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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.

study magnesium oxide (10% w/w) was used. The scans (2θ-θ) were recorded with copper radiation using D8 X-ray powder diffractometer equipped with D8 TOOLS software, a theta compensating slit and a silicon detector (Bruker AXS, Germany). The scans were recorded from 2 to 50°, with a step size of 0.03°2θ and a count time of 0.5 s at 25 °C.

2.4. Dissolution studies of spherical agglomerates

The *in vitro* dissolution studies were carried out using 8 Station USP type-1 dissolution apparatus (Electrolab, Mumbai, India). The dissolution study was carried out in 900 ml of 0.1 N HCl, distilled water and phosphate buffer (pH 6.8). The dissolution medium was kept in a thermostatically controlled water bath, maintained at 37 ± 0.5 °C. The basket containing agglomerates (equivalent to 100 mg aceclofenac) within muslin cloth was rotated at 75 rpm. At predetermined time intervals between 0 and 180 min, 5 ml of dissolution medium was withdrawn and analyzed for the drug release. At each time of withdrawal, 5 ml of fresh corresponding medium was replaced into the dissolution flask.

2.5. Preparation of tablets and their in vitro evaluation

Aceclofenac tablets of optimized agglomerates (each containing 100 mg of aceclofenac) were prepared. The tablet formulation A contained only spherical agglomerates. Tablets B and C contained sodium starch glycolate, 2 mg and 4 mg, respectively, along with spherical agglomerates. The ingredients were blended and compressed into tablets employing direct compression method (10 Station compression machine, Cadmach, Ahmedabad, India) using 8 mm flat-faced punches.

The thickness and diameter of the tablets were measured using digital vernier calipers. The crushing strength, friability and disintegration time of the tablets were determined using Monsanto hardness tester, friabilator and disintegration test apparatus, respectively [20]. Weight variation test was carried out by weighing 20 tablets individually and then calculating the average weight. Drug content determination and *in vitro* dissolution study of tablets were carried out in phosphate buffer (pH 6.8) in a similar way as explained for agglomerates in the previous section.

2.6. Stability studies

After determining the drug content, the formulations (F-9 agglomerates and tablet formulation C) were charged for the accelerated stability studies according to ICH guidelines (40 ± 2 °C and 75 ± 5% RH) for a period of 6 months in a stability chamber (Thermolab, Mumbai, India). The optimized formulations were placed in USP type-1 flint vials and hermetically closed with bromobutyl rubber plugs and sealed with aluminum caps. The samples were withdrawn at 15, 30, 60, 90 and 180 days and evaluated for the drug content (for F-9 and C formulations) and *in vitro* drug release (for C formulation).

2.7. Preclinical studies

The preclinical studies (anti-inflammatory, analgesic, pharmacokinetic and sub-acute toxicity studies) were carried out in Wistar rats and Swiss albino mice. Male Wistar rats (weighing 200–250 g) and Swiss albino mice (weighing 25–30 g) were obtained from the Central Animal House, Manipal University, Manipal. They were housed in elevated wire cages, four animals per cage, with free access to food (Lipton Feed, Mumbai, India) and water. The preclinical study protocol was approved by the Institutional Animal Ethical Committee, Kasturba Medical College, Manipal (Approval No: IAEC/KMC/06/2005–2006).

2.7.1. Anti-inflammatory activity

Carrageenan-induced rat paw edema model was used to assess the anti-inflammatory effect of the drug when administered in plain form and as spherical agglomerates [21]. The overnight fasted rats were divided into 3 groups ($n = 6$) and treated as follows:

Group I: Pure aceclofenac (10 mg/kg) in 0.5% sodium carboxymethylcellulose (CMC); p.o.

Group II: Aceclofenac agglomerates (10 mg/kg) in 0.5% CMC; p.o.

Group III (Control): 2.5 ml of 0.5% CMC; p.o.

After 30 min of drug administration, rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan in saline into the plantar site of the left hind paw. The paw volumes were measured with a plethysmometer, prior to administration of carrageenan and after 1, 2, 3, 4 and 5 h of administration. The percent inhibition of edema for all time intervals was calculated.

2.7.2. Analgesic activity

The writhing test was used to evaluate the analgesic activity of the drug when administered in plain form as well as spherical agglomerates [21,22]. The overnight fasted mice were divided into 3 groups ($n = 6$) and treated orally as given in the procedure of anti-inflammatory activity. After 1 h of dose, they were injected with 1% acetic acid (0.1 ml/10 g; i.p.). Then the number of writhings was recorded for 30 min. The analgesic activity was evaluated in terms of the percentage of writhe inhibitions.

2.7.3. Pharmacokinetic study

The overnight fasted rats were divided into 3 groups ($n = 6$) and treated as follows:

Group I: Pure aceclofenac (10 mg/kg) in 0.5% CMC; p.o.

Group II: Aceclofenac agglomerates (10 mg/kg) in 0.5% CMC; p.o.

Group III: Powdered marketed aceclofenac formulation (10 mg/kg) in 0.5% CMC; p.o.

Then blood samples were collected at predetermined intervals of 0.5, 1, 2, 4, 6 and 8 h of post-dose into heparinized tubes from the orbital sinus. The plasma was separated immediately using cold centrifugation (Remi Equipments Ltd., Mumbai) at 3000 rpm for 15 min and the plasma was stored at −72 °C until analysis.

2.7.4. Sub-acute toxicity study

The sub-acute toxicity studies were carried out in Wistar rats [23]. The rats were divided into 3 groups ($n = 6$) and treated orally for 30 days as in Anti-inflammatory activity (Section 2.7.1). Hematological parameters such as total red blood cell count (RBC), white blood cell count (WBC) and hemoglobin were estimated in blood collected from the retro orbital sinus into heparinized tubes on 15 and 30 days of the study. On the first and last day of the treatment, blood glucose levels were checked using ACCU-CHEK® sensor (Roche, USA) in overnight fasted rats before administration of drug. On the 31st day, the blood samples were collected from each rat individually into non-heparinized tubes and serum levels of alanine transaminase (ALT), aspartate transaminase (AST), urea, creatinine, cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were analyzed using Autoanalyzer (Hitachi 911, Tokyo, Japan).

2.8. Pharmacokinetic study in healthy human volunteers

The pharmacokinetics of the developed formulation (Tablet C) in comparison with marketed tablet (Hifenac Tablets, Intas Laboratories, Mumbai, India) was carried out in six healthy human volunteers (age: 20–35 years; weight: 60 ± 5 kg). The study protocol was approved by Kasturba Hospital Ethics Committee, Kasturba Medical College, Manipal (Approval No. KHEC No: 57/2006). The study

design was randomized, crossover and single blinded. The overnight fasted volunteers were administered with the prepared tablets and 2 ml of blood sample was withdrawn immediately and at different time intervals (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 h post-dose). After 4 h of dosing, the volunteers were continued with controlled diet. A similar procedure was performed on same volunteers by administering marketed tablets after a time gap of 7 days. The blood samples were collected in the Vacutainers containing EDTA as anticoagulant. The plasma was separated immediately by using cold centrifuge at 3000 rpm for 15 min and it was stored at −70 °C till the analysis.

2.9. Analysis of drug

A sensitive high performance liquid chromatographic (HPLC) method was used to analyze aceclofenac in plasma [24]. The HPLC system (Shimadzu Class *VP* series having Class *VP* 6.12 version software) with two pumps (LC-10AT *VP*), a variable wavelength programmable UV/vis detector (SPD-10A *VP*), a system controller (SCL-10A *VP*) and an RP C-18 column (Hypersil BDS C_{18}; 250 cm × 4.6 mm; 5 μ) was used. Mobile phase was methanol + 0.3% TEA pH 7.0 (60:40 v/v) and flow rate was 1.0 ml/min. The detection wavelength was 275 nm.

Preparation of stock and working standard solutions: From the stock solutions (1 mg/ml), working standard solutions were prepared to contain 1, 2, 5, 10, 20, 30, 50 and 70 μg/ml of aceclofenac and 500 μg/ml of venlafaxine (internal standard) in methanol and water (80:20 v/v; diluent).

Preparation of calibration standards in plasma curve: The plasma (95 μl) was pipetted into a micro-centrifuge tubes and spiked with 5 μl of the working standard solutions of drug. To this, 25 μl of 500 μg/ml internal standard and 200 μl of acetonitrile were added and mixed for a minute. Then 675 μl of diluent was added to make up the volume up to 1.0 ml and vortexed for 60 s. After cold-centrifugation of plasma sample, the supernatant layer was separated and injected to the HPLC system. Standard curves were obtained by using drug/internal standard peak area ratio and theoretical concentration.

Preparation of sample solutions: To 100 μl of plasma, 25 μl of internal standard solution (500 μg/ml) and 200 μl of acetonitrile were added and mixed for a minute. To this, diluent was added (675 μl) up to 1 ml. The resulting solution was vortexed for 60 s and centrifuged at 10000 rpm for 10 min. The supernatant layer was separated and analyzed using HPLC system. The response factor (peak area ratio; drug peak area to the internal standard peak area) of the standard solution and the sample was calculated and the concentration of the aceclofenac present in the plasma samples was calculated from the calibration curve.

The blank plasma samples were analyzed prior to the analysis of aceclofenac standard preparations. No interference from the blank plasma was observed for the analysis of drugs. The peaks were well resolved in both rat (retention time: 10.20 min for aceclofenac and 18.51 min for venlafaxin) and human plasma (retention time: 9.80 min for aceclofenac and 17.58 min for venlafaxine). The method was validated with respect to accuracy (99.83 ± 0.87%), precision (within 1%), and level of detection (1 ng/ml) and level of quantification (5 ng/ml).

2.10. Statistical analysis

Student's *t*-test was employed to analyze the results (Graph Pad Instat Software-1.13 version). Difference below the probability level 0.05 was considered statistically significant. The pharmacokinetic parameters were calculated by using PK Solutions 2.0™ Non-compartmental pharmacokinetic data analysis software.

3. Results and discussion

3.1. Optimization of agglomerization process

The choice of the best solvent was done based on the available literature on solubility of aceclofenac and miscibility of the solvents. Acetone, DCM and water were selected as good solvent, bridging liquid and bad solvent, respectively. Selection of bridging liquid should be such that it should be immiscible with the poor solvent i.e. water and the drug should have slight solubility in it [14]. In order to impart strength and sphericity besides increasing the solubility and drug release from aceclofenac agglomerates, HPMC was selected [7].

Different formulations (F-1–F-3) were prepared to select optimum speed of rotation. The impact of agitation speed on formation of spherical agglomerates was such that on increasing the agitation speed beyond 800 ± 20 rpm (i.e. 1000 ± 20 rpm), spherical agglomerates with smaller diameter (mean particle size: 250.81 ± 37.34 μm) and rough surface were produced (Fig. 2A and B). Fine powder was present along with irregular shaped agglomerates, which could be due to high shear force of stirrer. The agglomerates with good sphericity and flowability were produced at agitation speed of 800 ± 20 rpm (mean particle size: 460.30 ± 54.26 μm) (Fig. 2C and D). When the agitation speed was reduced to 600 ± 20 rpm, large irregular agglomerates were produced, where the shear energy may not be sufficient for the formation of good crystals. F-2 agglomerates (prepared with 800 rpm) produced spherical agglomerates with uniform size and possessed good flowability and compressibility (mean particle size: 658.64 ± 30.70 μm; compressibility index: 8.55%; Hausner's ratio: 1.12; angle of repose: 21.56).

The agglomerates F-4, F-5 and F-6 were prepared to select favourable concentration of bridging liquid, DCM. The amount of bridging liquid is a critical parameter in the spherical crystallization technique. Addition of a very small amount (0.5 ml) of DCM led to improper wetting of the crystals and irregular shaped agglomerates were obtained (mean particle size: 312.25 ± 20.15 μm). When 1 ml of DCM was used, uniform spherical agglomerates were formed (mean particle size: 469.13 ± 26.57 μm). With 1.5 ml of DCM, again irregular and large agglomerates were produced (mean particle size: 550.28 ± 18.46 μm). This indicates that 1 ml of bridging liquid is optimum and agglomeration might result from the coalescence of crystals with the addition of bridging liquid [13]. This is supported by good flow properties of formulation F-5 (prepared with 1 ml DCM) (compressibility index: 9.01%; Hausner's ratio: 1.05; angle of repose: 20.58). Hence based on variations made with rpm and

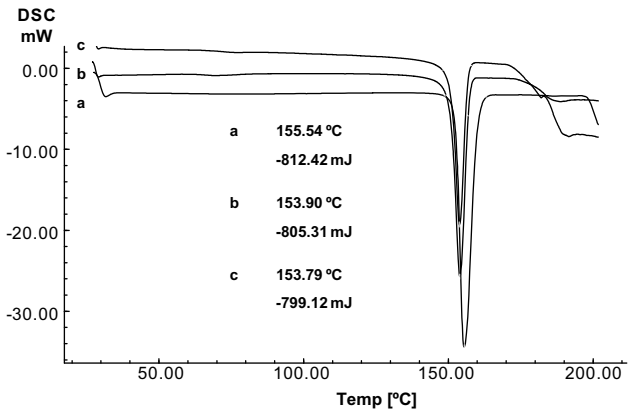

Fig. 1. DSC thermograms. a, Plain aceclofenac; b, physical mixture of aceclofenac and HPMC; c, F-9 agglomerates.

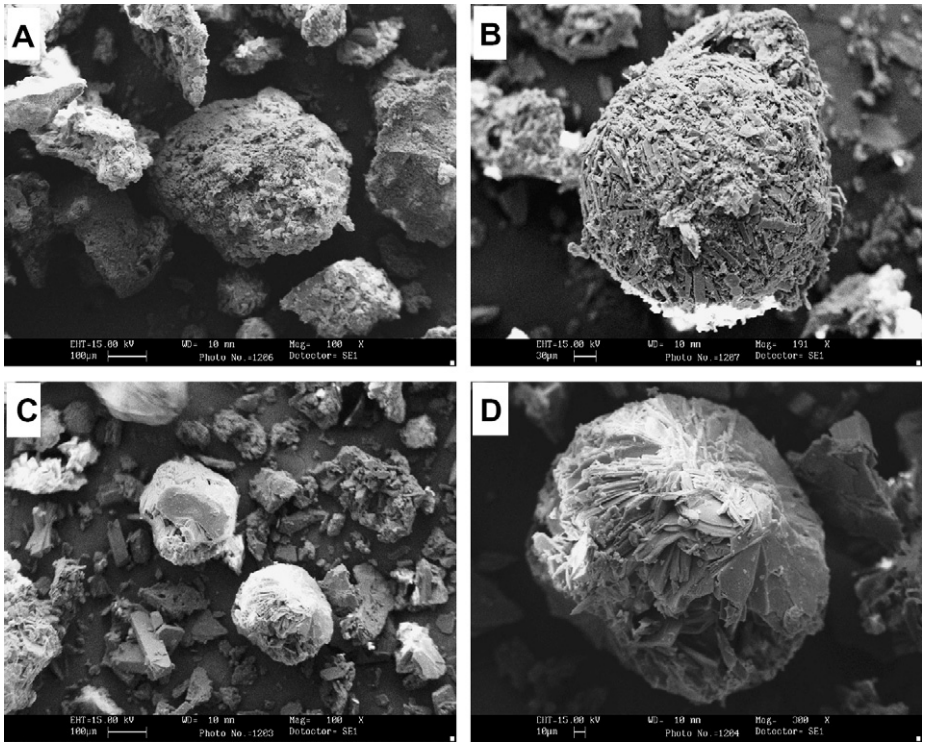

Fig. 2. SEM photomicrographs of aceclofenac agglomerates under low magnification (A and C) and high magnification (B and D) A and B, F-3 agglomerates (prepared at 1000 rpm); C and D, F-9 agglomerates.

DCM quantity, further formulation development was carried out at optimized speed of rotation (800 rpm) using 1 ml of bridging liquid by incorporating HPMC as hydrophilic polymers.

3.2. Physicochemical and micromeritic properties of agglomerates

The practical yield was found satisfactory and ranged from 85.72% to 90.47%. The drug content was decreased as polymer concentration in the agglomerates was increased. The value ranged from 90.15% to 98.55% and was higher in formulation F-11. The presence of HPMC in spherical agglomerates influenced the particle size of resultant agglomerates (Table 2). As the concentration of the HPMC increased, the size of the agglomerates increased. The presence of HPMC on the particle surface increases particle–particle interaction, causing faster squeezing out of DCM to the surface, resulting in increased particle size [7]. The primary particle size was also increased with an increase in polymer content. However, the primary particle size of all the tested agglomerates was lower than that of pure aceclofenac. These results are in accordance with previous report, where increased concentrations of HPMC produced an increase in the primary crystal size of paracetamol [25]. The porosity (%) values of the agglomerates decreased with an increase in HPMC content, but, however were greater in comparison with pure aceclofenac. The LOD values of the agglomerates ranged between 0.35 and 0.44% and indicated that the presence of HPMC reduced the residual moisture content of aceclofenac agglomerates [25].

Pure drug exhibited poor flowability and compressibility as indicated by high value of Carr's index (29.99%), Hausner's ratio (1.43) and angle of repose (46.92°). This could be due to the irregular shape and small size of powder, which put hurdles in the uniform flow of powder from the funnel. The agglomerates prepared with HPMC (F-7–F-10) showed improved flowability (Carr's index: 4.99–9.99%; Hausner's ratio: 1.04–1.11; Angle of repose: 19.64°–22.26°) when compared to pure drug. The improved flowability of spherical agglomerates may be due to good sphericity and more size of agglomerates. During the tapping process, smaller agglomerates might have infiltrated into the voids between larger particles, which could result improved packability. Among different agglomerates prepared, F-9 formulation showed maximum flowability as evident by low values of Carr's index, Hausner's ratio and angle of repose. The agglomerates without HPMC (F-11) did not show improvement in the flow properties.

The results of solubility study (Table 2) revealed that the spherical agglomerates showed increased solubility compared to the pure drug. In addition, as the concentration of HPMC was increased

Table 2
Physicochemical and micromeritic properties of spherical agglomerates

Formulations	Particle size (μm)	Primary particle size (μm)	Porosity (%)	Solubility		
				Water (μg/ml)	0.1 N HCl (μg/ml)	PB (mg/ml)
F-7	423.90 ± 11.40	1.45 ± 0.35	75.00 ± 2.50	85.65 ± 2.12	34.25 ± 1.15	13.41 ± 0.84
F-8	455.43 ± 12.82	3.89 ± 0.55	71.00 ± 2.00	87.99 ± 2.76	38.12 ± 1.55	13.54 ± 1.27
F-9	470.26 ± 10.25	5.41 ± 1.22	69.00 ± 1.50	93.32 ± 1.28	47.60 ± 1.25	13.88 ± 1.46
F-10	495.76 ± 13.47	6.55 ± 1.52	67.00 ± 1.50	98.07 ± 1.04	55.92 ± 2.02	14.27 ± 1.35
F-11	125.55 ± 45.54	0.99 ± 0.13	68.00 ± 2.00	78.85 ± 2.25	14.55 ± 1.25	11.73 ± 1.45
Aceclofenac	23.57 ± 10.46	21.50 ± 7.58	42.50 ± 1.50	75.59 ± 2.93	15.24 ± 1.55	10.58 ± 1.02

All the values are expressed as mean ± SEM, $n = 3$; PB, Phosphate buffer, pH 6.8.

in the agglomerates, the solubility of drug also increased. This may be due to the improved porosity, decreased primary particle size and partial amorphization of drug in agglomerates as demonstrated by DSC and XRD studies. This may also be due to the improved wettability of spherical agglomerates in the presence of HPMC. These results are in accordance with earlier report, where the solubility of tolbutamide was increased in its agglomerated form [3]. The F-3 agglomerates, prepared at high speed of rotation (water: 85.21 ± 2.65 μg/ml; 0.1 N HCl: 34.50 ± 1.75 μg/ml; phosphate buffer, pH 6.8: 13.08 ± 0.36 mg/ml), and the agglomerates prepared without HPMC (F-11) did not show noticeable improvement in solubility in spite of their less particle size (Table 2).

The prominent IR peaks (Wave numbers, cm^{-1}) of drug, drug-polymer physical mixture and spherical agglomerates are given below.

Aceclofenac: 3313.3, 2970.2, 2935.5, 1716.5, 1589.2, 1506.3, 1479.3, 1344.3, 1280.6, 1255.6 and 665.4; Aceclofenac + HPMC: 3278.8, 2970.2, 2935.5, 1710.7, 1589.2, 1506.3, 1479.3, 1344.3, 1280.6, 1255.6 and 665.4; F-9 agglomerates: 3278.8, 2970.2, 2935.5, 1714.6, 1589.2, 1506.3, 1479.3, 1344.3, 1280.6, 1255.6, 665.4. The IR spectra of all the tested samples showed the prominent characterizing peaks of pure aceclofenac which confirm that no chemical modification of the drug has been taken place.

In the DSC studies (Fig. 1), pure aceclofenac showed a sharp endotherm at 155.54 °C corresponding to its melting point. There was no appreciable change in the melting endotherms of physical mixture and spherical agglomerates compared to that of pure drug (Aceclofenac + HPMC = 153.90 °C; F-9 agglomerates = 153.79 °C). This observation also confirmed the absence of chemical interaction of drug with additives during agglomeration process, further supporting

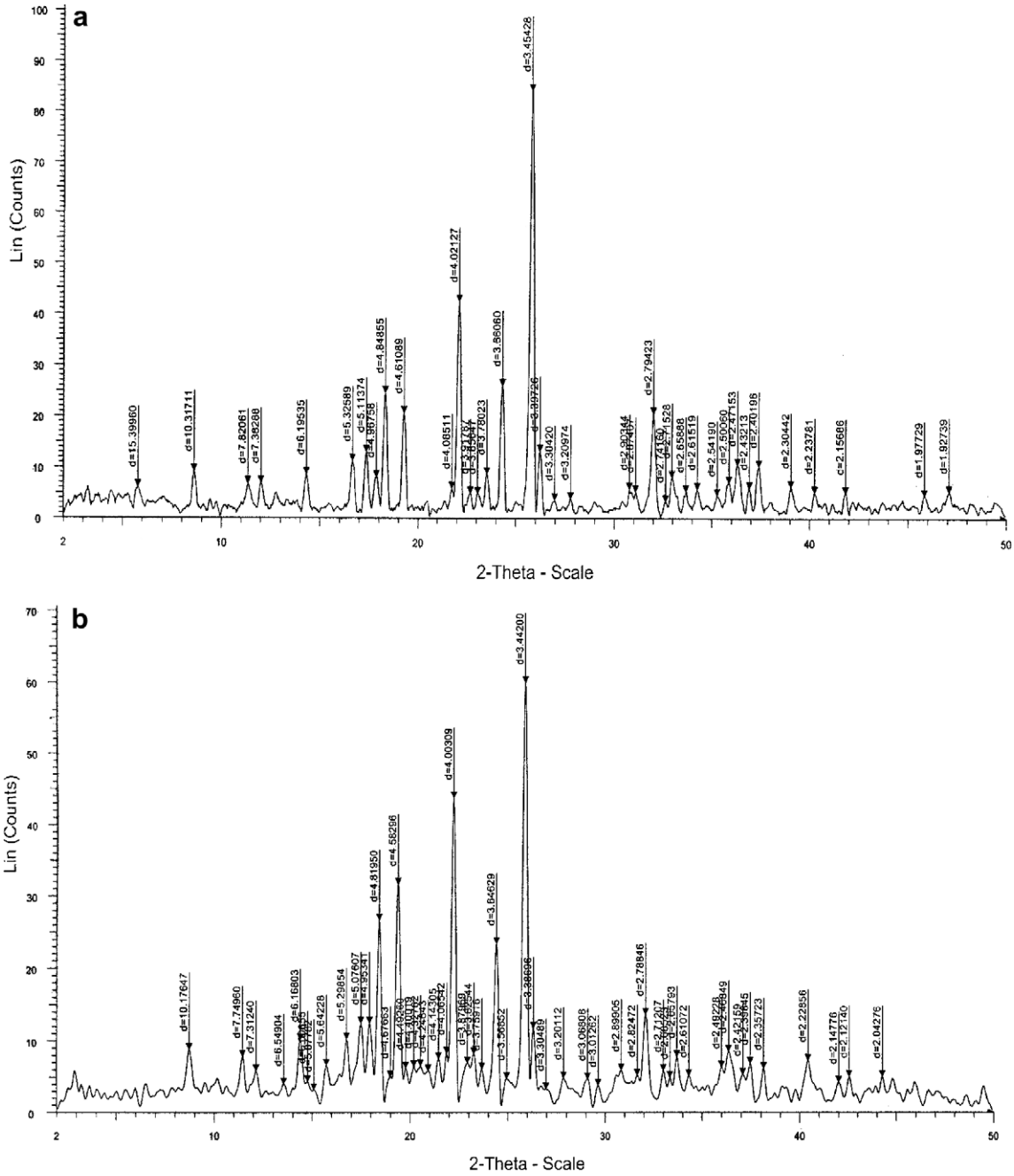

Fig. 3. XRD patterns. (a) Pure aceclofenac; (b) F-9 agglomerates.

the results of IR spectroscopy. The DSC results also revealed little amorphization of aceclofenac when prepared in the form of agglomerates with HPMC. This is evident by a decrease, although little, in the enthalpy changes of agglomerates when compared with that of pure drug (pure aceclofenac = −812.42 mJ/mg; Aceclofenac + HPMC = −805.31 mJ/mg; F-9 agglomerates = −799.12 mJ/mg) [7].

The SEM photographs of the agglomerates prepared at 1000 rpm are shown in Fig. 2A and B. The SEM photographs of F-9 agglomerates are shown in Fig. 2C and D. The photographs confirmed that the agglomerates formed were spherical in shape and the surface of the agglomerates was rough as they were formed by the cluster of aceclofenac crystals.

The XRD scan of plain aceclofenac showed intense peaks of crystallinity (Fig. 3a); whereas the XRD pattern of the agglomerates exhibited halo pattern with less intense and more denser peaks compared to plain aceclofenac indicating the decrease in crystallinity or partial amorphization of the drug in its agglomerated form (Fig. 3b). This further supports the DSC results which demonstrated partial amorphization of drug in agglomerates.

In relative crystallinity determinations, the XRD patterns of aceclofenac and magnesium oxide were clearly different (figures not shown). The intensity of the aceclofenac peaks at 24.43 2θ and 25.9 2θ, and in case of magnesium oxide at 43.06 2θ was calculated using D8 TOOLS software. The calculated relative crystallinity value for pure aceclofenac was 0.894 (89.40%) and that of F-9 agglomerates was 0.712 (71.20%), further indicating the partial amorphization of drug when formulated as spherical agglomerates. Inter-batch uniformity was found with respect to relative crystallinity (relative crystallinity value was 0.693 (69.30%) with another batch of F-9 agglomerates).

3.3. Dissolution studies of spherical agglomerates

The dissolution profiles of drug and its agglomerates are shown in Fig. 4(a, b and c). The aceclofenac agglomerates prepared with

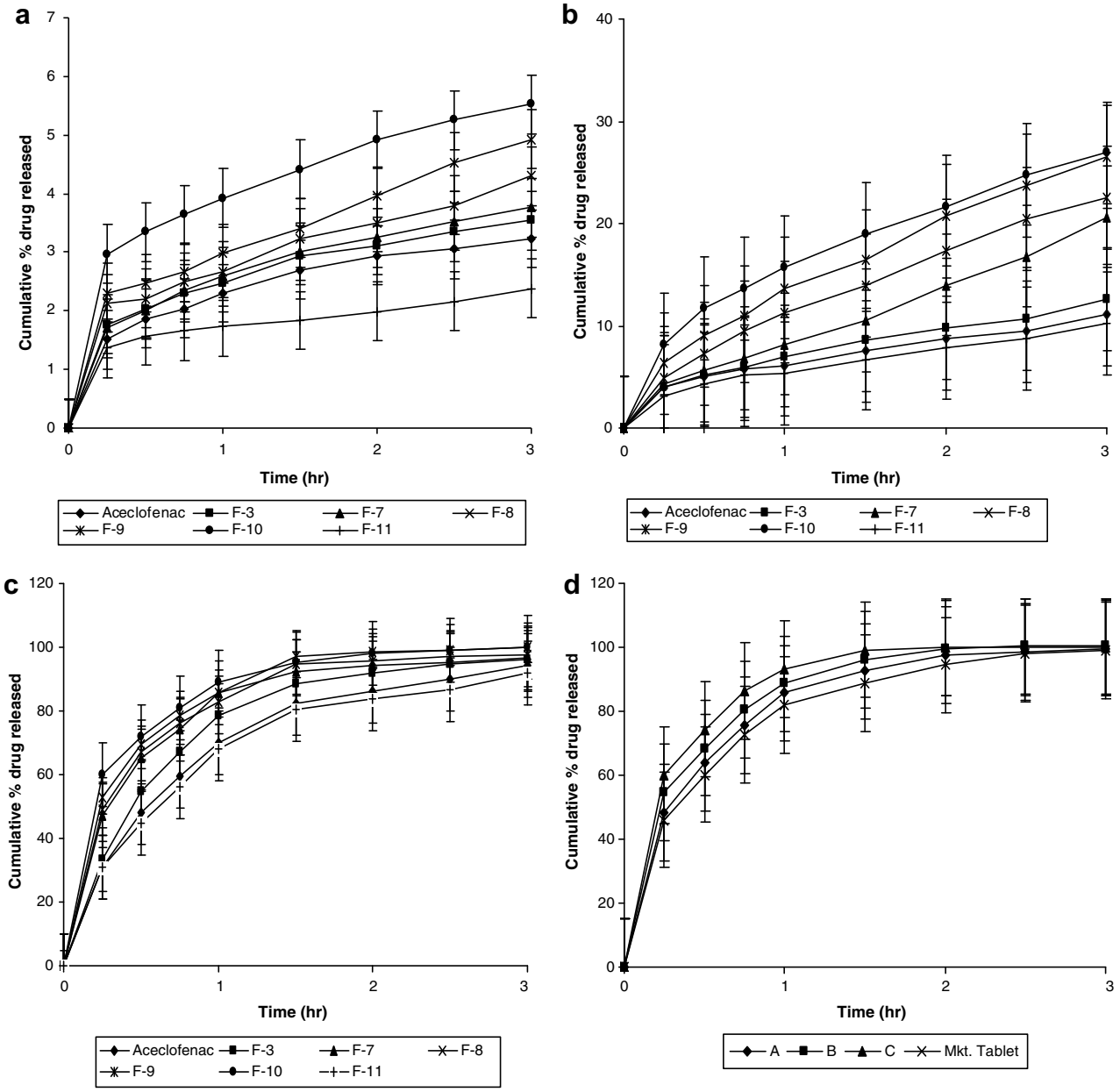

Fig. 4. Dissolution profiles of agglomerates in (a) 0.1 N HCl (b) water (c) phosphate buffer (pH 6.8) and (d) tablets in phosphate buffer (pH 6.8). Mkt. Tablet-Marketed tablet.

HPMC exhibited better dissolution rate when compared with plain aceclofenac. The cumulative drug release was increased with an increase in HPMC concentration, which could be attributed to deposition of polymer onto the drug surface. Among the formulations prepared, F-10 (prepared with 100 mg HPMC) showed highest drug release in 3 h. The cumulative percentage of drug released from different agglomerates was increased in the following order:

0.1 N HCl: F-11 (2.37 ± 0.15) < plain aceclofenac (3.23 ± 0.67) < F-3 (3.54 ± 0.45) < F-7 (3.76 ± 0.89) < F-8 (4.3 ± 0.66) < F-9 (4.92 ± 0.97) < F-10 (5.52 ± 1.06).

Distilled water: F-11 (10.2 ± 0.64) < plain aceclofenac (11.04 ± 0.52); F-3 (12.57 ± 1.53) < F-7 (20.63 ± 2.05) < F-8 (22.5 ± 1.64) < F-9 (26.54 ± 1.81) < F-10 (26.9 ± 2.56).

Phosphate buffer, pH 6.8: F-11 (92.03 ± 1.56) < plain aceclofenac (94.34 ± 1.20) < F-3 (95.98 ± 2.01) < F-7 (96.58 ± 0.84) < F-8 (97.80 ± 0.57) < F-9 (100.01 ± 0.25) < F-10 (100.16 ± 0.14).

The agglomerates prepared without HPMC (F-11) did not show any improvement in drug release; on the contrary, they showed decreased drug release when compared with that of pure drug. F-11 agglomerates were not spherical, rather they were in the form of lumps and fine powder. They exhibited less flowability, drug solubility and dissolution rate. Additionally, the crystals prepared with 1000 rpm (F-3) also did not improve the drug release rate in spite of their small particle size. These observations suggest the importance of HPMC in the agglomerates at an optimum concentration.

Among the different formulations, F-9 was selected for further compression into tablets and for *in vivo* studies. Although F-10 showed highest solubility and dissolution rate, there was a formulation problem during the preparation of these agglomerates. Because of very high concentration of HPMC (100 mg), it was difficult to form a solution with DCM. So F-10 agglomerates were just prepared to observe the effect of HPMC concentration on drug release and solubility. These points support the selection of F-9 agglomerates (prepared with 800 rpm and 75 mg HPMC) for further tablet development and evaluations.

Mainly two attributes to enhance drug dissolution rate from F-9 agglomerates can be considered; viz., surface treatment with HPMC and formation of partially amorphous aceclofenac during crystallization process. However from the studies like DSC, IR, XRD, solubility and stability, surface treatment could be major and significant factor in comparison with generating the amorphous form.

3.4. Evaluation of tablets

The compressed tablets remained within the desired limits (mean ± SEM) for quality control parameters such as thickness (A = 2.58 ± 0.01; B = 2.52 ± 0.05; C = 2.64 ± 0.02; marketed tablet = 2.60 ± 0.07 mm; $n = 6$), diameter (8.0 mm; $n = 6$), crushing strength (A = 2.80 ± 0.20; B = 3.00 ± 0.10; C = 2.90 ± 0.15; marketed tablet = 3.85 ± 0.30 kg/cm^2; $n = 6$), friability (A = 0.72 ± 0.04; B = 0.68 ± 0.07; C = 0.83 ± 0.03; marketed tablet = 0.57 ± 0.08%; $n = 6$), drug content (A = 98.50 ± 0.62; B = 98.0 ± 0.84; C = 99.20 ± 0.15; marketed tablet = 99.92 ± 0.05%; $n = 3$) and weight variation (A = 4.50 ± 0.34; B = 4.20 ± 0.51; C = 3.80 ± 0.37; marketed tablet = 3.50 ± 0.42%; $n = 20$). The disintegration time for prepared tablets was less than marketed tablet and Tablet C showed least disintegration time (A = 6.45 ± 0.04; B = 4.30 ± 0.06; C = 0.40 ± 0.15; marketed tablet = 10.50 ± 0.10 min; $n = 6$).

The results of *in vitro* dissolution studies in phosphate buffer (pH 6.8) are given in Fig. 4d. The dissolution profile of Tablet A (without sodium starch glycolate-SSG) was almost comparable with that of marketed tablet. In B and C tablets, a super-disintegrant (SSG) was added at 2 mg and 4 mg per tablet, respectively, in order to enhance the disintegration. Cumulative drug released (%) from different tablet formulations was 99.72 ± 1.18 (in 180 min), 100.04 ± 0.23 (in 150 min), 100.03 ± 0.37 (in 120 min) and 99.03 ± 1.06 in 180 min from A, B, C and marketed tablets, respectively (mean ± SEM; $n = 3$). The formulation C was found to be better since it showed highest drug release rate. This might be because of the presence of high concentration of SSG, which is highly efficient in disintegration because of its greater swelling capacity. Disintegration occurs by rapid uptake of water followed by enormous swelling. Based on these results, tablet formulation C was selected for pharmacokinetic studies in human subjects.

3.5. Stability studies

The agglomerates (F-9) and compressed tablets (Formulation C) did not show any significant change in the drug content during stability study. The drug content was more than 96% at the end of 6 months under accelerated conditions. The values for drug content (%) of agglomerates and compressed tablets, respectively, are 0 day: 99.92 ± 0.23 and 99.88 ± 0.45; 15 days: 99.15 ± 0.54 and 99.05 ± 0.34; 30 days: 98.63 ± 0.75 and 98.45 ± 0.23; 60 days: 97.95 ± 0.45 and 97.85 ± 0.66; 90 days: 97.05 ± 0.33 and 97.00 ± 0.27; 180 days: 96.25 ± 0.35 and 96.32 ± 0.55. No changes in the drug release profile of tablet C were observed during the stability study period and >98% of the drug was released in 120 min throughout the stability period. Hence the preparations are sufficiently stable as per the regulatory requirements.

3.6. Preclinical studies

The preclinical studies (anti-inflammatory, analgesic, toxicity and pharmacokinetic studies) were mandatory by the Kasturba Hospital Ethics Committee to conduct the clinical studies. There was no problem in administering orally the pure drug and agglomerates to the animals; but it was not meaningful to break the marketed tablet. However, as a prerequisite for clinical studies and to observe whether the optimized formulation composition retains similar activity in comparison with pure drug or marketed tablet composition, these studies were carried out with powdered marketed tablet.

Table 3
Effect of aceclofenac agglomerates on paw edema induced by carrageenan in Wistar rats

Time (min)	Paw volume (ml)			% Inhibition	
	Control	Pure drug	F-9	Pure drug	F-9
60	0.180 ± 0.044	0.140 ± 0.043	0.132 ± 0.038	23.00 ± 2.30	26.70 ± 1.63
120	0.250 ± 0.045	0.150 ± 0.044	0.147 ± 0.039	39.20 ± 1.50	41.20 ± 2.71
180	0.460 ± 0.053	0.190 ± 0.043*	0.180 ± 0.041*	57.20 ± 1.98	60.90 ± 2.32
240	0.620 ± 0.006	0.140 ± 0.010*	0.102 ± 0.012*,#	77.50 ± 2.54	83.60 ± 1.70
300	0.700 ± 0.063	0.120 ± 0.046*	0.150 ± 0.042*	82.90 ± 1.42	79.00 ± 1.58

All values are expressed as mean ± SEM, $n = 6$.
* Significant compared to control ($p < 0.05$).
# Significant compared to pure drug ($p < 0.05$).

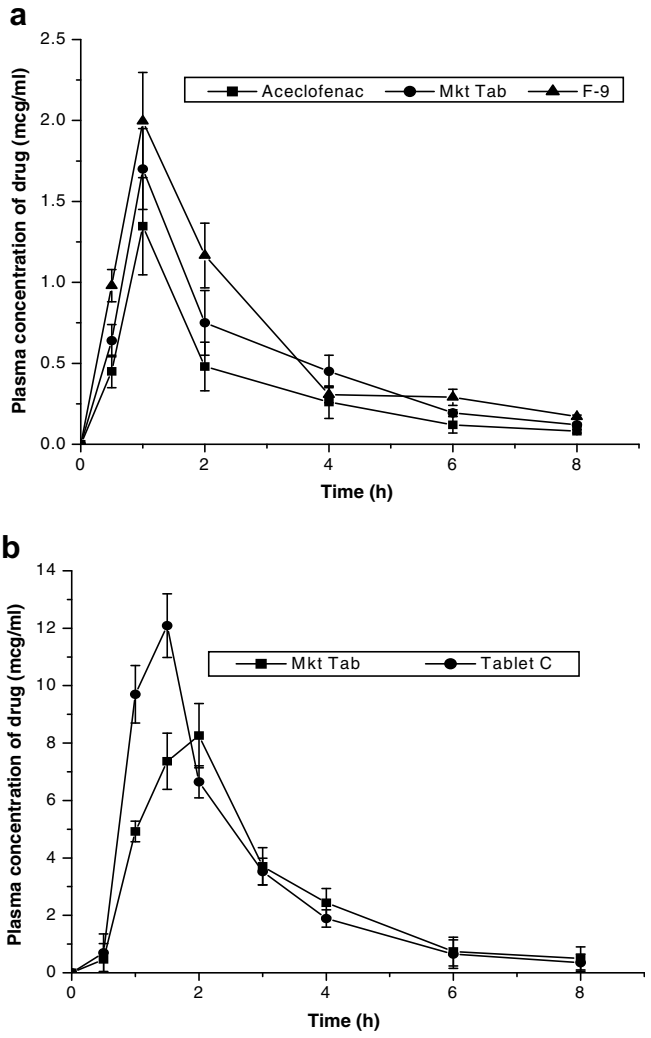

Fig. 5. Plasma drug concentration-time curves for pharmacokinetic study in (a) rats and (b) human volunteers. All points are presented as mean ± SEM, $n = 6$; Mkt Tab, Marketed tablet.

The results of anti-inflammatory activity are given Table 3. The drug in the form of spherical agglomerates showed maximum inhibition (%) of edema 83.6 ± 1.70 at 240 min; whereas pure drug provided maximum activity of 82.9 ± 1.42 at 300 min. The value of inhibition of edema exhibited by drug in the form of agglomerates was higher at all time points compared to that of plain drug. Thus F-9 agglomerates showed rapid and higher effectiveness in inhibiting rat paw edema when compared to pure drug.

The effect of pure aceclofenac and its agglomerates (F-9) on acetic acid-induced abdominal contractions in mice was compared to control group (no drug treatment). The control animals showed 34.83 ± 13.04 contractions. The analgesic activity from agglomerated drug was rapid (8.83 ± 1.42 contractions) and percentage inhibition of contractions (74.64 ± 1.52%) was high compared to those of pure drug (No. of contractions: 10.16 ± 1.90; Inhibition of contractions: 70.82 ± 1.25%). These observations confirmed the advantage of enhanced analgesic activity which might be due to improved rate of absorption and bioavailability of aceclofenac from spherical agglomerates.

The results of pharmacokinetics in rats are given in Fig. 5a and Table 4. Aceclofenac absorption after oral administration was rapid with all three groups as indicated by low T_{max} value of 1 h. However, the C_{max} value was high with spherical agglomerates indicating high absorption rate of drug. The elimination half-life ($t_{1/2}$) of aceclofenac with agglomerates was less indicating the drug is getting eliminated from the body rapidly. It was further supported by less mean residential time (MRT) and high elimination rate constant value (K_e) of agglomerates in comparison with marketed formulation and pure drug. The $t_{1/2}$, MRT and K_e values indicate that pure drug remains in the body for more time when compared with marketed and agglomerated formulations. Spherical agglomerates showed high AUC value indicating the greater extent of drug absorption from agglomerates. Thus low T_{max}, high C_{max} and high AUC values together indicate the improved bioavailability and rapid absorption of aceclofenac from agglomerates in comparison with marketed formulation composition and pure drug. This could be due to improved solubility and dissolution rate of drug from prepared tablets.

There were no deaths of the animals in the subacute toxicity study. No difference was observed in all the animals with respect to food and water consumption, general behaviour or other physiological activities. The results (not shown) did not reveal any changes in the tested hematological and biochemical parameters in drug-treated animals. All these observations indicated the non-toxic nature of F-9 formulation and hence it was found suitable for clinical studies in human volunteers.

3.7. Pharmacokinetic study in human volunteers

The plasma concentration of aceclofenac against time is shown in Fig. 5b. The pharmacokinetic parameters are recorded in Table 4. Absorption of aceclofenac after oral administration was rapid with both marketed and prepared tablets. However, T_{max} value of prepared tablet was less (1.42 h) when compared with marketed tablet indicating comparatively more rapid absorption of drug. Also

Table 4
Pharmacokinetic parameters from the plasma concentration-time curves

Parameters	Rats			Human volunteers	
	Pure drug	Marketed tablet	F-9 agglomerates	Prepared tablet (C)	Marketed tablet
C_{max} (μg/ml)	1.35 ± 0.22	1.70 ± 0.12*	2.00 ± 0.25*,#	12.08 ± 0.98♠	8.26 ± 0.91
T_{max} (h)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.42 ± 0.20♠	1.92 ± 0.20
$t_{1/2}$ (h)	3.41 ± 0.06	2.90 ± 0.04*	2.59 ± 0.28*,#	2.27 ± 0.26♠	3.53 ± 0.21
AUC_{0-8} (μg-h/ml)	2.80 ± 0.04	4.10 ± 0.03*	5.10 ± 0.05*,#	24.20 ± 1.55♠	21.90 ± 1.21
MRT (h)	3.70 ± 0.06	3.60 ± 0.08*	3.50 ± 0.76	2.70 ± 0.06♠	3.80 ± 0.10
K_e (h^{-1})	0.203 ± 0.00	0.239 ± 0.00	0.267 ± 0.00	0.306 ± 0.00♠	0.196 ± 0.00

All values are expressed as mean ± SEM, $n = 6$; C_{max}, Peak plasma concentration; T_{max}, Time of peak plasma concentration; $t_{1/2}$, Elimination half-life; AUC, Area under the curve; MRT, Mean residential time; K_e, Elimination rate constant.

* Significant compared to pure drug ($p < 0.05$).

\# Significant compared to marketed tablet in rats ($p < 0.05$).

♠ Significant compared to marketed tablet in human volunteers ($p < 0.05$).

C_{max} value of prepared tablet (12.08 μg/ml) was higher than that of marketed tablet. These C_{max} and T_{max} values together indicate improved rate of absorption of aceclofenac from agglomerated tablets. The increased extent of absorption was supported by higher AUC values observed with prepared tablet (24.2 μg-h/ml), which together with higher C_{max} and lower T_{max} values indicate the higher bioavailability of drug from prepared tablets. The difference in AUC between the prepared and marketed tablet was not very different which could be due to a sharp fall in the plasma concentration of drug in marketed tablet from its C_{max} point. The $t_{1/2}$ of aceclofenac with prepared tablet was less indicating the drug is eliminated from the body rapidly. It was further supported by MRT and high K_e of agglomerates in comparison with marketed tablet. The $t_{1/2}$, MRT and K_e values indicate that the drug remains in the body for more time when administered with marketed tablets. Hence the pharmacokinetic study indicates rapid absorption and higher bioavailability of drug from prepared tablet in comparison with marketed formulation. Further, the improved bioavailability achieved with F-9 formulation (higher AUC and C_{max}, and lower T_{max} values) may reduce the total dose of drug, which would result in cost effectiveness with respect to drug. Accordingly the toxicity or adverse effects related issues of the dug may be reduced which eventually would result in improved compliance.

4. Conclusion

In this study prepared aceclofenac agglomerates and tablets exhibited excellent physicochemical and micromeritic properties, solubility, dissolution rate, stability and *in vivo* (preclinical and clinical) performance when compared with pure drug as well as marketed formulation besides exhibiting no preclinical toxicity. If this process can be scaled-up to manufacturing level, this technology has the potential to provide the directly compressed aceclofenac tablets with improved bioavailability. However, extensive long-term stability, toxicity and clinical pharmacokinetic studies are required before commercialization.

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