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Study on degradation and systemic toxicity of multiblock poly(aliphatic/aromatic-ester) copolymers

Summary — The series of PED multiblock copolymers were synthesized. They are composed of poly(butylene terephthalate) (PBT) aromatic units (hard segments) and aliphatic sequences of dimer fatty acids (DFA) (soft segments). The composition of hard segments vary in the range from 26 to 70 wt. %. These polymers can be "tailor-made", and therefore, their properties can change along with the composition, from very soft and flexible materials to semi-rigid polymers. Their susceptibility to degradation is a function of hard/soft segment composition. Degradation test (buffer saline solution, pH = 7.4, temp. 37 ^{o}C, time 5 weeks) as well as *in vivo* implantation test for 6 months confirm this statement. Polymers containing higher concentrations of soft segments are more susceptible to degradation than the materials with higher concentrations of hard, aromatic segments as demonstrated by GPC and ATR FT-IR. The chloroformic extracts from PED copolymers were analyzed by GC/MS to evaluate the chemical composition of potential extractables, especially from the polymers demonstrating higher susceptibility to degradation. Prepared saline extracts were subjected to the pyrogenicity tests on rabbits. The influence of the polymer composition on skin irritation was also evaluated by the intracutaneous injections of polymer extracts. Additionally, hemolysis test in contact with bulk polymers was performed. Evaluating the nature of local tissue response to PED extracts and the results of hemolysis test, we did not detect any indication of systemic toxicity over the compositional range of PED copolymers. These novel copolymers were shown to be biocompatible and are very promising materials for biomedical applications.

Keywords: multiblock copolyesters, dimer fatty acid, degradation, toxicity test, hemolysis, mechanical properties.

Implantable synthetic polymer materials differ in their mechanical properties and the rate of degradation depending on the particular application (temporary or permanent prosthesis, drug delivery systems or others). Polymeric materials degrade in two ways: either by hydrolytic or by oxidative scissions of the polymer backbone releasing low molecular fragments, oligomers or monomers, which might be toxic [1, 2]. Other factors leading to degradation can be simple incorporation of low molecular weight compounds (water, lipids, organic acids) weakening secondary bonds within the polymer structure and acting as plasticizers. This process enlarges the distance between the polymer chains and causes the polymer swelling. The consequences are reduced mechanical strength and increased flexibility and softness [3].

To achieve good mechanical properties and thermal stability of polymers, they are usually synthesized in the presence of different additives (plasticizers or stabilizers). The presence of extractable substances (like antioxidants or low molecular weight molecules) lead to environmental stress cracking and the reduction in the polymer's molecular weight, as demonstrated by siloxane elastomers [4] or biocompatible polyolefines [5]. The chemical structures of segmented poly(etherurea-urethane)s [6] or segmented poly(ether-ester)s [7] contain the sites susceptible to oxidation, therefore, the ether soft segments have to be stabilized (phagocyte-gene-

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rated oxidants can also be involved in the environmental stress cracking of segmented polyether-based copolymers [8]). The application of synthetic antioxidants is often satisfactory (they usually have to be removed from the polymer by dissolution and precipitation). However, particularly for the biomedical applications, another stabilizer, such as natural antioxidant vitamin E, has recently been tested [6, 9, 10]. Therefore, the most recommended polymer compositions for medical applications should not contain any additives (antioxidants) and they also should not contain low molecular fragments, oligomers or monomers which might be toxic in contact with the human body.

Hydrolytic and oxidative stability of segmented polyesters can be improved by the introduction of dimer fatty acids (DFA) as a component of the soft segments in poly(butylene terephthalate) (PBT) block copolymers [11, 12]. This type of nonlinear hydrophobic dimers (aliphatic dibasic acids) has also been successfully applied to a new class of polyanhydride-based polymers developed for a drug delivery [13—15].

Multiblock poly(aliphatic/aromatic-ester) copolymers (PED) are prepared from dimer fatty acid and oligo(butylene terephthalate) *via* environmentally friendly method of polycondensation from the melt (there is no need to use any solvents; low molecular weight glycol is removed from the polycondensation products and can be used for the next synthesis after redistillation) [12]. The most important advantage, however, is the possibility to synthesize stable polymeric materials without the use of phenolic stabilizers, which can be irritating when the human body environment washes them out of the polymers. PED copolymers are representatives of thermoplastic elastomers and their microheterogeneous (crystalline-amorphous) structure is stabilized by physical, thermoreversible crosslinks, so they show good mechanical and elastomeric properties. Furthermore, they are stable during processing [12] and sterilization [16]. After subcutaneous long term implantation in rats they showed no adverse tissue reactions *in vivo* [17].

The aim of this work was to prepare a series of additive-free DFA-based PED copolymers, to evaluate the susceptibility to degradation, and to elucidate the dependence of biocompatibility (especially toxicity) and material properties on variable PED composition.

EXPERIMENTAL

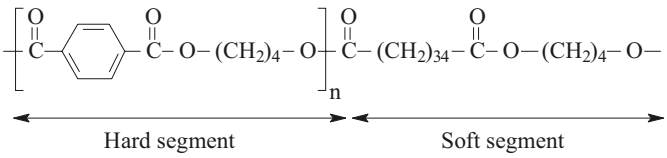
Materials

The following materials were used in this work:
— dimethyl terephthalate (DMT, Elana SA, Poland),
— 1,4-butanediol (BD, BASF, Germany),
— magnesium-titanate (characterized in [18]),
— dimerized fatty acid (DFA, "Pripol 1009", Uniquema B. V., The Netherlands).

Reagents were used as received.

Polymer synthesis

PED multiblock copolymers (I) were prepared in a two-stage process, namely transesterification and polycondensation from the melt [18]. The reaction mixture



n — degree of polycondensation
of hard segments (1, 1.9, 2.8, 4.3, 6.5) (I)

consisting of DMT, BD and magnesium-titanate catalyst was heated up to 200 °C with heating rate of 1.5 deg/min, in the reactor for transesterification. The molar ratio of BD and DMT was 1.8:1. During the second stage of the reaction, along with a catalyst, DFA was added. The polycondensation was carried out in a reactor for polycondensation and reaction mixture was heated to 225—230 °C at 0.066—0.080 kPa (0.5—0.6 mm Hg). Multiblock PED copolymers containing different concentrations of the hard, crystallizable segments varied in their physical appearance from semi-rigid plastics to soft elastomers (hard segments concentration was 26, 40, 50, 60 and 70 wt. %).

Samples preparation

Polymer films

Films were prepared by compression moulding of polymer discs (prepared previously by injection moulding at 50 MPa pressure) at temperature 20 deg higher than the melting point of the polymer. Polymer films of thickness 150 μm and 0.5 mm were cleaned with ethanol and washed with distilled water.

Polymer dumbbells

Dumbbells for mechanical testing were prepared by injection moulding according to the ASTM D 1897-77 Standard (S2 geometry).

Preparation of saline extracts

The saline extracts from polymer films were prepared according to US Pharmacopeia and ASTM F616 practice. Polymer films of 20x20 mm size and a total surface area of 1200 mm^2 were placed in a flask containing 20 ml of 0.9 % NaCl. Extracts were prepared for 24 hours at temperature of 70 °C. The saline extracts decanted from the polymer flakes were concentrated and analyzed using gas chromatography/mass spectroscopy method (GC/MS).

Method of testing

Degradation test

For degradation studies film specimens were incubated in 5 mL of buffer saline (PBS, pH 7.0) at 37 °C for up to 5 weeks. The buffer solution was changed every two weeks. At the end of each immersion period, the specimens were taken out, washed with distilled water three times, and dried at room temperature in a desiccator for 1 day. As a measure of hydrophilicity, the water absorption (A) was determined using the equation:

$$A = (W_s - W_d) \times 100\ \%/W_d \quad (1)$$

where: W_d, W_s — *weights determined at a given time, of the dried sample and of the swollen sample, respectively*.

The weight loss (W_l) of each film was determined by comparing the dry sample weight (W_d) of the degraded polymer with the initial weight (W_0):

$$W_l = (W_0 - W_d) \times 100\ \%/W_0 \quad (2)$$

Tensile testing

The tensile data were collected at room temperature using Instron TM-M tensile tester equipped with 500 N load cell at a cross-head speed of 200 mm/min. The stress data were calculated as the ratio of force and initial cross-section area. The strain was measured as the clamp displacement (the starting clamp distance was 25 mm). The stress at break and elongation values were averaged of 4—6 measurements for each sample.

Hardness

Hardness (H) was measured using Shore D apparatus (Zwick, type 3100) according to DIN 53505 (ISO 868) Standard.

Gel permeation chromatography (GPC)

Shodex (JM Science, Grand Island, NY, USA) linear GPC SE 61 column packed with 5 mm Pl-gel MIXED-C (Polymer-Laboratories) was employed for molecular weight analysis (GPC apparatus from Spectra Physics 8800).

Gas chromatography/mass spectroscopy (GC/MS)

GC/MS was used to study the migration of possible toxic products and non reacted monomer of dimethyl terephthalate from the polymer. 50 mL of saline extracts were extracted twice with 50 mL of chloroform by vigorous shaking for 10 min. Dimethyl- and diethyl terephthalate (DMT and DET, respectively) were used as internal standards. The chloroformic extract was dried and concentrated to 0.5 mL by evaporation in a rotary evaporator at 30 °C. Analysis was performed using Hewlett-Packard 6890 chromatograph coupled to a mass detector MSD 5973 and automatic injector. The following type of capillary and temperature program were used: 30 m × 0.25 mm I.D. coated with 0.25 μm HP-5MS (polydimethylsiloxane, 5 % of phenyl groups); from 60 °C (3 min isothermal) to 300 °C (10 min isothermal) at a ramp of 10 °C · min^{-1}. The capillaries were run in the constant flow mode (1.2 mL · min^{-1}).

Fourier Transform Attenuated Total Reflection Infrared Spectroscopy (ATR FT-IR)

ATR FT-IR spectra were obtained using Nexus FT-IR spectrometer (Nicolet Instrument Corporation, USA) equipped with the Golden Gate Single Reflection Diamond ATR (Specac INC, USA) scanning between 600 and 4000 cm^{-1}.

Scanning Electron Microscopy (SEM)

SEM micrographs of surfaces of polymer explants after 6 months of subcutaneous implantation into rats were analyzed using JEOL-JSM-IC848 microscope. Samples were vacuum dried and coated with 60 Å of gold prior to scanning.

Hemolytic property assessment

The hemolysis test of PED copolymers was performed according to ASTM F756 practice.

Subcutaneous implantation

Polymer films (10 × 10 × 0.5 mm) were implanted to the muscles of rabbits. Samples of polymers were retrieved after 6 months and used for SEM, GPC and ATR FT-IR analysis.

Intravenous injections

The saline extracts (3 mL/kg of animal weight) from polymer films were injected intravenously to three rabbits per each polymer extract and body temperature was measured after 60, 90, 120, 150 and 180 minutes after injection. According to the Polish Pharmacopeia, the examined extract is assumed to be free from pyrogens if the animal's body temperature doesn't increases more than 0.6 °C.

Intracutaneous injections

The saline extracts from polymer films were injected intracutaneously to the dorsal skin on the right side of New Zealand rabbits. The amount of 0.5 mL produced a bulla of a size 10 mm of diameter and 2 mm of height. Similar injections of a saline injected on the left side were the control ones. Every saline extract from a polymer sample was injected into two rabbits. All animals were carefully examined after 1, 2, 4, 8, 24, 48, and 72 hours in order to evaluate the skin colour, reddening diameter, temperature of the body and swelling extension.

RESULTS AND DISCUSSION

As it is known, in general all polymers are susceptible to degradation, but the conditions under which the polymers degrade vary within wide ranges. The biodegrada-

tion of polymers is predictable to a considerable degree based on *in vitro* experiments [19, 20].

T a b l e 1. Mechanical properties of multiblock PED copolymers

Sample of copolymer	Composition PBT/DFA (hard/soft) wt. %	Melting temperature °C	Elongation at break %	Stress at break MPa	Hardness Shore D
A	26/74	116	520	6	19
B	40/60	150	570	13	37
C	50/50	170	540	20	47
D	60/40	186	400	23	53
E	70/30	197	300	26	59

The compositions of multiblock PED copolymers, as well as their characteristic thermal and mechanical data are given in Table 1. Their thermal and mechanical properties change with hard/soft segment ratio: the higher the hard segment content creating the rigid phase, the higher hardness and stress at break. The softest polymer A (26 wt. % of hard segment) shows tensile strength comparable to silicone elastomer [21], while the hardest one (E) is comparable to poly(ether-ester)s of Arnitel type [22] thermoplastic elastomers.

PED copolymers are relatively stable materials due to hydrophobic surface as determined by water contact angle measurements (from 82° to 93°) [23]. Fig. 1 shows that water absorption increases gradually with increasing soft segment content from 8 % (sample E) to 20 % (sample A). In is worth to note that hydrophilic biodegradable copolymers based on poly(D,L-lactide) showed 800 % water absorption after 2 weeks [24].

The weight of the sample decreases along with increasing soft segment content as showed in Fig. 2. The maximal weight loss was not higher than 9 % for polymer A with 74 wt. % of soft segments. So the degradation test has indicated that polymers containing higher concentrations of amorphous phase (soft segments) show higher susceptibility to hydrolytic degradation. These observation correlates well with "boiling test" performed on these polymers [23]. Briefly, PED copolymers were boiled under reflux for up to 100 hours in water and the changes of their mechanical properties were monitored with respect to exposure to hot water. The remarkable susceptibility to hydrolytic degradation resulted in decrease in tensile strength of A sample containing the highest concentration of amorphous phase. As the hard segment content increased, the polymer resistance to accelerated degradation was much improved. Polymers containing high amounts of hard segments showed much better stability of mechanical properties

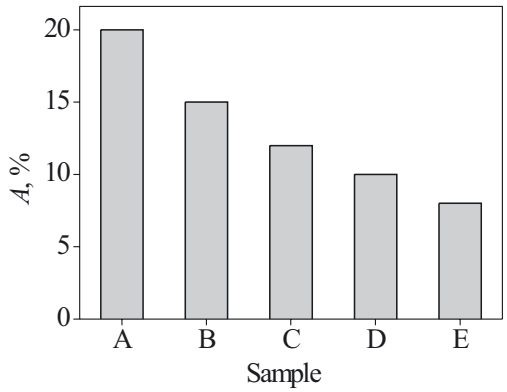


Fig. 1. Water absorption (A) of PED copolymer samples (A—E) in PBS buffer (pH 7.4) at 37 °C after 5 weeks

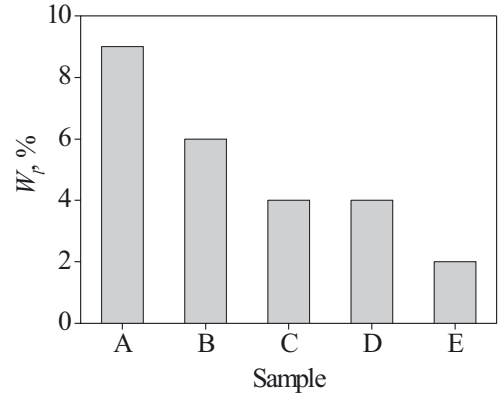


Fig. 2. Weight loss of PED copolymer samples in PBS buffer (pH 7.4) at 37 °C after 5 weeks

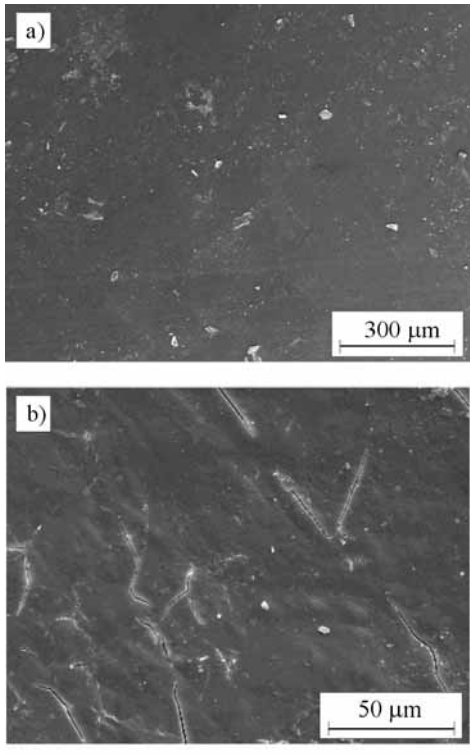


Fig. 3. SEM images of sample A surface after 6 months of implantation; magnification: a) ca. 100×, b) ca. 500×

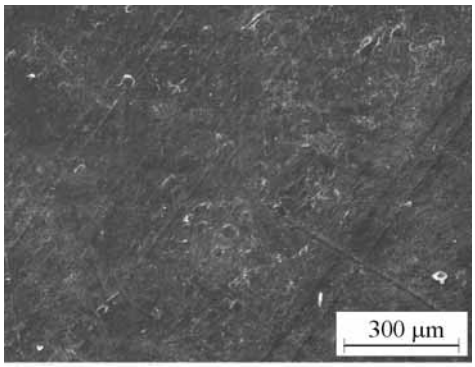


Fig. 4. SEM image of sample E surface after 6 months of implantation; magnification ca. 100×

(in our degradation experiments these polymers showed the smallest water absorptions and weight losses).

A higher susceptibility of soft polymer containing 26 wt. % of hard segment to the hydrolytic attack has been confirmed by *in vivo* degradation study. Two extreme polymers, containing 26 and 70 wt. % of hard segments (A and E, respectively), were implanted intracutaneously into rabbits for 6 month. SEM pictures of the explants show that both polymers remained intact (Figs. 3a, b and 4). However, upon higher magnification, the soft polymer shows the appearance of small crazes on the surface (Fig. 3b). This can be an indication that polymer can undergo degradation. Indeed, ATR FT-IR spectra (Fig. 5) show the appearance of a new band at 1550 cm^{-1}, which may correspond to acid C-O stretching asymmetric vibration in the carbonyl ion. There is also a broad band at 3306 cm^{-1}, which can be related to the stretching vibration of O-H group. Finally, GPC analysis (Table 2) confirmed decrease in molecular weight of this soft material (A) indicating slow degradation. Good stability *in vivo* (and after "boiling" test) of a polymer containing 70 wt. % of hard segments (E) was also confirmed by infrared spectroscopy — no changes of the chemical structure were detected (Fig. 6).

T a b l e 2. Molecular weight loss by GPC

Sample	M_n	M_w	M_w/M_n	$M_n^{*)}$	$M_w^{*)}$	$M_w/M_n^{*)}$
A	35 600	67 900	1.9	27 000	54 800	2.03

*) *after 6 months implantation*

Taking into account that PED copolymers can show susceptibility to prolonged degradation, it was important to assess the hemolytic property of polymers and to investigate the polymer extracts with respect to residual products, such as terephthalate units or another extractable products, mainly from aliphatic ester units. It is worth to point out, that aliphatic dimer fatty acid impart high hydrophobicity to the polymers, as illustrated by the water contact angle measurements of PED copolymers compared to poly(ester-ether) copolymers and poly(ether-urethane)s [23]. The incorporation of fatty acid terminal groups to polyanhydrides has been also increasing the polymer hydrophobicity which resulted in a slower degrading material [25, 26]. Therefore, PED material containing 26 wt. % of hard segments of dimmer fatty acid (sample A) will degrade much slower

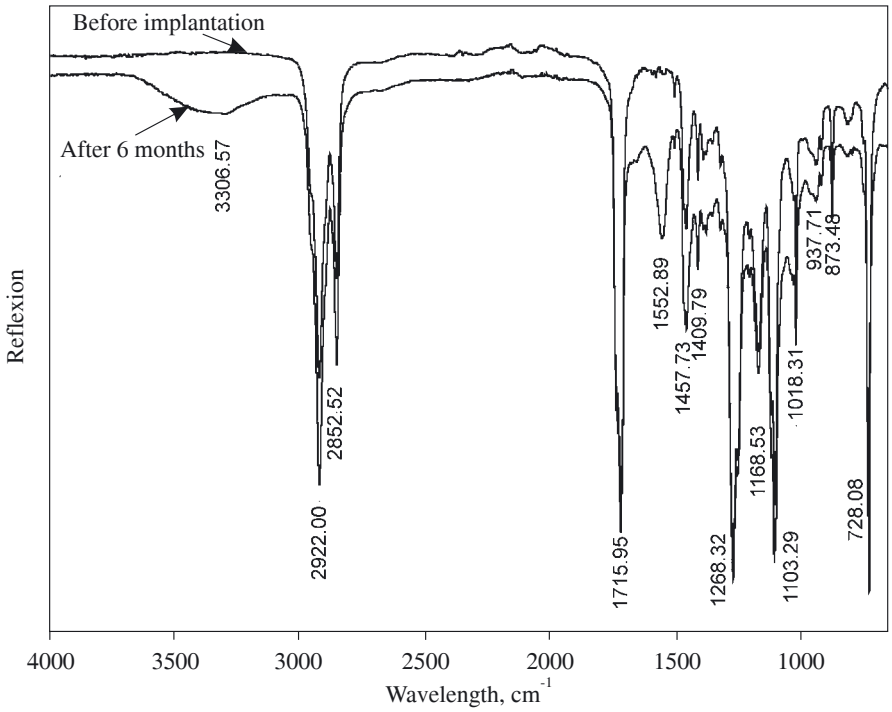


Fig. 5. ATR FT-IR spectra of sample A surface before and after 6 months of implantation

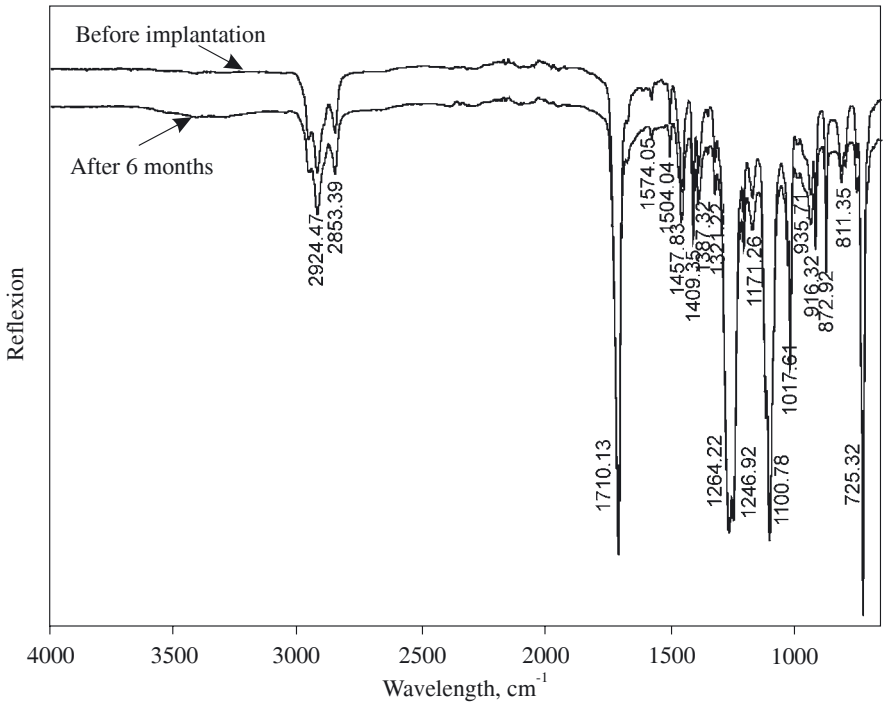


Fig. 6. ATR FT-IR spectra of sample E surface before and after 6 months of implantation

than its structural analogue, the Polyactive® copolymer, comprising aliphatic ether unit as a building block in soft segment (samples containing 30 wt. % of PBT hard segments showed disintegration after 3 week implantation in goats [27]).

In the case of polymers containing terephthalate or isophthalate units, the presence of non reacted monomer — dimethyl terephthalate (DMT) could be a problem. DMT however appears to show a very low order of toxicity [28]. Acute animal studies indicated that oral and dermal *LD*50 values in excess of 3 400 ppm, and subchronic oral (10 000 ppm DMT in the diet for 96 days) or inhalation exposures (2—10 ppm, 4 hr/day × 58 days) have not resulted in any hematologic, blood chemical, or pathologic alterations attributable to DMT. Results of other experiments with rats and rabbits demonstrated that DMT is rapidly absorbed and excreted (primarily in urine), and that no significant quantities accumulate in tissues following single or repeated oral, intratracheal, dermal, or ocular administration. DMT does not appear to irritate or sensitize the rodent skin [28].

The key problem in chemical method is a very high accuracy and determination of a specific migration limit [29]. In the case of polymers for food storage and according to Directive 90/128/EEC, the specific migration limit for dimethyl terephthalate from PET has been set at 0.05 ppm. Therefore, this migration limit has been selected as critical for migration of dimethyl terephthalate from PED copolymers.

Taking into account that the sensitivity of our method was found to be at 0.02 ppm of DMT, there was no evidence for dimethyl terephthalate extraction from PED copolymers within the limits of the assay sensitivity.

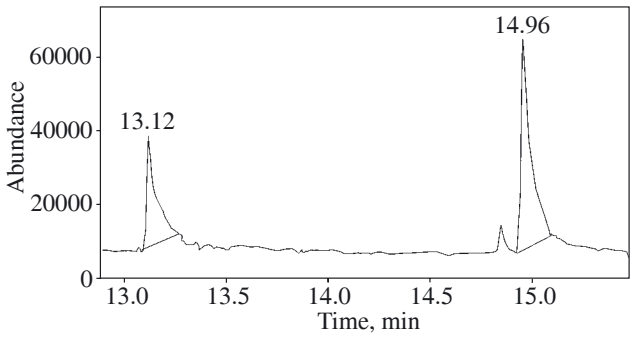


Fig. 7. Gas chromatogram of chloroformic extract from sample E

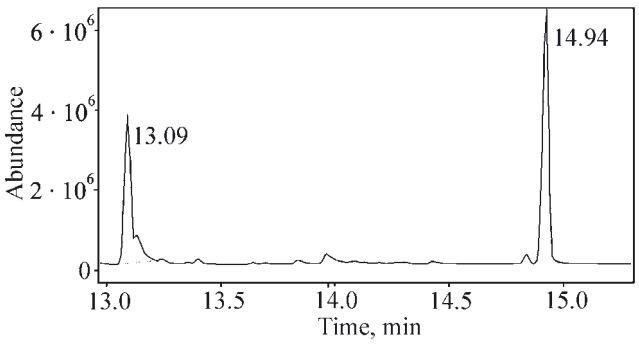


Fig. 8. Gas chromatogram of chloroformic extract from sample A.

Fig. 7 shows gas chromatograms of a chloroformic extract from sample E, and as can be seen, a very small amount of DMT (retention time 13.12 min) was detected. In the case of soft polymer containing higher amount of dimer fatty acids (sample A), a peak appearing at retention time of 13.09 min was detected (Fig. 8). This peak

was identified as dimethyl ester of 1,4-cyclohexanedicarboxylic acid. This kind of ester can be released from dimer fatty acids which contain 99.5 % of dimers, and importantly, is not included into the list of toxic or hazardous chemicals [26]. However, it is also possible that the peak identified at 13.09 min corresponds to DMT with a hydrogenated ring.

In order to evaluate whether trace amount of this chemical can act as pyrogen, saline extracts from all PED copolymers were intravenously injected into rabbits. The results of measurements of the body temperature of animals after 60, 90, 120, 150 and 180 minutes after injection are presented in Table 3. According to the Pharmacopeia recommendation, if the increase in the body temperature is up to 0.6 °C above the normal body temperature, and the overall increase from the measurements on three animals is not higher than 1.4 °C, the saline extract from candidate biomaterial is considered as being free from the fever-producing substances.

T a b l e 3. Rabbit's body temperature (in °C) after intravenous injection of saline extracts from PED copolymers

Sample of copolymer	Body temperature					
	initial	after injection				
		60 min	90 min	120 min	150 min	180 min
A	38.0	37.8	37.6	37.6	37.6	37.7
	38.3	38.1	38.3	38.4	38.4	39.0
	38.0	37.8	37.9	38.1	38.5	38.1
B	38.3	37.7	38.6	38.6	38.3	38.3
	38.2	38.4	38.4	38.4	38.6	38.7
	38.7	37.5	37.6	37.6	37.7	37.5
C	38.5	37.8	38.2	38.2	37.5	38.1
	38.8	37.5	37.6	37.2	37.2	37.6
	38.5	37.5	37.2	38.1	38.4	37.8
D	38.1	37.3	37.7	37.3	37.5	37.8
	38.9	37.7	37.5	37.5	37.7	37.7
	38.1	37.8	37.5	37.1	37.8	37.7
E	38.1	37.1	37.5	37.7	37.5	37.3
	38.1	37.3	38.2	38.7	37.4	38.1
	38.3	37.9	37.5	37.8	37.9	37.3

It can be seen from the results presented in Table 3, that the animals survived the experiment without an increase in the body temperature above the limit (0.6 °C above the initial body temperature). However, there were the animals which reacted differently on the same extraction vesicle, with their body temperature rising by 0.7 °C. In general, the animals' behavior after intravenous injections has been found on a normal level of feeding and activity, with no signs of blood pressure reduction, anoxaemia or clonus. This may testify that saline extracts, and particularly trace amounts of dimethyl ester of 1,4-cyclohexanedicarboxylic acid detected by GC/MS do not appear to have pyrogenic properties.

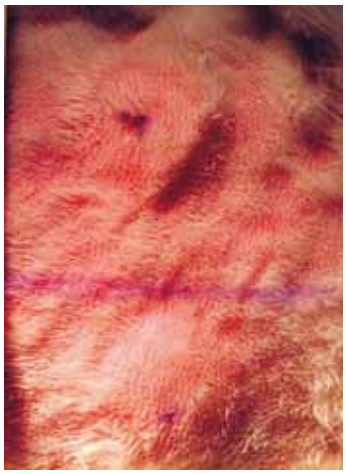


Fig. 9. Blisters produced after injection of 0.9 % NaCl (top) and saline extract from sample B (bottom)

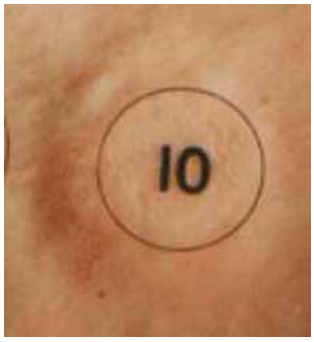


Fig. 10. Blister produced after injection of saline extract from polymer E

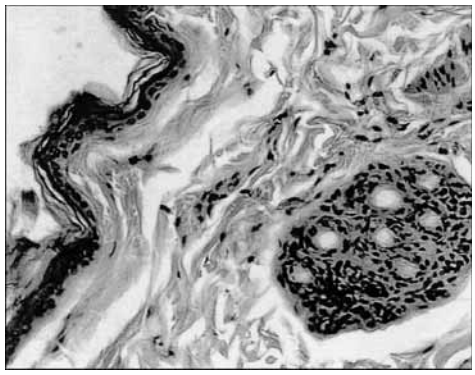


Fig. 11. Histological cross-section of a skin after injection of saline extract from polymer A

Saline extracts from PED copolymers were injected intradermally (in the dorsal skin of rabbit back) for skin irritation testing. The investigations of skin in the places of injections of saline extracts did not ascertain changes regarding colour and temperature in comparison to the area where 0.9 % NaCl as a control sample was injected (Fig. 9). Blisters produced in the places of injections remained several minutes and disappeared after 10—15 minutes not leaving any traces of skin irritation in macroscopic observations (Fig. 10).

The estimation of the toxicity of tested polymers was also made using microscopic analysis of histopathological slides. Detailed analysis of tissue reaction in the light of optical microscope did not ascertain any histological changes after injection of saline extracts as well as of 0.9 % NaCl solution. There was no inflammable infiltration, congestions, extravasations, swelling or oedema signs or focal necrosis in skin or subcutaneous tissue. Each layer of a skin behaved distinct in drawing and proportions (Fig. 11). PED copolymers were assessed to be nontoxic, since these materials exhibited no hemolytic responses (hemolytic indexes of PED multiblock copolymers were found to be "0" as compared to Teflon film used as negative control).

CONCLUSIONS

Poly(aliphatic/aromatic-ester) (PED) multiblock copolymers are very interesting group of materials. Due to the selection of dimer fatty acids as a building block of soft segments, it was possible to synthesize a series of additive-free multiblock copolymers of high hydrophobicity and variable physical appearance: from soft and flexible to semi-rigid copolymers. Their tensile properties are comparable to silicone rubber (for the soft PED) or poly(ether-ester)s (semi-rigid polymers). Their stability is attributed to the rigid phase content in the polymers: soft materials show a susceptibility to degradation *in vitro* and *in vivo*, while polymers containing high amount of hard segments (rigid phase) are very stable *in vivo*. It was not detected of any indication of systemic toxicity over the compositional range of PED copolymers. Presented characteristics could be valuable for PED applications either in biodegradable or biostable medical devices. PED copolymers with high content of amorphous phase are currently investigated for use in soft tissue reconstruction.

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