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PHYSICAL CHEMISTRY

Photodynamic Activity of a Hybrid Nanostructure Based on a Polycationic Fullerene Derivative and Phthalocyanine Dye Photosens

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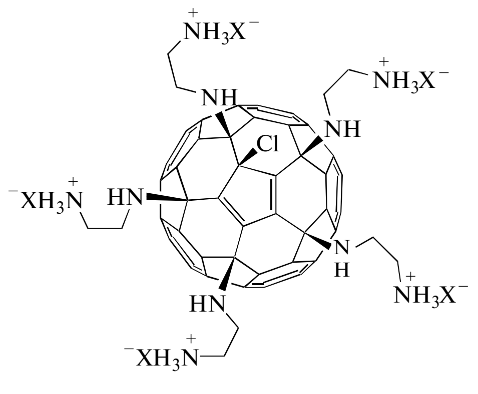
As known, fullerenes are photoexcited to a triplet state in 100% quantum yield, which then is deactivated to generate, depending on the polarity of a medium, either singlet oxygen $^1O_2$ or superoxide radical anions $O_2^{\cdot-}$ and other active radicals [1]. Numerous studies have addressed the photodynamic effect of fullerene and their derivatives: the generation of active radicals upon photoexcitation is responsible for damage of DNA, proteins, and membranes, as well as for the suppression of the reproduction of tumor cells, viruses, and bacteria [2]. However, the efficiency of the photodynamic action of fullerenes and the prospects for their application in medicine are essentially limited by the weak absorption by fullerenes of visible light, especially in the range 650–800 nm, which is most suitable for photodynamic therapy.

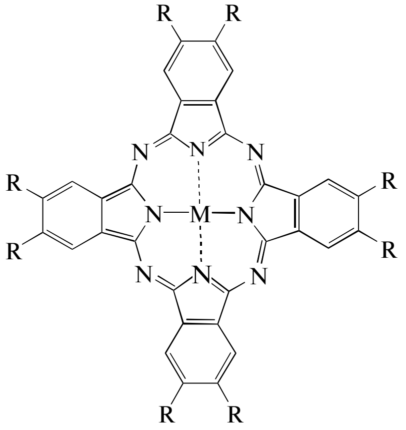
The photodynamic effect of fullerenes can be considerably enhanced by creating hybrid nanostructures (HNSs) via the formation of a fullerene complex with a dye that efficiently absorbs visible light [3]. In such a complex, the dye can efficiently absorb light and transfer excitation energy or electron to the fullerene. Further excitation transfer or electron transfer from the fullerene to molecular oxygen will generate active oxygen species. The creation of such an HNS based on fullerene and a dye can thereby significantly enhance the efficiency of photodynamic therapy.

Special challenges in creating photodynamic medicines are their solubility in water and the ability to selectively accumulate in therapeutic targets.

In this paper, we report the results of studying the spectral luminescence properties and photodynamic activity of HNSs based on the complex of a water-soluble polycationic fullerene C_{60} derivative (PFD) with the phthalocyanine dye Photosens, widely used in clinical practice as a photodynamic agent.



PFD



Photosens
(M = AlOH, R = SO_3^-)

A water-soluble PFD was synthesized by attaching five addends, ethylenediamine residues containing

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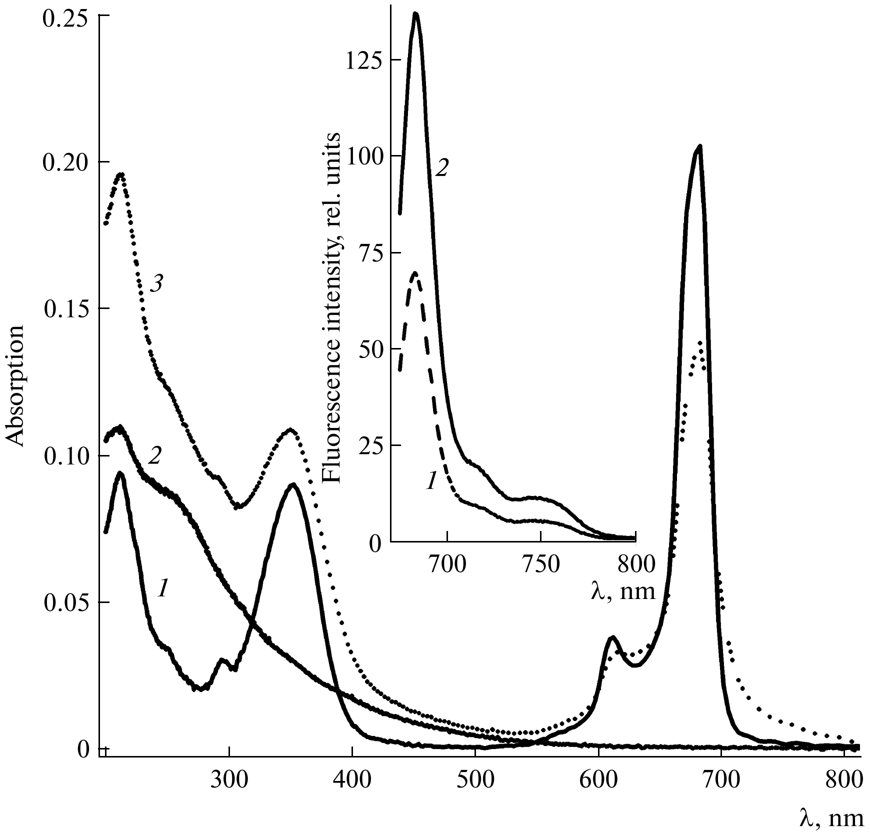

Fig. 1. Absorption spectra of aqueous solutions of (*1*) Photosens, 2×10^{-6} M; (*2*) PFD, 2×10^{-6} M; and (*3*) Photosens in the presence of PFD in an equimolar concentration of 2×10^{-6} M. Inset: Fluorescence spectra of Photosens (*1*) in the absence of the quencher, (*2*) in the presence of PFD in an equimolar concentration of 2×10^{-6} M. λ_{ex}= 683 nm.

water-solubilizing amino groups, to the fullerene spheroid [5]. Such fullerene derivatives are readily soluble in water (more than 100 mg/mL). The PFD structure was proved by IR spectroscopy, ^{1}H and ^{13}C NMR, and mass spectrometry.

The absorption spectra of PFD and Photosens taken in the same concentration ($2 \cdot 10^{-6}$ M) are shown in Fig. 1. In the absorption maximum of Photosens at 683 nm, the absorption of the dye is 420 times higher than that of the PFD.

The introduction of small amounts of PFD (10^{-6}–10^{-5} M) into a Photosens solution leads to changes in its absorption spectrum (Fig. 2), Photosens fluorescence quenching (Fig. 1), and a decrease in the intensity of the fluorescence decay curves (more than 100-fold), without a change in the fluorescence decay time (Fig. 2). These effects can be explained by the formation of static complexes of the PFD with Photosens.

Taking into account the spectral and donor–acceptor properties of fullerenes, two mechanisms of fluorescence quenching for Photosens incorporated into the complex with PFD can be suggested: inductive resonance energy transfer through dipole–dipole coupling and the electron transfer mechanism.

It is known that the former mechanism, inductive resonance excitation energy transfer through dipole–dipole coupling, is realized when the luminescence spectra of the donor (Photosens) and acceptor (PFD) are overlapped [6]. As follows from Fig. 1, such an overlap is observed, although PFD has rather low absorption in the range 550–800 nm. Using the formalism of the Förster theory [6], we can estimate, for the 100-fold fluorescence quenching of Photosens, the distance between the dye and the fullerene core should be less than 8 Å, which corresponds to the assumed structure of the complex.

The second mechanism of deactivation of the Photosens singlet state can by quenching through electron transfer. According to the theory of outer-sphere electron transfer, the efficiency of electron transfer between the donor and acceptor exponentially depends on the transfer distance [7, 8]. Under optimal reaction conditions, the electron transfer (*et*) rate can be estimated by the equation

$$k_{et} = k_0 \exp(-\alpha R), \quad (1)$$

which was derived by generalization of electron transfer data in different molecular structures, including those with the participation of photoexcited molecules

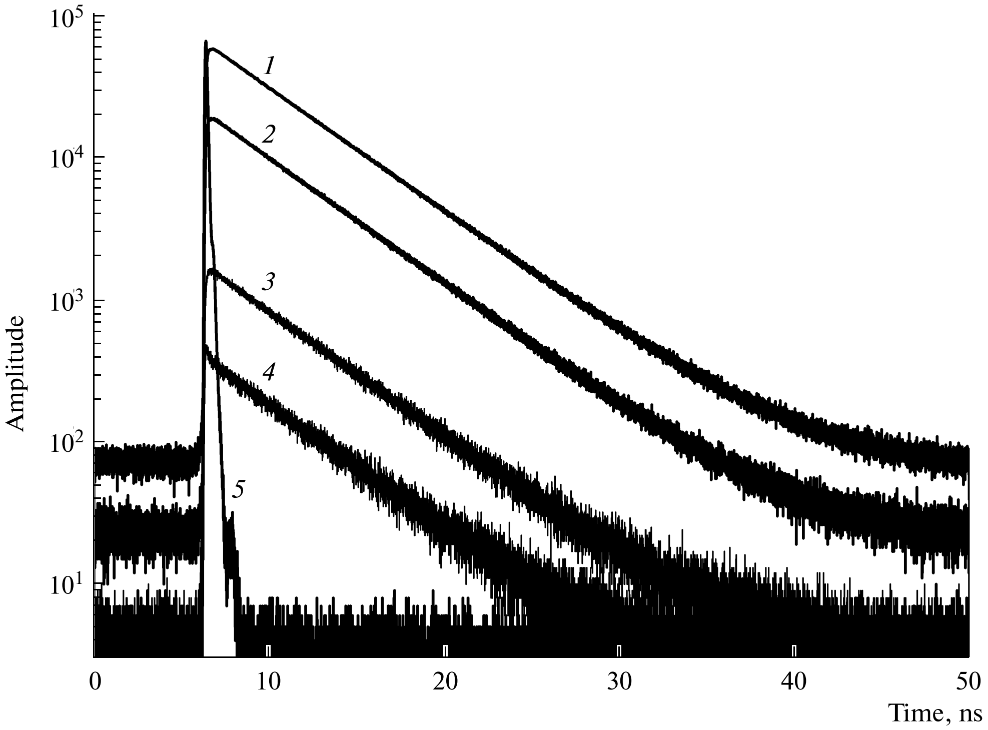

Fig. 2. Fluorescence decay kinetics of Photosens (aqueous solution, concentration 2.1×10^{-6} M) (*1*) in the absence of PFD; (*2–4*) in the presence of PFD in concentrations 2.4×10^{-6}, 6.9×10^{-6}, and 1.1×10^{-5} M, respectively; (*5*) apparatus function.

[9, 10]. Here, $k_0 = 10^{16}$ s^{-1}; R is the distance between the transfer sites, α is the parameter characterizing the effect of the medium on the overlap of the donor and acceptor wave functions due to superexchange interactions. Depending on the type of matrix separating the donor and acceptor, the α value can be 0.9 Å^{-1} for saturated hydrocarbon chains, 1.4 Å^{-1} for protein globules, and 1.8–2.4 Å^{-1} for water molecules [11].

The lifetime of the excited singlet state of free Photosens under the experimental conditions was measured to be 4.99 ± 0.01 ns (kinetic dependence *1* in Fig. 2). Let us assume that complex formation leads to the 100-fold fluorescence quenching. Then, it can be estimated by Eq. (1) that the electron transfer from the excited Photosens to the fullerene along the saturated hydrocarbon chain (α = 0.9 Å^{-1}) can occur to a distance of up to 25 Å in 0.05 ns, which is also consistent with the structure of the putative complex.

Thus, the change in the absorption spectrum and the fluorescence quenching of Photosens upon its introduction into a PFD solution point to the formation of the PFD–Photosens complex. In this complex, excitation energy or electron is transferred from the dye to the fullerene core, which should promote the generation of $O_2^{\dot{-}}$.

The photodynamic activity of the PFD, Photosens, and the PFD–Photosens complex was estimated from the generation of O_2^- under illumination with light at $\lambda > 650$ nm of a 10-mm cell with the reaction mixture containing an aqueous solution of PFD and/or Photosens in concentration 2×10^{-6} M, as well as NADH (nicotinamide adenine dinucleotide, Sigma), NBT (nitro blue tetrazolium chloride, MP Biomedical), and EDTA (ethylenediaminetetraacetic acid, Sigma), as described in [3]. The amount of the generated O_2^- was determined from the formation of formazan by monitoring the optical density at 560 nm [1].

Studying the photodynamic activity of PFD and Photosens as individual compounds showed that both reagents exhibit noticeable photodynamic activity under excitation with light at $\lambda > 650$ nm, the activity of Photosens being only 5.4 times higher than the activity of PFD (Fig. 3). With regard to the difference between the extinction coefficients ($\varepsilon_{683} = 10^5$ M cm^{-1} for Photosens and 0.23×10^3 M cm^{-1} for PFD), the photodynamic activity of PFD, as calculated per absorbed quantum, is about 77 times higher than the activity of commercially available Photosens.

The addition of an equimolar amount of PFD to a Photosens solution led to a considerable increase in the photochemical reaction rate as compared with the photodynamic activity of the individual compounds. In this case, the reaction rate exceeds more than tenfold the sum of the individual contributions of the compounds to the total reaction rate (Fig. 3). It is evident that this is due to the interaction of these compounds in solution.

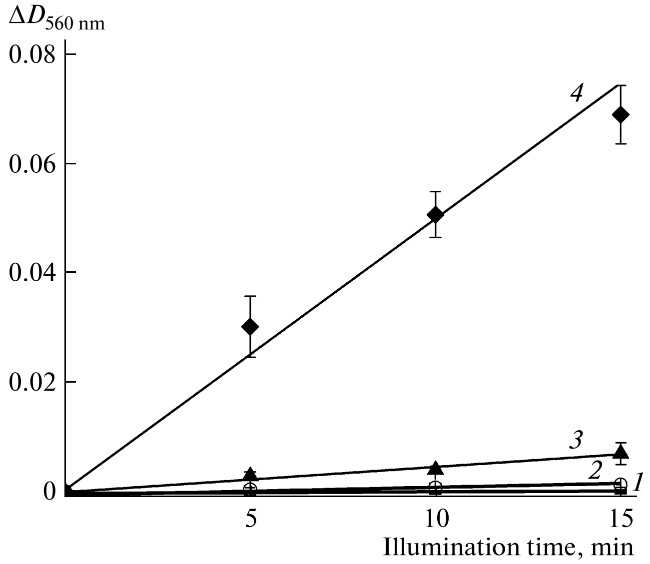

Fig. 3. Kinetics of $O_2^{\cdot -}$ generation in the photodynamic reaction under illumination with light with $\lambda > 650$ nm: (*1*) control, (*2*) PFD, (*3*) Photosens, and (*4*) PFD–Photosens complex.

It follows from the fluorescence quenching data that, under the experimental conditions, only 50% of PFD and Photosens molecules in solution are combined in the complex. Thus, it can be assessed that the photodynamic reaction rate of the PFD–Photosens complex increases 20 times as compared with the photodynamic activity of Photosens and 108 times as compared with the photodynamic activity of the PFD as individual compounds.

Thus, it has been experimentally shown that, under exposure to light with wavelengths exceeding 650 nm of aqueous solutions of PFD, the latter exhibit considerable photodynamic activity comparable with the activity of commercial dye Photosens at different concentrations and exceeds the latter by a factor of 77 in the quantum yield.

PFD and Photosens in solution form the complex that, being excited in the absorption band of Photosens, exhibits the photodynamic activity 20 times higher than that of Photosens and 108 times higher than that of PFD.

It is conventionally believed that the ability of Photosens and other dyes to generate active oxygen species is caused by the excitation or electron transfer to oxygen molecules from the triplet states of these dyes. In this work, we have shown for the first time that the high photodynamic activity of the PFD–Photosens complex is due to the efficient energy or electron transfer from the singlet level of the dye incorporated in the hybrid nanostructure to the fullerene core. This opens up the possibility of creating efficient photodynamic fullerene-based preparations of new generation with the use of dyes excited only to the singlet state, which considerably extends the range of suitable dyes.

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