DOI: 10.6060/mhc245786g

Antibacterial Activity of Chlorophyll and Hemin Polymeric Forms against *S. aureus* and *E. coli*

Dmitry V. Gruznov,[a@] Olga A. Gruznova,[a,b] Nikolay I. Popov,[a] Gulizar Sh. Shcherbakova,[a] Ekaterina N. Shuteeva,[a] Alina V. Konyashkina,[a] Irina P. Chesnokova,[b,c] Lilia F. Plaksina,[c] and Anton V. Lobanov[d]

[a]*All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology - Branch of Federal Scientific Center - K.I. Skryabin, Ya.R. Kovalenko All-Russian Research Institute of Experimental Veterinary Medicine of Russian Academy of Sciences, 123022 Moscow, Russian Federation*
[b]*N.N. Semenov Federal Research Center for Chemical Physics of Russian Academy of Sciences, 119991 Moscow, Russian Federation*
[c]*MIREA – Russian Technological University, 119454 Moscow, Russian Federation*
[d]*Moscow Pedagogical State University, 119991 Moscow, Russian Federation*
[@]*Corresponding author E-mail: 79164422245@yandex.ru*

The number of antibiotic-resistant microorganisms is increasing, and metal porphyrins are a promising and modern class of antimicrobial agents. Two well-known representatives of this class are chlorophyll (Chl) and hemin, which have been studied in this paper. The effectiveness of Chl and hemin complexed with poly-N-vinylpyrrolidone (PVP) against Staphylococcus aureus and Escherichia coli is presented. A method for producing polymeric forms of these compounds was developed. Using light microscopy, structural inhomogeneities in the form of aggregated particles were detected in Chl-PVP and hemin-PVP films. The binding constants for these substances to PVP were calculated to be $5.0 \cdot 10^4$ L/mol for Chl and $3.3 \cdot 10^4$ L/mol for hemin. Data on the release of these substances from the polymeric matrix were also obtained. It has been observed that complete release of Chl from PVP occurred after 13 h, and hemin after 10 h. Microbiological studies generated data on the relative antimicrobial effects of both the free and polymerized forms of these substances. These results can be used in the development of new medications for treating microbial infections.

Keywords: Chlorophyll, hemin, poly-*N*-vinylpyrrolidone, *Escherichia coli*, *Staphylococcus aureus*, antibacterial activity.

Антибактериальная активность полимерных форм хлорофилла и гемина в отношении *S. aureus* и *E. coli*

Д. В. Грузнов,[a@] О. А. Грузнова,[a,b] Н. И. Попов,[a] Г. Ш. Щербакова,[a] Е. Н. Шутеева,[a] А. В. Коняшкина,[a] И. П. Чеснокова,[c] Л. Ф. Плаксина,[c] А. В. Лобанов[d]

[a]*Всероссийский научно-исследовательский институт ветеринарной санитарии, гигиены и экологии - филиал ФГБУН «Федеральный научный центр - Всероссийский научно-исследовательский институт экспериментальной ветеринарии имени К.И. Скрябина и Я.Р. Коваленко Российской академии наук», 123022 Москва, Россия*
[b]*ФГБУН Федеральный исследовательский центр химической физики им. Н.Н. Семенова Российской академии наук, 119991 Москва, Россия*
[c]*ФГБОУ ВО «МИРЭА – Российский технологический университет», 119454 Москва, Россия*
[d]*ФГБОУ ВО «Московский педагогический государственный университет», 119991 Москва, Россия*
[@]*E-mail: 79164422245@yandex.ru*

Увеличение числа антибиотикорезистентных штаммов микроорганизмов приобретает все более массовый характер. Перспективными и современными антимикробными средствами представляются металлопорфирины. Известные представители металлопорфиринов – хлорофилл (Chl) и гемин. В работе представлены результаты исследований эффективности комплексов Chl и гемина с поли-N-винилпирролидоном (ПВП) в качестве антимикробного средства в отношении Staphylococcus aureus и Escherichia coli. Представлен способ получения полимерных форм Chl и гемина. С помощью световой оптической микроскопии полимерных пленок Chl-ПВП и гемин-ПВП были обнаружены структурные неоднородности в виде отдельных агрегированных частиц правильной формы размером 1.7–2 мкм, равномерно распределенные в полимерной матрице. Рассчитаны константы связывания этих веществ с полимером, которые составили $5.0 \cdot 10^4$ л/моль для Chl и $3.3 \cdot 10^4$ л/моль для гемина. Получены экспериментальные данные по релизу веществ из полимерной матрицы. Установлено, что полное высвобождение Chl из ПВП наблюдалось через 13 ч, а гемина – через 10 ч. В микробиологических исследованиях получены данные о сравнительном антимикробном действии свободной и полимерной форм веществ. В дальнейшем эти результаты могут быть использованы при разработке препаратов против микробных инфекций.

Ключевые слова: Хлорофилл, гемин, поли-*N*-винилпирролидон, *Escherichia coli*, *Staphylococcus aureus*, антибактериальная активность.

Introduction

In recent years, there has been an increasing number of microorganisms that are resistant to antibiotics and disinfectants, which has become a serious and global issue.[1-5] Therefore, it is essential not only to search for and develop new drugs, but also to explore other approaches such as photodynamic therapy (PDT) and light-independent therapies. Metal porphyrins, which have been widely used for many years in both fields, have proven to be effective.[3,4-8] Some well-known examples of porphyrins include chlorophyll (Chl) and hemin.[9-12]

Based on the available literature, it is possible to propose a hypothesis regarding the mechanism of antimicrobial action of metal porphyrins. Following the model of antibacterial activity mechanism proposed by I. Stojiljkovic and B.D. Evavold *et al*., it is suggested that metal porphyrins enter bacterial cells via heme receptors or through the outer membrane by passive diffusion. Metal porphyrins then bind to cytochrome heme sites, either directly in the periplasmic space (in the case of gram-positive bacteria) or after transportation into the cytoplasm and subsequent export back to the periplasm via the cytochrome assembly process. The binding of metal porphyrins to cytochromes prevents the transfer of electrons to O_2, leading to its incomplete reduction and, consequently, the formation of reactive oxygen species.[13,14]

The basic skeleton of Chl is a large, flat structure with a symmetrical arrangement. This structure consists of four pyrrole rings connected by methine (-CH=) bridges, with four nitrogen atoms coordinated to a central magnesium atom. In addition, Chl molecules contain a phytol group that imparts hydrophobic characteristics and the ability to integrate with the lipid layers of biological membranes.[15]

Chl was first isolated from plant leaves by J.B. Cavant and P.J. Pelletier. It was subsequently discovered that Chl consists of several components: *a*, *b*, *c1*, *c2*, *d* and *f*.[16,17] Chl is used in a variety of applications, such as the production of singlet molecular oxygen for PDT, photochemical and electrochemical catalysis, the food industry (as a food additive), the textile and paint industries (as a dye), semiconductor technology, and the thermal and photostabilization of fats and polymers.[9,11,18,19]

Hemin is a type of protoporphyrin IX molecule that contains a ferric iron ion and a coordinating chloride ligand.[20] It crystallizes from blood as dark brown, rhombic crystals.[21] The hemin reaction, also known as the Teichmann reaction, is used in forensic science to identify bloodstains.[22] Additionally, medications based on hemin have been developed to treat heme deficiency.[23,24] There is also evidence of successful use of hemin as an antimicrobial agent.[12,20,25]

However, due to the insolubility of both hemin and Chl in water and physiologic solutions, there is a concern regarding their low bioavailability. To address this issue, researchers have explored the incorporation of these compounds into polymeric matrices. Biocompatible and nontoxic polymers are widely used in medical applications,[26-31] and the increased efficacy of Chl and hemin when incorporated into these carriers has been demonstrated in several studies.[32-43]

This paper presents the results of a study on the antibacterial properties of Chl and hemin when incorporated into biodegradable PVP against both gram-negative and gram-positive microorganisms, specifically *Escherichia coli* and *Staphylococcus aureus*.

Experimental

The materials used in this work were chlorophyll (Chl) as Chl*a* and Chl*b* mixture (3:1), hemin (porcine, 99.5%, “BioFroxx), poly-*N*-vinylpyrrolidone (PVP,10 000 g/mol, “Ataman Kimya”), dimethyl sulfoxide DMSO (C_2H_6OS, “PanReac Applichem”), sterile physiological solution (0.9% NaCl, “Khimikom”), meat peptone agar (MPA, “Khimikom”), industry turbidity standard for determining the total concentration of microorganisms (BAK-10 kit, “Art-Medica”). Chl was extracted from dried nettle leaves (*Urtica dioica*).[44]

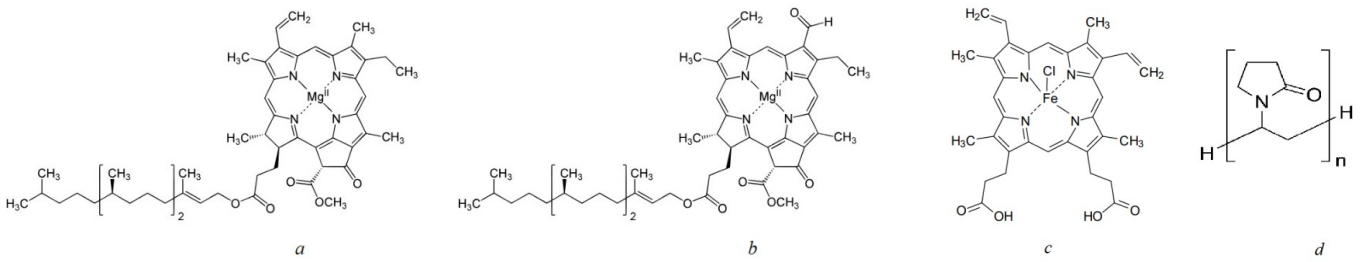

Figure 1. Structures of Chl*a* (*a*), Chl*b* (*b*), hemin (*c*) and poly-*N*-vinylpyrrolidone (n = 90) (*d*).

The structures of Chl*a*, Chl*b*, hemin and PVP are shown in Figure 1.

The electronic absorption spectra of Chl and hemin solutions were measured using a PE5400UF spectrophotometer (manufactured by Ekroskhim) with a spectral range of 190–1000 nm. The thickness of the absorbing layer was 10 mm. Optical density scans at a specified wavelength range were performed, and the resulting data were saved and loaded into tables using the SC5400 software (version 2.1).

To obtain a polymeric form of Chl, PVP was dissolved in water at a concentration of 5% (wt.%). In parallel, Chl solutions were prepared in dimethyl sulfoxide (DMSO) at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 wt.% relative to the weight of PVP, corresponding to concentrations of 15, 30, 45, 60, and 75 μg/mL. The resulting solutions of the polymer and the substances were mixed and kept at room temperature in darkness until experiments were carried out.

Hemin polymeric form was prepared using the following method. PVP was dissolved in water at a concentration of 6% (wt.%). Simultaneously, solutions of hemin were prepared in DMSO at concentrations ranging from 40 to 320 μg/mL. The resulting solutions of the polymer and the substance were then mixed. Thus, the final concentrations of hemin in the PVP ranged from 20 to 160 μg/mL, and the PVP concentration was 3% (wt.%). DMSO was not removed from the prepared solutions because these preparations are expected to be used as topical agents in further experiments, including on animal models. Additionally, we have previously conducted studies on the effects of DMSO solutions without active substances on test microorganisms. These studies found that an amount of DMSO equal to that contained in the formulations studied in this work did not have any effect on bacteria.

To conduct optical light microscopy, polymeric forms of metal porphyrins were applied to glass slides and allowed to air dry in the dark at room temperature. The resulting films were examined using a light triocular microscope, AmScope T390C (China), at a magnification of 400×. Photographs were taken using a Levenhuk M1000 PLUS digital camera (United States). The size of any structural inhomogeneities present in the samples was determined using the Levenhuk Lite Software for calibration purposes.

The binding constant (K_d) of porphyrins to PVP was calculated using mass concentrations.[45] For this purpose, Chl-PVP and hemin-PVP polymeric forms were prepared by dissolving metal porphyrins in DMSO and PVP in water. The DMSO:H_2O ratio in the resulting solutions was 1:1 (v/v). Chl concentration in the polymeric form was 14.4 μg/mL, and that of hemin – 2 μg/mL. The PVP concentration in the Chl-PVP complex varied from 0 to 5% (wt.%), and in the hemin-PVP complex from 0 to 3% (wt.%).

The mole fraction (Θ) of Chl or hemin was calculated using formula (λ_{max} = 650 and 403 nm, respectively):

$$\Theta = (A - A_o) / (A_\infty - A_o)$$

A_o is the optical density at a mass concentration of PVP equal to 0%, and A_∞ is the optical density at its maximum mass concentration.

Next, to construct a scatter plot along the *Y*-axis, Θ was subtracted from 1 ($1\text{-}\Theta$). The molar concentration of the polymer [*P*] was calculated using formula:

$$[P] = (m(PVP) / M_w) / V$$

Then, to construct a scatter plot, the value $-\Theta/[P]$ was calculated along the *X*-axis. After calculating all parameters in MS Excel, graphs were drawn, trend lines were drawn, and tgα was calculated. The binding constant (K_d) was calculated using the following formula:

$$K_d = 1/tg\alpha$$

To study kinetics of Chl or hemin release from the polymeric matrix *in vitro*, 6 mL of solution of the polymeric form of Chl or hemin (with Chl and hemin concentration of 14.4 and 2 μg/mL, respectively) was placed in a dialysis tubes (polypropylene with Mw = 7 000 g/mol, "LS Supplies Store") and dialysis against phosphate-buffered saline (PBS) with p*H* 7.2–7.4 at 37 °C. Then, every 20–40 min, a polymeric form was sampled from the dialysis tube to determine the concentration of Chl or hemin. For these purposes, a PE5400UF spectrophotometer (Ecrochem) and QA5400 software (version 2.1) were used.

To obtain daily cultures of *E. coli* (strain 1257) and *S. aureus* (strain 209-P), they were reseeded and further cultivated on a slanted MPA in a thermostat (37 °C, air-dry thermostat TV-80-1). Suspensions of 10^9 CFU/mL were prepared from daily cultures in sterile saline solution according to the turbidity standard. The obtained concentrations of suspended matter were confirmed spectrophotometrically (λ = 600 nm). Next, successive dilutions were prepared from suspensions of daily cultures of *E. coli* and *S. aureus* (10^9 CFU/mL) in 10-fold steps: 10^8 CFU/mL, 10^7 CFU/mL, 10^6 CFU/mL, 10^5 CFU/mL and 10^4 CFU/mL by titration in sterile saline solution.

To determine the minimum inhibitory concentration (MIC), a series of solutions of the active substance in DMSO with concentrations ranging from 150 to 4.7 μg/mL was prepared for use in the experiment. Additionally, a second series of solutions was prepared with concentrations from 300 to 9.4 μg/mL to create polymeric forms equivalent to the free active substance. 0.5 mL of a bacterial suspension containing approximately 10^5 colony-forming units (CFU) per milliliter was combined with 0.5 mL of each solution and the resulting mixtures were added to 5-milliliter tubes containing liquid nutrient medium (MPB). The tubes were then incubated at 37 °C for five days. Observations were made daily to assess the presence of visible turbidity in the medium, which indicated bacterial growth. These results were compared with those obtained from control samples containing sterile medium and control samples not treated with the active substance or its polymeric form.

To obtain more accurate results, the experiment was repeated using solutions with a 2.5-μg/mL increment in the active substance content. The minimum concentration of the preparation at which no turbidity (microorganism growth) was observed was taken as the minimum inhibitory concentration (MIC).

To calculate the percentage reduction in microbial cell growth in the presence of tested preparations, the absorbance of pure sterile medium MPB (sterility control) and control inoculation with microorganisms without the addition of preparations were measured, as well as sterile medium with the addition of prepared solutions and experimental inoculation of microorganisms with prepared solutions. The reduction in microbial growth in the presence of the tested preparation was calculated using the following formula:

$$\left(1-\frac{absorbance\ of\ test\ solution\ -\ absorbance\ of\ corresponding\ control}{absorbance\ of\ assay\ growth\ control\ -\ absorbance\ of\ sterility\ control}\right)\times 100$$

Determination of the antibacterial activity of Chl and hemin, in both free and polymeric forms, on a solid nutrient medium, was performed by measuring the diameters of inhibition zones. To this end, a dilution at a concentration of 10^4 CFU/mL was inoculated in Petri dishes containing sterile MPA medium. 100 µL of Chl or hemin complexed with PVP was then added to wells created with a sterile punch in the centre of the MPA plate. Incubation was then carried out in a thermostat at 37°C for 120 h, with daily readings taken for the diameter of the zone of growth inhibition (mm).

To avoid the impact of light on Chl, all experiments were conducted in a room with diffuse side lighting and without additional light sources.

All experiments were performed in triplicate.

Statistical analysis of the results was conducted using MS Excel 2010. All measurements were conducted in triplicate. Differences in mean values were determined using a t-test, with a significance level set at $p < 0.05$. The results are presented as mean values (M) with standard errors (±SEM).

Results and Discussion

At the initial stage, electronic absorption spectra of Chl and hemin solutions prepared in DMSO were recorded at concentrations from 1.8 to 28.8 µg/mL and from 0.25 to 4 µg/mL, respectively. As can be seen from Figure 2, the absorption maxima of the spectra were at wavelengths of 650 and 403 nm, which is typical for these compounds.

Using optical microscopy of the Chl-PVP and hemin-PVP polymeric films, structural inhomogeneities were detected in the form of individual aggregated particles with a regular shape (size 1.7–2 µm) uniformly distributed within the polymeric matrix. It should be noted that in hemin-PVP the structural inhomogeneities was expressed to a slightly greater extent (Figure 3).

Next, the binding constant of these compounds to PVP was calculated. Figure 4 shows the electronic absorption spectra of polymer complexes of Chl with PVP at concentrations from 0 to 5% (wt.%) and hemin with PVP in concentrations of 0 to 3% (wt.), as well as dot plots with drawn trend lines, constructed from the data obtained.

Based on the results obtained, the binding constants for the Chl-PVP and hemin-PVP complexes were calculated to be $5.0\cdot10^4$ L/mol and $3.3\cdot10^4$ L/mol, respectively. Additionally, it should be noted that the absorption spectra of Chl and hemin in Figures 4a and 4c show broadened absorption bands, which may indicate the presence of coordination and non-covalent interactions between the porphyrins and polymer macromolecules and possibly the local concentration of porphyrin molecules at the binding sites on the PVP macromolecule.

According to the data obtained during the study of literary sources, it was found that the values of binding constants in the ranges from 10^4 to 10^8 L/mol are taken as strong.[46-49] In the literature, which examined the interaction between ligands, in particular, porphyrins and various biopolymers, it was noted that on average the binding constants range from 10^4 to 10^6 L/mol.[50,51] For example, in the article of J.A. Strickland *et al.*[50] the interaction of *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin and its derivatives with DNA using equilibrium dialysis and stopped-flow dissociation kinetics as a function of [Na^+] was studied. As a result, it was found that the binding constants of porphyrins to biopolymers were quite high, for example, for *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin the values of binding constants with poly[d(G-C)]•poly[d(G-C)] [poly[d(G-$C)_2$]] were in the intervals from $7.5\cdot10^5$ to $6.7\cdot10^4$, decreasing with increasing of [Na^+] from 0.215 to 0.515 M. B.J. Hwang[51] analyzed the spectroscopic and thermodynamic properties of porphyrin complexes with the PVP. One aspect of the work was the investigation of the binding constant, the value of which was about 10^4–10^6 L/mol. Hence, it can be inferred that porphyrins have binding constants in the order of 10^4–10^6 L/mol, as confirmed by our own findings.

.

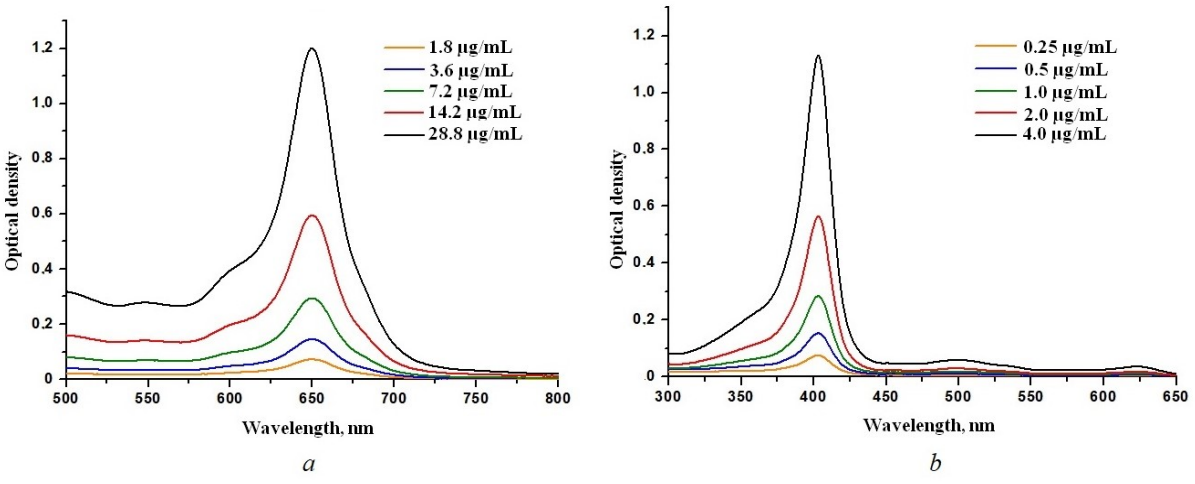

Figure 2. Electronic absorption spectra of Chl (*a*) and hemin (*b*) in DMSO at room temperature (22±2 °C).

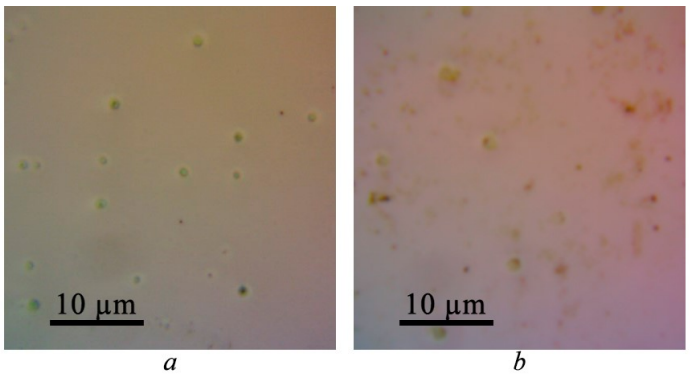

Figure 3. Microphotographs of polymeric films: Chl-PVP (*a*) and hemin-PVP (*b*).

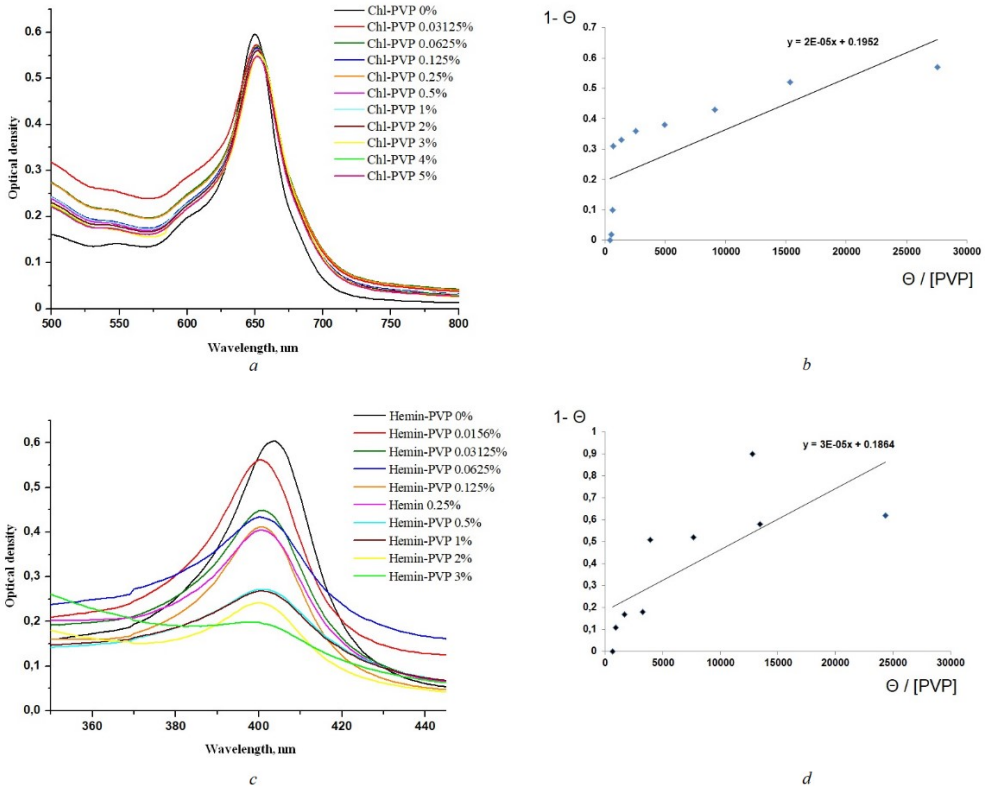

Figure 4. Electronic absorption spectra of the Chl-PVP complex (*a*) and hemin-PVP (*c*), dot plots with trend lines (*b*, *d*) at room temperature (22±2 °C).

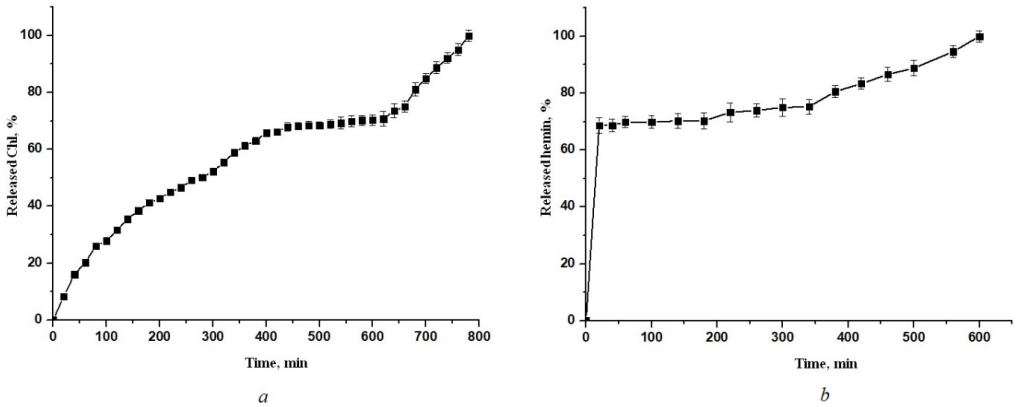

Figure 5. Chl (*a*) and hemin (*b*) release profile from the PVP polymeric matrix at p*H*=7.4 and 37 °C in phosphate-buffered saline (1×PBS).

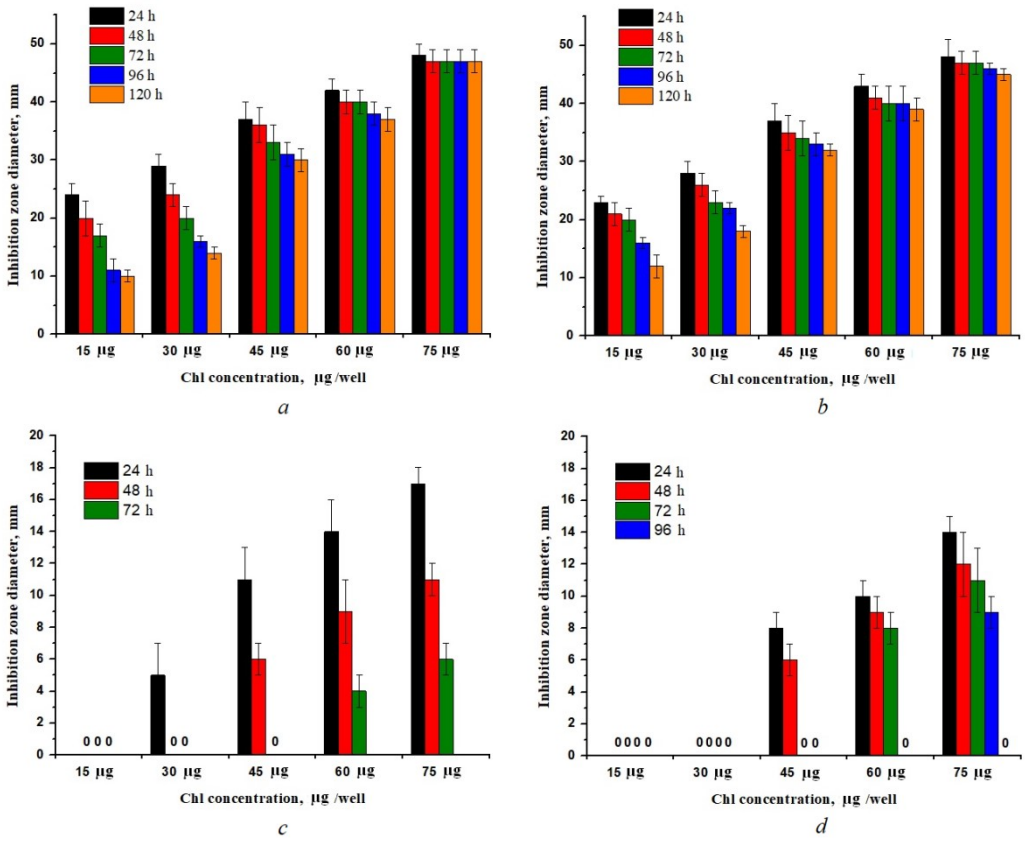

Figure 6. Inhibitory effect of Chl (*a*, *c*) and Chl-PVP (*b*, *d*) against *S. aureus* (*a*, *b*) and *E. coli* (*c*, *d*).

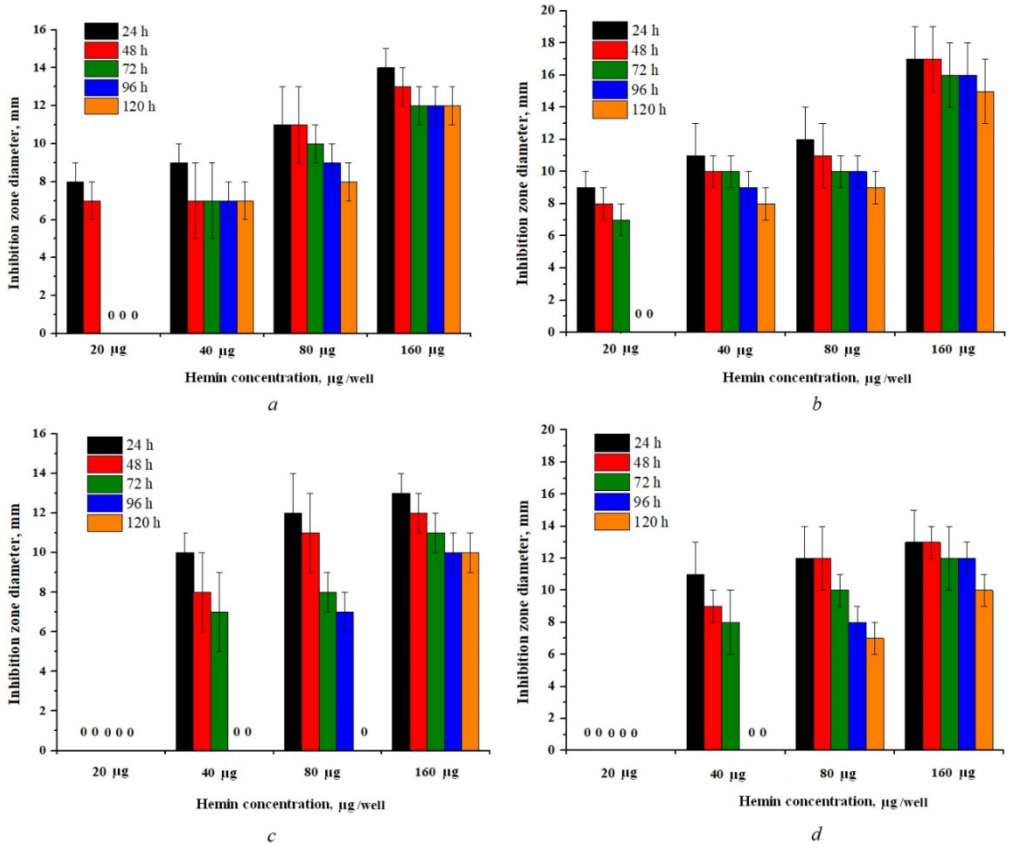

Figure 7. Inhibitory effect of hemin (*a*, *c*) and hemin-PVP (*b*, *d*) against *S. aureus* (*a*, *b*) and *E. coli* (*c*, *d*).

At the next stage, the kinetics of Chl and hemin release from the PVP polymeric matrix was studied (Figure 5).

As can be seen in the data presented, a rather gradual release of Chl from PVP was observed. 99.95% of the substance was released in 780 min (13 h). In the first 20 min, a rapid release of hemin from PVP was detected (about 70%). Then a stage of its slower exit was observed. As a result, 99.98% of hemin was released from the polymer in 600 min (10 h).

Based on the literature data, it can be concluded that the release of the active substance from the polymer matrix is influenced not only by its own physicochemical properties but also by the pH of the environment and the structure of the polymer carrier.[52-54] These factors can be modified to achieve optimal results. For PDT of malignant tumors, polymer depots are being developed that release porphyrins over a long period of time. For example, M.R. Faustova *et al*.[55] used a copolymer of lactic and glycolic acid (PLGA) in their work. They studied the kinetics of the release of Fe^{III}Cl-tetraphenylporphyrin under physiological conditions (p*H* close to blood p*H*) as well as at p*H* 5. A rapid release of the metal porphyrin occurred in the first 24 h, followed by a slower stage: less than 40% of the substance was released after 144 h. The fastest degradation of the polymer occurred at p*H* 5. U. Bazylińska *et al*.[56] presented data on the study of the release of porphyrin dyes – verteporfin and *meso*-tetraphenylporphyrin – from various types of sodium alkyliminobisacetate-stabilized nanosystems, including biocompatible poly(L-glutamic acid)/poly(L-lysine), multilayer nanocapsules. The analysis was conducted both at physiological p*H* (7.4) and in the presence of human serum albumin (1% HSA) dissolved in PBS. The nanocarriers, obtained on a nanoemulsion template, released porphyrins approximately 15–20% more quickly (after 180 h in PBS) compared to solid-core nanocapsules. The presence of HSA in the release medium increased the retention of verteporfin and meso-tetraphenylporphyrin in the nanocapsules by approximately 8–10%, regardless of the type of nano-capsule template. In our study, the release of Chl and hemin from PVP was 13 and 10 h, respectively. As the resulting polymeric forms will be used as an antimicrobial agent for the treatment of superficial wounds and not for a localized focus of infection, the time of release of substances from the matrix appears to be appropriate for the intended purpose.

We then determined the minimum concentrations of the active substance at which no growth of microorganisms was observed. The results are shown in Table 1.

Based on the data obtained, a decision was made on the choice of preparations concentrations for further experiments. Thus, both ineffective and excessive concentrations were cut off.

At the next stage, the reduction in microorganism growth (%) in the presence of the tested preparations was calculated (Table 2).

The data obtained demonstrated that the MIC did not completely inhibit the growth of the test microorganisms. A concentration twice the MIC resulted in at least a 99% level of inhibition of bacterial growth. This suggests that it may be advisable to use concentrations of preparations exceeding the MIC in future experiments.

Previously, we developed polymeric forms of hemin based on poly(3-hydroxybutyrate), with active substance concentrations ranging from 1–5%.[20] Additionally, the incorporation of Chl into the polymer matrix of poly(lactic acid) (PLA) has been investigated.[57] The Chl concentrations were 0.1–0.5 wt.% relative to the PLA mass. In this study, we transitioned to a different polymer form based on PVP. The concentration of active substances in PVP was selected for studies of antibacterial activity on solid nutrient media (MPA), based on several factors. Hemin precipitated when its concentration in the polymer matrix exceeded 160 μg/mL. As a result, the resulting polymeric form was unstable and could not be used for further work. Chl was stable at comparable concentrations, although this did not lead to enhanced antibacterial effects. Therefore, we used polymeric forms of Chl with a maximum concentration of 0.5 wt.% (75 μg/mL).

To determine the antibacterial activity of the preparations on MPA, a method of measuring the diameters of the inhibition zones was used (Figures 6 and 7). From the diagrams in Figure 6, it can be seen that with a comparable diameter of the growth inhibition zone for microorganisms at the start of the experiment, this decrease occurs more slowly when the Chl-PVP complex is used. This indicates that this complex has a longer-lasting effect compared to the free substance.

Based on the results of the study into the antimicrobial properties of hemin as shown in the graphs (Figure 7), it can be concluded that when combined with the polymer, hemin exhibits increased activity and a longer duration of effect against *S. aureus* and *E. coli*. This can most clearly be seen at a dosage of 160 μg hemin, where the intensity of the antimicrobial effect of the hemin-polymer combination on *S. aureus* is 25–30% higher than that of free hemin. No significant increase in antimicrobial activity against *E. coli* was observed in the experiments.

Table 1. Determination of Chl and hemin in free and polymeric forms MICs relative to *S. aureus* and *E. coli.*

Preparations	MIC, μg/mL	
	S. aureus	*E. coli*
Chl	12.5±0.4	37.5±0.9
Chl-PVP	12.5±0.5	40.0±1.1
Hemin	17.5±0.8	35.0±0.7
Hemin-PVP	17.5±0.6	35.0±0.9

Table 2. Preparations-induced reduction in CFU growth of *S. aureus* and *E. coli*.

Preparations	Reduction in CFU growth, %			
	MIC		2×MIC	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
Chl	91.6±0.3	90.8±0.2	99.8±0.1	99.3±0.2
Chl-PVP	92.4±0.4	91.5±0.4	99.8±0.1	99.5±0.1
Hemin	92.5±0.2	91.2±0.3	99.7±0.1	99.4±0.2
Hemin-PVP	93.8±0.3	92.2±0.4	99.8±0.1	99.6±0.1

Conclusions

This study examines the polymeric forms of Chl and hemin obtained by incorporating them into PVP matrix. Working concentrations of these compounds were selected based on an analysis of their stability and antimicrobial

activity. The resulting electronic absorption spectrum profiles of Chl and hemin dissolved in DMSO are characteristic of these substances.

In the morphology of the polymeric films Chl-PVP and hemin-PVP, individual particles of heterogeneity were identified in the form of particles with a size of up to 1.7–2 μm, evenly distributed in the structure of the matrix. In hemin-PVP films, aggregated particles are more pronounced. Most likely, this is due to the difference in the coordinating properties of the two metal complexes in the polymeric matrix. However, the observed features of the morphology of the films nevertheless provide their antimicrobial action and possibly higher stability.

Based on the literature data, it was concluded that the obtained values of the binding constants of Chl and hemin to the polymer are relatively strong and typical for metal porphyrins.

The release of substances from polymeric matrices is influenced by various factors, such as the pH of the environment and the structure of the polymer itself. For the treatment of localized pathologies that are deeply situated, it may be beneficial to use formulations that ensure the sustained release of the active substance for a period of time sufficient to allow it to enter the bloodstream and achieve its therapeutic targets. The aim of our research is to investigate the inhibitory effects of Chl and hemin, as well as their respective polymeric forms, against *S. aureus* and *E. coli*, both of which are common causes of infections in damaged skin and mucous membranes. Accordingly, a release time of 13 h for Chl and 10 h for hemin has been found to be adequate and sufficient for inhibiting the growth of these bacteria, as confirmed by microbiological tests conducted *in vitro*. However, a definitive conclusion can only be drawn following *in vivo* studies.

During the study, data was obtained on the antibacterial properties of Chl and hemin in both free and polymeric forms. The minimum inhibitory concentration (MIC) for each preparation and its effect on the growth of test microorganisms were determined. An inhibition of bacterial cell growth with an efficiency greater than 99% was achieved using preparations at a concentration of 2×MIC.

Based on the data obtained in microbiological experiments on MPA, the following conclusions can be drawn: Chl in free form and the Chl-PVP complex have an antibacterial effect at a dosage of 15 μg for *S. aureus* and from 45 μg on *E. coli* growth. Hemin has an effect on *S. aureus* growth starting from a dosage of 20 μg in free form or as part of a polymeric complex, and on *E. coli* growth from 40 μg. Moreover, the antimicrobial effect of both Chl and hemin and their polymeric forms is directly dependent on the concentration of the active substance.

Based on the data collected from microbiological experiments conducted on MPA, it can be concluded that Chl in its free form and as a complex with PVP have an antibacterial effect on *S. aureus* at a dosage of 15 μg and on *E. coli* at a dosage starting from 45 μg. Hemin also has an effect on *S. aureus* starting from a dosage of 20 μg in its free form or as a component of a polymeric complex, while on *E. coli*, it is effective at dosages starting from 40 μg. Furthermore, the antimicrobial activity of both Chl and hemin in their free and polymeric forms is directly related to the concentration of the active substances.

Polymeric Chl- and hemin-containing complexes hold promise for practical use as an alternative to conventional antimicrobial agents, providing sustained action against both gram-positive and gram-negative microorganisms. These results can contribute to the development of new medicines against microbial infections. In our opinion, Chl and its polymeric complex have greater potential as an antimicrobial agent, since at a lower dosage it had a more intense inhibitory effect (*in vitro*) on test microorganisms than hemin and hemin-PVP. It will be possible to draw a more reasoned and substantiated conclusion after *in vivo* testing. Furthermore, we intend to evaluate the photodynamic activity of Chl, both in its free and polymeric forms, and compare these findings with current studies.

Acknowledgements. The work was carried out within the framework of the project «Research on the problems of recycling waste of natural origin for the practical use of the products obtained» (122122600056-9).

Author Contributions. Dmitry V. Gruznov: Methodology; Investigation; Formal analysis; Writing - Original Draft. Olga A. Gruznova: Conceptualization; Methodology; Investigation; Writing - Original Draft. Nikolay I. Popov: Methodology; Resources; Writing - Review & Editing; Supervision. Gulizar Sh. Shcherbakova: Investigation; Validation; Writing - Review & Editing. Ekaterina N. Shuteeva: Investigation; Visualization. Alina V. Konyashkina: Investigation. Irina P. Chesnokova: Investigation; Visualization. Lilia F. Plaksina: Investigation. Anton V. Lobanov: Supervision; Project administration.

References

1. Begunov R.S., Egorov D.O., Chetvertakova A.V., Savina L.I., Zubishina A.A. *Antibiotics and Chemotherapy* [Бегунов Р.С., Егоров Д.О., Четвертакова А.В., Савина Л.И., Зубишина А.А *Антибиотики и химиотерапия*] **2023**, *68(3–4)*, 19–24. DOI: 10.37489/0235-2990-2023-68-3-4-19-24.
2. Tovmasyan A., Batinic-Haberle I., Benov L. *Antioxidants* **2020**, *9*(972), 1–14. DOI: 10.3390/antiox9100972.
3. Dharmaratne P., Sapugahawatte D.N., Wang B., Chan C.L., Lau K.-M., Lau C.B.S., Fung K.P., Ng D.K.P., Ip M. *Eur. J. Med. Chem.* **2020**, *200*, 112341. DOI: 10.1016/j.ejmech.2020.112341.
4. Anderson G.G., O'Toole G.A. *Curr. Top. Microbiol. Immunol.* **2008**, *322*, 85–105. DOI: 10.1007/978-3-540-75418-3.
5. Wang D., Ning Q., Deng Z., Zhang M., You J. *Environ. Pollut.* **2022**, *307*, 119603. DOI: 10.1016/j.envpol.2022.119603.
6. Koifman O.I., Ageeva T.A., Beletskaya I.P., Averin A.D., Yakushev A.A., Tomilova L.G., Dubinina T.V., Tsivadze A.Yu., Gorbunova Yu.G., Martynov A.G., Konarev D.V., Khasanov S.S., Lyubovskaya R.N., Lomova T.N., Korolev V.V., Zenkevich E.I., Blaudeck T., von Borczyskowski Ch., Zahn D.R.T., Mironov A.F., Bragina N.A., Ezhov A.V., Zhdanova K.A., Stuzhin P.A., Pakhomov G.L., Rusakova N.V., Semenishyn N.N., Smola S.S., Parfenyuk V.I., Vashurin A.S., Makarov S.V., Dereven'kov I.A., Mamardashvili N.Zh., Kurtikyan T.S., Martirosyan G.G., Burmistrov V.A., Aleksandriiskii V.V., Novikov I.V., Pritmov D.A., Grin M.A., Suvorov N.V., Tsigankov A.A., Fedorov A.Yu., Kuzmina N.S., Nyuchev A.V., Otvagin V.F., Kustov A.V., Belykh D.V., Berezin D.B., Solovieva A.B., Timashev P.S., Milaeva E.R., Gracheva Yu.A., Dodokhova M.A., Safronenko A.V.,

Shpakovsky D.B., Syrbu S.A., Gubarev Yu.A., Kiselev A.N., Koifman M.O., Lebedeva N.Sh., Yurina E.S. Macroheterocyclic Compounds – a Key Building Block in New Functional Materials and Molecular Devices. *Macroheterocycles* **2020**, *13*, 311–467. DOI: 10.6060/mhc200814k.

7. Koifman O.I., Ageeva T.A., Kuzmina N.S., Otvagin V.F., Nyuchev A.V., Fedorov A.Yu., Belykh D.V., Lebedeva N.Sh., Yurina E.S., Syrbu S.A., Koifman M.O., Gubarev Yu.A., Bunin D.A., Gorbunova Yu.G., Martynov A.G., Tsivadze A.Yu., Dudkin S.V., Lyubimtsev A.V., Maiorova L.A., Kishalova M.V., Petrova M.V., Sheinin V.B., Tyurin V.S., Zamilatskov I.A., Zenkevich E.I., Morshnev P.K., Berezin D.B., Drondel E.A., Kustov A.V., Pogorilyy V.A., Noev A.N., Eshtukova-Shcheglova E.A., Plotnikova E.A., Plyutinskaya A.D., Morozova N.B.,. Pankratov A.A, Grin M.A., Abramova O.B., Kozlovtseva E.A., Drozhzhina V.V., Filonenko E.V., Kaprin A.D, Ryabova A.V., Pominova D.V., Romanishkin I.D., Makarov V.I., Loschenov V.B., Zhdanova K.A., Ivantsova A.V., Bortnevskaya Yu.S., Bragina N.A., Solovieva A.B., Kuryanova A.S., Timashev P.S. *Macroheterocycles* **2022**, *15*, 207–302. DOI: 10.6060/mhc224870k.
8. Zhao J., Xu L., Zhang H., Zhuo Y., Weng Y., Li S., Yu D.H. *Colloids Surf., B* **2021**, *207*, 111974. DOI: 10.1016/ j.colsurfb. 2021.111974.
9. Kustov A.V, Belykh D.V., Smirnova N.L., Venediktov E.A, Kudayarova T.V., Kruchin S.O., Khudyaeva I.S., Berezin D.B. *Dyes Pigm.* **2017**, *149*, 553–559. DOI: 10.1016/ j.dyepig. 2017.09.073.
10. Kim H., Yin K., Falcon D.M. Toxicol. Appl. Pharm. **2019**, *374*, 77–85. DOI: 10.1016/j.taap.2019.04.025.
11. da Silva Souza Campanholi K., Jaski J.M., da Silva Junior R.C., Zanqui A.B., Lazarin-Bidóia D., da Silva C.M., da Silva E.A., Hioka N., Nakamura C.V., Cardozo-Filho L., Caetano W. *J. Photochem. Photobiol., B* **2020**, *203*, 111763. DOI: 10.1016/ j.jphotobiol.2019.111763.
12. Ladan H., Nitzan Y., Malik Z. *FEMS Microbiol. Lett.* **1993**, *112*, 173–178. DOI: 10.1111/j.1574-6968.1993.tb06444.x.
13. Stojiljkovic I., Kumar V., Srinivasan N. *Mol. Microbiol.* **1999**, *31*, 429–442. DOI: 10.1046/j.1365-2958.1999.01175.x.
14. Stojiljkovic I., Evavold B.D., Kumar V. *Expert Opin. Inv. Drug.* **2001**, *10*, 309–320. DOI: 10.1517/13543784.10.2.309.
15. Pareek S., Sagar N.A., Sharma S., Kumar V., Agarwal T., González-Aguilar G.A., Yahia E.M. *Fruit and Vegetable Phytochemicals* **2018**, *2*, 269–284. DOI: 10.1002/ 9781119158042
16. Woodward R.B., Ayer W.A., Beaton J.M., Bickelhaupt F., Bonnett R. *J. Am. Chem. Soc.* **1960***, 82*, 3800–3802. DOI: 10.1021/ja01499a093.
17. Fleming I., *Nature* **1967**, *216(5111)*, 151–152. DOI:10.1038/ 216151a0.
18. Berezin B.D., Rumyantseva S.V., Moryganov A.P., Berezin M.B. *Russ. Chem. Rev.* **2004**, *73(2)*, 185-194. DOI: 10.1070/ RC2004v073n02ABEH000773.
19. Shlyakhtin S.V., Trukhachova T.V. *Bulletin of Pharmacy* [Шляхтин С.В., Трухачева Т.В. *Вестник фармации*] **2010**, *2*, 1–20.
20. Gruznov D.V., Gruznova O.A., Popov N.I., Alieva Z.I., Stepanova S.P., Shcherbakova G.Sh., Kitushina E.V., Tyubaeva P.M., Varyan I.A., Olkhov A.A., Popov A.A., Klimenko I.V., Lobanov A.V. *Macroheterocycles* **2023**, *16*, 261–265. DOI: 10.6060/mhc2345386g.
21. Grenoble D.C., Drickamer H.G. *Proc. Natl. Acad. Sci. USA* **1968**, *61*, 1177–1182. DOI: 10.1073/pnas.61.4.1177.
22. Martusevich A.K., Kamakin N.F. *Vyatka Medical Bulletin* [Мартусевич А.К., Камакин Н.Ф. *Вятский медицинский вестник*] **2011**, *3–4*, 54–60.
23. Senderova O.M., Glinskaya E.V., Glinsky A.A., Gorohova L.A. *Siberian Medical Journal* [Сендерова О.М., Глинская Е.В., Глинский А.А., Горохова Л.А. *Сибирский медицинский журнал*] **2009**, *2*, 108–110.
24. Andreychenko S.A., Sukonnikov T.A., Bychinin M.V., Klypa T.V. *Russian Journal of Anaesthesiology and Reanimatology* [Андрейченко С.А., Суконников Т.А., Бычинин М.В., Клыпа Т.В. *Анестезиология и реаниматология*] **2019**, *3*, 90–96. DOI: 10.17116/anaesthesiology201903190.
25. Malik Z., Ladan H., Nitzan Ye., Ehrenberg B. *J. Photochem. Photobiol., B* **1990**, *6*, 419–430. DOI: 10.1016/1011-1344(90) 85115-D.
26. Tsatsakis A., Stratidakis A.K., Goryachaya A.V., Tzatzarakis M.N., Stivaktakis P.D., Docea A.O., Berdiaki.Ai., Nikitovic D., Velonia K., Shtilman M.I., Rizos A.K., Kuskov A.N. *Food Chem. Toxicol.* **2019**, *127*, 42-52. DOI: 10.1016/ j.fct.2019. 02.041.
27. Biglari N., Zare E.N. *Alexandria Engineering Journal* **2024**, *87*, 277-299. DOI: 10.1016/j.aej.2023.12.041.
28. Ma R., Shi L. *Giant* **2021**, *8*, 100074. DOI: 10.1016/ j.giant.2021.100074.
29. Mendrek B., Oleszko-Torbus N., Teper P., Kowalczuk A. *Prog. Polym. Sci.* **2023**, *139*, 101657. DOI: 10.1016/ j.progpolymsci. 2023.101657.
30. Nath P.C., Sharma R., Debnath S., Nayak P.K., Roy R., Sharma M., Inbaraj B.C., Sridhar K. *Int. J. Biol. Macromol.* **2024**, *259(1)*, 129129. DOI: 10.1016/j.ijbiomac.2023.129129.
31. González-Ulloa G., Jiménez-Rosado M., Benhnia M.R.E.I., Romero A., Ruiz-Mateos E., Ostos F.J., Perez-Puyana V. *J. Mol. Liq.* **2023**, *384*, 122224. DOI: 10.1016/ j.molliq.2023.122224.
32. Guo J., Liu Y., Zha J., Han H., Chen Y., Jia Z. *Polym. Chem.* **2021**, *12(6)*, 858 *Alexandria* 866. DOI: 10.1039/d0py01465f.
33. Hajizadeh S., Dicko C., Bülow L. *Front. Bioeng. Biotechnol.* **2022**, *10*, 1072153. DOI: 10.3389/fbioe.2022.1072153.
34. Tyubaeva P.M, Zykova A.K *News of higher educational institutions. Textile Industry Technology* [Тюбаева П.М., Зыкова А.К. *Изв. высш. учебн. завед. Технол. Текст. Пром.*] **2022**, *4(400)*, 223–233.
35. Bi S., Zhao T., Jia X., He P. *Biosens. Bioelectron.* **2014**, 57, 110–116. DOI: 10.1016/j.bios.2014.01.025.
36. Span K., Verhoef J.J., Hunt H., van Nostrum C.F., Brinks V., Schellekens H., Hennink W.E. *Eur. J. Pharm. Biopharm.* **2016**, *108*, 226-234. DOI: 10.1016/j.ejpb.2016.09.002.
37. Rizzi V., Fini P., Semeraro P., Cosma P. *Colloids Surf., B* **2016**, *142*, 239–247. DOI: 10.1016/j.colsurfb.2016.02.062.
38. Rizzi V., Gubitosa J., Fini P., Fraix A., Sortino S., Agostiano A., Cosma P. *Mat. Sci. Eng., C* **2021**, *119*, 111593. DOI: 10.1016/j.msec.2020.111593.
39. Kang Y.R., Lee Y.K., Kim Y.J., Chang Y.H. *Food Chem.* **2019**, *272*, 337-346. DOI: 10.1016/j.foodchem.2018.08.063.
40. Zhang Z.H., Peng H., Ma H., Zeng X.A. *J. Food Eng.* **2019**, *245*, 149-156. DOI: 10.1016/j.jfoodeng.2018.10.011.
41. Sharifi K.A., Pirsa, S. *Mater. Chem. Phys.* **2021**, *267*, 124580. DOI: 10.1016/j.matchemphys.2021.124580.
42. Soltaninejad V., Maleki A. *J. Photochem. Photobiol., A* **2021**, *404*, 112906. DOI: 10.1016/j.jphotochem.2020.112906.
43. Soltaninejad V., Ahghari M.R., Taheri-Ledari R., Maleki A. *Langmuir* **2021**, *37(15)*, 4700–4713. DOI: 10.1021/ acs.langmuir.1c00501.
44. Lobanov A.V., Klimenko I.V., Nevrova O.V., Zhuravleva T.S. *Russ. J. Phys. Chem. A* **2014**, *88*, 875-880. DOI: 10.1134/ S0036024414050173.
45. Lavrik O.I. *Physical chemistry of biopolymers: Course of lectures.* Novosibirsk: Novosibirsk National Research State University [Лаврик О.И. *Физическая химия биополимеров. Курс лекций*. Н.: ФГАОУ ВО "Новосибирский национальный исследовательский государственный университет" Факультет естественных наук], **2014**. 107 p.
46. Sanders C.R. *Biomolecular Ligand-Receptor Binding Studies: Theory, Practice, and Analysis*. Nashville: Vanderbilt University, **2010**. 43 p.
47. Orstan A., Wojcik J.F. *J. Chem. Educ.* **1987**, *64(9)*, 814–816. DOI: 10.1021/ed064p814.

48. Glasgow B.J., Abduragimov A.R. *MethodsX* **2018**, *5*, 345–351. DOI: 10.1016/j.mex.2018.04.007.
49. Sheehan D. *Physical Biochemistry: Principles and Applications*. Southern Gate: John Wiley & Sons, **2009**. 424 p.
50. Strickland J.A., Marzilli L.G., Gay K.M., Wilson W.D. *Biochemistry* **1988**, *27*(24), 8870–8878. DOI: 10.1021/bi0042 4a027.
51. Hwang B. *Porphyrin-Polymer Supramolecular Assemblies in Water: Spectroscopic and Thermodynamic Properties*. Fort Worth: Texas Christian University, 2014. 42 p.
52. Jin H., Dai X-H., Wu C., Pan J-M., Wang X-H., Yan Y-S., Liu D-M., Sun L. *Eur. Polym. J.* **2015**, *66*, 149–159. DOI: 10.1016/j.eurpolymj.2015.01.047.
53. Hamidian K., Barani M., Adeli-Sardou M., Sarani M., Daliran S., Oveisi A.R. *Heliyon* **2023**, *9(1)*, e12634. DOI: 10.1016/j.heliyon.2022.e12634.
54. Kuntsche J., Rajakulendran K., Sabriye H.M.T., Tawakal N., Khandelia H., Hakami Zanjani A.A. *J. Colloid Interface Sci.* **2023**, *651*, 750–759. DOI: 10.1016/j.jcis.2023.07.152.
55. Faustova M.R., Nikolskaya E.D., Mollaev M.D., Sokol M.B., Zabolotsky A.I., Zhunina O.A., Fomicheva M.V., Shvets V.I., Lobanov A.V., Yabbarov N.G. *Proceedings of the Academy of Sciences. Chemical Series* [Фаустова М.Р., Никольская Е.Д., Моллаев М.Д., Сокол М.Б., Заболотский А.И., Жунина О.А., Фомичева М.В., Швец В.И., Лобанов А.В., Яббаров Н.Г. *Изв. Акад. наук. Сер. Хим.*] **2019**, *12*, 2216–2224.
56. Bazylińska U., Frąckowiak R., Brzózka Z., Wilk K.A. *J. Photochem. Photobiol., B* **2017**, *166*, 169–179. DOI: 10.1016/j.jphotobiol.2016.11.012.
57. Gruznov D.V., Gruznova O.A., Lobanov A.V., Shcherbakova G.Sh., Chesnokova I.P. *BIO Web of Conferences* **2024**, *83*, 02001. DOI: 10.1051/bioconf/20248302001.

Received 28.03.2024
Accepted 02.11.2024