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Research paper

Preparation and, *in vitro*, preclinical and clinical studies of aceclofenac spherical agglomerates

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ABSTRACT

Aceclofenac agglomerates were prepared by spherical crystallization technique using a three solvent system comprising acetone: dichloromethane (DCM): water (bridging liquid, good solvent and bad solvent, respectively). Hydroxypropyl methylcellulose-50 cps (HPMC) in different concentrations was used as hydrophilic polymer. The effect of speed of rotation and amount of bridging liquid on spherical agglomeration were studied. The agglomerates were subjected to various physicochemical evaluations such as practical yield, drug content, particle size, loss on drying, porosity, IR spectroscopy, differential scanning calorimetry, X-ray diffraction studies, relative crystallinity, scanning electron microscopy, micromeritic properties, solubility and dissolution studies. The agglomerates showed improved micromeritic properties as well as dissolution behaviour in comparison to conventional drug crystals. The optimized agglomerates (F-9) showed good sphericity as well as high drug release, and hence they were compressed into tablets by direct compression. The tablets were found within the limits with respect to various physicochemical parameters. The dissolution rate of prepared tablets was better than that of marketed tablet and pure drug. The optimized agglomerates and tablet formulations were found to be stable for 6 months under accelerated conditions. The *in vivo* studies (preclinical pharmacokinetics, pharmacodynamics and toxicity studies, and clinical pharmacokinetics) of optimized agglomerates were carried out. The results of preclinical studies revealed that the agglomerates provided improved pharmacodynamic and pharmacokinetic profiles of drug besides being nontoxic. The results of pharmacokinetic studies of optimized tablet in human subjects indicated improved pharmacokinetic parameters of drug in comparison with that of marketed tablet.

1. Introduction

Direct tabletting of pharmaceutical materials is desirable to reduce the cost of production [1]. To succeed in direct compression, particle modification of a drug is required to impart the formula sufficient flowability and compressibility. The preparation of spherical agglomerates has come into the forefront of interest because the habit of the particles (form, shape, particle size distribution, surface, etc.) can be changed by the crystallization process [2]. The spherical crystallization is an efficient technique for particle design for direct tabletting, during which crystallization and agglomeration can be carried out in one-step. The physical properties of the agglomerated crystals can be controlled simultaneously without using any filler or binder. Spherical crystallization can be achieved by various methods such as spherical agglomeration, emulsion solvent diffusion, ammonia diffusion, and neutralization methods [3–5]. The spherical crystallization technique has already been successfully applied to improve the micromeritic properties of several drugs such as acebutolol hydrochloride, celecoxib, and mefenamic acid etc [6–8]. Besides modifying the size and shape, flowability, packability and bulk density of the particles, this technique can also be exploited to increase solubility, dissolution rate and hence bioavailability of poorly soluble drugs [9].

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. It is used in the treatment of osteoarthritis and inflammatory disease of the joints. It exhibits very slight solubility in water, poor flow and compression characteristics [10]. Previously we reported spherical crystallization of aceclofenac using polyvinylpyrrolidone and sodium alginate. The agglomerates were subjected only to

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physicochemical and preclinical studies [11]. It has been reported that hydroxypropyl methylcellulose is a good polymer for spherical crystallization [7,12]. Therefore the objectives of this study were (1) to prepare spherical agglomerates of aceclofenac using hydroxypropyl methylcellulose by solvent change method and (2) to conduct detailed physicochemical, preclinical (pharmacodynamic, pharmacokinetic and toxicological) and clinical pharmacokinetic investigations in order to aid direct compression, to improve solubility, dissolution rate and hence bioavailability, and consequently to achieve cost effectiveness and patient compliance for aceclofenac. Solvent change method was chosen as it is easy, common and faster related to other methods [13]. The solution of the material in a good solvent is poured in a poor solvent under controlled condition, to favour formation of fine crystals. The agglomerates are formed by agitating the crystals in a liquid suspension and adding a bridging liquid, which preferentially wets the crystal surface to cause binding. The agglomerates would be spherical if the amount of the bridging liquid and the rate of agitation are controlled [14].

2. Materials and methods

2.1. Materials

Aceclofenac and hydroxypropyl methylcellulose-50 cps (HPMC) were obtained as gift samples from Lupin Research Park, Pune, India. Acetone and dichloromethane (DCM) were purchased from Qualigens, Mumbai, India. All other chemicals used were of analytical grade.

2.2. Preparation of spherical crystals

The composition of different batches of spherical crystals is given in Table 1. A solution of aceclofenac in acetone (0.75 g in 3 ml) was added to a solution of HPMC in DCM. Drug was crystallized by adding the above solution to a 500 ml capacity beaker containing 100 ml of distilled water. The mixture was stirred continuously for a period of 0.5 h using a controlled speed stirrer (600–1000 rpm) to obtain spherical agglomerates. The agglomerates were separated by filtration and dried at room temperature. The amount of DCM, speed of agitation and amount of polymer were altered to get the agglomerates of desired properties. The range for each parameter was selected based on our previous studies [11,12].

2.3. Physicochemical and micromeritic properties of agglomerates

The practical yield of agglomerates was calculated by weighing the prepared agglomerates after drying stage. For the determination of drug content, agglomerates (100 mg) were powdered and dissolved in 10 ml phosphate buffer (pH 6.8) and vortexed for 20 min. The solution was filtered and after sufficient dilution with phosphate buffer (pH 6.8) analyzed for drug content. The average particle size was determined by using Ankersmid CIS-50 particle size analyzer (Ankersmid, USA). To determine the primary particle size, the agglomerates were disintegrated in an aqueous solution of Tween 80 (0.05%) using Ultrasonicator (VC 130, Sonics and Materials Inc., USA) for 30 s at 100 W before determining the particle size. Loss on drying (LOD) was determined by using Halogen Moisture Analyzer (Mettler Toledo, USA).

The loose bulk density (LBD) and tapped bulk densities (TBD) were determined by using Density apparatus (Serwell, Bangalore, India). The Carr's index (%) and the Hausner's ratio were then calculated by using LBD and TBD [15,16]. The angle of repose of drug powder and the agglomerates were assessed by fixed funnel method [16]. The porosity was calculated by determining bulk density and true density [17]. For solubility determination, an excess quantity (about 750 mg) of aceclofenac agglomerates was taken in 10 ml of distilled water or 0.1 N hydrochloric acid (HCl) in the vials. The vials were shaken in a water bath (100 agitations/min) for 24 h at room temperature. The solution was then passed through a 0.45 μ-membrane filter and the amount of the drug dissolved was analyzed after suitable dilutions.

Infrared (IR) spectroscopy was conducted using a Shimadzu FTIR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan) and the spectrum was recorded in the wavelength region of 4000–400 cm^{-1}. The procedure consisted of dispersing a sample (drug alone, physical mixture of drug and polymer or spherical agglomerates) in KBr and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained. Differential scanning calorimetry (DSC) analysis was performed using DSC-60 (Shimadzu, Tokyo, Japan) calorimeter. The instrument comprises calorimeter (DSC 60), flow controller (FCL 60), thermal analyzer (TA 60) and operating software (TA 60). The samples (drug alone, physical mixture of drug and polymer or spherical agglomerates) were heated in sealed aluminum pans under nitrogen flow (30 ml/min) at a scanning rate of 5 °C/min from 25 °C to 250 °C. Empty aluminum pan was used as a reference. The physical mixtures for IR and DSC studies were prepared by triturating drug and polymer in a dried mortar for 5 min. The X-ray diffraction (XRD) patterns of pure aceclofenac and F-9 agglomerates were recorded using Philips X-ray diffractometer (Model: PW 1710) with a copper target at 30 kV voltage and 30 mA current. The scanning speed was 1° per minute. The shape and surface morphology of the spherical agglomerates were studied by scanning electron microscopy (JEOL, JSM 50A, Tokyo, Japan).

The relative crystallinity of pure aceclofenac and F-9 agglomerates was determined by following the already reported method [18,19]. The crystalline substances show sharp peaks, but amorphous substances only show a "halo" and partially amorphous substances show both. So by comparing the intensity of the powder X-ray diffraction patterns the relative crystallinity can be determined. By mixing the drug powder with an internal standard, a quantification can be carried out eliminating the effects caused by differences in sample density or sample preparation. In this

Table 1
Composition of spherical agglomerates

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10	F-11
Aceclofenac (mg)	750	750	750	750	750	750	750	750	750	750	750
Acetone (ml)	3	3	3	3	3	3	3	3	3	3	3
HPMC (mg)	50	50	50	50	50	50	25	50	75	100	–
DCM (ml)	1	1	1	0.5	1	1.5	1	1	1	1	1
Water (ml)	100	100	100	100	100	100	100	100	100	100	100
Stirring speed (rpm)	600	800	1000	800	800	800	800	800	800	800	800

HPMC, Hydroxypropyl methylcellulose; DCM, Dichloromethane.