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Biocompatible copoly(urethane-imide)s containing flexible aliphatic blocks modified with castor oil

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Biocompatible copoly(urethane-imide)s containing flexible aliphatic blocks modified with castor oil

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Abstract

Biocompatible copoly(urethane-imide)s with and without castor oil triglyceride fragments in the macromolecules were synthesized using aliphatic polydiol and castor oil as monomers, aromatic diisocyanate, aromatic diamine, and aromatic dianhydride. The copolymers were studied by IR spectroscopy, thermogravimetry (TGA), differential scanning calorimetry (DSC), and dynamic mechanical analysis; the strength and deformation properties of samples in the form of films were investigated. It was shown that the copolymers exhibit the properties of highly heat-resistant elastomers (τ_5 up to 294-322°C, tensile strain ε, up to1275%).

Cytotoxicity of the copoly(urethane-imide) films that have been previously exposed to several organic solvents was assessed using the methyl tetrazolium test (MTT). The results of the MTT test revealed no cytotoxicity for the films soaked in all selected solvents, while the films not subjected to treatment with solvents demonstrated a noticeable toxic effect toward cultured cells. The viability of fibroblasts cultured in the presence of the nutrient medium obtained after incubation with these non-treated films decreased by more than 60% compared to the control sample (cells cultured under standard conditions).

Highlights:

1) Biocompatible copoly(urethane-imide)s based on castor oil triglycerides were obtained.

2) Copoly(urethane-imide)s are highly heat-resistant elastomers with a tensile strain of about 1300%.
3) The results of the MTT test showed the absence of cytotoxicity and showed the promise of using copolymers for biomedical purposes.

Keywords: synthesis, polyurethane, castor oil, copoly(urethane-imide), heat resistance, mechanical properties, biocompatibility, cytotoxicity, MTT test.

1. Introduction

Segmented (multi-block) copoly(urethane-imide)s represent a large family of modern high-strength and highly heat-resistant elastomers [**1-9**]. In terms of their structure, copoly(urethane-imide)s belong to polymers with a complex architecture. Each repeating unit of these polymers contains a rigid aromatic imide block (surrounded by urethane groups) and a flexible aliphatic polyester block. The rigid aromatic and flexible aliphatic blocks are poorly thermodynamically compatible, which causes microphase separation within the bulk of the polymer. As a result, the polymer acquires the properties of a thermoplastic elastomer: the glass transition temperature is below zero degrees Celsius; the dynamic mechanical analysis curves include areas where the storage modulus and loss modulus are practically independent of temperature (the so-called plateau of rubber-like high elasticity); the values of tensile strain are several hundred percent; such polymer can be processed into products using the equipment intended for processing thermoplastics [**8,10,11**].

Segmented (multi-block) copoly(urethane-imide)s are known since the 1980s [**9**]. These copolymers may be considered, on the one hand, as products of chemical modification of polyurethanes with aromatic imides (performed to increase strength and heat resistance of polyurethanes), and, on the other hand, as products of chemical modification of polyimides with polyurethanes (that tempers the processing conditions of polyimides). Initially, the driving force behind the development of copoly(urethane-imide)s was the possibility of using their films in petrochemical industry as pervaporation membranes for the separation of aromatic hydrocarbons from mixtures of aliphatic and aromatic hydrocarbons [**12-27**]. Currently, copoly(urethane-imide)s attract interest due to the need to find (develop) polymers that can be used in electrospinning technologies for preparation of nonwoven fabrics and in layer-by-layer application (3D printing) for producing three-dimensional products [**28,29**].

Polyurethanes are biocompatible polymers; they are widely used in manufacturing medical products (catheters, wound dressings, bioabsorbents, hydrogels, gloves, etc.) [**10, 30-32**]. Polyimides are also biocompatible [**33,34**]; at the Institute of Macromolecular Compounds, polyimide-based compositions with carbon fillers are being studied as flexible bioelectrodes [**35,36**]. As for copoly(urethane-imide)s, in our studies [**35,36**] it has been shown that copoly(urethane-imide) films are practically non-toxic toward human skin fibroblasts; thus, these polymers are promising objects in the search of modern materials for tissue engineering technologies. A preliminary assessment of cytotoxicity has been performed using the MTT analysis [**35,36**]. The development of polymer matrices with acceptable cytocompatibility for cell cultivation is of great practical importance [**37**].

In this regard, the research works on the synthesis of polyurethanes using castor oil triglycerides as a starting material are of interest, because the resulting polymers acquire useful properties: high biocompatibility, biodegradability, antimicrobial activity, and wound healing ability [**38-56**]. The results of these studies suggest that cytotoxicity of copoly(urethane-imide)s

can be decreased by introducing castor oil triglyceride fragments into flexible blocks of these copolymers.

The aim of this work was to synthesize copoly(urethane-imide)s with flexible blocks containing macroradicals of aliphatic polyesters and macroradicals of castor oil triglycerides, to characterize their thermal stability and heat resistance, investigate deformation, strength and dynamic mechanical properties of copolymer films, and to evaluate cytotoxicity of the films using the MTT test.

2. Methods

2.1.Chemical reagents

The following reagents were used in the polymer synthesis: 1,4-bis(4'-aminophenoxy)diphenylsulfone (CAS#13080-89-2), m.p. ~ 196–197°C, Sigma-Aldrich Co. LLC; 1,3-bis(3',4'-dicarboxyphenoxy)benzene dianhydride, m.p. ~ 163–165°C, Himprom, Yaroslavl; 2,4-toluene diisocyanate-terminated poly(propylene glycol) with a molecular weight of 2300 g/mol (PPG-2300) (CAS #9057-91-4), m.p. ~ -24°C, Sigma-Aldrich Co. LLC; technical castor oil GOST 6757-96, Rushim, Moscow, m.p. ~ -16°C, total fatty acid content 98-99.9%, OH value: 172.09 mg KOH/g. N,N-dimethylacetamide (DMA), Vecton Co. LLC, Russia, was used as a solvent.

2.2.Synthesis Polymer

Synthesis of polyurethane on the basis of castor oil (PU-CO). 2,4-Toluene diisocyanate-terminated poly(propylene glycol) (PPG-2300, 4.54 g, 1.98 mmol) was introduced at room temperature into a three-necked flask equipped with a top-drive stirrer and a nozzle for feeding and removing argon. Castor oil (0.41 g) and DMA (5 ml) were added at intensive stirring. The reaction mixture was heated at 75°C for 1 h.

Preparation of PU-CO films. The films were prepared by casting the reaction solution onto hydrophobized glass supports followed by drying in a thermostat at 80°C for 12 h. The films were easily removed from supports.

Synthesis of poly(urethane-imide) (PUI-CO) on the basis of 2,4-toluene diisocyanate-terminated poly(propylene glycol) and castor oil. Three grams (7.46 mmol) of 1,4-bis(4'-aminophenoxy)diphenylsulfone and 10 ml of DMA (to dissolve the diamine) were loaded into a three-necked flask equipped with a top-drive stirrer and a nozzle for supplying and removing argon. The flask was cooled to 5°C, then 1.61 g (3.73 mmol) of 1,3-bis(3',4'-dicarboxyphenoxy)benzene dianhydride was introduced in small portions over 30 min at stirring in argon flow; the contents of the flask were additionally stirred for 2 h, and the temperature was brought up to room temperature. Then, a previously prepared solution of 17.15 g (7.46 mmol) of 2,4-toluene diisocyanate-terminated poly(propylene glycol) (PPG-2300) in 10 ml of DMA was added to the reaction mixture. The temperature of the reaction mixture was raised to 120°C, and the contents of the flask were stirred for 2 h in argon flow until there was no longer any release of carbon dioxide bubbles (which were formed during the reaction of the terminal anhydride and isocyanate groups of the compounds in the reaction solution). The reaction mixture was then cooled down to 75°C; 2.43 g of castor oil was added, and the mixture was stirred for 1 h.

Preparation of PUI-CO films. The films were obtained by casting the prepared reaction solution onto hydrophobized glass supports followed by drying in a thermostat at 80°C for 12 h. The dried films were heated in a stepwise manner at 120, 140, 170, 190, and 220°C (for 1 h at each temperature). The films were easily removed from supports. The films intended for biological tests

were heated at 170°C, then exposed to organic solvents (hexane, heptane, petroleum ether, isopropanol) to remove impurities contained in castor oil; each sample was kept in one of the solvents for 12 h. Then the samples were washed with double-distilled water and dried under vacuum at room temperature.

2.3. Characterization methods

IR spectra of the films were recorded on an IRAffinity-1S Fourier transform infrared spectrometer (Shimadzu, Japan) in the mid-IR region (700–4000 cm^{-1}) using a MIRacle single-reflection attenuated total reflection (ATR) micro accessory (PIKE Technologies, USA).

Thermogravimetric analysis (TGA) was performed with the use of TG 209 F1 thermomicrobalances (NETZSCH, Germany) in the temperature range from 30°C to 800°C at a heating rate of 10°C/min in an inert atmosphere (argon). The weight of the samples was 2–3 mg.

The glass transition temperatures and melting temperatures of copolymer films were determined by differential scanning calorimetry (DSC) on a DSC 204 F1 setup (NETZSCH, Germany) at a heating rate of 10°C/min in the temperature range from -60°C to 300°C in an inert atmosphere (argon). The samples weighed 4–5 mg.

The temperature dependences of elastic modulus E', loss modulus E", and mechanical loss tangent tgδ of the films were recorded during the dynamic mechanical analysis (DMA) experiments on a DMA 242 C instrument (NETZSCH, Germany). The measurements were performed at a frequency of 1 Hz; the amplitude of film deformation was 0.1%, the heating temperature was 5°/min.

The strength and deformation properties of the films were studied at room temperature in the uniaxial tension mode using samples in the form of strips 2 mm wide with a working length of 25 mm. The tests were carried out on an Instron 5940 universal tensile testing machine (Instron, Switzerland). The stretching speed was 10 mm/min.

The contact angle values of the films were determined using the sessile drop method with a DSA30 device (Kruss, Germany). The measurements were performed at room temperature using 17 μl of distilled water. The values of contact angle were calculated 5 s after the application of the drop using DSA4 software. The values were determined in five areas randomly distributed over the surface.

The human skin fibroblast cell line DF1 obtained from Cell Culture Collection of the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg, Russia) were used in this work. The cells were cultured in a CO_2 incubator at 37°C in a humidified atmosphere containing air and 5% CO_2 in DMEM/F12 medium (Dulbecco's modified Eagle's medium; Gibco) containing 1% essential amino acids, 10% (v/v) thermally inactivated fetal bovine serum (FBS; HyClone, USA), 1% L-glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin.

Polynomial regression analysis in Microsoft Excel was used for the calculation.

3. Results and Discussion

The films of the target copoly(urethane-imide) (PUI-CO) and polyurethane (PU-CO) (the reference polymer) were selected as the objects of research in this work. Castor oil was used as one of the starting materials in the synthesis of both polymers. Vegetable oils (castor, soybean, sunflower, etc.) are currently used as raw materials for the development of bio-based polyurethanes in accordance with environmental requirements [**30, 31, 57, 58**].

Triglycerides contained in vegetable oils are chemically modified to form polyols, which then react with isocyanates to produce polyurethanes. Castor oil [**38-42**] has the advantage over

other vegetable oils: it contains reactive hydroxyl groups that can participate in the formation of urethane bonds in reactions with isocyanates, which simplifies the corresponding polymer syntheses.

Fig. 1 shows the scheme of the synthesis of polyurethane PU-CO based on 2,4-toluene diisocyanate-terminated poly(propylene glycol) and castor oil:



Fig. 1. Synthesis of PU-CO.

As can be seen from the scheme (Fig. 1), triglyceride of castor oil performs two functions simultaneously: it acts as a chain extender and a crosslinking agent for polyurethane macromolecules.

The scheme of synthesis of the target product PUI-CO based on 1,4-bis(4'-aminophenoxy)diphenylsulfone, 1,3-bis(3',4'-dicarboxyphenoxy)benzene dianhydride, 2,4-toluene diisocyanate-terminated poly(propylene glycol) and castor oil is shown in Fig. 2.

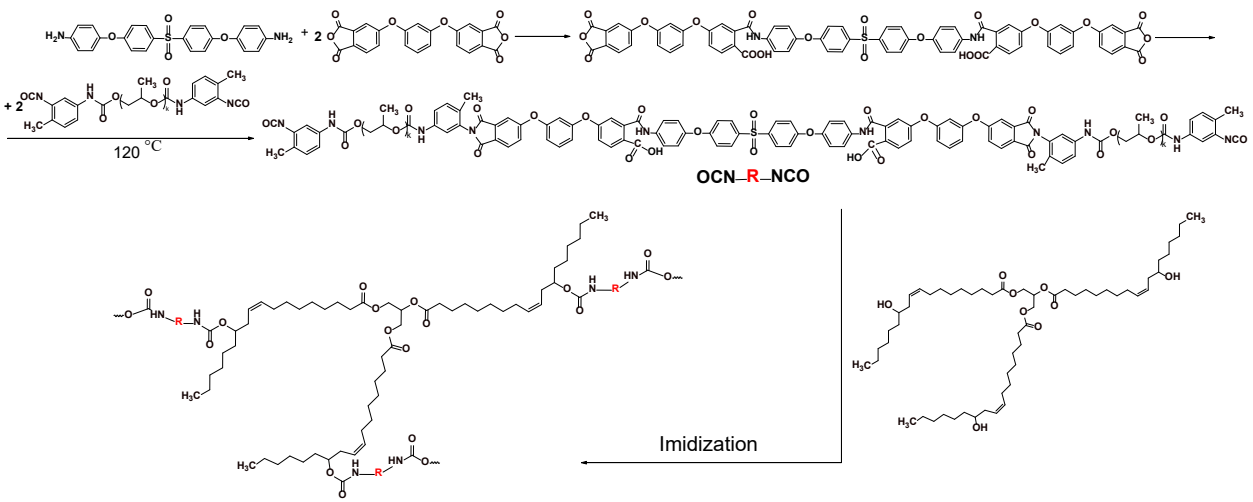


Fig. 2. Synthesis of PUI-CO.

The diagram (Fig. 2) shows the consequent stages of preparation of the target product: the synthesis of polyimide with terminal anhydride groups by acylation of aromatic diamine with an excess of aromatic dianhydride; the synthesis of poly(urethane-imide) based on poly(propylene glycol) terminated with diisocyanate; the extension of poly(urethane-imide) chain with terminal isocyanate groups during the reaction with the hydroxyl groups of castor oil triglyceride. Similarly to PU-CO, castor oil triglyceride also acts as a crosslinker for PUI-CO macromolecules. It should be noted that the synthesis of non-crosslinked poly(urethane-imide) was carried out using methods developed in [**59-63**].

IR spectra of the synthesized copolymers

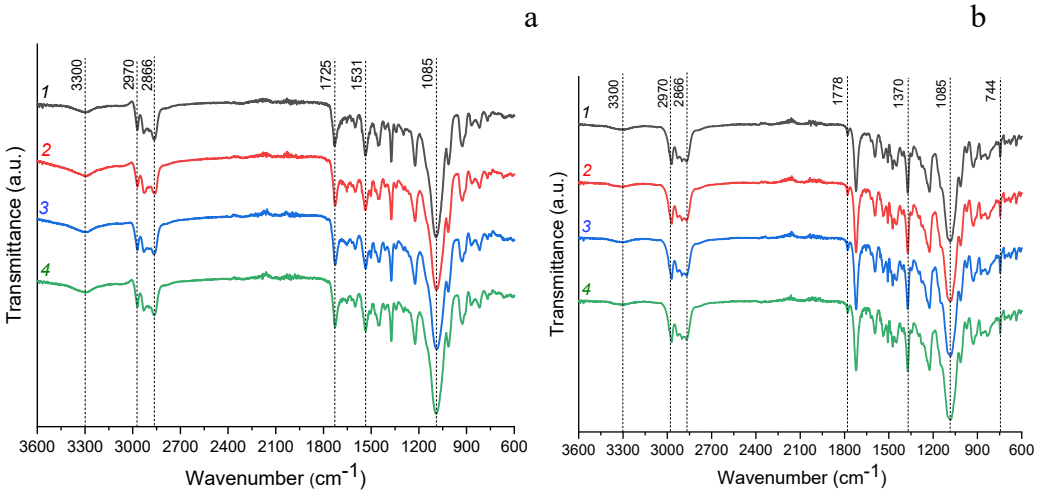


Fig. 3. (a): IR spectra of the PU-CO samples: dried at 80°C under vacuum for 6 h (1); dried at 80°C under vacuum for 6 h, then washed for 12 h with benzene (2), hexane (3), isopropanol (4); (b): IR spectra of the PUI-CO samples: dried at 80°C under vacuum for 6 h (1); dried at 80°C under vacuum for 6 h, then heated at 120°C (2); 140°C (3); 170°C (4); 220°C (5).

Fig. 3a presents the IR spectra of PU-CO films dried at 80°C and then washed with various solvents to remove impurities introduced by castor oil and other monomers. All spectra contain the identical set of absorption bands. The band at 3300 cm^{-1} is attributed to valence vibrations of the N-H bond of urethane group. The bands in the 3005-2823 cm^{-1} range are characteristic of C-H vibrations of the aliphatic part of castor oil, as well as poly(propylene glycol). The band at 1725 cm^{-1} corresponds to valence vibrations of the C=O bond of the ester group in castor oil. This band may also overlap with the band of C=O vibrations of the urethane group. The peak at 1085 cm^{-1}, characteristic of C-O vibrations, is observed in all spectra. A shoulder in this band may appear due to superposition of the C-O vibration bands of the ester group present in castor oil and the C-O vibrations of the urethane group. The band at 1531 cm^{-1} can be attributed to the deformation vibrations of N-H bond of the urethane group. Note that the spectra contain no bands in the 2275-2250 cm^{-1} range, which are typical of isocyanate groups; it may be concluded that virtually all isocyanate groups of the starting reagent have participated in the reaction.

Fig. 3b demonstrates the IR spectra of PUI-CO films subjected to heat treatment in the temperature range of 80–220 °C. This heat treatment is necessary for the imidization reaction (cyclization of O-carboxyamide groups) in PUI-CO prepolymers (see the diagram in Fig. 2). Unlike the spectra shown in Fig. 3a, the spectra of these samples contain the band at 1778 cm^{-1}

corresponding to C=O vibrations of the imide group. Additionally, the band at 1370 cm^{-1} can be attributed to C-N vibrations of imide groups. The peak assigned to the deformation vibrations of imide group is observed at 744 cm^{-1}. The bands corresponding to vibrations of castor oil fragments, poly(propylene glycol), and urethane bonds are present, similarly to the IR spectra of PU-CO shown in Fig. 3a.

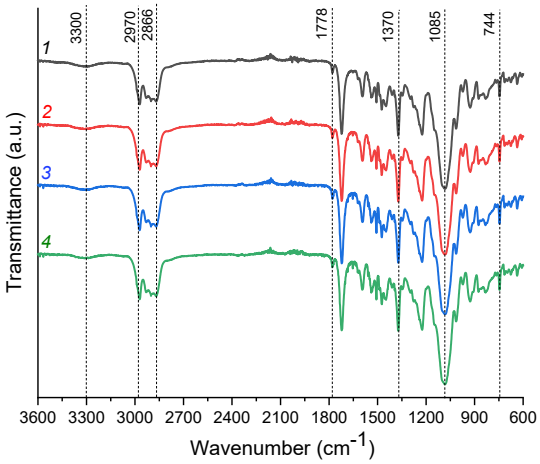


Fig. 4. IR spectra of PUI-CO samples heated at 170°C and washed for 12 h in hexane (1); heptane (2); isopropanol (3); petroleum ether (4).

The shapes of the spectra shown in Fig. 4 are similar to those displayed in Fig. 3b. It is reasonable to assume that the selected solvents remove the impurities present in the samples. (It is not possible to detect residual impurities inside the films by the single reflection attenuated total reflection (ATR) method used to record the IR spectra). The IR spectra presented above confirm the chemical structure of the target products PU-CO and PUI-CO (see Figs. 1, 2).

Thermal stability and heat resistance of PU-CO and PUI-CO films

Table 1 lists the TGA results for the PU-CO and PUI-CO films heated at different temperatures and exposed to several organic solvents.

Table 1. TGA results for the PU-CO and PUI-CO films heated and exposed to various organic solvents.

Sample preparation	Residual mass at 600°C, %	τ_5, °C
PU-CO		
Drying at 75°C	0.2	272.5
Drying at 75°C and exposure to hexane for 12 h	0.4	288.2
Drying at 75°C and exposure to isopropanol for 12 h	1.1	311.7
PUI-CO		
Drying at 80°C	14.4	311.0
Heating at 120°C	15.0	294.0
Heating at 140°C	14.9	312.0

Heating at 170°C	15.0	309.0
Heating at 190°C	14.9	311.0
Heating at 220°C	14.0	310.0
PUI-CO, heating at 170°C and exposure to organic solvents		
Petroleum ether (12 h)	15.2	314.0
Isopropanol (12 h)	15.1	314.0
Hexane (12 h)	14.1	310.0
Heptane (12 h)	14.7	310.0
Ethyl acetate (12 h)	14.8	322.0
Toluene (12 h)	14.9	318.0

According to Table 1, thermal degradation of the PU-CO and PUI-CO samples begins at 273°C and 294°C, respectively. The thermal stability indices τ_5 (the temperature at which a sample loses 5% of its mass) are quite high (τ_5 = 273–312°C for PU-CO and τ_5 = 294–322°C for PUI-CO); thus, PU-CO and PUI-CO can be classified as thermally stable polymers. During the experiment in the temperature range of 273–400°C, thermal degradation of the PU-CO sample proceeds more intensively; the residual mass at 600°C is 1.09% for the PU-CO sample and 14–15% for PUI-CO. The observed differences are most likely related to the presence of rigid imide blocks in the PUI-CO copolymer, which increases its thermal stability.

Heat resistance of a polymer (as a physical concept) is estimated according to its glass transition temperature (T_g) and melting temperature of the crystalline phase (T_m), if any. The DSC method used in this work allowed us to reveal the influence of preparation history of the studied samples on characteristic features of their structural organization. The DSC curves of PU-CO and PUI-CO films are shown in Figs. 5 and 6.

Fig. 5a illustrates the differences between the PU-CO and PUI-CO samples. While the DSC curves of the PU-CO sample contain only glass transition (Fig. 5b, curve 1), the results for PUI-CO samples (dried at 80°C and heated to facilitate imidization) show not only glass transitions (5b, curves 2-6), but also significantly blurred melting transitions of a mixed phase (Fig. 5a, curves 2-6).

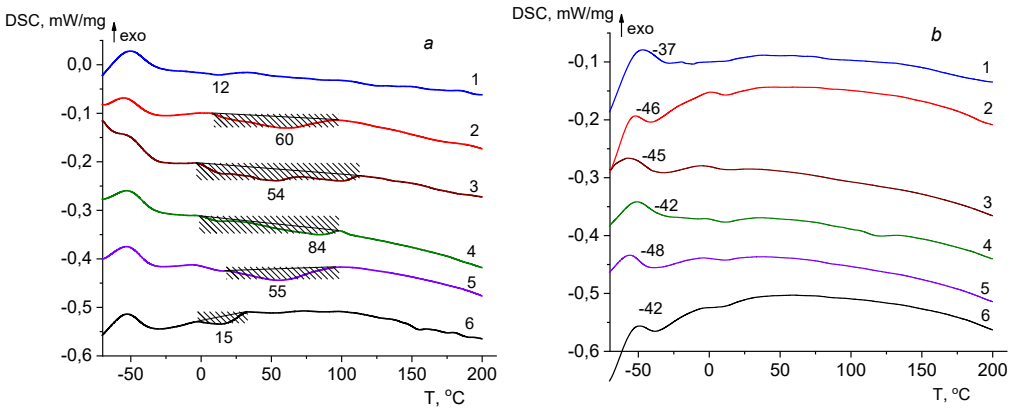


Fig.5. DSC curves (the first scan (*a*) and the second scan (*b*)) of the PU-CO film dried at 80°C (1) and the PUI-CO films dried and heated at 120 (2), 140 (3), 170 (4), 190 (5), and 220°C (6).

It should be noted that copoly(urethane-imide) PUI-CO, as a derivative of castor oil triglyceride, is a cross-linked product, and the so-called “castor oil cluster” in the PUI-CO

structure has a large molecular mass and occupies a large volume. By analogy with polyurethanes with a semi-interpenetrating network structure [**64-66**], it can be assumed that the rigid "linear" imide segments of PUI-CO (which also have high molecular masses) form mixed microphases with structural elements of the "castor oil cluster."

Fig. 6 demonstrates that treatment of copoly(urethane-imide) films with organic solvents can either destroy or strengthen interchain interactions (which lead to the formation of mixed microphases) due to the interaction of solvents with polymer chains. In the cases of ethyl acetate (Fig. 6a, curve 5) and toluene (Fig. 6a, curve 6), an increase in melting heat (6 J/g and 7 J/g, respectively) is observed compared to the samples in Fig. 5. In the cases of hexane, heptane, petroleum ether, and isopropanol (Fig. 6a, curves 1-4), melting transitions are practically undetectable.

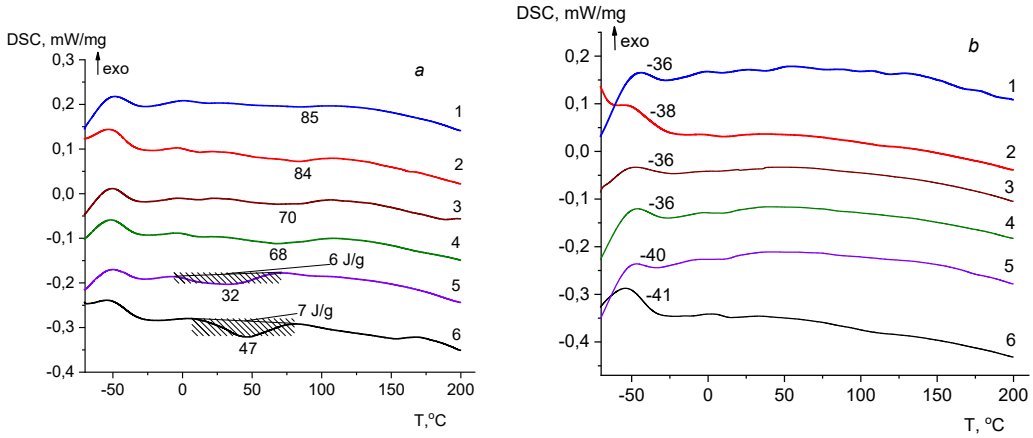


Fig. 6. DSC curves (the first scan (*a*) and the second scan (*b*)) for the PUI-CO films exposed to heptane (1), hexane (2), isopropanol (3), petroleum ether (4), ethyl acetate (5), and toluene (6).

Dynamic mechanical properties of copolymers

Investigations of dynamic mechanical properties of PU-CO and PUI-CO samples confirm the influence of their history on segmental mobility in polymer systems, which is manifested in changes in their T_g values. The comparative DMA curves for the initial PU-CO and PUI-CO films are shown in Fig. 7.

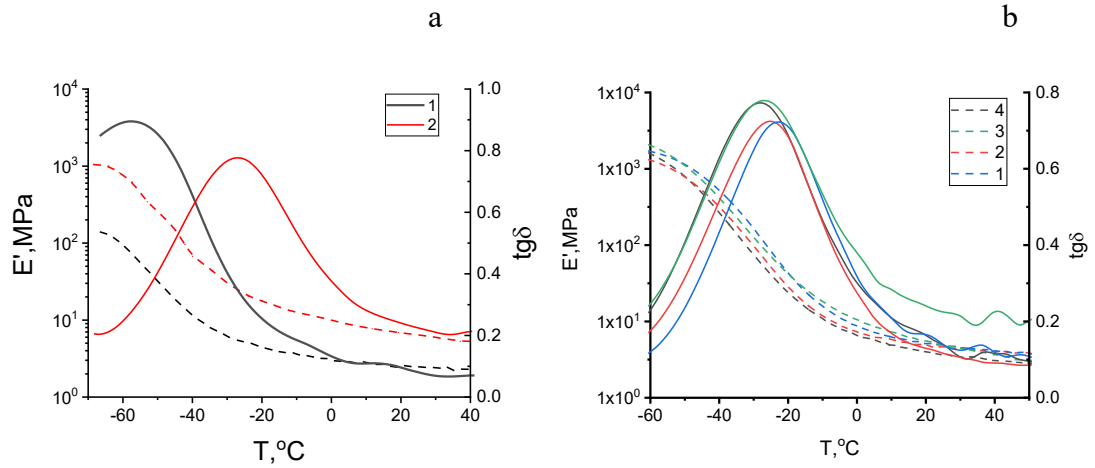


Fig. 7. (a): Temperature dependences of E′ (dotted curves) and tgδ (solid curves) for the PU-CO (curve 1) and PUI-CO (curve 2) films; (b): DMA curves for the PUI-CO samples heated at (1) 120, (2) 140, (3) 170, (4) 220°C.

In the temperature range from -70°C to +40°C, in the cases of PU-CO and PUI-CO, only one relaxation process is determined on the temperature dependences of E′ and tgδ, which is associated with unfreezing of segmental mobility of polymer chains. The values of T_g (-57°C for PU-CO and -26°C for PUI-CO) determined from the positions of maxima on temperature dependences of mechanical loss tangent tgδ show that the introduction of rigid imide blocks into the polymer system leads to a significant shift of glass transition to the region of elevated temperatures.

Fig.7b shows the DMA curves of PUI-CO samples dried at 80°C and additionally heated at elevated temperatures. Fig. 7b shows that as the heating temperature rises, the tgδ value of the samples increases from 0.7 to 0.8. The glass transition temperatures lie in the region of negative temperatures (on the Celsius scale), which is typical of elastomers. At the same time, the maxima on the tgδ(T) dependences shift to the region of lower temperatures with increasing the sample heating temperature. We attribute this phenomenon to a decrease in the interaction between flexible aliphatic and rigid aromatic segments of the copolymer.

It should be noted that the exposure of PUI-CO samples to selected organic solvents after heating also leads to an experimentally determined shift of T_g to the lower temperature range (Fig. 8).

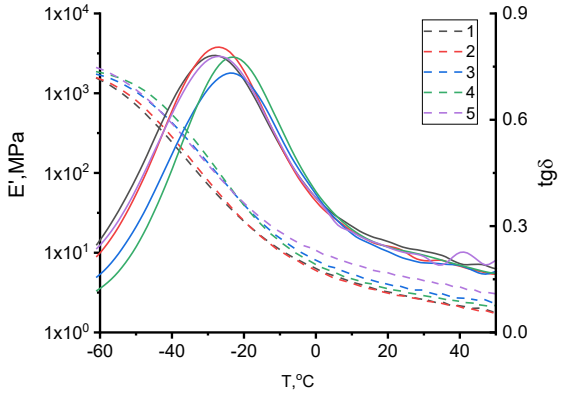


Fig. 8. DMA curves of PUI-CO films heated at 170°C and exposed to organic solvents for 12 h: (1) hexane, (2) heptane, (3) initial sample (not exposed to solvents), (4) petroleum ether, (5) isopropanol.

Fig. 8 illustrates the shift of the maxima on the tgδ temperature dependences resulting from exposure of PUI-CO to organic solvents: from -22°C (in the case of the sample heated to 170°C without subsequent treatment with organic solvents) to -28°C (in the case of the sample exposed to hexane) (Table 2).

Table 2. T_g values determined from the maxima on the curves in Fig. 8 for samples exposed to organic solvents.

Solvent	Hexane	Heptane	Petroleum ether	Isopropanol	Initial sample
T_g, °C (DMA)	-28	-27	-23	-27	-22

A comparison of the DMA results (Figs. 7b, 8) shows that for samples soaked in organic solvents (after heating at 170°C), the values of loss tangent tgδ increase significantly (from approximately 0.7 to almost 0.9) compared to the sample subjected only to heat treatment.

Strength and deformation properties of copolymer films

The effect of increased film strength due to the introduction of imide fragments into polymer macromolecules is clearly manifested when comparing the strength properties of PU-CO and PUI-CO samples.

The tensile curves of PU-CO films with different histories are shown in Fig. 9a. The curves have a shape characteristic of elastomers. It should be noted that, at virtually similar deformation, the strength parameters of the samples decrease upon exposure to hexane and isopropanol.

a b

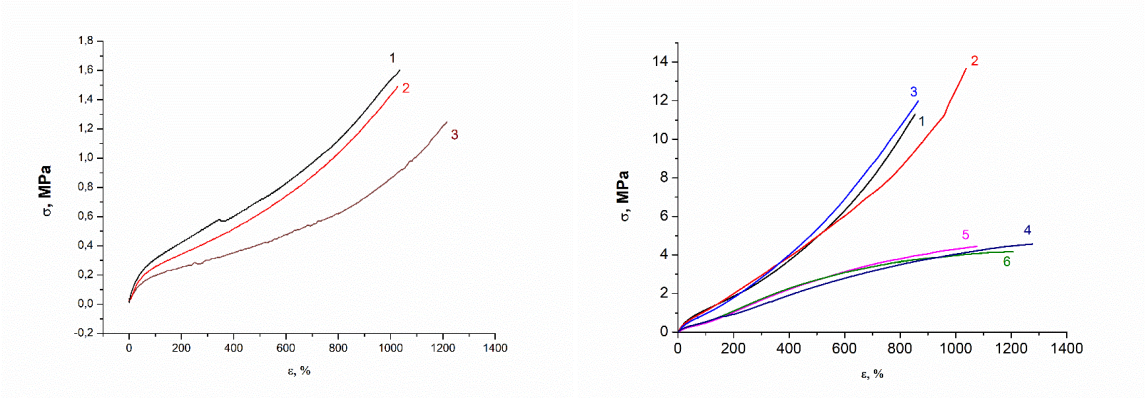


Fig.9. (a): Stress-strain curves for PU-CO films: the sample dried at 75°C (1); the sample exposed to hexane (2); the sample exposed to isopropanol (3). (b): Stress-strain curves for PUI-CO films: the sample dried at 80°C (1), the samples heated at 120 (2), 140 (3), 170 (4), 190 (5), 220°C (6).

The tensile curves of PUI-CO films demonstrate a strong influence of the heating temperature on strength characteristics of dried films (Fig. 9b). It is seen that starting from a heating temperature of 170°C, the tensile strength and Young's modulus decrease sharply at a constant deformation. Recall that the samples were annealed in order to provide cyclization of the amido acid groups in the chains of the PUI-CO prepolymer (see Fig. 2) and to improve structural integrity of the materials.

As can be seen in Fig. 10, exposure of the film heated to 170°C to organic solvents (hexane, heptane, and petroleum ether) significantly increases mechanical strength of the material.

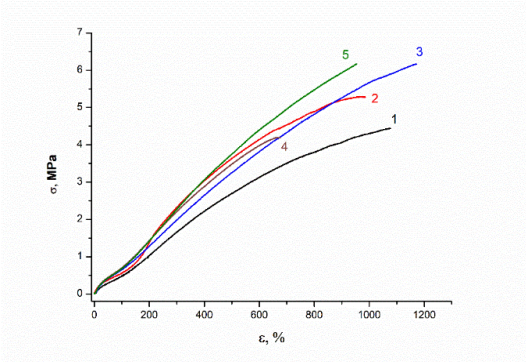


Fig. 10. Stress-strain curves for PUI-CO films: heated at 170°C (1), heated at 170°C and then kept in organic solvents for 12 h: hexane (2), heptane (3), isopropanol (4), petroleum ether (5).

The strength and deformation properties of the investigated samples are summarized in Table 3.

Table 3. Effect of the history of PU-CO and PUI-CO samples on the deformation and strength characteristics of films.

Sample	Sample history	Young's modulus E, MPa	Tensile strength σ_p, MPa	Tensile strain ε, %
PU-CO	Drying at 75°C	0.71±0.1	1.61±0.2	1033.22±64.4
	Drying at 75°C and exposure to hexane for 12 h	0.33±0.1	1.49±0.1	1027.61±78.1
	Drying at 75°C and exposure to isopropanol for 12 h	0.22±0.0	1.24±0.1	1218.12±81.6
PUI-CO	Drying at 80°C	2.67±0.2	11.28±1.3	853.16±73.8
	Heating at 120°C	2.24±0.1	13.67±0.6	1034.98±63.0
	Heating at 140°C	2.15±0.1	11.97±0.3	864.12±51.5
	Heating at 170°C	1.26±0.2	4.57±0.6	1275.37±35.0
	Heating at 190°C	1.18±0.11	4.44±0.1	1079.41±77.9
	Heating at 220°C	1.10±0.09	4.16±0.5	1204.33±86.5
PUI-CO	Exposure to hexane for 12 h	1.32±0.2	5.43±0.8	1146.33±109.1
	Exposure to heptane for 12 h	1.12±0.1	6.16±0.7	1169.33±95.6
	Exposure to isopropanol for 12 h	1.52±0.2	4.19±0.5	761.93±58.3
	Exposure to petroleum ether for 12 h	1.22±0.1	6.12±0.5	953.83±86.5

Analysis of the data given in Table 3 reveals certain differences in the characteristics of PU-CO and PUI-CO copolymer samples. In the case of PU-CO, the values of Young's modulus E and tensile strength σ_p are significantly lower; apparently, because their macromolecules do not contain imide units. The results of mechanical tests of PUI-CO films demonstrate that strength and elasticity of copolymer samples vary depending on the preparation conditions. These parameters can be adjusted according to the intended use of a copolymer. One may conclude that, when manufacturing materials for a specific purpose, it is advisable to combine annealing with subsequent exposure of a copoly(urethane-imide) to organic solvents.

In vitro Biological Assay of the films by the MTT method

An important parameter of the surface of a material intended for *in vitro* cell cultivation is hydrophilicity (wettability) of the substrate. To evaluate hydrophilicity of PU-CO and PUI-CO copolymer films, the contact angles between the film surface and water droplets were measured at five different locations on the sample surface. The average values of water contact angles are shown in Fig. 11 and Table 4.

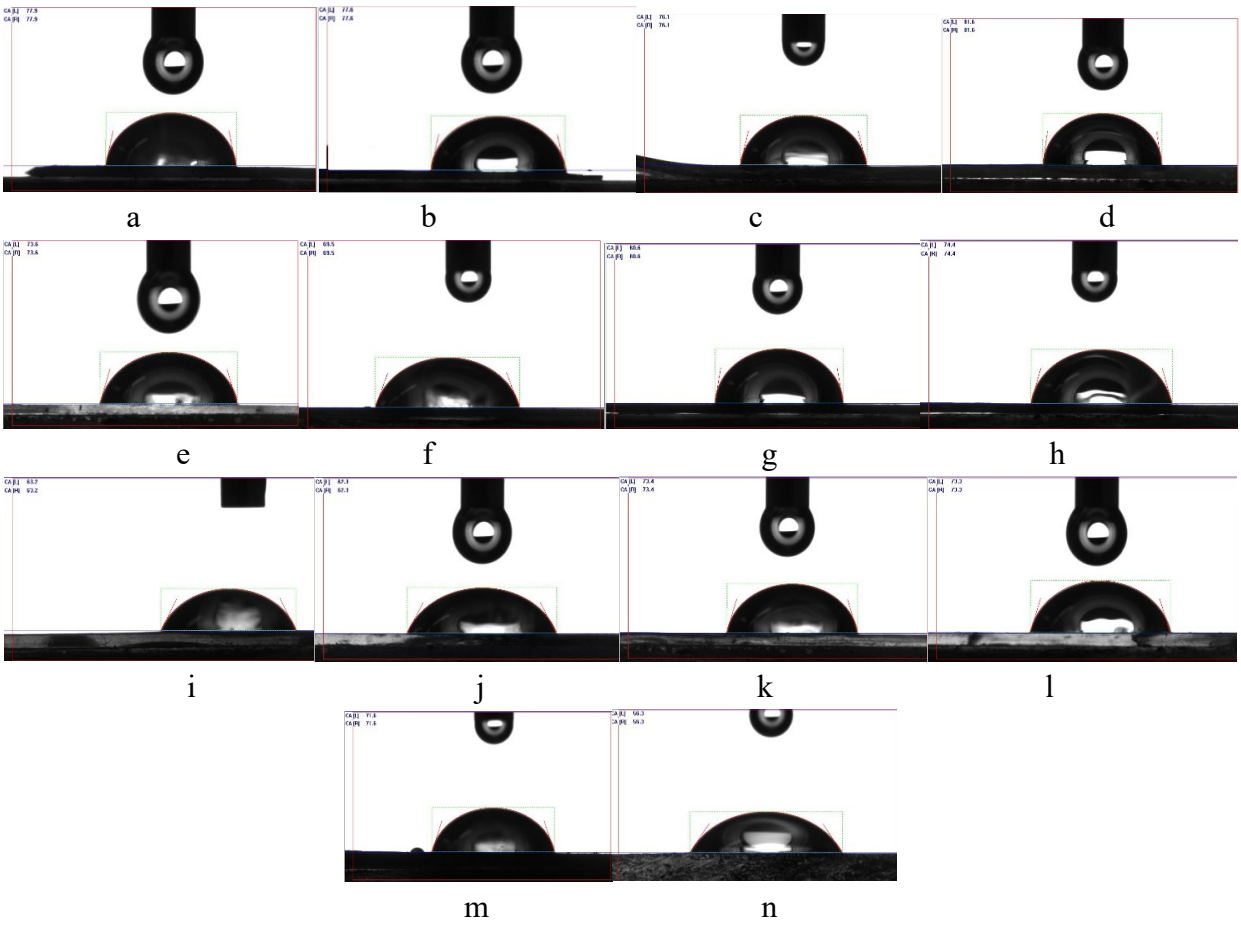


Fig. 11. Water contact angles of PU-CO and PUI-CO samples: a – PU-CO exposed to hexane; b – PU-CO exposed to isopropanol; c – PUI-CO heated at 80°C; d – PUI-CO dried under vacuum; e – PUI-CO dried at 120°C; f – PUI-CO dried at 140°C; g – PUI-CO dried at 190°C; h – PUI-CO dried at 220°C; i – PUI-CO dried at 170°C and exposed to hexane; j – PUI-CO dried at 170°C and exposed to heptane; k – PUI-CO dried at 170°C and exposed to isopropanol; l – PUI-CO dried at 170°C and exposed to petroleum ether; m – PUI-CO dried at 170°C; n – cell culture plasticware.

Table 4. Water contact angles of PU-CO and PUI-CO samples with different histories.

Sample	Sample history	Water contact angle (θ)
PU-CO	Drying at 75°C under vacuum for 6 h	78.2±0.0
	Drying at 75°C and exposure to hexane for 12 h	77.9±0.1
	Drying at 75°C and exposure to isopropanol for 12 h	77.6±0.0
PUI-CO	Drying at 80°C	81.6±0.2
	Heating at 120°C	73.6±0.1
	Heating at 140°C	69.5±0.1
	Heating at 170°C	71.6±0.0
	Heating at 190°C	80.6±0.1
	Heating at 220°C	74.4±0.2
PUI-CO heated at	Exposure to hexane for 12 h	63.2±0.1
	Exposure to heptane for 12 h	62.3±0.1

170°C and exposed to organic solvents	Exposure to isopropanol for 12 h	73.4±0.1
	Exposure to petroleum ether for 12 h	73.3±0.1
Control	Cell culture plasticware	56.3±0.0

It is known that when the contact angle is less than $\theta < 90°$, a solid surface is wetted by a liquid, and the surface itself is lyophilic (if the liquid is water, it is hydrophilic). If the contact angle is greater than $\theta > 90°$, a solid surface is poorly wetted by a liquid and is lyophobic (in the case of water, it is hydrophobic). According to Fig. 11 and Table 4, the values of water contact angle of the surface of the studied samples range from 62.3° to 81.6°, i.e., the samples are moderately hydrophobic. For polymer samples of virtually identical composition, this variation in θ values may be explained by fluctuations in their surface roughness. Note that in the case of adhesive cells (in our experiment, fibroblasts), moderately hydrophilic substrates ($\theta = 30–60°$) are used. The PUI-CO samples exposed to hexane and heptane approach this criterion.

Assessment of cytotoxicity of the films by the MTT method

Cytotoxicity of the synthesized polymers was assessed using the methyl tetrazolium test (MTT) [**33-36**]. For this purpose, the PUI-CO films preheated at 170°C were selected (both untreated and exposed to hexane, heptane, petroleum ether, and isopropanol).

The DF1 human skin fibroblast cell line (Institute of Cytology, St Petersburg) was used to study cytotoxicity. The cells were cultured in a CO_2 incubator at 37°C in a humidified atmosphere containing air and 5% CO_2 in DMEM/F12 nutrient medium (Dulbecco's modified Eagle's medium; Gibco), containing 1% essential amino acids, 10% (v/v) thermally inactivated fetal bovine serum (FBS; HyClone, USA), 1% L-glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin.

For the experiment, 5.0×10^3 cells/100μl/well were seeded in 96-well plates and cultured for 24 h for cell attachment. After 24 h, the initial medium was removed, and the nutrient medium obtained after incubation with the films (i.e., that has been in direct contact with copolymer samples) was added to the cells. To prepare this nutrient medium, the films with an area of 1 cm² were sterilized by UV irradiation for 30 min, then covered with 1 ml of complete nutrient medium and incubated for two days. The resulting incubation medium was added to the cells and cultured for 72 h. At the end of the incubation period (72 h), the medium was removed, and 50 μl/well of DMEM/F12 medium containing methyl tetrazolium (0.1 mg/ml) was added. The cells were incubated in a CO_2 incubator for 2 h at 37°C. After removal of the supernatant, the formazan crystals formed by metabolically viable cells were dissolved in dimethyl sulfoxide (50 μl/well) and transferred to clean wells; then optical density of the formazan solutions was measured at a wavelength of 570 nm on a plate spectrophotometer. This parameter reflects the amount of formazan present in the solution, which is proportional to the number of living cells. The results of the experiment are shown in Fig. 15.

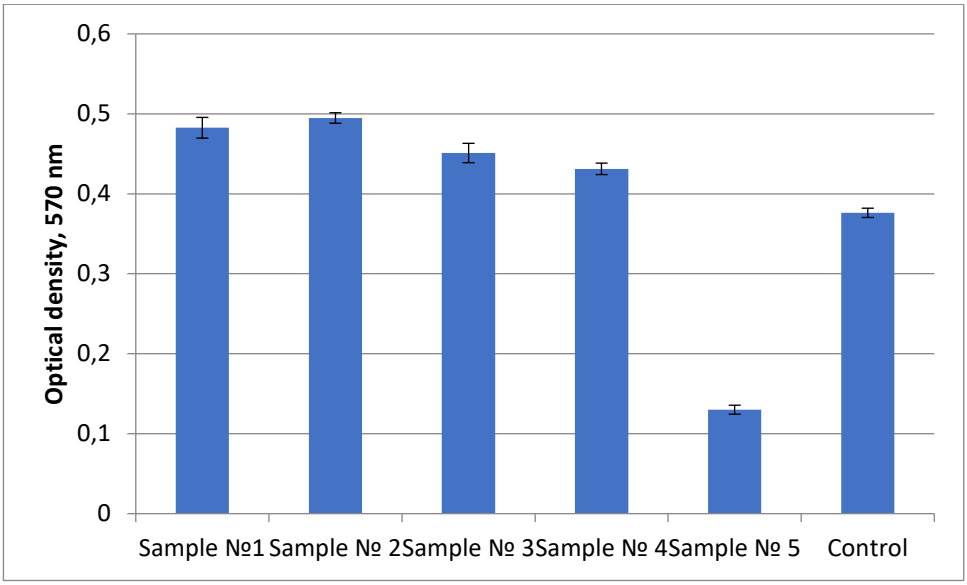


Fig.12. Optical densities of formazan solutions based on the results of the MTT test of PUI-CO films with different histories: Sample 1 - exposed to hexane; Sample 2 - exposed to heptane; Sample 3 - exposed to petroleum ether; Sample 4 - exposed to isopropanol; Sample 5 - without exposure; Control – cell culture plasticware (high-quality polystyrene, which ensures stability and compatibility with bacterial cultures).

In cell technologies, synthetic polymers may be a promising alternative to natural polymers [**67**]. They attract attention due to the possibility of varying different physical properties during their preparation and processing, which expands applications of these polymers in medicine. However, in the process of synthesizing polymers, various types of monomers, catalysts, and solvents are used, which can have a toxic effect on tissues after transplanting such materials into the damaged area. In this work, several solvents (hexane, heptane, petroleum ether, and isopropanol) were used to remove residual impurities from the studied polymers. After exposure of films of the synthesized PUI-CO copolymer to a number of solvents, the potential toxicity of the copolymer toward human skin fibroblasts was evaluated. The results of the MTT test (Fig. 12) showed no cytotoxicity of films exposed to all solvents, while the films that were not treated with these organic substances had a toxic effect on the cultured cells. The viability of fibroblasts cultured in the nutrient medium that contacted with untreated films decreased by more than 60% compared to that of the control sample (the cells cultured under standard conditions).

Conclusions

Thus, the results of experiments carried out in this work confirmed the importance and necessity of removing residual impurities during the preparation of films of biocompatible copoly(urethane-imide) that was synthesized on the basis of aliphatic polyester, aromatic dianhydride, aromatic diamine, diisocyanate, and castor oil.

Authorship contribution declaration

A.L. Didenko: Writing—original draft preparation, Investigation. Y.A. Nashchekina: Writing—original draft preparation, Methodology, Investigation. A.M. Kamalov: Formal analysis, Investigation. V.E. Kraft: Investigation. A.S. Ivanova: Resources, Formal analysis. E.N. Popova: Validation, Investigation. A.D. Artemeva: Resources, Validation. G.V. Vaganov: Methodology, Investigation. I.V. Polyakov: Resources, Investigation. V.M. Svetlichniy: Writing—original draft

preparation. V.E. Yudin: Resources, Writing—review and editing. V.V. Kudryavtsev: Conceptualization, Writing—review and editing.

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Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.
Conflict of interest. The authors declare that they have no conflict of interest.

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