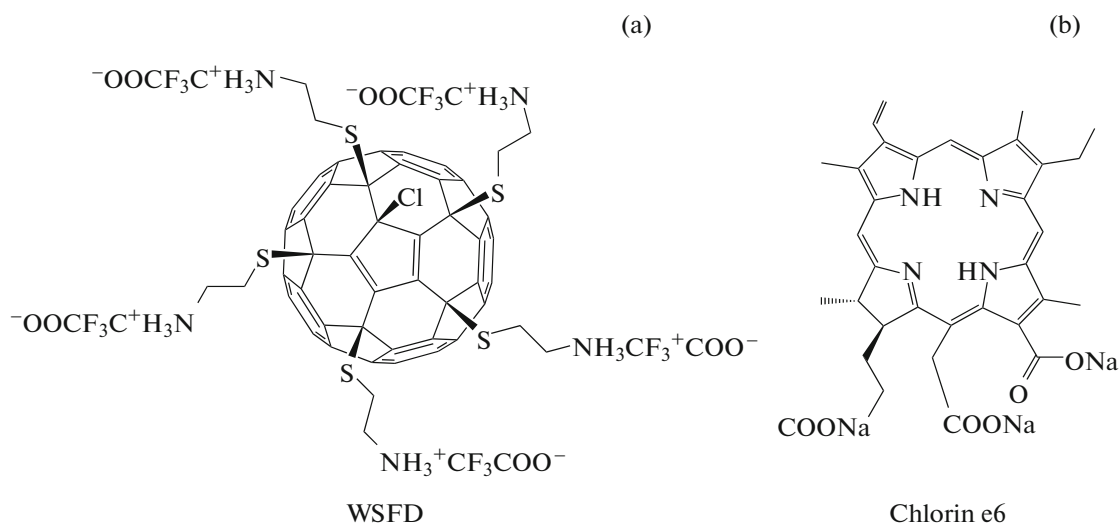
Scheme 1.

the photodynamic effect of the chlorin−fullerene dyad [9]. The possibility of occurrence of such an effect in complexes of fullerenes with chlorin was previously indicated in patent [17].

Creation of fullerene−chlorin hybrid nanostructures is a promising way to create new efficient photosensitizers, which attract attention from many researchers. The data that we obtained in this work may be of interest for studying mechanisms of interaction of WSFDs with chlorin e6 when combining them into a single hybrid nanostructure, as well as for analyzing the behavior of these structures in polar solvents.

MATERIALS AND METHODS

In this work, we investigated the photophysical properties of WSFD−chlorin noncovalent complexes. These complexes are formed when water-soluble C_{60} fullerene derivatives that have five positive charges on their addends and a water-soluble derivative of chlorin e6 that has three negative charges are introduced into a solution (Scheme 1).

In this work, water-soluble polycationic fullerene derivatives were used, which were obtained by attaching five addends to the fullerene spheroid, as was described in [18]. The synthesis of the chlorin e6 derivative was described in [9]. Fullerene derivatives of this type have a high solubility in water (more than 100 mg/mL). The structures of the WSFD and chlorin e6 were proven by using IR and UV spectroscopy, nuclear magnetic resonance spectroscopy on ¹H and ¹³C nuclei, and electrospray-mass-spectrometry. Absorption spectra were recorded on a Cary-60 spectrophotometer, while stationary fluorescence spectra were recorded on a Cary Eclipse spectrofluorometer.

The absorption spectra of the original dye in water and in the alcohol solution noticeably differ from each other, with the absorption maxima of the Q bands in water and alcohol being 643 and 663 nm, respectively. Our investigation of the stationary fluorescence spectra of chlorin showed their similar dependences on the kind of the solvent; the fluorescence maxima were observed at 651 and 668 nm for aqueous solutions and ethanol, respectively. The bathochromic shifts in the absorption and fluorescence of the chlorin derivative that occur upon changing the solvent from water to alcohol are associated with changes in the dielectric permittivity of the medium.

Our investigation of the absorption and stationary fluorescence spectra of the dye upon successive introduction of increasing volumes of the WSFD solution into the cell containing the chlorin e6 solution showed that, as the concentration of the WSFD increases, the absorption spectrum of chlorin e6 changes, shifting to the red range both in the aqueous solution and in ethanol (Figs. 1a, 2b). Simultaneously, upon addition of the WSFD, intense quenching of the stationary fluorescence of the dyes is observed (Figs. 1b, 2b).

The observed effects allow us to infer that nonfluorescent static WSFD−chlorin complexes are formed in the studied solutions. The red shift of the absorption spectrum of the dye in the structure of the complex indicates that electronic levels of the complex change as compared to their positions in the free dye, while the quenching of the stationary fluorescence indicates that there is an efficient electron transfer from the excited singlet state of the dye to the fullerene core.

From the analysis of the absorption and fluorescence spectra, one can calculate the ratio between the numbers of the chlorin and WSFD molecules in the complex. Assuming that a change in the fluorescence intensity is proportional to the number of quenched
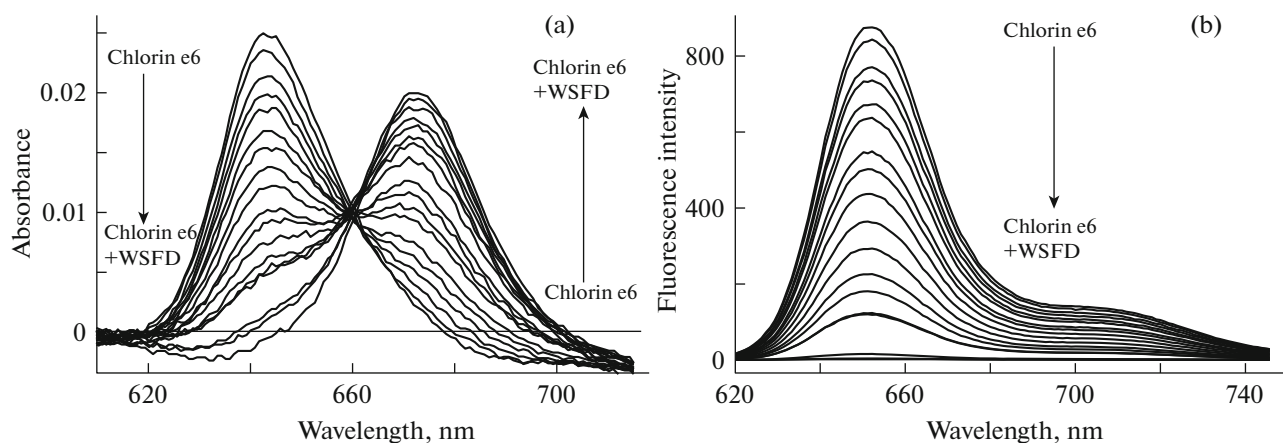
Fig. 1. Changes in the (a) absorption and (b) fluorescence spectra of chlorin e6 in an aqueous solution upon successive addition of WSFD. The concentration of chlorin e6 is 5×10^{-6} M, and the final concentration of WSFD is 1.8×10^{-6} M.
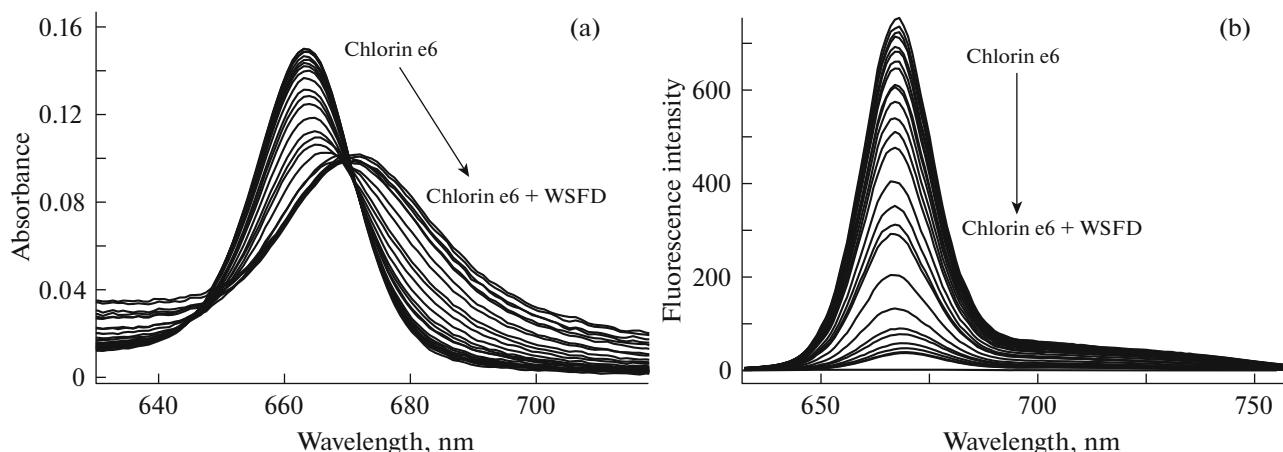

Fig. 2. Changes in the (a) absorption and (b) fluorescence spectra of chlorin e6 in ethanol upon successive addition of WSFD. The concentration of chlorin e6 is 5×10^{-6} M, and the final concentration of WSFD is 9.2×10^{-6} M.

(bound) chlorin molecules and assuming that their fluorescence is quenched completely, we calculated the ratio of the amount of added WSFD to quenched chlorin at each concentration of WSFD. We found that one WSFD molecule forms a complex with three chlorin molecules in water and with one chlorin molecule in ethanol (Fig. 3). As can be seen from Fig. 3, the data that were calculated independently from the absorption and fluorescence coincide, which also confirms the formation of nonfluorescent static complexes.

An analysis of the data on quenching the intensity of stationary fluorescence of dyes in Stern–Volmer coordinates (Fig. 4) makes it possible to determine the quenching constant of WSFD–chlorin complexes from the slope of the linear portion of the dependence at minimum values of the WSFD concentration, with

this constant being affected both by a change in the ionic strength of the solution and by the polarity of the solvent. The quenching constants for water and ethanol were found to be 7.3×10^5 and 2.0×10^5 M^{-1}, respectively (Fig. 4). High values of the constants indicate that the formation of complexes involves the participation of electrostatic interactions. The deviations of the plots in the Stern–Volmer coordinates from the linear behavior that was observed with increasing concentrations of the WSFD can be explained by the contribution from dynamic quenching or by the formation of larger dye–fullerene associates up to the formation of nanostructures of ~100 nm in size, as was shown by dynamic light scattering for fullerene derivatives of this type [5]. The entire set of the chlorin absorption spectra in water was decomposed into two components, using the initial and final absorption spectra as bases.
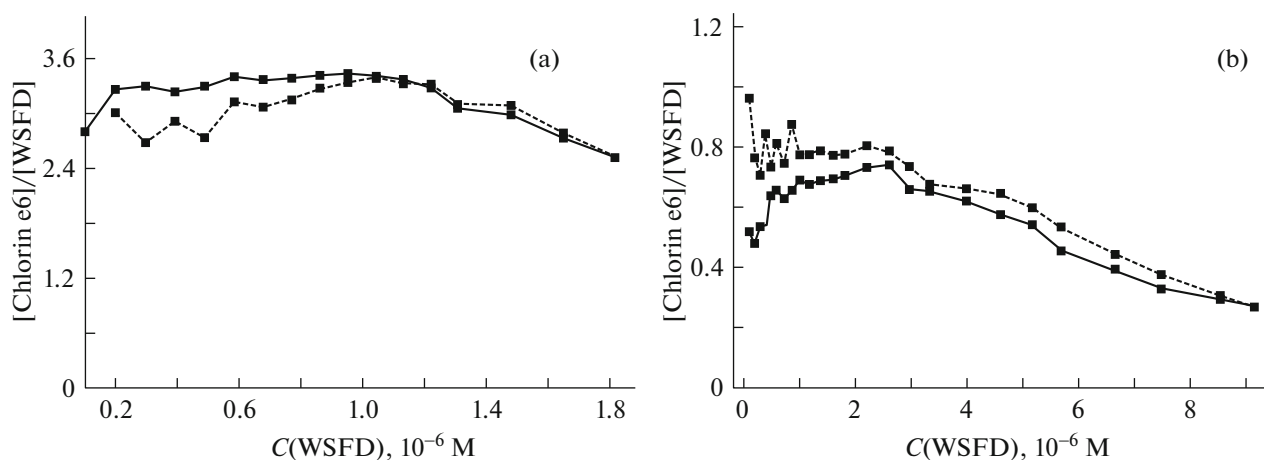
Fig. 3. Calculations of the WSFD : chlorin ratio in the composition of their complex vs. the concentration of WSFD in (a) water and (b) alcohol; the calculations were performed by the absorption (solid curves) and fluorescence (dashed curves) spectra.

The quality of decomposition was monitored by the total number of different forms of chlorin, which should be maintained throughout the dilution. In a similar way, the absorption spectra of chlorin in alcohol also were decomposed (Fig. 5). As can be seen from the absorption spectra of WSFD–chlorin complexes in water and ethanol presented in Fig. 5, as chlorin is combined with fullerene to form a complex, the Q absorption bands of chlorin are shifted to the red range of the spectrum, which indicates a change in the dielectric environment, indirectly confirming the formation of static complexes.

In this work, we described the results of our study of the spectral and photophysical properties of noncovalent complexes that are formed in polar solvents (water and ethyl alcohol) due to the interaction of a polysubstituted cationic fullerene derivative with a water-soluble derivative of chlorin e6. Using spectrophotometry and stationary fluorimetry, we showed that, in the aqueous solution and in ethanol, the interaction of the polycationic fullerene derivative and chlorin dye results in the formation of a complex due to electrostatic interactions, with the complexation efficiency and the structure of complexes depending on the polarity of the medium. The obtained data may be of interest from the point of view of studying the mechanisms of the WSFD interaction with chlorin e6 and further evaluation of their combined photodynamic action.
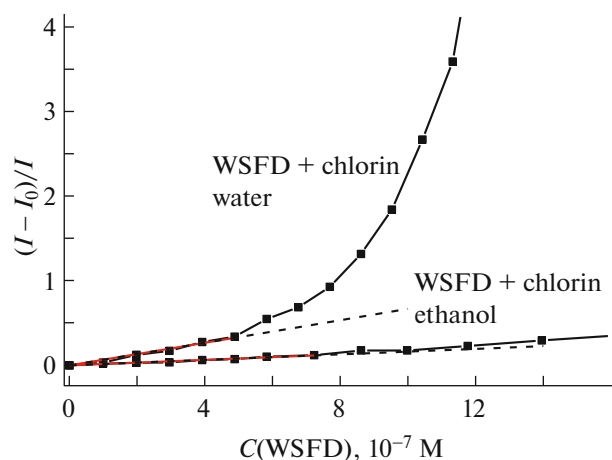
Fig. 4. Stern–Volmer constants of WSFD–chlorin complexes in water and alcohol.
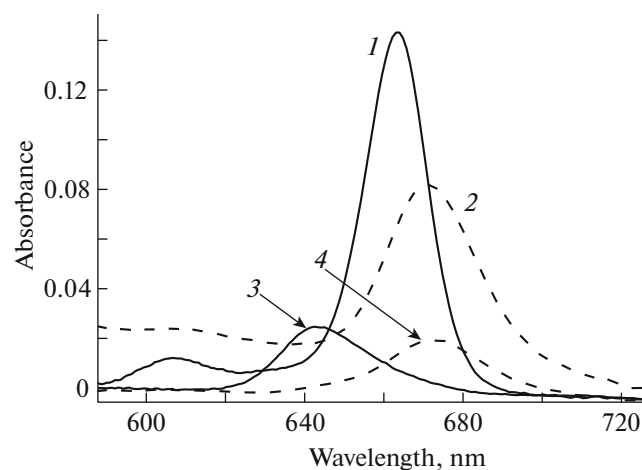
Fig. 5. Absorption spectra of free chlorin and WSFD–chlorin complexes. The concentration of chlorin e6 (free and in the composition of the complex) is 5×10^{-6} M. Curves: (*1*) chlorin e6 in ethanol, (*2*) WSFD–chlorin in ethanol, (*3*) chlorin e6 in an aqueous solution, and (*4*) WSFD–chlorin in an aqueous solution.

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