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Research paper

Fabrication of aceclofenac nanocrystals for improved dissolution: Process optimization and physicochemical characterization

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ABSTRACT

The purpose of the study is to optimize process variables and fabricate nanocrystals to improve dissolution rate of aceclofenac. Particle engineering was carried out to obtain pure drug nanocrystals of aceclofenac to overcome its poor dissolution behavior using different polymeric stabilizers. A Box-Behnken design was used to study the influence of process variables and further optimization was carried out. The physicochemical properties were evaluated including particle size distribution, powder X-ray diffractometry, scanning electron microscopy and dissolution studies. Preclinical investigation was also carried out in Wistar rats. All the identified process variables influenced the particle size and dissolution velocity of aceclofenac. Methyl cellulose (MC) and hydroxypropyl methyl cellulose (HPMC) were found very effective in preventing growth of crystals and improving the dissolution of aceclofenac. The optimized process variables predicted were 0.47%, 25 °C and 1070 rpm for stabilizer concentration, processing temperature and mixing speed respectively using MC as stabilizer. The optimized aceclofenac nanocrystals showed improved dissolution and reduced particle size (Q = 87.27 ± 0.83% and Mz = 54.23 ± 3.24 nm). Preclinical investigation using Wistar rats revealed statistically significant improvement of efficacy of optimized nanocrystals in terms of percentage inhibition of paw edema induced by carrageenan challenge indicating enhanced bioavailability through improved dissolution of aceclofenac nanocrystals.

1. Introduction

Delivering most of the new or already available pharmaceuticals to the human body has always been a technological and physical challenge. Specific characteristics of particles (size, shape, surface, crystal structure and morphology) are among the important factors to control technological and biopharmaceutical properties of drug products. Recent advances in powder and material processing and characterization methods allow formulators to design increasingly effective drug delivery systems. Moreover, the increasing demand for the targeted delivery of therapeutically active agents has been the key driver in particle engineering and processing within the pharmaceutical sector. One of the ongoing challenges for the formulation and preclinical evaluation of new chemical entities (NCEs) is that a significant number of lead candidates from drug discovery and high-throughput screening exhibit poor solubility [1–3]. With the increasingly lipophilic nature of the candidate drugs, solubility and dissolution rates have become the limiting factors that affect bioavailability of oral and parenteral formulations. While advanced technologies for enhancing delivery of poorly soluble drugs have included using prodrugs [4], polymorphs, solvates, co-crystals [5], salt formation [6], lipid based systems [7], micellar solubilization including self-emulsifying drug delivery systems [8], inclusion complexes [9], amorphous solid forms such as spray dried dispersions, etc. [10], a truly particle engineering-based solution remains particularly desirable to the pharmaceutical industry. Bosselmann and Williams III has recently reviewed contribution of nanotechnology, including nanonization of active pharmaceutical ingredients, in improving therapeutic outcome

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[11]. Among the top down methods for nanonization, high pressure homogenization, media milling and combination of these two strategies has been proved efficient in reducing particle size and improving solubility of poorly soluble drugs [12,13].

More recent research developments in engineering pharmaceutical nanosized drug particles have been based on a bottom-up approach, either by a droplet formation and evaporation approach, a solvent precipitation method or a combination [14–17].

Precipitation in the presence of special polymers to prevent crystal growth was successfully applied for some APIs, such as ibuprofen, itraconazole and ketoconazole [18,19]. The precipitation can also be performed at elevated temperatures (Evaporative Precipitation into Aqueous Solution, EPAS) [16]. Furthermore, organic drug solutions can be sprayed into cryogenic liquids using the SFL technology (SFL: spray freezing into liquid technology) [20]. Upon contact with the cryogenic liquid (e.g. liquid nitrogen) the droplets are frozen. A subsequent lyophilization step removes the organic solvent. Due to the mild process conditions this technology is suitable for temperature sensitive molecules, such as biological molecules [21]. Alternatively, precipitation can be performed in conjunction with centrifugation techniques (High gravity precipitation) [22].

Having particle dimensions in nanometer range and high particle surface area, nanocrystals can significantly increase the dissolution velocity and saturation solubility of insoluble drugs, therefore improving their bioavailability and biological effects. Another important advantage of nanocrystals is that when applied in oral administration, the increased adhesion to the gastrointestinal mucosa and the prolonged contact time will enhance absorption of drugs via the gastrointestinal tract [23]. Aceclofenac (2-[(2,6-dichlorophenyl) amine] phenylacetoxyacetic acid) is an orally effective non-steroidal anti-inflammatory drug (NSAID) of the phenyl acetic acid group, which possesses remarkable anti-inflammatory, analgesic and antipyretic properties [24,25]. Aceclofenac appears to be particularly well-tolerated among the NSAIDs, with a lower incidence of gastrointestinal adverse effects [26].

Aceclofenac exhibits very slight solubility in water and as a consequence it exhibits low bioavailability after oral administration [27,28]. Therefore, the improvement of aceclofenac dissolution from its oral solid dosage forms is an important issue for enhancing its bioavailability and therapeutic efficacy. Mutalik et al. [29], in an attempt to improve dissolution rate (and hence bioavailability) of aceclofenac, demonstrated the feasibility of co-crystals with chitosan. Surface solid dispersion of aceclofenac/microcrystalline cellulose also found to improve the dissolution rate of aceclofenac [30]. A careful literature review revealed insignificant attempts to prepare pure drug nanocrystals of aceclofenac for possible improvement of dissolution velocity and hence bioavailability.

In the present work, particle engineering was carried out to obtain pure drug nanocrystals of aceclofenac using a standard, simple and scalable bottom up technique to overcome its water insolubility and poor dissolution behavior. The stabilizers used in this study were semisynthetic non-ionic polymers [Methyl cellulose (MC) and Hydroxypropyl methyl cellulose (HPMC)]. The various process variables (factors) were identified and optimization was carried out to obtain stable aceclofenac nanocrystals. Principles of statistical design of experiments (DOE) were exploited to study the influence and optimization of the identified process variables during fabrication of the nanocrystals. The physicochemical properties were evaluated including particle size distribution, powder X-ray diffractometry (PXRD), scanning electron microscopy and *in vitro* dissolution studies. In vivo performance was also studied in Wistar albino rats.

2. Methods

2.1. Design of experiment

A Box-Behnken design was used to study the influence of process variables and their optimization for synthesis of aceclofenac nanocrystals. In this design three numeric factors were evaluated, each at three levels and one categoric factor was evaluated at two levels. Concentration of stabilizer (X_1), processing temperature (X_2), and mixing speed (X_3) were selected as numeric factors and stabilizer type (X_4) was selected as categoric factor. The graphic mean size (Mz) and percent dissolved at 1 h (Q) were chosen as dependent responses. Concentration of stabilizer was evaluated at 0.1% w/v (−1), 0.3% w/v (0) and 0.5% w/v (+1); processing temperature was evaluated at 5 °C (−1), 15 °C (0) and 25 °C (+1); mixing speed was evaluated at 100 rpm (−1), 600 rpm (0) and 1100 rpm (+1). The levels for these parameters were determined from preliminary trials. Design-Expert software (Version. 8.0.7.1, Stat-Ease Inc., Minneapolis, MN) was used for the generation and evaluation of the statistical experimental design. To avoid the bias, experiments were run in random order as suggested by the software.

2.2. Preparation of aceclofenac nanocrystals

Nanocrystals were produced by adding aqueous solution (0.1%, 0.3% or 0.5% w/v) of polymeric stabilizer (MC or HPMC) in acetone solution of aceclofenac (10% solution) under magnetic stirring (100, 600 or 1100 rpm) at different processing temperature (5°, 15° or 25 °C) as per Table 1. Rate of antisolvent addition was kept constant at 0.6–0.8 ml/min. The synthesized drug nanoparticles were separated by centrifugation followed by vacuum drying.

2.3. Particle size analysis

The size of drug nanoparticles was measured by dynamic laser light scattering (Nanoparticle size analyzer, Microtrac flex). Before analysis, the drug suspension was diluted by purified water to 0.2 mg/ml.

2.4. In vitro dissolution studies

The *in vitro* dissolution studies were carried out using USP Type I (basket type) dissolution apparatus. The dissolution media used was 900 ml of 1% sodium lauryl sulfate (SLS) solution in distilled water. The studies were carried out for 60 min. The dissolution medium was kept in thermostatically controlled water bath, maintained at 37 ± 0.05 °C. Basket rotation was adjusted to 100 rpm. At definite intervals, 5 ml samples were withdrawn and analyzed spectrophotometrically at 274 nm for the drug release. At each time of withdrawal, 5 ml of fresh corresponding medium was replaced into the dissolution flask to maintain the sink condition.

2.5. Scanning electron microscopy (SEM)

Scanning electron microscopy was used to characterize the particle morphology of the unprocessed drug as well as the fabricated drug nanoparticles. A small fraction of each drug powder sample was fixed on a double-sided conductive carbon tape and sputter-coated with 5 nm of a Pt–Pd alloy. Micrographs were obtained on a Zeiss DSM 982 Field Emission Gun Scanning Electron Microscope (Carl Zeiss AG, Germany).