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Research paper

Copolymers of pharmaceutical grade lactic acid and sebacic acid: Drug release behavior and biocompatibility

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Abstract

Pharmaceutical grade D,L-lactic acid, which is rather an economic source in comparison to lactide monomer, was utilized for synthesis of a series of copolymers with sebacic acid. Polymers were characterized by GPC, FTIR, NMR and DSC techniques, and formulated into blank and methotrexate (MTX) loaded microspheres by emulsion-solvent evaporation method. *In vitro* degradation of blank microspheres was studied by FTIR, GPC and SEM analysis. MTX loaded microspheres showed the encapsulation efficiency of 44–64% and were in the size range of 40–60 μm. These were used to study the release profile of the encapsulated drug. The release was found to be affected by the pH and buffer concentration of the release medium which was in turn revealed by solubility studies of MTX. The overall study demonstrates significance of drug as well as polymer properties on release. Biocompatibility of polymer was evaluated by injecting microspheres subcutaneously into Sprague–Dawley (SD) rat and no local histopathological abnormalities were found.

Keywords: PLA–PSA; Microspheres; Biodegradable polymers; Drug delivery; Biocompatibility; Drug release kinetics; Profile modeling

1. Introduction

Microspheres are a useful type of delivery system for administration of drugs, since at one fell swoop they can be used to encapsulate, protect and control the release of a wide variety of drugs [1,2]. By delivering the drug at a controlled rate over a prolonged time in the localized area, such devices can maintain optimal drug concentrations and aid patient compliance by reducing the frequency of administration. The main advantage of localized drug delivery is high locoregional concentration of therapeutic agents with prolonged retention and hence, chances of various adverse effects are reduced or completely eliminated due to the escape of high systemic dose to achieve the therapeutic concentration at diseased site [3]. In addition, biodegradable microspheres are easily administered by injection, and they do not require surgical removal after drug exhaustion. Since the drug loaded in a microsphere remains separated from that in other microspheres, a further advantage is the potential to administer multiple drugs in a single injection by mixing different drug loaded microspheres, which for compatibility reasons would otherwise need to be separated.

Besides, the drug release rates can be controlled by manipulation of the particle size, the polymer degradation and/or erosion rates, the type of polymer and polymer erosion mechanism (bulk vs. surface erosion), among other factors. Surface eroding polymers, such as polyanhydrides, may simplify the drug release kinetics because water penetration into the microsphere interior is minimized, and the drug release rate becomes dependent predominantly on the polymer erosion rate [4].

Foremost important criterion for any polymer used for drug delivery is biocompatibility and because the surface of the material is in immediate contact with the biological medium, the interfacial characteristics are often more

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significant for impermeable solid devices. In such cases, the surface structure governs the biological response. This is initially determined by cell and protein interactions. However, in case of biodegradable polymers used for drug delivery overall bulk properties are also important contributing factor [5]. Biocompatibility of the degrading polymer can be checked by histopathology at different time intervals of the tissue near implantation site in the animals.

Poly(ester-anhydride) copolymers have been synthesized to combine the individual properties of the two widely used biodegradable polymer classes. Once these are formulated into microspheres, bulk erosion would be expected for polyesters whereas polyanhydrides exhibit a surface erosion profile controlled by the hydrophobic nature of the polymer backbone and the lability of the anhydride unit. Depending on the poly(ester-anhydride) copolymer ratio, contrasts in degradation behavior can be achieved to influence controlled release profiles of encapsulated bioactive moiety [6]. In addition to adding versatility to poly(ester-anhydride) degradation and release behavior, the polyanhydrides content of formulated delivery devices provides a simple method for covalent surface modification. The lability of the anhydride bond allows for the addition of amine and acid containing compounds. This property would become especially meaningful in the context of poly(ester-anhydride) microspheres by extending the delivery potential for these devices [2,4,7,8]. Thus, by varying the polymer chemistry, a suitable degradation time can be achieved to meet delivery needs.

In the present study, PLA–PSA in varying ratios has been synthesized and formulated into microspheres with and without MTX. Their *in vitro* and *in vivo* degradation and drug release behavior have been studied. Biocompatibility study of the microspheres prepared from 50:50 PLA–PSA was carried out subcutaneously in SD rats.

2. Materials and methods

2.1. Materials

Sebacic acid (for synthesis) was purchased from Lobachemie (Mumbai, India). D,L-Lactic acid (90% aqueous solution, Merck, Mumbai, India) was the starting monomer for PLA synthesis. Acetic anhydride LR (Qualigens, Mumbai, India), chloroform GR (Merck, Mumbai, India), petroleum ether LR (Qualigens, Mumbai, India), diethyl ether stabilized (Lobachemie, Mumbai, India), dichloromethane (Lobachemie, Mumbai, India) and poly(vinyl alcohol) (Sigma, Germany) were used as received. Methotrexate was received as gift sample from Astron Pharmaceuticals (Ahmedabad, India).

2.2. Methods

2.2.1. Polymer synthesis

Poly(lactic acid) (PLA) was synthesized from pharmaceutical grade D,L-lactic acid by melt-polycondensation method. PLA was activated by refluxing with acetic anhydride at 150 °C for 30 min. Similarly, sebacic acid was prepolymerized by refluxing with acetic anhydride at 150 °C for 30 min. PLA–PSA copolymer was synthesized by melt-condensation of the two prepolymers at 150 °C for 1 h, using varying ratios of the two components; nomenclature of which is given in Table 1 (detailed procedure is described elsewhere) [9].

2.2.2. Characterization of polymers

The polymers were characterized by ^{1}H NMR (300 MHz spectrometer, Bruker Avane, Germany), FTIR (Perkin Elmer, USA), DSC (Mettler Toledo, Switzerland), SEM (Leo Electron Microscopy LTD, Cambridge, England) and GPC. GPC was carried out with Waters Styragel HR3 column and chloroform as the mobile phase. Molecular weight was determined with reference to polystyrene standards in the range of 682–28,000 Da. The system consisted of Shimadzu LC-10AT VP HPLC pump (Shimadzu Corporation, Kyoto, Japan), Shimadzu SIL-10AD VP autoinjector, and SIL-10AD VP refractive index detector. Dried microspheres were gold coated for electron microscopy.

2.2.3. Microsphere preparation

The synthesized polymers were formulated into blank and drug loaded microspheres using emulsion-solvent evaporation method [10–13].

2.2.3.1. Blank microspheres. Briefly, polymer was dissolved in methylene chloride (5%, w/v) (4 ml) and was added to 100 ml of 1% PVA solution at room temperature with stirring at 1200 rpm until all the methylene chloride evaporated (about 4 h). The solidified microspheres were collected by filtration using 0.45 μm filter paper (mdi, India) and air dried overnight.

2.2.3.2. MTX loaded microspheres. Polymer was dissolved in methylene chloride (5%, w/v). MTX (20% by weight) was added to polymer solution and probe sonicated (50% amplitude, Dr. Heilscher GmbH, Germany) for 1 min. After suspending the drug in polymer solution, the organic phase was added to aqueous phase of 1% PVA solution and magnetically stirred at 1200 rpm at room temperature to evaporate methylene chloride (about 4 h). The solidified microspheres were collected and dried in manner similar to blank microspheres.

Table 1
Nomenclature of synthesized polymers

Polymer	Composition
1	PLA–PSA; 100:0
2	PLA–PSA; 75:25
3	PLA–PSA; 50:50
4	PLA–PSA; 25:75
5	PLA–PSA; 0:100