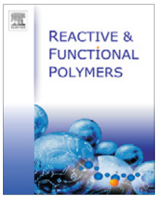
Reactive & Functional Polymers 70 (2010) 630–638

Contents lists available at ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react



REACTIVE & FUNCTIONAL POLYMERS

Synthesis, characterization and in vitro degradation of poly(ester-anhydride)s based on succinic acid and 1,6-bis-*p*-carboxyphenoxyhexane

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ARTICLE INFO

Article history:
Received 27 October 2009
Received in revised form 30 April 2010
Accepted 2 May 2010
Available online 10 May 2010

Keywords:
Poly(ester-anhydride)
Functional polymer
Hydrolytic degradation

ABSTRACT

This paper describes synthesis and characteristics of functional poly(ester-anhydride)s bearing allyl pendant groups. The polymers were obtained by polycondensation of 1,6-bis-*p*-carboxyphenoxyhexane (CPH) and oligo(3-allyloxy-1,2-propylene succinate) terminated with carboxyl groups (OSAGE). The carboxyl groups in OSAGE and in CPH were converted to mixed anhydride groups by acetylation with acetic anhydride. After that, prepolymers thus obtained were condensed in vacuum to yield poly(ester-anhydride)s. The structure of copolymers was confirmed by NMR spectroscopy. Influence of molecular weight of OSAGE as well as of the CPH and OSAGE content on selected properties of poly(ester-anhydride)s was examined. Poly(ester-anhydride)s were subjected to hydrolytic degradation at 37 °C, in aqueous phosphate buffer solution of pH 7.41 (PBS). The course of degradation was monitored by determination of weight loss of samples and ^{1}H NMR. Fracture surfaces of samples during degradation were examined by scanning electron microscopy.

1. Introduction

Polyanhydrides, as a class of surface-degradable polymers, have been investigated as potential vehicles for drug delivery [1,2] and other biomedical application [3–5]. They have been extensively researched for use in the controlled release systems of chemotherapeutics [6–10], antibiotics [11–14], anaesthesia [15,16], gene or hormone delivery [17–19].

The degradation rate of polyanhydrides can be controlled by use of diacids as comonomers, having different degradation rates in resulted copolymeric product [15,20–22]. Other developments in polyanhydride materials include the use of fatty acids [10,14,23–25] amino-acids [26], or poly(ethylene glycol)s [27–30] to modify the properties of polymers obtained. Recently, also poly(ester-anhydride) copolymers have been synthesized that combine the individual properties (such as biodegradation rate and mode) of these two classes of polymers [5,14,31–35].

Previously we have described the synthesis of functional poly(ester-anhydride)s with allyl pendant groups, based on succinic acid. They were obtained by polycondensation of oligo(3-allyloxy-1,2-propylene succinate) terminated with carboxyl groups (OSAGE) [36]. Succinic acid is naturally present in living tissues, its polymers could be considered to be potentially biocompatible and thus useful in medical applications. Pendant functionalities in such polymers create interesting perspectives for coupling chemically drugs. They may be also utilized to form crosslinked matrix with enhanced mechanical and other physical properties. Poly(ester-anhydride)s obtained from OSAGE exhibited rather fast degradation and weak solubility in organic solvents [36]. Solubility of polymers is a important factor influencing their application. For preparation of drug delivery systems the most commonly used organic solvents are dichloromethane and chloroform. They are volatile, non-flammable and inexpensive solvents that can be completely removed from the product under vacuum [37]. Some difficulties concerning bad solubility of poly(ester-anhydride)s in chlorinated solvents were successfully solved by copolymerization of OSAGE with sebacic acid. The copolymers containing more than 20% of sebacic acid appeared to be well soluble in methylene chloride. It allowed formulation of poly(ester-anhydride)s into microspheres. However, they still underwent rather fast hydrolytic degradation [38].

In this work, OSAGEs were copolymerized with 1,6-bis-*p*-carboxyphenoxyhexane (CPH) to yield poly(ester-anhydride)s with different properties. Poly(CPH) degrades on a time scale of years [39,40] while poly(OSAGE) on a time scale a week [36,38]. Thus, copolymers of OSAGE and CPH seem to be interesting due to a content of two components with vastly different degradation rate. Depending on the poly(ester-anhydride) composition ratio, a difference of degradation behaviour of both components can be used to influence release profiles of therapeutic molecules.

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doi:10.1016/j.reactfunctpolym.2010.05.003

2. Experimental

2.1. Starting materials

Succinic acid 99% (Aldrich), allyl glycidyl ether 99% (Aldrich), *p*-carboxy benzoic acid >99% (Acros Organics), 1,6-dibromoheksane 98% (Acros Organics) acetic anhydride (POCh S.A.) and phosphate buffer pH = 7.41 were used as supplied. Solvents were purified according to known procedures.

2.2. Instrumental and analytical methods

^{1}H NMR and ^{13}C NMR spectra were recorded using Varian UNITY/INOVA spectrometer (300 MHz and 75 MHz respectively) in $CDCl_3$ or DMSO–d_6 with TMS as an internal standard.

IR spectra (KBr pellet) were obtained using Carl Zeiss Jena UR-20 spectrophotometer.

Acid value (AV) and hydroxyl value (HV) were determined using standard acid–base titration methods. The number-average molecular weights Mn (EGA) of the oligoesters were calculated from results of end group analysis (AV and HV values) [36].

Viscosity measurements were performed in chloroform or THF solution, at 23 °C using Ubbelohde viscometer.

Vapor pressure osmometry (VPO) analyses were performed in chloroform using Knauer vapor pressure osmometer.

ESI-MS experiments were carried out using Finnigan MAT TSQ700 triple stage quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Finnigan, San Jose, CA, USA). The samples dissolved in chloroform (1.0 mg/mL) were introduced into electrospray source at rate 3 μl/min. Mass spectra were acquired over the range of *m/z* equal to 50–2000 in positive and negative ion mode.

The molecular weight of poly(ester-anhydride)s was determined in chloroform by gel permeation chromatography (GPC) using a Spectra-Physics 8800 chromatograph equipped with refractive index detector (Shodex SE 61) and calibrated with polystyrene standards.

Thermal analyses were performed using 822^e DSC Mettler Toledo differential scanning analyzer. Samples were tested in temperature range from −70 °C to 250 °C at a heating rate of 10 °C/min.

SEM microphotographs were recorded on a TESLA BS 340 scanning electron microscope.

2.3. Preparation of OSAGE

OSAGE was synthesised as described earlier [36] by melt condensation of succinic acid (SAc) and allyl glycidyl ether (AGE) using twofold excess of SAc. The reaction mixture was heated at 130 °C for 6 or 10 h. In the next step, the temperature was risen up to 150 °C and the reaction was carried out at 150 °C as long as the acid value (AV) decreased to a constant value.

The crude product was dissolved in chloroform and filtered to remove unreacted SAc. The oligoester was precipitated in diethyl ether/petroleum ether (1:1 v/v) mixture, separated by sedimentation and dried under vacuum. The yield of purified products was 50–70%. The structure of oligoesters was confirmed by ^{1}H and ^{13}C NMR spectroscopy. Molecular weight of OSAGE was determined by VPO and calculate from their AV (HV = 0) and from ^{1}H NMR spectra.

The NMR signals of the oligoesters can be assigned as follows:

^{1}H NMR ($CDCl_3$, ppm) δ: >10.00 (broad, —C(O)OH), 5.78–5.96 (m, —CH═), 5.11–5.30 (m, ═CH_2 and >CHO(O)C—), 4.18–4.38 (m, $CH_2O(O)C$—), 4.01 (d, $OCH_2CH{=}CH_2$), 3.58 (d, —CH_2O—), 2.67 (s, —$CH_2C(O)O$—).

^{13}C NMR ($CDCl_3$, ppm) δ: 176.91 (—C(O)OH), 171.55, 171.38, 171,28 (—C(O)O—), 133.96 (—CH═), 117.09 (═CH_2), 70.32 (>CH—O—), 72.05, 67.78, 62.79 (—CH_2—O—), 28.37 (—$CH_2C(O)O$—).

2.4. Synthesis of CPH

The 1,6-bis-*p*-carboxyphenoxyhexane (CPH) was synthesized according to procedure similar to described in the literature [41,42].

29.0 g (0.2 mol) of *p*-hydroxybenzoic acid was added to the NaOH solution (prepared by dissolution of 20 g (0.5 mol) NaOH in 100 cm^3 distilled water), placed in three-necked flask equipped with reflux condenser, droplet and magnetic stirrer. The reaction mixture was brought to reflux temperature before 1,6-dibromohexane (24.6 g, 0.1 mol) was added (dropwise ca. 2 h), the reaction mixture was refluxed for 4 h and next cooled. The precipitated product was isolated by filtration, washed twice with methanol and then dissolved in water at 60 °C. The solution was acidified with H_2SO_4 to pH = 2. Precipitated CPH was isolated by filtration, washed twice with distilled water (200 ml) and twice with acetone and dried in vacuum drier. The yield of purified product (white solid) was ca. 70%. It was characterized by IR spectroscopy and DSC technique. Melting point was T_m = 297 °C as determined by DSC.

The IR bands can be assigned as follows:

IR (KBr, cm^{-1}) ν: 3950 (O—H in acids), 1720 (C═O), 1615 and 1420 (C═C in aromatic compound), 860–800 (C—H in *p*-substituted aromatic ring), 770 (C—H in —O—CH_2).

2.5. Synthesis of CPH prepolymer

CPH prepolymer was prepared according to the literature [39,40]. 15 g of CPH was refluxed in 150 cm^3 of acetic anhydride under nitrogen for 60 min. Excess of acetic anhydride and acetic acid formed as a by-product were removed under vacuum. The prepolymer obtained was dissolved in methylene chloride, precipitated in diethyl ether/petroleum ether (1:1 v/v), separated and dried under vacuum. The prepolymer was stored at −18 °C and characterized by ^{1}H and ^{13}C NMR spectroscopy and DSC technique. Melting temperature was T_m = 103 °C as determined by DSC.

^{1}H NMR ($CDCl_3$, ppm) δ: 8.05 (d, 2H, ArH), 6.97 (d, 2H, ArH), 4.05 (t, 4H, OCH_2—$(CH_2)_4$—, 2.35 (s, 6H, $CH_3C(O)O$—C(O)—), 1.78 (m, 4H, —CH_2—), 1.52 (m, 4H, —CH_2—).

^{13}C NMR ($CDCl_3$, ppm) δ: 164.20 (—$CH_2O(O)C$—Ar), 162.00, 133.07, 121.00, 114.74 6C in Ar), 68.40 (—CH_2—O—), 29.20, 26.00(—CH_2—), 22.65 (CH_3— C(O)O—C(O)—).

2.6. Preparation of mixed (OSAGE and CPH) prepolymer

OSAGE was refluxed in acetic anhydride (1:10 w/v), under nitrogen for 30 min. After that CPH prepolymer was added to reaction mixture. OSAGE and CPH prepolymer were mixed in defined ratios (Table 2). Excess of acetic anhydride and acetic acid formed as a by-product were removed under vacuum. Mixed prepolymers formed were immediately condensed to yield poly(ester-anhydride)s.

2.7. Polycondensation of mixed prepolymers

Mixed (OSAGE and CPH) prepolymers (total amount ca. 10 g) were stirred magnetically and heated at 150 °C for 2 h under high vacuum conditions (0.10–0.01 mm Hg) to yield poly(ester-anhydride)s. The latter were crushed, washed with petroleum ether, dried under vacuum and stored in a freezer. The polymers were characterized by means of ^{1}H and ^{13}C NMR spectroscopy and

DSC technique. The content of acetyl end groups, determined by ^{1}H NMR was used to estimate the molecular weight.

The NMR signals (Fig. 2) can be assigned as follows:

^{1}H NMR ($CDCl_3$, ppm) δ: 8.10 (d, ArH in CPH-CPH sequences, m), 8.01 (d, ArH in CPH-OSAGE sequences, m), 6.90 (d, ArH, l), 5.80–5.88 (m, $-CH{=}$, b), 5.13–5.28 (m, $=CH_2$ and $>CHO(O)C-$, a, e), 4.18 (m, $-CH_2O(O)C-$, f), 4.05 (t, $-O-CH_2-(CH_2)_4$, i), 3.94 (d, $-OCH_2-CH=CH_2$, c), 3.53 (d, $-CH_2OCH_2-CH=CH_2-$, d), 2.91 (m, $-CH_2C(O)O(O)C-Ar$, h), 2.75 (m, $-CH_2C(O)O(O)C-CH_2-$, h), 2.66 (m, $-CH_2C(O)O-$, g) 2.35 (s, $CH_3C(O)O-C(O)-Ar$), 2.22 (s, $CH_3C(O)O-C(O)-CH_2-$), 1.87 (m, $-CH_2-$, j), 1.55 (m, $-CH_2-$, k).

^{13}C NMR ($CDCl_3$, ppm) δ: 171.55, 171.28 ($-CH_2O(O)C-CH_2$), 168.29 ($-C(O)O(O)C-CH_2$), 164.20 ($-(O)O(O)C-Ar$), 162.00, 133.07, 121.00, 114.74 6C in Ar), 133.96 ($-CH{=}$), 117.09 ($=CH_2$), 72.05, 70.32, 67.78, 62.79, 68.40 ($-CH_2-O-$ and $>CH-O-$), 29.86, 29.20, 26.00, 28.37 ($-CH_2C(O)O-$, $-CH_2C(O)O(O)C$, $-CH_2-$).

2.8. Hydrolytic degradation

Hydrolytic degradation was performed in phosphate buffer solution of pH = 7.41 (PBS) at 37 °C. Disc shaped samples (12 mm diameter, 1 mm thickness and 0.3 g weight) were placed in vials containing 20 ml of phosphate buffer solution. The vials were incubated at 37 °C for various time (7 h to 130 days). After incubation, the samples were separated and washed with water, dried in vacuum at 50 °C and weighted to estimate weight loss. Non-degraded parts of samples (solid residues after degradation) were characterized by ^{1}H NMR and SEM technique. For the SEM studies, disc shaped samples were broken at room temperature and gold sputtered to observe the fracture surfaces.

3. Results and discussion

3.1. Synthesis of poly(ester-anhydride)s

A series of functional poly(ester-anhydride)s was obtained by polycondensation of 1,6-bis-*p*-carboxyphenoxyhexane (CPH) and oligo(3-allyloxy-1,2-propylene succinate (OSAGE) prepolymers (scheme 1).

Two kinds of OSAGE (OSAGE-s and OSAGE-l), having different molecular weight (MW), were used in syntheses of poly(ester-anhydride)s. The number-average molecular weights (Mn) of OSAGE oligomers were determined by VPO and calculated from AV and ^{1}H NMR (Table 1). The Mn values determined by VPO had good correlation with those calculated from AV. Mn(VPO) values were 557 g/m for OSAGE-s and 1170 g/mol for OSAGE-l. This corresponds to oligomers consisting of ca. 2 repeating units in OSAGE-s and ca. 5 repeating units in OSAGE-l. ESI-MS mass spectra of OSAGE-s indicated presence of short oligomer chains consisting of 2–5 repeating units, whereas in ESI-MS mass spectra of OSAGE-l, signal assigned to longer chains with degree of polycondensation DP = 6–10 could also be found. Analysis of ESI-MS spectra confirmed the presence of oligomer chains ended with carboxyl groups only, respective signals were observed at low-field side of ^{1}H NMR spectra, at $\delta > 10$ ppm. The presence of carboxyl terminal groups only in OSAGE obtained was confirmed also by end groups analysis. The oligoesters obtained did not contain detectable amount of hydroxyl groups (HV = 0), while AV values were 215 mg KOH/1 g for OSAGE-s (shorter chains) and 107 mg KOH/1 g for OSAGE-l (longer chains).

Table 1
Characteristics of oligoesters obtained in condensation of SAc and AGE.

	Reaction time first/s step (h)	AV (mg KOH/ 1 g)	SA/AGE in oligoester	Mn (g/mol)			
				AV	^{1}H NMR		VPO
					SAc/ AGE[a]	Carboxyl[b] end groups	
OSAGE-s	10/18	215	4/3	522	753	734	557
OSAGE-l	6/14	107	13/12	1049	2670	1650	1170

VPO – vapor pressure osmometry.

AV – acid value.

[a] Calculated from intensities I_{SAc} and I_{AGE}.

[b] Calculated from intensities I_{AGE} and I_{COOH}.

The carboxyl end groups of the OSAGE and CPH were converted to mixed anhydride groups by refluxing the oligoesters and CPH in acetic anhydride. First, the CPH prepolymer was obtained, isolated and characterized. The complete conversion of acid groups was confirmed by ^{1}H NMR. There was no signal of carboxyl end groups protons at low-field side of ^{1}H NMR spectrum ($\delta > 10$ ppm) while the signal of methyl protons of the acetyl end groups appeared at $\delta = 2.35$ ppm. The ratio of intensity of methyl protons of the acetyl end groups to methylene or aromatic protons of CPH calculated from ^{1}H NMR spectrum indicated that CPH did not undergo self condensation process (number average degree of polymerization (DP) of CPH prepolymer was c.a 1).

Evaporation of acetic anhydride from OSAGE prepolymer solution always resulted in formation of oligoanhydrides with DP = 4–10. It would result in receiving block-like copolymers, whereas the random ones are more preferable. Since the former often exhibited microphase separation, which influence the mechanism of erosion and drug release kinetics [43]. Next, OSAGE prepolymer was obtained and prior to isolation from acetic anhydride, CPH prepolymer was added and dissolved in the reaction mixture. After that, acetic anhydride was removed and mixed (CPH and OSAGE) prepolymer was isolated. Such procedure was applied in order to prevent homopolycondensation of OSAGE during its isolation.

In the next step, mixed prepolymers (ca. 10 g) were heated under vacuum to yield higher molecular weight poly(ester-anhydride)s. The polycondensation was allowed to proceed for 2 h.

3.2. Characteristics of poly(ester-anhydride)s

The polymers obtained were solid materials, amorphous and elastic when containing more OSAGE and crystalline when containing more CPH fragments.

^{1}H and ^{13}C NMR sectroscopy of the final products confirmed expected structure (Fig. 1).

In ^{1}H NMR spectra (Fig. 2) signals at $\delta = 2.66$, 2.75 and 2.91 ppm are visible, which could be assigned to methylene protons close to ester and anhydride groups (in OSAGE–OSAGE or OSAGE–CPH sequences) respectively.

The ^{13}C NMR spectra of poly(ester-anhydride)s showed four different signals assigned to carbonyl carbon atoms in anhydride ($\delta = 168.29$ and 164.20 ppm) and ester groups ($\delta = 171.47$ and 171.78 ppm) as well as two different signals of methylene carbon atoms next to ester ($\delta = 29.86$ ppm) and anhydride ($\delta = 28.68$ ppm) groups respectively.

In ^{1}H (Fig. 2) and ^{13}C NMR spectra, besides of the signals confirming the presence of ester and anhydride bonds, also signals attributed to methylene and metine protons or carbon atoms in allyl groups were observed. The presence of the signals at $\delta = 5.11$–5.30 and at $\delta = 5.78$–5.96 ppm in ^{1}H NMR and at $\delta = 117.09$ and at $\delta = 133.96$ ppm in ^{13}C NMR spectra confirms presence of allyl groups in poly(ester-anhydride)s.

Table 2
Molecular weight and intrinsic viscosity [η] of poly(ester-anhydride)s.

Symbol	Kind of OSAGE	Feed ratio (w/w%)		[η] (dl/g)	Mn (^{1}H NMR)	Molecular weight (GPC)	
		OSAGE	CPH			Mn	Mw
PEA 100	–	0	100	0.118[c]	2480[a]	2100	3900
PEA 80/20S	OSAGE-s	20	80	0.128[c]	7840[a]	3800	8500
PEA 60/40S		40	60	0.135[c]	11400[a]	4100	10,800
PEA 40/60S		60	40	0.089[d]	26150[b]	n.d	n.d
PEA 20/80S		80	20	0.092[d]	34220[b]	n.d	n.d
PEA 80/20L	OSAGE-l	20	80	0.121[c]	4780[a]	2800	5100
PEA 60/40L		40	60	n.d.	n.d.	n.d	n.d
PEA 40/60L		60	40	0.075[d]	20170[b]	n.d	n.d
PEA 20/80L		80	20	0.073[d]	13440[b]	n.d	n.d

n.d. – not determined.

[a] ^{1}H NMR (300 MHz, $CDCl_3$).

[b] ^{1}H NMR (300 MHz, DMSO-deuterated).

[c] Determined in chloroform solution.

[d] Determined in THF solution.

^{1}H NMR was used to calculate the molecular weight (MW) of poly(ester-anhydride)s. MW of poly(ester-anhydride)s were calculated according to Eq. (1) assuming the structure of the polymers shown in scheme (Fig. 1). The results are summarized in Table 2.

$$\begin{aligned} MW &= n_{CPH}M_{CPH} + n_{OL}M_{OL} + M_T \\ n_{CPH} &= 3I_{CPH}/2I_T n_{OL} = 3I_{SAc}/2I_T \end{aligned} \tag{1}$$

M_{CPH} – molar mass of repeating unit of CPH in poly(ester-anhydride) equal to 340 g/mol, M_{OL} – Mn(VPO) of OSAGE equal to 557 g/mol for OSAGE-s or 1170 g/mol for OSAGE-l, M_T – molar mass of terminal groups in poly(ester-anhydride) equal to 102 g/mol (molar mass of acetic anhydride), I_T – intensity of the two signals of terminal methyl groups (δ = 2.35 and 2.22 ppm), I_{CPH} – intensity of signal of two aromatic protons (δ = 6.90 ppm), I_{SAc} – intensity of signals of methylene protons (from succinic acid) next to anhydride bonds —C(O)—O—(O)C—CH_2— (δ = 2.75 and 2.91 ppm).

Molecular weight values of poly(ester-anhydride)s calculated from ^{1}H NMR and determined by GPC, as well as their intrinsic viscosity [η] values, are summarized in Table 2. The molecular weight determined by GPC showed relatively broad dispersity (Mw/Mn were in the range of 1.8–2.6) and were much lower than Mn calculated from ^{1}H NMR spectra. The kind of OSAGE as well as the content of CPH and OSAGE in copolymers influenced on their MW. Generally MW of poly(ester-anhydride)s were higher when OSAGE-s was used and decreased as CPH content in polymer increased.

The ^{1}H NMR spectra of poly(ester-anhydride)s were recorded in $CDCl_3$ or DMSO–d_6 and their intrinsic viscosity [η] were determined in $CHCl_3$ or THF solution. Different solubility characteristic of poly(ester-anhydride)s (Table 3) did not measurements to be done in one solvent.

Lower values of [η] determined in THF, for (ester-anhydride)s containing less than 60% of CPH, can be a result of partial degradation. Poly(ester-anhydride)s obtained were unstable in polar

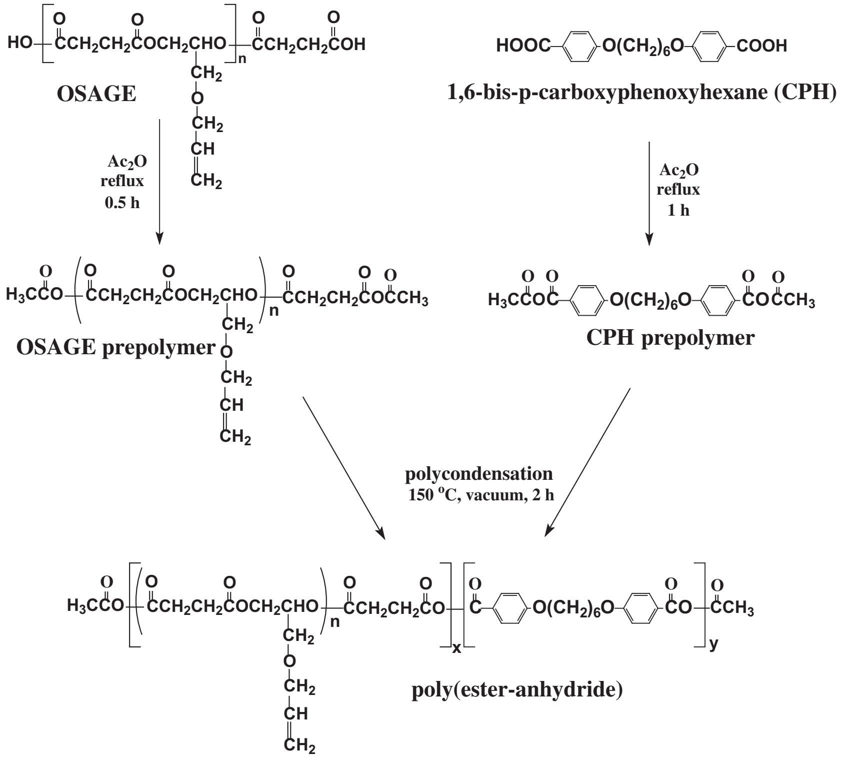

Fig. 1. Reaction scheme of synthesis of poly(ester-anhydride)s based on OSAGE and CPH.

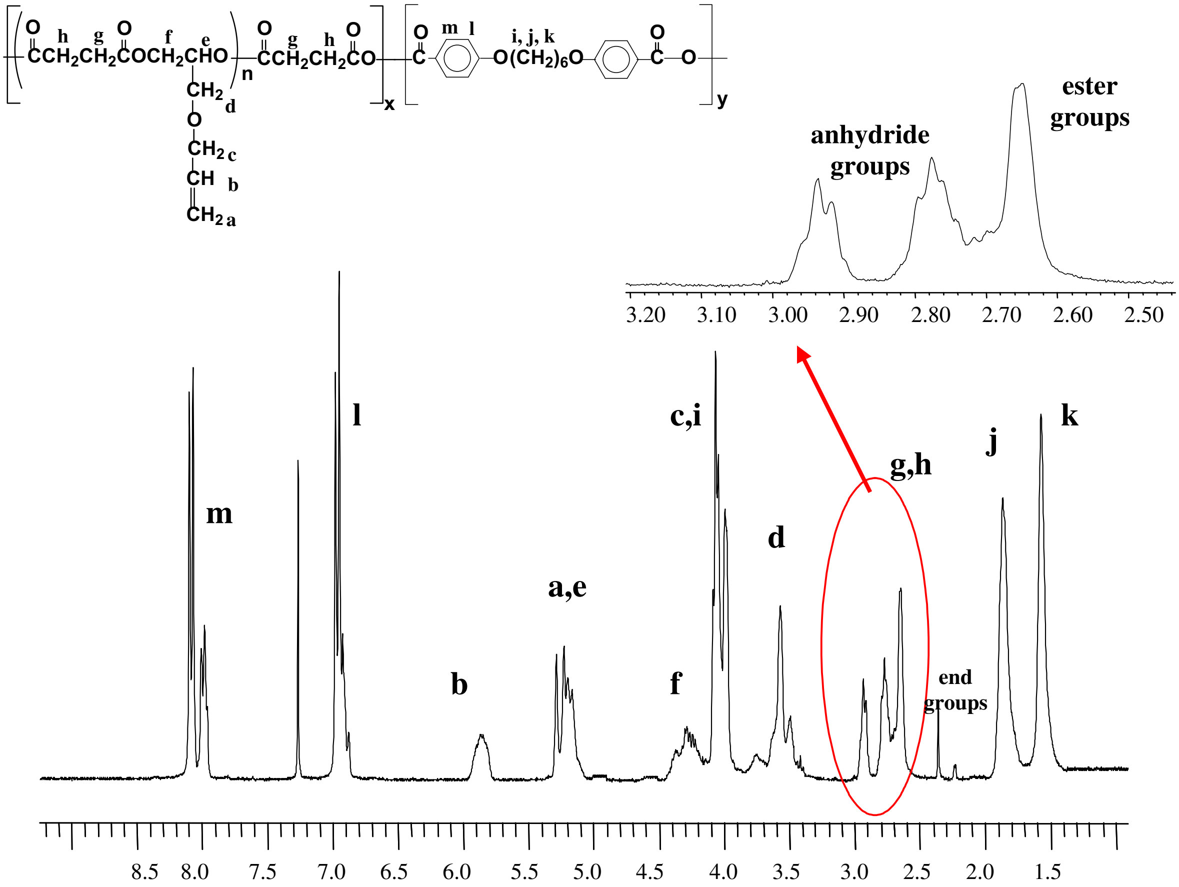

Fig. 2. ^{1}H NMR spectrum of poly(ester-anhydride) obtained by copolycondensation of 60% of CPH and 40% of OSAGE-s ($CDCl_3$).

aprotic solvents (DMSO, THF, DMF). The content of anhydride bonds in polymer were falling down after several hours of contact with such solvents. This phenomenon was more significant when CPH content in the copolymer was lower. The degradation was not observed in chloroform or methylene chloride, even for more than 2 weeks.

Hydrolytic unstability in THF or DMF was a reason of troubles with GPC analysis of the copolymers insoluble in chloroform.

Solubility characteristics of poly(ester-anhydride)s was dependent on CPH and OSAGE content as well as on the kind of OSAGE used in syntheses (Table 3). Poly(ester-anhydride)s obtained from OSAGE-s, containing more than 40% CPH exhibited solubility characteristics similar to that of poly(CPH). They were soluble only in chlorinated solvents. Increase of OSAGE-s content in copolymers resulted in worse solubility in chlorinated solvents but much better in polar aprotic solvents e.g. THF, DMF, DMSO as well as in methyl methacrylate.

Poly(ester-anhydride)s obtained with use of OSAGE-s were generally much better soluble than those containing OSAGE-l. Worse solubility of the latter could be eventually considered as a result of partial polymerization of double bonds present in pendant allyl groups during the synthesis. It would be consistent with results of DSC measurements (see Fig. 3 and Table 4), which revealed that thermal polymerization of double bonds in OSAGE-l based poly(ester-anhydride)s started always at lower temperature, than in the case of those synthesized from OSAGE-s. This explanation however, was not confirmed by measurements of molar mass of poly(ester- anhydride)s, which were always higher for polymers

Table 3
Solubility characteristics of poly(ester-anhydride)s.

	Solvent				
	$CHCl_3$	Acetone	DMSO	THF	MMA
PEA 100	+	–	–	–	–
PEA 80/20S	+	–	–	–	–
PEA 60/40S	+	–	–	+	–
PEA 40/60S	+/–	+/–	+	+	+
PEA 20/80S	+/–	+	+	+	+
PEA 80/20L	+	–	–	+/–	–
PEA 60/40L	+/–	–	–	+/–	–
PEA 40/60L	+/–	+	+/–	+	+/–
PEA 20/80L	+/–	+	+/–	+	+/–

+ Soluble, +/– partially soluble, – insoluble.

Table 4
Thermal properties of poly(ester-anhydride)s.

	T_{g1} (°C)	T_{g2} (°C)	T_m (°C)	ΔH_m (J/g)	T_c (°C)
PEA 100	–	54.1	142.6	−41.22	–
PEA 80/20S	–	54.9	135.2	−24.87	–
PEA 60/40S	−7.4	–	–	180.2	
PEA 40/60S	−18.1	48.4	–	–	177.8
PEA 20/80S	−24.4	47.0	–	–	171.4
PEA 80/20L	19.1	137.8	−21.9	–	
PEA 60/40L	−32.8	40.6	–	–	108.1
PEA 40/60L	−32.8	41.9	–	–	115.9
PEA 20/80L	−32.7	49.8	–	–	131.7

T_g – glass transition temperature, related to OSAGE (T_{g1}) or poly(CPH) (T_{g2}) segments.
T_m – melting temperature, ΔH_m – heat of fusion of melting process, T_c – curing temperature.

obtained from OSAGE-s, i.e. no increase of molar mass due to the polymerization of double bonds was observed for polymers synthesized from OSAGE-l. MW of the poly(ester-anhydride)s obtained from OSAGE-s were always higher than MW of poly(ester-anhydride)s contained the same amount of OSAGE but obtained from OSAGE-l. Another reason of differences in solubility observed may be some phase separation due to longer oligoester segments in poly(ester-anhydride)s obtained from OSAGE-l.

Thermal properties of the copolymers obtained, such as melting temperature (T_m), heat of fusion of melting process (ΔH_m), glass transition temperature (T_g), as well as curing temperature (T_c) and heat of curing process (ΔH_c) caused by polymerization of allyl pendant groups, were determined by DSC (Table 4).

Poly(CPH) was crystalline polymer with sharp melting peak at 142.6 °C and glass temperature $T_g = 54.1$ °C. The determined ΔH_m of poly(CPH) was equal to −41.22 J/g. The heat of fusion of poly(ester-anhydride)s obtained with OSAGE content equal to of 20% w/w, decreased by half which indicated lower crystallinity of copolymers. Melting temperatures of poly(ester-anhydride)s PEA 80/20S and PEA 80/20L were also lower than that of poly(CPH). Copolymers obtained with more than 20% of OSAGE were completely amorphous (no melting peaks was observed within the

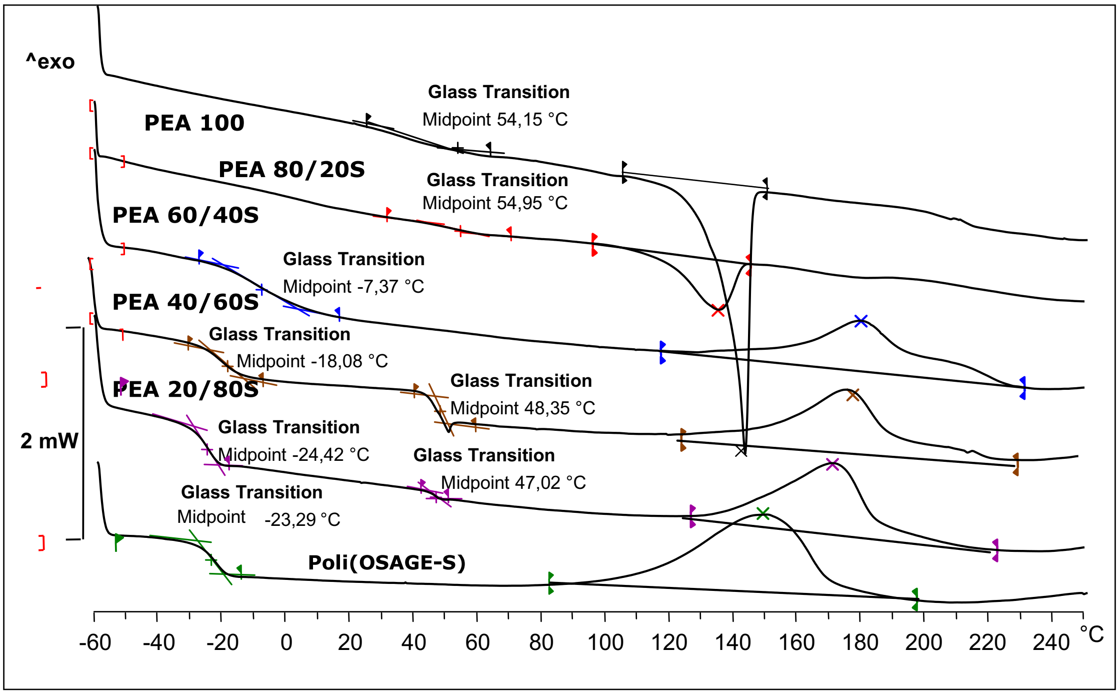

Fig. 3. DSC thermographs of poly(ester-anhydride)s obtained from CPH and OSAGE-s.

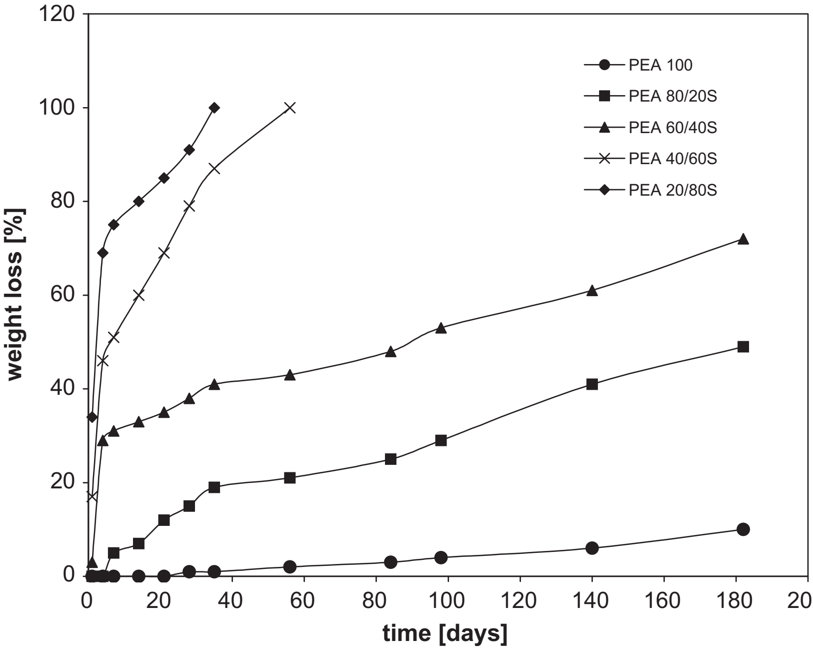

Fig. 4. The mass loss of samples during hydrolitic degradation of poly(ester-anhydride)s as a function of imersion time in buffer solution.

temperature ranging from −70 to 250 °C). In the DSC thermographs of copolymers usually two different glass temperatures were observed: one related to OSAGE segments (T_{g1}) and the second one related to poly(CPH) segments (T_{g2}). Along with increasing content of OSAGE, decrease of T_{g2} and increase of T_{g1} was observed. For the PEA 60/40S and PEA 80/20L copolymers however, only one T_g was found at −7.4 °C and 19.1 °C respectively (Table 4). Single T_g, especially for completely amorphous PEA 60/40, suggests phase homogeneity. It may have an influence on release kinetics from corresponding matrix system containing an effector. The rubbery state of that polymer (T_g below room temperature) is desirable for processing into tablets.

Exothermal transition in temperature above 100 °C, resulting from the polymerization of allyl groups, was observed on thermographs of poly(ester-anhydride)s obtained with more than 20% of OSAGE. Such transition was not observed in the range of tempera-

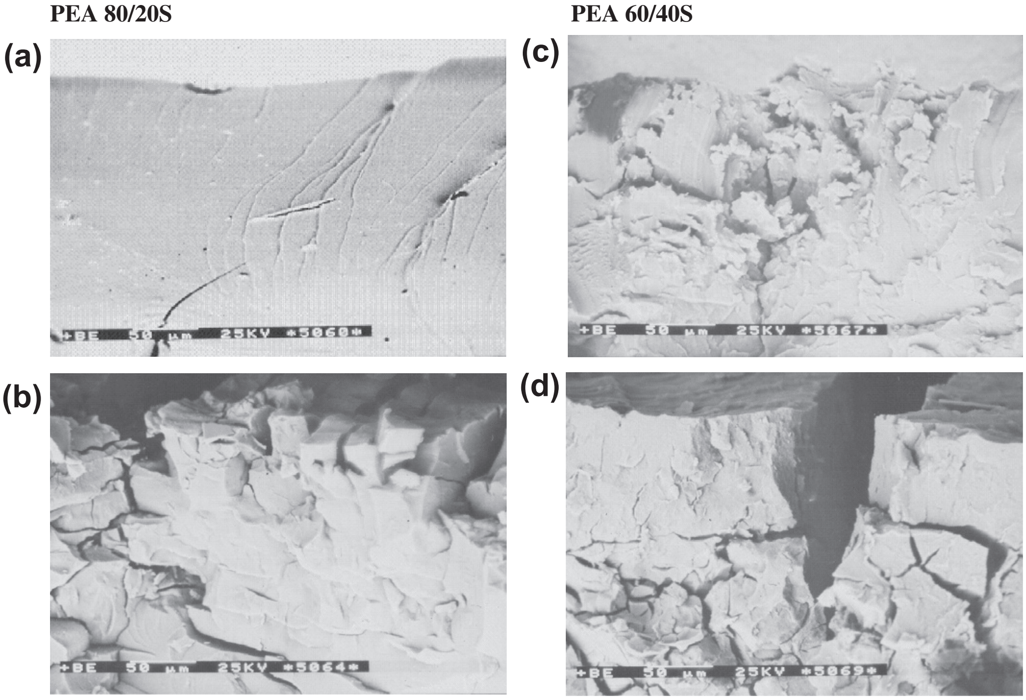

Fig. 5. SEM microphotographs of fracture surfaces (with visible outer layer) of samples of poly(ester-anhydride)s PEA 80/20S and PEA 60/40S after 4 days (upper) and 21 days (bottom) of hydrolytic degradation. Magnification 700×.

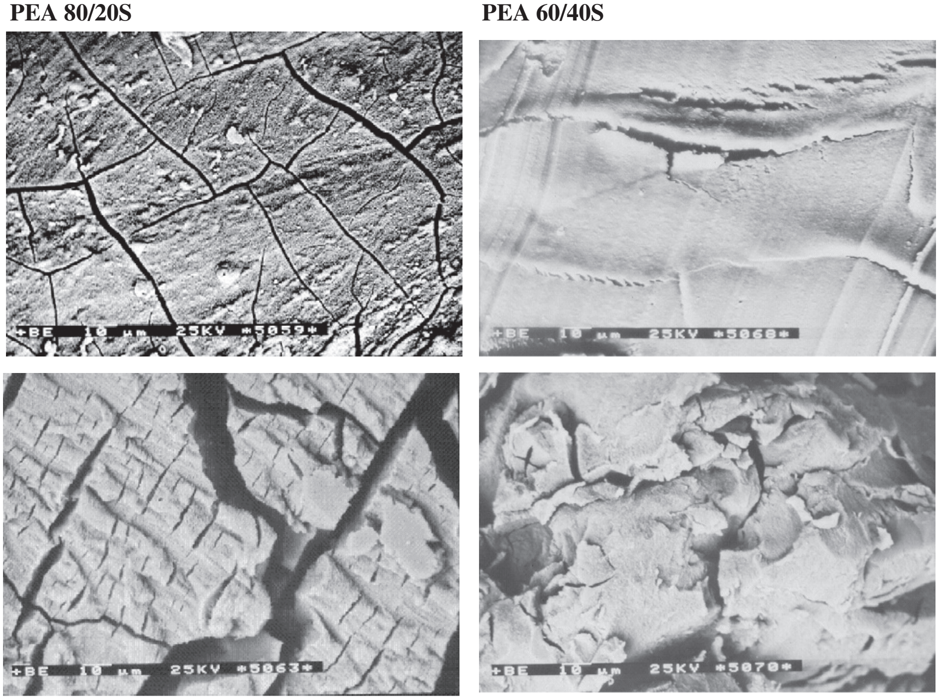

Fig. 6. SEM microphotographs of fracture surfaces (inner layer) of samples of poly(ester-anhydride)s PEA 80/20S and PEA 60/40S after 4 days (upper) and 21 days (bottom) of hydrolytic degradation. Magnification 1500×.

ture 100–250 °C, for PEA 100 and PEA 80/20 (Fig. 3). The kind of OSAGE used for the preparation of poly(ester-anhydride) had an influence on polymerization of allyl groups. As it was mentioned above thermal polymerization of double bonds in poly(ester-anhydride)s synthesized from longer oligoester (OSAGE-l) began at the temperatures much lower (T_c = 108–132 °C) than in the case of the polymers prepared from shorter ones (OSAGE-s, T_c = 171–180 °C). It may results in better stability of those polymers during storage, but on the other hand poly(ester-anhydride)s from OSAGE-l can be more useful for preparation of crosslinked materials.

3.3. Hydrolytic degradation

Poly(ester-anhydride)s obtained from OSAGE-s were degraded in PBS at 37 °C. The hydrolytic degradation was monitored mainly by determination of weight loss of the samples. The weight of all samples decreases as a function of time. Fig. 4 displays the weight loss of poly(ester-anhydride)s having different CPH to OSAGE-s ratios.

Degradation process of poly(ester-anhydride)s containing more than 40% of OSAGE was much faster than degradation of poly(CPH) or poly(ester-anhydride)s with 60% or higher CPH content. This is caused by the differences in crystallinity of copolymers as well as in the chemical structure of copolymers (the content of anhydride bond as well as aliphatic and aromatic fragments).

PEA100 and PEA 80/20S (partially crystalline), degraded very slowly at the beginning, whereas amorphous polymers containing more than 20% of OSAGE, degraded faster in the first few days, and next the degradation process slowed down. The first period of hydrolytic degradation of poly(ester-anhydride)s containing more than 20% of OSAGE, was bulk degradation process, different from that of PEA 100 or PEA 80/20S where mainly surface degradation was observed. Bulk degradation process caused fast hydrolysis of anhydride bonds in whole volume of a sample, what was confirmed by ^{1}H NMR analyses. In the first period, i.e. after 4 days of degradation time, the anhydride bond content decreased for ca. 5% in PEA 60/40S, 15% in PEA 40/60S and nearly 45% in PEA 20/80S. During this time PEA 60/40S lost about 30% of its initial weight, PEA 40/60S and PEA 20/80S lost about 50% and 70% respectively. Anhydride content in PEA 80/20S after 4 days of degradation process was the same as before degradation. Examination of the degrading samples revealed that the cross section of samples consisted of different layers, the outer layer (exposed to PBS solution) and the inner ones. The boundary between the layers moved from the surface to the center of the sample during degradation process. Morphology of fracture surface of samples of PEA 80/20S and PEA 60/40S during degradation process was examined by SEM. Microphotographs were made in magnification 700× (bar 50 μm) and 1500× (bar 10 μm). At each photos made in enlarging 700× (Fig. 5) there is a 1/3 of thickness of disc shaped sample (ca. 0.3 mm) with visible outer, more eroded layer. The microphotographs made in enlarging 1500× (Fig. 6) show more details of fracture surface of inner layer of samples. After 4 days of degradation process, the smooth surface of the outer layer of PEA 80/20S (Fig. 5) illustrating its dense structure in contrast with partially cracked surface of PEA 60/40S (Fig. 5). After three weeks of incubation in buffer solution, deep cracks penetrating from surface to inner part of samples could be seen on microphotographs of PEA 60/40S (Fig. 5d), whereas only small microcracks were visible in outer layer of PEA 80/20S (Fig. 5b). The inner surfaces of samples PEA 80/20S (Fig. 6) remained smooth and dense during degradation, but microcracks, visible on microphotographs, became deeper. The inner part of PEA 60/40S samples, relatively smooth and dense after 4 days of degradation process, become rougher and the image of fracture of sample suggested partial loss of consistency of the sample after three weeks of degradation.

Appearance of the samples during exposition in PBS changed as well, as it could be seen at Fig. 7. No crumbs or loose fragments were found to be generated during 98 days of degradation for PEA 60/40S and the size of those samples remained unvaried in spite of the fact that the weight loss was ca. 50%. The surface of the samples became however more cracked.

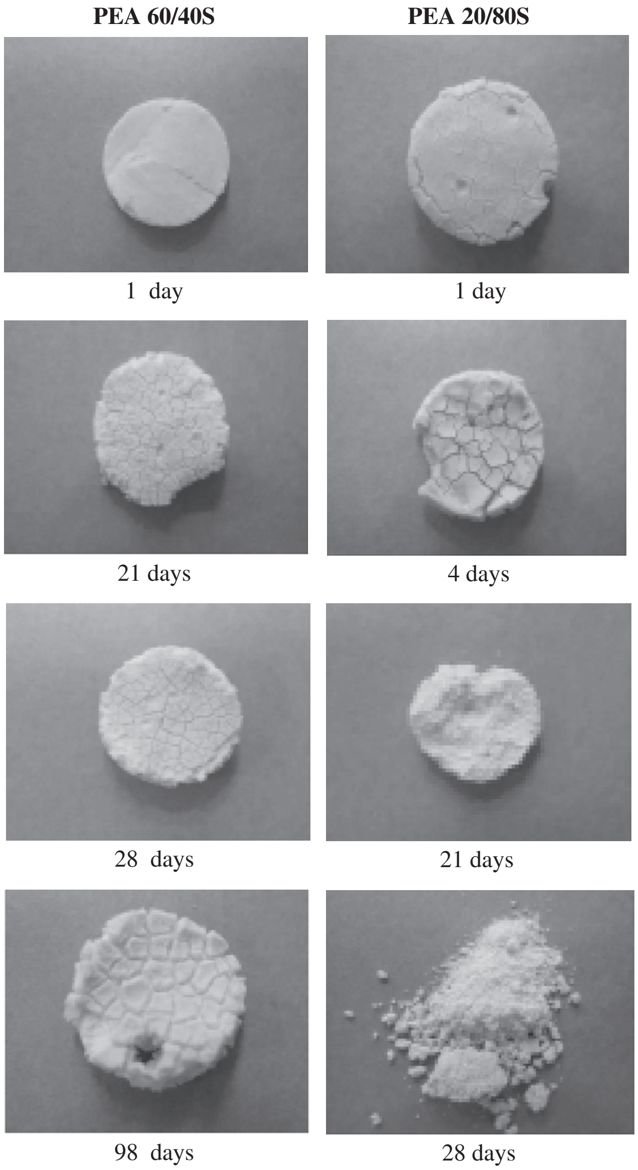

Fig. 7. Photographs of samples of PEA 60/40S and PEA 20/80S after different degradation time.

Fig. 8. Photograph of sample of PEA 80/20S after 98 days of immersion in phosphate buffer.

The samples of poly(ester-anhydride)s containing >60% CPH apparently have been changed even less (Fig. 8).

The samples of copolymers obtained with use of 80% or 60% of OSAGE underwent changes in shorter time period. They cracked and crumbled after 26 (PEA 20/80S) or 35 (PEA 40/60S) days of immersion in the buffer and after that time weight loss was found to be ca 90%.

4. Conclusion

Poly(ester-anhydride) copolymers, based on succinic acid and 1,6-bis-*p*-carboxyphenoxyhexane were obtained and characterized. The poly(ester-anhydride)s obtained with different amount of OSAGE and CPH, differ in their solubility and thermal properties. The studies indicate that poly(CPH) and its copolymers with OSAGE have vastly different degradation rate and mode depending on the content of both prepolymers. They could be considered as potential biodegradable carriers for controlled release of drugs. The amorphous state of most the copolymers and their glass temperature below room temperature are desirable for processing into tablets. Solubility of copolymers with higher amount of CPH in chlorinated solvent could allow to be fabricated into micro or nanospheres.

The obtained polymers contain allyl groups in the side chains, which could be used for copolymerization or modification. Due to solubility of some copolymers in methyl methacrylate, the application of poly(ester-anhydride)s in biodegradable bone cements could be also taken into consideration.

Acknowledgements

Financial support from the Polish State Committee for Scientific Research (Grant No. N205 016534).

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