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Laser delivery and spectral study of a modern chlorine-containing drug for the treatment of onychomycosis at laser radiation with a wavelength of 405 nm

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In an *in vitro* experiment, laser microporation of the nail plate and active laser delivery of Chloderm, a modern chlorine-containing photosensitizing drug, under the nail plate by laser radiation with a wavelength of 405 nm for the purpose of photodynamic therapy of onychomycosis is studied. The rate and efficiency of the nail plate ablation, as well as the rate of delivery of the drug under the nail plate are evaluated. The maximum ablation rate was $2600 \pm 200\,\mu$m/s, and the ablation efficiency was $2.6 \pm 0.2\,\mu$m/mJ. The maximum drug delivery rate was 5.3 ± 0.5 mg/s at $P = 1.0$ W and an exposure time of $t = 0.3$ s. The processes arising under the action of laser radiation on Chloderm and its aqueous solutions have been discovered and described for the first time. The results of studying extinction spectra of an aqueous solution of Chloderm in the range of 350–900 nm before and after exposure to laser radiation are presented. It has been shown that exposure to laser radiation with a wavelength of 405 nm and parameters sufficient for active laser delivery of an aqueous solution of the Chloderm preparation ($C = 5\%$) under the nail plate does not change the conformational state of the preparation, and therefore does not worsen its photodynamic and luminescent properties.

Keywords: laser delivery, microporation, ablation, nail plate, efficiency, onychomycosis, chlorine-containing photosensitizing drug, extinction spectrum, wavelength.

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Introduction

Lasers are widely used in modern physics, technology and medicine, including dermatology for the treatment of fungal diseases. Onychomycosis is a common dermatological nail disease caused by a fungal infection [1,2]. Oral antifungals are widely used to treat onychomycosis. However, in this case, therapy takes a long time, and there is a risk of hepatotoxicity. Surgical treatments of onychomycosis are very painful [3]. Local pharmaceutical preparations are also widely used in the treatment of onychomycosis. The effectiveness of this treatment is limited because the nail plate prevents the delivery of local drugs to the nail bed affected by the fungus [4,5].

The permeability of the nail plate can be increased by laser microporation. With an exposure to laser radiation, as a result of the conversion of the absorbed laser energy into heat, the biological tissue is heated and ablated forming micropores through which the drug delivery is possible [6]. Laser radiation with a wavelength of 405 nm can be used for effective laser microporation of a healthy and onychomycosis-affected nail, as it is effectively absorbed by keratin of the nail plate, as well as by the melanin produced by melanocytes of the nail matrix due to melanonychia (nail hyperpigmentation) in onychomycosis, as well as by the pathogens of onychomycosis located in the nail plate [7]. However, the efficiency and rate of the nail plate ablation by laser radiation with a wavelength of 405 nm has not been studied so far.

The efficiency of local drugs delivery under the microporated nail plate can be increased to a limited extent by improving the composition of the drug and to a greater extent by using external energy to improve the penetration of drugs into the biotissue (active delivery) [8,9]. In particular, laser-induced hydrodynamic processes can increase the rate of drug penetration into biological tissues [10,11]. In this case, it is important to avoid undesirable changes in the properties of the drug under the effect of laser radiation. It is known that laser radiation causes a change in the optical properties of photosensitizing drugs, including as a result of a change in their conformational state [12–15].

Modern effective photodynamic drugs for the treatment of onychomycosis are chlorine-containing photodynamic drugs of a new generation, including Chloderm (V.V. Ashmarov IE, Russia). Trismeglumine salt of chlorine e6, a photodynamic agent of this preparation, is a powerful photosensitizer. The absorption spectrum of chlorine e6 is characterized by the presence of the most intense absorption B-band (the Soret band), Qx 00-band and Qx 01-band, as well as Qy 00-band. The B-band is quite wide and extends from 320 to 480 nm at its base. In the photodynamic therapy, light sources are mainly used with wavelengths

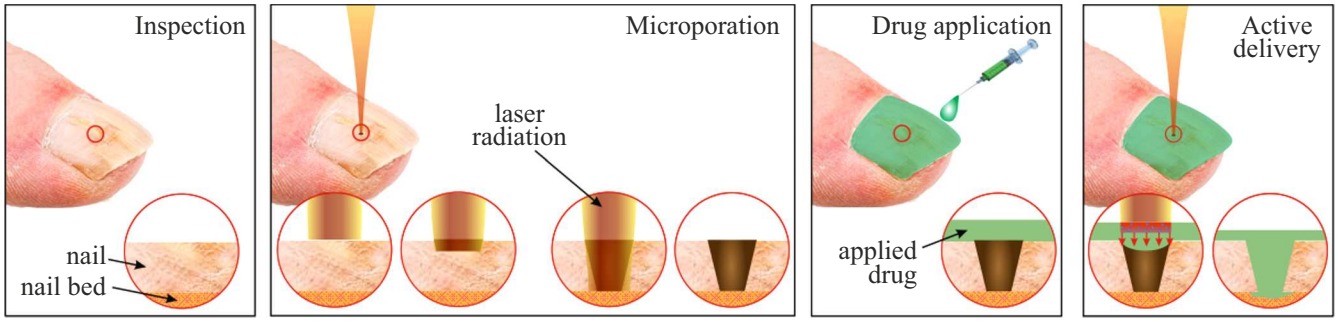

Figure 1. A method of microporation of the nail plate without a drug layer applied to the surface of the nail and active laser delivery of the drug after applying the drug to the dorsal surface of the microporated nail plate in the treatment of human nail diseases.

within the peak of the Qy 00-band. This is due to the fact that light with this wavelength penetrates deeply into biological tissues. However, this radiation is not absorbed by photosensitizer as effectively as the radiation within the peak of B-band. And the use of sources with wavelengths within the B-band is limited by the shallow penetration depth of their radiation into biological tissue. For a number of dermatological diseases and, above all, onychomycosis, the depth of light penetration should not exceed 0.3–0.5 mm, which opens up the possibility of using the radiation outside the Qy 00-band, including that with a wavelength of 405 nm, which is within the peak of the absorption B-band. It was shown in [15] that exposure of Chloderm to the laser radiation with a wavelength of 405 nm and an intensity of 200 mW/cm^2 effectively decolorizes the drug due to the generation of singlet oxygen, which has an explicit cytotoxic effect and leads to the destruction of cell membranes in the cells. However, the delivery rate of Chloderm preparation under the nail plate by laser radiation with a wavelength of 405 nm has not been studied so far. Also, the possibility of changing the conformational state of Chloderm under the effect of this radiation with parameters sufficient for active laser delivery of the drug under the nail plate has not been studied. In this regard, it is relevant to determine the average power and time of exposure to laser radiation with a wavelength of 405 nm, which, on the one hand, lead to the active delivery of the drug under the nail plate, and on the other hand, do not lead to the aggregation of chlorine molecules and, consequently, to a significant deterioration of their photodynamic and luminescent properties for subsequent photodynamic action.

Thus, the purpose of this study was to investigate *in vitro* the laser microporation of the nail plate and active laser delivery under the nail plate of Chloderm, a modern chlorine-containing photosensitizing drug for the treatment of onychomycosis by laser radiation with a wavelength of 405 nm, as a result of which the rate and efficiency of the nail plate ablation, as well as the rate of delivery of the drug under the nail plate are determined, the extinction spectra of Chloderm were studied before and after exposure to the laser radiation, and also the effect is evaluated of laser radiation with a wavelength of 405 nm and parameters sufficient for active laser delivery of Chloderm under the nail plate on the conformational state of the drug.

Materials and methods

Chloderm (V.V. Ashmarov IE, Russia), a modern chlorine-containing photosensitizing drug, and its aqueous solutions with different mass concentrations (C) of the preparation were studied. The processes occurring under the action of laser radiation with a wavelength of 405 nm on Chloderm were recorded using a DTX 50 digital USB microscope (Levenhuk, Inc., USA) with a 4x magnification in reflected light.

The method of microporation of the nail plate and active laser delivery of the drug under the nail plate was used, which involves inspection of the nail state, microporation of the nail plate by laser radiation without a layer of the drug applied to the nail surface, followed by application of the drug to the dorsal surface of the microporated nail plate and exposure to laser radiation through the layer of this drug for its active delivery under the nail plate (Fig. 1).

In the *in vitro* investigation, samples were used that were fragments of freshly extracted healthy human nail plates from three volunteers. A total of 20 samples were studied. Before the start of the experiment, the samples of nail plates with the average thickness of $370 \pm 20\,\mu$m were mechanically cleaned of dirt and washed with distilled water.

For the *in vitro* microporation of the nail plate and active laser delivery, radiation from a CW semiconductor InGaN laser by Xinrui technology (China) with a wavelength of 405 nm was used. Average power of the laser radiation was $P = 0.5$, 0.8 and 1.0 W and was limited by parameters of the laser diode used. The size of the laser beam on the dorsal surface of the nail plate was $140 \times 110\,\mu$m (at the level of e^{-2}). The time of exposure to laser radiation t corresponded to 0.04, 0.08, 0.1, 0.12, 0.15, 0.2, 0.3, 0.4 and 0.6 s. Schematic diagram of the experimental setup for the microporation of the nail plate and active delivery of

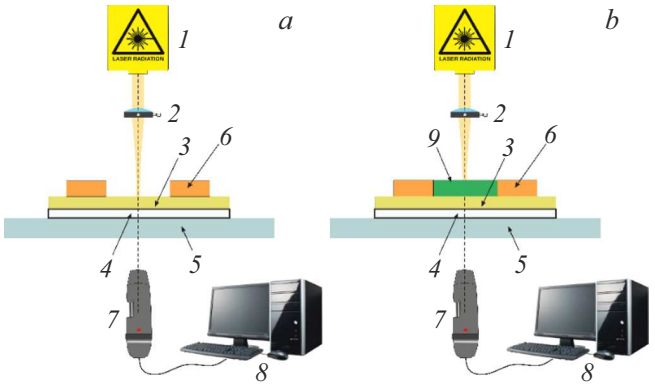

Figure 2. Schematic diagram of the experimental setup for microporation (a) and active laser delivery (b) of Chloderm under the nail plate: *50* — laser ($\lambda = 405$ nm); *2* — lens ($F = 120$ mm); *3* — nail plate fragment; *4* — paper substrate; *5* — glass slide; *6* — microcuvette; *7* — DTX 50 digital USB-microscope (Levenhuk, Inc., USA); *8* — computer; *9* — chlorine-containing photosensitizing drug.

photosensitizing drugs under the nail plate by laser radiation was similar to that described in [14] and is shown in Fig. 2.

A microcuvette was formed on the surface of the nail plate sample facing the laser, while the surface of the nail plate was the bottom of the microcuvette. Dimensions of the cuvette inner space were $\sim 1.5 \times 1.5 \times 0.1$ mm. When a chlorine-containing photosensitizing drug was placed in this inner space, a layer of the drug with a thickness of $100 \pm 10\,\mu$m was formed.

At the first stage (Fig. 2, a) the microcuvette was not filled with the photosensitizer, the laser radiation was focused on the nail plate surface faced toward the laser. As a result of the laser impact with a wavelength of 405 nm a micropore was formed in the nail plate. The process of nail plate microporation was monitored using a digital USB-microscope connected to a computer and located from the rear side of the paper substrate on which the nail plate sample was placed. The moment of microporation corresponded to the moment of defect emerging on the rear side of the paper substrate. At the second stage (Fig. 2, b) the microcuvette was filled with the photosensitizer drug. Immediately after that its active delivery was performed as a result of the laser impact with a wavelength of 405 nm. The moment of delivery of the photodynamic drug was recorded by a digital USB-microscope and corresponded to the moment of coloring the paper substrate with the drug.

The appearance of micropores created in the nail plate and their longitudinal sections obtained as a result of abrasive grinding along the micropore axis was recorded with a Zeiss Axio Scope.A1 microscope (Carl Zeiss, Germany). Analysis of the recorded images of micropores in the CorelDRAW Graphics Suite 2021 software package (Corel Corp., Canada) made it possible to determine their shape and depth, as well as the rate and efficiency of the nail plate ablation. The rate of ablation was determined as a ratio between the crater depth in the nail plate and the time of laser radiation action. The efficiency of ablation was determined as a ratio between the crater depth in the nail plate and the laser radiation energy required to create the crater. Also, the mass PM_{Chloderm} of photosensitizer drug penetrated through the single hole at the moment of its delivery under the nail plate and the rate V_{Chloderm} of drug delivery through the single microhole under the nail plate were determined similarly to the method described in [14].

To detect conformational changes in the Chloderm chlorine-containing photosensitizer during its active delivery by laser radiation with a wavelength of 405 nm, the extinction spectra of Chloderm were studied in the range of 350–900 nm before and after exposure to the laser radiation with a wavelength of 405 nm. Extinction spectra were recorded using a T90+ double-beam spectrophotometer (PG Instruments Ltd, UK). In the experiment, an aqueous solution of Chloderm ($C = 5\%$) was placed in a quartz cell with the same size as the inner space of the microcuvette created on the surface of the nail during microporation of the nail plate and active laser delivery (see above). A quartz cell with distilled water of the same size was placed in the reference arm of the spectrometer. The transmittance spectrum of Chloderm aqueous solution before the laser impact was recorded. The Chloderm aqueous solution was irradiated by scanning of the laser radiation with a wavelength of 405 nm along the surface of the photosensitizer drug. This was performed with maximum average intensity of the laser radiation equal to 1 W. The scanning was performed in a stepwise manner from point to point with a step equal to the laser beam diameter, until the whole surface of the drug was irradiated. Time of the laser impact on one point was 0.2, 0.4 or 0.8 s. Then, the irradiated aqueous solution of Chloderm inside the cuvette was stirred for 60 ± 1 s in a Multi Bio RS-24 multi-rotator (Biosan, Latvia), with rotational, reciprocating motion and shaking of the cuvette, then the transmission spectrum of the solution was recorded. Each measurement was carried out with a step of 1 nm and lasted about 3 min. For each sample, 10 measurements were performed.

Based on the obtained transmittance spectra, in accordance with Bouguer–Lambert–Beer law the extinction spectrum of the Chloderm aqueous solution before and after the laser impact was calculated. The extinction coefficient μ_t in this case took into account both absorption and scattering. In the experiments, absorption bands were investigated with a peak at a wavelength of 672 ± 3 nm (Qy 00-band of absorption of the drug) and with a peak at a wavelength of 697 ± 3 nm, which correspond to conformation states of chlorine e6 in the form of monomer and tetramer, respectively [16], as well as the extinction coefficient at the wavelength of 405 ± 5 nm nm (B-band of absorption of the drug). To evaluate the conformational state of chlorine e6 in Chloderm, the spectral transformation coefficient (k_t) was calculated as a ratio between the absorption coefficient of the drug at a wavelength of 672 nm corresponding to the absorption of monomers and the absorption coefficient at

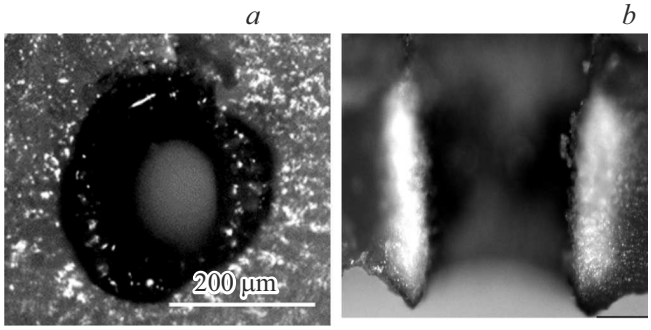

Figure 3. Appearance of the nail plate microporated by laser radiation with a wavelength of 405 nm: a photo of the dorsal side of the nail plate with a micropore on top (a) and a photo of a longitudinal section of the nail plate with a micropore (b /) ($P = 1$ W, $t = 0.2$ s).

a wavelength of 697 nm corresponding to the absorption of tetramers. The change in k_t as a function of the laser impact time t was investigated. It should be noted that in this case, the analysis of k_t makes it possible to understand only the trend in the change in the ratio between the amount of monomers and the amount of tetramers in the solution, but not to evaluate their amounts. To evaluate the amount of monomers and tetramers in the solution before and after exposure to the laser radiation, it is necessary to study the behavior of their molar absorption coefficients, which is beyond the scope of this work. Assuming constant molar absorption coefficients of monomers and tetramers, an increase in k_t indicates that the amount of monomers is increasing relative to the amount of tetramers. The statistical processing of the experimentally obtained data consisted in determining mean values and standard deviation of measured quantities and performed in the STATGRAPHICS Plus 5.0 software package (Statistical Graphics Corp., USA).

Results and discussion

The characteristic appearance of the nail plate microporated by radiation with a wavelength of 405 nm is shown in Fig. 3.

It can be seen that as a result of exposure to the laser radiation with a wavelength of 405 nm, through micropores with a diameter of the order of 200 μm can be created in the nail plate. The micropore walls are significantly deformed, the tissue adjacent to them is enlarged and carbonized, which indicates a significant heating of the nail plate during its microporation. Thickness of the enlarged carbonized area adjacent to the micropore wall is as high as 70 μm. It should be noted that the carbonization indicates the achievement of high temperatures, which can injure the nail bed as a result of microporation of the nail plate. At the same time, in [17], the authors studied the ablation of the nail plate by Er:YAG laser radiation, in which case a carbonization of the nail plate was observed, as it was in our case. The authors of this study have shown that such laser impact, in combination with the subsequent action of an antifungal drug (amorolfine), results in an increase in the effectiveness of local treatment of onychomycosis. In this regard, it can be expected that the carbonization will not have a negative effect on the result of the treatment in the case of exposure to laser radiation with a wavelength of 405 nm, although this statement, of course, needs additional verification, which is planned by the authors in the future, but is beyond the scope of this work.

Dependencies of rate and efficiency of the nail plate ablation on the time of exposure to the laser impact with a wavelength of 405 nm at different average power of the laser radiation are shown in Fig. 4, a and 4, b, respectively.

It can be seen that the presented dependencies have an extremum at the laser impact time of 0.1 s. The increase in the rate and efficiency of the nail plate ablation at times shorter than 0.1 s can be associated with an increase in the energy of laser radiation absorbed by the nail plate and the associated increase in the energy density exceeding the threshold level required to start the nail plate ablation. The decrease in the rate and efficiency of the nail plate ablation at times longer than 0.1 s can be associated with the decrease in the energy density of the laser radiation reaching the bottom of the micropore as a result of the increasing distance between the micropore bottom and the plane of the laser beam waist during the time of laser exposure, as well as with the accumulation of laser destruction products inside the crater that absorb the radiation. Also, it should be noted that the rate and efficiency of nail plate ablation increase with growth of laser radiation power, which is evidently caused by the increase in the volume of the nail plate where the laser radiation density exceeds the ablation threshold. At the laser impact time of $t = 0.6$ s, rates of the nail plate ablation for all three values of the average laser radiation power studied in the work become comparable with each other (Fig. 4, a) . This can be explained by the difference in the dynamics of changes in the efficiency of the nail plate ablation for these powers (Fig. 4, b). At $t = 0.3$ s, the ablation efficiency for the average laser radiation power of $P = 0.5$ W is less than the efficiency at $P = 0.8$ and 1.0 W, and at $t = 0.4$ s the ablation efficiency for the average power $P = 0.5$ W already exceeds the efficiency at $P = 0.8$ and 1.0 W. That is, for $P = 0.5$ W, the efficiency decreases more slowly over the exposure time than for $P = 0.8$ and 1.0 W, and at $t = 0.6$ s for $P = 0.5$ W the lower average power is compensated by the higher ablation efficiency.

The maximum rate of the nail plate ablation by the radiation with a wavelength of 450 nm was 2600 ± 200 μm/s, and the efficiency of the nail plate ablation was 2.6 ± 0.2 μm/mJ when the nail plate was exposed to the laser radiation with a power of $P = 1.0$ W for $t = 0.1$ s. It took $t = 0.2 \pm 0.02$ s to achieve through microporation of the nail plate with a thickness of 370 ± 20 μm with $P = 1.0$ W.

It is worth noting that the efficiency of the nail plate ablation by the CW InGaN laser radiation with a wavelength

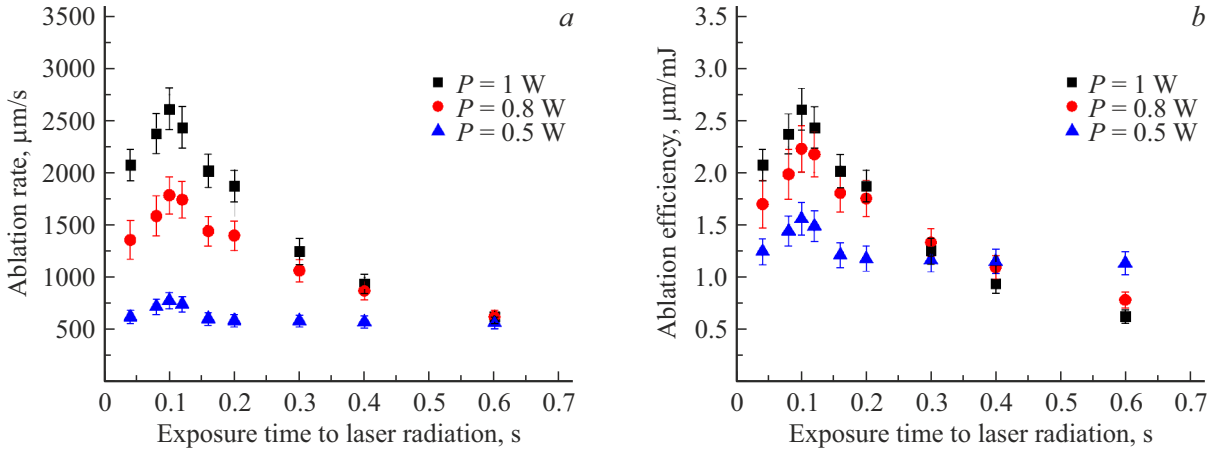

Figure 4. Dependencies of rate (*a*) and efficiency (*b*) of the nail plate ablation on the time of exposure to the laser impact with a wavelength of 405 nm at different average power of the laser radiation.

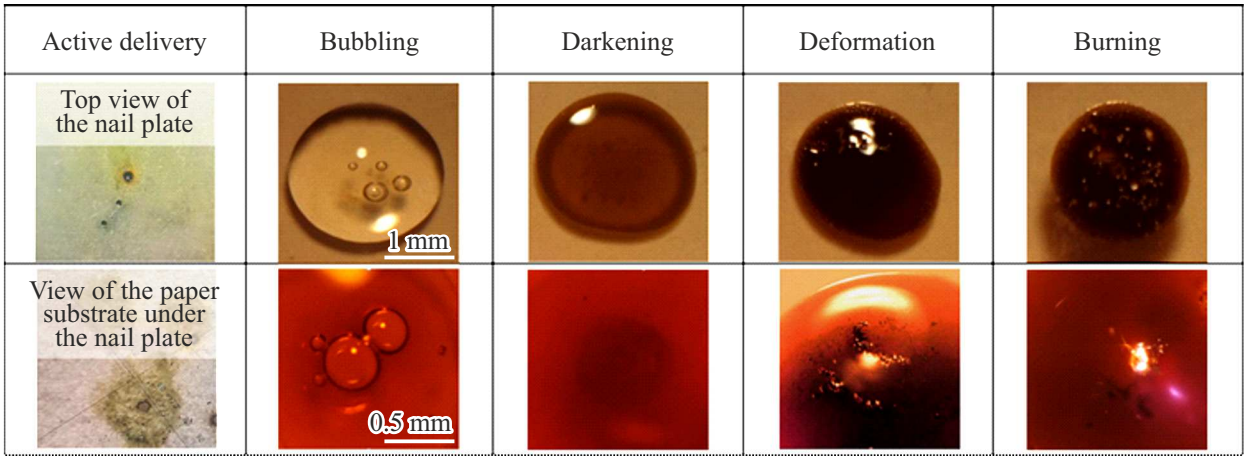

Figure 5. Processes that take place under the impact of the laser radiation with a wavelength of 405 nm on a drop of „Chloderm", chlorine-containing photosensitizer drug located on the surface of a microporated nail plate.

of 405 nm exceeds the efficiency of the nail plate ablation by the CW laser radiation with a wavelength of 450 nm (1.47 μm/mJ) [14] and is less than the efficiency of ablation by pulsed Er:YLF laser (4.6 μm/mJ) [18], which can be explained by both the difference in the absorption of radiation by the nail plate at these wavelengths, and the difference in the impulse power. The rate of the nail plate ablation by the CW InGaN laser radiation with a wavelength of 405 nm and $P = 1.0$ W is comparable to the rate of the nail plate ablation by the CW laser radiation with a wavelength of 450 nm with $P = 1.9$ W (2750 ± 30 μm/s) [14] and exceeds the rate of ablation by pulsed Er:YLF laser radiation with $P = 0.12$ W, a pulse energy of $E = 4$ mJ and a repetition rate of $f = 30$ Hz (360 μm/s) [18].

In the case of exposure to the laser radiation with a wavelength of 405 nm of a drop of Chloderm and its aqueous solutions located on the surface of a microporated nail plate, five processes were detected for the first time: active delivery, bubbling, darkening, deformation of the drug drop surface and burning (Fig. 5). The occurrence of these processes depended on the average power, the time of exposure to laser radiation and the concentration of Chloderm in the aqueous solution.

When a drop of Chloderm aqueous solution with a concentration of $C < 2\%$ on the surface of the microporated nail plate was exposed to the laser radiation with a wavelength of 405 nm with a power of $P = 1$ W for $t = 0.2$ s, only the active delivery of the solution under the nail plate was observed. When an aqueous solution of Chloderm with a concentration of $C = 2-25\%$ was exposed to the laser radiation, the active delivery of the solution under the nail plate and its bubbling were observed. When an aqueous solution of Chloderm with a concentration of $C = 25-55\%$ was exposed to the laser radiation, the active delivery of the solution and deformation of the drop surface were observed. When an aqueous solution of Chloderm with a concentration of $C = 55-60\%$ was exposed to the laser

radiation, the active delivery of the solution, darkening and deformation of the drop surface were observed. When an aqueous solution of Chloderm with a concentration of $C > 60\%$ and the drug were exposed to the laser radiation, the darkening and burning were observed. For further studies, the concentration of $C = 5\%$ was chosen, because in this case it was possible to evaluate the effect of the bubbling process only on the conformational state of chlorine e6 in Chloderm during its active laser delivery by radiation with a wavelength of 405 nm.

Dependencies of the mass PM_{Chloderm} of the aqueous solution of Chloderm ($C = 5\%$) penetrated through a single micropore in the nail plate at the time of its delivery under the nail plate, and the rate (V_{Chloderm}) of the active laser delivery of this aqueous solution under the nail plate on the time of exposure to the laser radiation with a wavelength of 405 nm and different average power are shown in Fig. 6, *a* and 6, *b*, respectively.

It can be seen that the presented dependences of PM_{Chloderm} and V_{Chloderm} have an extremum. The extremum for PM_{Chloderm} is located at $t = 0.3$ s with $P = 1$ W, at $t = 0.4$ s with $P = 0.8$ W, and no extremum was detected for $P = 0.5$ W in the studied range of exposure times t and average laser radiation power P . The extremum for V_{Chloderm} is located at $t = 0.3$ s for all P. The initial growth of PM_{Chloderm} and V_{Chloderm} is associated with an increase in the energy exposure of the laser radiation. The subsequent decrease in PM_{Chloderm} and V_{Chloderm} can be associated both with the partial expansion of the drop of the aqueous solution of Chloderm as a result of the formation of vapor-gas cavities (bubbles) in it under the action of laser radiation, and with the decrease in the absorption coefficient caused by this process at the wavelength of the laser radiation.

The maximum mass of $PM_{\text{Chloderm}} = 1.6 \pm 0.15$ mg and the delivery rate of $V_{\text{Chloderm}} = 5.3 \pm 0.5$ mg/s were registered at $P = 1.0$ W and $t = 0.3$ s. Minimum time required for the active laser delivery was observed at $P = 1.0$ W and was equal to $t = 0.1$ s.

It should be noted that the delivery rate V_{Chloderm} ($C = 5\%$) by the cw InGaN laser radiation with a wavelength of 405 nm exceeds the delivery rate V_{Chloderm} ($C = 0.65\%$) by the cw laser radiation with wavelength 450 nm (1.15 ± 0.10 mg/s) [14] and the delivery rate V_{Chloderm} ($C = 0.65\%$) by the Er:YLF laser radiation with a pulse duration of 270 μs, a pulse energy of $E = 4$ mJ and a repetition rate of $f = 30$ Hz is (1.40 ± 0.15 mg/s) [19], which can be explained by the difference in the amount of chlorine e6 in the drug and, respectively, the differences in the absorption of laser radiation with wavelengths of 405 and 450 nm by the drugs, as well as the difference in the pulse power of laser sources.

Typical extinction spectra of Chloderm aqueous solution ($C = 5\%$) before and after the exposure to the laser radiation with a wavelength of 405 nm and a power of $P = 1.0$ W (intensity of 6500 W/cm^2) are shown in Fig. 7, *a*. The dependence of spectral transformation coefficient k_t on the time of this laser impact is shown in Fig. 7, *b*.

The extinction spectra of aqueous solution of Chloderm ($C = 5\%$) have clearly distinguished absorption bands with peaks at wavelengths of 405 ± 5 nm (B-band), 672 ± 3 and 697 ± 3 nm (Qy 00-bands). Before the laser impact, the extinction coefficient μ_t of the aqueous solution of Chloderm at a wavelength of 405 nm was 24.24 cm^{-1}, at a wavelength of 672 nm it was 5.14 cm^{-1}, and at a wavelength of 697 nm it was 4.37 cm^{-1}. After the exposure of the aqueous solution of Chloderm ($C = 5\%$) to the CW laser radiation with a wavelength of 405 nm and a power of $P = 1.0$ W (Fig. 7, *a*) for $t = 0.1$, 0.2, 0.4 and 0.6 s the extinction coefficient μ_t at a wavelength of 405 nm has increased to 27.07, 28.86, 30.41 and 30.37 cm^{-1}, at a wavelength of 672 nm it has increased up to 5.52, 5.63, 6.04 and 6.25 cm^{-1}, and at a wavelength of 697 nm it has increased up to 4.63, 4.63, 4.96 and 5.18 cm^{-1}, respectively. The increase may be related to an increase in concentration of photosensitizer (chlorine e6) in the drug due to the water evaporation under the laser impact. The observed bubbling process obviously indicates that the solution achieves a temperature close to 100°C, which is necessary for water evaporation. It can also be noted that the peaks of the absorption bands at wavelengths of 405, 672, and 697 nm do not shift, which counts in favor of the absence of changes in the state of the chlorine e6 molecule.

Spectral transformation coefficient before and after exposure to laser impact $k_t > 1$. Transformation coefficient k_t at $t = 0.6$ s demonstrates a statistically significant (p-value < 0.05) increase from 1.18 ± 0.11 ($t = 0$ s) to 1.21 ± 0.12. This is indicative of the fact that with an increase in t the conformational state of the Chloderm aqueous solution changes and the amount of chlorine e6 monomers increases. An increase in the amount of chlorine e6 monomers leads to an increase in the quantum efficiency of singlet oxygen and an increase in the efficiency of the photodynamic therapy [20–22]. The growth of k_t may be associated with a change in temperature and pH of the drug as a result of light impact [16,20,21,23–25]. A change in the conformational state of chlorine e6 can occur both due to aggregation and due to changes occurring in the monomers and tetramers themselves. The spectral transformation coefficient studied in this work describes aggregation and can not be a gage of the changes taking place in monomers and tetramers, because it does not take into account changes in their molar absorption coefficients. With the minimum time required for active laser delivery of Chloderm by the laser radiation with a wavelength of 405 nm ($t = 0.1$ s), the k_t does not have statistically significant changes, which is indicative of the fact that with laser delivery of the Chloderm ($C = 5\%$) modern chlorine-containing drug by the radiation with a wavelength of 405 nm and an average power of $P = 1.0$ W, its conformational state remains unchanged, which means

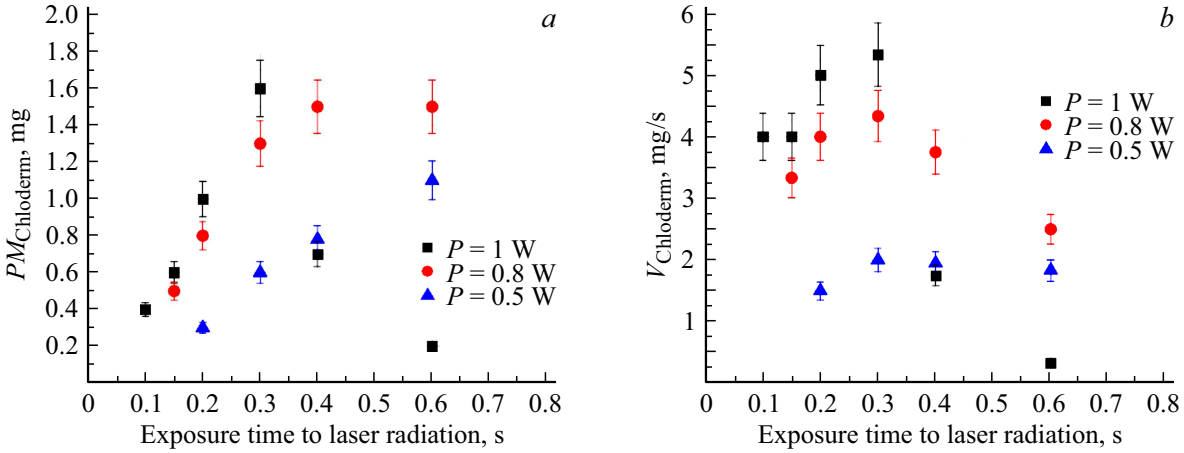

Figure 6. Dependencies of the mass PM_{Chloderm} (a) and the rate (V_{Chloderm}) of the active laser delivery (b) of Chloderm aqueous solution $(C = 5\%)$ on the time of laser radiation impact with a wavelength of 405 nm at different values of average power of the radiation.

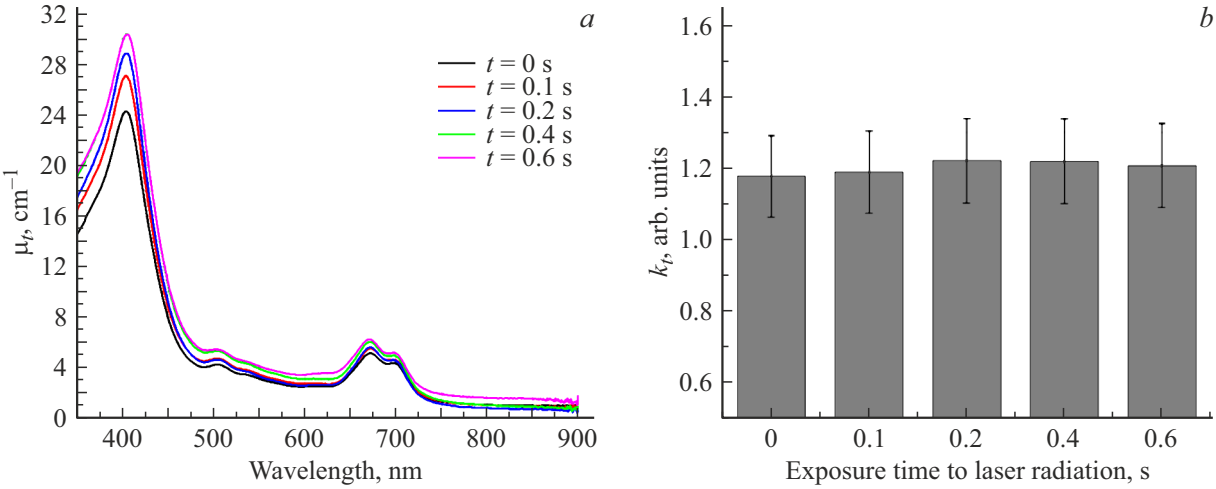

Figure 7. Extinction spectra (μ_t) of an aqueous solution of Chloderm $(C = 5\%)$ (a) and spectral transformation coefficients k_t (b) before and after the laser impact with a wavelength of 405 nm, a power of $P = 1.0$ W and different times of exposure to the laser radiation.

that its photodynamic and luminescent properties do not deteriorate.

Conclusion

Microporation of the nail plate and active laser delivery of a modern chlorine-containing drug Chloderm after its application to the dorsal surface of a microporated nail plate with CW InGaN laser radiation at a wavelength of 405 nm were studied *in vitro*. The following processes taking place in the case of exposure to the laser radiation with a wavelength of 405 nm of a drop of Chloderm and its aqueous solutions located on the surface of a microporated nail plate were detected for the first time: active delivery, bubbling, darkening, deformation of the drug drop surface and burning (Fig. 5). The occurrence of these processes depended on the average power, the time of exposure to laser radiation and the concentration of Chloderm in the aqueous solution. The rate and efficiency of the nail plate ablation by the radiation of a CW InGaN laser with a wavelength of 405 nm were determined. The maximum ablation rate was $2600 \pm 200\,\mu$m/s, and the ablation efficiency was $2.6 \pm 0.2\,\mu$m/mJ. The possibility of active delivery of an aqueous solution of Chloderm $(C = 5\%)$ under the nail plate microporated by this laser was demonstrated. It was shown that at $P = 1.0$ W and $t = 0.3$ s the rate of active laser delivery of Chloderm aqueous solution under the nail plate by radiation with a wavelength of 405 nm can be as high as 5.3 ± 0.5 mg/s, and the minimum time required for active laser drug delivery is 0.1 s. The extinction spectra of a Chloderm aqueous solution were studied before and after the exposure to the laser radiation with a wavelength of 405 nm. It has been established that at $P = 1.0$ W and $t = 0.6$ s its extinction coefficient increases, and the spectral transformation coefficient k_t has a statistically significant growth, but at $t = 0.1$ s, the minimum required for active

laser delivery of Chloderm, the k_t has no statistically significant changes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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