https://doi.org/10.15407/ujpe65.7.638

N. KUTSEVOL,[1] YU. KUZIV,[1] V. ZORIN,[2,3] I. KRAVCHENKO,[2] T. ZORINA,[2] A. MARYNIN,[4] L. BULAVIN[1]

[1] Taras Shevchenko National University of Kyiv, Faculty of Chemistry
(60, Volodymyrs'ka Str., Kyiv 01601, Ukraine; e-mail: garaguts.yulia.fox@gmail.com)
[2] Belarussian State University
(4, Nezavisimosti Pr., Minsk 220030, Belarus)
[3] A.D. Sakharov International State Ecological University
(23/1, Dolgobrodskaya Str., Minsk 220070, Belarus)
[4] National University of Food Technology, Problem Research Laboratory
(68, Volodymyrs'ka Str., Kyiv 01601, Ukraine)

EVALUATION OF A DEXTRAN-POLY(N-ISOPROPYLACRYLAMIDE) COPOLYMER AS A POTENTIAL TEMPERATURE-DEPENDENT NANOCARRIER FOR PHOTOSENSITIZERS WITH DIFFERENT PROPERTIES[1]

Thermosensitive polymer poly-N-isopropylacrylamide (PNIPAM) having a conformational transition in the interval of physiological temperatures was discussed last years as a novel drug delivery system. Chlorin e6 (Ce6) is a photosensitizer used in the photodynamic anticancer therapy. The comparative study of the encapsulation of Ce6 and its derivative, dimethylether of chlorine e6 (DME Ce6), into a water-soluble star-like PNIPAM-based copolymer to prevent the aggregation of a photosensitizer in the water medium is carried out. The photophysical properties of the copolymer/photosensitizer complexes as functions of the temperature in the region of the conformational transition of the polymer matrix have been studied and discussed. It is shown that Ce6 at low temperatures interacts weakly with the polymer phase. As a result, the absorption and fluorescence properties of Ce6 in aqueous and polymer solutions are practically identical. Fluorescence characteristics of Ce6 in a copolymer solution remain unchanged, when it is heated, which indicates the lack of a possibility for this sensitizer to bind in the bulk of the polymer phase. Following fluorescence data, all DME Ce6 molecules are bound with the polymer matrix, when a temperature is higher than the Lower Critical Solution Temperature (LCST) of the polymer. The formed complexes are quite stable. In the presence of serum proteins, the molecules of the photosensitizer remain associated for a long time with the polymer. At temperatures below LCST, DME Ce6 is not bound by the polymer. Moreover, the cooling of a solution of DME Ce6/polymer complexes leads to the rapid dissociation of photosensitizer molecules with subsequent aggregation or binding to biological structures in an aqueous medium. The obtained results show that the possibility of using the polymer PNIPAM as a temperature-dependent nanocarrier strongly depends on the properties of the loaded drug.

K e y w o r d s: poly-N-isopropylacrylamide, conformational transition, photosensitizer.

[1] This article is dedicated to the 75th anniversary of Academician L.A. Bulavin

1. Introduction

The growing progress in nanotechnology and life sciences demonstrates an urgent need for novel intelligent biocompatible polymers. Thermally responsible polymers based on poly(N-isopropylacrylamide) (PNIPAM) with Lower Critical Solution Temperature (LCST) became a subject of the study as a promising approach for the materials used in biomedical applications [1–7]. Such polymers exhibit a lower critical solution temperature (LCST), below which the polymers are soluble [7–8]. When the temperature is raised above LCST, these polymers firstly undergo a phase transition and become partially hydrophobic. This phenomenon is reversible [7–8]. Linear PNIPAM has a LCST value of approximately 32 °C. Thus, PNIPAM is soluble at room temperature, but it phase separates at the physiological temperature (37 °C). Therefore, it can be used as a nanoplatform for the controlled delivery of drugs in medicine. Any successful applications of such intelligent polymers-nanocarriers are dependent on the possibility to control the hydrophobic-hydrophobic balance of a macromolecule and its behavior within the region of LCST. A possible tuning of the hydrophobicity of these polymers and the regulation of the region of a phase transition and the size of hydrophobic domains would be a real achievement in pharmaceutical materials science and could extend their application. In our previous work, it was shown that LCST depends on the polymer molecular structure [9]. It was shown [10, 11], that the incorporation of an additional component into the polymer nanocarrier results in a change in the hydrophilic-hydrophobic balance of the polymer macromolecule. This process can lead to a significant aggregation of the polymer resulted in a loss of the pharmakinetic efficacy of the nanocomposite with incorporated antitumor drugs such as cisplatin. In the present work, we study the nanosystem containing some photosensitizers (PSs) incorporated into thermosensitive polymer-nanocarrier dextran-graft-Poly-N-isopropylacrylamide. Chlorin e6 (Ce6) and its derivatives generate singlet oxygen at the laser irradiation and may be used as photosensitizers for photodynamic therapy (PDT) [12, 13]. The application of Ce6 faces several challenges due to its inherent shortcomings [13–15], including the poor water solubility leading to its aggregation. The aggregation leads to the loss of a photodynamic efficacy [16]. To prevent the aggregation process, the PS can be loaded into the polymer water-soluble nanoplatform [17, 18]. It is known that the ideal drug delivery system can be switched-on by responding to biological stimuli. One of such stimuli is the temperature. Thus, the use of a thermoresponsive polymer with LCST in the region of physiological temperatures can create conditions for the appropriate photosensitizer release.

In the present work, we have made the comparative study of the behavior of nanosystems containing Photosensitizers of Chlorin's series (Ce6 and dimethylether of Ce6) incorporated into thermosensitive polymer-nanocarrier dextran-graft-Poly-N-isopropylacrylamide within the region of a Low Critical Solution Temperature (LCST) of the polymer. The purpose was to investigate the processes of binding and efflux from the polymer-nanocarrier of photosensitizers varying its hydrophobicity.

2. Materials and Methods

2.1. Polymer nanocarrier

Star-like copolymer with Dextran core ($M_w = 70 \times \times 10^5$ g/mol) and 15 grafted poly(N-isopropylacrylamide) chains was used as a polymer nanoplatform for the photosensitizer encapsulation. Further, this polymer will be designed as D70-*g*-PNIPAM15. The synthesis, characterization, and peculiarities of the copolymer molecular structure were discussed in [9] in detail. Molecular parameters of D70-*g*-PNIPAM15 are given in Table 1.

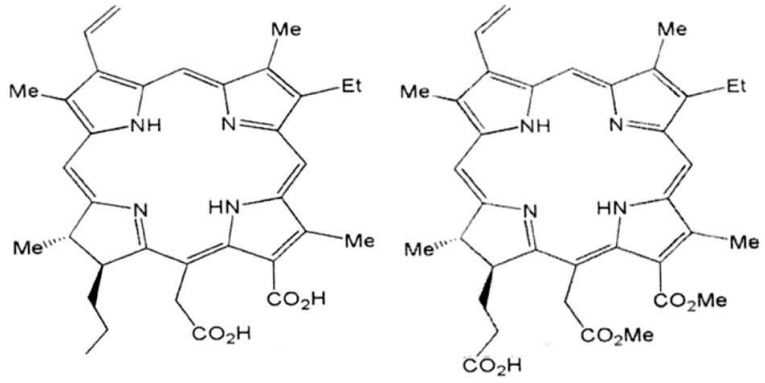
3. Photosensitizers

Photosensitizers Chlorine e6 (Ce6) and dimethylether of Ce6 (DME Ce6), whose chemical formulas are represented in Fig. 1, were obtained from the Institute of Physics of Belarusian Academy of Sciences. The properties of Ce6 and DME Ce6 were described ear-

Table 1. **Molecular parameters of D70-*g*-PNIPAM15 copolymer**

Sample	$M_w \times 10^{-6}$, g/mol	$M_n \times 10^{-6}$, g/mol	M_w/M_n
D70-g-PNIPAM15	1.03	0.674	1.52

M_w – average molecular weight; M_n – the number average molecular weight; M_w/M_n – polymer polydispersity.



a *b*

Fig. 1. Chemical formulas of Ce6 (*a*) and DME Ce6 (*b*)

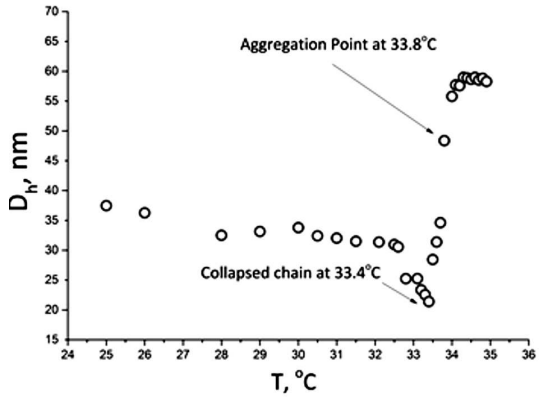


Fig. 2. Dependence of the hydrodynamic diameter on the temperature for a D70-*g*-PNIPAM15 solution

lier [15, 19]. Stock solutions of pigments (10^{-3} M) were prepared in ethanol and kept in dark at 4 °C.

4. Spectral Measurements

Corrected fluorescence spectra were recorded on an SFL-1211A spectrofluorimeter (Solar, Minsk). All spectral measurements were carried out with the thermostated cell unit equipped with a magnetic stirrer. Fluorescence polarization values were recorded by the standard methods.

5. Fluorescence Polarization Measurements

Measurements of PSs fluorescence polarization degree (P) were performed on Solar CM 2303 equipped with polarizers. In this case, samples were excited at 415 nm, and the fluorescence was registered at 670 nm as published before [16]. The polarization degree was calculated using a mean values of fluorescence intensities with the polarizers in parallel and perpendicular positions. The absolute error value for all measurements was less than 0.02.

6. Quasielastic Light Scattering (QELS)

QELS measurements were carried out using Zetasizer Nano ZS (Malvern Instruments Ltd., UK). The apparatus contains a 4 mW He–Ne laser with a wavelength of 632.8 nm, and the scattered light was detected at an angle of 173° (back scattering). For the accurate study of the transition, the correlograms of 0.1 mg/mL aqueous D70-PNIPAM15 were collected in the region of physiological temperatures, namely, at 25, 37, and 40 °C. Each temperature point was held for 5 min before measurements to equilibrate the sample. At least 10 correlation curves for each temperature point were treated by the CONTIN algorithm [20], which is known to be reliable for complicate systems to get hydrodynamic diameter (D_H) distributions.

7. Results and Discussion

It was shown [9, 21] that, above 33 °C, D70-g-PNIPAM15 undergoes a reversible LCST phase transition. Figure 2 demonstrates the results of analysis of the dynamic light scattering for a water solution of D70-*g*-PNIPAM15 (dilute regime) at the heating from 24 to 36 °C. The decrease of D_h from 31 to 22 nm in the temperature interval 32.6–33.4 °C and the sharp aggregation process at 33.7–34.1 °C were observed. The drastic change in the shape of the size distribution curves and an increase in the scattering intensity at 33.7–34.1 °C were registered. The further heating to 36 °C caused no additional aggregation. It

should be noted that the transition temperature is by 2–4 degrees higher than the typical LCST point for linear PNIPAM with similar molecular weight and polydispersity [22].

In the present study, we analyzed the comparative behavior of the individual D70-*g*-PNIPAM15 (Figure 3) and nanosystems with incorporated PSs Ce6 and DME Ce6 (Figs. 4 and 5) at 25, 34, and 40 °C. This approach helped us to understand the processes occurring in the polymer-PS nanosystem in the region of a conformational transition of the polymer at aa variation of the PS hydrophobicity. Figure 3 *a*–*c* represents the dependence of the normalized scattering intensity on the hydrodynamic diameter of the components in a D70-PNIPAM15 solution at 25, 34, and 40 °C, namely, before and after the conformational transition of the polymer and at LCST (34 °C).

As can be seen, at 25 °C, two peaks are detected on the size distribution curves. The peak in the region of 40–50 nm corresponds to the size of a polymer macromolecule and is consistent with the data on the static light scattering, as in our previous work [9]. The peak at 1000 nm is most likely due to the small number of macromolecule aggregates formed due to the ability of the amide groups of the grafted chains to form hydrogen bonds. Their number may be small. But, given the size and structure of objects, their contribution to the scattering intensity is significant. This peak can be also affected by a deviation of the scattering objects (macromolecules and aggregates) from the spherical shape during their motion, which, as noted, is due to the specificity of the dynamic light scattering experiment. These nodes cannot be caused by the aggregation, since the system was studied at the Guinier regime.

At 34 °C, the peak in the region of 35–40 nm corresponds to the observed size of the polymer macromolecule. But we indicate the narrowing of this peak as compared to the peak registered at 25 °C, which testifies to the beginning of a shrinking of the macromolecular coil. The second peak shifts to 120 nm and is also narrower than that at 25 °C. Thus, the aggregates, which can be present in the system, become more compact, and the shape of scattering objects approaches the spherical one.

At 40 °C, dramatic changes in the D70-*g*-PNIPAM15 size distribution curves in an aqueous solution are registered. One narrow peak is observed in

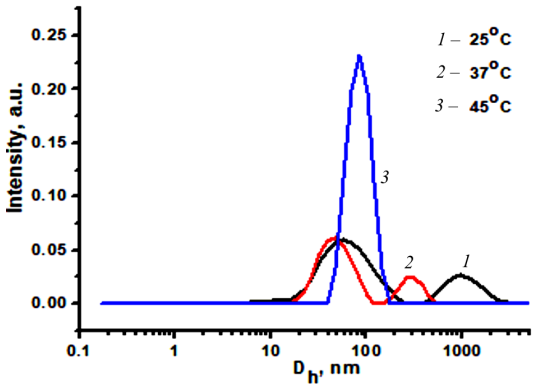


Fig. 3. Dependence of the normalized scattering intensity on the hydrodynamic diameter of the components in a D70-*g*-PNIPAM15 solution at 25 (*1*), 34 (*2*), and 45 °C (*3*)

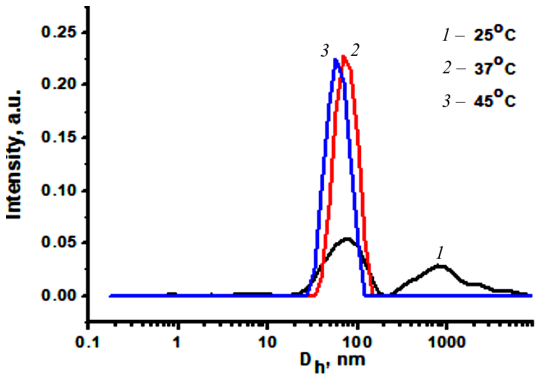


Fig. 4. Dependence of the normalized scattering intensity on the hydrodynamic diameter of the components in a D70-*g*-PNIPAM15/Ce6 solution at 25, 34, and 45 °C

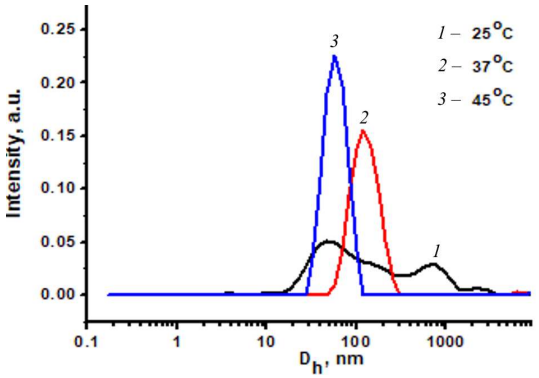


Fig. 5. Dependence of the normalized scattering intensity on the hydrodynamic diameter of the components in the D70-PNIPAM15/DME Ce6 nanosystem in a solution at 25, 34, and 45 °C

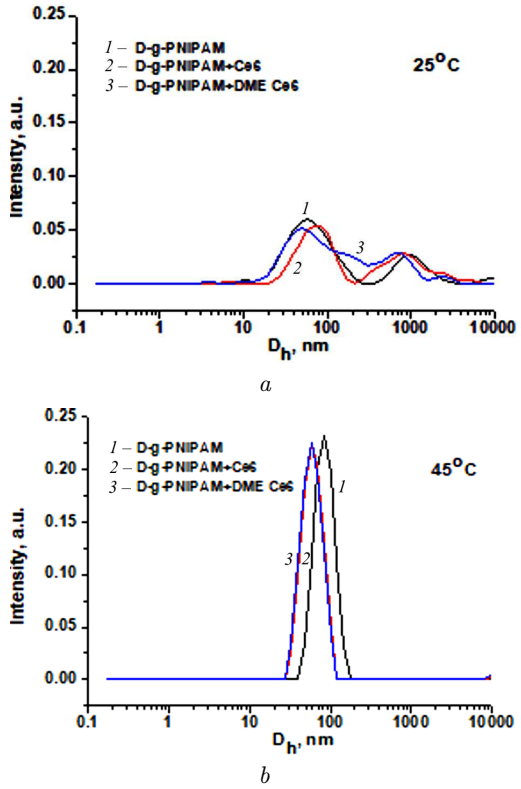


Fig. 6. Dependence of the normalized scattering intensity on the hydrodynamic diameter of the components for D70-PNIPAM15/Ce6; D70-PNIPAM15/DME Ce6 at 25 (*a*) and 45 °C (*b*)

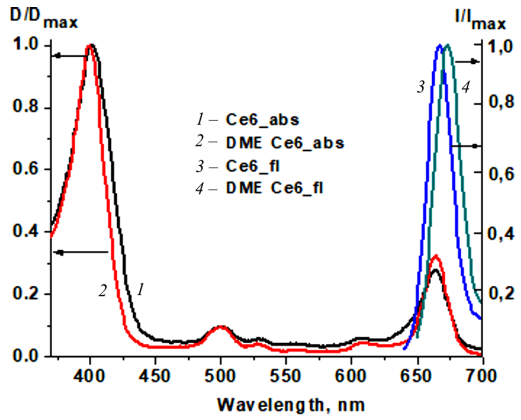


Fig. 7. Normalized absorption and fluorescence spectra in ethanol

the region of 100 nm. The intensity of this peak is much lower than those the peaks at 25 and 34 °C, which indicates a significant compaction of the scattering objects in the solution. The size of scattering objects testifies to the absence of individual macromolecules and the presence of aggregates. The aggregation is caused by an increase of the hydrophobicity of the polymer macromolecule after the conformational transition (Fig. 2).

Figures 4 and 5 demonstrate the results of studies of the nanosystem D70-PNIPAM15/PS at increasing the hydrophobicity of PS of chlorine series (Ce6 < TME Ce6) in the region of physiological temperatures, namely, before and after the conformational transition of the polymer nanoplatrform.

The polymodality of scattering objects is observed for the D70-*g*-PNIPAM/Ce6 system (Fig. 4) at 25 °C. Two peaks are registered at 50 nm and 800 nm, which is related to both the size of the scattering objects and their shape. When the temperature increases to 34 °C, only one peak in the region of 40–50 nm is observed, and its intensity increases. At 45 °C, a slight decrease in the size of scattering objects up to 40 nm and a slight decrease in the intensity were registered. We can conclude that a drastic change in the nanosystem occurs behind LCST of the polymer nanoplatform, when the polymer began partially hydrophobic. However, the QUELS method cannot detect the small molecules, that is why the released Ce6 can not be registered.

In Fig. 5, we observe the dependence of the normalized scattering intensity of several peaks on the hydrodynamic diameter of the components for the D70-PNIPAM15/DME Ce6 system at 25 °C – at 40, 110, 800, and 1100 nm. As the temperature increases to 37 ° C and further to 45 °C, the system becomes monomodal with one narrow peak. This peak is located at 100 nm at 37 °C and at 40 nm at 45 °C.

Figure 6 allows us to compare the QELS results for two nanosystems with Ce6 and DME Ce6 before and after LCTS of the polymer. Apparently, we observe significant changes in the nanosystems before and after the conformational transition of the polymer nanoplatform. These changes are more expressed for the nanosystem with DME Ce6. Obviously, they can be caused by the binding and release of PS. This assumption about the processes occurring in the nanosystems are only a hypothesis that needs to be confirmed by the study of the kinetics of a release of photosensitizers behind LCST.

However, the QELS results are very important, as they testify that, at physiological temperatures even after the conformational transition of a polymer na-

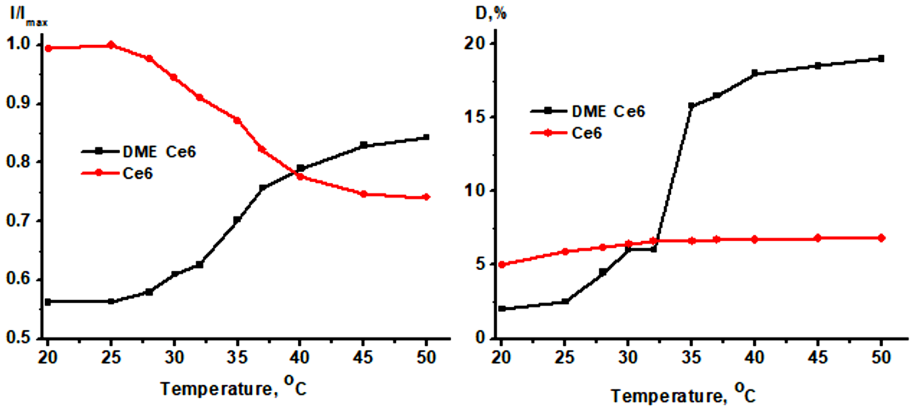


Fig. 8. Influence of the temperature on fluorescence characteristics of Ce6 and DME Ce6 in a D70-PNIPAM15 solution. Left panel – fluorescence intensity in maximum; right panel – fluorecence polarization $C_{D70-PNIPAM15}$ – 0.2 mg/ml; $C_{PS} = 1 \times 10^{-7}$ M

noplatform, the large aggregates which can annul the biological efficacy of nanosystems for PDT are not formed.

The results obtained from the spectral studies of Ce6 and DME Ce6 in neat solvents and polymer solutions are summarized below. The spectral characteristics of Ce6 and DME Ce6 are generally defined by PS belonging to chlorin-type compounds. Absorption spectra of both PS reveal most prominent peaks in the region of 402–405 nm (Soret band) and 660–680 nm (Q-band). But, for DME Ce6, the Q-band is more prominent (Fig. 7).

It is well known that, in organic solvents, all chlorins intensively fluoresce at 665–675 nm with a quantum yield around 0.15 [23] and the fluorescence lifetime of 5.5 ns. In different organic solvents, only minor spectral changes are observed: shifts of 5–7 nm in the peaks and the relative fluorescence quantum yield varying within 10% compared to an ethanol solution. Like many hydrophobic tetrapyrroles, chlorins in aqueous solutions tend to aggregate. The formation of aggregates leads to a significant change in the absorption and fluorescence properties [23]. The aggregated species are characterized by the low extinction coefficients for all bands and exhibit bathochromic shifts of the peaks.

In Fig. 8, *a, b* and in Table 2, the spectral characteristics of the nanosystem D70-*g*-PNIPAM15 + PS within the studied temperature interval are summarized. The aggregation results in decreasing the quantum yield of the fluorescence of chlorins in a water solution as compared with an ethanol solution. This effect is particularly pronounced in the case of apolar DME Ce6. For relatively polar Ce6, the fluorescence intensity decreases by 20–30%, which indicates a low probability of the formation of aggregates in dilute aqueous solutions. It is well documented that the aggregation of Ce6 derivatives is completely prevented via their binding with different colloid particles (liposomes, proteins, polymers) [24].

It is seen that, at the solution temperatures above 35 °C, the addition of the polymer causes a significant increase in the fluorescence intensity of DME Ce6, which suggests the destruction of molecular aggregates due to their interaction with the polymer. The shapes of the absorption and fluorescence spectra are close to the same characteristics of these photosensitizers in organic solvents. The formation of complexes

Table 2. **Influence of the temperature on the maximum fluorescence wavelength for Ce6 and DME Ce6 in a D70-PNIPAM15 solution**

T, °C	Polymer + Ce6 $^{fl}\lambda_{max}$, nm	Polymer + DME Ce6 $^{fl}\lambda_{max}$, nm
20	662	670
25	663	670
28	664	670
30	664	670
32	664	670
35	664	671
37	664	672
40	664	672
45	664	672

of DME Ce6 molecules with polymer macromolecules is also evidenced by the results of measurements of the fluorescence polarization degree for chlorins. The PS binding with a copolymer restricts the rate of rotational relaxation and enhances the degree of fluorescence polarization.

At temperatures below the critical point, adding a polymer has no effect on the fluorescence of DME Ce6 in aqueous solutions. Low values of the relative quantum yield of fluorescence and the fluorescence polarization degree, which practically coincide with the PSs characteristics in the absence of a polymer, allow us to conclude that, under such conditions, the processes of complex formation are ineffective.

The cooling of an aqueous solution of D70-*g*-PNIPAM15 with DME Ce6 to a temperature below the LCST point is accompanied by changes in the fluorescence characteristics that are typical of the aggregation of photosensitizers in an aqueous medium. Thus, the stimulus-dependent change in the phase state of the polymer is accompanied by the release of photosensitizer molecules from the complexes. It should be noted that if the solution contains structures that are able to bind chlorins, for example, serum proteins, the aggregation processes are not observed. In this case, DME Ce6 molecules released from the complexes can be bound by new acceptor structures [16]. The results on fluorescent characteristics indicate a significantly different behavior of Ce6 in the polymer solution. This photosensitizer is apparently uncapable to penetrate into and form stable complexes with D70-*g*-PNIPAM15. This results in that the Ce6 fluorescent characteristics are independent of the polymer phase state. In view of small values of P, the microviscosity of the photosensitizer environment in the polymer solution does not differ much from the same parameter in an aqueous solution.

8. Conclusions

The analysis of results on the fluorescent characteristics of chlorin derivatives allows us to draw a number of conclusions about the mechanisms of formation of the complexes photosensitizers/D70-*g*-PNIPAM15. According to the obtained data, nonpolar chlorins effectively bind to a polymer in a solution at temperatures above LCTS. DME Ce6 molecules are localized in the depth of the condensed polymer matrix. This leads to a restriction of the mobility of bound chlorin molecules, accompanied by an increase in the fluorescence polarization degree. As shown earlier, the spectral characteristics of fluorescence of Ce6 and its esterified derivative depend on the polarity of the microenvironment [19]. In this regard, the position of the maximum of the fluorescence spectrum of DME in the polymer at 672 nm corresponds to a significantly lower polarity compared to the aqueous medium. The change in the polymer structure during the cooling is accompanied by a decrease in the density of polymer nanoparticles, which provides a greater mobility of DME Ce6 molecules and, as a result, allows them to dissociate into the aqueous medium. With regard for the small degree of polarization of the fluorescence of Ce6 in the polymer solution, it can be assumed that the relatively polar molecules of this photosensitizer are not able to penetrate into the condensed polymer and, as a result, are located in an aqueous volume or on the surface of D70-*g*-PNIPAM15 nanoparticles regardless of the temperature. This is confirmed by the coincidence of the position of the maxima of the chlorin fluorescence spectra in the aqueous and D70-*g*-PNIPAM15 solutions regardless of the temperature. The results of our research with structurally similar chlorin-type photosensitizers clearly show that the applicability of D-*g*-PNIPAM as a smart drug-delivery system is dependent strongly on the properties of a loaded drug.

The authors declare that there is no conflict of interest regarding the publication of this paper.

This publication is supported in part by the Ministry of the Education and Science of Ukraine: joint Ukrainian-Belarusian research and development projects "Design and physico-chemical properties of novel multicomponent nanosystems for the treatment and diagnostics of solid tumors" M/110 (2019-2020), and with the financial support of the Belarusian Republican Foundation for basic research, grant No. M19UKRG-002.

1. S. Chen, H. Zhonh, B. Gu *et al.* Thermosensitive phase behavior and drug release of in situ N-isopropylacrylamide copolymer. *Mater Sci. Eng. C., Mater. Biol. Appl.* **32**, 2199 (2012).
2. J. Meid, F. Dierkes, J. Cui *et al.* Mechanical properties of temperature sensitive microgel/polyacrylamide composite hydrogels–from soft to hard fillers. *Soft Matter* **8**, 4254 (2012).

3. H.L. Bernice., A. Bismarck, M.B. Chan-Park. High internal phase emulsion templating with self-emulsifying and termoresponsive hitosan-graft-PNIPAM-graft-oligoproline. *Biomacromolecules* **5**, 1777 (2014).
4. Li-li Zhang, He Jia-song, Jun Zhang, Zhi-Hua Gan. Synthesis and characterization of temperature-sensitive cellulose-graft-poly (N-isopropylacrylamide)copolymers. *Chinese J. Polymer Sci.* **33**, 1640 (2015).
5. I. Bischofberger, V. Trappe. New aspects in the phase behavior of poly-N-isopropyl acrylamide: systematic temperature dependent shrinking of PNIPAM assemblies well beyong the LCST. *Sci. Rep.* **5**, 15520 (2015).
6. Rashmi R. Kokardekar, Vaibhav K. Shah, Hardik R. Mody. PNIPAM poly(N-isopropylacrylamide): A thermaresponsive "Smart" polymer in novel drug delivery systems. *Int. J. Medical Update* **7** (2), 60 (2012).
7. L.H.B. Lima, Y. Morales, T.B. Cabral. Poly-N-isopropylacrylamide (PNIPAM): A reversible bioadhesive for sclerotomy closure. *Int. J. Retina and Vitreous* **2**, 23 (2016).
8. Kenji Kubota, Kenzi Hamano, Nobuhiro Kuwahara *et al.* Characterization of poly(N-isopropylmethacrylamide) in water. *Polymer J.* **22**, 1051 (1990).
9. V. Chumachenko, N. Kutsevol, Yu. Harahuts *et al.* Starlike Dextran-graft-PNiPAM copolymers. Effect of internal molecular structure on the phase transition. *J. Molecular Liquids* **235**, 77 (2017).
10. N. Kutsevol, A. Naumenko, V. Chumachenko *et al.* Aggregation processes in hybrid nanosystem polymer/nanosilver/cisplatin. *Ukr. J. Phys.* **63** (6), 513 (2018).
11. G. Telegeev, N. Kutsevol, V. Chumachenko *et al.* Dextran-polyacrylamide as matrices for creation of anticancer nanocomposite. *Int. J. Polymer Sci.*, Article ID 4929857 (2017).
12. H. Liang, Z. Zhou, R. Luo *et al.* Tumor-specific activated photodynamic therapy with an oxidation-regulated strategy for enhancing anti-tumor efficacy. *Theranostics* **8** (18), 5059 (2018).
13. V.P. Zorin, I.I. Khludeev, T.E. Zorina. Study of tumoritropic capacity of chlorine-e6 and its derivatives on af mice with transplanted hepatoma-a. *Eksperiment. Onkologiya* **14** (3), 39 (1992).
14. Z.J. Zhou, J.B. Song, L.M. Nie, X.Y. Chen. Reactive oxygen species generating systems meeting challenges of photodynamic cancer therapy. *Chem. Soc. Rev.* **45** (23), 6597 (2016).
15. V.P. Zorin, I.I. Khludeyev, I.S. Mikhalovsky *et al.* Kinetic characteristics of porphyrin distribution in the blood. *Laser Use in Oncology II, Proc. SPIE* 4059 (1999).
16. V.P. Zorin. Kinetics of the redistribution of porphyrins between the proteins of blood and membranes. *J. Appl. Spectrosc.* **66**, 602 (1999).
17. P. Kumari, S. Jain, B. Ghosh, V. Zorin, S. Biswas. Polylactide-based block copolymeric micelles loaded with chlorin e6 for photodynamic therapy: In vitro evaluation in monolayer and 3D spheroid models. *Molecular Pharmaceutics* **14** (11), 3789 (2017).
18. N.B. Kutsevol, A.B. Naumenko, Yu.B. Harahuts *et al.* New hybrid composites for photodynamic therapy: synthesis, characterization and biological study. *Appl. Nanosci.* **9**, 881 (2019).
19. V.P. Zorin, I. Michalovsky, T.E. Zorina, I.I. Khludeyev. Distribution of chlorin-e6 derivatives in biological systems: investigation of pH-effect, photochemotherapy: Photodynamic therapy and other modalities. *Proc. SPIE* **2625** (1996).
20. A. Scotti, W. Liu, J.S. Hyatt *et al.* The CONTIN algorithm and its application to determine the size distribution of microgel suspensions, *J. Chem. Phys.* **142**, 234905 (2015).
21. O.A. Yeshchenko, A.P. Naumenko, N.V. Kutsevol *et al.* Anomalous inverse hysteresis of phase transition in thermosensitive dextran-graft-PNIPAM copolymer/Au nanoparticles hybrid nanosystem. *J. Phys. Chem. C* **122** (14), 8003 (2018).
22. A. Halperin, M. Krçger, F. Åoise, M. Winnik. Poly(N-isopropylacrylamide) phase diagrams: Fifty years of research. *Angew. Chem. Int. Ed.* **54**, 5342 (2015).
23. H. Gattuso, A. Monariab, M. Marazzi. Photophysics of chlorin e6: from one- and twophoton absorption to fluorescence and phosphorescence. *RSC Adv.* **7**, 10992 (2017).
24. R. Bachor, C.R. Shea, S.J. Belmonte, T. Hasan. Free and conjugated chlorin e6 in the photodynamic therapy of human bladder carcinoma cells. *J. Urol.* **146** (6), 1654-8 (1991).

Received 01.04.20

Н. Куцевол, Ю. Кузів, В. Зорін,
І. Кравченко, Т. Зоріна, А. Маринін, Л. Булавін

ДОСЛІДЖЕННЯ КОПОЛІМЕРУ ДЕКСТРАН-ПОЛІ(N-ІЗОПРОПІЛАКРИЛАМІДУ) ЯК ПОТЕНЦІЙНОГО ТЕМПЕРАТУРОЗАЛЕЖНОГО НАНОНОСІЯ ДЛЯ ФОТОСЕНСИБІЛІЗАТОРІВ З РІЗНИМИ ВЛАСТИВОСТЯМИ

Р е з ю м е

Останніми роками термочутливий полімер Декстран-полі(N-ізопропілакриламід) (ПНІПАМ), конформаційний перехід якого знаходиться в інтервалі фізіологічних температур, обговорюється як новітня система доставки ліків. Фотосенсибілізатор Хлорин е6 (Се6) використовується для фотодинамічної протиракової терапії. У цій роботі було проведено порівняльне дослідження інкапсуляції Се6 та його похідного Диметилового ефіру Хлорину е6 (ДМЕ Се6) у водорозчинний зіркоподібний кополімер на основі ПНІПАМу для запобігання процесу агрегації фотосенсибілізатора у водному середовищі. Досліджено та обговорено фотофізичні властивості комплексів кополімер/фотосенсибілізатор в залежності від температури в області конформаційного

переходу полімерної матриці. Було показано, що Ce6 при низьких температурах слабо взаємодіє з полімерною фазою. В результаті властивості поглинання та флуоресценції Ce6 у водних та полімерних розчинах практично однакові. Характеристики флуоресценції Ce6 в кополімерному розчині при нагріванні залишаються незмінними, що свідчить про відсутність у цього сенсибілізатора можливості зв'язуватися в основній масі полімерної фази. На підставі даних флуоресценції при температурі, вищій за нижню критичну температуру розчинення (НКТР) полімеру всі молекули ДМЕ Ce6 зв'язані з полімерною матрицею. Утворені комплекси досить стійкі. За наявності сироваткових білків молекули фотосенсибілізатора тривалий час залишаються зв'язаними з полімером. При температурі нижче НКТР ДМЕ Ce6 не зв'язується полімером. Крім того, охолодження розчину комплексів ДМЕ Ce6/полімер призводить до швидкої дисоціації молекул фотосенсибілізатора з подальшою агрегацією або зв'язуванням з біологічними структурами у водному середовищі. Отримані результати показали, що можливість використання полімеру ПНІПАМ в якості термочутливого наноносія дуже залежить від властивостей завантаженого препарату.