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Enhancement of the dissolution and bioavailability from freeze-dried powder of a hypocholesterolemic drug in the presence of Soluplus

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ABSTRACT

Ezetimibe (EZT) is a cholesterol lowering agent that is characterized by a low aqueous solubility resulting in unpredictable dissolution and bioavailability. Hence, the enhancement of dissolution is a way to improve its bioavailability. Freeze-drying of EZT with Soluplus was the approach used in this study. Different ratios of EZT: Soluplus were prepared using freeze-drying or using a mortar and pestle (physical mixing). All of the prepared ratios were characterized using Differential Scanning Calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electtrom microscopy (SEM to make sure there was no interaction between the drug and polymer. An in-vitro release study was performed, and the best ratio with the highest release was chosen for in-vivo release in hypercholesterolemic rats. The cholesterol level was measured before as well as 7 and 14 days after receiving EZT by oral gavage using three groups of rats; the first group received pure EZT, the second group received freeze-dried EZT with Soluplus and the third group was the control group. Blood samples were also withdrawn from these three groups of rats. It was found that the freeze-dried EZT with Soluplus gave the highest protection from cholesterol and the highest plasma concentration compared to pure EZT. Additionally, it was found that the EZT:Soluplus 1:5 ratio had higher dissolution resulting in greater absorption of EZT, and hence, it had greater bioavailability and a higher capability of lowering the cholesterol to its normal level.

1. Introduction

Ezetimibe (EZT) is the first member of a new class of hypolipidemic agents. It selectively inhibits the absorption of bile and dietary cholesterol as well as related phytosterols of the intestine. EZT does not affect the concentrations of fat soluble vitamins, triglycerides or bile acids [1]. It is rapidly absorbed orally, and >80% is metabolized in the small intestine and liver to its active glucuronide metabolite via UDP-glucuronosyl transferase. Both the drug and its active metabolite undergo enterohepatic recycling in humans. EZT is classified as a class II drug according to its low solubility and absorption profile [2]. It shows unpredictable dissolution in the gastrointestinal tract, thus making its bioavailability unpredictable [3]. Different methods were used to increase the water solubility of EZT such as nano-suspensions [4], nano-emulsions [5], self nano-emulsifying granules [6], nano-particle [4,7], and a liquisolid technique [8]. However, most of these methods involve a lot of surfactants and co-surfactants, making these methods costly and at the same time adding the possibility of toxicity. Additionally, some of these methods involve many solvents and procedures, making them time-consuming and expensive on an industrial scale. In addition, EZT has poor drug loading and recrystallization during the storage time. Freeze-drying or lyophilization is a method that can be used to enhance solubility; it is a process of drying in which water is sublimed from the product after its freezing. The lyophilization cycle design has been divided into three stages. The first stage involves freezing, during which the liquid sample is cooled until pure crystalline ice forms from part of the liquid and the remainder of the sample is freeze-concentrated into a glassy state, where the viscosity is too high to allow further crystallization. The second stage is the called the primary drying since the ice that is formed during the freezing is removed by sublimation under vacuum at low temperatures, leaving a highly porous structure in the remaining amorphous solute that is typically 30% water. The second stage is carried out at pressures of 10^{-4} to 10^{-5} atm and with a product temperature from −45 °C to 20 °C, where sublimation happens as a result of heat and mass transfer processes. The final stage is the secondary drying stage, where most of the remaining water is desorbed from the glass as the temperature of the sample is

Abbreviations: ACUC, animal care and use committee; DSC, differential scanning electron microscope; EZT, ezetimibe; EZT-D4, deuterated ezetimibe; FD-EZT, freeze-dried ezetimibe; FTIR, Fourier transform infrared spectroscopy; PHY, physical mixture of ezetimibe and soluplus; SEM, scanning electron microscope; XRD, X-ray diffraction.

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gradually increased while maintaining low pressures. Ideally, the final product is a dry and easily reconstituted porous powder with a high surface area (ca. 10 m^2/g) [9,10]. Compared to other methods, the freeze-drying (lyophilization) method has been the most successful in terms of sales volume and number of products available on the market.

Soluplus®, a polyethylene glycol-polyvinyl caprolactam acetate grafted copolymer, is a novel thermoplastic internally plasticized amphiphilic polymer particularly made for use in formulating solid dispersions. It has the potential of forming solid solutions with numerous drugs that have poor water solubility, hence enhancing their dissolution [11]. Based on the nature of this carrier, Soluplus® is classified as a fourth generation solid dispersion polymer [13–17]. It has many favorable properties such as a low bulk density, high molecular weight, excellent flow properties, a low glass transition temperature of 68 °C, and readily absorbs water from air [18].

In this study, the aim is to enhance the dissolution and bioavailability of EZT using a freeze-drying technique with the aid of Soluplus. The study involves three stages including the preparation of freeze-dried EZT using Soluplus in different ratios, characterization of the freeze-dried EZT using different methods, selecting the best formula for in-vitro release, and testing the selected formula for bioavailability and pharmacodynamic parameters in rats

2. Materials and methods

2.1. Materials

Ezetimibe was purchased from Biocon Pharmaceuticals (India). Soluplus® was kindly provided by BASF (Obegi Chemicals, Ludwigshafen, Germany). BG100® β-glucuronidase solution from genetically-selected *Haliotis rufescens* (30,000,000 U/g protein with activities of >100,000 units per ml for beta-glucuronidase and <8000 units per ml for sulfatase) was purchased from Kura Biotech (USA). Ezetimibe-D4 was purchased from TRC (Canada). All other chemicals and solvents were of reagent-grade and were used without further purification.

2.2. Preparation of the freeze-dried formula

Different weights of Soluplus were dissolved in 50 ml of water, and then, the aqueous solutions were added gradually to 50 ml of an ethanolic solution of EZT to obtain different EZT:polymer weight ratios (1:1, 1:2 and 1:3). The resultant dispersions were dried initially in a Rotovap to remove the ethanol at 70 °C and 90 rpm for five minutes. Then, the semi-dried dispersion was pre-frozen in a deep freezer at −20 °C for approximately 6 h. The frozen products were then placed in a freeze-dryer (Telestar, Spain) at −80 °C for 8 h, followed by another 24 h at the same temperature (−80 °C) but under vacuum. Then, the products underwent 10 h of drying at room temperature under vacuum to remove the marginally contained adsorptively-bound water. After removing the samples from the freeze-dryer, all of the samples were passed between sieves with mesh numbers of 180 and 250 μm and then placed in desiccators at room temperature until further use.

2.3. Preparation of physical mixtures

A calculated amount of EZT was weighed and mixed using a mortar and pestle with a calculated amount of Soluplus to give the same ratios used in the freeze-dried method, and then, the mixtures were passed between sieves with mesh numbers of 180 and 250 μm. The physical mixtures were stored in desiccators for further use.

2.4. Determination of the drug content

The freeze-dried mixture was titrated with 10 ml of water and allowed to rest for 10 min with occasional swirling, and water was then added to produce 100 ml. After suitable dilution, 10 ml of the solution was taken and filtered through a membrane filter (0.45 μm), and the amount of dissolved drug was measured at 232 nm using a UV spectrophotometer. The drug content was determined from a standard plot. Sampling was performed in triplicate.

2.5. In-vitro release study

In -vitro release studies of EZT, FD-EZT, and physical mixtures were carried out in a type II (paddle) USP dissolution test apparatus at 50 rpm in 500 ml of 0.45% (*w*/*v*) SLS in 0.05 M acetate buffer (pH = 4.5) at 37 °C (11). Dissolution studies were carried out with 10 mg of pure drug (EZT) or an equivalent amount of preparations (FD-EZT and physical mixtures). Aliquots of 5 ml were withdrawn at specified time intervals of 5, 10, 20, 30, 40, 50, and 60, 90, 105, and 120 min and replaced with fresh media. The samples were filtered, using 0.45 μm micro-syringe filter paper, diluted as needed and analyzed spectrophotometrically at 232 nm for the dissolved drug. The dissolution studies were carried out in triplicate, and the percentage of drug release was also calculated.

2.6. Characterization of FD-EZT and the physical mixtures

2.6.1. Fourier transform infrared spectroscopy

Further characterization of polymorphic changes and drug interactions was performed using Fourier transform infrared (FTIR) spectroscopy for EZT, Soluplus, freeze-dried samples and their physical mixture counterparts using an IRAffinity-1 instrument (Shimadzu, Japan). Samples were blended with potassium bromide powder, and the test was conducted over a frequency range of 4700–340 cm^{-1} with 0.04 cm^{-1} resolution.

2.6.2. Powder XRD

For identification of the crystallinity and amorphous characteristics, powder XRD (PXRD) patterns for EZT, Soluplus, freeze-dried samples and their physical mixture counterpart systems were recorded using an Ultima IV XRD diffractometer (Rigaku, Japan) with cobalt radiation at a voltage of 40 kV and a current of 40 mA with diffraction angles from 0 to 40° and a step scan size of 0.02°.

2.6.3. Differential scanning calorimetry

Thermal analysis was conducted to assess the thermotropic properties and any interactions between EZT and Soluplus. Samples of 3–4 mg were sealed in 50 μl aluminum pierced pans at a heating rate of 5 °C/min and a temperature range of 10–200 °C. Empty aluminum pierced pans were used as a reference, and DSC thermograms were recorded using a Shimadzu differential scanning calorimeter (DSC-50, Japan).

2.6.4. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the morphological characteristics and surface properties of pure EZT, Soluplus, freeze-dried samples and their physical mixture counterparts. The samples were mounted on aluminum stub by double-sided sticky discs of conductive carbon, and then, they were coated with platinum by a sputter coater to render them electrically conductive. The electron beam was scanned over the specimen to produce a digital image using a Philips scanning electron microscope (model Quanta 200, Holland).

2.7. Pharmacodynamic activity in rats

The experiments for determining the pharmacodynamic activity in rats were in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC), and they were approved by the ACUC committee for animal studies of Jordan University of Science and Technology. Sixty Sprague-Dawley male rats with weights