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Preparation and characterization of cefquinome sulfate microparticles for transdermal delivery by negative-pressure cavitation antisolvent precipitation

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ABSTRACT

The objective of this study was to prepare micronized cefquinome sulfate by negative-pressure cavitation antisolvent precipitation to improve its permeability and transdermal absorbability. Dimethylsulfoxide and ethanol were used as the solvent and anti-solvent, respectively. The effects of operating parameters, including cefquinome sulfate concentration, volume ratio of antisolvent to solvent, antisolvent precipitation time, and applied negative pressure on the characteristics of the precipitate crystals, were evaluated using an orthogonal array design. Under the optimized conditions, micronized cefquinome sulfate with a mean particle size of 415.8 nm was obtained at a yield of 78%. The micronized cefquinome sulfate was characterized by scanning electron microscopy, X-ray diffraction, and differential scanning calorimetry. No obvious change in its chemical structure was observed, but crystallinity was reduced. The physicochemical properties and transdermal absorbability of crystalline cefquinome sulfate improved as a result of the particle size reduction by physical modification. This negative-pressure cavitation antisolvent precipitation process offers a promising technique for improving the physicochemical properties and anti-inflammatory activity of cefquinome sulfate.

1. Introduction

Cow mastitis, the most commonly occurring inflammation in dairy cows, is difficult to cure, and is therefore a major topic of research by international scholars [1]. Cow mastitis symptoms include inflammations of udder parenchyma and interstitial tissue, which are usually caused by mechanical stimulation, immersion of pathogenic microorganisms, and chemicophysical damage [2]. The main symptoms manifest as redness, swelling, and heat or pain of the udder, which directly result from reduced or completely halted lactation [3]. Cows are reared widely in many countries, and they contribute to a major portion of milk production in countries like China. In China, the livestock industry suffers huge economic losses because of low milk production caused by bacterial diseases, including mastitis [4].

Oral anti-inflammatory drugs are often mixed in with the animal feedstocks, but, because the cow has a complex four-chambered stomach, this leads to excessive drug loss in the metabolic process [5,6]. Injection of drugs is difficult because of the thick hides of the animals and requires professional skill. Use of transdermal preparations for treating mastitis can reduce the drug in the body's metabolism, reduce injury to the liver, has the advantage of convenient use, and does not easily produce drug resistance [7]. The poor solubility and low dissolution rate of active pharmaceutical ingredients in water is one of the most difficult and as-yet unsolved problems in pharmaceutical technology [8]. This drawback limits medical developments, such as new dosage forms for transdermal absorption. Cefquinome sulfate (Fig. 1) is a synthetic compound, the poor solubility of which leads to low oral absorption [9].

Cefquinome sulfate, a fourth-generation cephalosporin, has been solely researched for veterinary use owing to its antimicrobial activity against a broad spectrum of gram-positive and gram-negative bacterial species [10,11]. It has been also applied for treatment of acute mastitis and foot rot in cattle, and respiratory tract diseases in pigs, cows, and horses [12]. Cefquinome sulfate has been shown to have wide antibacterial activity and can achieve the effect of sterilization by inhibiting cell synthesis. *In vitro* antibacterial use of cefquinome sulfate indicates that it can inhibit both positive and negative common bacteria: for example, *Escherichia coli*, Bacillus, Klebsiella bacteria, Pasteurella, Salmonella species, *Serratia marcescens*, Streptococcus, the *Bacteroides fragilis* group, *Clostridium prazmowski*, Actinobacillus, and *Erysipelothrix rhusiopathiae* [11]. Cefquinome sulfate is an effective agent against many *in vitro* and *in vivo* bacteria. It is mainly used for treating cow mastitis, which is caused by sensitivity to bacteria, respiratory infections in pigs, and mastitis–metritis–agalactia syndrome in sows [10].

Microparticles have been popularly applied in enhancing the dissolution rate of drugs that have poor solubility in water. Owing to the

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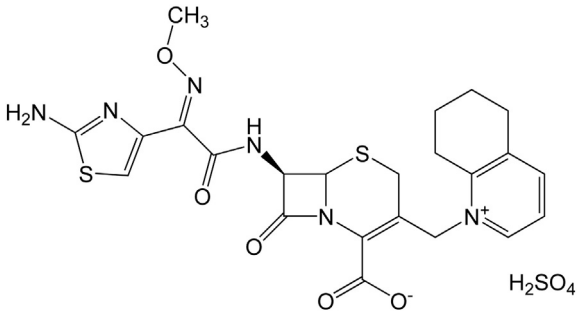

Fig. 1. Molecular structures of cefquinome sulfate.

reduced particle size of microparticles as compared with that of the raw drug particles, the interfacial surface area is increased, leading to improved solubility in water [13]. Bioavailability and oral efficacy of microparticles can be consequently improved by reduction in particle size, thereby allowing for direct usage of the poorly water-soluble drugs at smaller dosages or with more rapid response times [14,15]. Traditionally, many techniques have been applied for the preparation of microparticles, including ball milling [16], air jet pulverization [17,18], supercritical antisolvent technology [13–15], high pressure homogenization [19,20], reactive crystallization [21], and spray drying [22,23]. The process of mechanical comminution [16–18], a method of micronizing drugs, is now widely used in pharmaceutical manufacturing. Supercritical antisolvent technology has the disadvantage of large capital equipment investment, high pressure homogenization is limited by expensive equipment and solvent requirements, while reactive crystallization can only be applied to the preparation of acidic or alkaline drugs. In contrast, liquid antisolvent precipitation has the advantages of lower reagent consumption, higher process efficiency, and easy operation [24,25]. Cavitation is the phenomenon of sequential formation, growth and collapse of millions of tiny vapor bubbles (voids) in a liquid. It can occur whenever a liquid is used in a machine that induces pressure and velocity fluctuations in the fluid (e.g. pumps, turbines and propellers) [26]. Negative-pressure cavitation extraction has been used for extraction of biological active compounds from plant materials centuries. Negative-pressure cavitation antisolvent precipitation is based on a technique that uses negative pressure as its driving force. In this technique, a solvent and an anti-solvent are fully stirred by pumping air through the bottom of a reactor in which they are contained. The characteristic bubbles that form under conditions of negative pressure cavitation are used to keep the particle size of the powder uniform and tiny for a relatively long time.

The aim of this study was to prepare cefquinome sulfate microparticles using a negative-pressure cavitation antisolvent precipitation process and to evaluate their physicochemical properties and transdermal delivery *in vitro*. To optimize the negative-pressure cavitation antisolvent precipitation process, the effects of cefquinome sulfate concentration, volume ratio of antisolvent to solvent, antisolvent precipitation time, and applied negative pressure on the yield and mean particle size (MPS) of the cefquinome sulfate microparticles were studied using an orthogonal array design. The microparticles were further characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray diffraction (XRD). The transdermal delivery performance *in vitro* of cefquinome sulfate microparticles prepared was also evaluated.

2. Materials and methods

2.1. Drugs and chemicals

Cefquinome sulfate powder (purity of 98.5%) was provided by Zhejiang Hisun Pharmaceutical Co. Ltd. of China. Ethanol (analytically pure) was purchased from Tianli Chemical Reagent Company (Tianjin, China). N-Methyl-2-pyrrolidone, acetone, methanol, dichloromethane, trichloromethane, and dimethyl sulfoxide (DMSO) (analytically pure) were purchased from Bodi Chemical Reagent Company (Tianjin, China).

2.2. Preparation of skin

The skin permeation study was performed and approved by the Institutional Animal Ethics Committee. The thoracic skin of a dairy cow was obtained from Mengniu Pasture, Zhaodong, China. The skin was washed with normal saline and the hair on the skin was removed with an electric shaver before use.

2.3. Negative-pressure cavitation antisolvent precipitation apparatus

Micronized cefquinome sulfate was prepared by antisolvent precipitation. A schematic diagram of the apparatus is shown in Fig. 2. This consisted of an injection system (10–14), a negative-pressure cavitation reaction tank (8), and a vacuum system (1, 2, 6). The negative pressure valve (6) was first opened to maintain the pressure of the reaction tank (8) at −0.04 to −0.08 MPa, which measured by a vacuum gauge (YZ-40, Qingdao Dongfang Instrument Co., Ltd., China). The inlet valve (7) was then opened to allow the ethanol antisolvent to fill the negative-pressure cavitation reaction tank (8). The inlet valve (7) was then closed and the piston pump (2J-X 52/4.5, Zhijiang Science and Technology Instrument Factory, China) (19) was opened to control the air inflow speed in the range from 1 to 10 m^3/h. The intake valve (16) was then opened, which allowed air to flow through the intake branch pipeline (14), an annular conduit (10), and a nozzle (FD-1-1/8-Brass, Dalian Baoyuan Purification Technology Co., Ltd., China) (11). The liquid flow meter (LZZ-32, Changzhou Chengfeng Flowmeter Co., Ltd., China) (18) and piston pump (19) were then simultaneously opened to guide the mixed liquids (containing cefquinome sulfate and DMSO) into the negative-pressure cavitation reaction tank (8) by means of a branched pipeline (13), an annular conduit (12), and a nozzle (11). The mixed liquids then began to cavitate and become suspended. The intake valve (16) was used to control the air inflow and the piston pump (19) was employed to maintain the inlet fluid velocity. The mixed liquids were suddenly blended with ethanol antisolvent contained in reaction tank (8) when they were ejected through a microporous nozzle. Cefquinome sulfate submicroscopic particulates were finally separated out, suspended in the DMSO–ethanol solution under the effects of cavitation and suspension. During these procedures, secondary aggregation could not occur and so tiny, uniformly dispersed cefquinome sulfate particles were obtained. After importing the mixed liquids, the liquid flow meter (18) was closed and the piston pump (19) was switched off, having achieved its technical requirements with respect to the impacts of continuous cavitation and suspension. The intake valve (16), negative pressure valve (6), and relief valve (5) were then closed to balance the pressure in the reaction tank (8). The material discharge valve (15) was opened to export the residue solutions contained in the reaction tank. The suspension was centrifuged at 5000 rpm for 5 min, and the particles were then freeze-dried at −50 °C for 24 h. Each experiment was performed in triplicate.

2.4. Optimization of the antisolvent precipitation process

An $L_{16}(4)^5$ orthogonal experiment, performed with four factors and four levels, was selected for optimization of the operational parameters for cefquinome sulfate micronization using this process. Based on the results of preliminary experiments, we optimized the operating conditions and analyzed the statistical experimental results using an orthogonal array design with four factors applied using Design-Expert 7.0 software (Stat-Ease, Minneapolis, USA). The boundaries of the four factors were 100 to 400 mg/mL cefquinome sulfate concentration, 3 to 9 mL/mL volume ratio of antisolvent to solvent, −0.02 to −0.08 MPa