Photodynamic therapy (PDT) has lately been involved in the treatment of oncological, cardiovascular, skin and eye diseases. PDT is founded on the use of sensitizers, which have a longer retention time in cancerous cells. During the photoactivity of sensitizers, using visible radiation of suitable wavelength, the generation of cytoxic material is occurred which leads to irreversible damage in cancerous cells. In contrast to conventional treatment methods (surgery, radiotherapy and chemotherapy) PDT allows selective removal of cancerous cells without damaging the surrounding healthy tissue (1,2,7,8).

Today the quite often used sensitizer is Photofrin II. This sensitizer is one of the so-called "first generation sensitizers", which sets the standard for its class. Above all its selectivity is too narrow. Furthermore, thanks to its narrow extinction coefficient it is necessary to use a large amount of the sensitizer to achieve a photodynamic reaction.

The above mentioned problems lead to the creation so-called "second generation sensitizers". This includes porphyrin, phthalocyanines, naphtalocyanines, chlorins, and also polypyrrolic sensitizers TPSS, ZnTPPS and PdTPPS. "Second generation sensitizers" are considerably more selective which enhances the effectivity of PDT (3.5.6). Naturally they are hydrophobic, so they dissolve poorly in water. This problem can be solved by the use of a suitable sensitizer carrier (polymer particles, liposomes, antibodies, etc.).

Cyclodextrins (CD) are currently under intensive study as one of the suitable carriers for this entire class of medicine, and also of photodynamically active materials. Cyclodextrins are cyclic oligosacharades, compounded of 6, 7 a 8 (α, β, γ) α – 1, 4–D – glucopyranose units. Photodynamically active materials are bound in the cyclodextrin cavity with the help of hydrophobic and van der Waals forces. This binding with the CD allows a hydrophobic sensitizer to be transported in a water based medium. CD also has a significant monomerization effect, which inhibits the aggregation of sensitizers and increases the quantum yield and life span of the excited state of the sensitizer (4).

**Introduction**

Photodynamic therapy (PDT) has lately been involved in the treatment of oncological, cardiovascular, skin and eye diseases. PDT is founded on the use of sensitizers, which have a longer retention time in cancerous cells. During the photoactivity of sensitizers, using visible radiation of suitable wavelength, the generation of cytoxic material is occurred which leads to irreversible damage in cancerous cells. In contrast to conventional treatment methods (surgery, radiotherapy and chemotherapy) PDT allows selective removal of cancerous cells without damaging the surrounding healthy tissue (1,2,7,8).

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**Material and methods**

The absorption spectra of the cultivation medium (DMEM), the selected solution of porphyrin sensitizers TPPS, ZnTPPS and PdTPPS, and these sensitizers with cyclodextrin carriers HP-αCD, HP-βCD and HP-γCD (2-hydroxypropyl-α, β, γ-cyclodextrin) in a suitable environment for the cultivation of cancerous cell lines, and to determine the optimal radioactive conditions for maximizing photodynamic effects in cancerous cells.

**Summary:** The objective of our work was to describe the photophysical properties (absorption and fluorescence) of the sensitizers TPSS, ZnTPPS, a PdTPPS and above all the complexes of these sensitizers with cyclodextrin carriers HP-αCD, HP-βCD and HP-γCD (2-hydroxypropyl-α, β, γ-cyclodextrin) in a suitable environment for the cultivation of cancerous cell lines, and to determine the optimal radioactive conditions for maximizing photodynamic effects in cancerous cells.

**Key words:** Polypyrrolic sensitizer; Cyclodextrin carrier; PDT; Absorption spectrum; Fluorescence spectrum
Results

Fig. 1 shows the absorption spectrum of the polypyrrolic sensitizer solution. The wavelength absorption maximum for sensitizer TPPS₄ is 415 nm. The sensitizer ZnTPPS₄ expresses a shift in absorption maximum to the short wavelength of 2 nm, thus the level of 413 nm. The absorption maximum of sensitizer PdTPPS₄ is near the wavelength 423 nm. Fig. 2 shows the absorption spectra of the sensitizer ZnTPPS₄ in combination with the cyclodextrin carriers, which demonstrates that the type of used carrier only slightly affects the form of the spectrum.

In Fig. 3 the fluorescent emission spectra are obtained near the wavelength which corresponds to the sensitizer absorption maximum in the Soret region. The spectra illustrate a strong difference in the location of the maxima and the fluorescence intensity, where as with all of sensitizers there emerges two distinct fluorescent bands. TPPS₄ shows the highest fluorescence. The fluorescence intensity is near that of other sensitizers, after which sensitizers ZnTPPS₄ a PdTPPS₄ diminish along with it, such that it highlights the longer wavelength maximum with regard to the maximum short wavelength. Sensitizer PdTPPS₄ gains a long wavelength band with a higher intensity of fluorescence. Individual sensitizers also differ in the position of their fluorescent maxima. Fluorescent emission spectra of the sensitizer ZnTPPS₄ in combination with the cyclodextrin carriers, which demonstrates that the type of used carrier only slightly affects the form of the spectrum. We found similar behavior in the recordings of the spectra for sensitizers TPPS₄ a PdTPPS₄.

Discussion

The absorption spectra were measured in conditions parallel to that of the cultivated tumor cells. The spectra were measured in the cultivation medium; its character can influence the resulting exposure parameters because a portion of active radiation will likely be absorbed by the cultivation medium itself. The absorption spectra of the sensitizer solutions differ in absorption spectra of the cultivation media only in the region of short wavelength of light. Minor differences in the spectra of different sensitizers in this area fit their individual chemical structure (Fig. 1). The absorption of the sensitizer solutions in the ultraviolet area of the electromagnetic spectrum and the area around the wavelength 560 nm pertains to its cultivation medium, which was used as the solvent. The cyclodextrin carrier caused a shift in the absorption maxima of individual sensitizer solutions (Fig. 1 and 2). If we take a sensitizer in a cyclodextrin carrier then the absorption spectra of these complexes will not differ from the type of carrier used (Fig. 2). The fluorescent spectra of the sensitizers differ in their fluorescent intensity, in the long wavelength maxima (Fig. 3). These differences are attributed to their individual chemical structure.
Conclusion

The study of photophysical properties allows the establishment of irradiation parameters and conditions of the cultivation of cancer cells for combined studies of the cytotoxicity and phototoxicity of sensitizers bound in cyclo-dextrin carriers in vitro methods.

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References

1. Jori G. In vivo transport and pharmacokinetic behavior of tumour photosensitizers. Ciba Found Symp 1989;146:78–86.
2. Konan YN, Gurny R, Allemann E. State of the art in the delivery of photosensitizers for photodynamic therapy. J Photochem Photobiol B. 2002;66(2):89–106.
3. Leach MW, Higgins RJ, Autry SA, Boggan JE, Lee SJ, Smith KM. In vitro photodynamic effects of boil chlorin p6: cell survival, localization and ultrastructural changes. Photochem Photobiol 1993;58(5):653–60.
4. Moan J. Properties for optimal PDT sensitizers. J Photochem Photobiol B. 1990;5(3-4):521–4.
5. Mosinger J, Kliment V Jr, Sejbal J, Kubat P, Lang K. Host – guest complexes of anionic porphyrin sensitizers with cyclodextrins. J Porphyrins Phthalocyanines 2002;6:513–23.
6. Ochsner M. Photodynamic therapy: the clinical perspective. Review on applications for control of diverse tumorous and non-tumorous diseases. Arzneimittelforschung 1997;47(11):1185–94.
7. Strauss WS, Gschwend MH, Sailer R, Schneckenburger H, Steiner R, Ruck A. Intracellular fluorescence behaviour of meso – tetra (4-sulphonatophenyl) porphyrin during photodynamic treatment at various growth phases of cultured cells. J Photochem Photobiol B. 1995;28:155–61.
8. Wood SR, Holroyd JA, Brown SB. The subcellular localization of Zn (II) phthalocyanines and their redistribution on exposure to light. Photochem Photobiol 1997;65:397–402.

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