Investigation of the Changes in Extinction Spectrum of Modern Chlorine-Containing Photosensitizing Drugs under the Visible Light Action

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Abstract. The paper presents the results of a study of the extinction spectra (350–900 nm) of aqueous solutions of modern chlorine-containing photosensitizing drugs for photodynamic therapy “Chloderm" (Chloderm, Russia) and "Chloderm with hyaluronic acid" (Chloderm, Russia) before and after irradiation by visible light with wavelengths of 405 nm, 450 nm, and 656±10 nm, with exposure time 0–20 min and intensity 0–200 mW/cm^2. It is demonstrated that the addition of hyaluronic acid does not deform the shape of the extinction spectrum of the photosensitizing drug but reduces its absorption in proportion to the drop in the concentration of the photosensitizer in the drug. Photodynamic light action in the investigated range of parameters leads to a slight decrease in the extinction coefficient of both drugs at the wavelengths of the exposure, but significantly reduces extinction and deforms the Qy 00 absorption band (600–700 nm), thereby changing the ratio of monomers and tetramers in the drugs. This band is most significantly deformed after exposure to light with a wavelength of 656±10 nm, the least – with a wavelength of 450 nm.

Keywords: photodynamic therapy; light; extinction coefficient; chlorine e6; conformational state; transformation coefficient; monomers; tetramers.

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1 Introduction

Photodynamic therapy (PDT) is a modern effective treatment method, which is widely used in oncology, dentistry, dermatology, etc. [1–6]. The effectiveness of PDT depends on the choice of photosensitizer and light source. The photosensitizer penetrates into the biological tissue and, as a result of light absorption, passes into an excited singlet state, which can pass into an excited triplet state and/or relax with the emission of energy in the form of fluorescence, heat and/or other forms of photophysical energy. The excited triplet state promotes the formation of reactive oxygen species as a result of the participation of the photosensitizer in electron transfer reactions with the formation of radicals and radical ions (type I reaction) or as a result of energy transfer to molecular oxygen ($^3O_2$) with the formation of singlet oxygen ($^1O_2$) (type II reaction) [7–9].

Photosensitizers can be classified by generation. Disadvantages of first-generation photosensitizers based on hematoporphyrin (activation by light with wavelengths that do not penetrate deeply into biological tissues, complex synthesis, insolubility in water, etc.) stimulated the development of second-generation photosensitizers that are activated by visible and near-IR light, which penetrates deep into biological tissues, and have a high yield of singlet oxygen [8, 9–12]. Among the second-generation photosensitizers, the most common is chlorin e6 (Ce6), which is part of most modern photosensitizing drugs [13].

PDT uses laser sources (semiconductor lasers, metal vapor lasers, argon-pumped dye lasers, etc.) or broadband light from non-laser sources (fluorescent and xenon lamps, LEDs, etc.) [8, 14]. The choice of light source depends on the properties of the photosensitizer,

the depth of localization and the size of the object of treatment, the therapeutic dose of light, etc. [8, 14].

Chlorine e6 is a powerful photosensitizer with an absorption band in the red region of the spectrum. The absorption spectrum of Ce6 is characterized by the presence of several bands. The most intense is the B-band (Soret band) with a peak at a wavelength of about 400 nm. It is quite wide and extends from 320 to 480 nm along the base. The Qx 00 and Qx 01 bands (about 505–510 nm) are known, as well as the Qy 00 band with a peak at a wavelength of about 664 nm [15]. The shape of the Qy 00 band depends on the ratio of monomers and tetramers in the photosensitizer solution and can be significantly deformed when this ratio changes. It is known that monomers are characterized by a band with a peak at a wavelength of 664 ± 10 nm, and for tetramers, at a wavelength of 710 ± 10 nm [15, 16]. Peaks may slightly shift depending on the medium in which chlorin e6 is located. The ratio of absorption (extinction) coefficients at these wavelengths is called the spectral transformation coefficient and is used to describe the conformational state of a chlorine-containing photosensitizing drug, which in turn affects its photodynamic efficiency [15, 16].

In photodynamic therapy with the use of chlorine-containing photosensitizing drugs, light sources are mainly used whose wavelength align with the peak of the Qy 00 band, which is due to the fact that light with this wavelength penetrates deeply into biological tissues. For such an impact, the radiation of LEDs with a wavelength of 656 ± 10 nm is well suited [17]. At the same time, this radiation is not absorbed by the photosensitizer as efficiently as radiation aligning with the peak of the B absorption band. For such an impact, laser radiation with a wavelength of 405 nm can be used. It should be noted that the use of radiation with a wavelength aligning with the peak of the B absorption band is limited by the small depth of penetration of this radiation into the biological tissue [18]. Therefore, the use of radiation with a wavelength that aligning with the B absorption band, but does not correspond to its absorption peak, can make it possible to select the conditions, under which light will penetrate deeper into the biological tissue, but the absorption of light by the photosensitizer will be higher or at the same level as when exposed to light with a wavelength close to the peak of the Qy 00 band. This condition is satisfied by laser radiation with a wavelength of 450 nm [17]. It should be noted that exposure outside the peak of the absorption band (non-resonant exposure) can contribute to a decrease in the extinction coefficient of the photosensitizing drug at the excitation wavelength caused by its photodestruction during the exposure time, but to a lesser extent than exposure at the peak of the absorption band (resonance exposure). In addition, under non-resonant laser exposure, in contrast to resonant exposure, not only the process of photodestruction of the photosensitizer can occur, but also its heating and change in acidity, which affects the conformational state of the photodynamic agent [15]. This can ultimately compensate for the lower initial photodynamic efficiency of the non-resonant exposure. Thus, to excite chlorine-containing photosensitizing drugs, it is possible to use radiation of the visible range of the electromagnetic spectrum with wavelengths of 405 nm or 656 ± 10 nm (resonant exposure) and 450 nm (non-resonant exposure).

Photodynamic therapy can be used in dermatology in the treatment of fungal infections and skin rejuvenation [19–23]. In this case, the modern chlorine-containing photosensitizing drug Chloderm (Chloderm, Russia) is widely used [24]. Recently, to expand the range of applications, the use of a mixture of this drug with traditional dermatological agents, for example, a mixture with hyaluronic acid, has become relevant. Hyaluronic acid plays an important role in the regulation of various biological processes, such as skin repair, wound healing, tissue regeneration, and is also an anti-inflammatory and cosmetic agent [25]. Compositions based on hyaluronic acid are used against wrinkles, nasolabial folds, aging, etc. [26]. These effects are achieved by expanding and enlarging soft tissues, improving skin hydration, and stimulating collagen and elastin [27]. Thus, the addition of hyaluronic acid to "Chloderm" or its aqueous solution can lead to additional positive effects in photodynamic skin rejuvenation.

The effect of radiation with wavelengths of 656 ± 10 nm and 450 nm on chlorine-containing photosensitizing preparations, including Chloderm, has been studied in detail in Refs. [16–17]. Usually, PDT in dermatology uses light with an intensity of 0–200 mW/cm^2, and the exposure time does not exceed 20 min [28 29]. At the same time, data on the influence of exposure time and intensity of radiation with a wavelength of 405 nm on the extinction spectra and spectral transformation coefficients of "Chloderm" are not available in the literature. In addition, the extinction spectrum of the mixture "Chloderm with hyaluronic acid" (Chloderm, Russia) and its change depending on the concentration of the drug in water and when exposed to radiation with wavelengths of 405 nm, 450 nm and 656 ± 10 nm, have not been studied despite the importance of these data for the dosimetry of the process of photodynamic therapy. All of the above leads to a conclusion about the relevance of investigation of the changes in absorption spectrum modern chlorine-containing photosensitizing drugs under the visible light action.

The purpose of this study is to investigate the effect of concentration of chlorine-containing photosensitizing drugs "Chloderm" and "Chloderm with hyaluronic acid" (Chloderm, Russia) in an aqueous solution, as well as light exposure time and intensity of 405 nm, 450 nm and 656 ± 10 nm radiation on the extinction spectra and spectral transformation coefficients of these drugs.

2 Materials and Methods

Modern chlorine-containing photosensitizing preparations "Chloderm" (Chloderm, Russia) and "Chloderm with hyaluronic acid" (Chloderm, Russia) and their aqueous solutions were used in the study. The

mass concentration (C) of both drugs in water varied from 0.01% to 100%. The range of studied concentrations is wide, since during PDT the concentration of the drug can change as it penetrates into the biological tissue. In this regard, for adequate planning of the PDT procedure, it is important to have as complete information as possible about the dynamics of the extinction spectrum of the drug as its concentration changes and the associated changes in its conformational state.

For resonant photodynamic light action, a semiconductor InGaN laser (Xinrui technology, China) with a wavelength of 405 nm and an average power of up to 1.0 W and an LED source "LED Forester 660" (LLC "NELA", Russia) with a wavelength of 656 ± 10 nm and a power of 20 W were used. For non-resonant photodynamic light action, a semiconductor InGaN laser (Xinrui technology, China) with a wavelength of 450 nm and an average power of up to 2.0 W was used. The intensity of exposure varied in the range from 0 to 200 mW/cm^2 with a step of 50 mW/cm^2. For each intensity value, the exposure time was 0, 1, 5, 10, and 20 min.

In the experiment, the transmission spectra of samples of aqueous solutions of drugs were recorded before and after resonant or non-resonant photodynamic light action. The transmission spectra were recorded using a "T90" (PG Instruments Ltd, UK) double-beam spectrophotometer operating in the wavelength range from 200 nm to 900 nm. The studied drugs and their aqueous solutions were placed in a quartz cuvette with dimensions of 10×10×45 mm (WxDxH). A quartz cuvette with distilled water, identical in size, was placed in the reference arm of the spectrometer. Each measurement was carried out with a step of 1 nm and lasted about 3 min. The cuvette material poorly transmitted radiation in the range of 200–350 nm, which made it possible to record the transmission spectra of the samples only in the range of 350–900 nm. For each sample with different drug concentrations in water, ten measurements of the transmission spectrum were performed. According to the obtained transmission spectra, in accordance with the Beer-Lambert-Bouguer law, the extinction spectrum of the drug or its aqueous solution was calculated.

As a result of the analysis of the extinction spectra of the samples before and after photodynamic light action, the dependences of the extinction coefficients of the samples at the excitation wavelengths (405 nm, 450 nm and 656 ± 10 nm) and at the wavelengths corresponding to the recorded in the experiment peaks of the absorption bands of the monomers (672 nm) and tetramers (703 nm) on the time and intensity of photodynamic light exposure were determined.

Also, in order to assess the conformational state of the photosensitizing drug before and after photodynamic light action, the coefficient of spectral transformation (k_t) was calculated equal to

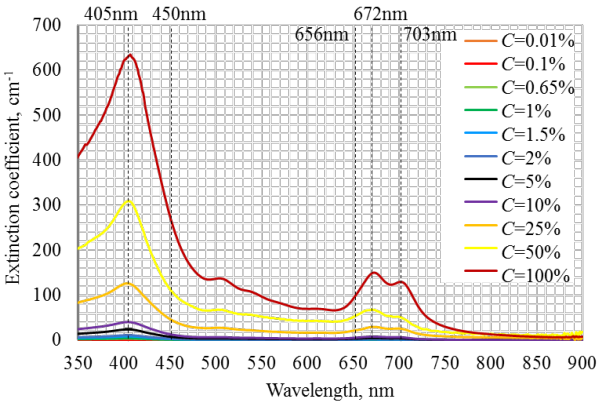
$$k_t = \frac{k_{672}}{k_{703}}, \quad (1)$$

where k_{672} – extinction coefficient of a photosensitizing drug at a wavelength of 672 nm, k_{703} – extinction coefficient of a photosensitizing drug at a wavelength of 703 nm.

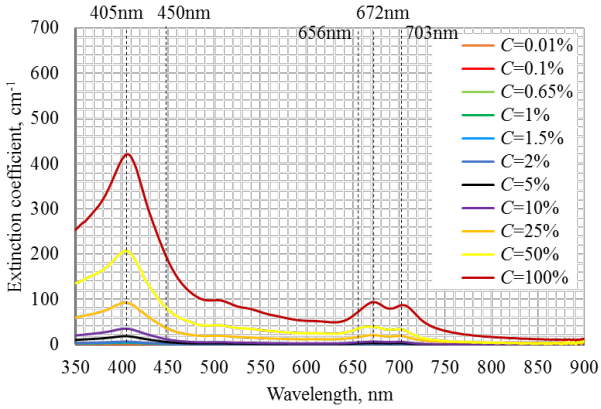
For statistical processing of the obtained data, the "StatGraphics Plus" (Statgraphics Technologies, Inc., USA) software package was used; as a result of processing, the average value and confidence interval of the extinction coefficient and spectral transformation coefficient were calculated for each value of the wavelength.

3 Results and Discussion

Extinction spectra of chlorine-containing photosensitizing preparations "Chloderm" and "Chloderm with hyaluronic acid" at different concentrations in an aqueous solution are shown in Fig. 1.



(a)



(b)

Fig. 1 Typical extinction spectra of chlorine-containing photosensitizing drugs "Chloderm" (a) and "Chloderm with hyaluronic acid" (b) at different concentrations (C) in aqueous solution.

It can be seen that the spectra of "Chloderm" and "Chloderm with hyaluronic acid" are similar to each other. Both drugs have pronounced absorption bands with peaks at wavelengths of 405 nm (B-band), 672 nm and 703 nm (Qy 00 bands).

The dependences of the extinction coefficients of chlorine-containing photosensitizing drugs “Chloderm” and “Chloderm with hyaluronic acid” at wavelengths of 405 nm, 450 nm, 656 nm, 672 nm, 703 nm on their concentration in an aqueous solution are shown in Fig. 2.

It can be seen that the addition of hyaluronic acid does not deform the shape of the extinction spectrum of the photosensitizing drug but reduces its absorption in the entire studied spectral range. The decrease in absorption is proportional to the increase in the proportion of hyaluronic acid relative to the amount of “Chloderm” in “Chloderm with hyaluronic acid”. In this case, assuming complete transparency of hyaluronic acid at the studied wavelengths, its concentration should be 42%, which satisfactorily matches the manufacturer’s data.

It can be seen that for both drugs, as their concentration in an aqueous solution increases, the extinction coefficients at the studied wavelengths increase linearly, which allows us to conclude that there are no effects of concentration deformation of the spectrum in the entire range of studied concentrations. Therefore, the choice of concentration for further studies should not affect the initial conformational state of the drug. In this regard, experiments to study the influence of the exposure time and the intensity of the photodynamic effect on the extinction spectra and the spectral transformation coefficient were carried out only at one concentration of the drug in an aqueous solution of $C = 5\%$.

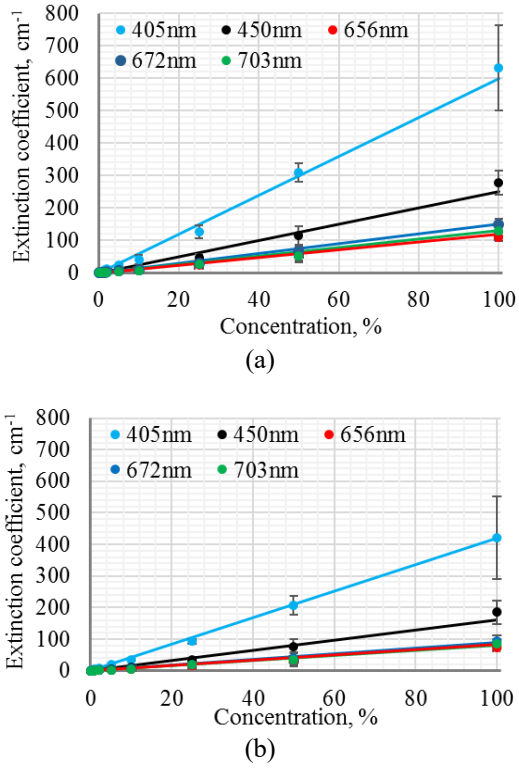


Fig. 2 Dependences of extinction coefficients of chlorine-containing photosensitizing drugs “Chloderm” (a) and “Chloderm with hyaluronic acid” (b) at wavelengths of 405 nm, 450 nm, 656 nm, 672 nm, 703 nm on their concentration in aqueous solution.

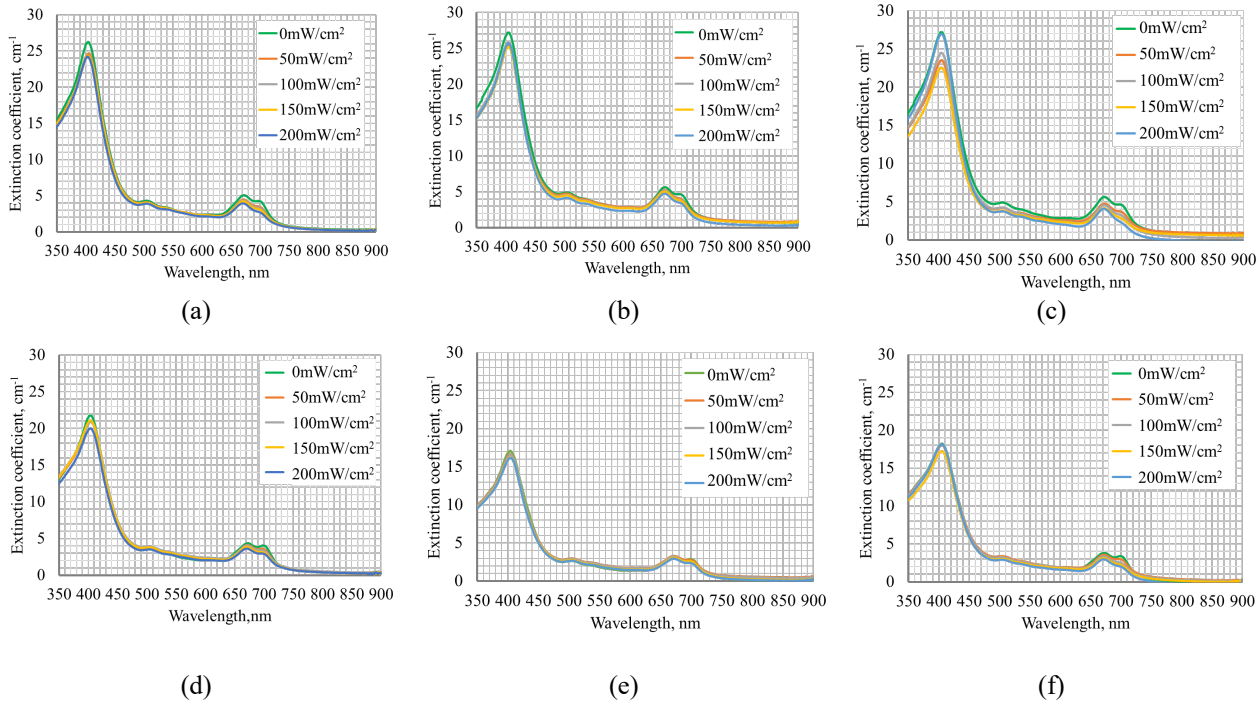


Fig. 3 Typical extinction spectra of chlorine-containing photosensitizing drugs “Chloderm” (a, b, c) and “Chloderm with hyaluronic acid” (d, e, f) with $C = 5\%$ after photodynamic light exposure with wavelengths of 405 nm, 450 nm and 656 ± 10 nm, respectively, at a constant exposure time (20 min) and with different intensities (0–200 mW/cm^2).

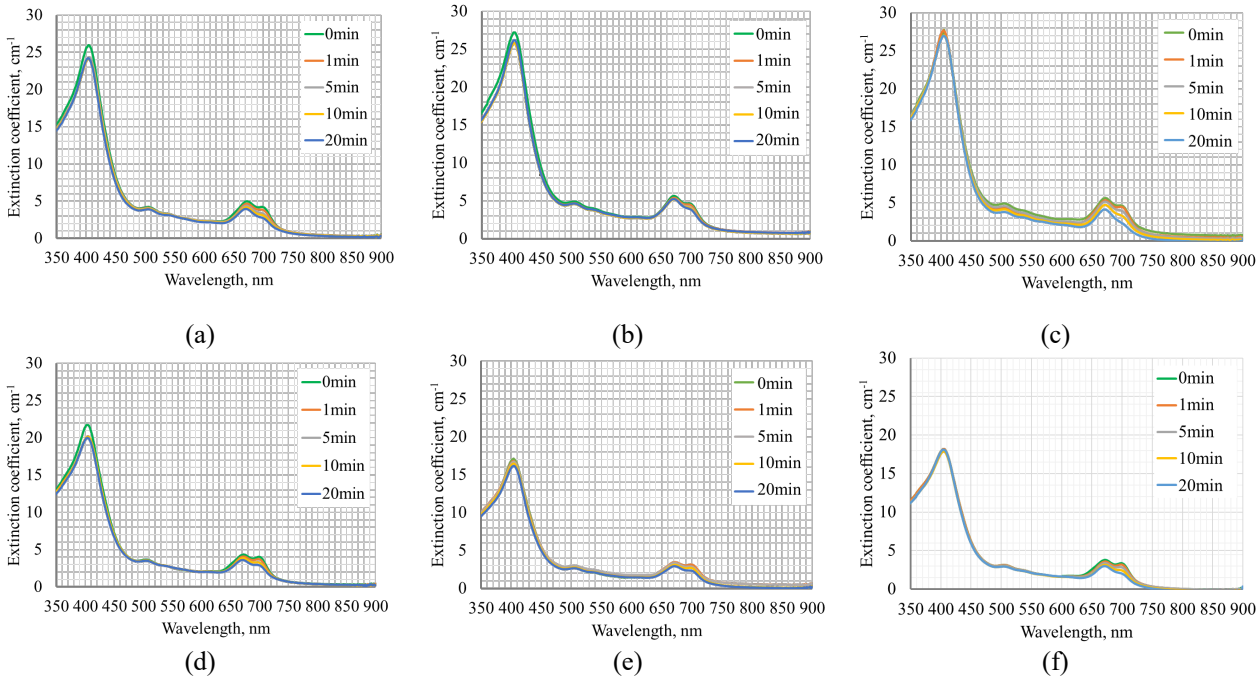


Fig. 4 Typical extinction spectra of chlorine-containing photosensitizing drugs “Chloderm” (a, b, c) and “Chloderm with hyaluronic acid” (d, e, f) with $C = 5\%$ after photodynamic light exposure with wavelengths of 405 nm, 450 nm and 656 ± 10 nm, respectively, at a constant intensity (200 mW/cm^2) and with different exposure times (0–20 min).

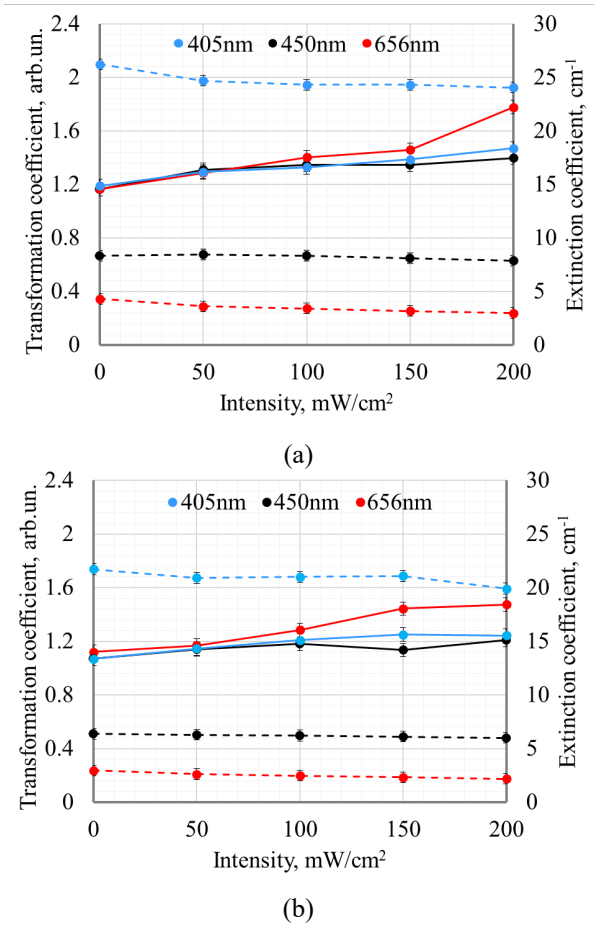


Fig. 5 Dependence of the spectral transformation coefficient (solid line) and the extinction coefficient at the wavelength of photodynamic light action (dotted line) of “Chloderm” (a) and “Chloderm with hyaluronic acid” (b) with $C = 5\%$ on the intensity of photodynamic light action with wavelengths 405 nm, 450 nm and 656 ± 10 nm at constant exposure time of 20 min.

Extinction spectra of chlorine-containing photosensitizing drugs “Chloderm” and “Chloderm with hyaluronic acid” with $C = 5\%$ after photodynamic light exposure with wavelengths of 405 nm, 450 nm and 656 ± 10 nm at a constant exposure time (20 min) and with different intensity (0–200 mW/cm^2) are presented in Fig. 3, and at constant intensity (200 mW/cm^2) and with different exposure times (0–20 min) – in Fig. 4.

It can be seen that photodynamic light action at any of the three studied wavelengths did not lead to a significant change in the extinction coefficient at wavelengths of 405 nm and 450 nm, which lie within the B absorption band of the drug. The main changes in the spectra are observed at wavelengths of 656 nm, 672 nm, and 703 nm lying within the Qy 00 absorption band of the drug. It can also be noted that the peaks of the absorption bands at wavelengths of 672 nm and 703 nm do not shift. It can be seen that after photodynamic light action, the extinction coefficients of both photosensitizing drugs at Qy 00 absorption band peaks are decreased which may be due to the photobleaching of Ce6. It should be expected that as a result of this photobleaching, the photodynamic efficiency of the drugs decreases. The strongest decrease of extinction coefficients at Qy 00 absorption band peaks was observed after exposure to radiation with a wavelength of 656 ± 10 nm.

Dependences of the spectral transformation coefficient and the extinction coefficient at the

wavelength of the photodynamic light action of "Chloderm" (a) and "Chloderm with hyaluronic acid" (b) with C = 5% on the light intensity (0–200 mW/cm^2) at a constant time of photodynamic light action (20 min) with wavelengths of 405 nm, 450 nm and 656 ± 10 nm are shown in Fig. 5, and on time (0–20 min) at a constant intensity (200 mW/cm^2) – in Fig. 6.

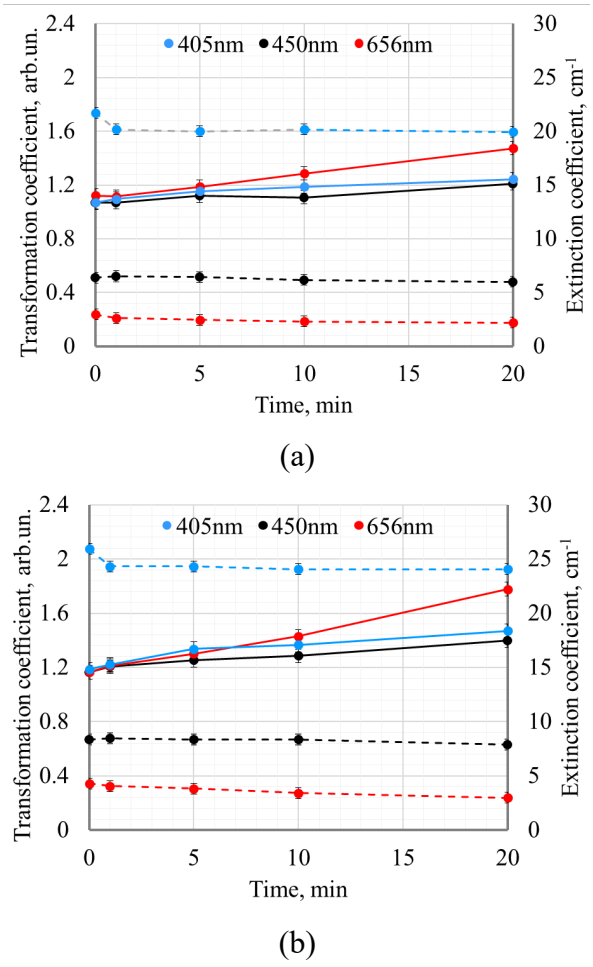


Fig. 6 Dependence of the spectral transformation coefficient (solid line) and the extinction coefficient at the wavelength of photodynamic light action (dotted line) of "Chloderm" (a) and "Chloderm with hyaluronic acid" (b) with C = 5% on the time of photodynamic light action with wavelengths 405 nm, 450 nm and 656 ± 10 nm at a constant intensity of 200 mW/cm^2.

It can be seen that after any type of photodynamic light action (non-resonant and resonant), the extinction coefficients of both photosensitizing drugs at the wavelength of photodynamic light exposure, although slightly, decrease. This may be due to the photobleaching of Ce6. The decrease occurs both with an increase in intensity and with an increase in the time of photodynamic light action. The most significant decrease is observed at a wavelength of 656 nm after resonant light exposure at a wavelength of 656 ± 10 nm, and the least significant at a wavelength of 450 nm after non-resonant light exposure at a wavelength of 450 nm.

For both drugs, the initial spectral transformation coefficient k_t >1. This suggests that the amount of monomers in the drug before photodynamic light exposure is higher than the amount of tetramers [15]. The spectral transformation coefficient of both drugs after any type of photodynamic light exposure (non-resonant and resonant) increases and, consequently, the amount of monomers increases. An increase in the amount of chlorin e6 monomers leads to an increase in the quantum yield of singlet oxygen and an increase in the efficiency of photodynamic therapy [15, 28–30]. An increase in k_t may be associated with a change in the temperature and pH of the drug as a result of light exposure [15, 28–33]. It was shown in Ref. [15] that the polymerization of chlorin e6 depends on pH. It is also known that the state of protonation of carboxyl groups of chlorin e6 depends on pH and, as a result, the state of intermolecular aggregation of chlorin molecules with each other may change [28–29]. It was shown in Refs. [31–32] that the effect of temperature leads to a change in the absorption spectra of the dyes under study, and an increase in temperature leads to an increase in the concentration of monomers due to the decomposition of dimers. It was noted in Ref. [34] that heating a chlorine-containing photodynamic preparation can change its conformational state and lead to an increase in the concentration of monomers. The initial and final values of the spectral transformation coefficient for "Chloderm" are higher than for "Chloderm with hyaluronic acid", which may be due to the difference in the initial concentration of chlorin e6 in the drugs. For both drugs, the increase in k_t occurs both with an increase in intensity and with an increase in the time of light action. The most significant increase in k_t is observed after resonant light action at a wavelength of 656 ± 10 nm, and the least significant after non-resonant light exposure at a wavelength of 450 nm.

4 Conclusion

The effect of the concentration of modern chlorine-containing photosensitizing drugs "Chloderm" and "Chloderm with hyaluronic acid" (Chloderm, Russia) in an aqueous solution, as well as the duration and intensity of non-resonant (with a wavelength of 450 nm) and resonant (with wavelengths of 405 nm or 656±10 nm) visible light action, on the extinction spectra of these drugs and spectral transformation coefficients was studied. It was established that a change in the concentration of drugs in an aqueous solution does not lead to a deformation of their extinction spectrum. The addition of hyaluronic acid does not deform the shape of the extinction spectrum of the photosensitizing drug but reduces its absorption in the entire studied spectral range (350–900 nm). The decrease in absorption is proportional to the increase in the proportion of hyaluronic acid relative to the amount of "Chloderm" in "Chloderm with hyaluronic acid". It was found that photodynamic light action does not lead to a significant change in the extinction coefficient within the B absorption band of the drugs, but significantly reduces extinction and deforms the Qy 00 band of their extinction spectrum. It is shown

that after photodynamic light action, the extinction coefficients of both photosensitizing drugs at the wavelength of photodynamic light exposure slightly decrease, while the spectral transformation coefficients increase. The extinction coefficient at the wavelength of photodynamic light action and the spectral transformation coefficient k_t change most significantly after resonant light exposure at a wavelength of 656 ± 10 nm, and the least significantly after non-resonant light exposure at a wavelength of 450 nm.

Disclosures

The authors declare no conflict of interest.

Acknowledgments

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