ANNOTATION

Dissertation for the degree of Doctor of Philosophy (PhD)

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Physico-chemical properties and quantum-chemical calculations of photosensitizers used in photodynamic therapy

Relevance of the research topic. PS used in PDT can change its physical and chemical properties depending on the environment after introduction into the human body. That is, it shows a variety of properties depending on the nature of the medium, temperature, viscosity, concentration, and pH. Therefore, one of the most pressing issues is the study of the physical and chemical properties of PS and the identification of effective conditions for their use in the treatment of PDT.

The transport of medicinal substances in the biological system is determined by lipophilicity. The ability to form a complex between the PS and the receptor is determined by this indicator,. Absorption, distribution, metabolism and excretion of drugs from the body, toxicity - they all relate to physicochemical, pharmacokinetic and pharmacodynamic properties. In order to fully understand these listed properties, it is important to determine lipophilicity.

It was noted that the basis of treatment by photodynamic therapy is the transition of PS to a triplet state, the formation of reactive oxygen species, the absorption and distribution of PS into the cell. In addition to experimental results, the study of these properties with quantum chemistry calculations provides access to important information. That is, the results of obtaining Spectra by studying the excited state of PS, designing structures, monitoring the distribution of drugs in the body, calculating the intensity and energy of electronic transitions allow for additional opportunities in photochemistry and photobiology.

Although photodynamic therapy has been used since the late 1970s, it began to arrive in our country in 2016. PDT was officially used for the first time in November 2016 in the walls of the Medical Center of the Office of the President of the Republic of Kazakhstan.

Currently, PDT is widely used in the field of oncology, as well as in other fields, such as gynecology, urology, and ophthalmology. Therefore, it is relevant to study the physical and chemical properties of drugs used in treatment, determine the effective parameters of their use by calculating them by experimental and quantum chemistry methods.

The purpose of the research is to study the physico-chemical properties of photosensitizers used in photodynamic therapy by experimental and quantum chemistry methods.

The objectives of the research:

To achieve this goal, the following tasks were set:

- to study of the physicochemical properties of photosensitizers in various solvents, at various viscosities, concentrations, temperatures and pH of the medium;

- selection and justification of effective parameters for determining the separation and distribution factor by spectrophotometric, chromatographic and quantum chemical methods;

- obtain the optical and vibrational spectra of photosensitizers, calculate the intensities and energies of electronic transitions using the quantum chemistry method

Research methods. The following modern physico-chemical and quantum-chemical research methods were used in the research work: UV-vis spectroscopy, IR spectroscopy, fluorescence, phosphorescence, time-resolved fluorescence, high-performance liquid chromatography (HPLC), density functional theory (DFT).

Scientific novelty of the research work and main results:

- The physicochemical properties of photosensitizers of the I and II generations were studied by UV-vis, IR, fluorescence, phosphorescence, and fluorescence with time resolution. Although the change in the polarity of the medium did not significantly change the UV-visible absorption spectrum of FF, a sharp change was observed for PpIX and PF. It is known that PpIX should be excited at a wavelength of 630 nm in vitro or in vivo. However, the wavelength of this excitation was chosen based on the absorption spectrum in ethanol. The QI band was located at 641 nm near the physiological medium in the phosphate buffer solution (PBS) and in the fetal bovine serum (FBS). Among the three PSs, PPa showed the highest value of Φ_f, i.e. 0.39 in toluene and EtOH. The value of τ_f for PPa was between 6.1-7.5 ns for monomers and 0.3-2.1 ns for aggregates, the value of τ_f for PpIX was between 10.3-15.9 ns for monomers and 2.5-3.0 ns for aggregates and for FF was between 8.7-15.0 ns for monomers and 2.2-3.4 ns for aggregates. The highest value of Φ_Δ was determined in ethanol, that is, for PPa, PpIX and FF, it was 0.53, 0.92 and 0.80, respectively. The value τ_Δ indicated an approximate value in each solvent for all PSs and was approximate to the values given in the literature. The local viscosity may vary depending on the location of the FS in the cells. Although the change in the viscosity of the solvent did not have a strong effect on the maximum wavelength of the PpIX and FF QI bands, it was noted for PF that the band was shifted to the short-wavelength zone of 10 nm (from 678 nm to 668 nm). The fluorescence of the three PSs decreased with the addition of water, proving that the non-irradiated decrease is low when the viscosity is high. Unfortunately, the relationship between the viscosity of the medium and the monomer/aggregate fractions has not been established. The reason for this is that when different amounts of glycerin and water are mixed, the polarity of the medium can also change. Therefore, the changes observed in W/G mixtures may not only be related to

the viscosity of the solution. The change in concentration showed a pattern of decreasing and increasing fluorescence for FS. That is, if the intensity of fluorescence decreased in the PBS, and aggregation was high, then the opposite phenomenon was observed in the FBS. This means that it can be seen that the protein molecules present in the FBS reduce aggregation. Although the temperature change did not show significant changes in the absorption spectra of PpIX and FF, but the UV-vis spectrum underwent significant changes in the range of 10-40°C for PPa. Therefore, when using photosensitizers, the most effective temperature is below 40°C. For PPa in PBS, the intensity of the Soret band decreased and the shape of the QI band changed, shifting from 680 nm to 712 nm in the red zone, where the intersection (isobestic) point is 685 nm. The intersection point registered in the FBS also showed 685 nm. Also, an increase in temperature leads to a decrease in the aggregate/monomer ratio. The pH change also showed that for PPa, the QI band shifts by a short region of 25 nm (from 704 nm to 679 nm). As the most surprising result, we can talk about the values of Φ_{Δ} obtained in various solvents. The values obtained depending on the solvent were also different. Although it is difficult to determine $^1O_2$ in toluene for FF, the value of Φ_{Δ} in this solvent was 0.68 and 0.49, respectively, for PpIX and PPa. In ethanol, the ΦΔ value was 0.92, 0.53, and 0.80 for PpIX, PFa, and FF, respectively. When using D_2O as a solvent, $^1O_2$ for PpIX and PPa was not determined and was equal to 0.15 for FF. In real conditions, the presence of proteins, lipids and other biomolecules affects the physicochemical properties of FS. This means that the use of the solvent should be monitored for in vitro studies;

- For the first time, separation and distribution coefficients were determined by spectrophotometry, chromatography, and quantum chemistry. As a result of the studies carried out, it was shown that the HPLC method for determining the partition coefficient seems to be highly accurate, and the execution time is fast. The obtained LogD values for PS obeyed the laws of lipophilicity of molecules and proved their high hydrophobicity in accordance with the values of PFa 0.30-0.44. The Log*D* value of PpIX according to the methods of flask shaking and HPLC showed positive and negative values, respectively, but these values were close to each other (0.19 and -0.49). Interestingly, three bands were formed for FF, as a result of which the values of three Log*D* were calculated, and this proved the amphiphilic nature of this PS. Two calculated values showed the polar properties of FF, and one value-nonpolar (-2.73, -1.23 and 0.15, respectively). The fact that LogP values could be predicted by various methods of theoretical chemistry showed that this was an excellent chance. It was found that this approach plays a big role when there is no sample of the PS compound required for the study.. In other words, the density functional theory (DFT) with hybrid (B3LYP) and long-dimensional (ωB97X-D) functionals was defined as an important tool for calculating the lipophilicity of FS. Solvent models based on the solvation model based on density (SMD), the conductor-like polarization continuum model (C-PCM) and integral equation formalism polarizable continuum model (IEF-PCM) were used to determine the Log*P* values of PpIX and PPa molecules using 6-31G(d), 6-31+G(d,p)

and 6-311++G(d,p) basis sets. The complexity of the FF structure did not allow calculating the Log*P* value of this PS. According to the SMD in the 6-31+G(d,p) and 6-311++G(d,p) basis sets, it was shown that Log*P* values determined using the functional ωB97X-D can be better calculated;

- Optical and vibrational spectra of photosensitizers were obtained by quantum chemistry, the intensity and energy of electronic transitions were calculated. By the method of quantum chemistry, it is possible to obtain optical and vibrational spectra of PpIX and PPa photosensitizers in toluene and water and calculate the intensity and energy of electronic transitions. The calculation of the UV-visible and IR spectra of PPa and PpIX using the solvent models of SMD, C-PCM on two theories of ωB97X-D, B3LYP and the basic set of 6-31+G(d,p) allowed us to obtain values very close to the experimental result. Experimentally, sore and four Q bands were obtained, and as a result of the calculation, an intense sore and two low-intensity Q_x and Q_y bands were obtained. B3LYP compared to functional ωB97X-D showed that the Spectra found as a result were similar to experimental Spectra. That is, the energy levels of orbitals calculated for Gouterman's four-orbital model had shown a higher value when using the ωB97X-D functional. Therefore, even in the calculation of electronic spectra, it has been found that functional is of great importance. The intensity of the bands obtained as a result of the experiment and calculation showed a direct trend. That is, the Soret band showed a high intensity both experimentally and computed way. Due to the large differences in energy between the homo, homo-1 and lumo, lumo+1 orbitals of the PPa molecule, the spectra obtained by calculation were obtained with high accuracy. In addition, the total IR spectrum of the PPa molecule calculated in vacuum and water according to the SMD and C-PCM solvent model at the B3LYP theory level and the basis set of 6-31+G(d, p) showed a result close to experimental results in comparison with the spectra obtained according to the ωB97X-D theory. Later it was proved that this method plays an important role in the study of the electronic and optical properties of synthesized new PS.

Practical significance of the work. The obtained results on the study of the physicochemical properties of drugs used in the treatment of tumor diseases revealed the high practical significance of determining the optimal conditions for their use. In addition, the main proposed effective parameters for determining lipophilicity and the technique of quantum chemical calculations open up great opportunities for use in PDT in the future.

The main provisions submitted for defense:

1. Significant shift changes were recorded in the absorption bands of PpIX and PPa. So far, *in vitro* or *in vivo* studies with PpIX have shown that the excitation wavelength was 630 nm, while in phosphate buffer solution, which is similar to the physiological environment, and fetal bovine serum solvents, the QI band is at a wavelength of 641 nm. In addition, the effect of viscosity, temperature, and pH is great for PPa molecules. For the mentioned photosensitizers, the ability to form singlet oxygen in an aquatic environment is determined with singlet oxygen sensor green to

detect singlet oxygen, and this sensor allows to determine the quantum yield and lifetime of singlet oxygen in different solvents.

2. As a result of the conducted research, the HPLC method for determining the distribution coefficient is recommended as a method with high accuracy and fast execution time. The partition coefficient for PpIX and PPa molecules using the DFT method is calculated for the first time in solvent models such as the solvation model based on density (SMD), the conductor-like polarization continuum model (C-PCM) and integral equation formalism polarizable continuum model (IEF-PCM) and shows a value close to the results obtained experimentally. As a result of HPLC, PF has three lipophilic values and two of them are hydrophilic and one is hydrophobic, i.e. amphiphilic PS. Also, for the first time in determining this parameter, the use of DFT as a quantum chemistry method for PpIX and PPa allows to get results with higher accuracy than other methods.

3. By the method of quantum chemistry, it is possible to obtain the optical and vibrational spectra of photosensitizers PpIX and PPa in toluene and water and calculate the intensity and energies of electronic transitions. Also, as a result of the analysis of optical spectra obtained in the SMD and C-PCM solvent models, the C-PCM solvent model shows a result that is close to the experimental results.

The relationship of the topic of the dissertation work with research work and various state programs. Part of the dissertation work was carried out at the University of Lorraine under the International Program Erasmus+, created on the initiative of the European Union and with progressive funding, aimed at improving the quality of higher education for students on academic mobility.

Personal contribution of the doctoral student to the preparation of each publication:

1. Larue L., Myrzakhmetov B., Ben-Mihoub A., Moussaron A., Thomas N., Arnoux P., Baros F., Vanderesse R., Acherar S., Frochot C. Fighting Hypoxia to Improve PDT. *Pharmaceuticals* (**Q1, IF=5.4**) 2019; 12:163;
2. Myrzakhmetov B., Arnoux P., Mordon S., Acherar S., Tsoy I., Frochot C. Photophysical Properties of Protoporphyrin IX, Pyropheophorbide-a, and Photofrin® in Different Conditions. *Pharmaceuticals* (**Q1, IF=5.2**) 2021; 14:138;
3. Myrzakhmetov, B., Honorien, J., Arnoux, P., Fournet, R., Tsoy, I., Frochot, C., *Luminescence* (**Q2, IF=3.7**) 2022, 37, 1597.

The results of the study also passed the clinical approbation in the laboratory of ONCOTHAI "Laser and Immunotherapy in Oncology", the French University Hospital of Lille and the National Institute of Health and Medical Research.

The doctoral student analyzed publications in the field of research and wrote his part in a review article (Fighting Hypoxia to Improve PDT). The articles (Photophysical Properties of Protoporphyrin IX, Pyropheophorbide-a, and Photofrin® in Different Conditions and Lipophilicity prediction of three photosensitizers by liquid–liquid extraction, HPLC, and DFT methods) present the results of the experiments, as well as

the analysis of the data obtained and the formulation of the conclusions made by the dissertator personally.

Currently, in connection with the obtained promising results on quantum chemical calculations, work is underway to design the article in accordance with the requirements of the journal included in the Q1 quartile.