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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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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], anaesthesis [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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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