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Original article

Formulation and investigation of 5-FU nanoparticles with factorial design-based studies

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Abstract

This study describes an orthogonal experimental design to optimize the formulation of 5-fluorouracil (5-FU) loaded poly D,L (lactide-co-glycolide) (PLGA) nanoparticles (5FU-NP) by a nanoprecipitation-solvent displacement technique. The type of surfactant, amount of acetone and molecular weight of the polymer with three levels of each factor were selected and arranged in an $L_{18}(3^5)$ orthogonal experimental table. From the statistical analysis of the data polynominal equations were generated. Optimized formulations have the particle size ranging from 160 to 250 nm. Smallest nanoparticles (161 ± 1.22 nm) were obtained using Resomer PLGA 755 and pluronic F-68 with 10 ml acetone amount. Under these conditions the 5-FU entrapment percentage was maximum 78.30%, suggesting 5-FU might be entrapped and adsorbed on the nanoparticle surface. In vitro release of three formulations with maximum drug entrapment efficiency and minimum particle size, were also investigated by release kinetics. According to the determined coefficients, release data fit to Higuchi's diffusion kinetics. The in vitro release of 5FU-NP in phosphate buffered saline (PBS, pH 7.4) is suggested to be controlled by a combination of diffusion with slow and gradual erosion of the particles. Also, the antimicrobial activity was observed even on the end of seventh day with all formulations.

Keywords: Poly(lactide-co-glycolide); Biodegradable nanoparticles; 5-Fluorouracil; In vitro distribution; Nanoprecipitation technique

1. Introduction

The lymphatics are present in the various organs of the body, and are recognized as an important vascular system for the maintenance of normal function of the body. The lymphatic route is an important pathway in some metastatic cancers, and the lymph nodes along this route are potential targets for cancer chemotherapy [1].

It is widely accepted that the biodistribution of intravenously administered colloids is greatly influenced by their interaction with biological environment which is mediated by their physicochemical properties. The effect of the size of the colloids has been shown to be of primary importance [2–6]. Small particles (≈ 100 nm) and small particle fragments cannot easily cross the blood capillary endothelium and are preferentially drained into the regional lymphatic capillaries. Large particles (> 1 μm) are too big to diffuse through the barrier and are generally retained at the site of injection. The fate of intermediate-sized particles is determined by their size and surface character. These particles drain from the regional lymphatic capillaries into the regional lymph nodes and into the central lymph before returning to the systemic circulation via through thoracic lymph duct [7]. Also, some studies demonstrated that if the size of these particles is larger than about 250–300 nm, then the particles are largely captured by filtration in the red pulp of the spleen and phagocytosed within the cells of the reticuloendothelial system [8,9].

In addition to the size of the particle, after injection, the surface character also determines the disposition of particular system. Generally, microparticulate systems that present a hydrophilic surface are more rapidly cleared from the site of injection and less effectively retained in the regional lymph nodes than microspheres with essentially hydrophobic surface character [7]. Pluronics are copolymers of polyethylene oxide and polypropylene oxide. Pluronics which have more hydrophobic polyproplene oxide segments, can altered surface hydrophobicity. Moghimi et al. [10] demonstrated that hydrophilic polyethylene oxide chains of poloxamine 901 and

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904 presumably facilitate drainage from the injection site but are too short to revert opsonization and phagocytosis of particles in the lymph nodes. Also Hawley et al. [11] demonstrated that PLGA nanospheres coated with poloxamine 908 have a maximal lymph node uptake of administered dose.

The lymphatic route is known as one of the primary pathways for tumor metastasis. Tumor cells that have detached from the tissue or have invaded a lympatic vessel become trapped in the mesh work of a lymph node. For effective cancer chemotherapy, an optimal concentration of anticancer agent must reach the tumor tissues and remain there for required period of time [12]. For this purpose, attempts have been focused on the development of drug delivery systems containing antineoplastic drugs. Promising results have been reported for bleomycin [13], mitomycin [14], peptides [1], pepleomycin [15,16] and 5-fluorouracil (5-FU) [17–20] loaded particles in clinical studies. Biodegradable polymers such as PLGA have been extensively studied in controlled release technology with respect to their biodegradability and biocompatibility. In formulation or process optimization problems, a great number of controlled variables such as amount of materials or process parameters, may have an influence on the studied characteristics so that; analyzing all these factors generally leads to a prohibitive number of experiments. A screening experimental design is the most convenient way to reduce this number.

The objective of this study was to develop a mathematical model in order to deduce the adequate conditions to prepare colloidal systems of desired characteristics, which could improve the cancer chemotherapy. In vitro release and microbial activity of the optimized formulations with maximum drug entrapment efficiency and minimum particle size, were also examined.

2. Materials and methods

2.1. Chemicals

Poly (D,L-lactide-co-glycolide) was kindly supplied by Boehringer Ingelheim (Germany); named as Resomer® RG 755 and Resomer® RG 752. PLGA with average molecular weights of 50,000 and 20,000, whose copolymer ratio of D,L-lactide to glycolid is 74:26 and 73:27, respectively. 5-FU was a gift from Hoffmann-La Roche (Basle, Switzerland). Poly (ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide), EO_{76}-PO_{29}-EO_{76} (Pluronic F-68), and EO_{100}-PO_{65}-EO_{100} (Pluronic F127) was purchased from Sigma Chemical Company (St Louis, USA). Poly (vinyl alcohol) (PVA) was obtained from Merck, Germany. All other chemicals were reagent grade and were used without any further purification.

2.2. Preparation of the nanoparticles

The PLGA nanoparticles were prepared by a precipitation-solvent evaporation method with modifications employed by Fessi et al. [12]. Briefly, an organic solution, which contains the chemotherapeutic agent (10 mg), 2–10 ml of PLGA 1% (w/v) in acetone, was added to an aqueous solution 10 ml of pluronic 1% (w/v) or PVA 10% (w/v) with moderate stirring. The desolvation of polymeric material occurred instantaneously in form of colloidal particles. Acetone was then evaporated from the colloidal suspension by an adapted system. This system is consist of a desiccator with a magnetic stirring. Reduced pressure was supplied by a vacuum-pump. Nanoparticle suspension was filtered through a 0.8 μm nitrocellulose membrane (Millipore) filter and concentrated to a final volume of 10 ml by removal of water under the same conditions. Mannitol 0.1% (w/v) was added to the nanoparticle suspension and it was lyophilized (Fig. 1).

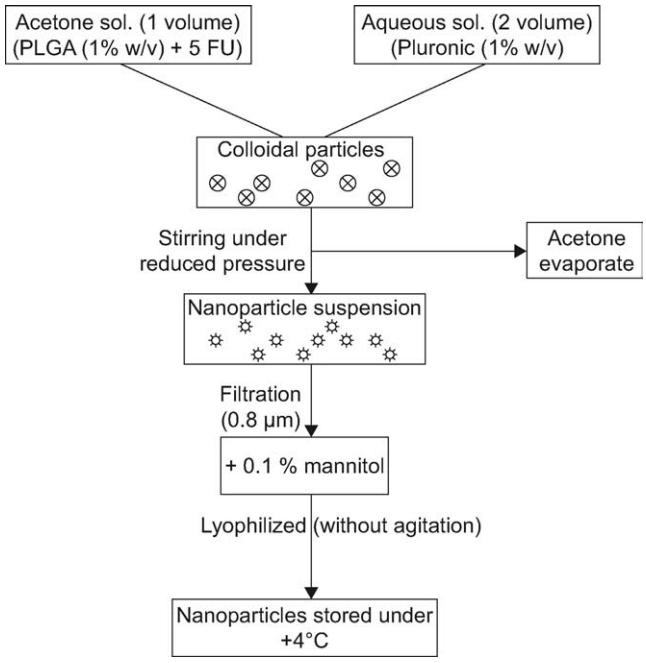

Fig. 1. Schematic representation of the nanoparticle production.

2.3. Experimental design

Most formulation studies involve variation of one factor at a time, keeping other factors constant. Factorial design enables all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. In this study, an orthogonal experimental design was introduced to optimize the formulation of nanoparticles. In order to optimize the preparation of formulations, the amount of acetone (X_1), the molecular weight of the polymer (X_2), and the type of surfactant (X_3), were chosen as independent variables. These three factors that might affect the nanoparticle formulation and three levels of each factor were selected (Table 1) and arranged according to an $L_{18}(3^5)$ orthogonal experimental table (Table 2). At each experimental level, two batches of nanoparticles were prepared. Possible interactions between X_1–X_2 and X_2–X_3 were also analyzed.