составит соответственно около 7 и 16 мкг/кг массы тела в сутки в пересчете на стандартную массу тела 60 кг и 6 и 14 мкг/кг массы тела в сутки пересчете на 70 кг массы тела. Риски оцениваются как приемлемые (до 5 %).

В суммарное суточное поступление бария для 1-2 моделей алиментарной экспозиции (реалистичный сценарий) и концентрации его в воде на уровне медианы удельный вклад воды не превышал 50 % (40–42 %).

Результаты исследований послужили доказательной базой при актуализации норматива бария в питьевой воде и позволили обосновать возможность корректировки ПДК в сторону его «смягчения» по критериям риска здоровью с учетом региональных сценариев воздействия – 0,7 мг/л. Подготовлен проект постановления Совета Министров Республики Беларуси «О внесении изменения в постановление Совета Министров Республики Беларусь от 25 января 2021 г. № 37» в части внесения изменений в таблицу 2 гигиенического норматива «Показатели безопасности питьевой воды», согласован с ключевыми заинтересованными. Планируемый срок утверждения – 2023 год.

Дополнительно внесено предложение о дополнении формы ведомственной отчетности 17(18)-(Сведения о санитарном состоянии территории)) в части требований о предоставления данных о соответствии проб воды нормативным требованиям по показателям «барий» и «бор» (ранее данные по указанным показателям на уровне республики отсутствовали).

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FLUORESCENCE ANALYSIS OF THE TEMOPORFIN DISSOCIATION KINETICS FROM COMPLEXES WITH POLYMER AND MONOMERIC β-CYCLODEXTRIN IN LIPOSOMES

ФЛУОРЕСЦЕНТНЫЙ АНАЛИЗ КИНЕТИКИ ДИССОЦИАЦИИ ТЕМОПОРФИНА ИЗ КОМПЛЕКСОВ С ПОЛИМЕРНЫМ И МОНОМЕРНЫМ β-ЦИКЛОДЕКСТРИНОМ В ЛИПОСОМЫ

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Nanosized materials based on cyclodectrins (CD) are a promising platform for creating new pharmacological forms of drugs. Today, it has been shown that β-CD is characterized by a high affinity for several tetrapyrrole compounds, including Temoporfin, an effective photosensitizer (PS) for photodynamic therapy. In this work, we studied the fluorescence characteristics of Temoporfin in solutions and in complexes with monomeric/polymeric

β-CD and lipid vesicles. Differences in the spectral characteristics of PS fluorescence in complexes with β-CD derivatives, and liposomes were established. Based on these established differences, an analysis method has been developed, which makes it possible to monitor the distribution dynamics between different binding sites of Temoporfin molecules in complex systems.

Наноразмерные материалы на основе циклодектринов (ЦД) являются перспективной платформой для создания новых фармакологических форм лекарственных препаратов. К настоящему времени показано, что для β ЦД характерно высокое сродство к ряду тетрапиррольных соединений, в том числе к Темопорфину, эффективному фотосенсибилизатору (ФС) для метода фотодинамической терапии. В данной работе были исследованы флуоресцентные характеристики Темопорфина в растворах и в составе комплексов с мономерными/полимерными β-ЦД, липидными везикулами. Установлены различия в спектральных характеристиках флуоресценции ФС в комплексах с производными β-ЦД и липосомами. На основании установленных отличий разработан метод анализа, позволяющий отслеживать в динамике распределение между различными центрами связывания молекул Темопорфина в сложных системах.

Keywords: photodynamic therapy, photosensitizer, cyclodextrrin, liposome, inclusion complexes, polymer.

Ключевые слова: фотодинамическая терапия, фотосенсибилизатор, циклодекстрин, липосомы, комплексы включения, полимер.

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Nanosized materials based on cyclodextrin derivatives (CD) attract interest as a possible platform for the development of new pharmacological forms of drug compounds. CDs are supramolecular cyclic structures, formed by glucose oligomers linked by α-1,4 glycosidic bonds. The spatial structure of the CD can be represented as a truncated hydrophilic cone with a hydrophobic region inside (Fig. 1). This feature of the structure of CD molecules makes it possible to form inclusion complexes with various organic and inorganic compounds.

Studies conducted with various CD derivatives have shown that they form inclusion complexes with tetrapyrrole compounds with high efficiency [1]. Such molecules are widely used in clinical practice for photodynamic therapy (PDT) - a new minimally invasive treatment method based on the application of the effect of selective laser photodestruction of target cells/tissues, pre-stained with special substances - photosensitizers (PS).

The introduction of PS in complexes into CD prevents aggregation, increases the stability and bioavailability of PS, and influences the processes of biodistribution and localization of photoactive compounds [2]. It should be noted that, despite the high values of the complex formation constants of PS with CD, the rate of dissociation of PS molecules from inclusion complexes is high. This is a significant limitation of the possibility of their use for the regulation of the biodistribution processes of PS and other drug compounds in the patient's body.

One of the effective ways to increase the lifetime of inclusion complexes is the polymerization of CD, by taking the advantage of crosslinking agents. Thus, the presence of two reactive groups in epichlorohydrin allows it to bind bivalently with CD, and consequently, to form polymeric structures (nanosponges). It is believed that such materials have a high associative capacity (Fig. 1), and significantly increase the lifetime of complexes with a drug compound compared to native CD [3].

To compare objectively between the PS dissociation from inclusion complexes, and monomeric and polymeric CDs, it is necessary to develop quantitative methods that allow quantifying the rate of PS release from inclusion complexes, and subsequent binding to biological structures.

Our research aimed to develop a fluorescence method for the analysis of redistribution processes between inclusion complexes with monomeric/polymeric β-CD, and liposomes of the well-known photosensitizer Temoporfin (mTHPC). It was previously shown in our laboratory that Temoporfin successfully forms inclusion complexes with both monomeric and polymeric CDs [1].

Photosensitizer mTHPC was provided by Biolitec research GmbH (Jena, Germany). Soluble carboxymethyl-β-cyclodextrin polymer (CM-β-CDPS) and soluble cyclodextrin polymer (β-CDPS) were purchased from CYCLOLAB R&D. Ltd., (Budapest, Hungary). CM-β-CDPS and β-CDPS are formulated by random crosslinking of the cyclodextrin monomers with epichlorohydrin, and have an approximate molecular weight of 153 kDa and 152 kDa with an estimated cyclodextrin content of 50%–70%. Methyl-β-cyclodextrin (M-β-CD) was obtained from AraChem (Tilburg, Nederland). Dipalmitoylphosphatidyl choline (DPPC) was purchased from Sigma Chemical Co. (Avanti, USA).

DPPC unilamellar liposomes were made by filter extrusion technique as described in Ref. [4]. After extrusion liposomes were stored at 4 °C.

Aqueous solutions of monomeric/polymeric CDs and liposomes were prepared on the basis of phosphate-buffered saline (PBS) (8 g NaCl, 0.20 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 per 1 L of solution; pH = 7.4). The stock buffer solution during the operation was diluted with distilled water in a ratio of 1:4.

Spectral and fluorescent characteristics were measured using a Solar CM-2203 spectrofluorimeter (SOLAR, Republic of Belarus) equipped with a temperature-controlled cell with a magnetic stirrer. Measurement of mTHPC concentration was carried out photometrically at a wavelength of λ=650 nm (extinction coefficient, ε=30000 M-1 cm-1).

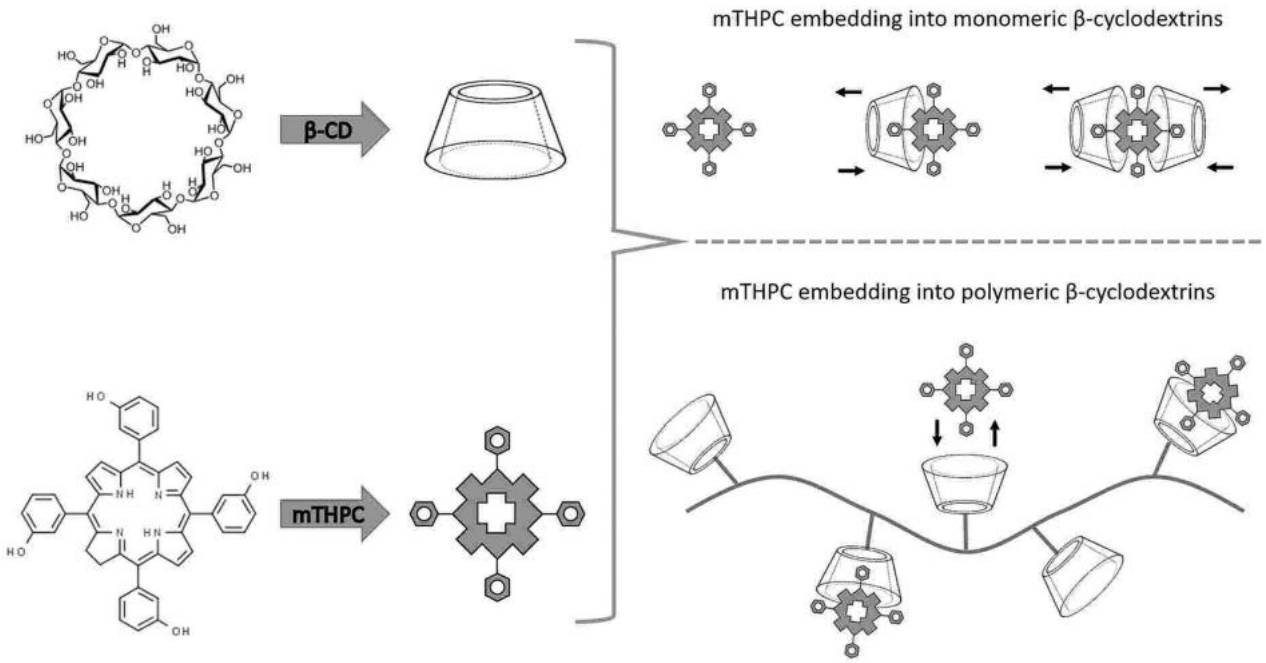


Figure 1 – Schematic representation of the formation of an inclusion complex between a monomeric and polymeric CDs (host) and a mTHPC (guest)

We studied the fluorescent characteristics of mTHPC in solutions, in complexes with monomeric/polymeric CDs, and also as part of lipid vesicles. In organic solvents, mTHPC fluoresces intensely (the fluorescence quantum yield is about 10%). When mTHPC is transferred into an aqueous solution, nonfluorescent aggregates of PS molecules are formed. The addition of M-β-CD, β-CDPS, CM-β-CDPS, and liposomes is accompanied by the monomerization of mTHPC molecules and restoration of their fluorescent properties.

The binding of Temoporfin to the monomeric M-β-CD does not lead to noticeable changes in the shape of the bands in the fluorescence excitation spectra relative to the organic solvent, but is accompanied by a slight (2 nm) shift towards longer wavelengths (Fig. 2).

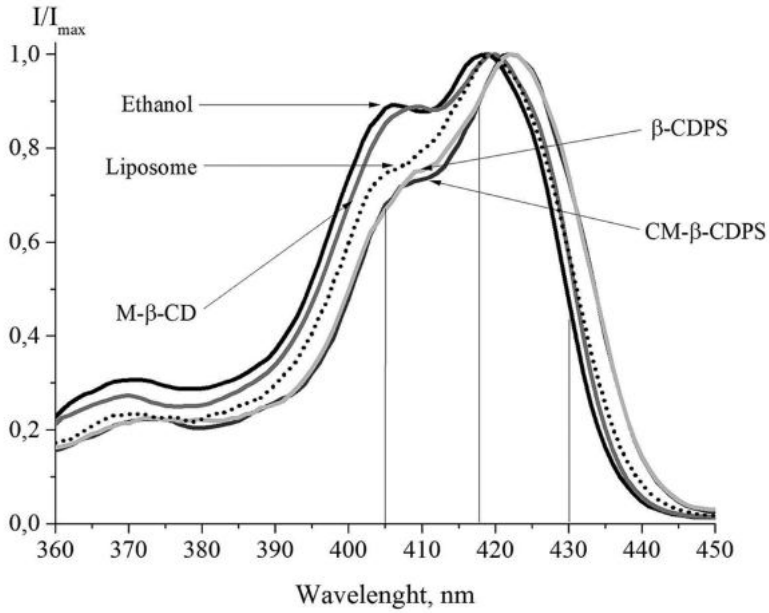
When mTHPC is added to a solution of polymeric CDs, the maximum of the fluorescence spectrum is shifted to the long wavelength region by 4–5 nm, the spectra become narrower, and the intensity of the short-wavelength shoulder decreases (Fig. 2). Similar changes are also observed in the solution of liposomal vesicles; however, their amplitude is less pronounced.

The characteristic features of spectral characteristics of mTHPC in the studied systems can be used to control the nature of Temoporfin binding centers [5]. The value of the ratio of fluorescence intensities at two wavelengths in the Soret band ($I_{\lambda 1}/I_{\lambda 2)}$ can act as an indicator of the nature of the binding centers of this PS in various structures.

To study the processes of mTHPC redistribution between DPPC liposomes and cyclodextrin derivatives, the following characteristic fluorescence excitation wavelengths were used $\lambda_1 = 417$ nm and $\lambda_2 = 420$ nm for redistribution with CM-β-CDPS (β-CDPS) and with $\lambda_1 = 405$ nm and $\lambda_2 = 430$ nm for redistribution with M-β-CD. Fluorescence registration was carried out at a wavelength $\lambda = 653$ nm.

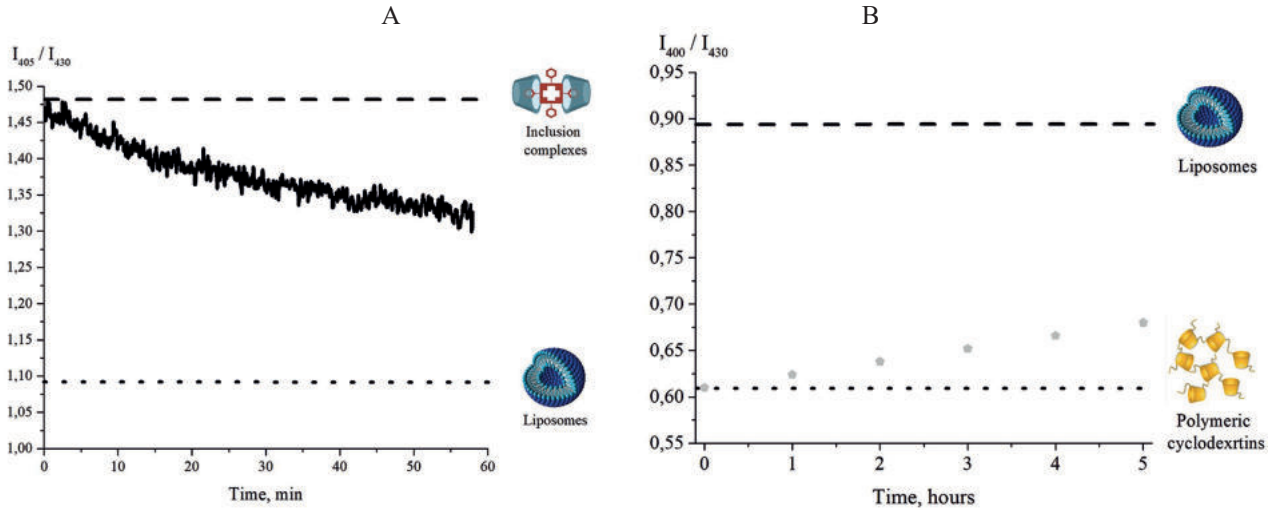
For mTHPC in complex with CM-β-CDPS (β-CDPS), the value of parameter $I_{417}/I_{430} = 0.61$ (0.60) corresponds to complete binding to PS with polymeric CDs. Values $I_{417}/I_{430} = 0.89$ indicate the presence of mTHPC in lipid vesicles. In the case of monomeric M-β-CD (Fig. 3), the values of the parameter $I_{405}/I_{430} = 1.47$ correspond to the location of Temoporfin in inclusion complexes, and $I_{405}/I_{430} = 1.08$ – in liposomes.

In the course of the study, differences were found in the fluorescence characteristics of mTHPC in complexes with monomeric/polymeric β-CDs and liposomes. It has been demonstrated that the spectral method based on the analysis of the features of the Soret band in the mTHPC fluorescence excitation spectrum can be used to analyze the nature of binding sites. The same developed method can be used to analyze the processes of dissociation and association of Temoporfin molecules in the system monomeric CD vs liposomes, and polymeric CD vs liposomes.



Concentration of mTHPC – 0,1 μM.
Concentration of DPPC liposomes – 0,1 mM.
Concentration of polymeric and monomeric CDs – 10 μM.
Temperature – 20 °C.

Figure 2 – Normalized fluorescence excitation spectra of mTHPC in ethanol and various biological media



Concentration of mTHPC – 0,1 μM.
Concentration of DPPC liposomes – 0,1 mM.
Concentration of polymeric and monomeric CDs – 10 μM.
Temperature – 20 °C.

Figure 3 – Redistribution of mTHPC from A) M-β-CD and B) to liposomes

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