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Photodynamic Effect of Iron(III) Oxide Nanoparticles Coated with Zinc Phthalocyanine

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Abstract—The possibility of using iron oxide(III) nanoparticles covered with photoactive derivative of zinc phthalocyanine for diagnostics and treatment of malignant tumors was studied experimentally.

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Over the past 30 years, molecular photosensitizers are used in medicine for fluorescence diagnostics and photodynamic therapy of cancer and other than cancer diseases. The most widespread molecular photosensitizers are based on derivatives of phthalocyanines, porphyrins, and chlorin e6. The main disadvantages of molecular photosensitizers as medicinal products include low differentiation between the pathological and healthy tissues and long time of circulation of residual doses of the drug throughout the body. The use of photosensitizers in the composition of nanoparticles can not only eliminate these disadvantages, but also significantly expand the scope of their application. This may be done by using, in addition to light sources, therapeutic drugs activation means (magnetic field, ultrasound, low intensity electric current, etc.) and new methods of the accumulation of the drug in the desired region of biological tissue.

We studied the possibility of using iron(III) oxide nanoparticles coated with 2,3-dicarboxy-substituted zinc phthalocyanine (ZnPc) for diagnostics and treatment of malignant neoplasms. Zinc phthalocyanine derivatives in molecular form are considered by researches as very promising drugs for fluorescence diagnostics and photodynamic therapy of malignant tumors. The physicochemical and biomedical characteristics of zinc phthalocyanine and its derivatives are described in [1–4].

For preparing iron(III) oxide nanoparticles covered with zinc phthalocyanine (*n*Fe_2O_3@ZnPc), we used γ-Fe_2O_3 (particle size of 20–40 nm, NanoArc®) from Alfa Aesar®.

Phthalocyanine product, zinc 2,3-dicarboxyphthalocyanine anhydride, was synthesized by the statistical condensation of unsubstituted phthalonitrile with 4,5-bis(hexaxycarbonyl) phthalonitrile with following hydrolysis of the ester groups and transformation of the resulting dicarboxylic acid into anhydride [5]. Nano-particles of iron(III) oxide and ZnPc were conjugated by the original Klimov's procedure (Department of Chemistry, Lomonosov Moscow State University). The Fe_2O_3 to ZnPc weight ratio in the resulting nano-particles of composition *n*Fe_2O_3@ZnPc was 40 : 1. Contrary to molecules, ZnPc nanoparticles do not fluoresce in aqueous solution (see Fig. 1) [4, 6].

Mice (females, weight 25–30 g, age 9–10 weeks) vaccinated intramuscularly with Ehrlich carcinoma in the femoral region of the right paw were experimental model; the second paw of the animal served as reference. The tests were carried out within 10 days after tumor inoculation.

The animal fur in the studied sites was removed with a special hair removal cream within 24 h before the experiment. In the tail vein of the animal, a colloidal solution of nanoparticles at a dose of 40 mg/kg (1 mg/kg in terms of ZnPc) was injected.

For recording and analyzing the spectral data we used a LESA-01-BIOSPEC fiber optic spectrum analyzer (Biospek) [7]. Zinc phthalocyanine fluorescence in the red region of the spectrum was excited with a laser radiation (He-Ne laser, wavelength 632.8 nm, power 20 mW).

The tumor was irradiated within 24 h elapsed after introducing nanoparticles. We used laser sources emitting in the red spectral range: a cw semiconductor laser (wavelength 670 nm) and a pulsed gold vapor laser (wavelength 627.8 nm, pulse length 20 ns, pulse repetition rate 8–16 kHz). The irradiation power density was 300 mW/cm^2, the treatment time, 15 min, and the total light dose 270 J/cm^2.

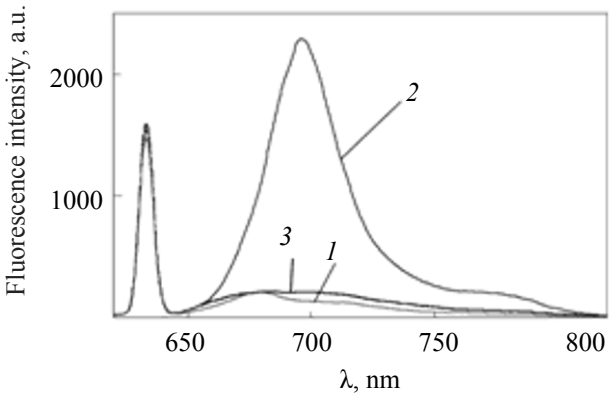

Fig. 1. Fluorescence spectra of model solutions of (*1*) *n*Fe_2O_3@ZnPc nanoparticles (concentrations 10 and 0.25 mg/L in terms of ZnPc) and (*2*) ZnPc nanoparticles (water soluble form, concentration 0.1 mg/L). Into the samples (*1*) and (*2*), a scattering medium (fat emulsion "Lipofundin MCT / LCT" (B. Braun Melsungen AG) was introduced in order to bring the optical properties of the samples to those of biological tissues; (*3*) autofluorescence spectrum of healthy skin of the human hand.

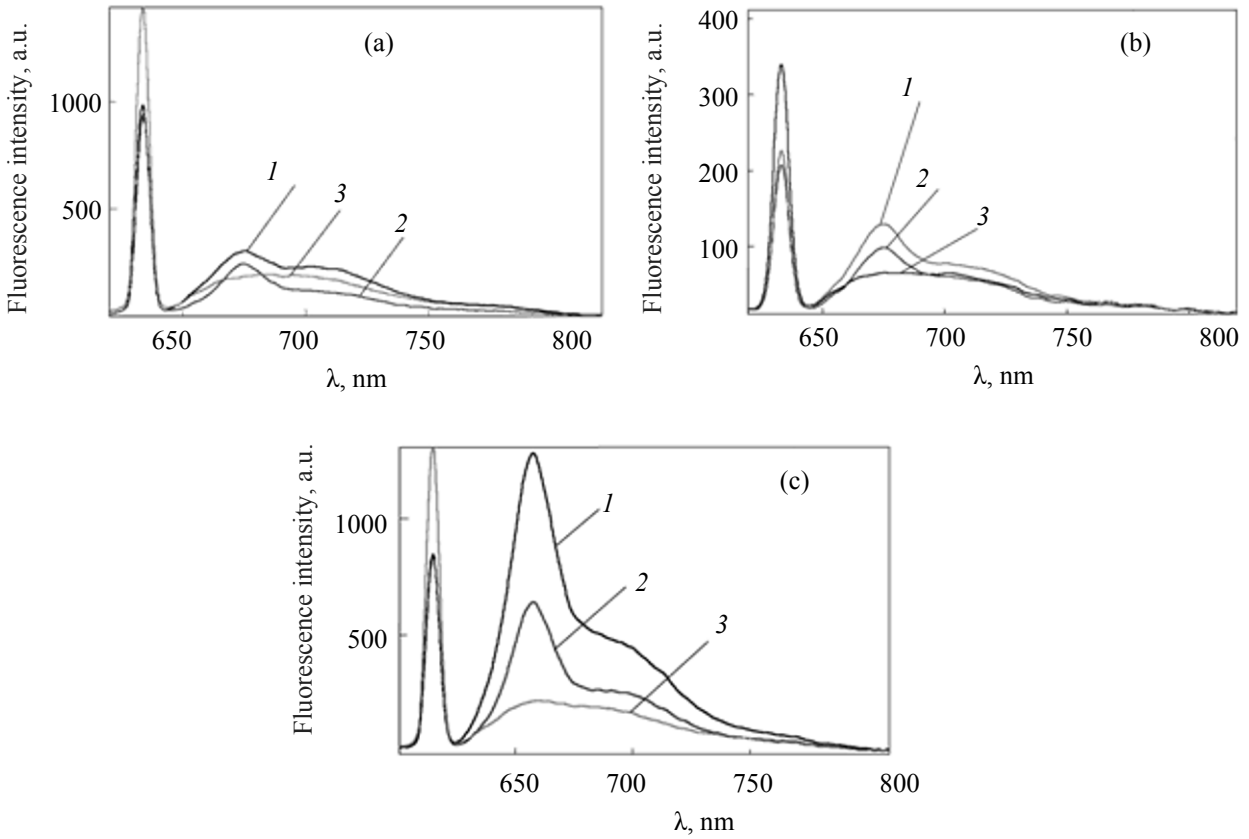

Fig. 2. Autofluorescence spectra of (*1*) tumor and (*2*) normal tissue (a) before introduction of *n*Fe_2O_3@ZnPc nanoparticles and (b, c) within 2 and 24 h after introduction, respectively. (*3*) Autofluorescence spectrum of healthy skin of the human hand.

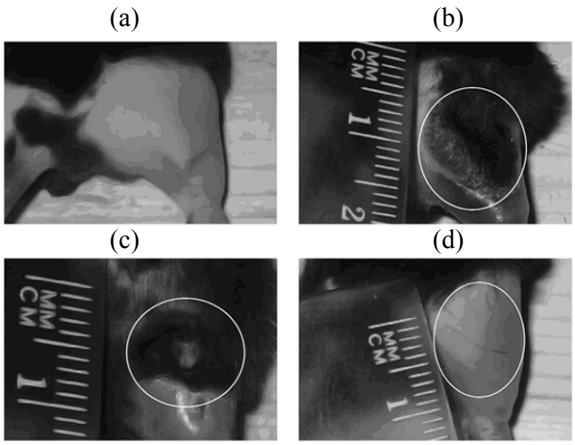

Fig. 3. The photograph of the tumor (right paw of the animal). (a) 1 day after introduction of the nanoparticles directly prior to irradiation; (b) 5 days after irradiation (pulse mode); (c) 5 days after irradiation (cw mode); (d) 5 days after irradiation (pulse mode) of the control group [iron(III) oxide nanoparticles without ZnPc coating were introduced]. The irradiation region is designated by white oval.

The control group consisted of animals subjected to irradiation before or after the Fe_2O_3 nanoparticles not covered with ZnPc were introduced.

Figure 2 demonstrates fluorescence spectra of the tumor tissue and normal muscle tissue. The spectra were measured in the absence of nanoparticles and after 2 and 24 h elapsed after introducing nanoparticles. Peak at a wavelength of 632.8 nm corresponds to the diffusely reflected laser radiation and a broad peak in the range 645–800 nm, to fluorescence.

As can be seen from these spectra, contrary to ZnPc molecular forms, ZnPc $n$$Fe_2O_3$@ZnPc nanoparticles do not increase the fluorescence intensity of the tumor and normal tissues. As known [1–3], in the presence of water-soluble ZnPc derivatives, the fluorescence intensity of the tumor tissue relative to the intensity of the intrinsic fluorescence of a biological tissue is increased significantly (by 2–3 orders of magnitude).

Figure 3 demonstrates photographs of the irradiated tumors. The irradiation of tumors in the presence of $n$$Fe_2O_3$@ZnPc nanoparticles causes strong photodynamic effect, which is not observed in the presence of Fe_2O_3 nanoparticles not covered with ZnPc and in the absence of nanoparticles.

The increase in the probability of transition of the surface ZnPc molecules to the triplet state owing to the distortion of the electric field, which occurs from the interaction of the ZnPc molecule with the Fe_2O_3 nanoparticle (increase in the intersystem crossing probability) and the resonance energy transfer onto phonons of the Fe_2O_3 nanoparticle or onto phonons formed due to the change in the bond length in the ZnPc molecules upon their interaction with the Fe_2O_3 nanoparticle may be responsible for the fact that, compared to molecular hydrophilic ZnPc forms, the fluorescence intensity of the $n$$Fe_2O_3$@ZnPc nanoparticles is low.

Strong photodynamic effect exerted by irradiation of biotissue supports the first mechanism.

To conclude, $n$$Fe_2O_3$@ZnPc nanoparticles can be considered as an effective agent for photodynamic therapy. Unusually strong photodynamic effect, resulting in a significant tumor necrosis at the considerably decreased fluorescence of ZnPc molecules, requires detailed experimental study. The study of the mechanisms of interaction of $n$$Fe_2O_3$@ZnPc particles with optical radiation in the presence of biotissues is underway.

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