International Journal of Nanomedicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

RETRACTED ARTICLE: Formulation of Aceclofenac Tablets Using Nanosuspension as Granulating Agent: An Attempt to Enhance Dissolution Rate and Oral Bioavailability

This article was published in the following Dove Press journal:
International Journal of Nanomedicine

Haroon Rahim[1]
Abdul Sadiq[2]
Riaz Ullah[3]
Ahmed Bari[4]
Fazli Amin[1]
Umar Farooq[5]
Naeem Ullah Jan[1]
Hafiz Majid Mahmood[6]

[1]Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Khyber Pakhtunkhwa, Pakistan; [2]Department of Pharmacy, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan; [3]Department of Pharmacognosy (MAPPRC), College of Pharmacy, King Saud University Riyadh, Riyadh, Saudi Arabia; [4]Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University Riyadh, Riyadh, Saudi Arabia; [5]Legacy Pharmaceutical (Pvt) Ltd., Peshawar, Khyber Pakhtunkhwa, Pakistan; [6]Department of Pharmacology, College of Pharmacy, King Saud University Riyadh, Riyadh, Saudi Arabia

Purpose: The aim of the studies was to fabricate aceclofenac (AC) tablets using nanosuspension as granulating fluid to boost its rate of in vitro dissolution and eventually its oral bioavailability.

Methods: The optimized nanosuspension with particle size of 112±2.01 nm was fabricated using HPMC 1% (w/v), PVP-K30 1% (w/v) and SLS 0.12% (w/v) at 400 watts of ultrasonication energy for 15 min duration and 3 sec pause. Then, the optimized aceclofenac nanosuspension was used as granulating fluid for aceclofenac tablets formulation. The characterization was performed using Malvern zetasizer, SEM, TEM, DSC and P-XRD. The granules were evaluated for the bulk and tapped densities, Hausner's ratio, angle of repose and their resulted values were found within limits. The prepared tablets were tested for average weight, hardness, friability, disintegration, dissolution and in vivo bioavailability in rabbits.

Results: The in vitro dissolution data showed the boosted rate of nanosuspension-based tablets compared to the microsuspension-based tablets. The in vivo bioavailability (in rabbits model) of aceclofenac nanosuspension-based tablets (ACN-1, ACN-2) proved an improved absorption as in comparison to the marketed formulation. The C_{max} and $AUC_{0\rightarrow24}$ of ACN-1 and ACN-2 were 1.53-fold, 1.48-fold and 2.23-fold, 2.0-fold greater than that of the marketed drug, and were 1.74-fold, 1.68-fold and 2.3-fold, 2.21-fold greater in comparison to raw drug.

Conclusion: This boosted in vitro and in vivo bioavailability may be attributed to reduced particle size of aceclofenac nanoformulations used in tablets. Finally, this will result in faster absorption of these fabricated tablets.

Keywords: nanosuspension-based tablets, release kinetics, enhanced oral bioavailability

Introduction

Aceclofenac, [2-[[2-[2- [(2, 6-dichloro phenyl) amino] phenyl] acetyl] oxy] acetic acid], is a well-known non-steroidal anti-inflammatory and analgesic drug compound as shown in Figure 1.[1] The main problem in the therapeutic response of aceclofenac in orally taken dosage form is its poor aqueous-solubility as it belongs to Class 2 drug of the BCS (biopharmaceutical classification system).[2] Nanosuspension technology has been applicable to improve the poor aqueous solubility and bioavailability issues.[3] Anti-solvent precipitation (a bottom-up approach) is an effective way which involved dissolving the drug compound in

Correspondence: Haroon Rahim
Department of Pharmacy, Sarhad University of Science and Information Technology Peshawar, Peshawar 25000, Khyber Pakhtunkhwa, Pakistan
Tel +92 332 946 1642
Email hrahimpk@gmail.com

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/IJN.S270746

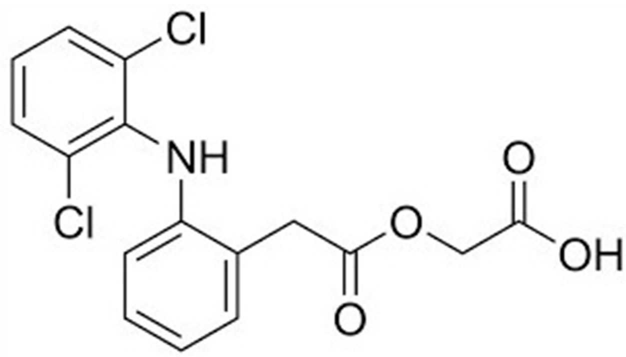

Figure 1 Chemical structure of aceclofenac.

solvent followed by incorporating into the anti-solvent phase, finally leading to the precipitation of drug.[4] But still, this technology is facing some issues including the maintenance of particle size, stability problem after precipitation process and scale-up of batch. In earlier period, precipitation-ultrasonication has gained great focus for controlling both the nucleation and crystallization due to the efficient transfer of mass to hasten molecular diffusion.[4–7] HPMC (hydroxypropyl methylcellulose), PVA (polyvinyl alcohol), PVP (polyvinylpyrrolidone) etc., are some polymers used to achieve stability.[8,9]

Despite the progress in this area of research, nanosuspension technologies have an instability problem, provoked by the nucleation and particle size growth. In the nonexistence of a stabilizer, the high surface energy of nanosized particles can induce Ostwald's ripening.[10] The solid dosage forms are preferred due to ease of administration, accurate dosing and stability. Nanosuspensions are usually dried to enhance their stability, allowing the conversion into solid dosage forms, either tablets or capsules.[11] The literature reports various techniques for transformation of nanosuspension into solid dosage forms (tablets) including: spray drying, layering of nanosuspension onto the pellets or granules, freeze drying or wet granulation, etc.[12–14] It is estimated that approximately 33% of all prescribed medications are dispensed in the form of tablets.[15,16]

Therefore, it is imperative to fabricate stable aceclofenac-tablets to resolve these problems of nanosuspension and ultimately enhanced bioavailability. The WG (Wet granulation) process was selected as it is very simple and fast to execute, which makes it a cost-effective and time saving procedure. Therefore, tablets were produced by using nanosuspension as granulating fluid with other suitable excipients with the objective of increasing the dissolution rate which will ultimately boost bioavailability of the chosen drug candidate.

Materials and Methods

Materials

Aceclofenac (AC), sodium lauryl sulphate (SLS), HPMC (Hydroxypropyl methylcellulose) Grade: 6cps, PVP K30 (polyvinylpyrrolidone), ethanol, corn starch (CS), microcrystalline cellulose (MCC) pH 102, Sodium starch glycolate (NaStG), Talcum (Tal), magnesium stearate (Mg) and all the other chemicals used were received as a generous gift from Bryon Pharmaceuticals Private Limited, Peshawar, KPK (Khyber Pakhtunkhwa), Pakistan and Legacy Pharmaceuticals Private Limited, KPK, Pakistan.

All the experiments conducted on animals were approved from Departmental ethical committee, Scientific Procedure Issued by Animal Ethics Committee at University of Malakand, KPK and related Bye-Laws 2008 vide approved protocol number UOM/PHARM/EC/01/A[illegible]/2017. The 1996 guidelines by National Research Council were followed for the welfare of laboratory animals.[17]

Formulation of AC Tablets

Optimized AC-N (aceclofenac nanosuspension) was fabricated as per our previously reported work using "precipitation-ultrasonication method".[18] Simply by dissolving aceclofenac (30 mg/mL) in organic solvent i.e. ethanol followed by injecting it to antisolvent phase i.e. water cooled at 4°C. The antisolvent phase containing polymers/stabilizers including HPMC, PVP-K30 and aqueous SLS solution already prepared at speed of 1500 rpm by the magnetic stirrer. Then, the ultrasonication processing of the prepared suspension was carried at different time length and ultrasonic inputs at 3 sec pause. The AC-N (aceclofenac nanosuspension) was added as granulating fluid to other suitable excipients to prepare granules for conversion to the tablets as listed in Table 1. After optimization, two batches (ACN-1, ACN-2) containing nanosuspension as granulating liquid were prepared. Simply, the corn starch, lactose and MCC were mixed and passed through mesh 30. Binder solution was prepared using PVP K30 and IPA (Isopropyl alcohol), then the aceclofenac nanosuspension was added to this (binder) solution. The mixture (binder + nanosuspension) is further incorporated to the already prepared mixture (corn starch, lactose, MCC) and passed through mesh 08. The granules were

Table 1 Composition of aceclofenac tablets

Formulation Code	AC-N	AC-M	Excipients Used							
			CSt	Lactose	MC pH 102	PVP	IPA	NaStG	Talc	Mag. S
ACN-1	50	—	20	70.75	31.25	8.0	150.0	15.0	3.0	2.0
ACN-2	50	—	15	75.75	31.25	8.0	150.0	15.0	3.0	2.0
ACM-3	—	50	20	60.75	40.25	8.0	150.0	15.0	3.0	2.0

Abbreviations: CSt, corn starch; MC, microcrystalline cellulose pH-102; PVP, polyvinylpyrrolidone; IPA, isopropyl alcohol; NaStG, sodium starch glycolate; Talc, talcum powder; Mag. S, magnesium stearate.

dried in oven at 60ºC, sifted with extra-granular excipients. The micronized particles batch (AC-M) was prepared by passing the corn starch, lactose, MCC, and micronized drug through the mesh 30 and then blended thoroughly. The binder solution was prepared in the same way as mentioned earlier for fabrication of the ACN-1 and ACN-2 batches, then incorporated it to the first mixture. The blend was passed via mesh 30, dried in oven at 60ºC and sifted with extra-granular excipients. All the prepared and dried granules were evaluated and finally compressed using a compression machine.

Evaluation of Nanosuspensions and Formulated Tablets

Particle Size Determination

The particle size of the diluted sample (nanosuspension) was determined in triplicate using Zetasizer (Malvern, UK), where the Brownian motion of the particles are measured which is converted to size and size distribution by the application of Stokes-Einstein relation.[18]

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

Scanning electron microscope was used to evaluate the morphology of freshly prepared raw drug, which was deposited on glass slides followed by evaporating the solvent. Fabricated nanosuspension was evaluated using TEM. Sample (AC liquid nanosuspension) was dropped on a copper 200 mesh formvar/carbon coated grid and allowed to dry.[18]

FTIR Studies

For studying compatibility between raw drug and other additives used in formulation, the FTIR analysis was carried out. The drug and formulation blend compatibility were evaluated using Thermoscientific Nicolet, FTIR Instrument, USA. A small quantity of raw drug and blend of formulation were directly placed on germanium piece of the infrared (IR) spectrometer with constantly applied pressure. The IR absorbance scanning range was 4000–500 cm^{-1}.[19]

Powder X-Ray Diffractometry (P-XRD) and Differential Scanning Calorimetry (DSC)

For crystallinity, the samples were evaluated using P-XRD (Panalytical, X'pert), by scanning detector over 2θ angles at a scan rate of 0.0[illegible]°. The melting point of aceclofenac raw drug, AC-N and fabricated tablet batch were performed by DSC (TA-60, Shimadzu). All the samples, which were about 3 mg, were placed in pans made of aluminum for heating, under 50 mL per min nitrogen flow rate and the scanning was kept at 10°C per min, from 40–200°C.

Evaluation of the Prepared Granules

The densities (tapped, bulk), HR (Hausner's ratio), Carr's index and AOR (angle of repose) were determined for dried granules.

Bulk Density (ρ_B)

The accurately weighed amount (W) of the granules was placed in a 10 mL graded cylinder, V_0 (untapped volume) was recorded and the ρ_B (bulk density) was obtained (g/mL) by using the following equation:[20]

$$\rho_B = W/V_0$$

Tapped Density (ρ_T)

The 10 mL graduated cylinder containing the accurately weighed quantity (W) of prepared granules were tapped onto a hard surface till there was no further change in the volume. Then, the V_T (tapped volume) was recorded and ρ_T (tapped density) was determined by using the below formula:[19]

$$\rho_T = W/V_T$$

Carr's Compressibility Index

Carr's compressibility index was determined by using the formula:[19]

$$\text{Carr's Compressibility Index} = [\rho_T - \rho_B/\rho_T] \times 100$$

Where, ρ_T is tapped and ρ_B is the bulk densities.

Hausner's Ratio (HR)

The HR values were determined by the formula given below:[19]

$$\text{Hausner's ratio} = \rho_T/\rho_B$$

Where, "ρ_T" is the tapped and "ρ_B" is the bulk densities.

AOR (Angle of Repose)

Using stabilized funnel method, granules (5 g) formed heap with "h" height and "r" i.e. radius of base. The AOR were determined by equation:[19,21]

$$\text{AOR} = \tan^{-1}(\text{h/r})$$

Compression of Prepared Granules into Tablet Dosage Form

The granules prepared are shown in Table 1. They were compressed to tablet dosage form by a compression machine (ZP19, China) fitted with 11-mm biconcave punches for aceclofenac tablets.

Evaluation of Fabricated Tablets

The post-compression properties (weight variation, % friability, hardness, disintegration time) of the prepared tablets were determined. The hardness of formulated ten tablets was determined using Pharma test hardness test machine. The DT (disintegration time) of prepared tablets was measured in the purified water keeping temperature at 37±2°C, using DT apparatus (Model: DT-0607, Curio) with disks. Drug contents of tablets were evaluated as per HPLC procedure used by Rahim et al.[22] Tablets' friability was calculated for 20 tablets after completion of 100 revolutions in the Friabilator using formula:

$$\%\text{Friability} = [W_1 - W_2/W_1] \times 100$$

Where, W_1 is weight of tablets before completing rotations and W_2 is final weight after completing revolutions.

Stability Studies of Compressed Tablets

The formulated tablet dosage form was evaluated for the in vitro dissolution by storing at accelerated temperature 40±2°C and RH 75%±5% and at room 30±2°C and keeping the RH 65%±5% for three months.[23]

In vitro API Release Studies

The in vitro API release studies of both AC-N (aceclofenac nanosuspension) as granulating fluid and microsuspension-based tablets' batches were performed using dissolution apparatus (USP Type-2) and 0.1N hydrochloric acid containing 2% Tween 80 was used as dissolution medium at speed of 75 rpm keeping the temperature at 37±0.5°C. After 10 minutes, sample of 5 mL, withdrawn up to an hour, were filtered through 0.02 µm syringe filter. The equal volume (5 mL) of the medium was replaced for maintaining sink conditions.[22,23] The quantity of active compound (aceclofenac) in the sample was determined by HPLC as method used by Rahim et al.[22]

Release Kinetics

To investigate the mechanism of drug release from the formulated tablets, the drug release data were fitted into zero-order, first-order, Higuchi and Korsmeyer's equation. The Korsmeyer's equation, Equation (A), describes the drug release behaviour from the polymers.

$$\text{Log}(\text{Mt} \div \text{Mf}) = \text{Logk} + \text{nLogt} \quad (\text{A})$$

Where,

Mt = the quantity of drug release at time "t";

Mf = the quantity of drug release after infinite time;

k = release rate constant incorporating structural and geometric characteristics of the tablet;

n = the diffusional exponent indicating the mechanism of drug release.

To clarify the release exponent for formulated tablets, the log value of % drug release was plotted against log time for each formulation according to Equation (A).[19]

In vivo Bioavailability Studies

The bioavailability studies were conducted in white albino rabbits (2.5–3.0 kg). Animals were housed in wire cages, offered food and water freely as per protocols earlier mentioned in the *Materials and Methods* section. Fabricated tablet groups ACN-1 and ACN-2, marketed product and raw drug were administered orally in a dose of 10 mg/kg to animals (n=6 rabbits in each group). Venous blood was collected in the

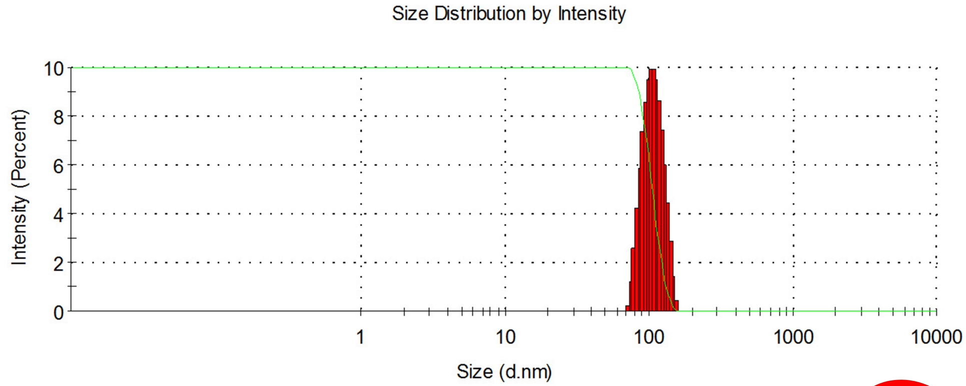

Figure 2 Particle size distribution of aceclofenac nanosuspension.

heparinized tubes at different intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hrs) after oral administration. The blood samples were centrifuged at 3000 rpm for 20 min to separate the plasma and stored at −20ºC. The plasma samples were analyzed using the HPLC method by Rahim et al.[22] The chromatographic conditions were: mobile phase—methanol: 0.3% TEA pH 7.0 (60:40, v/v), Hypersil BDS C18 (250 cm×4.6 mm), 5µm column was used at 1.0 mL/min flow rate, keeping injection volume 20 µL; at 25°C; Run time: 25 min; 275 nm as detection wavelength; and venlafaxine as internal standard. The pharmacokinetic parameters were determined by PK solutions 2.0 non-compartmental pharmacokinetic analysis.

Statistical Analysis

All the results were given as mean ± standard deviation (SD), mean values were compared using ANOVA and differences were considered significant at the level of $P<0.05$ using GraphPad Prism 5.

Results and Discussions

Optimized AC-N (aceclofenac nanosuspension) was fabricated as per previously reported work using "precipitation-ultrasonication method".[18] Then, the optimized batch was used as granulating fluid for conversion to the tablets' formulations using the AC-N and AC-M (aceclofenac suspension containing unprocessed/raw microparticles) as granulating fluid with other excipients. The optimized batch formulated with particle size found was 112±2.01 nm, keeping the ultrasonic energy input at 200 watts with 15 min duration and 3 a sec pause. The particle size of fabricated optimized batch of nanosuspension is shown in Figures 2 and 3A. All the particles displayed in Figure 3B reveal well-defined morphology related with crystalline material. The nanosuspension was stabilized using polymers/stabilizers, i.e. 1.0% (w/v) of each HPMC and PVP K-30 while 0.12% (w/v) SLS. The formulations of the tablets were shown in the Table 1.

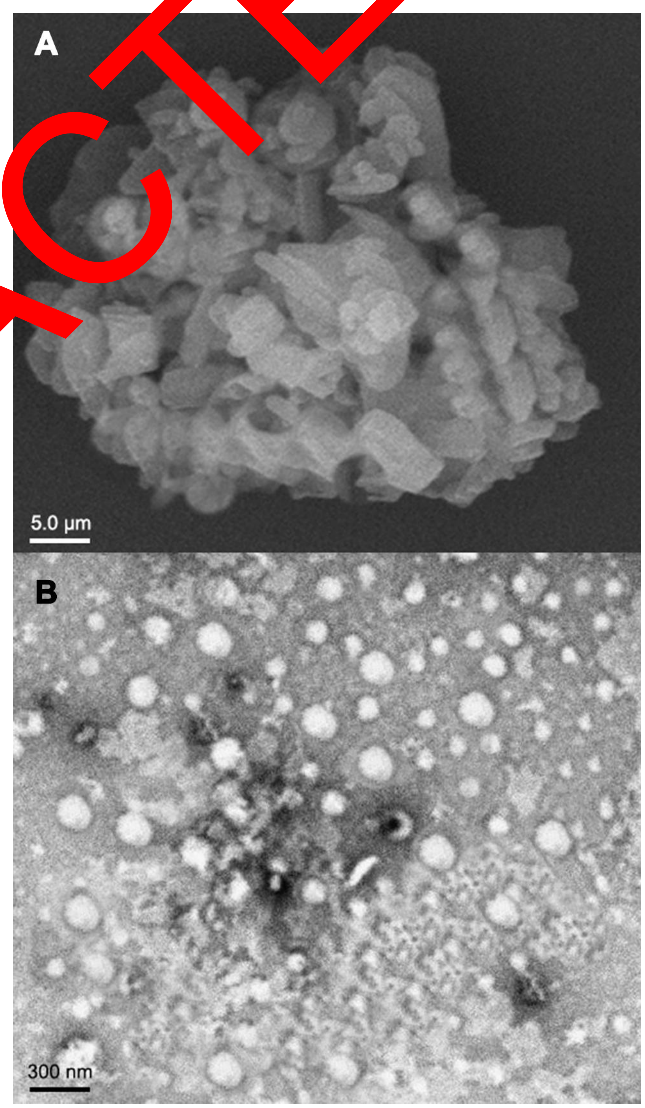

Figure 3 Scanning electron micrographs of raw drug (**A**); transmission electron micrographs of drug nanoparticles ().

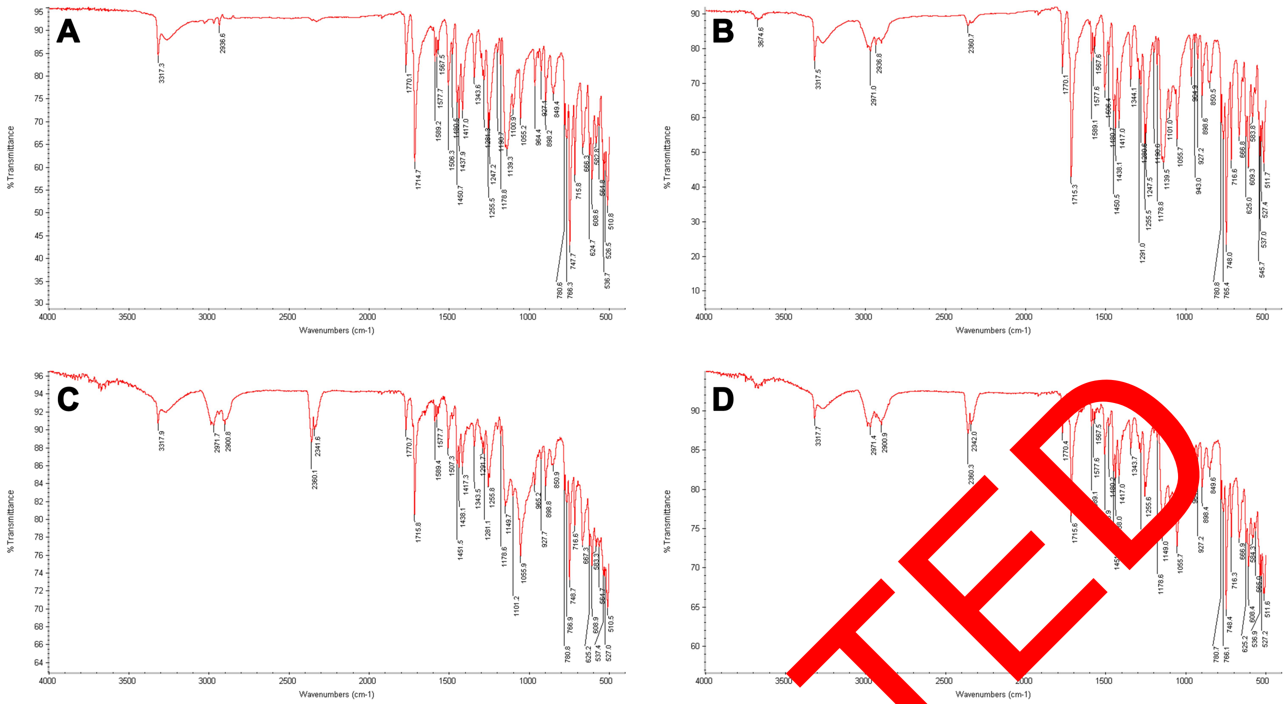

Figure 4 FT-IR of aceclofenac (**A**), ACN (**B**), ACN-T (**C**), and marketed tablets (**D**).
Abbreviations: ACN, aceclofenac nanosuspension; ACN-T, aceclofenac nanoparticles-based tablets.

FTIR Spectra Analysis

The FTIR studies showed that the spectrum of raw drug compound (aceclofenac), aceclofenac nanosuspension and nanoformulation-based tablets are displayed in Figure 4A–D respectively. The raw AC presented distinctive peaks at 3317.3 cm^{-1} assigned to N–H stretching, 2936.6 cm^{-1} are due to stretching of O–H, the peak 1770.1 cm^{-1}, 1714.7 cm^{-1} are assigned to C=O stretching, band 1589.2 cm^{-1} is due to the skeleton vibration of aromatic C=C stretching, 1506.3 cm^{-1} is assigned to in plane bending for N–H, band 1343.6 cm^{-1} is due to O–H in plane bending, 1291.3 cm^{-1} (C–N aromatic amine), 964.4 cm^{-1} (O–H out plane bending) and 750.3 cm^{-1}. The nanosuspension blend exhibited spectra (cm^{-1}) at 3317.9, 2936.8, 2310.7, 1770.1, 1506.4, 1344.1, 1291.0 and 943.0. Whereas the fabricated optimized tablet batch exhibited distinct bands (cm^{-1}) at 3317.9, 2971.7, 2900.8, 1770.7, 1715.8, 1507.3, 1343.5, 1291.2, 766.9. FTIR spectra results showed a lack of any interaction between the aceclofenac and additives employed in the nanoformulation as well as in formulated tablets.

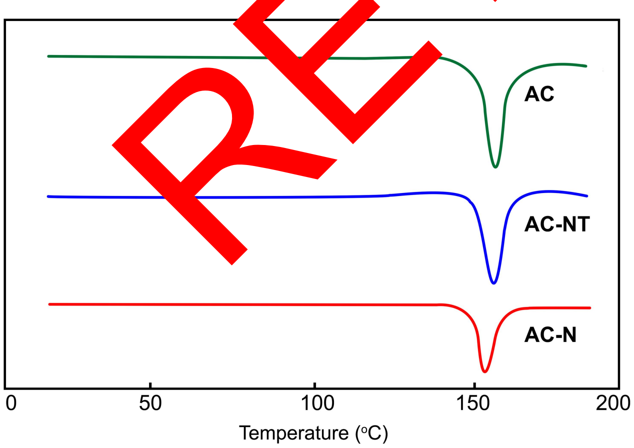

Figure 5 DSC thermogram of raw AC, AC-NT and AC-N.
Abbreviations: DSC, differential scanning calorimetric; AC, raw aceclofenac; AC-NT, aceclofenac nanosuspension-based tablets; AC-N, optimized nanosuspension of aceclofenac.

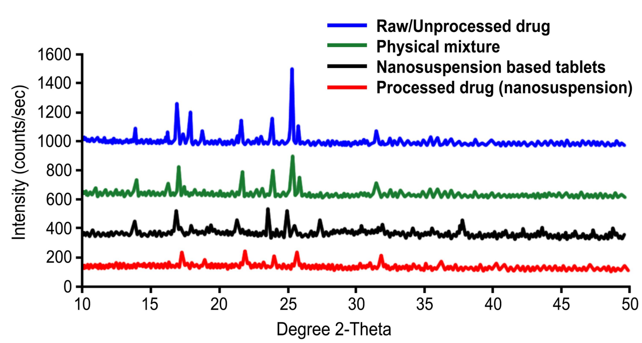

Figure 6 PXRD patterns of raw drug, physical mixture and drug nanosuspension.
Abbreviation: PXRD, powder X-ray diffraction.

Table 2 Pre-compression parameters of various blends

Batch	Angle of Repose (°)	Bulk Density (gm/mL)	Tapped Density (gm/mL)	Carr's Index (%)	Hausner's Ratio
ACN-1	27.25±1.05	0.541±0.01	0.632±0.01	15.18±0.98	1.17±0.01
ACN-2	28.42±1.25	0.552±0.01	0.662±0.01	16.69±1.28	1.19±0.01
ACM-3	29.45±1.45	0.574±0.01	0.692±0.01	16.94±0.58	1.20±0.01

Note: All the values are expressed as mean ±S.D, n=3.

Table 3 Post-compression evaluation of AC tablets

Formulations with Codes	Uniformity of Weight (mg)	Hardness (kg/cm^2)	% Friability	DT (min)	% Drug Content
ACN-1	199.57±1.42	6.65±0.52	0.46±0.42	6.45±[illegible]	99.25±2.84
ACN-2	200.15±1.75	8.42±0.18	0.58±0.37	7[illegible]4±1.36	[illegible].68±2.55
ACM-3	199.57±2.58	9.25±0.25	0.62±0.31	[illegible]55±1.16	1[illegible]04±1.55

Abbreviation: DT, disintegration time.

Powder X-Ray Diffractometry (P-XRD) and Differential Scanning Calorimetry (DSC)

The DSC thermograms as displayed in Figure 5. The raw drug (i.e. aceclofenac), showed an endometrial peak at 154.49°C, conforming the melting point.[1] Nanosuspension-based tablets and the prepared nanosuspension of the selected drug candidate indicated a slight change of melting point to 154.12 and 153.67°C respectively. The difference [illegible] the particle size among the samples is the leading caus[illegible] of these alterations. The presence of stabilize[illegible] [illegible]es on [illegible] surface of particles of the drug compou[illegible] may re[illegible]lts in th[illegible] peaks' broadening.[25,26] Hence, no new [illegible]al in the [illegible]C thermogram formed, proving th[illegible] [illegible]ack of any [illegible]hemical reaction taking place.

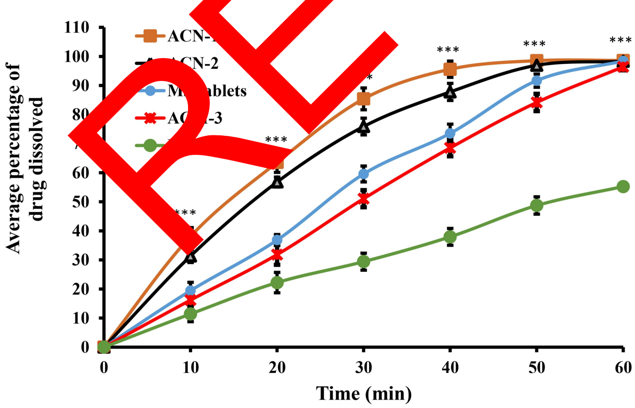

Figure 7 In vitro dissolution of formulations and M. Tablets. Values represent mean ±SD, n=3. ***P<0.001 compared with raw drug.

Abbreviations: ACN-1, ACN-2, aceclofenac nanoparticles-based tablets; ACM-3, unprocessed/raw aceclofenac-based tablets; M. Tablets, marketed aceclofenac tablets.

The result[illegible] obtain[illegible] from P[illegible]RD displayed that the prepared [illegible]suspension [illegible] drug (aceclofenac) were crystalli[illegible]e in na[illegible]e as shown in Figure 6. However, peaks' int[illegible]s of nanop[illegible]ticles were comparatively low to the [illegible]w drug, this may the effect of nanonization.

The smal[illegible]r PS (particle size) and presence of amor[illegible]ous stabili[illegible]rs in trace amounts may be the reason for the peak [illegible]ction of AC nanoparticles as displayed in the [illegible]ure 6.[27–29] Moreover, the X-ray diffractogram of the PM (physical mixture) and nanosuspension-based tablets showed a dominant peak as shown in Figure 6, while the peaks for the small amount of the used stabilizers and other additives in the formulation of tablets were amorphous in nature and did not appear.

Pre-Compression Parameters of Formulation Blends

The granules of ACN-1 and ACN-2 (nanosuspension-based tablets) showed the values of angle of repose ranges from 27.25±1.05 to 28.42±1.25 while the batch ACM-3 (microsuspension-based batch) granules showed the values of 29.45±1.45. All the formulation blends presented excellent to good flow properties.[30] The prepared granules exhibited bulk density (mg/mL) value 0.541±0.01 for ACN-1, 0.552±0.01 for ACN-2 and for ACM-3 results 0.574±0.01. The tapped density (mg/mL) recorded for ACN-1 was 0.632±0.01, ACN-2 was 0.662±0.01 and ACM-3 was 0.692±0.01, showing that the prepared granules have good packability. The Carr's index values of the ACN-1 and ACN-2 batches range from 15.18±0.98 to 16.69±1.28 whereas the microsuspension-based granules

Table 4 In vitro release kinetics of fabricated tablets

Code of Formulation	Zero Order	First Order	Higuchi	Hixson Crowell	Korsmeyer		Release Mechanism
	r^2	r^2	r^2	r^2	r^2	N	
ACN-1	0.8496	0.968	0.9693	0.9668	0.9531	0.476	Fickian
ACN-2	0.9113	0.9615	0.9807	0.9935	0.9302	0.571	Non-Fickian
ACM-3	0.9939	0.8836	0.9243	0.9606	0.7939	0.951	Non-Fickian
Marketed	0.9874	0.8613	0.9378	0.9584	0.8175	0.476	Fickian

(ACM-3) resulted in a value of 16.94±0.58, proving that all batches exhibited good compressibility. The nanosuspension-based granules were found to be Hausner's ratio values ranging from 1.17±0.01 to 1.19±0.01 and 1.20±0.01 for the micronized/unprocessed batch, these results presented good to fair flow property exhibited by the formulations. All these results are shown in Table 2.

Compression of Granules into Tablet Dosage Form

The different formulation batches (ACN-1, ACN-2, ACM-3) of tablets resulted in hardness (kg) values from 6.65 ±0.52 to 9.25±0.25, average weight 199.57±1.42 mg to 200.15±1.75 mg and friability values from 0.46±0.42% to 0.62±0.31%. The DT (disintegration times) recorded were 6.45±1.55 for ACN-1, 7.24±1.36 for ACN-2 and 9.55±1.16 for ACM-3. The compressed batches showed values of performed tests complied with official limits as shown in Table 3. All the formulated batches had uniformity in weight which complied with USP specifications, i.e. ±7.5% allowed limit.[31,32]

In vitro Release of Aceclofenac Tablets

The in vitro API release data presented a substantial improvement in dissolution rate of batch ACN-1 in comparison to marketed tablets and unprocessed aceclofenac-based tablet formulation. The graph showed that in the first 30 min, more than 85% of ACN-1 were dissolved compared to 75.89% for ACN-2, 51.06% for unprocessed micronized drug formulation (i.e. ACM-3), 59.56% for the M. Tablets and 29.41% for raw drug. Boosted in vitro release rate of ACN-1 was observed while comparing to ACN-2, unprocessed drug containing [illegible] i.e. ACM-3, M. Tablets and raw drug, all the results are illustrated in Figure 7. The solubility of drug compound will be enhanced when the particle size of the drug is reduced to nanosized range as described by Xia et al.[33] The release data showed the $P<0.001$ compared with raw drug.

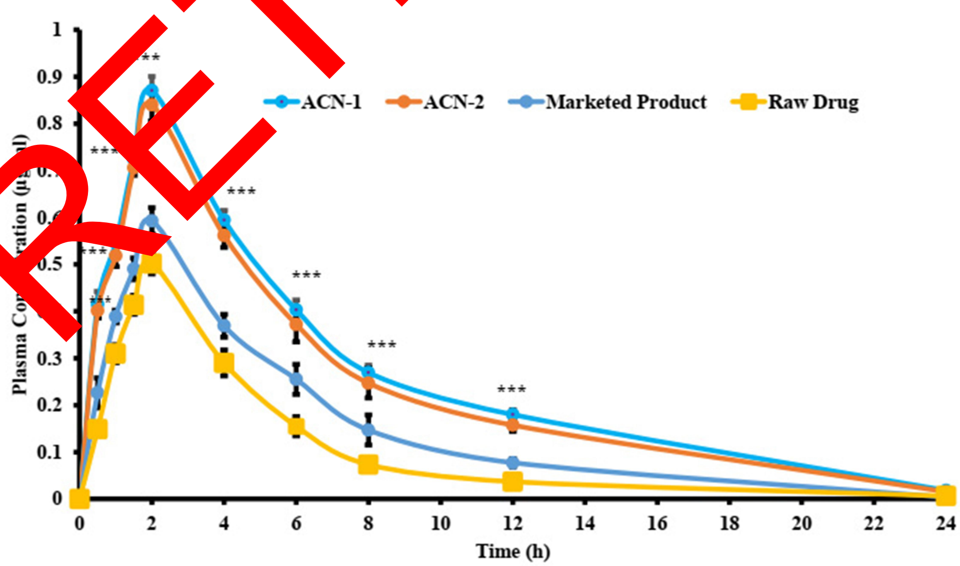

Figure 8 Average plasma drug concentration versus time profiles after oral administration of formulations to rabbits (n=6), ***$P<0.001$ compared with raw drug.
Abbreviation: ACN-1, ACN-2, aceclofenac nanosuspension-based tablets.

Table 5 Pharmacokinetic parameters from the plasma concentration versus time

Parameters	ACN-1	ACN-2	Raw Drug	Marketed Product
C_{max} (µg/mL)	0.870±0.03**	0.840±0.03**	0.501±0.02	0.567±0.02^{ns}
T_{max} (h)	2.0±0.00	1.0±0.00	2.0±0.00	2.0±0.00
$AUC_{0–24}$ (µg-h/mL)	5.756 ±0.17***	5.531 ±0.14***	2.501±0.15	2.752±0.16*

Notes: All the values are represented as mean ±S.D, n=6. ns=non-significant, *P<0.1, **P<0.01, ***P<0.001 compared with raw drug.
Abbreviations: ACN-1, ACN-2, aceclofenac nanosuspension-based tablets; C_{max}, maximum plasma concentration; T_{max}, time for maximum plasma concentration; AUC, area under the curve.

It has evidently been confirmed and support in the development of solid dosage forms BCS Class-II drug compounds i.e. poorly soluble drug candidates.[34]

Release Kinetics

Two batches, i.e. ACM-3 and marketed product, obey zero order kinetics with values of r^2 0.9939 and 0.9874 respectively. While the formulation batches ACN-1 and ACN-2 follow first order kinetics with values of r^2 0.9680 and 0.9615 respectively. Fickian (Case-I) release was obeyed by ACN-1 and marketed product, while ACN-2 and ACM-3 obey the Non-Fickian type release behavior. The value of "n" equal to 0.45 indicates Fickian (Case-I) release, more than 0.45 but less than 0.89 for non-Fickian (anomalous) release and "n" more than 0.89 indicates Case-II type of release. Case-II refers to the erosion of the polymeric chain while non-Fickian (anomalous transport) illustrate a combination of both diffusion and erosion controlled-drug release as shown in Table 4.[31]

Bioavailability Study

The in vivo study of aceclofenac nanosuspension-based tablets (ACN-1, ACN-2) showed an enhanced absorption in comparison to the marketed drug formulation, as displayed in Figure 8. The C_{max} and $AUC_{0\rightarrow24}$ of ACN-1 and ACN-2 were 1.53-fold, 1.48-fold and 2.23-fold, 2.0-fold greater than that of the marketed drug product (P<0.001), as displayed in Table 5. While, the C_{max} and $AUC_{0\rightarrow24}$ of ACN-1 and ACN-2 were 1.74-fold, 1.68-fold and 2.3-fold, 2.21-fold greater than that of the raw drug (**P<0.01).

The enhanced bioavailability of aceclofenac nanosuspension-based tablets after oral administration will possibly be owed to the faster absorption of the aceclofenac nanosuspension used in the formulation.[22]

Stability Studies of Fabricated Tablet Dosage Form

The stability and dissolution of the fabricated tablets of aceclofenac fabricated by utilizing AC nanosuspension in the form of a granulating liquid, was carried out at both accelerated (40°C/75% Relative Humidity) as well as room temperature conditions (25°C/60% Relative Humidity) for three months. The in vitro dissolution rate for fabricated solid dosage form (tablets) was stable at aforementioned storage conditions, as represented in Figure 9.

Hence, it is evidently proved from the results of in vitro dissolution profiles that the optimized

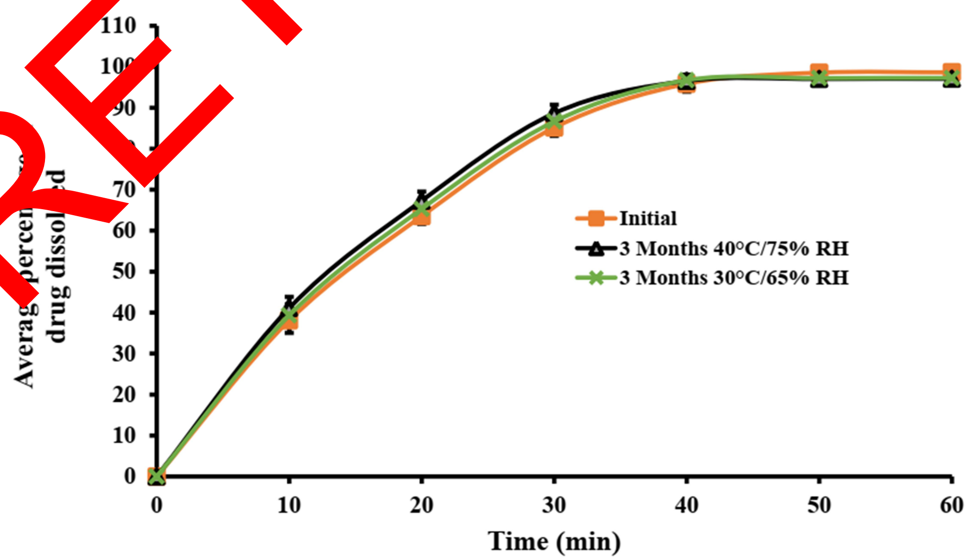

Figure 9 Stability of ACN-1 batch formulation at different storage conditions.

Figure 10 Visual image of ACN-1 batch tablets.

nanosuspension-based tablets showed remarkable improved dissolution rate compared to the microsuspension-based (raw drug) tablets. The ACN-1 batch tablets (as depicted in Figure 10) showed stability at two different conditions (30°C/65%RH, 40°C/75%RH).

Conclusion

The conducted research proved that aceclofenac tablets can be prepared using optimized nanosuspension as granulating fluid and micronized drug with other suitable excipients. The stable formulated tablets with improved in vitro dissolution and oral improved bioavailability in rabbits is achieved by using optimized nanosuspension as granulating fluid compared to micronized drug-based and marketed tablets. The C_{max} and $AUC_{0\rightarrow24}$ of ACN-1 and ACN-2 were 1.53-fold, 1.[illegible]8-fold and 2.23-fold, 2.0-fold greater than that of the marketed drug product ($P<0.001$). The studies proposed using similar techniques for other poorly-soluble drug compounds to improve the in vitro rate of dissolution and ultimately their oral in vivo bioavailability.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University, Saudi Arabia for funding this work through research group no. RG-1440-009. We are thankful to Department of Pharmacy, Sarhad University of Science and Information Technology Peshawar, Khyber Pakhtunkhwa, Pakistan and Department of Pharmacy, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan for providing facilities.

Disclosure

Umar Farooq is an employee of Legacy Pharmaceutical (Pvt.) Ltd. The authors report no other potential conflicts of interest for this work.

References

1. Mutalik S, Anju P, Manoj K, Usha AN. Enhancement of dissolution rate and bioavailability of aceclofenac: a chitosan-based solvent change approach. *Int J Pharm*. 2008;350(1–2):279–290. doi:10.1016/j.ijpharm.2007.09.006
2. Soni T, Nagda C, Gandhi T, Chotai N. Development of discriminating method for dissolution of aceclofenac marketed formulations. *Dissolut technol*. 2008;15(2):31. doi:10.14227/DT150208P31
3. Dizaj SM, Vazifehasl Z, Salatin S, Adibkia K, Javadzadeh Y. Nanosizing of drugs: effect on dissolution rate. *Res Pharm Sci*. 2015;10(2):95.
4. Li H, Wang J, Bao Y, Guo Z, Zhang M. Rapid sonocrystallization in the salting-out process. *J Cryst Growth*. 2003;247(1–2):192–198. doi:10.1016/S0022-0248(02)01942-5
5. Louhikultanen M, Karjalainen M, Rantanen J, Huhtanen M, Kallas J. Crystallization of glycine with ultrasound. *Int J Pharm*. 2006;320(1–2):23–29. doi:10.1016/j.ijpharm.2006.03.054
6. De Castro ML, Priego-Capote F. Ultrasound-assisted crystallization (sonocrystallization). *Ultrason Sonochem*. 2007;14(6):717–724. doi:10.1016/j.ultsonch.2006.12.004
7. Merisko-Liversidge EM, Liversidge GG. Drug nanoparticles: formulating poorly water-soluble compounds. *Toxicol Pathol*. 2008;36(1):43–48. doi:10.1177/0192623307310946
8. Douroumis D, Fahr A. Nano- and micro-particulate formulations of poorly water-soluble drugs by using a novel optimized technique. *Eur J Pharm Biopharm*. 2006;63(2):173–175. doi:10.1016/j.ejpb.2006.02.004
9. Lindfors L, Forssén S, Westergren J, Olsson U. Nucleation and crystal growth in supersaturated solutions of a model drug. *J Colloid Interface Sci*. 2008;325(2):404–413. doi:10.1016/j.jcis.2008.05.034
10. Patravale V, Date AA, Kulkarni R. Nanosuspensions: a promising drug delivery strategy. *J Pharm Pharmacol*. 2004;56(7):827–840. doi:10.1211/0022357023691
11. Malamatari M, Taylor KMG, Malamataris S, Douroumis D, Kachrimanis K. Pharmaceutical nanocrystals: production by wet milling and applications. *Drug Discov Today*. 2018;23(3):534–547. doi:10.1016/j.drudis.2018.01.016
12. Bartos C, Ambrus R, Katona G, et al. Transformation of Meloxicam Containing Nanosuspension into Surfactant-Free Solid Compositions to Increase the Product Stability and Drug Bioavailability for Rapid Analgesia. *Drug Des Devel Ther*. 2019;13:4007. doi:10.2147/DDDT.S220876
13. He W, Lu Y, Qi J, Chen L, Yin L, Wu W. Formulating food protein-stabilized indomethacin nanosuspensions into pellets by fluid-bed coating technology: physical characterization, redispersibility, and dissolution. *Int J Nanomedicine*. 2013;8:3119.
14. Salazar J, Müller RH, Möschwitzer JP. Application of the combinative particle size reduction technology H 42 to produce fast dissolving glibenclamide tablets. *Eur J Pharm Sci*. 2013;49(4):565–577. doi:10.1016/j.ejps.2013.04.003
15. Mahida MV, Gupta M. Immediate release tablet of antihypertensive drug olmesartan medoxomile. *J Drug Deliv Ther*. 2013;3(2). doi:10.22270/jddt.v3i2.427
16. Bhakay A, Rahman M, Dave RN, Bilgili E. Bioavailability enhancement of poorly water-soluble drugs via nanocomposites: formulation–processing aspects and challenges. *Pharmaceutics*. 2018;10(3):86.

17. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. The 1996 guide for the care and use of laboratory animals. *ILAR J*. 1997;38(1):41–48. doi:10.1093/ilar.38.1.41
18. Tran TT-D, Tran PH-L, Nguyen MNU, et al. Amorphous isradipine nanosuspension by the sonoprecipitation method. *Int J Pharm*. 2014;474(1–2):146–150. doi:10.1016/j.ijpharm.2014.08.017
19. Rahim H, Khan MA, Badshah A, Chishti KA, Khan S, Junaid M. Evaluation of prunus domestica gum as a novel tablet binder. *Braz J Pharm Sci*. 2014;50(1):195–202. doi:10.1590/S1984-82502011000100020
20. Reddy KR, Mutalik S, Reddy S. Once-daily sustained-release matrix tablets of nicorandil: formulation and in vitro evaluation. *AAPS PharmSciTech*. 2003;4(4):480–488. doi:10.1208/pt040461
21. Shah RB, Tawakkul MA, Khan MA. Comparative evaluation of flow for pharmaceutical powders and granules. *AAPS PharmSciTech*. 2008;9(1):250–258. doi:10.1208/s12249-008-9046-8
22. Rahim H, Sadiq A, Khan S, et al. Aceclofenac nanocrystals with enhanced in vitro, in vivo performance: formulation optimization, characterization, analgesic and acute toxicity studies. *Drug Des Devel Ther*. 2017;11:2443. doi:10.2147/DDDT.S140626
23. Organization WH. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. *WHO Tech Rep Ser*. 2009;953:87–123.
24. Rahim H, Sadiq A, Khan S, et al. Fabrication and characterization of glimepiride nanosuspension by ultrasonication-assisted precipitation for improvement of oral bioavailability and in vitro α-glucosidase inhibition. *Int J Nanomedicine*. 2019;14:6287. doi:10.2147/IJN.S210548
25. Bunjes H, Koch MH, Westesen K. Effect of particle size on colloidal solid triglycerides. *Langmuir*. 2000;16(12):5234–5241.
26. Valleri M, Mura P, Maestrelli F, Cirri M, Ballerini R. Development and evaluation of glyburide fast dissolving tablets using solid dispersion technique. *Drug Dev Ind Pharm*. 2004;30(5):525–534. doi:10.1081/DDC-120037483
27. O'Mahony M, Leung AK, Ferguson S, Trout BL, Myerson AS. A process for the formation of nanocrystals of active pharmaceutical ingredients with poor aqueous solubility in a nanoporous substrate. *Org Process Res Dev*. 2014;19(9):1109–1118. doi:10.1021/op500262q
28. Khan S, Matas M, Zhang J, Anwar J. Nanocrystal preparation: low-energy precipitation method revisited. *Cryst Growth Des*. 2013;13(7):2766–2777. doi:10.1021/cg4000473
29. Ali HS, York P, Ali AM, Blagden N. Hydrocortisone nanosuspensions for ophthalmic delivery: a comparative study between microfluidic nanoprecipitation and wet milling. *J Control Release*. 2011;149(2):175–181. doi:10.1016/j.jconrel.2010.10.007
30. Aulton ME. *Powder Flow. Pharmaceutics. The Design and Manufacture of Medicines*. *4th* ed. Edinburgh: Churchill Livingstone; 2013:187–199.
31. Rahim H, Sadiq A, Khan S, et al. Is[illegible]nd preliminary evaluation of Mulva Neglecta mucilage: [illegible]ovel table[illegible]nder. *Braz J Pharm Sci*. 2016;52(1):201–210. doi:1[illegible]590/S1984-825[illegible]016000100022
32. Rahim H, Khan MA, Sadiq A, [illegible]n S, Chishti [illegible]A, Rahman IU. Comparative studies of [illegible]ding pot[illegible]ial of Pru[illegible]s armeniaca and Prunus domestica gu[illegible] in tablets form[illegible]tio[illegible]. *Pak J Pharm Sci*. 2015;28:909–914.
33. Xia D, Quan [illegible] Pia[illegible]H, [illegible]al. Prepa[illegible]tion of stable nitrendipine nanosuspens[illegible]s using [illegible] precipitat[illegible]n–ultrasonication method for enhance[illegible] of dissolution [illegible]d o[illegible]bioavailability. *Eur J Pharm Sci*. 2010;[illegible](4):32[illegible]334. doi:10.10[illegible]/j.ejps.2010.04.006
34. Takano R, Furumo[illegible]K, Shiraki K, et al. Rate-limiting steps of oral absorption [illegible]ly water-solub[illegible]rugs in dogs; prediction from a miniscale dissolution test and a physiologically-based computer simulation. *Pharm Res*. 2008;25(10):2334–23[illegible]4. doi:10.1007/s11095-008-9637-9