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Upon synthesis of a polymeric matrix with pH and temperature responsiveness and antioxidant bioactivity based on poly(maleic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5.5] undecane) derivatives☆

Aurica P. Chiriac [a],*, Loredana E. Nita [a], Nita Tudorachi [a], Iordana Neamtu [a], Vera Balan [a], Liliana Tartau [b]

[a] *"Petru Poni" Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, 700487 Iasi, Romania*
[b] *"Gr.T. Popa" University of Medicine and Pharmacy, Iasi, Romania*

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ABSTRACT

Poly(maleic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5.5] undecane), acquired through radical polymerization, was synthesized with the aim to prepare an alternant copolymer with precise placement of functional groups along the polymer backbones. The new structure owing to the suitable and specific functionalities is anticipated to be used as reactive polymer to link bioactive compounds via maleic anhydride moiety. The copolymer was improved in its functionality by maleic anhydride ring opening with different amounts of erythritol in order to confer antioxidant characteristics to the polymeric structure. The chemical structure of the new prepared polymers was confirmed by FTIR and ^{1}H NMR spectra, and the polymers were also characterized from the viewpoint of their thermal stability. The dual sensitivity of the polymeric structure, at temperature and pH, was evaluated by determining the hydrodynamic radius and zeta potential in interdependence with the environment conditions. The polymer morphology was investigated by SEM. The antioxidant character was evaluated measuring the scavenger properties of the functionalized copolymer with erythritol against the 2,2-diphenyl-1-picrylhydrazyl radicals. The acute toxicity investigation, realized in vivo for the copolymer and the derivatives, allows the inclusion of the compounds into the group of moderately toxic accordingly to Hodge and Sterner toxicity scale owing to the lethal dose 50 determined values.

1. Introduction

In recent years, synthetic polymers and polymeric assemblies become an important tool for biological sciences in order to interact with biological moieties to improve the efficiency of therapeutics delivery, or for diagnostics and biological assays. In this context, the interest for copolymers of maleic acid and its derivatives is entirely justified taking into consideration the usefulness of these compounds in a wide range of applications in various fields, including biomaterials and drug delivery systems [1,2].

The maleic anhydride copolymers applied in medical or pharmaceutical field were classified as active by themselves and for controlled delivery systems in drug formulations or as drug polymer systems (conjugates). The alternant copolymers of maleic anhydride fulfill the requirements for drug carrier: biocompatibility, reproducible chemical structure, suitable molar mass, narrow molar mass distribution, availability of attachment sites, water solubility [3–5]. Conjugation of bioactive compounds onto these polymeric matrices can be easily carried out by reactions of the anhydride cycle thus, because the advantages brought by the maleic anhydride co-polymers are between others the possibilities to modify the maleic anhydride ring through suitably reactions [6–9]. The specific reactions to the copolymer rings, as for example amidation or esterification with various amino- or hydroxy-functionalized compounds, allow the preparation of the new polyfunctional polymers with tuned and tailored structure for requested properties.

In our previous studies copolymers of 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane with 2-hydroxyethyl methacrylate or N,N-dimethyl-acrylamide, used as polymeric matrices to link drugs, were presented [10–12]. The incorporation of spiroacetal groups in polymer structures improves the solubility and the adhesive properties induce good oxidative and thermal stability, are formers of good fiber or films with good flexibility and tensile strength [13]. Also, the spiroacetal rings induce interactions on ether oxygen such as hydrogen bonds or coordinate bonds with other functional groups determining bulkiness and stiffness [14]. Developments in synthesis of alternating

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* Corresponding author.
E-mail address: achiriac1@yahoo.com (A.P. Chiriac).

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poly(ester–ether)s from spiroortho-esters were described and they are considered biodegradable and useful for biomedical applications [15–18]. Spiroacetals, the structural element key, in many bioactive polyketide natural products and related analogues are characterized by having diverse arrays of stereocenters, combined with a high level of oxygenation [19].

The present study is devoted to the poly(anhydride maleic-co-3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane) copolymer synthesis. Both comonomers are able for generating special effects as for example network formation, biodegradability and biocompatibility, gel formation capacity, binding properties, amphiphilicity, good oxidative and thermal stability, good film formers, and acid pH sensitivity, and in this context the interest in the development and studying these polymeric structures is entirely justified. These new copolymers are considered to have potentially rigid backbones due first to the unsaturated double bond of 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane, whose reactions can give crosslinked macromolecular chains with network type structures. Secondly the steric repulsion between spiroacetal rings along the chains can offer the space needed to host and immobilize through physical bonds bioactive substances. In addition the rich chemistry available through the maleic anhydride ring capable to be modified through suitably reactions makes possible the preparation of a variety of functional derivatives from these copolymers. In this context, the maleic anhydride ring was opened with erythritol in order to obtain a polymeric structure with antioxidant characteristics [20]. The new structures owing to their suitable functionalities are anticipated to be used as reactive polymers to link further biological molecules via the maleic anhydride moiety. Thus, the synthesized copolymers may function as building-blocks for the preparation of micro- and nanostructured materials through self-organization.

2. Experimental section

2.1. Chemicals

The chemicals maleic anhydride (MA) (product for synthesis, from Merck), 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane — (U) (98%, from Aldrich,) and meso-erythritol (E) (≥99%, from Sigma) were used without further purification. The free-radical initiator 2,2′-azobis(2-methylpropionitrile) (AIBN) (from Sigma-Aldrich) was recrystallized from methanol prior to use. The solvents used 1,4 dioxane (≥99.0%) and diethyl ether (for precipitation) were obtained from Sigma-Aldrich and were ACS grade. The water used in other experiments was purified using an Ultra Clear TWF UV System.

2.2. Synthesis processes

2.2.1. Synthesis of poly(maleic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5.5] undecane) (PMAU)

PMAU was synthesized through thermally initiated free-radical polymerization of maleic anhydride with 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane. Equimolar amounts of maleic anhydride (2.52 mg) and 3,9-divinyl-2,4,8,10-tetra-oxaspiro[5.5]undecane (5.47 mg) were combined in a 50 mL round bottom flask with AIBN and 1,4 dioxane (D) as solvent. 0.008% amount of radical initiator relative to total monomer and 60 wt.% D as solvent, were used. The continuous polymerization process was conducted under nitrogen atmosphere, at 70 °C, in a constant temperature bath, with a stirring rate of 250 rpm, and it was carried out for 24 h. The polymerization product was a homogeneous viscous liquid; by dropwise addition of diethyl ether pure PMAU polymer was precipitated and washed 2–3 times also with diethyl ether, and then filtered and dried under vacuum at room temperature.

2.2.2. Preparation of erythritol derivatives of poly(maleic anhydride-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5.5] undecane) copolymer: PMAU_E

Erythritol derivatives were prepared through the reaction of the polyol with the anhydride groups of the PMAU backbone. Gravimetric ratio from 1/1 up to 1/5 between copolymer and erythritol for synthesizing the derivatives were used. In a typical reaction of 1/1 copolymer to erythritol ratio, 0.3 mg PMAU and 0.3 mg erythritol were dissolved in 16.5 mL of dioxane in a 50 mL round-bottom flask. Then, the obtained solution was sparged with nitrogen before increasing the temperature up to 80 °C. Adding the predetermined amount of erythritol is related on the intended degree of antioxidant capacity and crosslinking for the final product. The reaction was carried out for 24 h, at 80 °C, and 200 rpm stirring rate. The erythritol derivative of the copolymer was recovered by precipitation in cold diethyl ether (followed by filtration) several times and after that dried under vacuum at room temperature.

2.3. Characterization

2.3.1. FTIR spectra

FTIR spectra of the prepared polymeric samples – *PMAU* and *PMAU_Es* – were recorded on a Vertex Brucker Spectrometer in an absorption mode ranging from 400 to 4000 cm^{-1}. The polymeric sample was grounded with potassium bromide (KBr) powder and compressed into a disc to analysis. Spectra were acquired at 4 cm^{-1} resolution as an average of 64 scans.

2.3.2. ^{1}H NMR experiments

^{1}H NMR experiments were performed with a Bruker Avance DRX 400 NMR spectrophotometer Rheinstetten (Germany) with 16 scans and 0.1 Hz FID resolution, equipped with a 5 mm broad band probe. The samples were dissolved in dimethyl sulfoxide (DMSO). The analyses were carried out at 25 °C and chemical shifts were reported in ppm using tetramethylsilane (TMS) as the internal reference, and confirm *PMAU* and *PMAU_E* compound composition.

2.3.3. Molecular weights

The molecular weights of PMAU and derivatives up to PMAU-E (1/3) were determined by GPC analyses and are presented in Table 1; for derivatives with higher content of erythritol the determinations have not been achieved due to crosslinking. The determinations were run at room temperature in THF (at a flow rate of 1 μl × min^{-1}) on a Waters 510 HPLC pump with a series of columns (TSK-GEL G5000-HXL, G3000-HXL: 7.5 mm i.d. × 30 cm, Tosoh Ltd.) using a Waters 410 RI-detector. The injection volume was 20 ml of 2-wt.% copolymer solutions in THF. Molecular weight (Mn) calculations were based on a linear calibration curve obtained using narrow molecular weight of standard polystyrene (Mn = 800; 2000, 4000, 9000, 30,000, 50,000, 10,900, and 233,000) (from Polysciences).

2.3.4. Thermal analysis

Thermal analysis of polymers and evolved gas analyses (EGA) was performed using an online TG-DSC/FTIR/MS system. The system is equipped with an apparatus of simultaneous thermogravimetric and differential scanning calorimetry analyses model STA 449F1 Jupiter (Netzsch—Germany), FTIR spectrophotometer Vertex-70 model (Bruker-Germany) and mass spectrometer QMS 403C Aëolos model (Netzsch—Germany). *PMAU* and *PMAU_E* samples ranging between 7.4 and 7.6 mg were heated from 30 °C to 650 °C, at heating rate of

Table 1
GPC results of PMAU copolymer and derivatives.

Polymer	Mn(Da)	Mw(Da)	PDI
PMAU	40,500	61,000	1.51
PMAU-E (1/1)	44,500	75,000	1.69
PMAU-E (1/3)	46,500	85,000	1.83