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Controlled release kinetics of p-aminosalicylic acid from biodegradable crosslinked polyesters for enhanced anti-mycobacterial activity

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ABSTRACT

Unlike conventional polymeric drug delivery systems, where drugs are entrapped in polymers, this study focuses on the incorporation of the drug into the polymer backbone to achieve higher loading and sustained release. Crosslinked, biodegradable, xylitol based polyesters have been synthesized in this study. The bioactive drug moiety, p-aminosalicylic acid (PAS), was incorporated in xylitol based polyesters to impart its anti-mycobacterial activity. To understand the influence of the monomer chemistry on the incorporation of PAS and its subsequent release from the polymer, different diacids have been used. Controlled release profiles of the drug from these polyesters were studied under normal physiological conditions. The degradation of the polyesters varied from 48% to 76% and the release of PAS ranged from 54% to 65% of its initial loading in 7 days. A new model was developed to explain the release kinetics of PAS from the polymer that accounted for the polymer degradation and drug concentration. The thermal, mechanical, drug release and cytocompatibility properties of the polymers indicate their suitability in biomedical applications. The released products from these polymers were observed to be pharmacologically active against Mycobacteria. The high drug loading and sustained release also ensured enhanced efficacy. These polymers form biocompatible, biodegradable polyesters where the sustained release of PAS may be tailored for potential treatment of mycobacterial infections.

Statement of significance

In the present work, we report on novel polyesters with p-aminosalicylic acid (PAS) incorporated in the polymer backbone. The current work aims to achieve controlled release of PAS and ensures the delivered PAS is stable and pharmacologically active. The novelty of this work primarily involves the synthetic chemistry of polymerization and detailed analysis and efficacy of active PAS delivery. A new kinetic model has been developed to explain the PAS release profiles. These polymers are biodegradable, cytocompatible and anti-mycobacterial in nature.

1. Introduction

The burden of mycobacterial infections worldwide and particularly in the developing countries continues to be a major healthcare challenge. Mycobacterial infections like tuberculosis (TB) [1] and leprosy [2] claim million lives worldwide. Other common mycobacterial diseases include buruli ulcer caused by *Mycobacterium ulcerans* [3] and respiratory diseases caused by rapidly growing Mycobacteria (RGM) [4,5]. Some diseases have also been reported to be caused by *Mycobacterium avium* and *Mycobacterium xenopi* [6]. These diseases can be effectively treated by multi-drug therapy.

p-Aminosalicylic acid (PAS) is a well-known drug used in conjunction with first line drugs like isoniazid, rifampicin, etc. [7] that is active against mycobacterium and also finds use in treating TB. In recent years, this drug has found various other applications including the treatment of cancer and inflammatory bowel disease (IBD) [8], etc. PAS exhibits modest activity against *Influenza virus* A [9,10]. PAS has also been derivatized to more potent forms to treat diseases such as influenza and manganism, etc.

In spite of its widespread potential as a cure for various diseases, its short serum half-life (15 min to 1 h), makes it difficult

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to use PAS for treatment. As an alternative, PAS loaded into different polymers to be delivered at the site of interest for enhanced efficacy has been proposed. In addition, owing to its anti-inflammatory properties, PAS loaded polymers may be well suited for regenerative medicine such that the release of PAS can suppress implantation-induced inflammation and potential bacterial infections.

As discussed in a recent review [11], loading of the drug in the polymer backbone ensures controlled release, higher loading and better processability. PAS has been incorporated in methacrylic polymers with maximum loading of ~14% [12]. The low aminosalicylate uptake of these polyesters is a major limitation and has resulted in their limited success as therapeutics for mycobacterial diseases. Previous studies [13,14] have reported the incorporation of aminosalicylates (PAS and 5-aminosalicylic acid) in poly(anhydride esters) (PAEs) backbone for drug release applications. PAEs are a preferred class of polymers for drug delivery applications. However, the stability of the drug in these polymers is questionable [13]. Moreover, the anhydride bonds, being extremely labile to hydrolysis, degrade very fast in the body [15]. There has, therefore, been an increasing demand for delivery systems that offer sustained release of such drugs while minimizing the usual side-effects associated with high dosage. Crosslinked polymers have gained increasing importance in this regard owing to their easily tunable properties with regards to drug/biomolecule delivery [16,17]. Since, it is imperative to incorporate PAS in a polymer backbone with efficient loading to mediate its sustained release over time; polyesters may serve as suitable alternatives. Crosslinked polyesters offer sustained drug delivery [18] and are also capable of supporting tissue regeneration [19].

In the present study, we have synthesized crosslinked polyesters from xylitol and a diacid. Two different diacids, namely, adipic acid or sebacic acid were selected to study the effect of chain length on the polymer properties and release of PAS. It has been studied earlier how these slight modifications in the polymer chemistry offer a plethora of release rates and properties [20,21]. As the stability of PAS is dependent on thermal conditions and the three functional groups on PAS are susceptible to conjugation, the reaction scheme was specially designed to successfully incorporate PAS in the polymer backbone and it remained pharmacologically active after release. The activity of these PAS-based polyesters against *Mycobacterium smegmatis* was also studied.

2. Experimental

2.1. Materials

Adipic acid (AA), sebacic acid (SA) (SRL laboratories, India), p-aminosalicylic acid (PAS, I), benzyl chloroformate (Cbz-Cl) and xylitol (all from Sigma Aldrich) were used for the synthesis of crosslinked polyesters. The di-carboxylic acids were recrystallized in ethanol (Merck, India) and kept at 4 °C prior to use in order to remove organic impurities. Solvents used at various stages of the work include ethyl acetate (EtoAc), chloroform, dimethylsulfoxide (DMSO) and N,N-dimethylformamide (DMF), acetone (all from S.D. Fine Chemicals, India).

2.2. Synthesis

2.2.1. Step I: amine protection of PAS

Cbz acts as a protecting agent that reacts with the $-NH_2$ group of PAS to prevent its further interference in the esterification reaction. PAS (1.5 g, 10 mmol) was dissolved in methanol (8 mL) and allowed to solubilize. $NaHCO_3$ was added to this reaction mixture in slight excess (1.0 g, 12 mmol) to make the reaction pH basic. Ten minutes after the addition of $NaHCO_3$, Cbz-Cl (1.7 mL, 12 mmol) was added to the reaction mixture at 0 °C (over ice). The reaction was performed for 5 h at 25 °C. After evaporation of the solvent, the product was obtained by partitioning the product into 1 N HCl and EtoAc. The extracted organic layer was washed with brine and dried over anhydrous Na_2SO_4. The product (NCbz-PAS, II) obtained was dried in a rotary evaporator.

2.2.2. Step II: synthesis of diacid

NCbz-PAS (2 g, 7 mmol) was dissolved in DMF (5 mL). K_2CO_3 (5.8 g, 42 mmol) was added to it and allowed to stir for several minutes. After this, adipoyl chloride (500 μL, 3.5 mmol) or sebacoyl chloride (746 μL, 3.5 mmol) was added dropwise to a stirred mixture over ice. The reaction was performed for 12 h at 25 °C. After completion of the reaction, the product was obtained by partioning in a 1:1 mixture of EtoAc and water. The organic layer was extracted and dried over anhydrous Na_2SO_4. The diacid product (III) obtained was dried using a rotary evaporator and subsequently, in high vacuum.

2.2.3. Step III: polymerization of diacid with xylitol

The reagents, diacid (IIIa or IIIb) and xylitol were mixed in a round bottomed (RB) flask. Polymerization was conducted by melt condensation at 150 °C with continuous stirring under nitrogen atmosphere for 2 h (Schematic 1). Subsequently, after 2 h, a liquid nitrogen trap connected to vacuum (6 mm Hg) was used to remove the byproduct (water) of esterification and to increase the pre-polymer yield for 12–16 h. The prepolymer was further cured at 130 °C under vacuum (60 mm Hg) for 6 days to obtain PAS loaded crosslinked polyesters. The polyesters are named as PX4-PAS (IVa) and PX8-PAS (IVb) where PX4 and PX8 denotes poly(xylitol adipate) and poly(xylitol sebacate), respectively, wherein the numbers represent the number of carbon atoms between the acids groups in the diacid.

2.3. Materials characterization

The polymers were chemically characterized using Fourier transform infrared (FTIR) spectroscopy and proton-nuclear magnetic resonance (NMR) spectroscopy, mechanical properties were obtained by dynamic mechanical analysis (DMA) and thermal properties acquired using differential scanning calorimetry (DSC). Other than NMR spectroscopy, all characterizations were performed on completely cured samples.

2.3.1. FTIR spectroscopy

Universal attenuated total reflectance (uATR-FTIR) mode was used for FTIR analysis of the polyesters. FTIR spectra were recorded on a Perkin-Elmer Frontier FT-NIR/MIR spectrometer. An average of 16 scans was taken with a resolution of 4 cm^{-1} over the range 4000–650 cm^{-1}. The 6 day cured polymer discs were placed on the instrument without further processing.

2.3.2. Dynamic mechanical analysis

The 6 day cured samples of PX4-PAS and PX8-PAS were characterized for mechanical properties by dynamic mechanical analysis (DMA, TA Instruments Q800). A dynamic frequency ranging from 1 to 100 Hz with fixed amplitude of 15 μm was applied to a rectangular sample (dimensions: 30 mm × 5 mm × 2 mm) held under tension isothermally at 37 °C.

2.3.3. Differential scanning calorimetry

Thermal properties were obtained from a differential scanning calorimeter (DSC, TA Instruments Q 2000). The samples were subjected to heat–cool–heat temperature program. The first heating cycle removes all thermal history due to processing. All the