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Epoxidation of pendant allyl groups in poly(ester-anhydride)s proposed for application in drug delivery

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ABSTRACT

This paper describes the synthesis and modification of functional poly(ester-anhydride)s that have potential for a variety of applications in drug delivery. Functional poly(ester-anhydride)s with pendant allyl groups were synthesized by two step polycondensation of oligo(3-allyloxy-1,2-propylene succinate) terminated with carboxyl end groups (OSAGE) and one of diacids (DA): sebacic (SBA) or dodecanedicarboxylic acid (DDC). Poly(ester-anhydride)s with molecular weights with range of 3100–7300 Da and with varying degree of functionality were oxidized with *m*-chloroperbenzoic acid (MCPBA) to yield respective polymers with epoxy pendant groups. The OSAGE to DA ratio in the poly(ester-anhydride)s, polymer concentration in the reaction solution, excess of MCPBA and duration of the reaction, had influenced the conversion of allyl groups into glycidyl ones. For all types of poly(ester-anhydride)s conditions were set up in which 100% of epoxidation could be achieved. Completely oxidized poly(ester-anhydride)s were characterized by means of ^{1}H NMR spectroscopy and DSC technique. Their thermal and solubility characteristics were compared with characteristics of initial poly(ester-anhydride)s. Initial and oxidized polymers containing 80% of SBA or DDC were formulated into microspheres using O/W emulsion technique. The presence of epoxy groups at the surface of microspheres were confirmed by ATR IR spectroscopy. Microspheres obtained were subjected to hydrolytic degradation at 37 °C, in aqueous phosphate buffer solution of pH = 7.41 (PBS).

1. Introduction

Polyesters and polyanhydrides are widely-studied biodegradable polymers with numerous medical and pharmaceutical applications [1–6]. They may be especially useful for biomedical purposes when products of their hydrolysis are naturally present in living tissues (like lower hydroxyacids or succinic acid involved in Krebs cycle) or at least if they are not harmful and their elimination from a body is possible [7]. For this reason polyesters constituting of glycolic and lactic acids are commonly used as biomaterials or as matrices in physical polymer-drug combinations [8,9]. However, these polymers undergo bulk erosion and the rate of drug release was found to be controlled rather by diffusion, not by the rate of degradation. Therefore, these matrices do not show well-defined drug release kinetics, particularly for water–labile low molecular weight drugs and high molecular weight peptides or proteins [10]. Polyanhydrides, on the other hand, are the most promising polymers for controlled drug delivery, since they displace predominantly surface erosion mechanism and they are relatively non-toxic and biocompatible. Aliphatic homo-polyanhydrides have been found to have limited applications due to their crystalline structures and fast degradation [5]. The degradation rate and mode can be controlled by manipulation with the polymer composition, thus poly(ester-anhydride) combine the individual properties of these two classes of polymers. Recently, we have described the synthesis of functional poly(ester-anhydride)s with allyl pendant groups, based on oligosuccinates and various diacids [11–13]. Pendant functionality in such polymers creates an interesting perspectives for chemical coupling of drugs to such a carrier, but makes the synthesis more complex. That problem may be solved by use of reagents with dormant functional groups, which are not reactive during the synthesis of polymers. Double bonds may be considered as relatively stable precursors of other functional groups since they can be transformed to oxiranes or glycols. That idea was realized by us previously in the synthesis of functional polyesters based on succinic or carbonic acid and allyl glycidyl ether, followed by the oxidation of pendant unsaturated groups [14,15]. In this work, we present the effects of modification of poly(ester-anhydride)s realized by epoxidation of pendant allyl groups.

The aim of this work was to evaluate the influence of various parameters on the conversion of allyl groups, to achieve total oxidation in possibly mild conditions (not to cause degradation of the

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polymer) and additionally to examine the possibility of formulation of oxidized polymers into microspheres.

2. Experimental

2.1. Materials

Succinic acid 99% (Aldrich), allyl glycidyl ether 99% (Aldrich), sebacic acid 99% (Aldrich), dodecanedicarboxylic acid (Aldrich), acetic anhydride (POCh S.A.), *m*-chloroperbenzoic acid 70% (Fluka), poly(vinyl alcohol) (MW = 88,000 g/mol, 88% hydrolyzed) (ACROS Organic) and phosphate buffer solution pH = 7.41 (LabStand Poznań) were used as supplied. Solvents were purified according to known procedures.

2.2. Instrumental and analytical methods

^{1}H NMR spectra were recorded using Varian UNITY/INOVA spectrometer (300 MHz) in $CDCl_3$ with TMS as an internal standard.

Acid value (AV) and hydroxyl value (HV) were determined using standard acid–base titration methods. The number-average molecular weight of the oligoester was calculated basing on results of end group analysis (AV values) [12].

Vapour pressure osmometry (VPO) analyses were performed in chloroform using Knauer vapor pressure osmometer. The molecular weight of initial 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. The molecular weight of initial and oxidized copolymers were also estimated from acetyl end groups content, determined by ^{1}H NMR [13]. ATR IR spectra of microspheres were obtained using BIO-RAD 175S spectrophotometer.

Thermal analyses were performed using 822^e DSC Mettler Toledo differential scanning analyzer. Samples were tested at the temperature ranging 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. Synthesis of poly(ester-anhydride)s

Poly(ester-anhydride)s were synthesized by polycondensation of diacid (DA): sebacic (SBA) or dodecanedicarboxylic (DDC) ones with oligo(3-allyloxy-1,2-propylene succinate) terminated with carboxyl groups (OSAGE). OSAGE was synthesised as described earlier [12] by melt condensation of succinic acid and allyl glycidyl ether. The structure of OSAGE was confirmed by ^{1}H NMR spectroscopy. The oligoester obtained did not contain detectable amount of hydroxyl groups (HV = 0), while its AV was 235 mg KOH/1 g ($Mn_{(AV)}$ = 477 g/mol). Molecular weight of OSAGE determined by VPO was $Mn_{(VPO)}$ = 570 g/mol whereas that calculated from ^{1}H NMR was $Mn_{(NMR)}$ = 620 g/mol, corresponding to an oligomer consisting of ca. 2 repeating units. Thermal properties of the oligoester were evaluated using DSC technique. T_g = −39 °C and no melting transitions were observed for the oligomer obtained. Exothermal transition was observed however above 115 °C due to polymerization of allyl groups.

In the first step of polycondensation, OSAGE and one of dicarboxylic acids (SBA or DDC) were reacted with an excess of acetic anhydride to yield mixed prepolymers. OSAGE and DA (total amount 10 g) were refluxed in 100 ml of acetic anhydride, under nitrogen for 30 min. Excess of acetic anhydride and acetic acid formed as a by-product were next removed under vacuum. In the second step, mixed prepolymers were condensed to yield poly(ester-anhydride)s. Polycondensation was carried out at 150 °C for 2 h under high vacuum (finally 0.01 mm Hg). The poly(ester-anhydride)s obtained were crushed, washed with petroleum ether, dried under vacuum and stored at −18 °C prior to be used for oxidation process.

The poly(ester-anhydride)s were characterized by means of ^{1}H NMR spectroscopy. The ^{1}H NMR signals (Fig. 1a) can be assigned as follows:

^{1}H NMR ($CDCl_3$, ppm) δ: **b** 5.80–5.88 (m, $-C\mathbf{H}=$), **a, e** 5.12–5.28 (m, $=C\mathbf{H}_2$ and $>C\mathbf{H}O(O)C-$), **f** 4.25 (m, $-C\mathbf{H}_2O(O)C-$), **c** 3.98 (d, $-OC\mathbf{H}_2-CH=CH_2$), **d** 3.56 (d, $-C\mathbf{H}_2OCH_2-CH=CH_2-$), **h** 2.78 (m, $-C\mathbf{H}_2C(O)OC(O)-$), **g** 2.67 (m, $-C\mathbf{H}_2C(O)O-$), **i** 2.51 (m, $-CH_2C\mathbf{H}_2C(O)OC(O)-$), **z** 2.22 (s, $C\mathbf{H}_3C(O)O-C(O)-$), **j** 1.70 (m, $-\mathbf{CH}_2CH_2C(O)OC(O)-$), **k** 1.29 (m, $-CH_2-$).

2.4. Epoxidation of pendant allyl groups

Oxidation of allyl groups of the poly(ester-anhydride)s was carried out in CH_2Cl_2 solution with an molar excess (30–150%) of *m*-chloroperbenzoic acid at room temperature. Concentration of the polymers in reaction solution (3–7% w/w) and duration of the reaction (72–120 h) were varied in order to achieve total oxidation.

The precipitate formed during oxidation process was separated by filtration, the resulting solution was stirred with anhydrous K_2CO_3 as long as CO_2 was evolving and the potassium *m*-chlorobenzoate was separated by centrifugation. This operation was repeated 3–4 times and then the oxidized poly(ester-anhydride) was isolated by evaporation of solvent. The yield of oxidized products was ca. 50%.

The conversion of double bonds in the epoxidized poly(ester-anhydride)s were calculated from intensities of respective signals in ^{1}H NMR spectra according to the following equation:

$$\text{“C=C” conversion } [\%] = 100.Ib'/(Ib + Ib') \qquad (1)$$

I_b, $I_{b'}$ denote relative intensity of the signals of methine protons of allyl groups (δ = 5.80–5.88 ppm) and methine protons of the oxirane ring (δ = 3.14 ppm).

Completely oxidized polymers were characterized by means of ^{1}H NMR spectroscopy and DSC technique. The changes in molecular weight (calculated from acetyl end groups) and ester to anhydride groups content in polymers after oxidation were controlled by ^{1}H NMR spectra.

The ^{1}H NMR signals (Fig. 1b) can be assigned as follows:

^{1}H NMR ($CDCl_3$, ppm) δ:, **e** 5.10–5.30 (m,$>C\mathbf{H}O(O)C-$), **f** 4.25 (m, $-C\mathbf{H}_2O(O)C-$), **d** 3.56 (d, $-C\mathbf{H}_2OCH_2-$), $\mathbf{c}'_1$ 3.81, $\mathbf{c}'_2$ 3.39 (dd, $-OC\mathbf{H}_2-$ attached to epoxy groups), *b′* 3.14 (m,$>CH-O-$ in epoxy groups, **h**, $\mathbf{a}'_1$ 2.88–2.75 (m, $-C\mathbf{H}_2C(O)OC(O)-$; one of $-C\mathbf{H}_2-$ in epoxy groups), **g** 2.67 (m, $-C\mathbf{H}_2C(O)O-$), $\mathbf{c}'_2$ 2.59 (d, one of $-C\mathbf{H}_2-$ in epoxy groups), **i** 2.51 (m, $-CH_2C\mathbf{H}_2C(O)OC(O)-$), **z** 2.28; 2.22 (two singlets, $C\mathbf{H}_3C(O)O-C(O)-$), **j** 1.70 (m, $-\mathbf{CH}_2CH_2C(O)OC(O)-$), **k** 1.29 (m, $-CH_2-$).

2.5. Formulation of microspheres using emulsion solvent evaporation method

The polymer solution in methylene chloride (20 ml with concentration of 50 mg/ml) was emulsified with 400 ml of 0.5% w/w aqueous solution of poly(vinyl alcohol) (PVA 88% hydrolyzed, MW = 88,000), using ULTRA-TURRAX T18 homogenizer (9000 rpm), for 30 s. The emulsion was then stirred with magnetic stirrer at 1100 rpm at room temperature for 3 h to evaporate the organic solvent. After that the microspheres were collected by centrifugation at 5000 rpm for 5 min, washed three times with distilled water, lyophylized and stored in a freezer.