ORIGINAL ARTICLE

IMPROVED BIOAVAILABILITY OF ACECLOFENAC FROM SPHERICAL AGGLOMERATES: DEVELOPMENT, *IN VITRO* AND PRECLINICAL STUDIES

MUATLIK S*, USHA AN, REDDY MS, RANJITH AK AND PANDEY S

Department of Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal 576104, Karnataka State, India

ABSTRACT

The objective of present study was to improve the solubility, dissolution rate, micromeritic properties and bioavailability of aceclofenac (NSAID) by formulating its spherical agglomerates. They were prepared with different water soluble polymers (polyvinylpyrrolidone-K30, polyvinylpyrrolidone-K90 and sodium alginate) by using acetone-water-dichloromethane solvent system. The agglomerates were subjected to various physicochemical properties, DSC, IR spectroscopy, scanning electron microscopy (SEM), micromeritic properties and dissolution studies. The *in vivo* studies (anti-inflammatory, analgesic and pharmacokinetic studies) were conducted in Wistar rats and Swiss albino mice. SEM studies showed that agglomerates were spherical in structure and formed by cluster of small crystals. The agglomerates prepared with polyvinylpyrrolidone-K90 exhibited improved solubility, dissolution rate and micromeritic properties compared to those prepared with other polymers and pure drug. These optimized agglomerates showed rapid analgesic and anti-inflammatory activity besides exhibiting improved bioavailability of drug when compared to pure drug.

Keywords: Aceclofenac, spherical crystallization, agglomerates, anti-inflammatory, analgesic, pharmacokinetic

INTRODUCTION

Spherical crystallization has been developed by Yoshiaki Kawashima and co-workers as a novel particulate design technique to improve the processibility such as mixing, filling, tableting characteristics and the bioavailability of pharmaceuticals. Spherical crystallization is a multiple unit process in which crystallization, agglomeration and spheronization can be carried out simultaneously in one step. The resultant crystals can be designated as spherical agglomerates (Kulkarni and Nagavi, 2002). Apart from modifications in the micromeritic properties of the particles, this technique offers advantage in terms of reduction in the number of unit operations and in turn, processing cost of tablet compression (Pawar *et al*., 2004). The bioavailability of insoluble pharmaceuticals can be increased by mechanical micronization of crystals or preparation of fine crystals by the addition of surfactants to the system. However, the micronization leads to a decrease in flowability and packability (Sano *et al*., 1987). Spherical crystallization is an effective alternative to improve the bioavailability of drugs. This can be achieved by various methods such as spherical agglomeration, emulsion solvent diffusion, ammonia diffusion and neutralization methods (Paradkar *et al*., 1998; Kawashima *et al*, 1989). Aceclofenac (2-[(2,6-dichlorophenyl) amine] phenylacetoxyacetic acid) is a new orally effective NSAID of the phenyl acetic acid group, which has remarkable anti-inflammatory, analgesic and antipyretic properties. It exhibits very slight solubility in water, poor flow and compression characteristics (Alvarez-Larena *et al*., 1992). The purpose of the present work was to improve the micromeritic properties, aqueous solubility and dissolution rate, and finally *in vivo* performance of aceclofenac by preparing its spherical agglomerates with hydrophilic polymers like polyvinylpyrrolidone-K30 (PVP K-30), polyvinylpyrrolidone-K90 (PVP K-90) and sodium alginate.

EXPERIMENTAL

Materials

Aceclofenac (Lupin Research Park, Pune, India), PVP K-30 and PVP K-90 (Hi Media Laboratories, Mumbai, India), sodium alginate (S.D. Fine Chemicals, Mumbai, India), acetone and dichloromethane (Ranbaxy, Mumbai, India) were principally used. All other chemicals used were of AR grade.

Spherical agglomerization process

A solution of aceclofenac (0.75 g) in 3 ml acetone was added to a solution of polymer in 1 ml of dichloromethane (DCM). Drug was crystallized by adding the drug solution to a 500 ml capacity beaker containing 100 ml distilled water. The mixture was stirred continuously for a period of 0.5 h using a controlled speed mechanical stirrer at 800 rpm to obtain spherical agglomerates (Paradkar *et al*., 2002). The agglomerates were separated by filtration and dried at room temperature. The amount of polymers was altered to get desired agglomerates. The composition

Corresponding author: E-mail: ssmutalik@yahoo.com - Tel: +91-820-2922482; Fax: +91-820-2571998

is given in the table 1.

Infrared spectroscopy and Differential Scanning Caloriemetry (DSC)

Infrared spectroscopy was conducted using a Shimadzu FTIR 8300 Spectrophotometer and the spectrum was recorded in the region of 4000 to 400 cm^{-1}. The procedure consisted of dispersing a sample (drug, drug-polymer mixture and agglomerates) in KBr (200-400 mg) 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.

The DSC study was performed using DSC-60 caloriemeter (Shimadzu, Tokyo, Japan). The instrument was comprised of calorimeter (DSC 60), flow controller (FCL 60), thermal analyzer (TA 60) and operating software (TA 60). The samples were sealed in aluminum pans and heated under nitrogen flow (30 ml/min) at a scanning rate of 5ºC/min from 25ºC to 250ºC. Empty aluminum pan was used as a reference.

Particle size, shape and surface morphology

The size of agglomerates was determined by microscopic method using stage and eyepiece micrometers. The shape and surface morphology of the spherical agglomerates was studied by scanning electron microscopy (SEM) (JEOL, JSM 50A, Tokyo, Japan).

Physicochemical and micromeritic properties

The practical yield was determined by weighing the agglomerates after drying. For the determination of drug content, agglomerates (100 mg) were powdered and dissolved in 10 ml phosphate buffer pH 6.8 (PB) and vortexed for 20 min. The solution was filtered and after sufficient dilution with PB, analyzed spectrophotometrically (UV-1601PC, Shimadzu, Japan) at 275nm.

Loss on drying (LOD) was determined by using Halogen Moisture Analyzer (Mettler Toledo, USA). The sample (1 g) was kept on pan for about 10 min at 105ºC. The percentage LOD was displayed on the analyzer at the end of the process.

Table 1: Composition of spherical agglomerates

Ingredients	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12
Aceclofenac (mg)	750	750	750	750	750	750	750	750	750	750	750	750
Acetone (ml)	3	3	3	3	3	3	3	3	3	3	3	3
PVP K-30 (mg)	25	50	75	100	-	-	-	-	-	-	-	-
PVP K-90 (mg)	-	-	-	-	25	50	75	100	-	-	-	-
S. Alginate (mg)	-	-	-	-	-	-	-	-	25	50	75	100
DCM (ml)	1	1	1	1	1	1	1	1	1	1	1	1
D. Water (ml)	100	100	100	100	100	100	100	100	100	100	100	100
Stirring speed (rpm)	800	800	800	800	800	800	800	800	800	800	800	800

S. Alginate=Sodium alginate; D. water=Distilled water.

Table 2: Micromeritic properties, solubility and dissolution data of spherical agglomerates

Samples	Carr's index (%)	Hausner's Ratio	Angle of repose (°)	Particle size (µm)	Solubility		Drug release (%) in 180 min	
					Water (µg/ml)	PB (mg/ml)	Water	PB
Drug	29.99	1.42	46.92	23.57±1.46	81.89±2.34	12.42±0.83	11.04±0.52	94.34±1.20
C-1	6.79	1.07	20.14	421.39±10.58	83.42±2.23	13.49±0.57	18.68±0.25	95.46±1.61
C-2	6.71	1.07	20.12	430.50±10.24	85.08±2.47	13.85±1.0	27.07±1.64	96.24±2.00
C-3	7.32	1.07	21.54	430.82±9.42	85.98±2.14	14.89±0.38	30.63±1.32	96.90±1.98
C-4	9.42	1.1	22.31	440.24±11.56	86.24±1.76	15.01±0.97	32.32±1.08	99.03±0.50
C-5	6.87	1.07	20.36	430.47±9.47	99.83±2.42	12.47±0.84	19.40±1.36	96.25±1.37
C-6	6.79	1.07	20.20	438.70±12.01	106.34±1.54	12.69±1.06	24.94±2.14	96.82±1.43
C-7	6.62	1.07	20.02	441.75±11.25	113.75±1.63	13.87±0.84	30.86±1.76	97.99±2.14
C-8	6.90	1.06	21.15	448.40±10.23	115.42±1.28	13.94±0.79	32.97±2.67	99.34±0.31
C-9	6.12	1.06	19.96	480.40±10.54	79.76±2.45	12.34±0.24	11.18±0.98	94.41±1.20
C-10	5.87	1.06	19.13	512.47±17.43	79.82±2.14	12.30±0.21	11.00±1.14	93.07±1.66
C-11	4.33	1.04	18.32	520.40±14.82	80.12±0.86	11.98±0.43	10.79±2.06	92.67±2.40
C-12	4.77	1.05	19.12	552.48±13.57	79.50±2.08	11.31±0.58	10.41±1.50	92.32±1.65

PB=Phosphate buffer pH 6.8.

The Carr's Index and Hausner's ratios were calculated by determining loose and tapped bulk density using bulk density apparatus. The flowability of the agglomerates was assessed by determining the angle of repose by following a fixed funnel method (Liebermann *et al*., 1990; Aulton, 1990).

Solubility determination

An excess quantity of drug or agglomerates was shaken with 10 ml of distilled water or PB in a shaking water bath (100 agitations/ min) for 24 h at room temperature. The solution was then passed through a 0.45µ-membrane filter and the amount of the drug dissolved was analyzed spectrophotometrically (Wells, 1988).

Dissolution studies

The *in vitro* dissolution study was carried out in USP Type 1 dissolution apparatus using 900 ml of PB or distilled water as dissolution media. The dissolution medium was kept in a thermostatically controlled water bath, maintained at 37±0.5 ºC. The basket tied with muslin cloth, containing spherical agglomerates equivalent to 100 mg aceclofenac, was lowered so that the lower end of the stirrer was 25 mm above from the base of the beaker. The basket was rotated at 75 rpm. At predetermined time intervals (0 to 180 min), 5 ml of samples were withdrawn and analyzed spectrophotometrically. At each time of withdrawal, 5 ml of fresh corresponding medium was replaced into the dissolution flask.

Stability studies

The formulations were charged for the accelerated stability studies in accordance with the ICH guidelines (40±2ºC and 75±5% RH for a period of 6 months). The initial drug content of optimized agglomerates was determined. Then they were placed in USP type I flint glass vials, closed with bromobutyl rubber bung and sealed with aluminum caps. The vials were placed in stability chamber (Thermolab, Mumbai, India) and samples were withdrawn at predetermined time intervals (15, 30, 60, 90 and 180 days) and evaluated for the drug content.

Preclinical studies

The preclinical studies (anti-inflammatory, analgesic and pharmacokinetic studies) were carried out in Wistar rats (200-250 g) and Swiss albino mice (25-30 g), obtained from Central Animal House, MAHE, Manipal. They were housed in elevated wire cages, 4 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).

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

Table 3: Effect of spherical agglomerates of aceclofenac on the paw edema induced by carrageenan in Wistar rats.

Time (min)	Paw volume (ml)			% Inhibition	
	Control	Pure drug	C-8	Pure drug	C-8
60	0.18±0.044	0.14±0.043	0.12±0.027	23.00±2.30	28.80±1.47
120	0.25±0.045	0.15±0.044	0.14±0.019	39.20±1.50	42.40±2.62
180	0.46±0.053	0.19±0.043*	0.17±0.017*	57.20±1.98	61.90±3.35
240	0.62±0.006	0.14±0.040*	0.09±0.031*	77.50±2.54	84.20±1.98
300	0.70±0.063	0.12±0.046*	0.13±0.042*	82.90±1.42	83.0±0.84

All values are the expressed as Mean±SE, n=6. * Significant compared to control ($p<0.05$).

Table 4: Pharmacokinetic parameters from the plasma concentration-time curves.

Parameters	Pure drug	Marketed tablet	C-8
C_{max} (µg/ml)	1.35±0.22	1.70±0.12*	2.03±0.28*#
T_{max} (h)	1.00± 0.00	1.00±0.00	1.00±0.00
$T_{1/2}$ (h)	3.41± 0.06	2.90±0.04*	2.55±0.31*#
AUC_{0-8} (µg-h/ml)	2.80± 0.04	4.10±0.02*	5.50±0.04*#
MRT (h)	3.70± 0.06	3.60±0.08*	3.48±0.85
K_e (h^{-1})	0.203±0.001	0.239±0.00	0.276±0.00

All values are the expressed as Mean±SE, 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 ($p<0.05$).

Group I: Pure aceclofenac (10 mg/kg) in 0.5% sodium carboxymethylcellulose (CMC); p.o.
Group II: Spherical agglomerates (C-8) containing equivalent of aceclofenac (10 mg/kg) in 0.5% CMC; p.o.
Group III: 2.5 ml of 0.5% CMC; p.o.

After 30 min of drug administration, rats of all groups 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 (Kulkarni, 1997).

Analgesic activity

Writhing test in mice was used to evaluate the analgesic activity of the drug when administered in its plain form as well as spherical agglomerates (Kulkarni, 1997; Mutalik *et al*., 2003). The overnight fasted mice were divided into 3 groups (n=6) and treated orally as given in above procedure (Anti-inflammatory activity). After 1 h post-dose, they were injected with 1% acetic acid (0.1 ml/10 g) intraperitonially. Then the number of writhings was recorded for 30 min. The analgesic activity was evaluated in terms of the percentage of writhe inhibitions.

Pharmacokinetic study

The pharmacokinetic studies were carried out in Wistar rats. The overnight fasted animals were divided into 3 groups (n=6) and treated orally as shown below.

Group I: Pure aceclofenac in 0.5% CMC
Group II: Spherical agglomerates (C-8) equivalent quantity of aceclofenac in 0.5% CMC
Group III: Powdered marketed aceclofenac formulation in 0.5% CMC.

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

Analysis of drug in plasma

An analytical method based on HPLC was used to analyze the aceclofenac in plasma (Mutalik *et al*., 2007). The HPLC system (Shimadzu Class *VP* series having Class *VP* 6.12 version software) consisted of 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 x 4.6 mm; 5µ). 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.

From the stock solution (1 mg/ml), working standard solutions were prepared (1-70 µg/ml of aceclofenac and 500 µg/ml of venlafaxine, an internal standard) in methanol and water (80:20 v/v). The rat plasma (95 µl) was pipetted into 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 was added and mixed for a min. Then 675 µl of diluent was added to make up the volume to 1.0 ml and vortexed for 60 sec. 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. In the preparation of sample solutions, 25 µl of internal standard solution (500 µg/ml) and 200 µl of acetonitrile was added to 100 µl of rat plasma and mixed for a min. To this, diluent was added (675 µl) upto 1 ml. The solution was vortexed for 60 sec and centrifuged at 10000 rpm for 10 min. The supernatant layer was separated and analyzed using HPLC system. The response factor (peak area ratio of drug peak area to the internal standard peak area) of the standard solution and the sample were calculated and the concentration of the aceclofenac present in the plasma samples was calculated from the calibration curve. When blank rat plasma samples were analyzed, no interference was observed in the analysis of drugs. The peaks were well resolved (retention time: 10.20 min for aceclofenac and 18.51 min for venlafaxin).

STATISTICAL ANALYSIS

Student's *t*-test was used to analyze the data (Graph Pad Instat Software-1.13 version) and $p<0.05$ was considered statistically significant. The pharmacokinetic parameters were calculated by using PK Solutions 2.0^{TM} software.

RESULTS AND DISCUSSION

Formulation development

Spherical agglomerates of aceclofenac were prepared by solvent change method. This process is easy, common and fast in comparison to other methods and hence this process was used for the preparation of spherical crystals. In the first step, a choice of three appropriate solvents was undertaken based on the available solubility data. These solvents must be as following: the first one must be a good solvent of the drug; the second solvent must be a liquid in which the drug is insoluble (poor solvent); and the third solvent must be a liquid in which the drug is insoluble (bridging liquid), but which can wet the drug crystals (Chourasia and Jain, 2003). The selection of bridging liquid should be such that it should be immiscible with the poor solvent. Bridging liquid plays a vital role in the process of spherical crystallization. In the present study acetone, water and dichloromethane were

used as good solvent, poor solvent and bridging liquid. The addition of the drug solution to the non-solvent phase (water) caused aceclofenac precipitation and a weak tendency to particle agglomeration was observed. The addition of bridging liquid favored the transfer of drug to this emulsified phase in which crystal agglomerates were densified and developed spherically. In order to modify drug release from aceclofenac agglomerates, hydrophilic additives like PVP K30, PVP K90 and sodium alginate were used.

IR spectroscopy and DSC studies

The major IR peaks (wave number, cm^{-1}) of drug, drug and polymer mixture and agglomerates are given below.

Pure aceclofenac: 3313.3, 2970.2, 2935.5, 1716.5, 1589.2, 1506.3, 1479.3, 1344.3, 1280.6, 1255.6, 665.4; Aceclofenac + PVP (K30): 3265.3, 2964.1, 2935.5, 1716.5, 1577.7, 1506.3, 1479.3, 1344.3, 1282.6, 1255.6, 665.4; Aceclofenac + PVP (K90): 3319.3, 2970.2, 2935.5, 1716.5, 1589.2, 1506.3, 1479.3, 1344.3, 1280.6, 1255.6, 665.4; Aceclofenac + sodium alginate: 3319.3, 2970.2, 2935.5, 1716.5, 1589.2, 1508.2, 1479.3, 1344.3, 1280.6, 1255.6, 665.4; C-8 agglomerates: 3276.8, 2979.8, 2935.5, 1714.6, 1589.2, 1506.3, 1479.3, 1344.3, 1282.6, 1255.6, 665.4. The infrared spectrum is extremely sensitive to structure and conformation of a compound and thus can be used to compare the structure of a compound in different solid states (Garekani *et al*., 1999). The results showed that the principal absorption bands of pure drug, its physical mixture with polymers and agglomerates were almost similar, suggesting no interaction between drug and polymers during preparation of agglomerates.

DSC thermograms of pure drug, drug and polymer mixture and agglomerates are shown in fig. 1. The melting points of pure aceclofenac, aceclofenac + PVP-K30, aceclofenac + PVP-K90 and aceclofenac + sodium alginate were 155.54, 152.71, 153.54 and 153.02 °C, respectively. The melting endotherms of agglomerates C-3, C-8 and C-9 were 153.12, 153.25 and 153.90 °C, respectively. These results showed that melting point of drug was not altered considerably in the presence of polymers as well as after formulating it into agglomerates.

Particle size, shape and surface morphology

The size of agglomerates is given in Table 2. The particle size of pure aceclofenac was 23.57±1.46 µm. The particle size of agglomerates prepared in the presence of PVP was in the range of 421.39±10.58 µm to 448.4±10.23 µm where as the particle size of agglomerates prepared in the presence of sodium alginate was in the range of 480.4±10.54 µm to 552.48±13.57 µm. It was found that the presence of polymer in the spherical agglomerates influenced the particle size of resultant agglomerates. The size of particles increased with an increase in polymer content which could be due to increase in deposition of polymer on the surface of the crystals. The SEM results revealed the spherical structure of the agglomerates (Fig. 2). The surface morphology studies also revealed that the agglomerates were formed by very small crystals, which were closely compacted into a spherical form. The agglomerates prepared with sodium alginate were more spherical compared to PVP agglomerates of drug when observed under SEM.

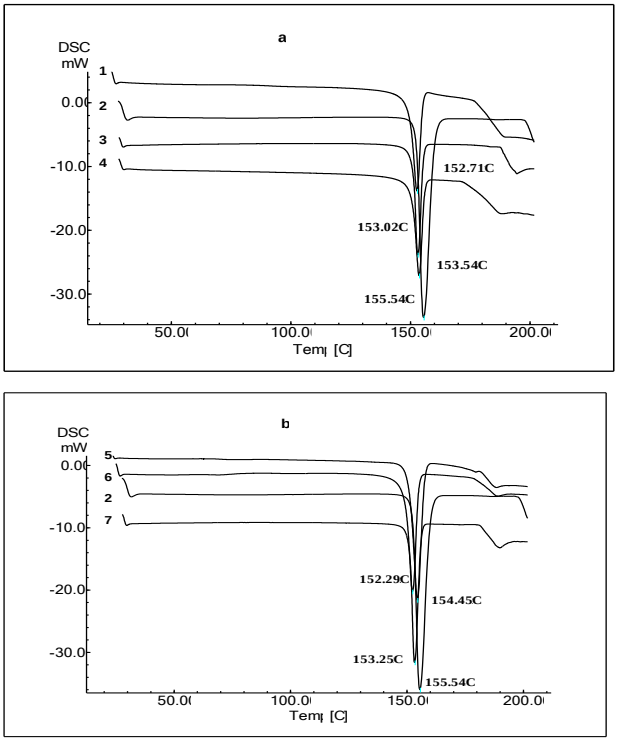


Fig. 1: DSC thermograms of physical mixtures (a) and agglomerates (b) 1=Aceclofenac+PVP-K30 (152.71ºC); 2=Aceclofenac (155.54ºC); 3= Aaceclofenac+ sodium alginate (153.02ºC); 4= Aaceclofenac+PVP-K90 (153.54ºC); 5=Spherical agglomerates prepared with PVP-K30 (154.45ºC); 6=Spherical agglomerates prepared with sodium alginate (152.29ºC); 7=Spherical agglomerates prepared with PVP-K90 (153.25ºC).

Physicochemical and micromeritic properties

The practical yield and drug content of agglomerates were found to be 78.47 to 87.98% and 91.72±2.49 to 98.34±1.21%, respectively. This indicates the negligible loss of drug during preparation.

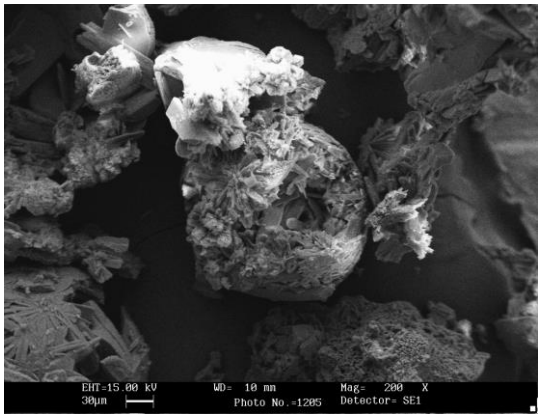
The LOD value for pure aceclofenac was 0.42% and for spherical agglomerates ranged from 0.34 to 0.42%. The results indicated that the presence of polymers reduced the residual moisture content of aceclofenac agglomerates.

The micromeritic properties of drug and the agglomerates are given in table 2. The Carr's index and Hausner's ratio values for pure drug were 29.99 % and 1.42, respectively.

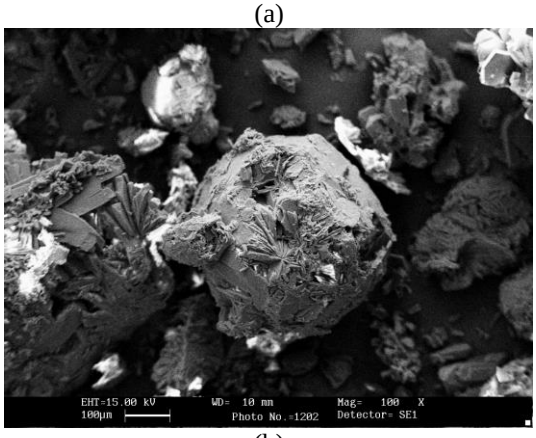
For agglomerates those values were ranged from 4.33 to 9.42% and 1.04 to 1.10, respectively, indicating considerable improvement of flow and compressional properties of drug in the form of agglomerates (Kachrimanis *et al*., 1998). In evident to this, angle of repose values of agglomerates were decreased noticeably when compared to pure drug, indicating further good flow properties of agglomerates (Aulton, 1990). The drug alone showed angle of repose of 46.92° and that of agglomerates was in the range of 18.32° to 22.31°. The high flowability of agglomerates may be due to spherical structure which exhibited higher packing ability than pure drug powder. The improvement in flowability contributes to making the filling of the die easier and more precise and thus gives more reproducible results. This fact, added to an increase in tablettability and compactability properties, helps to obtain a material for direct compression. These properties are probably due to the characteristics of agglomerated crystals which consist of an agglomeration of very small particles, favorable to compression.

Solubility and dissolution studies

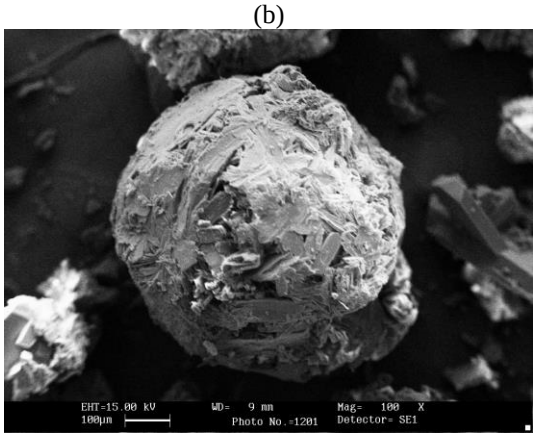
The results of solubility and dissolution study are given in Table 2 and the dissolution profiles are shown in Fig. 3 and 4. The solubility of pure drug was increased in its agglomerated forms from 81.89±2.34 µg/ml to 115.42±1.28 µg/ml in water and from 12.42±0.83 mg/ml to 15.01±0.97 mg/ml in phosphate buffer. Pure drug released 11.04±0.52% of drug at the end of 180 minutes, whereas the agglomerates (C-8) released 32.97±2.67% drug in water. Also in phosphate buffer, the drug release from agglomerates (C-8; 99.34±0.31%) was much higher than that of pure drug (94.34±1.20%). The results revealed that the spherical agglomerates showed increase in aqueous solubility and drug release compared to the pure drug. Among different polymers tested, PVP-90 showed better effect on solubility and dissolution rate compared to other polymers. As the concentration of PVP was increased, the solubility of drug was also increased, which could be due deposition of PVP onto the surfaces of agglomerates which improves the wettability. The results also revealed that the amount of PVP adsorbed onto the particles may increase by increasing the molecular weight and/or the concentration of PVP in the crystallization medium (Garekani *et al*., 2000). Meanwhile the drug solubility from sodium alginate agglomerates was not improved. The maximum drug release from sodium alginate agglomerates (C-9) was 94.41% at the end of 3 h and drug release was decreased as the concentration of sodium alginate was increased. This could be due to the formation of gelled layer of sodium alginate on the surface of the crystals. The gelation of spherical agglomerates caused a decrease in the specific surface area and the elongation of the diffusive path, led to the retardation in the drug release (Kawashima *et al*., 1995).



(a)



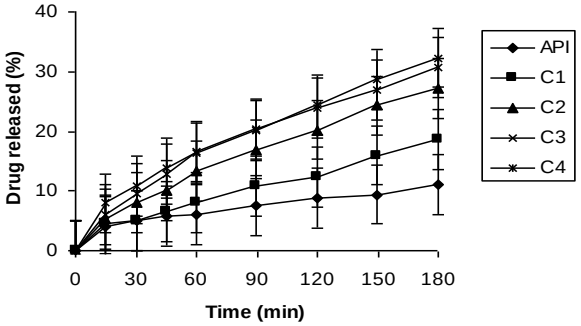
(b)



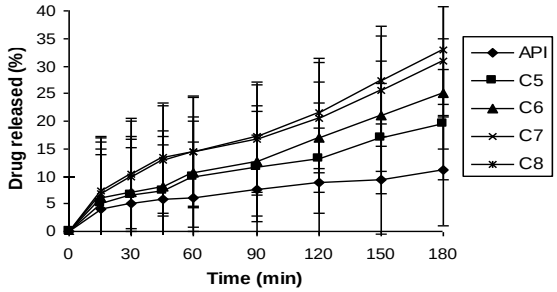
(c)

Fig. 2: Scanning electron microscopy of spherical agglomerates containing (a) PVP K-90 (b) sodium alginate- low magnification and (c) sodium alginate.

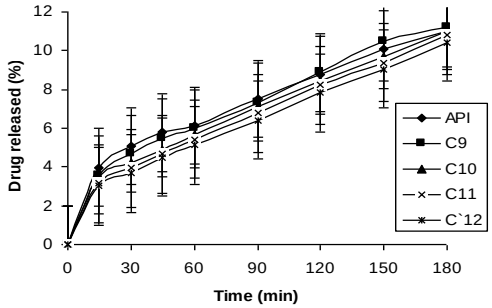
Among the prepared agglomerates, formulation C-8 was found to be better since they exhibited good solubility, high dissolution rate along with good flowability, compressibility and sphericity. C-8 agglomerates were selected for *in vivo* studies. Although agglomerates prepared using sodium alginate showed good flowability, compressibility and sphericity compared to PVP agglomerates, there was no increase in solubility and dissolution rate of drug from agglomerates.



(a)



(b)



(c)

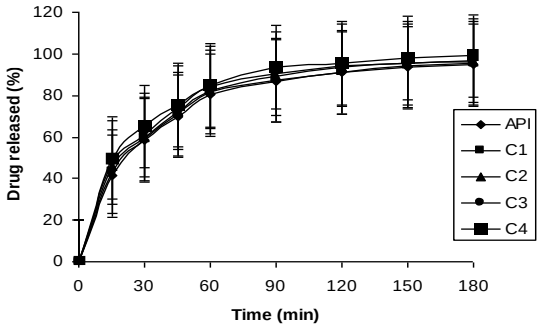
Fig. 3: Dissolution profile of pure drug and agglomerates in water (a) PVP K30 (b) PVP K90 (c) sodium alginate.

Stability study

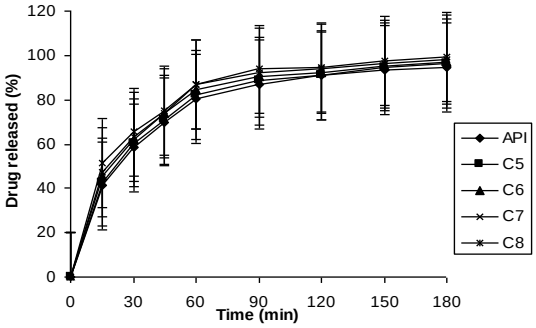
The values for drug content of spherical agglomerates were: 0 day: 99.89±0.19%; 15 days: 99.24±0.35%; 30 days: 98.87±0.26%; 60 days: 98.31±0.58%; 90 days: 97.75±0.60%; 180 days: 97.00±0.52%. On storage, the C-8 agglomerates did not show considerable change in the drug content for a period of 6 months at accelerated conditions when compared to initial value, which indicates good stability of the agglomerates.

Preclinical studies

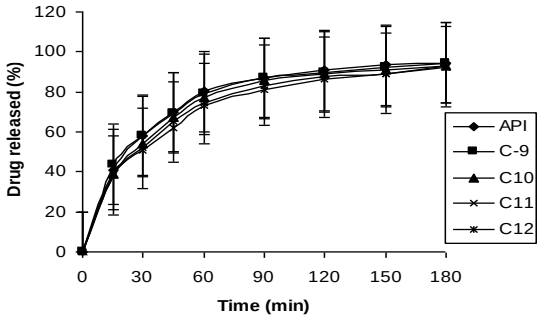
The results of anti-inflammatory activity are given table 3. C-8 agglomerates showed highest inhibition of edema 84.2±1.98 at 240 min, whereas pure aceclofenac provided maximum activity of 82.9±1.42 at 300 minutes. The value of inhibition of edema exhibited by drug in the form of agglomerates was higher at all time points compared to that of plain drug. Thus optimized agglomerates (C-8) showed rapid and higher performance in inhibiting rat paw edema when compared to pure aceclofenac.



(a)



(b)



(c)

Fig. 4: Dissolution profile of pure drug and agglomerates in phosphate buffer pH 6.8 (a) PVP K30 (b) PVP K90 (c) sodium alginate.

The effect of plain aceclofenac and C-8 agglomerates on acetic acid induced abdominal contractions in mice was compared to control group (no treatment). The control animals showed 34.83±13.04 contractions. The analgesic activity from agglomerated drug was faster (No. of contractions: 8.40±1.92) and % inhibition of contractions (78.46±2.52%) was high compared to pure aceclofenac (No. of contractions: 10.16±1.90; Inhibition of contractions: 70.82±1.25%). These results suggest the advantage of enhanced analgesic activity due to improved rate of

absorption and bioavailability of aceclofenac from its agglomerates.

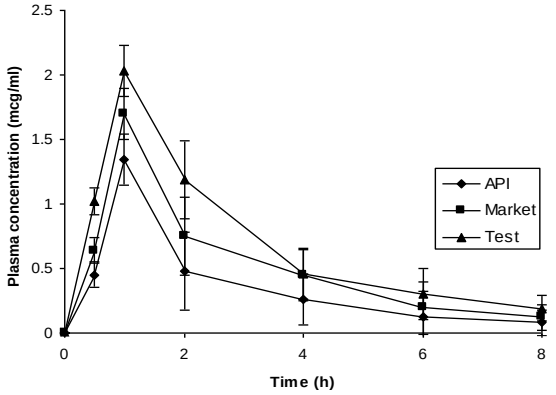


Fig. 5: Plasma drug concentration-time curve for pharmacokinetic study in rats API=Pure aceclofenac; Test=C-8 agglomerates.

In the pharmacokinetic study, the plasma concentration of aceclofenac against time is shown in fig. 5. The pharmacokinetic parameters were calculated from the plasma concentration-time curves and presented in Table 4. Low T_{max} value (1 h) was observed with all 3 groups indicated rapid absorption of drug. On the other hand, C_{max} value was high with C-8 agglomerates indicating maximum absorption of drug. The elimination half-life ($t_{1/2}$) of aceclofenac with agglomerates was less which suggests the rapid elimination of drug from the body. Low mean residential time (MRT) and high elimination rate constant values (K_e) of agglomerates in comparison with marketed tablet composition and pure drug further supported this result. The $t_{1/2}$, MRT and K_e values of plain aceclofenac indicated that pure drug remains in the body for more time. C-8 agglomerates showed high area under the curve (AUC) value when compared with marketed tablet composition and pure drug, which indicates the higher extent of absorption of drug. The higher value of AUC along with higher C_{max} and lower T_{max} values observed with C-8 agglomerates clearly indicated improved bioavailability of drug, which could be due to improved solubility and dissolution rate of drug from C-8 agglomerates.

CONCLUSIONS

The present study revealed that spherical agglomerization process is an interesting technique to improve the micromeritic properties, solubility, dissolution rate and bioavailability of aceclofenac. The study also demonstrated that PVP-K90 is an effective additive during crystallization of aceclofenac, which improved the *in vitro* and *in vivo* performance of aceclofenac. However, clinical investigation of the optimized agglomerates and real time stability studies are necessary to confirm these results.

REFERENCES

Alvarez-Larena A, Piniella JF and Carrasco E (1992). Crystal structure and spectroscopic study of 2-[(2,6-dichlorophenyl) amino] phenylacetoxyacetic acid (Aceclofenac). *J. Chem. Cryst.*, **22**: 323-328.

Aulton EM (1990). Pharmaceutical technology. Pharmaceutics. The science of dosage form design. English Language Book Society/ Churchill/livingstone, pp.610-612.

Chourasia MK and Jain NK (2003). Preparation and characterization of agglomerates of flurbiprofen by spherical crystallization technique. *Indian J. Pharm. Sci*., **May-June**: 287-291.

Garekani HA and Ford JL, Rubinstein MH and Rajabi-Siahboomi AR (1999). Formation and compression characteristics of prismatic polyhedral and thin plate-like crystals of paracetamol. *Int. J. Pharm.*, **187**: 77-89.

Garekani HA, Ford JL, Rubinstein AR and Rajabi-Siahboomi AR (2000). Highly compressible paracetamol: I: crystallization and characterization. *Int. J. Pharm.*, **208**: 87-99.

Kachrimanis K, Ktistis G and Malamataris S (1998). Crystallisation conditions and physicomechanical properties of ibuprofen–Eudragit® S100 spherical crystal agglomerates prepared by the solvent-change technique. *Int. J. Pharm.*, **173**: 61-74.

Kawashima Y, Takeuchi H, Niwa T, Hino T, Yamakoshi M and Kihara K (1989). The development of an emulsion-solvent-diffusion preparation method of agglomerated crystals for direct tableting and evaluation of the compressibilities. *Powder Technol.*, **26**: 659-665.

Kawashima Y, Cui F, Takeuchi H, Niwa T, Hino T and Kiuchi K (1995). Improved static compression behaviors and tablettabilities of spherically agglomerated crystals produced by the spherical crystallization technique with a two-solvent system. *Pharm. Res.*, **12**: 1040-1044.

Kulkarni SK (1997). Handbook of experimental pharmacology, 2nd revised and enlarged edition, Vallabh Prakashan, pp.52-56.

Kulkarni PK, and Nagavi BG (2002). Spherical crystallization. *Indian J. Pharm. Edu.*, **36**(2): 66-73.

Liebermann HA, Lachman L and Schwartz JB (1990). Pharmaceutical dosage forms: Tablets. Volume 2. Marcel Dekker. New York, pp.201-243.

Mutalik S, Paridhavi K, Mallikarjuna RC and Udupa N (2003). Analgesic and antipyretic effect of *Solanum melongena. Indian J. Pharmacol.*, **35**: 312-315.

Mutalik S, Naha A, Usha AN, Ranjith AK, Musmade P, Manoj K, Anju P and Prasanna S (2007). Preparation, *in vitro*, preclinical and clinical evaluations of once daily sustained release tablets of aceclofenac. *Arch. Pharm. Res.*, **2**: 222-234.

Paradkar AR, Pawar AP, Mahadik KR and Kadam SS (1998). Spherical crystallization: A novel particle design technique. *Indian Drugs*, **31**: 229-233.

Paradkar AR, Pawar AP, Chordiya JK, Patil VB and Ketkar AR (2002). Spherical crystallization of celecoxib. *Drug Dev. Ind. Pharm.,* **28**: 1213-1220.

Pawar A, Paradkar A, Kadam S and Mahadik K (2004). Agglomeration of Ibuprofen with talc by novel crystallo-co-agglomeration technique. *AAPS Pharm. Sci. Tech.*, **5**: 55.

Sano A, Kuriki T, Handa T, Takeuchi H and Kawashima Y (1987). Particle design of tolbutamide in the presence of soluble polymer or surfactant by the spherical crystallization technique: Improvement of dissolution rate. *J. Pharm. Sci.,* **76**: 471-474.

Wells JI (1988). Pharmaceutical preformulation, In: M.M. Rubinstein (Ed.), The physicochemical properties of drug substances. Ellis Horwood Limited. Chinchester.UK, pp.209-214.