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ACECLOFENAC SOLUBILITY ENHANCEMENT BY SONO-PRECIPITATION METHOD: FORMULATION OPTIMIZATION, CHARACTERIZATION AND *IN-VITRO* EVALUATION

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ABSTRACT
Aceclofenac is a commonly used NSAID drug for treatment of pain and inflammation. Aceclofenac has poor water solubility, hence it corresponds to BCS class II drug. Poor solubility leads to poor oral bioavailability, so a challenge for drug delivery. The objective of the present research work is to improve solubility of aceclofenac by using a novel combination of anti-solvent and sono-precipitation technique. Sonoprecipitation has several advantages over conventional methods, like it is cost effective, produces small particle, process is clean, etc. To make this formulation, we dissolved aceclofenac in ethanol and applied 250W sonification for 5 sec followed by 5 sec of interval. This process was repeated for 12 mins and produced micro-sized Aceclofenac dispersion. Further, we characterized and evaluated Aceclofenac dispersion by X-ray powder diffraction (XRPD), Fourier transform infrared (FT-IR) spectroscopy, differential scanning calorimetric (DSC) and *in vitro* solubility and dissolution rate. The results shows that sonoprecipitation process led to the development of partially amorphous microparticles. The particle size range of 1-3 µm with mean diameter of 1.59 µm. The *in-vitro* studies indicated that the saturation solubility and dissolution rate of aceclofenac microcrystals were enhanced by 2.5 folds and 2-fold respectively, as compared to crude aceclofenac. In conclusion, the process of combining the antisolvent precipitation under sonication produced small, uniform, and stable aceclofenac microparticles with enhanced dissolution, solubility, and bioavaibility.

Keywords: Aceclofenac, Microparticles, Sonoprecipitation, Solubility, Dissolution rate.

1. INTRODUCTION
Solubility of drugs is a major hurdle for drug development. Most of the drugs has poor solubility in water and hence results in poor bioavaibility [1]. Several approaches applied for enhancement of solubility like increasing surface area of particles, solid deposition, solid dispersion etc. [1, 2]. Among these the most common, efficient, and economical approach is to increase particle surface area by particle size reduction [3, 4]. According to the Noyes-Whitney equation, a marked reduction in particle size down to the submicron level would dramatically increase the effective surface area of the particles available for dissolution [1, 5].
Microparticle precipitation by the anti-solvent method is a direct and simple procedure for the formulation of drug microcrystals [6]. The process of antisolvent precipitation is carried out by mixing organic drug solution with an aqueous antisolvent solution in presence of stabilizing surfactants or polymers to form submicron particle. But controlling the particle size is difficult and cause particle instability [7]. In order to overcome this problem of physical instability, the process of anti-solvent precipitation under sonoprecipitation can be a better approach. Sonoprecipitation causes cavitation, which assists nucleation, this helps in generating more uniform particle size and morphology. Sonoprecipitation has several advantages like better mixing efficiency, atomize the drug solution into fine droplets, formation of smaller particles, and particle agglomeration can be inhibited [8-11].
Aceclofenac is a commonly used NSAID [12]. It has poor solubility and dissolution rate [13, 14]. Researchers have applied several approaches to improve aceclofenac solubility, but none of the study applied a novel combination of anti-solvent with sonoprecipitation [15-18]. In the present research work we formulated

aceclofenac microparticle using this novel approach. Further we evaluated aceclofenac microparticles for different particle characteristics, solubility and *in-vitro* dissolution. The resultant formulation was uniform with mean diameter of 1.59 µm. The saturation solubility and dissolution rate of aceclofenac microcrystals were enhanced by 2.5 folds and 2-fold respectively.

2. MATERIAL AND METHODS

2.1. Material

Aceclofenac was used as a model drug and was a generous gift from Aarti Drug Distributors, Mumbai (India). HPMCE5LV which was used as a stabilizing agent was obtained from Loba Chemie Pvt. Ltd, Mumbai. Ethanol of chromatographic grade was purchased from Finar Chemicals Ltd. All other chemicals and solvents were of reagent grade.

2.2. Methods

2.2.1. Formulation of microparticles

Sonoprecipitation was used to produce the aceclofenac microparticles. An apparatus (DP120 sonifier, PCi Analytics) consisting of a probe and sonifier was employed to provide the source of the ultrasound and a power of 250W was applied in the precipitation process. A glass vessel possessing the stabilizer solution was fixed under the probe (a tip of 8 mm in diameter) which was immersed 10 mm in the liquid. Ethanol and water were employed as solvent and anti-solvent, respectively, and the volume ratio of solvent and anti-solvent was 1:12. In brief, 0.5 g aceclofenac was dissolved in 5 ml ethanol to attain an organic solution. One ml of the solution was inserted into 12 ml 0.1% stabilizer solution through syringe under ultrasonic conditions at 250W. This process led to the generation of milky white aceclofenac microdispersions. The dispersion was sonicated intermittently by sonication for 5 seconds followed by 5 seconds intervals leading to total sonication time of 12 min. Spray-drying was used for solidification of aceclofenac suspensions to study saturation solubility and dissolution rate. The parameters, inlet temperature (80°C), outlet temperature (60°C), aspirator flow rate (45 Nm^3/hr) and feed pump flow rate (3 ml/min) were set for spray drying.

2.2.2. Experimental design

The levels for the designing of the experiment were selected by carrying out the process by designing the trial batches at various levels on trial-and-error basis. Experimental batches for the study were designed by using central composite design (CCD) by considering two independent variables such as:

I) X 1: Solvent / antisolvent ratio

II) X 2: Stabilizer concentration (% w/v)

III) X3: Sonication time (min)

The dependable variables selected for the study are average particle size, solubility, drug content, *in vitro* drug release. Detailed summary of experimental design is given in table 1 and the response values for the design are mentioned in Table 2 and the actual designed batches are given in Table 3.

2.2.3. Process efficiency

The weight used for formulation is considered as theoretical yield and dried formulation weight was reported as practical yield [22]. The percentage yield of the experiment was determined by using the formula given as:

Process efficacy = (Practical yield of drug in gm/ theoretical yield in gm) X 100

Table 1: Summary for designing of batches

Factor	Name	Units	Type	Subtype	Min	Max	-1	+1
A	Antisolvent/solvent	-	Numeric	Continuous	10	20	12.03	17.97
B	Stabilizer Conc.	% w/v	Numeric	Continuous	0.05	0.3	0.1	0.25
C	Time	Min	Numeric	Continuous	1	15	3.84	12.16

Table 2: Responses selected for optimization

Response	Name	Unit	Analysis
Y1	Particle size	µm	Polynomial
Y2	Solubility	mg/ml	Polynomial
Y3	Drug Content	%	Polynomial
Y4	In vitro drug release	%	Polynomial

Table 3: Experimental batches designed by using central composite design (CCD)

Batch No.	STDS	Runs	Antisolvent/Solvent ratio	Stabilizer Concentration (g)	Time (min)
S1	9	1	10	0.17	8
S2	20	2	15	0.17	8
S3	17	3	15	0.17	8
S4	10	4	20	0.17	8
S5	18	5	15	0.17	8
S6	3	6	12	0.25	4
S7	12	7	15	0.3	8
S8	19	8	15	0.17	8
S9	16	9	15	0.17	8
S10	11	10	15	0.05	8
S11	8	11	18	0.25	12
S12	6	12	18	0.10	12
S13	2	13	18	0.10	4
S14	13	14	15	0.17	1
S15	1	15	12	0.10	4
S16	15	16	15	0.17	8
S17	7	17	12	0.25	12
S18	5	18	12	0.10	12
S19	4	19	18	0.25	4
S20	14	20	15	0.17	15

2.2.4. Drug content

To analyze drug content 10 mg of aceclofenac drug sample and dissolved in 100 ml of phosphate buffer pH 7.5 and absorbance was measured on UV-visible spectrophotometer at 275nm [22]. Below formula was used for calculation:

Percent Drug content = (Test absorbance/Standard absorbance) X Standard concentration/weight of drug) X dilution factor X 100

2.2.5. Particle size

The mean particle size of aceclofenac microparticles was measured by the method of laser light diffraction using Malvern Mastersizer Micro Ver. 2.19 (Malvern Instruments Ltd, UK). Prior to measurements, about 50 mg of each sample were dispersed with 100 ml of hexane. The particle size distributions were estimated by setting the intensity of scattered light at a wavelength of 750 nm and the scattering angle (θ) of 90° [12,13].

2.2.6. Saturation solubility

Saturation solubility of aceclofenac microparticles was determined in distilled water. Excess of drug 200 mg was added in each cap vial containing 5 ml distilled water. Each vial was in sonicated for about 15 min so that excess amount of drug gets dissolved up to super saturation and some drug is kept in suspended form. The vials were kept in orbital shaker for 48 hrs for stirring. The selected quantity of sample was centrifuged at 7500 rpm for 15 min so that excess amount of supernatant obtained. The drug in supernatant was analyzed by making proper dilution with selected ratio of solvent by UV-Spectrophotometer at 272 nm to calculate the solubility of drug [4-6].

2.2.7. Fourier Transform Infrared Spectroscopy

Infrared spectrum of aceclofenac and its microparticles was determined on Fourier Transform Infrared Spectrophotometer (FT/IR 4100, Jasco) using KBr dispersion method. The base line correction was done using dried potassium bromide. The samples to be examined and KBr were previously dried in oven for 30 min and mixed meticulously with potassium bromide in 1:25 (sample: KBr) ratio in a glass mortar. These samples were then placed in a sample holder and scans were obtained at a resolution of 2 cm^{-1} from 4000 to 400cm^{-1} [21].

2.2.8. Zeta Potential

Formulation (0.5 ml) was diluted to 50ml with distilled water in glass beaker with constant stirring. Zeta-potential of the resulting suspension was determined using the Zetasizer (model: Nano ZS, Malvern Instruments, Westborough, MA, USA). Electrophoretic

mobility (μm/s) was assessed using small volume disposable zeta cell and converted to zeta potential by in-built software using Helmholtz-Smoluchowski equation. All determinations were performed in triplicate [22].

2.2.9. Flow properties of product

The obtained powder sample by SAA processing was further evaluated for its flow properties like angle of repose, bulk density, tapped density, hausner's ratio and compressibility index. These were the important properties which were required to be studied before formulation into dosage form. Reports of these studies were given in results [18].

2.2.10. Polydispersity index

The PDI determination was done using photon correlation spectroscopy with in-built Zetasizer (model: Nano ZS, Malvern Instruments, Westborough, MA, USA) at 633 nm [22].

2.2.11. Differential Scanning Calorimetry (DSC)

DSC measurements were executed on a differential scanning calorimeter equipped with an intra-cooler (DSC Mettler STAR SW 9.20, Switzerland). Inert atmosphere was sustained by ejecting nitrogen gas at a flow rate of 50 ml/min. All accurately weighed samples (about 5-10 mg of samples) were placed in a sealed aluminum pan, and the samples were heated under nitrogen gas flow (20 ml/min) at a scanning rate of 10°C per min from 40 to 300°C. An empty aluminum pan was used as reference [19, 20].

2.2.12. Powder X-Ray Diffraction Study (XRD)

X-ray diffraction patterns of the powdered samples of the drug and carrier were recorded using Philips PW3710 Analytical XRD B. V. X-ray diffractometer using Cu K 2α rays with a voltage of 40 kV and a current of 25 mA. Samples were scanned for 2θ from 5 to 80°. Diffraction pattern for aceclofenac micro-particles were obtained [6, 12].

2.2.13. Scanning Electron Microscopy (SEM)

Morphological characteristics of sonoprecipitated aceclofenac powder were analyzed by a Scanning Electron Microscope (JEOL, JSM-6360A, Japan). Powder was dispersed on a carbon tab previously stuck to an aluminum stub. Samples were coated with gold-palladium (layer thickness 250Å) using a sputter coater [12, 14].

2.2.14. Optical microscopy

Optical microscopy of the drug sample was carried out using Motic Digital Microscope. Slight quantity of the powder sample was spread on the glass slide by using fine haired brush. This slide was focused under various magnification lenses and the pictures were captured [22].

2.2.15. In vitro drug release study

In vitro drug release study was performed according to the guidelines given in USP 30- NF24. Test was performed by using USP apparatus II (Basket type). The volume of medium used was 900ml of Distilled water. The temperature was sustained at 37± 2°C. The RPM of the study was kept at 50. 5ml aliquots were withdrawn at time interval of 10 min and the study was carried for duration at which drug shows almost complete release from the capsule. According to the USP standards at least not less than 70% (Q) of the labeled amount of $C_{16}H_{13}Cl_2NO_4$ should get released (USP30) [22].

3. RESULTS

3.1. Process efficiency

The percent yield was calculated from the theoretical and practical yield. Since spray drying was employed to obtain the microparticles in dried form, the percent yield was affected. The percent yield was observed to be in the range of 59-70% (Table 4).

Table 4: Percent yield of batches S1-S20

Batch	Percent Yield	Batch	Percent Yield
S1	65.22±0.568	S11	62.87±0.014
S2	64.08±0.718	S12	63.56±0.115
S3	70.16±0.623	S13	64.12±0.117
S4	67.12±0.967	S14	67.12±0.216
S5	64.07±0.728	S15	67.44±0.701
S6	67.42±0.811	S16	63.45±0.215
S7	63.2±0.425	S17	66.21±0.315
S8	65.45±0.331	S18	63.53±0.325
S9	66.8±0.397	S19	61.98±0.218
S10	59.36±0.501	S20	66±0.115

3.2. Drug Content

Drug content in the microparticle batches (S1-S20) were analysed by UV spectrophotometer at 272 nm. The results of drug content are given in table 5. Drug content in batch S5, S12, S17, S18 was detected to be highest. Batch S6 and S15 showed lowest % drug

content in microparticles. This may be due to the stabilizer concentration and antisolvent/solvent ratio [22]. Thus, it was observed that higher the stabilizer concentration and antisolvent/ solvent ratio, higher the encapsulation of the drug and hence the drug content was observed to be maximum for S18.

Table 5: Drug content of batches S1-S20

Batch	Drug Content (%)	Batch	Drug Content (%)
S1	76.67±0.68	S11	76.46±0.50
S2	74.86±0.8	S12	85.74±0.65
S3	78.8±0.72	S13	79.45±0.50
S4	81.43±0.50	S14	74.41.43±0.520
S5	85.91±0.50	S15	72.62±0.54
S6	71.62±0.54	S16	73.66±0.57
S7	83.49±0.50	S17	84.42±0.51
S8	72.56±0.51	S18	85.64±0.55
S9	80.48±0.50	S19	83.42±0.51
S10	73.54±0.50	S20	76.4±0.52

3.3. Particle Size Determination

The average particle diameter of the aceclofenac after complete processing of the drug is shown in table 6. The mean particle size of aceclofenac when processed by sonoprecipitated technique was considerably smaller than the unprocessed aceclofenac. The unprocessed aceclofenac showed particle diameter average up to 200 mm while after processing it showed particle diameter ranging from 1-3 mm which was suitable for many of the formulations like powder for inhalation. This may be due to the fact that as the antisolvent/ solvent ratio increases, the supersaturation and nucleation rate of drug is enlarged, and the particle size became smaller [22].

Table 6: Average particle size of batches S1-S20

Batch	Particle Size (µm)	Batch	Particle Size (µm)
S1	1.583±0.04	S11	3.14±0.707
S2	1.77±0.03	S12	2.38±0.18
S3	2.41±0.42	S13	2.34±0.233
S4	1.96±0.15	S14	1.61±0.101
S5	1.386±0.102	S15	1.53±0.15
S6	2.79±0.1	S16	2.91±0.101
S7	2.52±0.12	S17	1.64±0.15
S8	2.11±0.16	S18	1.56±0.15
S9	2.51±0.12	S19	1.48±0.12
S10	1.89±0.167	S20	1.71±0.8

3.4. Particle Size Distribution

The fig. 1 represents particle size distribution of sonoprecipitated drug. The mean diameter was found to be 1.591 µm. The particle size distribution was found to be narrow which is indicated by the sharp peak. Thus, all the particles were found to be uniform in size. The reason behind uniform particle size distribution may be due to increased supersaturation which leads to increased nucleation and inhibition of crystal growth [3].

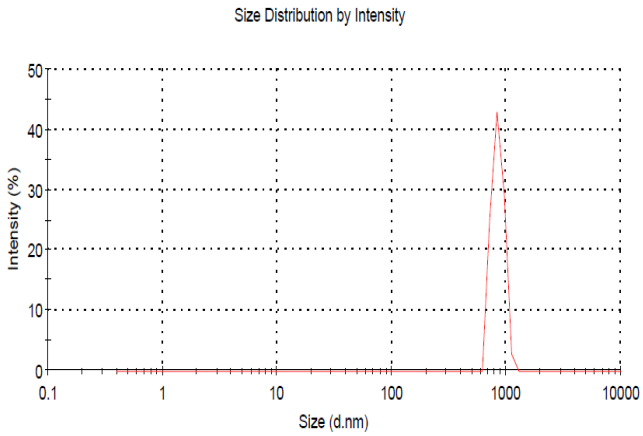

Fig. 1: Particle Size Distribution of optimized batch (S18)

3.5. Saturation Solubility

Saturation Solubility of the microparticle batches (S1-S20) was analysed by UV spectrophotometer at 272 nm. The results of saturation solubility are given in Table 7. The values of solubility showed that there was significant change in solubility of aceclofenac after processing by sonoprecipitation. Initially unprocessed aceclofenac showed saturation solubility of 0.15 mg/ml in distilled water and later it was increased significantly ranging between 0.285 to 0.396 mg/ml as. Thus, the results indicate that the solubility has increased by 2.5 times than the original. When process was carried out at higher antisolvent/solvent ratio, higher stabilizer concentration and under sonicating conditions for long period of time, the product obtained showed the greater solubility in comparison with that of lower values. S18 showed the maximum solubility in water while batches S11 and S7 showed the minimum solubility amongst all other batches. This may be due to a higher antisolvent/ solvent ratio, particle size of the drug obtained was smaller, which exposes higher surface area to solvent which increase the solubility of the drug in water [1] and ethanol has good diffusivity property which generates the porous powder, which has good water uptake

capacity and hence the solubility of the powder obtained was higher [27].

3.6. FTIR

Sonicated microparticles along with drug and polymer were studied by FT-IR spectrum. It has been observed that all the characteristic peaks (Table 8) were retained but the intensity has been reduced in graphs of FTIR spectra, confirming compatibility of aceclofenac with all excipients. These results indicate that there are no chemical or structural changes in drug (Fig. 2).

3.7. Zeta Potential Determination

The fig. 3 represents the zeta potential of sono-precipitated drug. The average zeta potential was found to be -23.5mV. This value indicates that the formulation has medium stability [16].

Table 7: Saturation solubility of batches S1-S20

Batch	Solubility (mg/ml)	Batch	Solubility (mg/ml)
S1	0.351±0.09	S11	0.296±0.015
S2	0.324±0.014	S12	0.372±0.013
S3	0.343±0.009	S13	0.343±0.008
S4	0.325±0.007	S14	0.368±0.009
S5	0.394±0.014	S15	0.355±0.009
S6	0.347±0.012	S16	0.346±0.014
S7	0.274±0.013	S17	0.366±0.018
S8	0.33±0.008	S18	0.38±0.014
S9	0.366±0.01	S19	0.375±0.004
S10	0.366±0.009	S20	0.35±0.005

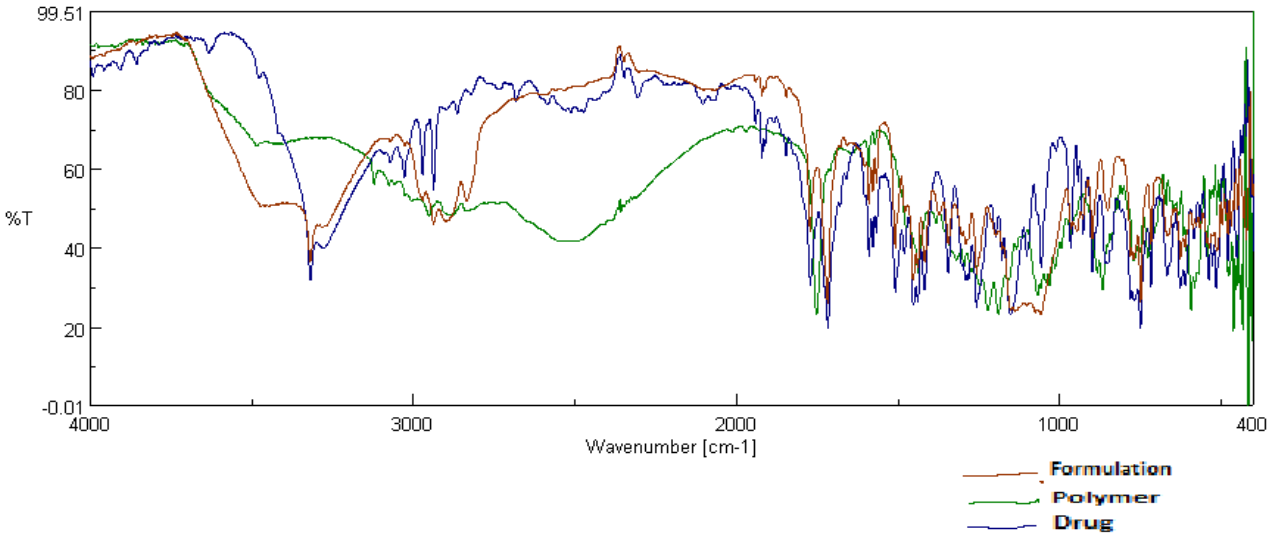

Fig. 2: IR spectra of drug, polymer, and formulation

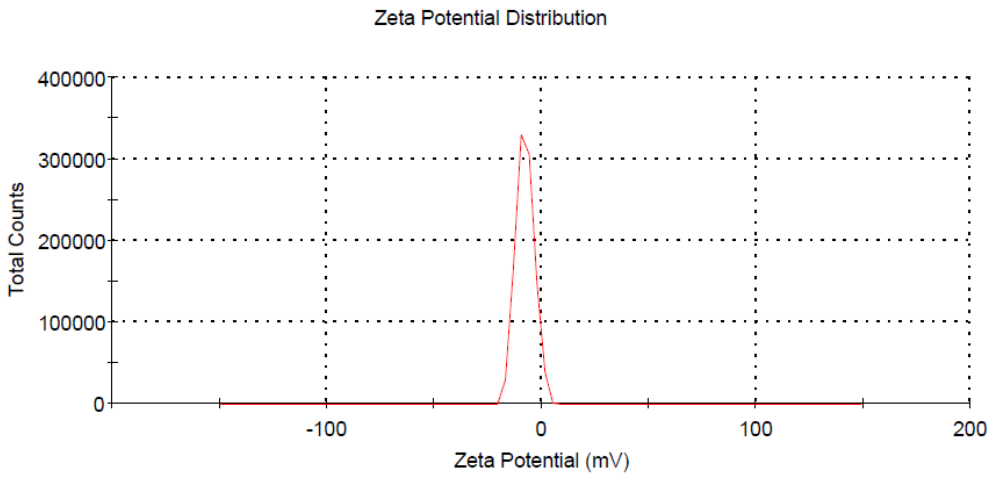

Fig. 3: Zeta potential distribution curve of formulation

Table 8: IR interpretation data of microparticles formulation

Sr. No.	Remarks	Peak cm^{-1} (Observed)
1.	N-H Stretching	3327.57
2.	O-H Stretching	3025.76, 2094.32
3.	C-O stretching	1707.66
4.	skeleton vibration of aromatic C-C stretching for NH	1513.85
5.	O-H in plane bending	1339.32
6.	CN aromatic amine	1250.61
7.	O-H out plane bending	908.308

3.8. Flow Properties

Microparticles of the aceclofenac were evaluated for its flow properties like Bulk density, tapped density, Angle of repose, Compressibility properties and Hausner's ratio [6]. The results of this analysis were shown in Table 9. These reports shows that the formed particles have the flow properties within the passable range, and which is best suitable in both tablet as well as powder for inhalation formulations. This may be probably due to formation of spherical particles by sonoprecipitation technique. Since the powder shows uniform distribution, the flow properties of drug are modified [15].

3.9. Polydispersity Index

The polydispersity index was observed to be 0.561 which was observed to be less than 1. Thus, it indicates that the suspension is monodispersed [8].

3.10. Differential scanning calorimetry

The DSC measurements were carried out to further investigate the properties of the crystals and their DSC thermograms were shown in Fig. 4. The thermograms of crude aceclofenac indicated a single endothermic peak at 152°C (ΔH = -92.29 J/g) ascribed to drug melting. Microparticles exhibited a melting point of 125.7°C (ΔH = -7.36 J/g), indicating that the intrinsic crystalline properties had been retained. The endothermic curve was decreased in size indicated that amorphous character has been introduced in the microparticles. The decreasing in melting ΔH can be understood first by the fact that, at a fast nucleation rate, the drug solute lacks sufficient time to be incorporated into the growing crystal lattice to form perfect crystals which leads to lower lattice energy. Second, the lower melting point can be attributed to the reduction in crystal size. The decreased crystallinity of aceclofenac confirmed by DSC results might contribute to the increase of solubility, dissolution, and bioavailability [22].

Table 9: Flow properties of batches S1-S20

Batch No.	Angle of repose (^{0})	Bulk density (gm/cm^3)	Tapped Density (gm/cm^3)	Carr's index	Hausner's ratio
S1	32.67±0.5950	0.314±0.0017	0.405±0.0048	22.33±0.538	1.287±0.0087
S2	31.80±0.5773	0.323±0.0012	0.413±0.0024	21.35±0.541	1.276±0.015
S3	30.84±0.5980	0.319±0.0012	0.410±0.0017	22.16±0.732	1.284±0.0118
S4	31.94±0.2950	0.315±0.0008	0.407±0.0021	22.57±0.366	1.291±0.0062
S5	31.64±0.4927	0.324±0.0008	0.415±0.0020	21.96±0.671	1.280±0.011
S6	30.48±0.4899	0.342±0.0012	0.422±0.0020	18.97±0.407	1.231±0.012
S7	30.34±0.7715	0.338±0.0012	0.416±0.0020	18.71±0.137	1.229±0.0011
S8	29.71±0.5456	0.333±0.0012	0.409±0.0028	18.59±0.536	1.228±0.0078
S9	31.36±0.5543	0.343±0.0012	0.425±0.0021	19.18±0.502	1.27±0.0596
S10	32.01±0.5161	0.313±0.0012	0.404±0.0036	22.43±0.704	1.288±0.0104
S11	29.85±0.5278	0.323±0.0012	0.414±0.0014	21.55±0.242	1.27±0.0121
S12	32.20±0.6923	0.318±0.0008	0.413±0.0016	22.99±0.338	1.29±0.0046
S13	31.93±0.6655	0.318±0.0016	0.408±0.0008	22.36±0.951	1.278±0.0005
S14	32.98±0.7539	0.323±0.0016	0.415±0.0020	22.20±0.775	1.286±0.0143
S15	30.09±0.8172	0.320±0.0017	0.418±0.0016	23.25±0.581	1.304±0.0075
S16	28.96±0.5532	0.342±0.0016	0.422±0.0020	19.05±0.531	1.235±0.0080
S17	28.87±0.8229	0.337±0.0016	0.415±0.0023	18.90±1.011	1.233±0.0151
S18	31.05±0.3231	0.356±0.0012	0.434±0.0026	17.99±0.918	1.193±0.0388
S19	31.02±0.4325	0.334±0.0020	0.411±0.0020	18.67±0.228	1.229±0.0036
S20	31±0.3417	0.343±0.0012	0.426±0.0016	19.43±0.566	1.275±0.0562

**Flow properties represent mean ± SD, n = 3 determinations*

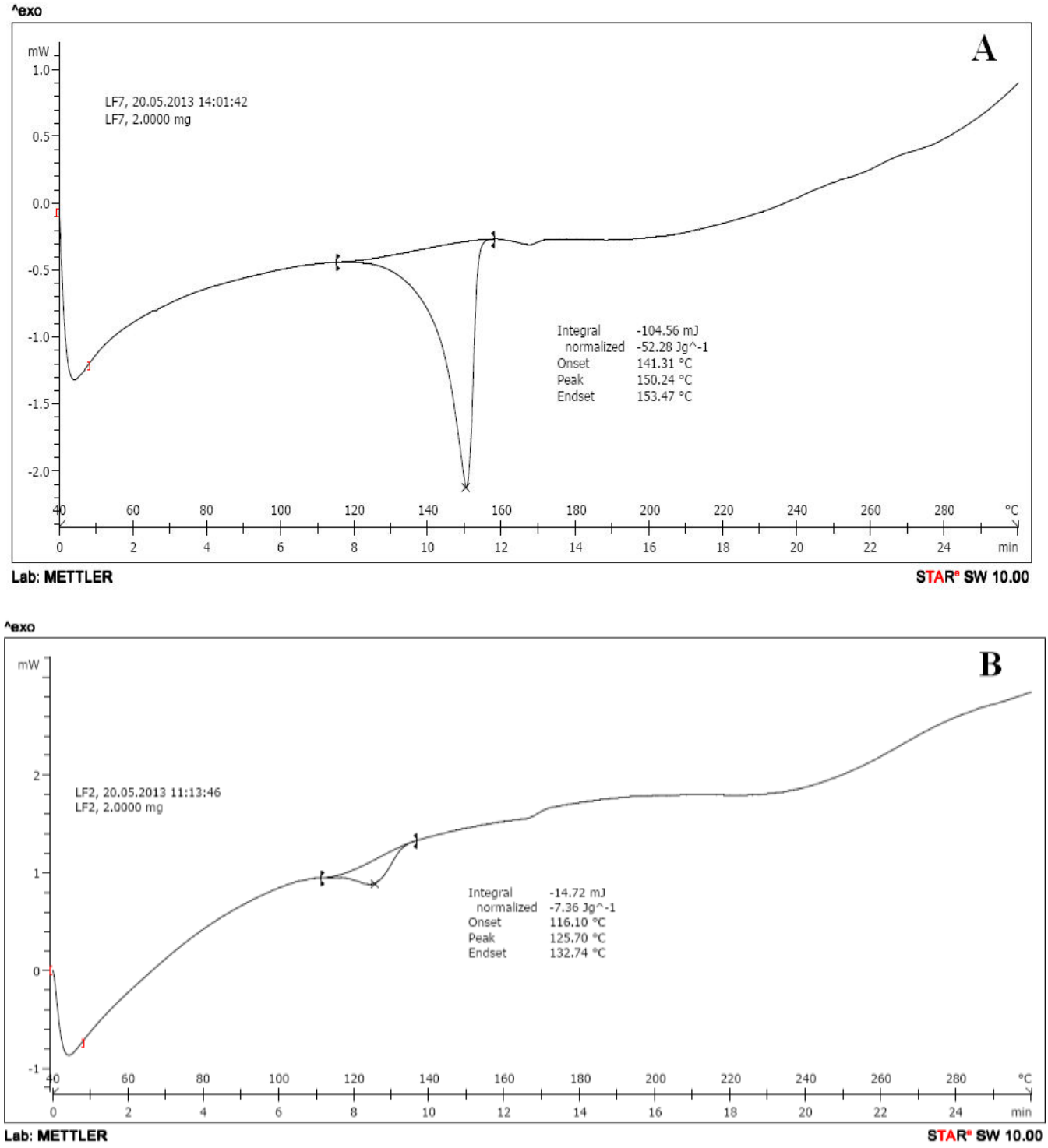

Fig. 4: DSC curves of A) drug B) formulation

3.11. Powder X-ray diffraction study

X-ray diffraction patterns of Fig. 5 revealed that pure drug was in crystalline state as it showed sharp distinct peaks notably at 2θ diffraction angles of 17.53, 18.53, 19.44, 22.27, 24.5, 25.94, 32.210 with 838, 1450, 904, 1868, 1604, 1175 intensities for aceclofenac respecttively. The reflections (specific peaks) corresponding to the drug were found in the formulation diffractogram with reduced intensity as 489,1015,699,1580,965,3048 and 326 respectively as compared to drug alone.

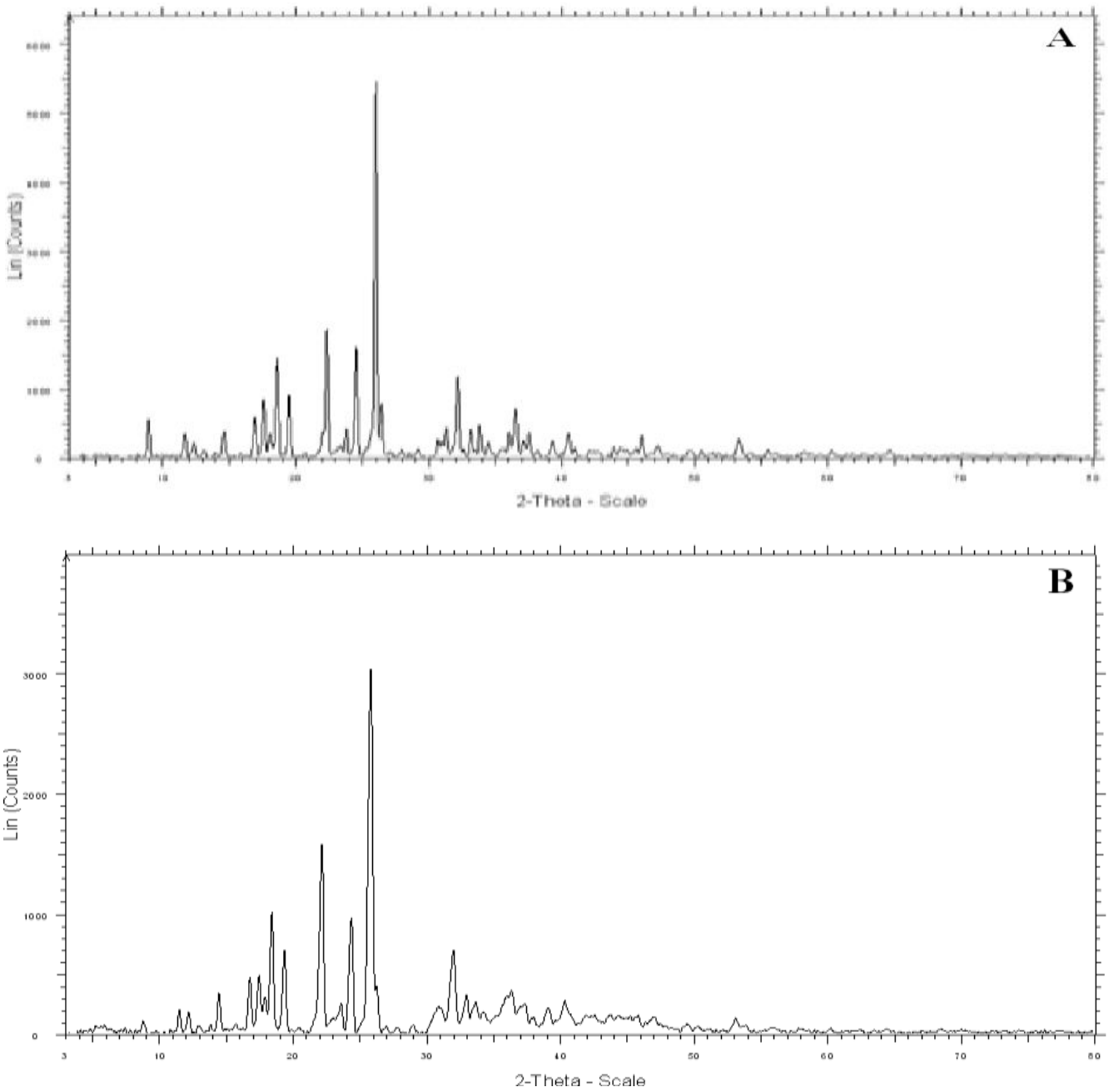

Fig 5: XRD graph of A) drug B) formulation

3.12. Scanning Electron Microscopy (SEM)

The uniformity and spherical nature of discrete microparticles without agglomeration was noticed in the photomicrograph of sonoprecipitated drug (Fig. 6 A). It was confirmed from the photomicrographs of unprocessed drug that the fine particles aggregated into large micron-sized porous aggregates. (Fig.6 B). When the drug solution was added into the anti-solvent, it did not disseminate immediately and produced local zones of excessive supersaturation. When anti-solvent process uses mechanical stirring, poor micro mixing is inevitable which raises the precipitation rate locally resulting in agglomeration and surge in particle size as observed in conventional precipitation technique. In contrast homogeneous micro mixing during ultrasonication maintains reasonably uniform conditions throughout the vessel causing the formation of nanosized discrete microparticles [22].

3.13. Optical microscopy

Fig. 7 shows the optical microscopic (Motic Microscope) images of the Aceclofenac after processing

by the sonoprecipitation. The image shows the sharp distribution of drug particles after processing; there appears some clusters of the particles due to generation of static charges aroused due to micronization. All S1-S20 batches showed very fine and narrow distribution of the particles [3].

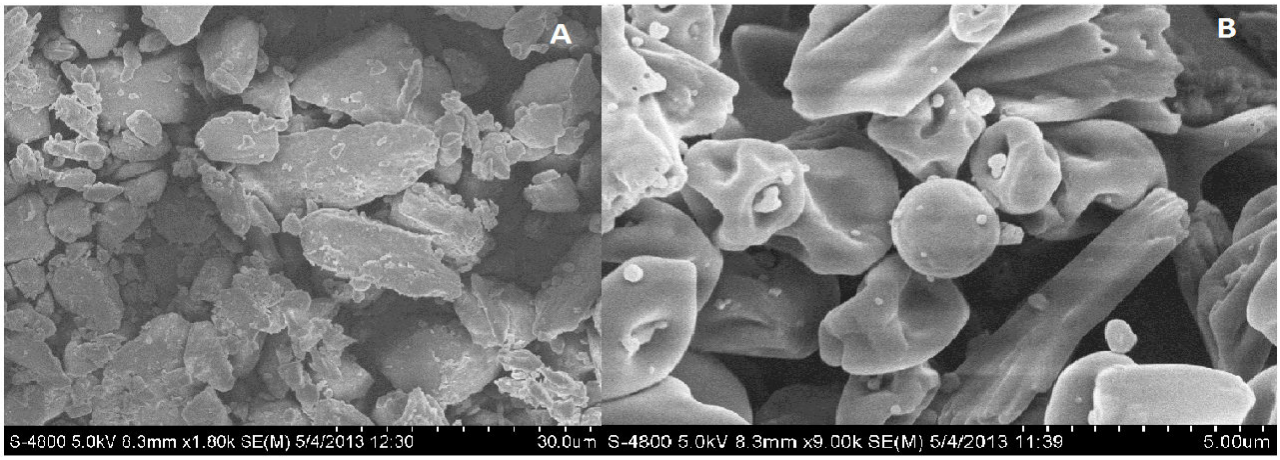

Fig. 6: SEM images of A) drug B) formulation

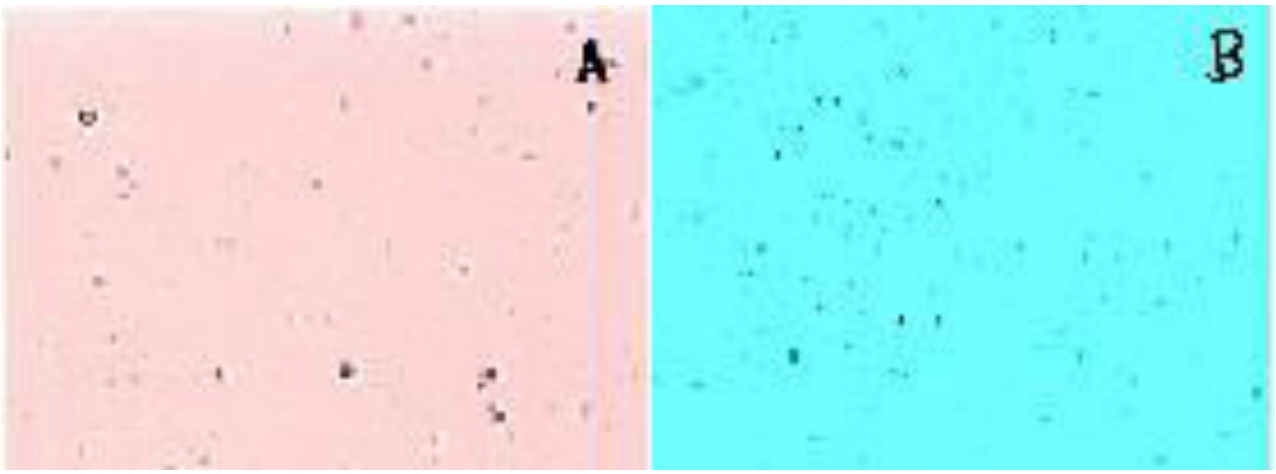

Fig. 7: Optical microscopic image of A) drug B) formulation

3.14. *In Vitro* Drug release profile

The *in vitro* dissolution profile of the drug (aceclofenac) was carried as per the USP I (Basket type) apparatus (Electrolab-TDL-08L) specifications. All the batches S1-S20 shows the better release profile than the pure unprocessed drug. Batches S1, S5 and S18 show the rapid release of drug within 30 min of study. Batches S1 and S18 showed the maximum release. All the batches S1-S20 shows the release kinetics by first order drug release. It showed the r^2value up to 0.9646 and 'K' value was 13.4574, which indicate anomalous transport by erosion mechanism**.** These results are passing the USP specifications for the aceclofenac [20]. These batches show the rapid release of drug from its formulations. The batches with greater solubility showed rapid dissolution of drug. As the particle size of the sonoprecipitated aceclofenac is lesser, drug shows higher solubility which results into rapid release of the drug. Aceclofenac shows complete release of the drug from formulation, but its onset of action is late. After processing, onset was detected to be quicker which may reduce dose requirement [28]. Graphs of the drug release from capsules were given in the Fig. 8. proving the release rate and kinetics of drug release. This rapid release may be due to micronization of drug particles and increased solubility of drug, which ultimately enhances the dissolution of drug. Solubility depends on particle size, generated porosity, and increased water uptake capacity of drug, which rapidly dissolves drug in medium [3].

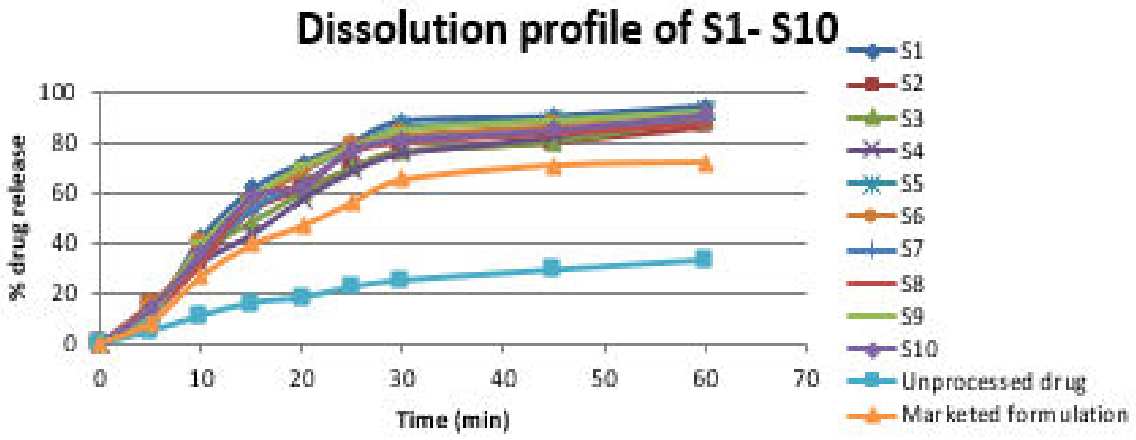

Fig. 8: Dissolution profile of batches S1-S10 along with unprocessed and drug and marketed formulation

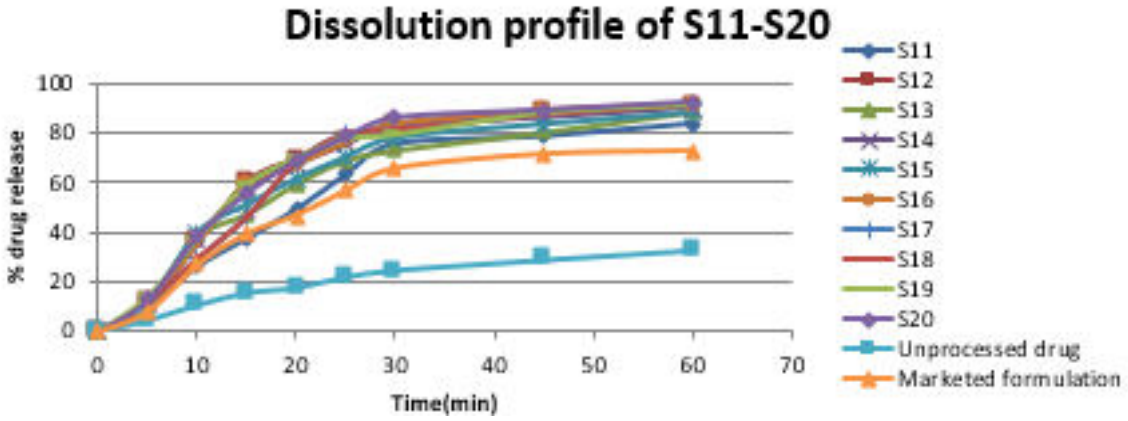

Fig. 9: Dissolution profile of batches S11-S20 along with unprocessed and drug and marketed formulation

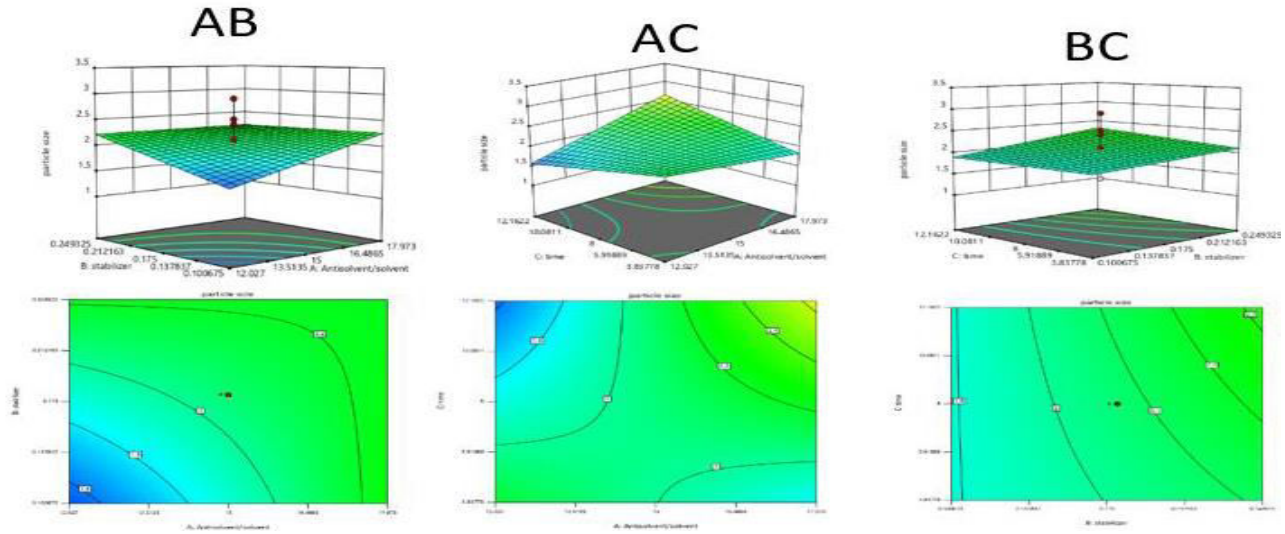

Fig. 10 Effect of Antisolvent to solvent ratio (A), stabilizer concentration (B) and time (C) on particle size

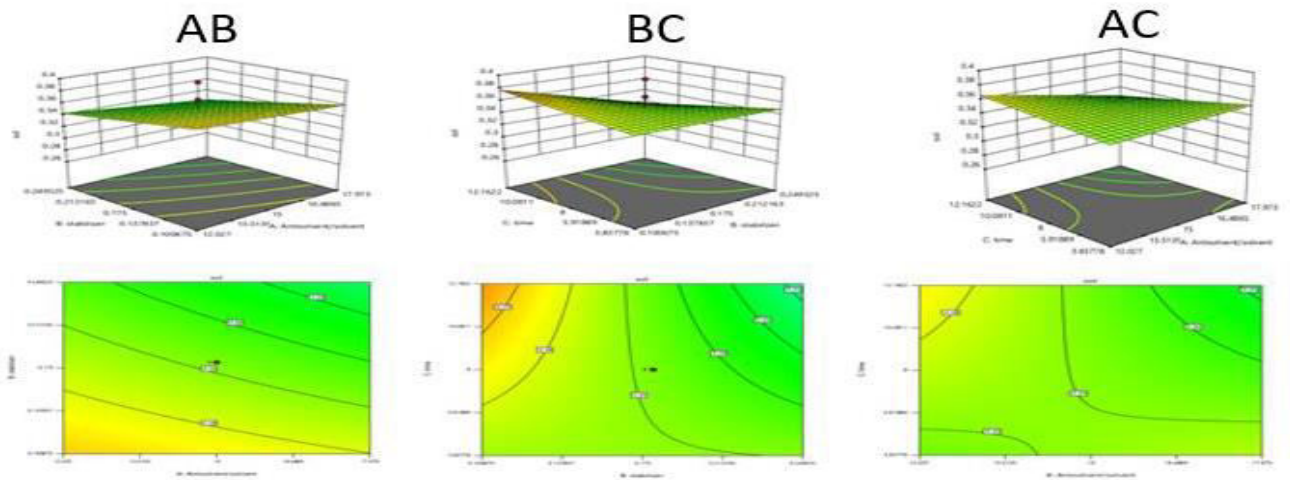

Fig. 11: Effect of Antisolvent to solvent ratio (A), stabilizer concentration (B) and time (C) on solubility

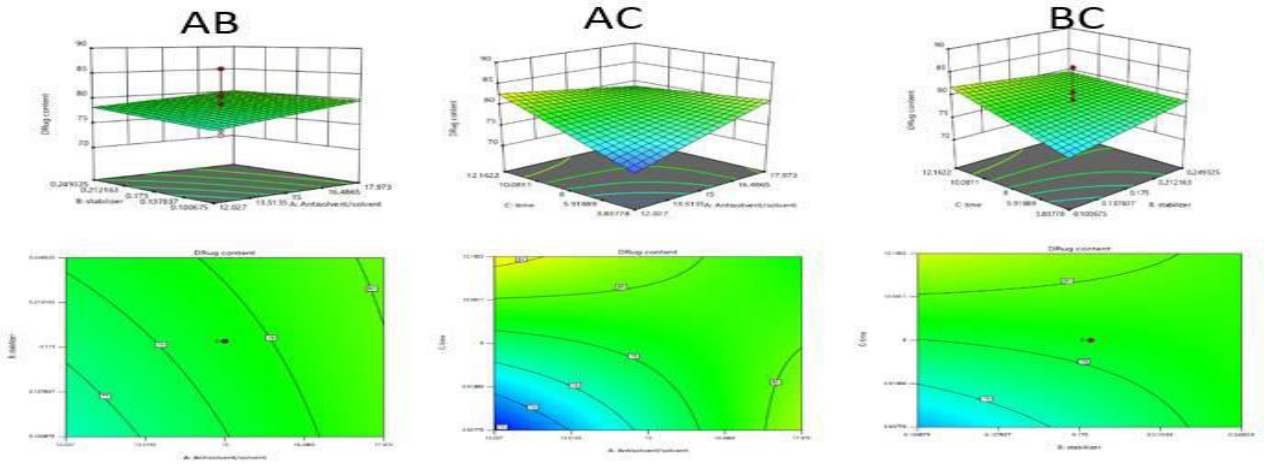

Fig. 12: Effect of Antisolvent to solvent ratio (A), stabilizer concentration (B) and time (C) on drug content

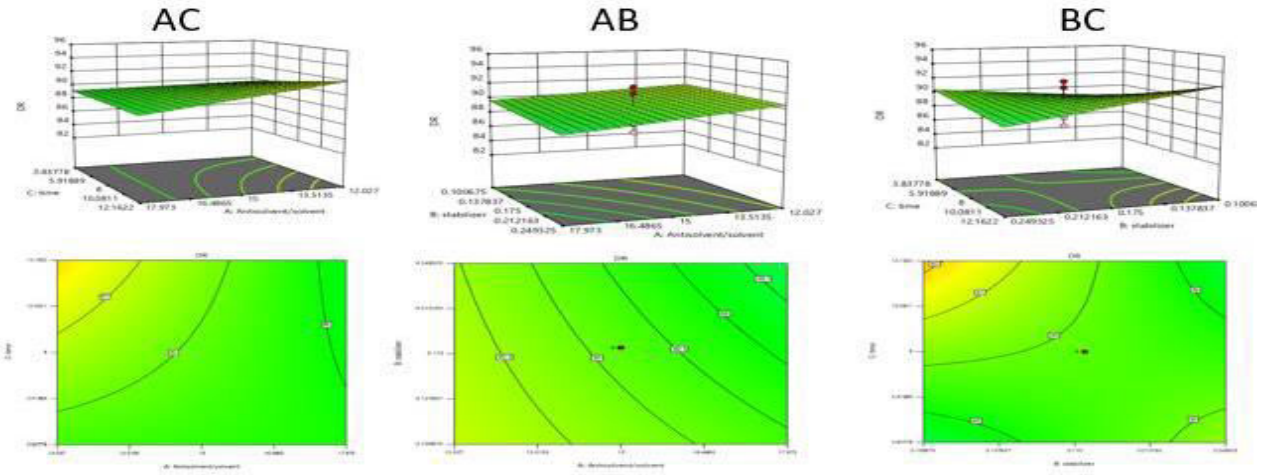

Fig. 13: Effect of Antisolvent to solvent ratio (A), stabilizer concentration (B) and time (C) on drug release

Table 10: Regression results of measured responses after application of CCD

Responses	Standard Deviation	R Squared	Adjusted R Squared	Adequate precision	Model
Particle Size	0.45	1.98	2.253	6.347	2 FI
Solubility	0.025	0.337	0.3001	8.705	Linear
Drug Content	3.62	0.5797	0.3858	6.187	2 FI
Drug Release	2.21	0.2376	0.1953	6.817	Linear

Table 11: ANOVA results for response surface reduced 2fi model for particle size

Source	Sum of Square	Degree of freedom	Mean Sum of Square	F value	p- value Prob> F
Model	2.06	4	0.52	2.58	0.0496 significant
A- Antisolvent/Solvent ratio	0.13	1	0.13	0.68	0.4239
B- Stabilizer Conc.	0.82	1	0.82	4.09	0.0614
C- Time	0.046	1	0.046	0.23	0.6362
AC	1.07	1	1.07	5.34	0.0354
Lack of fit	1.37	10	0.14	0.42	0.8838 Not Significant

Table 12: ANOVA results for response surface reduced linear model for solubility

Source	Sum of Square	Degree of freedom	Mean Sum of Square	F value	p- value Prob> F
Model	5.737E-003	1	5.737E-003	9.15	0.0073 significant
B- Stabilizer Conc.	5.737E-003	1	5.737E-003	9.15	0.0073
Lack of fit	7.950E-003	13	6.115E-004	0.92	0.5898 Not significant

Table 13: ANOVA results for response surface 2fi model for drug content

Source	Sum of Square	Degree of freedom	Mean Sum of Square	F value	p- value Prob> F
Model	235.31	6	39.22	2.99	0.0463 significant
A-Antisolvent/solvent ratio	7.223E-003	1	7.223E-003	5.304E-004	0.9816
B- Stabilizer Conc.	35.8	1	35.8	2.73	0.1225
C-Time	22.52	1	22.52	1.72	0.2129
AB	7.88	1	7.88	0.60	0.4522
AC	167.81	1	167.81	12.79	0.0034
BC	1.30	1	1.30	0.099	0.7583
Lack of fit	80.14	8	10.02	0.55	0.7821 Not significant

Table 14: ANOVA for response surface reduced linear model for drug release

Source	Sum of Square	Degree of freedom	Mean Sum of Square	F value	p- value Prob> F
Model	27.32	1	27.32	5.61	0.0293 significant
B- Stabilizer Conc.	27.32	1	27.32	5.61	0.0293
C-Time	22.52	1	22.52	1.72	0.2129
Lack of fit	68.35	13	5.26	1.36	0.3889 Not significant

4. DISCUSSION

The drug content of various batches was affected with alteration in processing factors. This may be due to the stabilizer concentration and antisolvent / solvent ratio. The unprocessed aceclofenac shows particle diameter average up to 200 mm while after processing it shows particle diameter ranging from 1-3 mm which is suitable for the many of the formulations like powder for inhalation. This may be due to the fact that as the antisolvent/ solvent ratio increases, the supersaturation and nucleation rate of drug is enlarged, and the particle size became smaller. Additionally, fewer collisions and

less agglomeration occurred in diluted system. At the lowest stabilizer concentration, the average particle size was around 3-3.5 μm, which can be explained as the coverage of stabilizer on the microcrystal surface was inadequate to arrest particle growth. The particle size reduced with the increasing of stabilizer concentration. In the initial time, the average particle size was around 1-2μm, which was obtained since it was the nucleation period. Sonication reduces the elapsed period. After 15 min, nucleation stops which leads to increase in crystal growth [14]. The reason behind uniform particle size distribution may be due to increased supersaturation which leads to increased nucleation and inhibition of crystal growth.

Since the particle size was greatly reduced, so was the solubility affected. The results indicate that the solubility has increased by 2.5 times than the original. When process was carried out at higher antisolvent /solvent ratio, higher stabilizer concentration and under sonicating conditions for long period of time, the product obtained showed the greater solubility in comparison with that of lower values. S18 showed the maximum solubility in water while batches S11 and S7 showed the minimum solubility amongst all other batches. This may be due to a higher antisolvent/ solvent ratio, particle size of the drug obtained was smaller, which exposes higher surface area to solvent which increase the solubility of the drug in water [1] and ethanol has good diffusivity property which generates the porous powder, which has good water uptake capacity and hence the solubility of the powder obtained was higher [17]. Micronization leads to the development of static charges. These static charges give rise to strong attractive forces which results into agglomeration of particle. The zeta potential determines the stability of microparticles by measuring the magnitude of these forces. The value obtained for sonicatedmicroparticles was found to be in stability range. The flow property results report that the formed particles have the flow properties within the passable range, and which is best suitable in both tablet as well as powder for inhalation formulations. This may be probably due to formation of spherical particles by sonoprecipitation technique. Since the powder shows uniform distribution, the flow properties of drug were modified [14]. The reduction in intensity and number of typical diffraction peaks in formulation diffractogram suggests reduction in crystalline nature of drug and may be converted from crystalline to amorphous form. The decreased crystallinity of aceclofenac confirmed by DSC results might contribute to the increase of solubility, dissolution, and bioavailability [21-24].

The SEM images confirmed the morphology of the microparticle. They were found to be almost spherical with smooth surface. The reason behind preparation of spherical particles is supersaturation zones. When drug solution is added into the anti-solvent, it does not disperse immediately and produces local zones of excessive supersaturation. When anti-solvent process uses mechanical stirring, poor micro mixing is unavoidable, which increases the precipitation rate locally leading to agglomeration and increase in particle size as observed in conventional precipitation technique. In contrast homogeneous micro mixing during ultrasonication maintains reasonably uniform conditions throughout the vessel causing the formation of nanosized discreet microparticles.

The *in vitro* drug release was found to be greater as compared to crude drug due to increase solubility. As the particle size of the sonoprecipitatedaceclofenac is lesser, drug shows higher solubility which results into rapid release of the drug. Aceclofenac shows complete release of the drug from formulation, but its onset of action is late. After processing, onset was found to be quicker which may reduce dose requirement. Graphs of the drug release from capsules were given in the Fig. 12 and 13 proves the release rate and its kinetics of drug release. This rapid release may be due to micronization of drug particles and increased solubility of drug, which ultimately enhances the dissolution of drug. Solubility depends on particle size, generated porosity, and increased water uptake capacity of drug, which rapidly dissolves drug in medium.

5. CONCLUSION

The data obviously shows that formulated Aceclofenac microparticles using sonoprecipitation approach were in size range which ultimately leads to improved*in-vitro* solubility and dissolution rate. These results also suggest that combination of antisolvent and sonoprecipitation approach can be applied for industrial manufacturing of Aceclofenac microparticles, as this will improve therapeutic performance in humans.

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Conflicts of interest

None declared

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