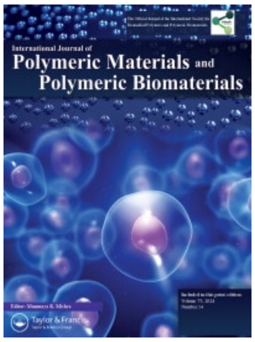

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Surface active polyesters based on N-substituted glutamic acid as promising materials for biomedical applications

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ABSTRACT

In the present work, N-substituted glutamic acid, polyethylene and polypropylene glycols have been used to design biocompatible copolyesters via Steglich reactions. Due to the presence of alternating hydrophilic and hydrophobic blocks in their structures, these copolyesters are able to form self-stabilized nanoparticle dispersions in aqueous media. The lipophilic core of these nanoparticles can solubilize poorly water-soluble compounds and release them into a model of lipids in a human body. Moreover, the obtained copolyesters possess no cytotoxic effects over a wide concentration range. Thus, we conclude that obtained copolyesters show significant promise for further development as drug delivery systems.

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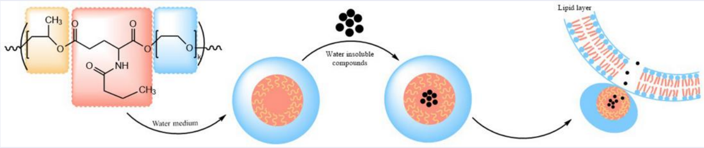
Glutamic acid; polyesters; polyethylene glycols; steglich reaction

GRAPHICAL ABSTRACT



1. Introduction

The development of new therapeutic agents is an expensive process and is currently being worked on by a large number of scientists around the world. There is a growing drive to develop new drug delivery systems for existing therapeutic agents, to improve their efficacy, and to treat different diseases. Drug delivery systems need to be safe, transport drugs within the body, and release the bioactive species only at the specified target site. This improves the pharmacokinetic and pharmacodynamic properties of the therapeutic by increasing the circulation period in blood, increasing therapeutic enzyme stability, increasing the solubility of low molecular weight pharmaceuticals, and introduces the potential to target drug delivery to the site of interest[1–3]. Particular classes of polymers are excellent candidates for drug delivery systems since they possess many characteristics that make them ideally suited for these applications. At the time of writing, polymers based on polyethylene glycol (PEG), poly(D,L-lactide-co-glycolide) (PLGA), poly(lactic acid) (PLA), poly(glutamic acid) (PGA), poly(caprolactone) (PCL), N-(2-hydroxypropyl)-methacrylate (HPMA) copolymers, and poly(amino acids) are widely reported and used in various medical applications[4]. These polymers have already been shown to be able to transport bioactive species, can be non-toxic, do not trigger an immune response, and are biodegradable or controllably biodegradable[5,6].

BASF have reported the development of "Pluronics", a new class of ABA triblock copolymers. These copolymers consist of a hydrophilic polyethylene oxide (PEO) block (A), and a lipophilic polypropylene oxide (PPO) block (B). Pluronic copolymers form well dispersed micelle-like structures in aqueous media that are able to solubilize and stabilize pharmaceuticals. The therapeutic agent is in the lipophilic core and surrounded by the hydrophilic shell, which protects the therapeutic agent from cells and proteins. It is important that the chemical and physical properties of the system can be easily modified by changing the ratio of hydrophilic and hydrophobic blocks in the polymer structure[7,8]. This approach has the potential to be used for the synthesis of new copolymers for the delivery of various drugs. Pluronic-based systems have been utilized to deliver drugs: to the central nervous system across the blood-brain barrier[9,10], in anticancer therapy[11–14], via oral drug

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delivery[15]; as well as having been used in cosmetics as surfactants, emulsifiers, and solubilizing agents[16].

Pluronic molecules have been shown to be able to penetrate cell membranes and affect intracellular mechanisms such as: ATP synthesis, apoptotic signal transmission, gene expression, etc.)[17]. This improves the transport of therapeutic drugs across the intestine and blood-brain barrier and enhances the efficiency of cancer drugs.

However, the use of Pluronics in biomedical applications is not without limitations or drawbacks. PEO-PPO-PEO structures are not biodegradable, this fact significantly limits the molecular weight of Pluronics that can be used for drug delivery applications, since non-biodegradable polymers need to be excreted from the body via glomerular filtration. As a general rule, polymers with molecular weights in the range of 30–50 kDa can be removed via glomerular filtration, although the molecular conformation and chemical properties of the polymer can alter this[1]. Polymers whose size and chemistry prevent their removal by glomerular filtration and can avoid capture by macrophages can circulate in the body for a long time and accumulate in organs[18]. Although PEG is the most widely used polymer for in vivo and in vitro biomedical applications, and has been approved by the FDA for such systems[19]; PEG does have a degree of immunogenicity and toxicity in certain organs[20–22].

As the molecular weight of the polymer is reduced it is more rapidly removed from the body. Experiments on animals revealed that 20% of Pluronic-188 was excreted in their urine 1 h after intravenous injection, and 40% was excreted within 24 h. Assessment of the excretion and safety profile of Pluronics indicates their fairly rapid excretion from the body in urine, as well as some accumulation in the lung, liver, brain, and kidney[17]. These results significantly limit the utilization of Pluronics for drug delivery systems.

This work aimed to develop a new class of polymers forming pseudomicellar structures in aqueous media, similar to Pluronic micelles but possessing controllable biodegradability. The block copolyesters presented in Figure 1 were found to meet these requirements, to a certain extent.

The ester bond, unlike the ether bond in Pluronics, is biodegradable; therefore, the proposed polyesters should be biodegradable. The connecting fragment in the proposed structure is an N-alkanoyl derivative of glutamic acid Glu(Alk), which is highly lipophilic. Combining Glu(Alk) fragments with hydrophilic PEG fragments and weakly lipophilic PPG fragments (or without them) in one block provides surface-active properties for separate blocks and the entire macromolecule. Regulation of the surface-active properties can be obtained by changing the molecular weight of any of the three constituent monomers (PEG, PPG, Glu(Alk)). The incorporation of α-amino acids by non-peptide bonds makes the proposed class of polymers similar to pseudo-poly(amino acid)s – a class of polymers extensively studied for medical applications. Polymers constituting amino acids combined by non-peptide bonds acquire pyrogenic properties, do not cause an immune reaction when introduced into the body, and their biodegradation does not result in toxic products[23–25] as expected biodegradation products of proposed polyesters are building blocks of amino acid derivatives and PEG and/or PPG fragments. The residence time for these delivery systems can be controlled by tuning their molecular weight and knowing the rate of their biodegradation. Furthermore, the products of the degradation are either compounds inherent in the human body (derivatives of amino acids) or PEG and PPG molecules with a molecular weight not exceeding 1.5–2.0 kDa, the removal of which from the body are known to be facile. Moreover, recently we have confirmed the increased efficiency of oxytetracycline in a complex with Glu(Alk)/PEG based copolyesters[26,27].

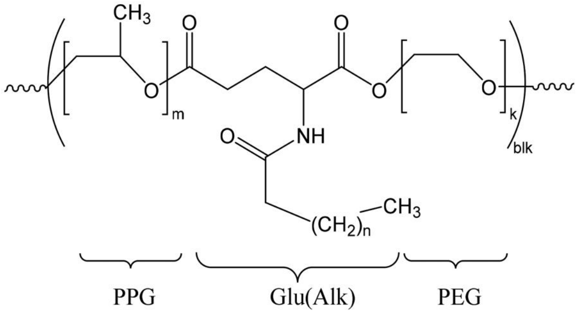


Figure 1. Structure of the polyesters created with N-derivatives of L-glutamic acid (Glu(Alk)), polyethylene glycol (PEG), and polypropylene glycol (PPG).

2. Materials and methods

2.1. Materials

Glutamic acid and polyoxyethylene/polyoxypropylene diols were obtained from Sigma-Aldrich and further dried. Glutamic acid was dried over P_2O_5 under a vacuum. Polyoxyethylene/polyoxypropylene diols were dried by refluxing in benzene with the azeotropic removal of water. Stearoyl chloride, thionyl chloride, triethylamine, and solvents were obtained from Sigma-Aldrich and used as received. N,N′-Dicyclohexylcarbodiimide (DCC), and 4-dimethylaminopyridine (DMAP) were obtained from Alfa Aesar and used as received.

2.2. Synthesis

N-derivatives of L-glutamic acid (GluSt, GluL) were synthesized using the procedure described in Ref. [28, 29].

Dicopolyester and tricopolyester synthesis was carried out as follows: the glutamic acid derivative (Glu(St) or Glu(L)) (10 mmols), glycol, or a mixture of two different glycols (11 mmols) and a solvent (dimethylformamide/dichloromethane mixture) were loaded into the reactor. Then DCC (21 mmols) and a catalyst — 4-dimethylaminopyridine (DMAP) (1.25 mmols), in solution, were added using a drop funnel at a temperature of 7 °C. After the addition of the reagents was complete the mixture was sequentially held at 15 °C for 3h and 35 °C for 3 h. Finally, a side product of the reaction, dicyclohexylurea (DCU), was filtered off and the reaction mixture was evaporated.

Reactive blocks with terminal carboxyl groups were synthesized using the procedure described in Ref. [30].

Block copolyester synthesis was carried out as follows: First, reactive blocks with terminal carboxyl groups Glu(Alk)-PPG-Glu(Alk) (10 mmols), glycol (PEG) (11 mmols), and solvent (dimethylformamide/dichloromethane mixture) were loaded into the reactor. Then a solution of DCC (21 mmols) and catalyst – 4-dimethylaminopyridine (DMAP) (1.25 mmols) was added using a drop funnel at a temperature of 7 °C. After the addition of the reagents, the mixture was held at 15 °C for 3 h and then at 35 °C for 3 h. Finally, the side product of the reaction, dicyclohexylurea (DCU), was filtered off and the reaction mixture evaporated.

2.3. Characterization

2.3.1. ^{1}H NMR spectroscopy

Monomer and copolymer samples were dissolved in the corresponding deuterated solvent (deuterobenzene, deuterochloroform, deuterated water). ^{1}H NMR spectra of the samples were recorded using JEOL's ECA Series Nuclear Magnetic Resonance (NMR) Spectrometer at a frequency of 400 MHz in automatic scanning mode.

FTIR spectroscopy was conducted with KBr pellets or in a thin layer using a Thermo Scientific Nicolet 8,700 spectrometer. IR spectra were recorded in transmittance mode within the wave number range of 500–4,000 cm^{-1} with 4 cm^{-1} resolution.

2.3.2. Determination of molecular weight

The average MW of the copolymers was determined using a WatersCorporation chromatograph with a Waters 2,410 refractive index detector, a Waters 515 HPLC pump, and an UltrahydrogelTM 500 7.8 × 300 mm column. Tetrahydrofuran was used as the eluent. The consumption rate was 0.1 ml/min. Calibration curves of the molecular weight were measured according to low polydispersity polystyrene standards of the Polymer Laboratories (USA) type.

2.3.3. Determination of particle size

Determination of the effective hydrodynamic radius of self-stabilized micellar structures in polyester solutions was conducted by dynamic light scattering (DLS). Particle charge was measured on a Zeta Sizer Nano-ZS90 (Malvern) instrument at 20 °C. The polyester concentration was 0.1–1wt% in deionized water (pH = 6.6–6.8). All measurements were made in triplicate.

The surface tension of pseudo-poly(amino acid) water solutions was measured at 20 °C and pH = 6.6–6.8 using the Du Noüy ring method. All measurements were made in triplicate and averaged.

The solubilization of water-insoluble dyes (Sudan III) by polyester dispersions in water was studied by spectrophotometry. First, the calibration dependence of the different dyes (in acetone) in the presence of different, appropriate, concentrations of the polyesters were obtained. Then a constant amount (0.01 g) of dye was added to aqueous dispersions of the polyesters with concentrations between 0.002 and 4 g/L. These systems were stirred at room temperature for 24 h. The excess dye was filtered off and the resulting solutions were diluted with acetone. The optical density (D) was measured using a photoelectrocolorimeter LMP-72M using light filter No. 4 ($\lambda = 490$ nm) and cuvettes with a thickness of 10.18 mm. Using the obtained calibration dependences, the "S" value (g/g) corresponding to the weight of the dye (g) solubilized by 1 gram of the polyester was determined. All measurements were made in triplicate and averaged.

The release of the dye into the model lipophilic medium of 1-octanol was determined using a Yuniko 1,201 spectrophotometer. Aqueous dispersions containing solubilized Sudan III and 1-octanol were stirred in a cuvette with a thickness of 5.11 mm at a speed of 120 rpm, preventing the formation of an emulsion. The content of Sudan III in 1-octanol was determined spectrophotometrically according to the calibration curve obtained in octanol. All measurements were made in triplicate and averaged.

2.3.4. Cytotoxicity

To investigate the cytotoxicity of the copolyesters, they were each dispersed in deionized water before being added to a solution of bull sperm in a citrate-yolk diluent (in a ratio of 1:6). 10 µL, 25 µL, and 50 µL of a 1% polymer dispersion was added to 1 ml of diluted sperm. The survival time (progressive movement of sperm) was recorded visually under a microscope (×200) in comparison with a control sample of sperm without the addition of polymer dispersion. The time (hours) from the moment the polymer dispersion is introduced into the diluted sperm until the sperm motility stops (m) was taken as the duration of sperm survival. All measurements were made in 5 replicas and averaged.

3. Results and discussions

The polymers presented in Figure 1 are polyesters that can be synthesized via the polycondensation of N-alkanoyl substituted glutamic acid, polyethylene and/or polypropylene glycols. E. Fisher developed a method of synthesizing amino acid esters in solution. Using this approach, high yields of esters can be obtained by adding high concentrations of hydrogen chloride (10 times greater than the alcohol concentration). One peculiar property of dicarboxylic amino acids is the unequal reactivity of the two carboxyl groups. This accounts for the scarcity of scientific publications on polyester synthesis using this approach.

According to the literature, esters of glutamic and aspartic acid can be obtained via selective activation of the ω-carboxylic group by hydrochloric acid at room temperature[31]. The dications, formed by the protonation of carboxyl group 2 (Figure 2)

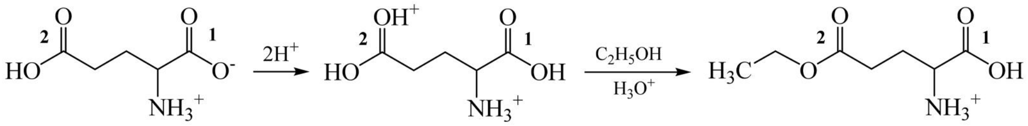


Figure 2. Esterification of glutamic acid esters using hydrogen chloride.

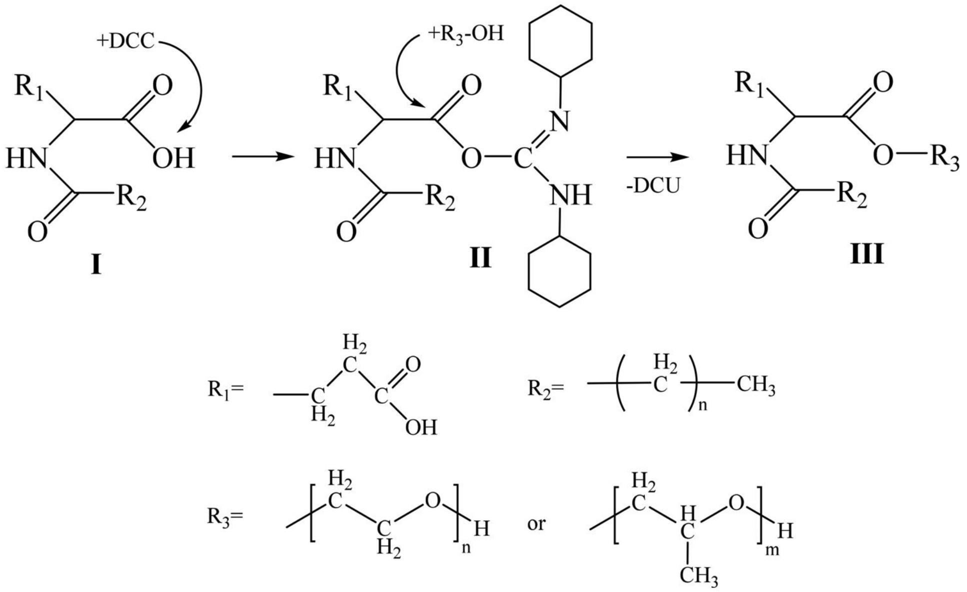


Figure 3. Formation of an ester bond when using the DCC activator, with the participation of N-alkanoylglutamic acid.

are more stable, as the atoms bearing positive charges are located further away from each other.

Ester formation on carboxyl group 1 (a-carboxyl group) could be achieved at elevated temperatures (100–140 °C). However, the temperature at which this reaction can be performed is limited by the thermolability of dicarboxylic amino acids. In this work, the reaction between N-alkanoyl dicarboxylic amino acids, polyethylene and polypropylene glycols (in benzene/toluene media) was performed with equimolar ratios on the reactants. This was done to assess the possibility of polyesterification occurring by the Fisher reaction. Significant tarring of the mixture was observed and several products were identified after the reaction. The most abundant product of the reaction was an ester formed by a single PEG molecule binding to the ω-carboxylic group. Thus, it was concluded that the Fisher method is not a promising synthetic approach for N-alkanoyl dicarboxylic amino acid polyester formation.

Won et al.[32] have reported being able to modify the Fisher reaction, to obtain polyesters based on the N/derivatives of aspartic acid and polyethylene glycols, with molecular weights between 200 and 2,000 Da. This synthesis was conducted in four steps, via the formation of an intramolecular amino acid anhydride. In said work, the authors conclude that their multistep, high-temperature synthetic approach only leads to the formation of polyester with PEG-200 with the average polycondensation degree 4,5–6; for samples containing PEG-600 or PEG-2000 the degree of polycondensation was only 2–3.

The low degrees of polycondensation presented in this work confirm that the synthesis of esters and polyesters based on dibasic a-amino acids are limited due to: (1) the decarboxylization and cyclization processes that can occur at high temperatures, (2) the unequal reactivity of carboxyl groups located at different distances from the amino group. Thus, approaches of direct esterification, re-esterification, and migration polymerization should not be considered as promising methods. Therefore, the synthesis of the desired polyesters should be conducted under mild conditions using coupling agents.

One promising method of esterification is the Brenner reaction, which is widely used for the synthesis of polyesters from dibasic acids and diols. This reaction occurs in mild conditions and could conceptually be used to synthesize dibasic amino acid-based polyesters. However, this reaction is performed in different solvents, over a wide temperature range, and with different reagent stoichiometries. When we tried this method, we found that it led to the tarring of the reaction mixture without polyester formation. However, a small quantity of the carboxyl groups was consumed during the reaction. Further investigation revealed the formation of reactive blocks with terminal carboxyl groups – Glu(Alk)-PEG(or PPG)-Glu(Alk)[30]. Thus, we have developed a convenient approach to amino acid diester synthesis under mild conditions (45 °C) with a yield of 85–88%.

Irreversible polycondensation via the Steglich reaction occurs under mild conditions (between −20 °C and +50 °C) and results in the formation of an ester bond between the hydroxyl group and carboxyl group activated by N,N-dicyclohexylcarbodiimide (DCC). An additional advantage of this activator is the insolubility of the reaction byproduct (dicyclohexylurea, DCU) in the majority of organic solvents. Thus, DCU can easily be removed after the reaction has been completed. The Steglich reaction on α-amino acids can only be conducted if the amino group is protected, as the activated carboxyl group (I) reacts with the amino group faster than with the hydroxyl group. We have previously investigated the Steglich reaction using amino acids with different N-substitutions, including temporary protecting groups. In this paper only N-alkanoyl derivatives are discussed, as they not only protect the amino group, but also influence the hydrophilic-lipophilic nature of the macromolecular products.

The general mechanism of ester bond formation via the Steglich reaction of N-alkanoylglutamic acid is presented in Figure 3.

According to the scheme shown in Figure 3, the formation of an ester bond occurs via the nucleophilic attack of the DCC activated carbonyl carbon shown as intermediate (II) When the ratio of dicarboxylic acid to diol is close to equimolar, and a high degree of conversion (more than 95–97%) is achieved; the given reaction should lead to the formation of polyesters with the structure shown in Figure 1. However, the reaction of the activated carboxyl group (II, Figure 3) with the hydroxyl occurs at a rate comparable with that of the rearrangement of the activated carboxyl group into the passive N-isoacylurea (III), according to the scheme in Figure 4. This rearrangement reaction can be inhibited by using an excess of alcohol.

Since an excess of one of the reagents is not suitable for polycondensation reactions, tertiary amines are used as nucleophilic agents to promote polyesterefication. In this senario, the nucleophilic agent reacts with the activated carboxyl group (II, Figure 3) forming an intermediate compound with strong acylating properties.

The rearrangement occurs with the incorporation of the DCC fragment into the structure of the passive product and, therefore, leads to a decrease in DCU yield. Therefore, the efficiency of the nucleophilic agent could be evaluated by the quantifying the amount of DCU that could be isolated after the reaction. When 4-dimethylaminopyridine was used as a nucleophilic agent, it provided a DCC conversion of 98% or greater, whereas DMAP yielded a 5–6% DCC conversion (Figure 5a).

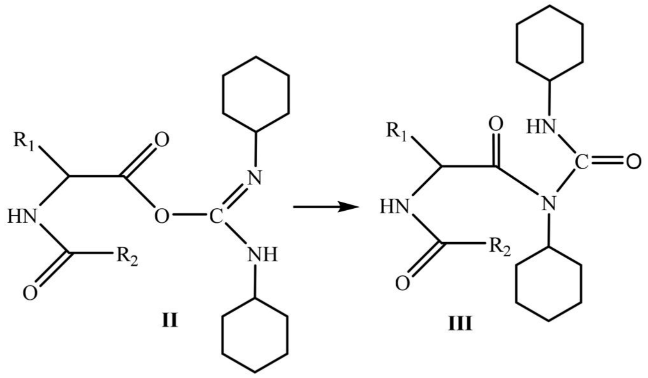


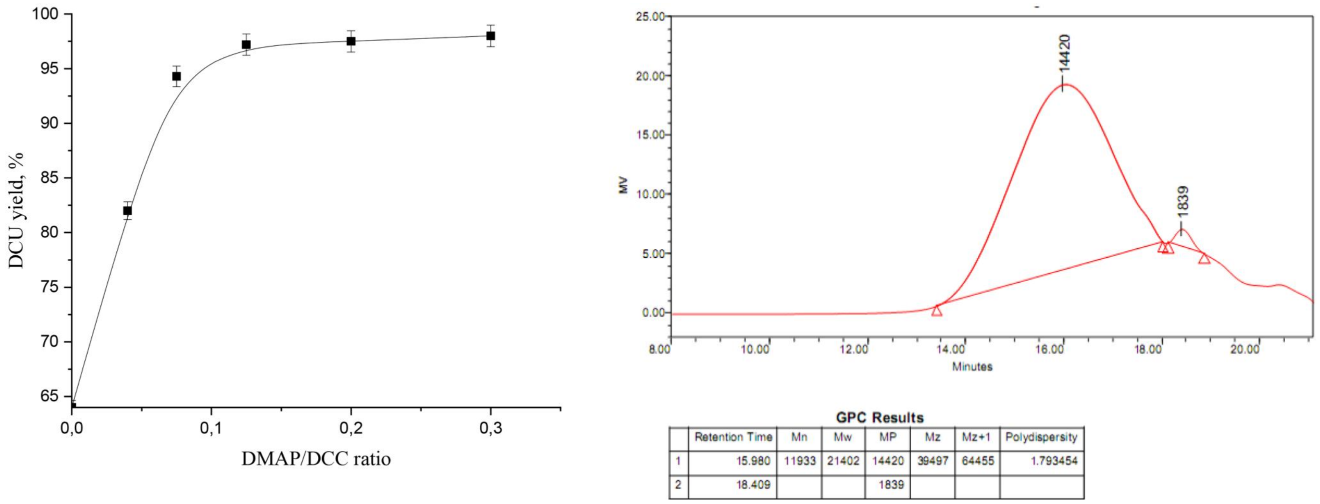
Figure 4. Rearrangement of the activated carboxyl group (II) into a less reactive form, which is unable to participate in the Steglich reaction.

GPC data confirmed that the nucleophilic agent not only leads to an increased DCC conversion, but also leads to an increased degree of polycondensation (Figure 5b). The introduction of DMAP (12.5–15% mol.) results in a high degree of conversion in the polyesterification of N-alkanoyl derivatives of glutamic acid, PEG and PPG diols. The structure of the polyesters obtained was confirmed by NMR and FTIR spectroscopy (see SI).

Since the polymers described were developed for biomedical applications, in particular for drug delivery, a set of experiments were designed to establish the colloidal and cytotoxic properties of these polymers.

Using the synthetic approach developed, three types of copolymers were synthesized and investigated: (1) copolymers based on N-substituted glutamic acid and one alcohol (PEG or PPG diol, labeled dicopolyesters); (2) copolymers based on N-substituted glutamic acid and two different alcohols (PEG and PPG diols, labeled tricopolyesters); (3) copolymer blocks with terminal carboxyl groups that had been pre-synthesized via the Brenner reaction and PPG diols (block copolyesters). Stirring for 15 minutes or ultrasonication of the copolyesters obtained (in an aqueous medium) led to the formation of dispersions without the need for additional stabilizers or emulsifiers. The aggregate stability of such dispersions largely depends on the composition of the polyesters (ratio of Glu(A), PEG, and PPG fragments), their structure, and the synthetic approach used.

Formation of the dispersion is accompanied by a decrease in the surface tension at the water-air interface, this was attributed to the presence of hydrophilic PEG fragments and lipophilic alkyl fragments in the N-substituent of glutamic acid in the polyesters. The data in Figure 6 compares the



GPC Results

	Retention Time	Mn	Mw	MP	Mz	Mz+1	Polydispersity
1	15.980	11933	21402	14420	39497	64455	1.793454
2	18.409			1839			

Figure 5. (a) Dependence of DCU yield on the ratio of DCC and DMAP; (b) molecular weight of the copolyester co-Glu(St)-co-PEG600 synthesized at a DCC/DMAP ratio 1:0.125.

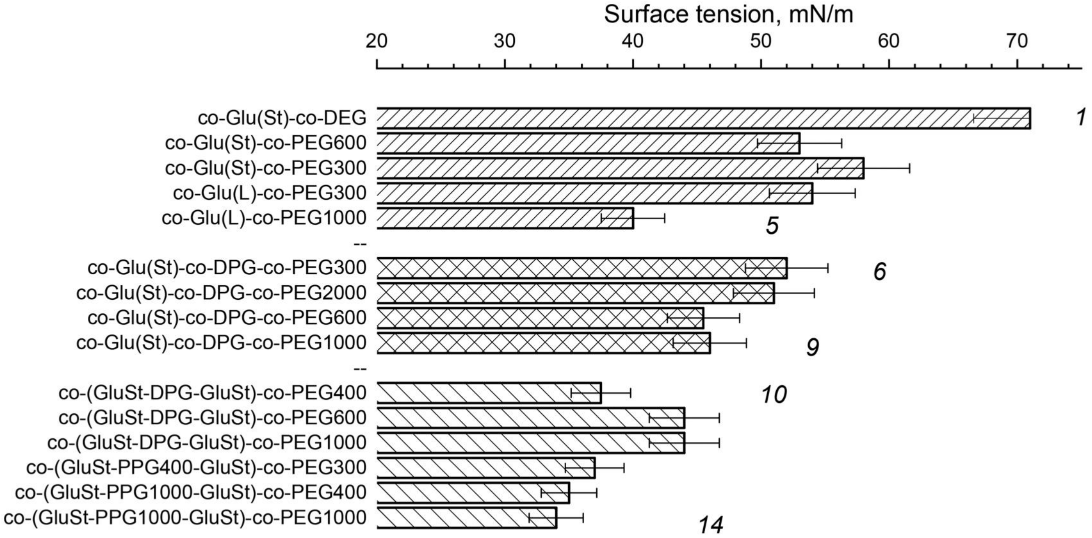


Figure 6. Surface tension of water dispersion with 0,2% of dicopolyester (bars 1–5), tricopolyesters (bars 6–9), and block copolyesters (bars 10–14).

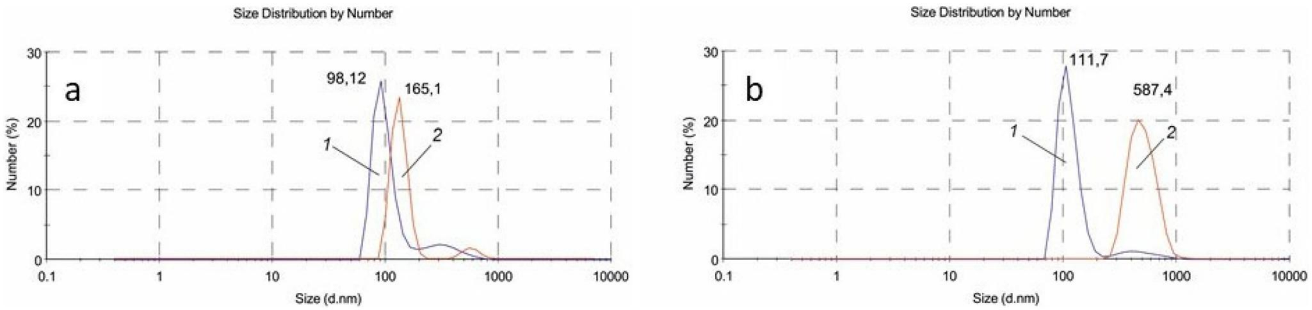


Figure 7. Histograms of particle size distribution for the aqueous dispersions of tricopolyester co-Glu(St)-co-DPG-co-PEG1000 (blue line) and block copolymers co-(Glu(St)-DPG-Glu(St))-co-PEG1000 (red line) at concentrations of 0.02% % (a) and 0.1%(b) (20 °C, pH = 6.6–6.8).

surface tension of water dispersions containing the different copolyesters synthesized in this work. The surface tension spans a relatively wide range of values, from 30 to 60 mN/m and primarily correlates to the composition and structure of the copolyesters.

As would be expected, the molecular weight of the polyoxyethylene fragment significantly influences the ability of polyesters to decrease the surface tension of the water (comparing bars 2 and 3, and bar 4 with bar 5). A less significant factor is the molecular weight of the alkyl moiety in the N-substituent of glutamic acid (bar 3 compared to bar 4). The combination of these two factors makes it possible to achieve a large decrease in surface tension (bar 5). As expected, the sample with a short polyoxypropylene fragment of dipropyleneglycol (DEG) (bar 1) does not possess any surface-active properties.

The tricopolyesters, samples 6–9, were obtained via the simultaneous addition of both polyoxyethylene and dipropylene glycols into the reaction mixture in an equimolar ratio. The introduction of dipropylene glycol fragments into the polyester structure, instead of half of the polyoxyethylene fragments, contributes to a significant decrease in surface tension (samples in bars 6–9 compared to samples represented by bars 2–5). It is worth noting that NMR studies confirm a close to the equimolar ratio of these monomers in the polyester structure. However it should be stressed that this synthetic approach does not guarantee a regular alternation of the different fragments (-Glu(St)-PEGn- and -Glu(St)-PPGm-), their frequency and spacing in the macromolecules is statistically random.

The most significant decrease in surface tension was observed for the block copolyester samples, obtained via the copolymerization of pre-synthesized blocks (-Glu(St)-PPGn-Glu(St)-) with polyethylene glycols of different molecular weights (bars 10–14). The -Glu(St)-PPGn-Glu(St)- blocks are “triblocks” with terminal carboxyl groups, detailed studies of these blocks are presented in the literature[25]. Polyesters synthesized by this method have a strict repeating structure of alternating -Glu(St)-PEGn- and -Glu(St)-PPGm- fragments. Such regularity of the polyester chemical structure provides the most favorable macromolecule conformation for surface tension reduction. Moreover, increasing the molecular weight of polyoxypropylene glycol fragments leads to a decrease in surface tension, which confirms the afore mentioned conclusion.

Thus, both the composition and the structure of the copolyesters determine their ability to reduce surface tension by aqueous dispersions. The surface active properties of the copolymers correlate well with their ability to form self-stabilized aqueous dispersions. Copolyesters possessing surface

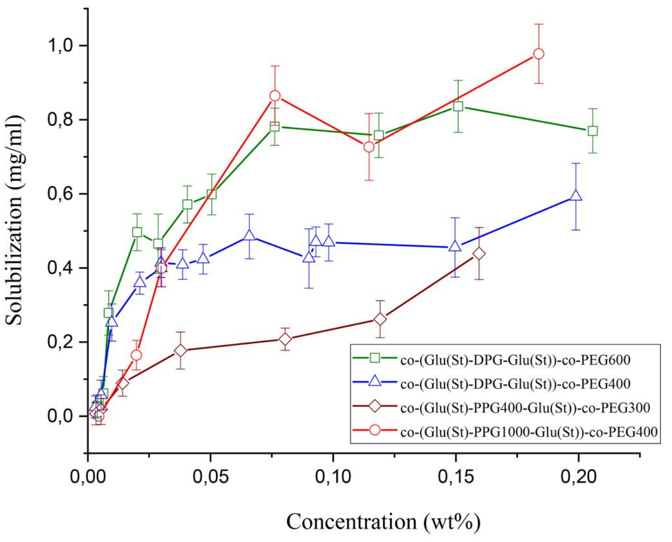


Figure 8. Dependence of the amount of solubilized Sudan (III), per unit mass of polyester, on the concentration of polyester in the aqueous dispersion.

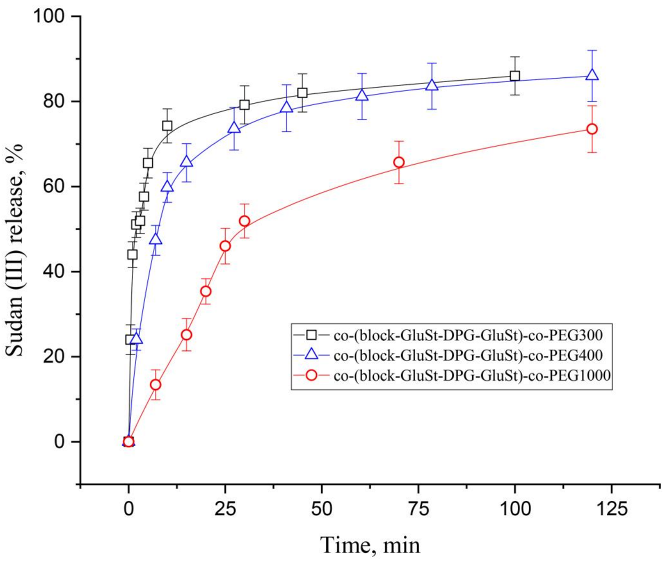


Figure 9. Release of Sudan III from the aqueous micellar phase into octanol for various copolymer structures (phase ratio water:octanol-1–2.5:1).

tensions above 55 mN/m do not form aqueous dispersions at all. Copolyesters with surface tensions between 48 and 55 mN/m were found to form sedimental suspensions, or dispersions with low aggregate stability. In most cases, dicopolyesters do not form stable dispersions, with the exception of co-Glu(St)-ko-PEG1000 and co-Glu(L)-co-PEG1000 which lowered the surface tension below 48 mN/m, and form relatively stable aqueous dispersions. The majority of tricopolyesters and block copolyesters form colloidally stable aqueous dispersions.

Surface activity is not the only factor that determines the ability of polyesters to form stable dispersions over wide concentration ranges. This fact is best evidenced by the stability of aqueous tricopolyester dispersions of co-Glu(St)-co-DPG-co-PEG1000 and the block copolyester co-(Glu(St)-DPG-Glu(St))-co-PEG1000, which have the same monomer composition but different structures. Both copolyesters

Table 1. Study of the cytotoxic effect of the copolyester co-(Glu(St)-PPG1000-Glu(St))-co-PEG400.

Sample no	Volume of dispersion added, µL	Study duration, h									Survival time, h	% of survival compared to control samples
		0	24	48	72	96	120	144	168	192		
1	Control sample	+	+	+	+	+	–	–	–	–	96 ± 4	100
	10	+	+	+	+	+	–	–	–	–	96 ± 4	100
	25	+	+	+	+	+	+	–	–	–	120 ± 5*	125
	50	+	+	+	+	+	+	–	–	–	120 ± 5	125
2	Control sample	+	+	+	+	+	–	–	–	–	96 ± 4	100
	10	+	+	+	+	+	–	–	–	–	96 ± 4	100
	25	+	+	+	+	+	–	–	–	–	96 ± 4	100
	50	+	+	+	+	–	–	–	–	–	72 ± 6	75
3	Control sample	+	+	+	+	+	+	+	+	–	168 ± 6	100
	10	+	+	+	+	+	+	+	+	–	168 ± 7	100
	25	+	+	+	+	+	–	–	–	–	96 ± 6*	57,1
	50	+	+	+	+	+	+	+	+	–	168 ± 7	100
4	Control sample	+	+	+	+	+	+	–	–	–	120 ± 7	100
	10	+	+	+	+	+	–	–	–	–	120 ± 7	100
	25	+	+	+	+	+	+	+	–	–	144 ± 7*	120
	50	+	+	+	+	+	+	–	–	–	120 ± 7	100

"+": translational motion of spermatozoa is observed; "–": no motion of spermatozoa is observed.
*The result is statistically significant $p < .05$.

exhibit a significant decrease in surface tension at the air-water interface (bars 9 and 12 in Figure 6), and form particles of approximately the same size at a concentration of 0.02% (98 and 168 nm, respectively, Figure 7a). However, the size of the block copolyesters was seen to increase as their concentration within the dispersion increased. This is particularly well exemplified by the co-(Glu(St)-DPG-Glu(St))-co-PEG1000 sample, which had a particle diameter of 110 nm, Figure 7b; and for tricopolyesters, where a 5-fold increase in the size of the dispersed phase is observed (co-Glu(St)-co-DPG-co-PEG1000– 587 nm, Figure 7b) at higher concentrations. Strict alternation of hydrophilic and lipophilic fragments in block copolyester structures ensures their stability at higher concentrations.

The ability to solubilize and release lipophilic compounds from the copolymer composition is one of the main requirements for materials under consideration of being used for drug delivery systems. In this work, the ability of the copolymers to solubilize lipophilic molecules was studied using poorly water-soluble dye Sudan III. Figure 8 shows the solubilization capacity (g of dye per 1 ml of polymer dispersion) of block copolyesters. The optimal concentration values lie across a narrow range of low concentrations, beyond these values the curves plateau. The maximum amount of Sudan III solubilized varies between 0.6 and 0.9 mg of dye per 1 ml of dispersion and depends on the copolyester composition.

The release patterns of solubilized Sudan III were studied by assessing the octanol-water partition coefficient. Figure 9 displays release rate curves for copolyesters with different polyoxyethylene fragment lengths. Copolyester co-(GluSt-DPG-GluSt)-co-PEG-300 releases the dye with the highest rate – 75% of the solubilized dye passes into the lipophilic phase after 10 min. For the copolyester, co-(GluSt-DPG-GluSt)-co-PEG-1000, only 50% of solubilized Sudan III is released after 25 min and 75% – after 140 min.

Finally, we evaluated the cytotoxicity of the dispersions using the spermatozoa survival method, it is convenient and effective *in vitro* system for studying acute lethality to cells[33,34]. Typically, the sperm survival rate is quite long, 2–10 d[33,34]. Table 1 presents the results of the cytotoxic evaluation of spermatozoa survival treated with a different copolyesters dispersions.

The data presented indicates that copolyesters based on the N-substituted glutamic acid, PEG and PPG diols do not inhibit sperm survival over the concentration range tested, and in some cases even extend survival. It can therefore be concluded that are not cytotoxic toward these spermatozoa.

4. Conclusions

In summary, nonionic surface-active polymers, suitable for use in biomedical applications, can be synthesized from N-substituted glutamic acid, PEG, and PPG diols. As demonstrated in this study, the most promising approach to synthesizing this type of polyester under mild conditions is the Steglich reaction. Although this is a relatively well-known esterification reaction, polyesterification via the Steglich reaction has been limited due to its low degrees of polycondensation. However, this work has revealed that, under optimized conditions, this approach can be used for the preparation of glutamic acid and PEG/PPG-based copolyesters.

Copolyesters obtained via the proposed method are able to: form self-stabilized water dispersions, form nanoscale disperse phase particles, solubilize poorly water-soluble compounds in their lipophilic cores and release them into an octanol phase as a model of a lipid bilayer. Furthermore, N-substituted glutamic acid and PEG/PPG copolyesters possess no cytotoxic effects against spermatozoa across a wide concentration range.

The synthetic approach developed allows the synthesis of copolyesters with different compositions and structures, which can be tuned in the intelligent design of flexible drug delivery systems with optimized specified capacities, stabilities, and release parameters. In conclusion, aqueous dispersions of obtained copolyesters possess properties necessary for potential application as polymer systems for passive drug delivery.

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References

[1] Duncan, R. The Dawning Era of Polymer Therapeutics. *Nat. Rev. Drug Discov.* **2003**, *2*, 347–360. DOI: 10.1038/nrd1088.

[2] Duncan, R. Polymer Conjugates as Anticancer Nanomedicines. *Nat. Rev. Cancer* **2006**, *6*, 688–701. DOI: 10.1038/nrc1958.

[3] Roberts, M. J.; Bentley, M. D.; Harris, J. M. Chemistry for Peptide and Protein PEGylation. *Adv. Drug Deliv. Rev.* **2002**, *54*, 459–476. DOI: 10.1016/s0169-409x(02)00022-4.

[4] Acharya, S.; Sahoo, S. K. PLGA Nanoparticles Containing Various Anticancer Agents and Tumor Delivery by EPR Effect. *Adv. Drug Deliv. Rev.* **2011**, *63*, 170–183. DOI: 10.1016/j.addr.2010.10.008.

[5] Sung, Y. K.; Kim, S. W. Recent Advances in Polymeric Drug Delivery Systems. *Biomater. Res.* **2020**, *24*, 12. DOI: 10.1186/s40824-020-00190-7.

[6] Martinho, N.; Damgé, C.; Reis, C. Recent Advances in Drug Delivery Systems. *JBNB* **2011**, *02*, 510–526. DOI: 10.4236/jbnb.2011.225062.

[7] Bodratti, A. M.; Alexandridis, P. Formulation of Poloxamers for Drug Delivery. *J. Funct. Biomater.* **2018**, *9*, 11. DOI: 10.3390/jfb9010011.

[8] Chiappetta, D. A.; Sosnik, A. Poly(Ethylene Oxide)-Poly(Propylene Oxide) Block Copolymer Micelles as Drug

Delivery Agents: improved Hydrosolubility, Stability and Bioavailability of Drugs. *Eur. J. Pharm. Biopharm.* **2007**, *66*, 303–317. DOI: 10.1016/j.ejpb.2007.03.022.

[9] Kabanov, A. V.; Batrakova, E. V.; Miller, D. W. Pluronic Block Copolymers as Modulators of Drug Efflux Transporter Activity in the Blood-Brain Barrier. *Adv. Drug Deliv. Rev.* **2003**, *55*, 151–164. DOI: 10.1016/s0169-409x(02)00176-x.

[10] Spitzenberger, T. J.; Heilman, D.; Diekmann, C.; Batrakova, E. V.; Kabanov, A. V.; Gendelman, H. E.; Elmquist, W. F.; Persidsky, Y. Novel Delivery System Enhances Efficacy of Antiretroviral Therapy in Animal Model for HIV-1 Encephalitis. *J. Cereb. Blood Flow Metab.* **2007**, *27*, 1033–1042. DOI: 10.1038/sj.jcbfm.9600414.

[11] Krupka, T. M.; Weinberg, B. D.; Wu, H.; Ziats, N. P.; Exner, A. A. Effect of Intratumoral Injection of Carboplatin Combined with Pluronic P85 or L61 on Experimental Colorectal Carcinoma in Rats. *Exp. Biol. Med.* **2007**, *232*, 950–957. DOI: 10.3181/00379727-232-2320950.

[12] Kabanov, A.; Alakhov, V. Pluronic Block Copolymers in Drug Delivery: From Micellar Nanocontainers to Biological Response Modifiers. *Crit. Rev. Ther. Drug Carrier Syst.* **2002**, *19*, 1–72. DOI: 10.1615/critrevtherdrugcarriersyst.v19.i1.10.

[13] Kwon, S. H.; Kim, S. Y.; Ha, K. W.; Kang, M. J.; Huh, J. S.; Im, T. J.; Kim, Y. M.; Park, Y. M.; Kang, K. H.; Lee, S.; et al. Pharmaceutical Evaluation of Genistein-Loaded Pluronic Micelles for Oral Delivery. *Arch. Pharm. Res.* **2007**, *30*, 1138–1143. DOI: 10.1007/BF02980249.

[14] Zhao, L.-Y.; Zhang, W.-M. Recent Progress in Drug Delivery of Pluronic P123: pharmaceutical Perspectives. *J. Drug Target* **2017**, *25*, 471–484. DOI: 10.1080/1061186X.2017.1289538.

[15] Akash, M. S.; Rehman, K. Recent Progress in Biomedical Applications of Pluronic (PF127(Sad) Pharmaceutical Perspectives. *J. Control Release* **2015**, *209*, 120–138. DOI: 10.1016/j.jconrel.2015.04.032.

[16] Singh-Joy, S. D.; McLain, V. C. Safety Assessment of Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, Poloxamer 105 Benzoate, and Poloxamer 182 Dibenzoate as Used in Cosmetics. *Int. J. Toxicol.* **2008**, *27*(Suppl 2), 93–128. DOI: 10.1080/10915810802244595.

[17] Batrakova, E. V.; Kabanov, A. V. Pluronic Block Copolymers: evolution of Drug Delivery Concept from Inert Nanocarriers to Biological Response Modifiers. *J. Control Release* **2008**, *130*, 98–106. DOI: 10.1016/j.jconrel.2008.04.013.

[18] Marasini, N.; Fu, C.; Fletcher, N. L.; Subasic, C.; Er, G.; Mardon, K.; Thurecht, K. J.; Whittaker, A. K.; Kaminskas, L. M. The Impact of Polymer Size and Cleavability on the Intravenous Pharmacokinetics of PEG-Based Hyperbranched Polymers in Rats. *Nanomaterials* **2020**, *10*, 2452. DOI: 10.3390/nano10122452.

[19] D'souza, A. A.; Shegokar, R. Polyethylene Glycol (PEG): a Versatile Polymer for Pharmaceutical Applications. *Expert Opin. Drug Deliv.* **2016**, *13*, 1257–1275. DOI: 10.1080/17425247.2016.1182485.

[20] Kaminskas, L. M.; McLeod, V. M.; Kelly, B. D.; Cullinane, C.; Sberna, G.; Williamson, M.; Boyd, B. J.; Owen, D. J.; Porter, C. J. H. Doxorubicin-Conjugated PEGylated Dendrimers Show Similar Tumoricidal Activity but Lower Systemic Toxicity When Compared to PEGylated Liposome and Solution Formulations in Mouse and Rat Tumor Models. *Mol. Pharm.* **2012**, *9*, 422–432. DOI: 10.1021/mp200522d.

[21] Kubicka-Wołkowska, J.; Kędzierska, M.; Lisik-Habib, M.; Potemski, P. Skin Toxicity in a Patient with Ovarian Cancer Treated with Pegylated Liposomal Doxorubicin: A Case Report and Review of the Literature. *Oncol. Lett.* **2016**, *12*, 5332–5334. DOI: 10.3892/ol.2016.5309.

[22] Suk, J. S.; Xu, Q.; Kim, N.; Hanes, J.; Ensign, L. M. PEGylation as a Strategy for Improving Nanoparticle-Based Drug and Gene Delivery. *Adv. Drug Deliv. Rev.* **2016**, *99*, 28–51. DOI: 10.1016/j.addr.2015.09.012.

[23] Mallakpour, S.; Tirgir, F.; Sabzalian, M. R. Synthesis, Characterization and in Vitro Antimicrobial and Biodegradability Study of Pseudo-Poly(Amino Acid)s Derived from N,N′-(Pyromellitoyl)-bis-L-Tyrosine Dimethyl Ester as a Chiral Bioactive Diphenolic Monomer. *Amino Acids* **2011**, *40*, 611–621. DOI: 10.1007/s00726-010-0686-0.

[24] Pulapura, S.; Kohn, J. Tyrosine-Derived Polycarbonates: Backbone-Modified "Pseudo"-Poly(Amino Acids) Designed for Biomedical Applications. *Biopolymers* **1992**, *32*, 411–417. DOI: 10.1002/bip.360320418.

[25] Zavradashvili, N.; Puiggali, J.; Katsarava, R. Artificial Polymers Made of α-Amino Acids-Poly (Amino Acid) s, Pseudo-Poly (Amino Acid) s, Poly (Depsipeptide) s, and Pseudo-Proteins. *Curr. Pharm. Des.* **2020**, *26*, 566–593. DOI: 10.2174/1381612826666200203122110.

[26] Kozak, M.; Stasiuk, A.; Vlizlo, V.; Ostapiv, D.; Bodnar, Y.; Kuz'mina, N.; Figurka, N.; Nosova, N.; Ostapiv, R.; Kotsumbas, I.; et al. Polyphosphate Ester-Type Transporters Improve Antimicrobial Properties of Oxytetracycline. *Antibiotics* **2023**, *12*, 616. DOI: 10.3390/antibiotics12030616.

[27] Stasiuk, A. V.; Fihurka, N. V.; Tarnavchyk, I. T.; Nosova, N. G.; Pasetto, P.; Varvarenko, S. M.; Samaryk, V. Y. Influence of Structure and Nature of Pseudo-Poly(Amino Acid)s on Size and Morphology of Their Particle in Self-Stabilized Aqueous Dispersions. *Appl. Nanosci.* **2023**, *13*, 5011–5019. DOI: 10.1007/s13204-022-02664-7.

[28] Varvarenko, S. M.; Ferens, M. V.; Samaryk, V.; Nosova, N. G.; Fihurka, N. V.; Ostapiv, D. D.; Voronov, S. A. Synthesis of Copolyesters of Fluorescein and 2-(Dodecanamino) Pentanedionic Acid via Steglich Reaction. *Vopr. Khimii Khimicheskoi Tekhnologii* **2018**, *2*, 5–15.

[29] Nagornyak, M.; Figurka, N.; Samaryk, V.; Varvarenko, S. V.; Ferens, M.; Oleksa, V. Modification of Polysaccharides by N-Derivatives of Glutamic Acid Using Steglich Reaction. *ChChT* **2016**, *10*, 423–427. DOI: 10.23939/chcht10.04.423.

[30] Fihurka, N.; Tarnavchyk, I.; Samaryk, V.; Varvarenko, S.; Nosova, N.; Voronov, A.; Nagornyak, M.; Ferens, M.; Voronov, S. A Study of an Irreversible Condensation of Glutamic Acid and Polyoxyethylene/Polyoxypropylene Diols Using Thionyl Chloride. *OPPI* **2018**, *50*, 502–508. DOI: 10.1080/00304948.2018.1525674.

[31] Chen, C.; Wang, Z.; Li, Z. Thermoresponsive Polypeptides from Pegylated Poly-L-Glutamates. *Biomacromolecules* **2011**, *12*, 2859–2863. DOI: 10.1021/bm200849m.

[32] Won, C.-Y.; Chu, C.-C.; Lee, J. D. Novel Biodegradable Copolymers Containing Pendant Amine Functional Groups Based on Aspartic Acid and Poly(Ethylene Glycol). *Polymer* **1998**, *39*, 6677–6681. DOI: 10.1016/S0032-3861(98)00032-9.

[33] Kemp, R. B.; Beck, N. F.; Meredith, R. W.; Gamble, S. H. Bull Sperm as a Potential Model System for Cytotoxicity Testing in Vitro. *Toxicol. in Vitro* **1990**, *4*, 47–50. DOI: 10.1016/0887-2333(90)90008-h.PMID: 20702283.

[34] Muzyka, V. P.; Atamanyuk, I. S.; Chaikovska, O. I.; Panich, O. P.; Serhienko, O. I.; Ostapiv, D. D.; Yaremchuk, I. M.; Kuzmina, N. V.; Ostapiv, R. D. To the Assessment of Cytotoxic Effect of Polymers Using Bull Sperm as Testing Objects. *Anim. Biol.* **2011**, *13*, 2–6.