Biol. Stud. 2025, 19(1), 47–60 • doi: https://doi.org/10.30970/sbi.1901.814
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 57.08; 541.64; 544.773.432

FLUORESCEIN-CONTAINING AMPHIPHILIC COPOLYMERS AS PROMISING OBJECTS FOR BIOMEDICAL RESEARCH

Maria Yakoviv [1], ***Nataliia Nosova*** [1], ***Dmytro Ostapiv*** [2], ***Iryna Yaremchuk*** [2], ***Zoriana Nadashkevych*** [1], ***Volodymyr Samaryk*** [1], ***Serhii Varvarenko*** [1]

[1] *Lviv Polytechnic National University, 12 S. Bandera St., Lviv 79013, Ukraine*

[2] *Institute of Animal Biology NAAS, 38 V. Stus St., Lviv 79034, Ukraine*

Yakoviv, M., Nosova, N., Ostapiv, D., Yaremchuk, I., Nadashkevych, Z., Samaryk, V., & Varvarenko, S. (2025). Fluorescein-containing amphiphilic copolymers as promising objects for biomedical research. *Studia Biologica*, 19(1), 47–60. doi:10.30970/sbi.1901.814

Background. Polymeric nanoparticles are increasingly used as drug carriers. They demonstrate a significant improvement in the therapeutic efficacy of drugs and are widely studied as components of drug transport and release systems. Unlike other types of nanoparticles, depending on the nature and properties, polymeric carriers can be designed to target specific organs, tissues, or cells and ultimately biodegrade with minimal systemic toxicity. The study aimed to investigate the *in vitro* cytotoxicity of drug delivery systems based on nanoparticles of fluorescein-containing amphiphilic copolymers, as well as to assess their ability to penetrate the cell and the possibility of controlling this process.

Materials and Methods. Copolymers obtained on the basis of 2-(dodecanoylamino) pentanedioic acid and 2-(octadecanoylamino)pentanedioic acid, polyethylene etherdiols, and fluorescein were used for the study. The surface-active properties of the copolymers and the solubilization ability of their colloidal solutions were examined. The cytotoxicity of fluorescein-containing copolymers and the activity of cellular enzymes were studied on live spermatozoa obtained from bull ejaculates of 2–6 mL, with a sperm concentration of 0.6–1.5·10^9 cells/mL and an activity of 7.5–8.0 points.

Results. Two groups of amphiphilic copolyesters, with molar weights of polyethylene etherdiols from 600 to 1500 and different content of fluorescein were studied. Their surface-active properties and ability to solubilize lipophilic substances – drug analogs – were determined. It was found that the obtained copolyester dispersions do not exhibit cytotoxicity. During direct contact with germ cells, copolymers can penetrate the cell

membrane and decompose with the release of fluorescein. This allows us to track their location in the structures of germ cells.

Conclusion. The relationship between the structure of amphiphilic fluorescein-containing copolyester and the degree of their effect on living objects *in vitro* has been established. The composition of copolymers that do not exhibit cytotoxicity and can be used as drug transporters has been determined. It has been shown that the synthesized copolymers can penetrate the membrane of germ cells and are decomposed during metabolic processes in sperm with the release of fluorescein.

Keywords: amphiphilic copolyester, fluorescein, cytotoxicity, enzyme activity

Registration number – 0124U000727,
The source of funding is the Ministry of Education and Science of Ukraine

INTRODUCTION

Recently, there has been an intensive development of technologies related to nano-objects of various nature, primarily in the fields of pharmacy and medicine (Anestopoulos *et al.*, 2020; Lucarini *et al.*, 2018; Eom *et al.*, 2020). Dispersed systems of nanoparticles based on amphiphilic polymers are particularly noteworthy due to their unique physical and chemical properties (Shamma *et al.*, 2022; Yu *et al.*, 2021; Vakilzadeh *et al.*, 2018; Varshosaz *et al.*, 2018). Among them, the most interesting are nanomedical forms that combine the possibilities of therapy and diagnosis of diseases (Haleem *et al.*, 2023; Patra *et al.*, 2018; Bhat *et al.*, 2019). The main advantage of such polymeric systems is that, in addition to delivering and releasing therapeutic compounds, they can also provide information on their distribution in living tissues, which allows studying and controlling the delivery efficiency (Yang *et al.*, 2020; Wang *et al.*, 2020; Stetsyshyn *et al.*, 2020).

However, the primary requirement for materials that are supposed to come into direct contact with the tissues of living organisms is their non-toxicity and biological compatibility (Wu *et al.*, 2020; Lam *et al.*, 2017). Experimental evaluation of *in vitro* cytotoxicity is aimed at determining the effect on cell metabolism and viability. The possibility of *in vivo* observation by microscopy makes live cell cultures a convenient model for research in biology, medicine, pharmacology, and cosmetology (Tihauan *et al.*, 2021; Nicolas *et al.*, 2020). As a rule, at the first stage of such research, model cells are used, in particular cancer cells, or such cells as germ cells (sperm) (Chekh *et al.,* 2017; Cho *et al.*, 2023).

The aim of the study was to investigate *in vitro* the cytotoxicity of a number of amphiphilic copolymers based on N-derivatives of glutamic acid and polyethylene etherdiole, the macromolecules of which contain covalently bound fluorescein. The aim was also to evaluate their ability to penetrate into the cell and the possibility of controlling this process using the luminescence of fluorescein released as a result of the enzymatic decomposition of copolyester macromolecules.

EXPERIMENTAL PART

Materials. Copolymers based on 2-(dodecanoylamino) pentanedionic acid (GluLa) and 2-(octadecanoylamino) pentanedionic acid (GluSt) of polyethylene etherdioles (PEG600, PEG1000, PEG1500) and fluorescein were obtained according to the methods described in (Chekh *et al.*, 2017; Yakoviv *et al.*, 2020; Varvarenko *et al.*, 2018).

Saline as a 0.9% aqueous solution of sodium chloride was produced by Aldrich, and used without additional purification.

Phosphate-salt buffer was of the following composition: NaCl – 0.8 g; KCl – 0.02 g; Na_2HPO_4 – 0.11 g; KH_2PO_4 – 0.02 g; $MgCl_2$ – 0.01 g; H_2O – to100 mL.

Bull sperm. Ejaculates with a volume of 2–6 mL, with a sperm concentration of 0.6–$1.5 \cdot 10^9$ cells/mL and an activity of 7.5–8.0 points were selected for the study (Morrell *et al.*, 2018).

Methods. The procedure for obtaining fluorescein copolyesters via the Steglich reaction is described in the article (Yakoviv *et al.*, 2020; Yakoviv *et al.*, 2018; Varvarenko *et al.*, 2018). The content of fluorescein in polymers was determined after their hydrolysis in alkaline medium by spectrophotometry (Yakoviv *et al.*, 2020).

To prepare the dispersion, an aliquot of fluorescein-containing copolyester was dispersed in water to create a concentration of 1.0%.

The surface tension of the substances was determined by the du Noüy ring method (Lee *et al.*, 2012). The value of surface tension and the critical micelle concentration (CMC) of copolyester were calculated by the method described in (Stasiuk *et al.*, 2023).

The amount of Sudan III lipophilic dye solubilized by colloidal solutions of amino functional copolymers in water was determined by spectrophotometry according to the procedure given in (Stasiuk *et al.*, 2023).

To evaluate the cytotoxicity of fluorescein-containing copolymers, sperm diluted with phosphate-salt buffer (at a ratio of 4÷1) was divided into parts. The calculated amount of 1% fluorescein-containing copolyester dispersion was added to the experimental part to obtain specified concentrations and the appropriate amount of water was added to the control part. The activity of the enzymes – succinate dehydrogenase (SDH units/h·0.1 mL of diluted sperm) and cytochrome oxidase (COX units/h·0.1 mL of diluted sperm) was determined by the methods given in (Kornyat *et al.*, 2021; Bukartyk *et al.*, 2022).

To obtain reliable data, the experiments were repeated six times for each type of copolyester. The statistical analysis of the results was carried out according to the procedure described in (Liubas *et al.*, 2022; Stasiuk *et al.*, 2023). Data shown in graphs and tables are mean values with indicated errors. The significance value is within $P \leq 0.05$.

The materials of the presented article on laboratory, scientific, and experimental studies meet the requirements of the bioethical examination standards by the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18.03.1986 and the Directive of the 2010/63/EU of the European Parliament and the Council of September 22, 2010 on the Protection of Animals Used for Scientific Purposes. As well as approved by the Ethics Committee of Institute of Animal Biology NAAS of Lviv, (Protocol No 144 of 16.01.2024).

RESULTS AND DISCUSSION

A significant amount of published data shows that polymers obtained by polycondensation, such as pseudo-polyamino acids, are capable of meeting the set of requirements for drug transporters (Wu *et al.*, 2015; Xiaowei *et al.*, 2021; Caddeo *et al.*, 2013). The advantages of amphiphilic polymers compared to low-molecular surfactants are their lower toxicity and greater stability of their micelles *in vivo* (Torchilin 2001; Parker *et al.*, 2003; Ghezzi *et al.*, 2021). In particular, new copolyesters of N-derivatives of dibasic natural amino acids and polyoxyethylene glycols are among the most promising materials for biomedical use due to their biocompatibility, biodegradability, and non-toxicity (Yakoviv *et al.*, 2020; Stasiuk *et al.*, 2023; Varvarenko *et al.*, 2018).

In a number of previous studies, we have described the synthesis of amphiphilic copolyesters of glutamic acid N-derivatives and their analogues via the Steglich reaction using fluorescein as a component of the monomer mixture (Yakoviv *et al.*, 2020; Yakoviv *et al.*, 2020). The synthesized copolyesters are oligomeric products with a polycondensation degree of 3–10. The presence of all monomeric units in their macromolecules has been proved by PMR spectroscopy and functional analysis (Yakoviv *et al.*, 2020; Varvarenko *et al.*, 2018).

The macromolecules of the obtained products contain both hydrophilic (polyoxyethylene glycols) and hydrophobic (N-derivatives of glutamic acid and higher fatty acids) fragments and naturally exhibit surface-active properties in aqueous media. Some of them are capable of forming self-stabilized dispersions with particles ranging in size from 180 to 400 nm (the size depends on the nature of the monomeric links and their ratio in the copolyester) (Yakoviv *et al.*, 2020; Varvarenko *et al.*, 2018). At the same time, to ensure the ability of fluorescein-containing copolyester dispersions to self-stabilize in water, the MM value of the hydrophilic comonomer (PEG) should be at least 600.

Therefore, two groups of amphiphilic copolyesters (synthesized on the basis of 2-(dodecanoylamino) pentanedionic acid (GluLa) and 2-(octadecanoylamino) pentanedionic acid (GluSt)) were selected for the study, which differed in the molar mass of polyethylene etherdiols (PEG 600, PEG 1000, PEG 1500) and fluorescein content (**Table 1**). For comparison, the characteristics of copolyesters of a similar composition but without fluorescein, synthesized by the same method are given.

Table1. **Composition and colloidal-chemical properties of copolyester aqueous dispersions**

N	Composition of copolyesters of general formula $Glu(A)_m$-$(PEG)_l$-(DPG)q-(F)g	Fluorescein content in copolyester, %	CMC, %	Maximum decrease in the surface tension, mN/m	Maximum solubilization, g sudan/g copolyster
1	$[GluLa]_{0,5}$-$[PEG1000]_{0,315}$-$[DPG]_{0,207}$	0	0.0095	37.4	5.01
2	$[GluLa]_{0.5}$-$[PEG1000]_{0.262}$-$[DPG]_{0.185}$-$[F]_{0.077}$	5.56	0.0105	36.6	5.85
3	$[GluLa]_{0.5}$-$[PEG1500]_{0.286}$-$[DPG]_{0.187}$-$[F]_{0.035}$	3.18	0.0095	41.1	4.57
4	$[GluSt]_{0,5}$-$[PEG1000]_{0,31}$-$[DPG]_{0,15}$-$[F]_{0,037}$	2.87	0.0128	42.6	6.0
5	$[GluSt]_{0.5}$-$[PEG1000]_{0.258}$-$[DPG]_{0.162}$-$[F]_{0.05}$	5.87	0.0052	46.5	3.9
6	$[GluSt]_{0,5}$-$[PEG1000]_{0,325}$-$[DPG]_{0,18}$	0	0.0109	45.5	1.3
7	$[GluLa]_{0.5}$-$[PEG600]_{0.264}$-$[DPG]_{0.205}$ $[F]_{0.065}$	5.2	0.0069	36.05	-
8	$[GluLa]_{0.5}$-$[PEG600]_{0.264}$-$[DPG]_{0.106}$ $[F]_{0.13}$	10.8	0.0192	39.05	-

The surface-active properties of copolymers of this type are manifested in the fact that in their aqueous solutions the surface tension decreases to 35–47 mN/m (**Fig. 1** ***A***, ***B***). An increase in the molecular weight of the acyl substituent of the nitrogen atom in glutamic acid naturally leads to an increase in the lipophilicity of the synthesized polymers, resulting in the deterioration of their colloidal solubility in water. At the same time, the CMC values of copolyester synthesized using both GluSt and GluLa are close to 0.01±0.002 %. Nevertheless, the plateau value of the maximum decrease in the surface tension of aqueous dispersions for products obtained using GluSt is 5–10 mN/m was higher than their analogues obtained using GluLa.

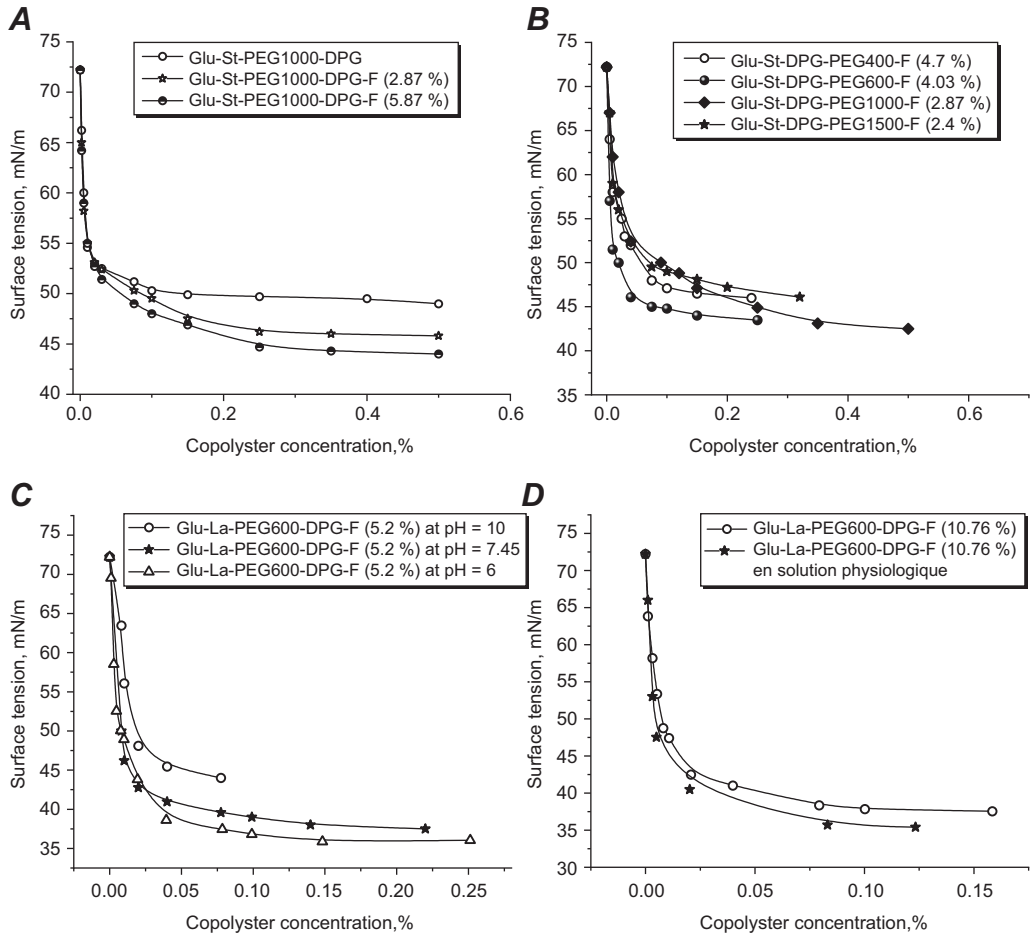

Fig. 1. Surface tension isotherms of copolyester GluSt, PEG1000 with different fluorescein contents (***A***), GluSt and polyethylene diols of different molecular weights (***B***). Surface tension isotherms of GluLa-PEG600-DPG-F (5.2 %) copolyester in distilled water (pH = 6.7), phosphate-salt buffer solution (pH = 7.4), and in a solution with pH = 10.2 (***C***) and GluLa-PEG600-DPG-F (10.76 %) in water and in saline (***D***)

The practical use of dispersions of the synthesized copolymers is associated with the prospect of their introduction into living organisms as drug carriers (Gheorghita

et al., 2021; Udayakumar *et al.*, 2021). Drugs administered parenterally are usually diluted with saline, and studies on living cells (sperm) are performed in phosphate-salt buffered saline (at pH 7.4). Therefore, the surface-active properties of copolyester were also investigated in these media. Although the synthesized copolyesters belong to non-inogenous surfactants, a certain influence of the environment on their surfactant properties was observed (**Fig. 1** ***C***, ***D***).

In saline, the general appearance of the surface tension isotherm did not change, with only a slight decrease in the plateau values by 1-2 mN/m (**Fig. 1*D***). The plateau values also changed slightly in the buffered solution with blood pH. In particular, at pH = 7.45, a slight increase in plateau values by 2–3 mN/m was observed (**Fig. 1*C***). It can be assumed that at pH higher than neutral, a partial opening of the lactone cycle in the fluorescein fragment occurs. The appearance of ionized groups (carboxyl groups) in copolyester obviously has a significant effect on the overall hydrophilic-lipophilic balance of the macromolecule and, accordingly, on its surface activity. This is confirmed by the fact that the most significant changes were observed in a solution with pH = 10, resulting in an increase of the CMC values and plateau to 45 mN/m.

The successful practical use of amphiphilic polymers in biomedical applications is largely determined by their ability to form micelles capable of low-molecular compounds' solubilization (Myers 2006). In this study the solubilization was examined using Sudan III, a poorly water-soluble dye typical of therapeutic drugs modeling. **Table 1** demonstrates rather high values of Sudan III solubilization by the dispersions of the synthesized copolymers and indicates their prospects as drug transporters.

The synthesized fluorescein-containing copolymers, in addition to the transportation function, can perform the function of visualization due to the ability to fluorescent response under certain conditions. In the structure of the synthesized copolyesters, fluorescein is covalently bonded and does not exhibit fluorescence. However, the ester bond through which the monomeric links are bounded in the synthesized copolymers, provides the possibility of its hydrolytic cleavage, including that under the action of cellular enzymes with the release of fluoroscein (Gref *et al.*, 1995; Lyu *et al.*, 2009). The high intensity of fluorescence within the wavelengths of visible light, and the absence of tissue fluorescence in this range allows us to control this process without using ultrasensitive devices (Rui-Lin *et al.*, 2022; Hang *et al.*, 2022).

The ability to penetrate into the structures of germ cells and decompose there with the release of fluorescein was confirmed by direct contact of the copolyester dispersion with germ cells. This is evidenced by the appearance of their fluorescence under UV irradiation, the highest intensity of which was detected in the metabolically active parts of sperm: acrosome, body and neck, and tail (**Fig. 2**).

The *in vitro* cytotoxicity of the obtained amphiphilic copolymers was evaluated according to the method described above. The following parameters were determined during the study: (1) activity of succinate dehydrogenase (SDH, units/h·0.1 mL of sperm); (2) activity of cytochrome oxidase (COX, units/h·0.1 mL of sperm); (3) sperm survival time in hours at a temperature of 2–4 °C (until their straightforward movement stopped). The study was carried out by creating a concentration of 0.0001 %, 0.0005 %, 0.001 % of the corresponding amphiphilic fluorescein-containing copolyester in a mixture with a sperm (the procedure is given in the experimental part).

Figure 3 shows the activity of succinate dehydrogenase for a number of copolymers. It should be noted that the introduction of fluorescein into the macromolecules of

the amphiphilic copolymers GluLa-PEG1000-DPG-F (sample 2, **Table 1**) and GluSt-PEG1000-DPG-F (sample 5, **Table 1**) did not change the enzyme activity compared to the control sample at all studied concentrations of the copolymers. In the selected range a negative correlation of enzyme activity with an increase in its concentration was observed only for the copolyether GluLa-PEG1500-DPG (sample 3, **Table 1**). All fluorescein-containing copolymers caused a slight increase in the SDH activity compared to the copolyesters without fluorescein, which, however, remained at the control level.

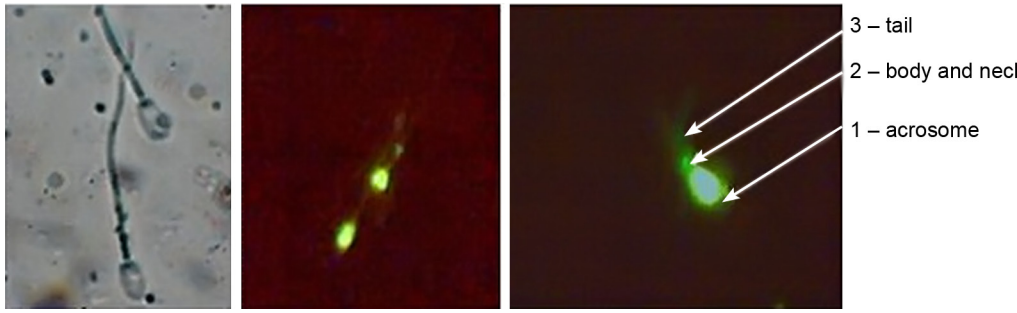

Fig. 2. Photographs of sperm with the addition of the amphiphilic fluorescein-containing copolyester GluSt-PEG1000-DPG-F (2.87 %)

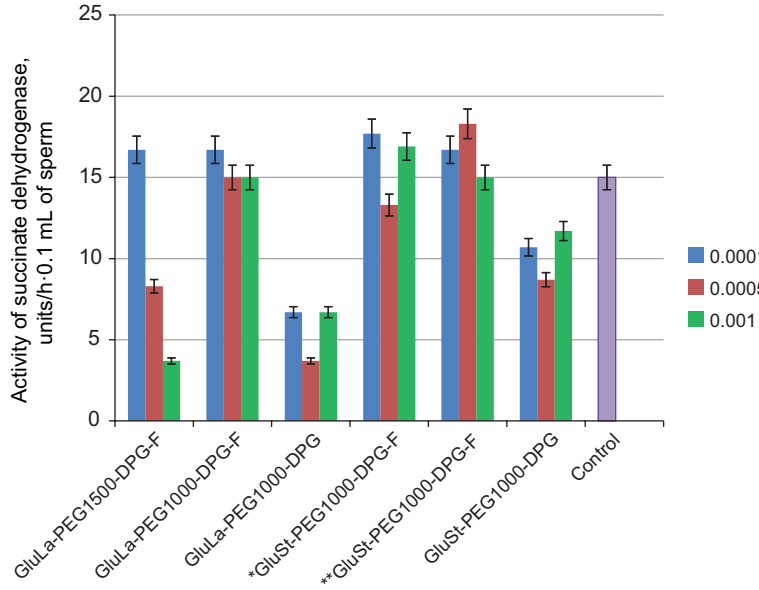

Fig. 3. Dependence of succinate dehydrogenase activity in germ cells on the amphiphilic copolyester concentration in a mixture for different copolyesters. * – copolymer contains 2.87 % of fluorescein; ** – copolymer contains 5.87 % of fluorescein. The difference between control and experimental groups is statistically significant at $p < 0.05$

Figure 4 shows the effect of copolyester concentration on the activity of germ cell cytochrome oxidase. The minimum concentration of copolymers did not decrease the activity of this enzyme, which remained at the control level. With increasing concentration, a decrease in enzyme activity was observed, with the least effect observed for the

copolyesters GluLa-PEG1000-DPG (sample 1, **Table 1**), which did not contain fluorescein, and the greatest effect for its analog containing fluorescein GluLa-PEG1000-DPG-F (sample 2, **Table 1**). It is worth noting that fluorescein-containing copolymers synthesized using GluLa have a significantly stronger negative effect on the enzyme activity with increasing concentration in the mixture.

The results of determining the sperm survival time depending on the amphiphilic copolyester concentrations are represented in **Table 2**.

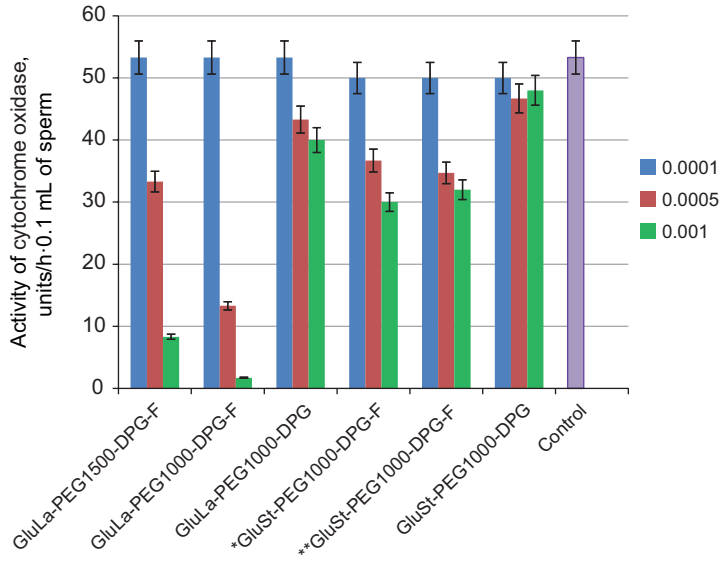

Fig. 4. Dependence of cytochrome oxidase activity in germ cells on the amphiphilic copolyester concentration in a mixture for different copolyesters * – copolymer contains 2.87 % of fluorescein; ** – copolymer contains 5.87 % of fluorescein. The difference between control and experimental groups is statistically significant at p <0.05

Table 2. **Sperm survival time depending on the amphiphilic copolymer concentrations n = 6; M±m**

Copolyester	Sperm survival (hours) at different copolyester concentrations, %			η
	0.0001	0.0005	0.001	
GluLa-PEG1500-DPG-F (3.18 %)	112.0±9.24	104.0±4.62	64.0±7.30*	0.704
GluLa-PEG1000-DPG-F (5.56 %)	72.0±9.80	44.0±8.79*	40.0±7.30***	0.736
GluLa-PEG1000-DPG	76.0±10.46	68.0±8.79*	68.0±6.73*	0.477
GluSt-PEG1000-DPG-F (2.87 %)	84.0±9.38	72.0±8.00	56.0±9.24**	0.601
GluSt-PEG1000-DPG-F (5.87 %)	80.0±7.30	68.0±8.79*	56.0±4.62***	0.634
GluSt-PEG1000-DPG	88.0±5.30	75.0±8.79*	59.0±4.62***	0.644
Control	96.0±8.00			

Note: difference is statistically significant compared to the control sample *– p <0.05; **– p <0.01; ***– p <0.001

For GluLa-based copolyester a 2.5-fold increase in fluorescein content (from 3.18 to 5.56 %) caused a 37 % deterioration in survival. For copolyester with a fluorescein content of 3.18 %, a 50 % decrease in survival was observed with an increase in its concentration in the mixture. For the copolyester synthesized using GluSt, the fluorescein content had virtually no effect on cell survival. In general, only the GluLa-PEG1500-DPG-F sample at concentrations of 0.0001 and 0.0005 % was within the control range and did not reduce the survival time.

The addition of the same dose of the studied copolyesters also had an ambiguous effect on sperm survival. Thus, at a GluLa-PEG1500-DPG-F concentration of 0.0001 %, the value of the physiological indicator was maximum (112.0±9.24 h), and at the same concentration of other copolyesters lowered by 21.5–35.8 % ($p < 0.05–0.01$). It does not differ from the control sample. Similar results were obtained at a copolyester concentration of 0.0005 %: high sperm survival was established with the addition of GluLa-PEG1500-DPG-F (104.0±4.62 h). This was by 27.9–67.3 % ($p < 0.05–0.001$) lower than with the addition of other copolyesters. When the concentration of GluLa-PEG1500-DPG-F and GluLa-PEG1000-DPG increased up to 0.001 %, higher sperm survival rate (64.0–68.0 h) was observed. Moreover, the indicator value diminished by 41.2 % ($p <0.05$) when GluLa-PEG1000-DPG-F was added and lowered by 13.3–17.3 % when other copolyesters were introduced. At the same time, at a concentration of 0.0005 and 0.001 % GluLa-PEG1000-DPG-F, survival almost did not change and remained at the same level (40.0–44.0 h), which indicates an excess dose of copolyester.

CONCLUSION

The interrelation between the structure of amphiphilic fluorescein-containing copolyester and the degree of their influence on living objects *in vitro* has been established. Copolymers containing polyethylene glycol chains with a molecular weight of 1000 and above, when used in concentrations of 0.0001 % or 0.0005 %, do not exhibit cytotoxicity and can be used as drug transporters.

It has been shown that the synthesized copolymers are able to penetrate the membrane of germ cells and are decomposed during metabolic processes in sperm with the release of fluorescein, which makes it possible to create drug delivery systems based on them with the ability to monitor their localization in the body.

ACKNOWLEDGMENTS AND FUNDING SOURCES

The publication presents the results of the research project “Creation of medical devices based on new polymerhybrid hydrogel systems for the treatment and rehabilitation of military personnel with therapeutic and surgical pathology” funded from the state budget (the Ministry of Education and Science of Ukraine), 2024–2026.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

Animal Studies: all international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [S.V.; V.S.]; methodology, [M.Y.; D.O.]; investigation, [M.Y.; D.O.; N.N.]; resources, [Z.N.; I.Y.]; writing – original draft preparation, [S.V.; N.N.]; writing – review and editing, [S.V.; M.Y.; N.N.]; visualization, [N.N.]; supervision, [S.V.; V.S.].

REFERENCES

Anestopoulos, I., Kiousi, D. E., Klavaris, A., Galanis, A., Salek, K., Euston, S. R., Pappa, A., & Panayiotidis, M. I. (2020). Surface active agents and their health-promoting properties: molecules of multifunctional significance. *Pharmaceutics*, 12(7), 688. doi:10.3390/pharmaceutics12070688
Crossref ● PubMed ● PMC ● Google Scholar

Bhat, I. A., Roy, B., & Kabir-ud-Din. (2019). Micelles of cleavable gemini surfactant induce fluorescence switching in novel probe: industrial insight. *Journal of Industrial and Engineering Chemistry*, 77, 60–64. doi:10.1016/j.jiec.2019.04.035
Crossref ● Google Scholar

Bukartyk, M., Nosova, N., Maikovych, O., Bukartyk, N., Stasiuk, A., Dron, I., Fihurka, N., Khomyak, S., Ostapiv, D., Vlizlo, V., Samaryk, V., & Varvarenko, S. (2022). Preparation and research of properties of combined alginate/gelatin hydrogels. *Journal of Chemistry and Technologies,* 30(1),11–20. doi:10.15421/jchemtech.v30i1.242230
Crossref ● Google Scholar

Caddeo, C., Manconi, M., Fadda, A. M., Lai, F., Lampis, S., Diez-Sales, O., & Sinico, C. (2013). Nanocarriers for antioxidant resveratrol: formulation approach, vesicle self-assembly and stability evaluation. *Colloids and Surfaces B: Biointerfaces*, 111, 327–332. doi:10.1016/j.colsurfb.2013.06.016
Crossref ● PubMed ● Google Scholar

Chekh, B. O. C., Ferens, M. V., Ostapiv, D. D., Samaryk, V. Y., Varvarenko, S. M., & Vlizlo, V. V. (2017). Characteristics of novel polymer based on pseudo-polyamino acids GluLa-DPG-PEG600: binding of albumin, biocompatibility, biodistribution and potential crossing the blood-brain barrier in rats. *The Ukrainian Biochemical Journal*, 89(4), 13–21. doi:10.15407/ubj89.04.013
Crossref ● Google Scholar

Cho, I. K., & Easley, C. A. (2023). Recent developments in *in vitro* spermatogenesis and future directions. *Reproductive Medicine*, 4(3), 215–232. doi:10.3390/reprodmed4030020
Crossref ● Google Scholar

Eom, S., Choi, G., Nakamura, H., & Choy, J.-H. (2020). 2-Dimensional nanomaterials with imaging and diagnostic functions for nanomedicine; a review. *Bulletin of the Chemical Society of Japan*, 93(1), 1–12. doi:10.1246/bcsj.20190270
Crossref ● Google Scholar

Haleem, A., Javaid, M., Singh, R. P., Rab, S., & Suman, R. (2023). Applications of nanotechnology in medical field: a brief review. *Global Health Journal*, 7(2), 70–77. doi:10.1016/j.glohj.2023.02.008
Crossref ● Google Scholar

Hang, Y., Boryczka, J., & Wu, N. (2022). Visible-light and near-infrared fluorescence and surface-enhanced Raman scattering point-of-care sensing and bio-imaging: a review. *Chemical Society Reviews*, 51(1), 329–375. doi:10.1039/c9cs00621d
Crossref ● PubMed ● PMC ● Google Scholar

Gheorghita, R., Anchidin-Norocel, L., Filip, R., Dimian, M., & Covasa, M. (2021). Applications of biopolymers for drugs and probiotics delivery. *Polymers*, 13(16), 2729. doi:10.3390/polym13162729
Crossref ● PubMed ● PMC ● Google Scholar

Ghezzi, M., Pescina, S., Padula, C., Santi, P., Del Favero, E., Cantù, L., & Nicoli, S. (2021). Polymeric micelles in drug delivery: an insight of the techniques for their characterization and assessment in biorelevant conditions. *Journal of Controlled Release*, 332, 312–336. doi:10.1016/j.jconrel.2021.02.031
Crossref ● PubMed ● Google Scholar

Gref, R., Domb, A., Quellec, P., Blunk, T., Müller, R. H., Verbavatz, J. M., & Langer, R. (1995). The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. *Advanced Drug Delivery Reviews*, 16(2–3), 215–233. doi:10.1016/0169-409x(95)00026-4
Crossref ● PubMed ● PMC ● Google Scholar

Kornyat, S., Sharan, M., Ostapiv, D., Korbeckij, A., Jaremchuk, I., & Andrushko, O. (2021). Quality of deconserved bull sperm for the action of nanosuccinates Zn, Cu and Mn in the diluents. *The Animal Biology*, 23(1), 23–29. doi:10.15407/animbiol23.01.023 (In Ukrainian)
Crossref ● Google Scholar

Lam, P.-L., Wong, W.-Y., Bian, Z., Chui, C.-H., & Gambari, R. (2017). Recent advances in green nanoparticulate systems for drug delivery: efficient delivery and safety concern. *Nanomedicine*, 12(4), 357–385. doi:10.2217/nnm-2016-0305
Crossref ● PubMed ● Google Scholar

Lee, B. B., Chan, E. S., Ravindra, P., & Khan, T. A. (2012). Surface tension of viscous biopolymer solutions measured using the du Nouy ring method and the drop weight methods. *Polymer Bulletin*, 69(4), 471–489. doi:10.1007/s00289-012-0782-2
Crossref ● Google Scholar

Liu, R.-L., & Cai, R.-Q. (2022). Recent advances in ultrasound-controlled fluorescence technology for deep tissue optical imaging. *Journal of Pharmaceutical Analysis*, 12(4), 530–540. doi:10.1016/j.jpha.2021.10.002
Crossref ● PubMed ● PMC ● Google Scholar

Liubas, N., Iskra, R., Stadnytska, N., Monka, N., Havryliak, V., & Lubenets, V. (2022). Antioxidant activity of thiosulfonate compounds in experiments *in vitro* and *in vivo*. *Biointerface Research in Applied Chemistry*, 12(3), 3106–3116. doi:10.33263/briac123.31063116
Crossref ● Google Scholar

Luan, X., Pan, Y., Gao, Y., & Song, Y. (2021). Recent near-infrared light-activated nanomedicine toward precision cancer therapy. *Journal of Materials Chemistry B*, 9(35), 7076–7099. doi:10.1039/d1tb00671a
Crossref ● PubMed ● Google Scholar

Lucarini, S., Fagioli, L., Cavanagh, R., Liang, W., Perinelli, D. R., Campana, M., Stolnik, S., Lam, J. K. W., Casettari, L., & Duranti, A. (2018). Synthesis, structure–activity relationships and *in vitro* toxicity profile of lactose-based fatty acid monoesters as possible drug permeability enhancers. *Pharmaceutics*, 10(3), 81. doi:10.3390/pharmaceutics10030081
Crossref ● PubMed ● PMC ● Google Scholar

Lyu, S., & Untereker, D. (2009). Degradability of polymers for implantable biomedical devices. *International Journal of Molecular Sciences*, 10(9), 4033–4065. doi:10.3390/ijms10094033
Crossref ● PubMed ● PMC ● Google Scholar

Morrell, J. M., Valeanu, A. S., Lundeheim, N., & Johannisson, A. (2018). Sperm quality in frozen beef and dairy bull semen. *Acta Veterinaria Scandinavica*, 60(1), 41. doi:10.1186/s13028-018-0396-2
Crossref ● PubMed ● PMC ● Google Scholar

Myers, D. (2006). Polymeric surfactants and surfactant–polymer interactions. In: *Surfactants science and technology* (pp. 220–244). New Jersey: Wiley. doi:10.1002/047174607X.ch7
Crossref ● Google Scholar

Nicolas, J., Magli, S., Rabbachin, L., Sampaolesi, S., Nicotra, F., & Russo, L. (2020). 3D extracellular matrix mimics: fundamental concepts and role of materials chemistry to influence stem cell fate. *Biomacromolecules*, 21(6), 1968–1994. doi:10.1021/acs.biomac.0c00045
Crossref ● PubMed ● Google Scholar

Parker, A. L., Newman, C., Briggs, S., Seymour, L., & Sheridan, P. J. (2003). Nonviral gene delivery: techniques and implications for molecular medicine. *Expert Reviews in Molecular Medicine*, 5(22), 1–15. doi:10.1017/s1462399403006562
Crossref ● PubMed ● Google Scholar

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. del P., Acosta-Torres, L. S., Diaz-Torres, L. A., Grillo, R., Swamy, M. K., Sharma, S., Habtemariam, S., & Shin, H.-S. (2018). Nano based drug delivery systems: recent developments and future prospects. *Journal of Nanobiotechnology*, 16(1), 71. doi:10.1186/s12951-018-0392-8
Crossref ● PubMed ● PMC ● Google Scholar

Shamma, R. N., Sayed, R. H., Madry, H., EL Sayed, N. S., & Cucchiarini, M. (2022). Triblock copolymer bioinks in hydrogel three-dimensional printing for regenerative medicine: a focus on pluronic F127. *Tissue Engineering Part B: Reviews*, 28(2), 451–463. doi:10.1089/ten.teb.2021.0026
Crossref ● PubMed ● Google Scholar

Stasiuk, A. V., Fihurka, N. V., Tarnavchyk, I. T., Nosova, N. G., Pasetto, P., Varvarenko, S. M., & Samaryk, V. Y. (2022). Influence of structure and nature of pseudo-poly(amino acid)s on size and morphology of their particle in self-stabilized aqueous dispersions. *Applied Nanoscience*, 13(7), 5011–5019. doi:10.1007/s13204-022-02664-7
Crossref ● Google Scholar

Stetsyshyn, Y., Raczkowska, J., Harhay, K., Awsiuk, K., Shymborska, Y., Nastyshyn, S., Ohar, H., Vasilyev, V., Ostapiv, D., Sharan, M., Sharan, O., Voronov, S., & Budkowski, A. (2020). Grafted polymer brush coatings for growth of cow granulosa cells and oocyte-cumulus cell complexes. *Biointerphases*, 15(3), 031006. doi:10.1116/6.0000183
Crossref ● PubMed ● Google Scholar

Tihauan, B.-M., Marinas, I.-C., Bleotu, C., Dolete, G., Onisei, T., Serbancea, F., Mateescu, C., & Rascol, M. R. (2021). Evaluation of cytotoxicity, nutritional and antioxidative status of lyophilized plant extracts used in dietary supplements. *Romanian Biotechnological Letters*, 26(2), 2396–2405. doi:10.25083/rbl/26.2/2396.2405
Crossref ● Google Scholar

Torchilin, V. P. (2001). Structure and design of polymeric surfactant-based drug delivery systems. *Journal of Controlled Release*, 73(2–3), 137–172. doi:10.1016/s0168-3659(01)00299-1
Crossref ● PubMed ● Google Scholar

Yakoviv, M. V., Varvarenko, S. M., Samaryk, V. Ya., Nosova, N. G., Fihurka, N. V., Maikovych, O. V., Dron, I. A., & Voronov, S. A. (2020). Peculiarities of the molecular weight distribution of fluorescein-containing copolyesters synthesized by the steglich reaction. *Journal of Chemistry and Technologies*, 28(1), 10–16. doi:10.15421/082002
Crossref ● Google Scholar

Yakoviv, M. V., Nosova, N. G., Samaryk, V. Y., Pasetto, P., & Varvarenko, S. M. (2020). Study of physical interactions of fluorescein-containing amphiphilic copolyesters with albumin in aqueous dispersions. *Applied Nanoscience*, 10(8), 2655–2663. doi:10.1007/s13204-019-00987-6
Crossref ● Google Scholar

Yakoviv, M., Fihurka, N., Nosova, N., Samaryk, V., Vasylyshyn, T., Hermanovych, S., Voronov, S., & Varvarenko, S. (2018). Researches of amphiphilic properties of copolyesters with chromophore groups. *Chemistry & Chemical Technology*, 12(3), 318–325. doi:10.23939/chcht12.03.318
Crossref ● Google Scholar

Yang, B., Chen, Y., & Shi, J. (2020). Tumor-specific chemotherapy by nanomedicine-enabled differential stress sensitization. *Angewandte Chemie International Edition*, 59(24), 9693–9701. doi:10.1002/anie.202002306
Crossref ● PubMed ● Google Scholar

Yu, J., Qiu, H., Yin, S., Wang, H., & Li, Y. (2021). Polymeric drug delivery system based on pluronics for cancer treatment. *Molecules*, 26(12), 3610. doi:10.3390/molecules26123610
Crossref ● PubMed ● PMC ● Google Scholar

Vakilzadeh, H., Varshosaz, J., & Minaiyan, M. (2018). Pulmonary delivery of triptorelin loaded in pluronic based nanomicelles in rat model. *Current Drug Delivery*, 15(5), 630–640. doi:10.2174/1567201815666180209113735
Crossref ● PubMed ● Google Scholar

Varvarenko, S. M., Ferens, M. V., Samaryk, V. Ya., Nosova, N. G., Fihurka, N. V., Ostapiv, D. D., & Voronov, S. A. (2018). Synthesis of copolyesters of fluorescein and 2-(dodecanamino) pentanedionic acid via Steglich reaction. *Voprosy Khimii i Khimicheskoi Tekhnologii*, 2, 5–15. (In Ukrainian)
Google Scholar

Varshosaz, J., Taymouri, S., Minaiyan, M., Rastegarnasab, F., & Baradaran, A. (2018). Development and *in vitro*/*in vivo* evaluation of HPMC/chitosan gel containing simvastatin loaded self-assembled nanomicelles as a potent wound healing agent. *Drug Development and Industrial Pharmacy*, 44(2), 276–288. doi:10.1080/03639045.2017.1391832
Crossref ● PubMed ● Google Scholar

Wang, M., Chen, M., Niu, W., Winston, D. D., Cheng, W., & Lei, B. (2020). Injectable biodegradation-visual self-healing citrate hydrogel with high tissue penetration for microenvironment-responsive degradation and local tumor therapy. *Biomaterials*, 261, 120301. doi:10.1016/j.biomaterials.2020.120301
Crossref ● PubMed ● Google Scholar

Wu, J., Gao, H., Shi, D., Yang, Y., Zhang, Y., & Zhu, W. (2020). Cationic gemini surfactants containing both amide and ester groups: synthesis, surface properties and antibacterial activity. *Journal of Molecular Liquids*, 299, 112248. doi:10.1016/j.molliq.2019.112248
Crossref ● Google Scholar

Wu, Y., Zhou, D., Qi, Y., Xie, Z., Chen, X., Jing, X., & Huang, Y. (2015). Novel multi-sensitive pseudo-poly(amino acid) for effective intracellular drug delivery. *RSC Advances*, 5(40), 31972–31983. doi:10.1039/c5ra03423j
Crossref ● Google Scholar

Udayakumar, G. P., Muthusamy, S., Selvaganesh, B., Sivarajasekar, N., Rambabu, K., Banat, F., Sivamani, S., Sivakumar, N., Hosseini-Bandegharaei, A., & Show, P. L. (2021). Biopolymers and composites: properties, characterization and their applications in food, medical and pharmaceutical industries. *Journal of Environmental Chemical Engineering*, 9(4), 105322. doi:10.1016/j.jece.2021.105322
Crossref ● Google Scholar

ФЛУОРЕСЦЕЇНОВМІСНІ АМФІФІЛЬНІ КОПОЛІЕСТЕРИ – ПЕРСПЕКТИВНІ ОБ'ЄКТИ ДЛЯ БІОМЕДИЧНИХ ДОСЛІДЖЕНЬ

***Марія Яковів*[1], *Наталія Носова*[1], *Дмитро Остапів*[2], *Ірина Яремчук*[2], *Зоряна Надашкевич*[1], *Володимир Самарик*[1], *Сергій Варваренко*[1]**

[1] *Національний університет "Львівська політехніка" вул. С. Бандери, 12, Львів 79013, Україна*

[2] *Інститут біології тварин НААН, вул. В. Стуса, 38, Львів 79034, Україна*

Обґрунтування. Полімерні наночастинки дедалі частіше використовують як носії лікарських препаратів. Вони продемонстрували значне покращення терапевтичної ефективності лікарських препаратів і широко досліджуються як компоненти систем транспорту й вивільнення ліків. На відміну від інших типів наночастинок,

залежно від природи та властивостей, полімерні носії можуть конструюватися зі здатністю орієнтуватись на певні органи, тканини чи клітини та, в остаточному підсумку, біологічно розкладатися з мінімальною системною токсичністю. Метою було дослідити *in vitro* цитотоксичність систем доставлення лікарських препаратів на основі наночастинок флуоресцеїновмісних амфіфільних кополіестерів, а також оцінити їхню здатність проникати в клітину та можливість забезпечити контроль за цим процесом.

Матеріали та методи. Для досліджень використовували кополімери, отримані на основі 2-(додеканоїламіно)пентандіової кислоти та 2-(октадеканоїламіно) пентандіової кислоти, поліетиленетердіолів і флуоресцеїну. Було оцінено поверхневоактивні властивості кополімерів і солюбілізаційну здатність їхніх колоїдних розчинів. Дослідження цитотоксичності флуоресцеїновмісних кополіестерів і активності клітинних ензимів проводили на живих сперматозоїдах, отриманих з еякулятів бугаїв, об'ємом 2–6 мл, з концентрацією сперміїв 0,6–1,5·10^9 клітин/мл та активністю 7,5–8,0 балів.

Результати. Проведено дослідження двох груп амфіфільних кополіестерів, отриманих на основі 2-(додеканоїламіно)пентандіової кислоти та 2-(октадеканоїламіно)пентандіової кислот, з різною молярною масою поліетиленетердіолів (від 600 до 1500) та з різним вмістом флуоресцеїну. Визначено їхні поверхневоактивні властивості й визначено здатність до солюбілізації ліпофільних речовин – аналогів лікарських препаратів. Встановлено, що отримані дисперсії кополіестерів не проявляють цитотоксичності та, за безпосереднього контакту зі статевими клітинами, кополіестери мають здатність проникати крізь клітинну мембрану й розкладатися з вивільненням флуоресцеїну, що дає змогу відстежувати їхнє місце у структурах статевих клітин.

Висновки. Встановлено взаємозв'язок будови амфіфільних флуоресцеїнвмісних кополіестерів зі ступенем їхнього впливу на живі об'єкти *in vitro*. Встановлено склад кополіестерів, які не проявляють цитотоксичності й можуть бути використані як транспортери лікарських препаратів. З'ясовано, що синтезовані кополіестери здатні проникати крізь мембрану статевих клітин і руйнуються під час метаболічних процесів у сперміях з вивільненням флуоресцеїну.

Ключові слова: амфіфільний кополіестер, флуоресцеїн, цитотоксичність, активність ензимів

Received / Одержано 16 July, 2024 | Revision / Доопрацьовано 04 October, 2024 | Accepted / Прийнято 19 March, 2025 | Published / Опубліковано 26 March, 2025