New amino acid based biodegradable polymers *via* bis-azlactone chemistry

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Abstract: New biodegradable amino acid based biodegradable (**AABB**) ester polymers having a potential for constructing resorbable surgical and therapeutical devices were synthesized by solution step-growth polymerization (SGP) of bis-azlactones (**BALs**) with di-*p*-toluenesulfonic acid salts of bis-(α -amino acid)- α,ω -alkylene diesters (**TDADs**). Relatively low-molecular-weight homopolymers - poly(ester amide)s with M_w within 2,800-19,600 Da (GPC in DMF) were obtained. To increase molecular weights of the goal **AABB** ester polymers two additional synthetic strategies were applied – either activated diester such as di-*p*-nitrophenyl sebacate was used as a comonomer of **BAL**, or alkylene diamine such as 1,6-hexamethylenediamine was used as a comonomer of **TDAD**. As a result high-molecular-weight **AABB** ester polymers with M_w up to 103,500 Da were synthesized depending on co-monomers' structure and mole ratios. All the **BAL**-based polymers obtained after SGP were practically^[1] amorphous. The thermograms (DSC) of the polymers made of the **BAL^F** showed wide endotherms within 55-120 °C. These endotherms were assigned to melting hydrophobic domains formed by highly hydrophobic fragments - residues of phenylalanine-based bis-azlactone (**BAL^F**) in the polymeric backbones. The obtained new **BAL**-based ester polymers substantially expands a family of **AABB** polymers destined for constructing resorbable surgical and pharmaceutical devices.

Keywords: bis-azlactones, amino acids, bis-diamine-diester, biodegradable polymers, homo-poly(ester amide)s, co-poly(ester amide)s, poly(ester amide-co-amide)s, synthesis, thermal properties, *in vitro* biodegradation

1. Introduction

The synthesis and study of biodegradable polymers is at forefront of modern polymer chemistry because of the technological challenges and a high commercial potential. For many medical, agricultural and environmental purposes it is important to have biodegradable polymers which degrade under the action of physiological environment or in soil “without traces”. Materials that can decompose and disappear “without traces” from the body are desirable for a variety of biomedical applications such as surgical and pharmaceutical devices, wound closure/dressing materials, vascular grafts, nerve guidance tubes, absorbable bone plates, orthopedic pins and screws, body-wall/hernia repair, sustained/controlled drug delivery systems, to name a few. Different materials with tailored properties are required for each of these applications. Therefore, biodegradable polymers with a variety of hydrophilicity/hydrophobicity, permeability, morphology, degradation rates, chemical and mechanical properties are needed.

Among a huge variety of biodegradable polymers destined for applications as biodegradable biomaterials, one of the most promising look a relatively new family of the ester polymers on the basis of naturally occurring α -amino acids and other nontoxic building block such as fatty diols and dicarboxylic acids [1-10]. Three main classes of Amino Acid Based Biodegradable (AABB) ester polymers were designed: (i) poly(ester amide)s (**PEAs**) composed of α -amino acids, fatty diols and dicarboxylic acids, (ii) poly(ester urea)s (**PEUs**) composed of α -amino acids, one diol and carbonic acid, and (iii) poly(ester urethane)s (**PEURs**) composed of α -amino acids, one or two diols and carbonic acid.

One of the most promising among **AABB** ester polymers are **PEAs**. These polymers combine all advantages of “parent polymers” - aliphatic polyesters and polyamides: ability of biodegradation with a reasonable rate that comes from polyesters, along with a high affinity to and, hence, good compatibility with tissues and a wide range of desired mechanical properties that comes from polyamides. The scopes of applications of **AABB PEAs** and related polymers can substantially be expanded by their functionalization through the incorporation of various

groups and sites into the backbones or lateral chains [11-14]. One of the most facile ways to render polymers functional is the incorporation of hydrophobic fragments into the macromolecules [15]. The **PEAs** with hydrophobic site in the macromolecules can bind various bioactive substances *via* hydrophobic forces. The existence of hydrophobic sites in the macromolecules can promote their interaction with surfactants that, as a result, can facilitate the preparation of macro- and nanoparticles. Besides, such sites are convenient for surface PEGylation of the beads¹ by firm hydrophobic anchoring of properly modified PEG molecules [16]. The enhanced hydrophobicity also decreases the stickiness (caused by the plasticizing influence of water) of particulates thus preventing their aggregation. Hydrophobic modification of biodegradable polymers is highly reasonable also for modifying their hydrophobic-hydrophilic balance and, hence, to tune drug release from and biodegradation rates of appropriate polymeric devices.

The present paper deals with the synthesis and study of new hydrophobically modified **AABB** ester polymers. For the hydrophobic modification of the ester polymers we have selected so called “azlactone method” for the following reasons: (i) starting bis-electrophilic monomers - *p*-phenylene-bis-oxazolinons (bis-azlactones, **BALs**) are highly active compounds which interact with fatty diamines in organic solvents under mild conditions resulting in high-molecular-weight polyamides (**PAs**) [17-21], (ii) the reaction is polyadditive, and consequently, after synthesis of the polymer, a low molecular weight by-product is not released, which reduces the impurities in the target polymers, and (iii) the **BALs** are easily synthesized in high yields by chemical cyclization of corresponding N,N'-terephthaloyl-bis- α -amino acids [19,20]. The **BALs** based **PAs** [20] are characterized by excellent film-forming properties and biocompatibility, however, their biodegradation rates are too low to be used as biodegradable biomaterials [22]. To render **BALs** based polymers biodegradable with appropriate rates, easily hydrolysable linkages such as ester linkages have to be incorporated into their backbones - in other words to synthesize **PEAs** or related ester polymers. For incorporating ester linkages into the polymeric backbones one of the most convenient monomers are amino acid based diamine-diester [1] - bis-(α -amino acid)- α,ω -alkylene diesters which are normally used as stable di-*p*-toluenesulfonic acid salts. These

¹ The PEGylation of drug nanocarriers increases their bioavailability. Besides, it helps to overcome the problems connected with the formation of protein “corona”, which represents proteins and lipids rapidly adsorbed on their surface when nanosystems are in a physiological environment [17]

salt monomers, referred below as **TDADEs**, represent key compounds for synthesizing all classes of **AABB** ester polymers reported previously [1-15].

In the present paper we have used three strategic approaches to the synthesis of three types of new **AABB** ester polymers made of **TDADEs** and **BALs**: 1) the interaction of the **TDADEs** with the **BALs** that resulted in homo-poly(ester amide)s (**homo-PEAs**), 2) the interaction of the **TDADEs** with a mixture of the **BALs** and activated diester - di-p-nitrophenyl sebacate (**NPS**) that resulted in corresponding co-poly(ester amide)s (**co-PEAs**), and 3) the interaction of a mixture of **TDADEs** and 1,6-hexanediamine (**HDA**) with **BALs** that resulted in corresponding poly(ester amide-*co*-amide)s (**PEA-co-PAs**). The elaboration of such synthetic strategy was stipulated by the synthesis of relatively low-molecular-weight **homo-PEAs** with poor material properties by interaction of **BALs** (100 mol %) with **TDADEs** monomers (100 mol %) due to the reasons discussed below. Therefore, to increase molecular weights of the goal polymers the basic monomers - **BALs** and **TDADEs** were in part replaced by comonomers such as **NPS** and **HDA**, accordingly. Copolymers - **co-PEAs** and **PEA-co-PAs** were synthesized at mol % ratios of comonomers: **BAL/NPS** 50/50, 30/70, 10/90 and **TDADEs/HDA** 90/10, 50/50, 30/70, 10/90, accordingly.

Experimental part

Materials and methods

L-Valine (**V**), L-leucine (**L**) and L-phenylalanine (**F**), 1,6-hexanediol, 12-dodecanediol, 1,6-hexanediamine (**HDA**), terephthaloyl chloride, sebacoyl chloride, p-nitrophenol, p-toluenesulfonic acid monohydrate, acetic acid anhydride, solvents such as acetone, benzene, toluene, ethyl acetate, chloroform, dichloromethane (DCM), pyridine, glacial acetic acid, 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), trifluoroethanol (TFE), trifluoroacetic acid (TFA), lithium chloride were purchased from Sigma-Aldrich and used without further purification. Dimethylformamide (DMF), dimethylacetamide (DMA), N-methylpyrrolidone (NMP) and triethylamine (from Sigma-Aldrich) were dried and purified according to standard methods prior to use in the SGP reactions.

The chemical structures of the materials synthesized were characterized by FT-IR and NMR spectra. Thermo Nicolet Avatar 370 FT-IR spectrophotometer (coupled with EZ OMNIC

software) was used for IR analysis. The FT-IR spectra of the polymers were recorded in thin films cast from HFIP solution directly on an Avatar Multi-Bounce Flat Plate 45 degree Ge. The 300 MHz ^1H -NMR spectra were recorded on a Varian Mercury 300 spectrometer at 303 °K using DMSO- d_6 /CCl $_4$ (1:3 v/v) containing tetramethylsilane (TMS) as an internal standard; the ^1H NMR-data was used for the assessment of the **co-PEAs** composition. The number-average (M_n), and weight-average (M_w) molecular weights, and molecular weight distribution (Dispersity, D) of the polymers were determined on a GPC machine (Waters Associates, Inc., Milford, United States. Styragel columns in DMF: HR4, HR3, HR0.5 all 7.8 mm $\times$ 300 mm) equipped with a high-pressure liquid chromatography pump (Waters 1525 Binary HPLC) and a Waters refractive index detector 2414 and UV-detector (Waters 2487 dual absorbance detector, $\lambda = 240$ nm). A solution of KBr (0.1 M) in DMF was used as an eluent. Injected volume 100 μL , sample concentration 5.0 mg/mL, flow rate 1.0 mL/min. The columns were calibrated with PMMA standards. In parallel the molecular weight characteristics were measured in HFIP solution using a GPC machine (Shimadzu, model LC-8A) equipped with an Empower computer program (Waters). A PL HFIP gel column (Polymer Lab) and a refractive index detector (Shimadzu RID-10A) were employed. The polymer was dissolved in and eluted with HFIP containing CF $_3$ COONa (0.05 M, to suppress polyelectrolyte effects). Injected volume 100 μL , sample concentration 2.0 mg/mL, flow rate 0.5 mL/min. The columns were calibrated with PMMA standards. The thermal properties of new polymers were measured by NETZSCH DSC 200 PC Phox within the temperature range 20 – 200 °C under a dry nitrogen gas flow at a heating rate of 10 °C/min.

The *in vitro* biodegradation was studied by both automatic potentiometric titration (APT) [23] and gravimetry (weight loss, WL) [24] using two hydrolases – α -chymotrypsin and lipase. Conditions: pH 7.4, $t = 37^\circ\text{C}$, enzymes in solution at a concentration 0.4 mg/mL. Substrates: in case of **co-PEAs** – circular disc-shaped films (cast from 10% chloroform solutions) of diameter 4.0 cm and an average weight 400-500 mg, in case of **homo-PEAs** – 100 mg of the grinded polymer powders sieved through 50 μm sieve mesh. In case of the polymeric disc-substrates both APT and WL methods were applied; as far as the hydrophobic polymeric discs were „non-contractive“ they were used without backings recommended in Ref. [23]. In all WL experiments, the film discs were periodically (every 20 h after the incubation in the enzymes' solution) weighed following to the procedure described in [24] and the weight change per unit surface area

of the sample was calculated. After each weighing the samples were placed in freshly prepared enzymes' solutions. In case of **homo-PEAs'** powdery-substrates only APT method was used. In case of APT the biodegradation rate of the polymers was assessed by the quantity of hydrolysed ester bonds (in μmole) per minute, which was calculated after 60 min hydrolysis of the polymer as $Q_{\text{NaOH}}/60$ where Q_{NaOH} is a quantity of NaOH in μmole consumed for neutralizing carboxyl groups released after the hydrolysis of ester bonds. A Metrohm automatic potentiometric titrator 842 Titrando was used in these experiments.

Monomer synthesis

Bis-electrophilic monomers

a) *Synthesis of the **BALs**.* 2,2'-p-phenylene-bis-(4-isopropyl-oxazolin-5-one) - **BAL^V** and 2,2'-p-phenylene-bis-(4-benzyl-oxazolin-5-one) - **BAL^F** were synthesized as reported previously [20] by the cyclization of N,N'-terephthaloyl-bis- α -amino acids (diamide-diacids, DADAs) as depicted in Scheme 1:

Scheme 1. The synthesis of the **BALs**.

The intermediate DADAs were synthesized using Shotten-Baumann procedure. The cyclization of the DADAs was carried out in acetic anhydride at 100°C [20]. The **BALs** were recrystallized from benzene. The yield of the **BAL^V** was 60%, m.p. 163-164°C, formula weight ($\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_4$) 328.36; The yield of the **BAL^F** was 80%, m.p. 180-181°C, formula weight ($\text{C}_{26}\text{H}_{20}\text{N}_2\text{O}_4$) 424.14. The results well coincide with reported data [20]. As reported previously the **BALs** are racemic compounds [19,20].

b) *Synthesis of the **NPS**.* The synthesis of the activated diester - **NPS** was carried out as reported previously [25] by the interaction of p-nitrophenol (2.0 mol) with sebacoyl chloride (1.0 mol). The yield of **NPS** was 89% (from acetone), m.p. 103-104 °C (reported m.p. 103-104 °C [25]), formula weight ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$) 444.43.

Bis-nucleophilic monomers - TDADEs

The synthesis of **TDADEs** was carried out as reported previously [25,26] by direct condensation of an α -amino acid with a diol in refluxed toluene, as depicted in scheme 2:

where: **AA** = L- α -amino acid (**F** or **L**); $x = 6, 12$. Labelling of the **TDADEs AAx**: **F6**, **L6**, and **L12**

Scheme 2. The synthesis of the TAADs - **F6**, **L6**, and **L12**.

The yield of the **TDADE F6** was 94%, m.p. 213-215 °C (reported m.p. 213-215 °C [25]), formula weight ($\text{C}_{38}\text{H}_{48}\text{N}_2\text{O}_{10}\text{S}_2$) 756.94. The yield of the **TDADE L6** was 89%, m.p. 190-192 °C (reported m.p. 190-192 °C [25]), formula weight ($\text{C}_{32}\text{H}_{52}\text{N}_2\text{O}_{10}\text{S}_2$) 688.91. The yield of the **TDADE L12** was 88%, m.p. 178-179 °C (reported m.p. 178-179 °C [26]), formula weight ($\text{C}_{38}\text{H}_{64}\text{N}_2\text{O}_{10}\text{S}_2$) 773.07. The results completely coincide with previously reported data [25,26].

Polymer synthesis

For synthesizing **BAL**-based **AABB** polymers *via* the SGP we used three strategic approaches: (i) the **homo-PEAs** were obtained by interaction of the **BAL** with the **TDADE**, (ii) the copoly(ester amide)s (**co-PEAs**) were obtained by interaction of mixtures of the **BAL** and **NPS** with the **TDADE**, and (iii) the poly(ester amide-*co*-amide)s (**PEA-co-PAs**) were obtained by interaction of the **BAL** with mixtures of the **TDADE** and **HDA**.

(i) Synthesis of the homo-PEAs via the SGP

Below are given procedures describing the synthesis of **homo-PEA** *via* SGP of the **TDADE L12** and **BAL^V** at variable parameters to establish optimal reaction conditions. At that the following reaction components were invariable: the quantities of **BAL^V** (3.2836 g, 0.01 mol) and **L12** (7.7307 g, 0.01 mol) and the volume (2.8 mL, 0.02 mol) of TEA. These components were placed in a three-necked 50 mL round-bottom flask equipped with a stirrer (for the sequence of the components introduction see below); an organic solvent (varying both the nature and volume) was used to carry out the reaction.

The influence of the order of addition of components. The invariable reaction components above (**BAL^V**, **L12**, TEA) and a solvent (DMA) were introduced into the flask in three various sequences:

Sequence “a”: (powdery **L12** + powdery **BAL^V**) + (DMA) + (TEA);

Sequence “b”: (powdery **L12** + TEA) + (powdery **BAL^V**) + (DMA);

Sequence “c”: (powdery **BAL^V** + TEA) + (powdery **L12**) + (DMA).

The influence of the sequence was studied at the starting temperature 0 °C, monomer concentration 1.2 mole/L (2.8 mL of TEA + 5.5 mL DMA = 8.3 mL). After adding TEA the reaction mixtures became red in colour that is typical for 4-monosubstituted **BALs** in the presence of amines [18,20]. The reaction mixture was stirred at 0 °C until its homogenisation and discoloration (1-2 h), then the reaction temperature was increased up to 80 °C and the solution was stirred again for 18 h. The sequence “a” gave the best results and therefore was applied for the subsequent experiments.

The influence of the solution concentration. To the invariable reaction components above (**BAL^V**, **L12**, TEA) a predetermined volume of DMA was added with the purpose to vary a monomer concentration from 0.30 to 1.2 mole/L (the volume of TEA 2.8 mL was taken into consideration). In all cases the starting temperature was 0 °C and the components were added according to the sequence “a” upon stirring. The reaction mixture was stirred at 0 °C until its homogenisation and discoloration (1-2 h), then the reaction temperature was increased up to 80 °C and the solution was stirred again for 18 h.

The influence of the starting reaction temperature. In this series of experiments the SGP with participating the invariable reaction components above (**BAL^V**, **L12**, TEA) was carried out in DMA at a monomer concentration 1.2 mole/L (2.8 mL of TEA + 5.5 mL DMA = 8.3 mL) using predetermined starting temperatures such as 0, 20, 40 and 80 °C and the sequence “a”. The reaction mixture was stirred at the selected starting temperature until it became homogeneous and colourless (1-2 h) and then the reaction was continued at 80 °C for 18 h.

The influence of an organic solvent. Three polar aprotic solvents such as DMF, DMA and NMP were examined in the SGP with participating the invariable reaction components above (**BAL^V**, **L12**, TEA) at the following reaction conditions: starting temperature 0 °C at the sequence “a”, solution concentration 1.2 mole/L (2.8 mL of TEA + 5.5 mL of an organic solvent = 8.3 mL). In case of each of the selected solvents the reaction mixture was stirred at 0 °C until

its homogenisation and discoloration (1-2 h), then the reaction temperature was increased up to 80 °C and the solution was stirred for 18 h.

Optimal reaction conditions. The parameters of the reaction between **BAL^V** and **L12** were varied to establish optimal conditions for the SGP of a new type of monomer pair **BAL** + salt monomer **TDAD**E. For other **BAL-TDAD**E monomer pairs the SGP was carried out under optimal conditions found: sequence “a”, solution concentration 1.2 mole/L, starting reaction temperature 0 °C, solvent DMA, the reaction temperature and duration 80 °C and 18 h, accordingly. The results are listed in Tab. 1. The parameters which were found as optimal for the monomer pair **BAL^V** / **L12** (solution concentration, starting reaction temperature, solvent, the reaction temperature and duration) were applied to the synthesis of all other **BAL**-based polymers. In some cases, when the gelation of the reaction solutions took place, LiCl was added to homogenize the solutions (see below).

(ii) Synthesis of the co-PEAs

The synthesis of the **co-PEAs** *via* SGP was carried out between the **BALs**, **NPS** and **TDAD**Es at predetermined mole ratios of **BAL/NPS** (0.05/0.05, 0.03/0.07, 0.01/0.09, in sum 0.01 mol.). For synthesizing the **co-PEAs** the following order of addition of components was applied: in a three-necked 50 mL round-bottom flask equipped with a stirrer, 0.01 moles of a **TDAD**E, x moles of **NPS** ($x = 0.005, 0.007, \text{ and } 0.009$ moles) and $2x$ moles of TEA were placed, 5.5 mL of DMA was added and stirred at r.t. until a complete homogenisation of the reaction mixture (1-2 h). Afterwards the reaction solution was chilled to 0 °C and $(0.01 - x)$ moles of a **BAL** and $2(0.01 - x)$ moles of TEA were added (in total 2.8 mL of TEA, TEA + DMA = 2.8 + 5.5 = 8.3 mL that corresponds to the solution concentration of 1.2 mole/L). After adding the **BAL**, as it was expected, the reaction mixture became red in colour. The reaction mixture was stirred at 0 °C until its homogenisation and discoloration (1-2 h), then the reaction temperature was increased up to 80 °C and the solution was stirred for 18 h. In case when the gelation of the reaction solution was observed, 5% w/v of LiCl (calculated per DMA's volume) was added and the reaction was finalized at 80 °C under the homogeneous condition. The **co-PEAs** obtained under the said conditions are listed in Tab. 2.

(iii) Synthesis of the PEA-co-PAs

The synthesis of **PEA-co-PAs** *via* SGP was carried out between the **BAL^F**, **TDAD L6** and **HDA** at predetermined mole ratios of **L6/HDA** (0.09/0.01, 0.05/0.05, 0.03/0.07, 0.01/0.09,

in sum 0.01 mol.). For synthesizing the **PEA-co-PAs** the following order of addition of components was applied: in a three-necked 50 mL round-bottom flask equipped with a stirrer, x moles ($x = 0.009, 0.005, 0.003$, and 0.001 moles) of **L6** and $(0.01 - x)$, moles of **HAD** (as free base) and 0.01 moles of the **BAL^F** were placed, a precalculated volume of DMA (see below) was added and chilled to 0 °C upon stirring and then $2x$ moles of TEA were added. The solution concentration was 1.2 mole/L, i.e. total volume of DMA and TEA was 8.3 mL as above. Accordingly, the volume of DMA was calculated as $(8.3 - 2x \cdot 139.48)$ mL, where 139.48 mL is the volume of 1.0 mole of TEA. The reaction mixture was stirred at 0 °C until its homogenisation and discoloration (1-2 h), and then the reaction temperature was increased up to 80 °C and the solution was stirred for 18 h. In case when the gelation of the reaction solution was observed 5% w/v of LiCl (calculated per DMA's volume) was added and the reaction was finalized at 80 °C under the homogeneous condition (additionally stirred for 1.0 h). The **PEA-co-PAs** obtained under the said conditions are listed in Tab. 3.

Separation of the polymers

In all cases the reaction solution was filtered and poured into water. The separated polymer was filtered off, thoroughly washed with water, then with ethyl acetate and dried in a vacuum at 60 °C. The yields of the polymers obtained under the optimal reaction conditions were within 81-97% depending on the class and structure (See Tabs. 1-3).

Table 1. Properties of **homo-PEAs** (GPC data in DMF and HFIP)

#	homo-PEA*	Yield, %	GPC in DMF			GPC in HFIP		
			Mw	Mn	<i>Đ</i>	Mw	Mn	<i>Đ</i>
1	BAL^F-F6	84	2,800	2,600	1.07	Incalculable	Incalculable	-
2	BAL^V-F6	81	10,100	6,500	1.54	5,400	1,800	3.00
3	BAL^F-L6	85	14,000	8,400	1.67	9,900	3,300	3.00
4	BAL^V-L6	85	7,700	5,100	1.49	4,200	1,500	2.80
5	BAL^F-L12	92	19,600	10,400	1.88	11,700	3,100	3.77
6	BAL^V-L12	92	12,200	7,400	1.64	n/a	n/a	n/a

* For labelling the **homo-PEAs** see Scheme 4.

Table 2. Properties of the **co-PEAs** (GPC data in DMF and HFIP)

#	co-PEA*	Yield, %	GPC in DMF			GPC in HFIP		
			Mw	Mn	<i>D</i>	Mw	Mn	<i>D</i>
1	[BAL ^F / 8] _{50/50} - F6	82	35,500	18,900	1.87	16,000	6,300	2.54
2	[BAL ^F / 8] _{30/70} - F6	85	97,500	53,600	1.82	n/a	n/a	n/a
3	[BAL ^F / 8] _{10/90} - F6	83	108,700	62,500	1.74	n/a	n/a	n/a
4	8-F6 (PEA)	86	78,600	41,400	1.89	25,000	10,400	2.40
5	[BAL ^F / 8] _{50/50} - L6	90	61,100	36,700	1.67	26,300	11,600	2.27
6	[BAL ^F / 8] _{30/70} - L6	93	62,300	36,600	1.70	n/a	n/a	n/a
7	[BAL ^F / 8] _{10/90} - L6	92	64,300 ^[J2] [RbD3] ^[J4]	36,000	1.78	28,000	14,200	1.97
8	8-L6 (PEA)	90	46,500	23,400	1.98	19,800	9,900	2.00
9	[BAL ^F / 8] _{50/50} - L12	90	57,900	31,300	1.85	27,400	9,600	2.85
10	[BAL ^F / 8] _{30/70} - L12	93	101,300	56,500	1.79	45,300	18,000	2.52
11	[BAL ^F / 8] _{10/90} - L12	92	103,500	60,000	1.72	48,100	18,000	2.67
12	[BAL ^V / 8] _{50/50} - L12	90	35,500	20,400	1.74	12,900	5,300	2.43
13	[BAL ^V / 8] _{30/70} - L12	93	45,800	24,900	1.84	n/a	n/a	n/a
14	[BAL ^V / 8] _{10/90} - L12	92	63,900	32,600	1.95	24,200	7,600	3.18
15	8-L12 (PEA)	93	76,200	42,700	1.78	n/a	n/a	n/a

* For labelling the **co-PEAs** see Scheme 6.

Table 3. Properties of the PEA-co-PAs (GPC data in DMF)

#	PEA-co-PA*	Yield, %	Content of BAL ^F residue, % (ΔH, J/g)	GPC in DMF		
				Mw	Mn	<i>D</i>
1	BAL ^F -L6**	85	59.6 (28.2)	14,000	8,400	1.67
2	BAL ^F -[L6 / HDA] _{90/10}	94	61.4 (28.5)	18,800	11,400	1.65
3	BAL ^F -[L6 / HDA] _{50/50}	96	70.0 (35.7)	45,500	27,200	1.67
4	BAL ^F -[L6 / HDA] _{30/70}	95	75.3 (49.7)	65,800	37,400	1.76
5	BAL ^F -[L6 / HDA] _{10/90}	94	81.7 (69.3)	89,000	49,200	1.81
6	BAL ^F -HDA	97	84.8 (73.6)	110,400	61,300	1.80

* For labelling the **PEA-co-PAs** see Scheme 7.

** Sample #3 from Tab.1.

Results and Discussion

Monomer Synthesis

Three bis-electrophilic monomers used in the present study for synthesizing the goal polymers were: two bis-azlactones – phenylalanine based 2,2'-p-phenylene-bis-(4-benzyl-oxazolin-5-on (**BAL^F**) and valine based 2,2'-p-phenylene-bis-(4-isopropyl-oxazolin-5-on (**BAL^V**) were synthesized according to Scheme 1, as described previously [20], and one activated diester – di-p-nitrophenyl sebacate (**NPS**) obtained according to Ref. [25]. The characteristics of bis-electrophilic monomers were in full accordance with the reported data [20,25].

Four bis-nucleophilic monomers used in the present study for synthesizing the goal polymers were: three **TDADEs** - **F6**, **L6** and **L12**, and one alkylene diamine (free base) **HDA**. The **TDADEs** were synthesized according to Scheme 3, as described previously [25,26] and their characteristics were in full accordance with the reported data [25,26]. The **HDA** was used as free base (as purchased). Labelling of the obtained polymers is given in Schemes 4, 6 and 7 below.

Polymer Synthesis

(i) Synthesis of the homo-PEAs

The SGP reaction between BALs and diamines proceeds with opening the azlactone cycle after interaction with amino groups that leads to the formation of two amide links. **TDADEs** in a salt form (di-tosic acid salt) does not interact with azlactone. To generate the free amino groups from the salt monomer to start the chain growth process, an acid acceptor (e.g. TEA) should be applied (Scheme 4).

The SGP with participating BALs and salt monomers such as **TDADEs**, depicted in Scheme 4, is a new reaction and we studied its regularities in details. This study comprised the influence of various factors on the molecular weights of the resulted polymers which were assessed by reduced viscosities. First of all we studied the influence of the sequence of the components addition using the monomer pair **BAL^V** / **L12**. We applied three sequences (reduced viscosities of the obtained PEAs are given in parentheses): “**a**” when to a mixture of powdery **TDADE** and **BAL^V** first was added an organic solvent and then TEA ($\eta_{\text{red.}} = 0.38 \text{ dL/g}$); “**b**”

when to a powdery **TDADE** first was added TEA (to pre-liberate free amino groups), stirred for 5 min and then added powdery **BAL^V** and DMA ($\eta_{\text{red.}} = 0.35 \text{ dL/g}$); “c” when to a powdery **BAL^V** first was added TEA, stirred for 5 min and then powdery **L12** and DMA were added ($\eta_{\text{red.}} = 0.27 \text{ dL/g}$). In all cases the reaction mixtures ($c = 1.2 \text{ mole/L}$) started at 0 °C and stirred at this temperature until they became homogeneous and colourless that means the **BAL** was completely consumed in the SGP. Afterwards, the reaction temperature was increased up to 80 °C and continued at this temperature for 18 h (see Experimental Part) to finalize the chain growth. According to the obtained data (reduced viscosities) the best was found the Sequence “a” which was subsequently applied to all experiments when synthesizing **homo-PEAs**.

We have also studied the influence of the starting reaction temperature which was varied from 0 to 80 °C. The reaction mixtures ($c = 1.2 \text{ mole/L}$) were stirred at a selected temperatures until it became homogeneous and colourless. Afterwards, the reaction temperature was increased up to 80 °C and continued at this temperature for 18 h. The used starting temperatures and reduced viscosities of the obtained **homo-PEAs** were as follows: 0 °C - 0.38 dL/g, 20 °C - 0.31 dL/g, 40 °C - 0.28 dL/g, 80 °C - 0.20 dL/g. Hence, 0 °C was found as the best starting temperature and, therefore, it was subsequently applied for the synthesis of all classes of the polymers.

Labelling the **homo-PEAs**: **BAL^V-AA_x**, **BAL^F-AA_x**, where **AA** = **F** or **L**, $x = 6$ or **12**

Scheme 4. The synthesis of the **BAL**-based **homo-PEAs**

As to the organic solvent very little difference was found between three polar aprotic solvents used, in terms of reduced viscosities of the resulting polymers: DMA ($\eta_{\text{red.}} = 0.38 \text{ dL/g}$),

DMF ($\eta_{\text{red.}} = 0.36 \text{ dL/g}$), and NMP ($\eta_{\text{red.}} = 0.38 \text{ dL/g}$). Subsequently DMA was used for the synthesis of all classes of the polymers.

From the reaction solutions the polymers were separated by precipitation in water (see Experimental part). The yields and molecular weight characteristics of the **homo-PEA** obtained on the basis of two **BALs** - **BAL^V** and **BAL^F** and three **TDADs** **F6**, **L6**, and **L12** according to Scheme 4, are listed in Tab.1. The yields of the **homo-PEAs** were rather good – within 81-92%. The **homo-PEAs** are relatively low-molecular-weight polymers not revealing film-forming properties. Films made of these polymers were brittle and grindable into fine powders. This property makes them promising as biodegradable matrices for preparing sustained releasing drug loaded powdery formulations, e.g. prolonged acting bactericidal powders which could be promising to treat infected deep wounds and cavities.

One of the reasons of the synthesis of relatively low-molecular-weight **homo-PEAs** could be a steric hindrance between lateral substituents of amino acids – R in **BALs** and R' in **TDADs**; the lowest molecular weight of **BAL^F-F6** – the strongest steric repulsion between the most bulky benzyl groups of **BAL^F** and **F6** - could speak for this assumption. However, we assume that one of the main reasons could be a side reaction - TEA-catalysed dimerization of bis-azlactones [18], depicted in Scheme 5. This reaction leads to breaking equimolar ratio of electrophilic/nucleophilic functional groups that, in turn, leads to the restriction of chain-growth (i.e. decreasing molecular weight) in SGP.

Scheme 5. One of the possible side reactions of azlactone cycle catalysed by tertiary amine.

To decrease the contribution of the side reactions in the SGP with the participation of **BALs** and **TDADs**, we decided to dilute the **BAL** or **TDAD** with other monomers. In other words, to replace a part of **BAL** with **NPS** or a part of **TDAD** (salt monomer) by **HAD** (free base). The reason of these replacements was as follows: (a) *p*-Nitrophenyl esters of carboxylic

acids are less prone to side reactions with tertiary amines in polar aprotic solvents [27] and led to high-molecular-weight PEAs after the SGP in DMA in the presence of TEA [25,26]; we assumed, that diminishing the **BAL**'s mole-portion in the SGP by substituting with corresponding mole-equivalent of **NPS** will decrease the contribution of possible side reactions, e.g. depicted in Fig. 5. (b) The contribution of possible side reactions can also be decreased by replacing a part of a salt monomer - **TDAD**E with a free base – **HDA** and, hence, by diminishing the quantity of an acid acceptor (TEA). Accordingly, we have synthesized **co-PEAs** and **PEA-co-PAs** which are discussed below.

(ii) Synthesis of the co-PEAs

The **co-PEAs** were synthesized as depicted in Scheme 6. According to this approach, at the initial stage of SGP a predetermined part - 0.50, 0.70, or 0.90 mole/equivalent of the **NPS** and, accordingly, 1.00, 1.40, or 1.80 mole/equivalents of TEA were added to the 1.00 mole/equivalent of the **TDAD**E monomers - **F6**, **L6**, or **L12**. This means that actually corresponding quantity - 0.50, 0.70, or 0.90 mole/equivalents of the initial 1.00 mole/equivalent of the **TDAD**E were interacted with the **NPS**. The initial stage was carried out at r.t upon stirring until the homogenisation of the reaction mixture (1-2 h). Afterwards, the obtained solution was chilled to 0 °C and 0.50, 0.30, or 0.10 mole/equivalents of the second bis-electrophilic monomer - either **BAL**^V or **BAL**^F, and additional 1.00, 0.60, or 0.20 mole/equivalents of TEA (in total 2.0 mole/equivalents of TEA per one mole/equivalent of the **TDAD**E) were added. The reaction mixture was stirred at 0 °C until homogenization, and discoloration of the reaction mixture (1-2 h) that means **BAL** was completely consumed in the SGP reaction. Then the reaction temperature was increased up to 80 °C and the solution was stirred for 18 h to finalize the chain growth.

From the reaction solutions the polymers were separated by precipitation in water (see Experimental part). The yields and molecular weight characteristics of the obtained **co-PEA** are listed in Tab.2. The **co-PEAs** were obtained in good yields ranged from 82 to 93%. The substitution of 50 mol.% of the **BAL** by **NPS** drastically increased the molecular weights (MWs) of the polymers (cf. the following samples: #1 in Tab.1 and #1 in Tab. 2, #3 in Tab. 1 and #5 in Tab.2, #5 in Tab.1 and #9 in Tab.2, and #6 in Tab.1 and #12 in Tab.2). As it was expected, the increase of mole portion of **NPS** up to 70 and 90 mol.% led to further increase of the MWs of the **co-PEAs**. Most of the **co-PEAs** listed in Tab.2 showed excellent film-forming properties.

$R = \text{CH}(\text{CH}_3)_2$ (**V**), $\text{CH}_2\text{C}_6\text{H}_5$ (**F**); $R' = \text{CH}_2\text{CH}(\text{CH}_3)_2$ (**L**), $\text{CH}_2\text{C}_6\text{H}_5$ (**F**); $x = 6$ or **12**.

Labelling the polymers:

$[\text{BAL}^{\text{F}}/8]_{k/l}\text{-AAx}$, $[\text{BAL}^{\text{F}}/8]_{k/l}\text{-AAx}$, $[\text{BAL}^{\text{V}}/8]_{k/l}\text{-AAx}$, where **AA** = **F** or **L**, $x = 6, 12$. **8** means **NPS**.
 $k + l = 1$; $k/l = 0.5/0.5, 0.3/0.7, 0.1/0.9$ for **co-PEAs**, and $0.0/1.0$ for **PEAs 8-AAx**.

Scheme 6. The synthesis of the **BAL**-based **co-PEAs**

It has to be noted here that some **co-PEAs**, especially obtained at a mole ratios **BAL^F/NPS** = 30/70 and 10/90 (when the contribution of chain termination side reactions during the **co-PEAs** synthesis is minimal), showed higher values of the observed MWs compared to **PEAs** obtained on the basis of less tend to side reactions **NPS** only (*cf.* the following samples listed in Tab.2: ## 2 and 3 with #4, ## 5, 6 and #7 with #8, ## 10 and 11 with #15). This could be ascribed to higher chain rigidity caused by the bulky and rigid residues of **BAL^F** (see Fig. 4 below) in the **BAL^F**-based **co-PEAs** (which absents in the **PEAs 8-AAx**) that presumably increases the excluded volume of the **BAL^F**-based polymers (*cf.* the GPC data with DSC data below). For this assumption could speak lack of such kind of correlation in **BAL^V**-based **co-PEAs** containing less bulky residues of **BAL^V** (samples ## 12-15 in Tab. 2).

(iii) Synthesis of the **PEA-co-PAs**

The **PEA-co-PAs** were synthesized as depicted in Scheme 7. According to this approach, a predetermined quantity – 0.90, 0.70, 0.50, or 0.10 mole/equivalent of the salt monomer **TDAD** (**L6**) and 0.10, 0.30, 0.50, or 0.90 mole/equivalent of free-base monomer **HDA** (in sum **L6+HAD**=1.00 mole/ equivalent) was interacted with 1.00 mole/equivalent of the **BAL^F**. For this, to a mixture of the dry monomers a precalculated volume of DMA was added, chilled to 0 °C upon stirring and a precalculated volume of 0.18, 0.14, 0.10, or 0.20 mole/equivalent of TEA were added (the volumes of DMA and TEA were calculated so that to keep the solution concentration 1.2 mole.L; the TEA was used in quantities 2.0 mole/equivalent per 1.00 mole/equivalent of **TDAD**). The subsequent reaction conditions were as above. The stirring at 0 °C was continued until the homogenisation and discoloration of the reaction mixture (1-2 h) that means **BAL** monomer was completely consumed. Afterwards, the reaction temperature was increased up to 80 °C and the solution was stirred for 18 h to finalize the chain growth. If the gelation of the reaction solution was observed, 5% w/v of LiCl (calculated per volume of DMA) was added and the reaction was finalized by an additional stirring (1.0 h) under the homogeneous conditions.

Labelling the polymers: **BAL^F**– [L-6 / HDA]_{k/l}; **k / l = 0.9/0.1, 0.5/0.5, 0.3/0.7 and 0.1/0.9** for co-PEAs, and **0.0/1.0** for homo-PEAs 8-AA-x. **k + l = 1**;

Scheme 7. The synthesis of the copolymers - **PEA-co-PAs**

From the reaction solutions the polymers were separated by precipitation in water (see Experimental part). The yields and molecular weight characteristics of the obtained **PEA-co-PAs** are listed in Tab. 3. The **PEA-co-PAs** were obtained in good yields ranged from 85 to 96%. The substitution of 50 mol.% of the **L6** by **HDA** significantly increased molecular weights of the polymer (*cf.* the samples #3 in Tab.1 and #2 in Tab. 3). As it was expected, the increase of mole portion of **HDA** up to 70 and 90 mol.% led to further increase of the MWs of the **PEA-co-PAs**. All the **PEA-co-PAs**, containing ≥ 50 mol.% of **HDA**, showed excellent film-forming properties.

Solubility of the polymers

The solubility of the obtained polymers (10.0 mg) in organic solvents (1.0 mL) was evaluated (Tab. 4). All of the obtained **BAL**-based polymers were well-soluble in acidic solvents such as trifluoroacetic acid (TFA) and 1,1,2,2-tetrachloroethane:phenol (3:1 w/w) mixture. Some of them were soluble in chloroform and trifluoroethanol (TFE), and very few - in ethanol

Table 4. Solubility of the polymers in organic solvents

#	Polymer	Solubility of 10 mg polymer in 10 mL solvent at room temperature (25°C)						
	Homo-PEAs	CHCl ₃ or DCM	TFE	DMF	EtAc	EtOH	TFA	TCE/PhOH*
1	BAL^F-F6	-	+t	+	-	-	+	+
2	BAL^V-F6	Swells	+t	+	-	-	+	+
3	BAL^F-L6	-	+	+	-	-	+	+
4	BAL^V-L6	Swells	+t	+	-	+t	+	+
5	BAL^F-L12	-	-	+	-	-	+	+
6	BAL^V-L12	Swells	+t	+	-	+t	+	+
	Co-PEAs							
1	[BAL^F / 8]_{50/50} - F6	-	Swells	+	-	-	+	+
2	[BAL^F / 8]_{30/70} - F6	-	Swells	+	-	-	+	+
3	[BAL^F / 8]_{10/90} - F6	-	-	+	-	-	+	+
4	8-F6 (PEA)	+	+	+	-	-	+	+
5	[BAL^F / 8]_{50/50} - L6	+	-	+	-	-	+	+
6	[BAL^F / 8]_{30/70} - L6	+	-	+	-	+t	+	+
7	[BAL^F / 8]_{10/90} - L6	+	+t	+	-	+t	+	+
8	8-L6 (PEA)	+	+	+	-	+	+	+
9	[BAL^F / 8]_{50/50} - L12	+	±	+	-	-	+	+
10	[BAL^F / 8]_{30/70} - L12	+	±	+	-	-	+	+
11	[BAL^F / 8]_{10/90} - L12	+	±	+	-	-	+	+
12	[BAL^V / 8]_{50/50} - L12	+	+t	+	-	-	+	+
13	[BAL^V / 8]_{30/70} - L12	+	+t	+	-	-	+	+

14	[BAL ^V / 8] _{10/90} -L12	+	+t	+	-	-	+	+
15	8-L12 (PEA)	+	+	+	-	+	+	+
	PEA-co-PAs							
1	BAL ^F - [L6 / HDA] _{90/10}	-	+t	+	-	-	+	+
2	BAL ^F - [L6 / HDA] _{50/50}	-	+t	+	-	-	+	+
3	BAL ^F - [L6 / HDA] _{30/70}	-	+t	+	-	-	+	+
4	BAL ^F - [L6 / HDA] _{10/90}	-	+t	+	-	-	+	+
5	BAL ^F - HDA	-	Swells	+	-	-	+	+

* 3:1 (w:w). «+» soluble, «-» insoluble, «+t» soluble upon heating.

(leucine based ones) in which completely aliphatic analogues such as **PEAs 8-L6** and **8-L12** showed good solubility. Decreasing the solubility of the **BAL**-based polymers could be ascribed to rigid terephthalic acid fragments in the polymeric backbones. It is very important that all the polymers were soluble in DMF that allowed to determined their MWs using GPC. All the polymers were insoluble in ethyl acetate (EtAC) which was used for washing the polymers and removing organic impurities from them.

The polymers structure and composition

The obtained polymers contain dipeptide fragments (see Schemes 4,6 and 7) – **AA₁**-**AA₂**, where **AA₁** (F or V) comes from the **BAL** and **AA₂** (F or L) comes from the **TDAD**. These dipeptide fragments are formed as a result of the chain propagation reactions. The structure of the obtained polymers were confirmed by a good coincidence between calculated and found elemental analysis (not given in this paper) and FTIR data: in the spectra of the obtained **BAL**-based polymers carbonyl absorption bands at 1740-1742 cm⁻¹ (-CO- ester), 1640-1650 cm⁻¹ (-CO- amide), 1190-1195 cm⁻¹ (-O- ether), 3300 cm⁻¹ (-NH- amide) were observed. At the same time in the spectra no absorption band at 1810-1820 cm⁻¹ responsible for azlactones CO was found that meant a complete consumption of the azlactone cycles during the polymers synthesis (discoloration of the reaction solutions (see experimental part) also witnessed for a complete consumption of the **BAL**-monomer).

The composition of the **BAL**-based polymers and their fit with the co-monomers feed ratio was studied for representative samples of **BAL/L12** based **co-PEAs** (samples 12-14 in Tab. 2) using ¹H NMR data. The calculation was carried out using integral intensities of the NH protons of the amide bonds of both **BAL-L12** and **8-L12** blocks of the copolymers, CH protons of the

aromatic ring (terephthalic acid's residues), and CH₃ protons of isopropyl group (valine residues). The obtained data listed in Tab. 5 showed satisfactory coincidence between the comonomers feed ratio and calculated compositions of the **co-PEAs** studied.

Table 5. The composition of the **co-PEAs** according to the feed ratios of the comonomers **BAL/NPS** and calculated from the ¹H NMR data

#	co-PEA	The composition of the BAL-L12 and 8-L12 blocks	
		The feed ratio of BAL/NPS	Calculated from ¹ H NMR data
1	[BAL ^V / 8] _{50/50} - L12	50:50	48:52
2	[BAL ^V / 8] _{30/70} - L12	30:70	29:71
3	[BAL ^V / 8] _{10/90} - L12	10:90	12:88

Molecular weights of the polymers by GPC

The molecular weight characteristics of the obtained polymers – M_w, M_n and M_w/M_n (Dispersity, *D*) were determined using GPC in two organic solvents – DMF containing LiBr (0.1 M solution) and HFIP containing CF₃COONa (0.05 M solution). The results of these measurements, listed in Tabs. 1-3 show that the MWs determined in DMF are two-three times higher than MWs determined in HFIP. The dispersity *D* measured in DMF ≤ 2.0 (it is within 1.5-2.0) that is typical for **AABB** polymers [1-5] and in general for polymers obtained *via* SGP. The dispersity *D* measured in HFIP in the most cases ≥ 2.0 and in some cases even ≥ 3.0 that are high values for polymers obtained *via* SGP.

To explain the contradictory data obtained after the GPC measurement in two different solvents, we assume that LiBr/DMF is a good solvent and CF₃COONa/HFIP is a poor solvent for the obtained macromolecular systems. LiBr/DMF as a good solvent provides high excluded volumes of macromolecules whereas CF₃COONa/HFIP causes the shrinkage of the macromolecules to compact globule with lower excluded volumes. It has to be noted that MWs, in spite of the difference in the values, are changed parallelly - the higher is MW in DMF the higher is MW in HFIP, and vice-versa. High values of the dispersity *D* in HFIP could be connected with the formation of compact globules and this compacting does not happen uniformly with constant factor – we assume that for smaller molecules the shrinkage is more

pronounced. In other words, in GPC measurements decreasing M_n values is more substantial than decreasing M_w values that leads to increasing the fraction M_w/M_n , i.e. $\bar{D}$.

Hydrophobicity assessment of the polymers

The hydrophobicity of the **BAL**-based polymers was assessed by weight gain of the polymeric films immersed in water. Four **co-PEAs** on the basis of **L12**, were selected for this study: **[BAL^F/8]_{30/70}-L12**, **[BAL^F/8]_{10/90}-L12**, **[BAL^V/8]_{30/70}-L12**, and **[BAL^V/8]_{10/90}-L12**; the **PEA 8L12** was also used for a comparative study. The obtained results given in Fig.1 reveals that among the polymers studied the **PEA 8L12** showed the lowest and the **co-PEA [BAL^F/8]_{30/70}-L12** the highest hydrophilicity. The **co-PEAs** made of the **BAL^F** are more hydrophobic than the **co-PEAs** made of the **BAL^V** – e.g. the **co-PEA [BAL^F/8]_{10/90}-L12** composed of 10 mol.% of **BAL^F** shows almost the same hydrophobicity as the **co-PEA [BAL^V/8]_{30/70}** composed of 30 mol.% of **BAL^V** (cf. curves **c** and **d**). This could be ascribed to a higher hydrophobicity of phenylalanine compared to valine: the Hansch hydrophobicity constant of isopropyl group of valine is 1.30, whereas for benzyl group of phenylalanine it is twice as higher - 2.63 [28].

Fig. 1. Water absorption by the polymeric films (37 °C): The films made of the **PEA 8-L12** (a) and **co-PEAs**: **[BAL^V/8]_{10/90}-L12** (b), **[BAL^V/8]_{30/70}-L12** (c), **[BAL^F/8]_{10/90}-L12** (d), **[BAL^F/8]_{30/70}-L12** (e).

Hence, the hydrophobicity of the polymers can be tuned by varying the structure and content of the **BALs** in the polymeric backbones.

Thermal properties

Thermal properties of the polymers were studied within the temperature range of 20 – 200 °C. The thermograms showed wide endotherms in the range 55-120 °C with maximums within 86-96°C (Figs. 2 and 3). The endotherms were observed in case of all the **BAL^F**-based polymers: homopolymers such as **BAL^F-L6** and **BAL^F-HDA** (Figs. 2, curves **I** and **II**, accordingly), and **BAL^F-F6** (Fig. 3, curve **I**), as well as in case of corresponding copolymers - **PEA-co-PAs** (Figs. 2, curves **III-VI**) and **co-PEAs** (Fig. 3, curves **II** and **III**). This wide endotherm was not observed in the thermogram of **8-F6** (Fig. 3, curve **IV**) which represents a fatty **PEA** free of rigid aromatic residues in the backbones – a sharp endothermal peak, which corresponds to melting crystalline phase, was observed instead at 106 °C (reported m.p. of the **PEA 8-F6** 108 °C [25]).

Fig.2. The thermograms of the **BAL^F** based homopolymers **BAL^F-L6** (**I**) and **BAL^F-HDA** (**II**), and corresponding copolymers **PEA-co-PAs** (**III-VI**); **a** – the first scan, **b** – the second scan.

Both the broadened form and low temperature range (55-120 °C) of the endotherms allow to assign them rather to amorphous or [J5]small crystalline domains (involving probably lateral groups) than to crystalline phase [J6]. This assumption is in conformity with our previous finding that all the **BAL**-based polymers (even relatively more symmetric homo-polyamides such as e.g. **BAL^F-HDA**) according to x-ray analysis are amorphous [20]; this is because of racemic nature of **BALs** that leads to statistical distribution of L and D amino acids' residues which come after the opening the azlactone cycles. Taking into account that these types of endotherms were not observed in case of the **BAL^V**-based polymers (not given in this paper), we suppose that responsible for these domains are highly hydrophobic fragments - residues of **BAL^F** in the polymeric backbones (Fig. 4) which are formed as a result of opening the azlactone rings after the reaction of **BAL^F** with primary amino groups of **TDADEs** or **HAD** monomers. The thermograms of the **co-PEAs** the most likely represent an overlapping the melting of the hydrophobic domains formed by the **BAL^F** residues and crystalline phase formed by **8-L6** blocks (see Fig. 3, *cf.* thermogram **II** and, especially, thermogram **III** with thermogram **IV**).

Fig.3. The thermograms of the **BAL^F** based **homo-PEABAL^F-F6** (**I**) and Corresponding **co-PEAs** **[BAL^F/8-F6]_{30/70}** (**II**) and **[BAL^F/8-F6]_{10/90}** (**III**), and aliphatic **PEA 8-F6** (**IV**); **a** – the first scan, **b** – the second scan.

This assumption regarding the melting of the hydrophobic domains rather than the crystalline phase is confirmed by the representation of the melting enthalpy (ΔH in J/g) of the hydrophobic domains in **BAL^F** based **PEA-co-PAs** and homopolymers **BAL^F-L6** and **BAL^F-HDA** vs. **BAL^F** residues content in the polymeric backbones (Fig. 5) – the higher is the **BAL^F** content the higher is the melting enthalpy of the polymer.

Fig.5. Melting enthalpy (ΔH in J/g) of the hydrophobic domains in **BAL^F** based **PEA-co-PAs** (2-5) and homopolymers **BAL^F-L6** (1) and **BAL^F-HDA** (6) vs. **BAL^F** residues (see in Fig. 4) content in the polymeric backbones. The figures on the curve correspond to the numbers of the polymer samples in Tab. 3.

It has to be noted here that after melting and cooling (see the second scan in Figs. 2 and 3) the hydrophobic domains were not rebuilt. This behaviour is similar to semi-crystalline **AABB PEAs** [25] which also were not recrystallized after melting-cooling cycles – the crystalline phase was rebuilt only after dissolving of the amorphous melt in an organic solvent (e.g. chloroform) and casting as a film and drying.

Biodegradation

The *in vitro* biodegradation of films of the selected samples of the obtained polymers (**co-PEAs**) was studied using both APT (Figs. 6-8) and weight-loss (Fig. 9) methods under the conditions close to physiological (pH 7.4, 37 °C). Two hydrolases such as α -chymotrypsin and lipase were used in these experiments. The potentiometric titration study showed that after incorporating rigid and highly hydrophobic terephthalic acid residues into the polymeric backbones the polymers retained biodegradability, though these polymers showed somewhat lower biodegradation rate as compared with the fully aliphatic analogue **8-L12**. The quantity of terephthalic acid residues in both **BAL^V** (Fig. 6) and **BAL^F** (Fig. 7) polymers influenced the biodegradation rate - the higher was its content in the backbones the lower was the rate (*cf.* **a** and **b** in Figs. 6 and 7). The lower biodegradation rate of the **co-PEAs** on the basis of **BAL^F** (*cf.* data given in Figs. 6 and 7) could be in part ascribed to the formation of the hydrophobic domains above which impede the access of the enzymes to the ester bonds (analogously to crystalline phase in polymers). The study of the **homo-PEAs** made of **BAL^V** powders showed that the α -chymotrypsin catalyzed biodegradation rate of the polymer on the basis of more hydrophobic **F6** is lower than on the basis of less hydrophobic **L6** (*Cf.* **a** and **b** in Fig. 8,A). It has also to be noted that the enzyme (α -chymotrypsin) catalysed hydrolysis was much faster compared to nonspecific (chemical) hydrolysis (*Cf.* **b** and **c** in Fig. 8).

Fig. 6. *In vitro* biodegradation of **co-PEAs** films: **[BAL^V/8]_{30/70}-L12** (a), **[BAL^V/8]_{10/90}-L12** (b) and **PEA 8-L12** (c) catalysed by: **A** - α -chymotrypsin and **B** - lipase. The APT method.

Fig. 8. *In vitro* degradation **homo-PEAs** powders: **A** –hydrolysis of **BAL^V-F6** (a) and **BAL^V-L6** (b) catalysed by α -chymotrypsin; nonspecific (chemical) hydrolysis of **BAL^V-L6** in 0.1 N NaCl (c). The APT method.

The results of the APT express method was confirmed by long term (140 h) WL method. In Fig. 9 are given the results of *in vitro* WL kinetics study for two selected samples of the **co-PEA** **[BAL^V/8]_{30/70}-L12** (the curves **a**) and **[BAL^V/8]_{10/90}-L12** (the curves **b**) using α -chymotrypsin (**A**) and lipase (**B**). For a comparison the degradation curves of the **PEA 8-L6** are given as well (the curves **c**). The WL study confirmed that **BAL**-based polymers biodegraded slowly as compared with fully aliphatic **8-L6**, and the higher was the terephthalic acids content in the polymeric backbones the lower was the biodegradation rate.

Thus we can conclude that the chain rigidity and strong intermolecular hydrophobic interactions though decrease the biodegradation ability of the **BAL**-based ester polymers, however, they still retain biodegradability that makes them promising for practical applications. The amorphous structure of the polymers could be a guarantee of their complete degradation in the organism.

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