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Comparative study on diffusion and evaporation emulsion methods used to load hydrophilic drugs in poly(ortho ester) nanoparticle emulsions

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ABSTRACT

Poly(ortho ester) (POE) nanoparticles were prepared by two different emulsification techniques: novel double emulsion solvent diffusion (DESD) and double emulsion solvent evaporation (DESE). We investigated the effect of sonication time/speed, type of organic phase solvents, polymer concentration, organic/aqueous phase ratio, surfactant type and concentration on the mean particle sizes of obtained POE nanoparticles by both DESD and DESE methods. Different organic solvents [ethyl acetate, methyl ethyl ketone, acetone, dichloromethane and chloroform] were used with several stabilizers [pluronic F 68, didodecyl dimethyl ammonium bromide, poly(vinyl alcohol), and sodium dodecyl sulfate]. The particle size of nanoparticles was observed by the dynamic light scattering method and transmission electron microscopy. We assumed that combining the double emulsion system with a partially water-soluble organic solvent, ethyl acetate, would result in better encapsulation efficiency of water-soluble drugs in nanoparticles and the utilization of both biocompatible surfactants and solvents. As a model drug, we used vancomycin hydrochloride, a hydrophilic low molecular weight glycopeptide antibiotic. The new nanoparticle preparation technique, DESD, resulted in improved formulation characteristics including smaller size, lower size distribution, higher encapsulation yield, and more biocompatible ingredients with unaltered bioactivity of vancomycin hydrochloride in comparison to classical DESE method used to load hydrophilic molecules.

1. Introduction

The interest in nano-emulsions has experienced a continuous increase in the last decade, as evidenced by several publications and comprehensive review articles on this subject [1–3]. This enormous interest is triggered by a wide range of applications, for example in the pharmaceutical [4–6], cosmetic [7,8], food [9–11], and chemical industries [12–14]. Nano-emulsions (≤1 μm size droplets) have advantages over micro-emulsions (micrometer-size droplets) due to their small droplet size, which confers them stability against sedimentation or creaming and a transparent or translucent optical aspect. However, nano-emulsions, in contrast to micro-emulsions which are thermodynamically stable, are non-equilibrium systems, which may undergo flocculation, coalescence and/or Ostwald ripening. Nevertheless, with an appropriate selection of system components, composition and preparation method, nano-emulsions with high kinetic stability can be achieved. It is generally accepted [11,15] that nano-nano-emulsion's main breakdown process is Ostwald ripening (diffusion of molecules of the disperse phase from small to big droplets, through the continuous phase, as a consequence of their different Laplace pressures). However, recent reports have shown flocculation as a possible breakdown mechanism for nano-emulsions formulated with mixed nonionic and ionic surfactants [16,17].

The choice of a suitable nanoparticle emulsion technique is dependent on the physicochemical properties of the drug to be encapsulated. While several methods have been successfully reported and applied for the incorporation of hydrophobic compounds into biodegradable nanoparticles [5,18], encapsulation of hydrophilic compounds involving these carriers is more problematic, since the drug is expelled from the hydrophobic matrix into the dispersing water phase during nanoparticle preparation [4,19].

Water-in-oil-in-water (W/O/W) type multiple emulsions are three phase systems in which oil droplets containing an internal aqueous phase are dispersed in an external aqueous phase [20,21]. A W/O/W emulsion was discovered serendipitously, when an emulsion occurred during the preparation of a macro-emulsion at the phase conversion from a W/O emulsion to an O/W emulsion [22]. Methods of preparation of W/O/W emulsions include a phase inversion method [22,23], a one-stage emulsification method [24] and a two-stage emulsification method [20,21,25]. Of these methods, the two-stage emulsification method can encapsulate drugs most effectively, and many researchers

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have reported the development of formulation and application of W/O/W emulsions prepared by this method. Recently, a membrane emulsification procedure, which is another two-stage emulsification method, has been evaluated and applied to preclinical or clinical studies [26,27].

In this work, we describe a novel poly(ortho ester) (POE) nanoparticle preparation technique, double emulsion solvent diffusion (DESD), and compare it to the double emulsion solvent evaporation (DESE) technique. As a model drug we used vancomycin hydrochloride, a hydrophilic small molecule. It is known from the literature that vancomycin hydrochloride was quite stable under stirring by a homogenizer for at least 20 min. On the contrary, it was not stable under sonication, the remaining decreased to 90% and 80% after 2 and 5 min of sonication, respectively [28]. Therefore temperature and sonication time are important factors to be considered while entrapping vancomycin hydrochloride in nanoparticle emulsions. POE polymer is a biocompatible, biodegradable and hydrophobic polymer, degraded by surface erosion confined to the polymer–water interfaces, which follows zero-order drug release kinetics, rather than drug diffusion, when placed in a biological environment. Drugs encapsulated in biodegradable polymeric nanoparticles with properly designed vehicles are clinically viable options for the sustained delivery of drug molecules for biomedical applications. A previous report from our laboratory showed the physico-chemical and in vitro drug release and cytocompatibility properties of POE nanoparticles designed for intraocular treatments [4]. In the present study, we executed detailed comparison studies between the novel DESD and classical DESE methods, the effect of formulation variables, and the mechanism of POE nanoparticle formation. One of the purposes of this research was to prepare stable W/O/W emulsions, which show small mean particle sizes, and good drug entrapment efficiency.

2. Materials and methods

2.1. Materials

Poly(vinyl alcohol) (PVA), didodecyl dimethyl ammonium bromide (DMAB), sodium dodecyl sulfate (SDS), 1,10 decanediol, D,L-Lactone, poloxamer 188, and vancomycin hydrochloride were obtained from Sigma Aldrich (St. Louis, MO). 3,9-Divinyl-2,4,8,10-tetraoxaspiro[5.5] undecane was obtained from AK Scientific Inc., USA. Analytical grade solvents such as, ethyl acetate (EA), dichloromethane (DCM), methyl ethyl ketone (MEK), acetone and chloroform were purchased from Sigma Aldrich (St. Louis, MO) and used as obtained. Poly(ortho ester) polymer was synthesized and characterized as described in the literature [29]. Deionized water (DIW) was used in all experiments.

2.2. Preparation of POE nanoparticles

2.2.1. Double emulsion solvent diffusion (DESD) method

The preparation of POE nanoparticles by using the DESD method [30,31] with some modifications is as follows: POE was dissolved in 1 ml of various organic solvents (EA, DCM and MEK). The organic phase was added to an aqueous phase containing various stabilizers. After mutual saturation of organic and aqueous phases, the mixture was emulsified with a probe-tip sonicator (probe-tip diameter: 1.3 cm, Sonics & Materials Inc., Danbury, CT, USA) operating at 45–65% amplitude intensity. In order to allow for the diffusion of the organic solvent into water, a constant volume (6 ml) of water containing 2.5% of polyvinyl alcohol was subsequently added to the W/O emulsion, leading to the formation of W/O/W emulsion of POE nanoparticles. The organic solvent was allowed to evaporate overnight by stirring over a magnetic stir plate. Nanoparticles thus formed were collected by ultracentrifugation at 50,000 rpm, 60 min, and 25 °C, and then washed three times with distilled water to remove unincorporated drug and emulsifiers. The final product was dried by lyophilization at 0.002 mbar, −50 °C for 48 h (Freezone, Labconco Corporation, Kansas, MO).

2.2.2. Double emulsion solvent evaporation (DESE) method

The preparation of POE nanoparticles by using the DESE method was described in our previous publication [4] and used the same method to prepare empty and vancomycin-loaded nanoparticles in this study.

2.3. Methods

2.3.1. Physico-chemical characterization of POE nanoparticles

Particles were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). DLS measurements were conducted with particle suspensions (1 mg/ml) in Millipore water with a Malvern Nano-ZS (Malvern Instruments, Worcestershire, UK). The morphology of POE nanoparticles was documented using a transmission electron microscope (JEM-2000 EX II Electron Microscope, JEOL, LTD, Tokyo, Japan) using an acceleration voltage of 60 kV. Two microliters of POE nanoparticles (0.1 mg/ml) were placed at the center of a copper grid and dried in a dessicator for 24 h. Grids were visualized under the electron microscope at a magnification of 100,000×.

2.3.2. Determination of encapsulation efficiency

The encapsulation efficiencies of vancomycin in the drug-loaded nanoparticles were determined by dissolving 10 mg of drug-loaded nanoparticle powder in 2 ml of DMSO:deionized water (DIW) (1:1). Samples were rotated for at least 24 h at 50 rpm to ensure complete dissolution in aqueous DMSO solution. Empty nanoparticles were treated identically. The concentration of vancomycin in the resulting solution was determined by measuring the absorbance at 238 nm in a spectrophotometer (MQX 200, Bio-Tec Instruments, Winooski, VT, USA). The obtained values were then subtracted from the absorbance values of empty POE nanoparticles. All samples were analyzed in triplicate.

3. Results

The features of empty and vancomycin-loaded nanoparticles prepared by DESD and DESE techniques under various experimental conditions are explained here. This study describes the effect of various parameters such as sonication time/speed, organic solvents, organic to aqueous phase ratio and surfactants on the particle size of POE nanoparticles prepared by DESD and DESE methods. Parameters used to prepare POE nanoparticles were optimized based on their nano-size range, surface charge, spherical shapes and high encapsulation efficiencies in both methods. Comparison between the DESD and the DESE techniques was also discussed in detail.

3.1. Effect of sonication time and speed

To optimize the sonication speed, three kinds of emulsions were prepared by DESD method by using 45%, 55%, and 65% power intensity, when EA as a solvent, F 68 as an emulsifier and 333 μL of internal aqueous phase were used. Among these emulsions, nanoparticles prepared from 65% power intensity gave smaller size distribution (Table S1) than the rest. Sonication time was also optimized by sonicating the emulsions at various time points, such as, 90 s, 180 s and 360 s. POE nanoparticle emulsions sonicated for 90 s, gave smaller size distribution than the rest of the emulsions (Table S1). Hence, 65% power intensity and 90 s of sonication time were optimal factors of speed and time to prepare the POE nanoparticles by DESD method.

To optimize sonication speed and time in the preparation of POE nanoparticle emulsions by DESE method, various sonication intensities and time points were tested. Table S2 shows the average particle distributions and various process conditions. By increasing the sonication speed from 45% to 65%, mean particle size decreased from 607 nm to