International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 5, 2023

Original Article

MODULATING THE DRUG SOLUBILITY OF ACECLOFENAC BY DESIGN AS SOLID LIPID PARTICLES: *IN VITRO/IN VIVO* CORRELATION

SIHAM A. ABDOUN[1], AMAL M. ALSUBAIYEL[1], DALIA A. GABER[1*], BAYAN S. ALSAUDI[2], HAIFA A. ALRASHED[2], NORAH F. ALJARBOOA[2], RANEEM H. ALRADDADI[2], RAZAN R. ALHARBI[2], HEBA A. MOHAMED[3]

[1]Department of Pharmaceutics, College of Pharmacy, Qassim University, Buraidah, Kingdom of Saudi Arabia. [2]College of Pharmacy, Qassim University, Buraidah, Kingdom of Saudi Arabia. [3]Department of Organic Chemistry, National Research Center, Giza, Egypt
[*]Corresponding author: Dalia A. Gaber; [*]Email: dr_daliaahmed@hotmail.com

Received: 17 Apr 2023, Revised and Accepted: 15 Jun 2023

ABSTRACT

Objective: The main objective of the study was to enhance the dissolution and hence the oral bioavailability of Aceclofenac (ACF).

Methods: ACF was formulated as solid lipid particles (SLPs), which compressed into a tablet form for immediate release purpose and certain formulations were then coated by Eudragit RS100 polymer for sustained release action. SLPs of ACF were prepared by melt fusion method under the optimum conditions, using Compritol ATO 888 (Cr), Precirol ATO 5 (Pr), glyceryl monstearate, polyethylene glycols 4000, and Poloxamer 188 at different ratios SLP formulations were characterized for particle size, flow characteristics. The compressed tablets were identified in term of hardness, friability, content, moisture uptake, and *in vitro* release. Oral pharmacokinetics of the optimum tablet formulation and marketed tablet as reference were studied in rabbits.

Results: SLP of acecloenac (ACF) showed accepted flowing properties, and the dissolution rate of the ACF from tablets was significantly enhanced compared to unprocessed drug. The results showed that about 45.5±2.5% of AC was released within 30 min from F1 while 12.7±4.5% was released from commercial AC tablets. The *in vivo* studies verified that the C_{max} was 1.98±0.29, 2.10±0.33, and 4.83±86 µg/µl for the optimized immediate, sustained formula and commercial tablet, respectively. While the area under the curve from zero time to 24 h for the immediate and sustained release formula was 1.79, and 2.41 fold greater than the marketed formulation.

Conclusion: The results showed that solid lipid particles under optimized conditions might be an efficient method for improving the solubility and hence the bioavailability of poorly soluble drugs likes ACF. The proper coating of the formula helps to achieve a convenient release of the drug.

Keywords: Aceclofenac, Dissolution rate, *In vivo* studies, Analgesic, NSAID, Anti-inflammatory, Solid lipid

DOI: https://dx.doi.org/10.22159/ijap.2023v15i5.48108. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Pharmaceutical active drugs are classified according to biopharmaceutics classification system based on their solubility and absorption where more than 40% of the commonly used drugs are poorly water-soluble [1, 2]. Many techniques have been developed to enhance the solubility of such drugs, but these techniques have many limitations, either for the cost or for the difficulty to apply the technique on the industrial scale [3]. New Pharmaceutical techniques are now developing to use conventional polymers and technologies to increase the solubility of class II drugs (Drugs with poor solubility) [4]. One of these technologies for improving the solubility of poorly soluble drugs involves the use of excipient-like lipid polymers [5]. Nowadays, many new lipid polymers are widely used as a base for improving the solubility of water-insoluble drugs, it seems to have a particular advantage in the preparation of oral dosage forms due to their gastric protective effect, chemical inertness, better characterization, and formulation versatility [6-8]. Recently, focusing on the use of gelucires (Gu) as carriers in delivery systems have increased [9]. Gu is a solid material that has amphiphilic characters and is identified through melting points and hydrophilic–lipophilic balance (HLB) [10]. Gu are saturated polyglycolic glycerides consisting of either mono-, di-or tri-glycerides and mono-and/or di-fatty acid esters of polyethylene glycol [11]. Gu has been widely used for increasing the solubility of poorly soluble drugs and subsequently enhancing their bioavailability [12]. Gu gives molten semisolid mass upon heating with the drug, which can be easily ground and pressed in a compact tablet form, a process that is more efficient and economical than other solubility-enhancing methods [13]. It can be considered a simple process that can be easily scaled up to an industrial platform Cr, Pr, and Glycerol monostearate (GMS) are types of Gu that are widely used as glyceride base polymers for enhancing the solubility of poorly soluble drugs [14]. ACF is a non-steroidal anti-inflammatory drug with effective analgesic and anti-inflammatory characteristics [15]. It is widely used for different types of pain, toothache, osteoarthritis, rheumatoid arthritis, and other acute and chronic musculoskeletal injuries, which increases the requirement for immediate and prolonged release formulations [15]. ACF (fig. 1) is a class II drug as it is practically insoluble and its absorption is limited by its low dissolution rate [16]. This study aimed to investigate the role of lipid bases and their different blends in altering the dissolution rate of water-insoluble drugs to obtain release profiles for immediate and Eudragit RS100 coated sustained release formulations. A new concept of solid lipid particle (SLP) formulations, followed by compressing into immediate-release or coated sustain-release tablets, was implemented for this purpose. ACF a weakly acidic and poorly soluble drug was used as a model drug. SLP of ACF was formulated using different materials like Cr, Pr, GMS, PEG 4000, PEG 6000, PVP K-30, and Poloxamer 188, which were then compressed into either uncoated tablets for immediate release purposes and then coated by Eudragit RS100 solutions with different concentrations (o.5, 1, and 2%) for sustained release action. The tablets were evaluated for drug content, *in vitro* dissolution, release kinetics, and moisture pickup and were compared with convention-marketed tablets. The optimized formula was subjected to *in vivo* pharmacokinetic study in a rabbit model.

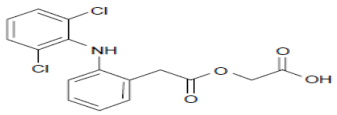

Fig. 1: Chemical structure of Aceclofenac (ACF)

MATERIALS AND METHODS

Aceclofenac, and Amoflam®200 mg (Batch no. 2510) were obtained as a gift sample from Amoun Pharmaceutical Company-Human

pharmaceutica, Egypt. Cr (glyceryl behenate NF; Gattefosse´ s. a., Lyon, France), Pr (glyceryl palmitostearate; Gattefosse´ s. a.), Glyceryl monostearate GMS (Loba Chemie Pvt. Ltd., Mumbai, India), Eudragit RS100 RS100 (Adwic Pharmaceuticals, Cairo, Egypt). PEG 4000, PEG 6000, (Loba Chemie Pvt. Ltd., Mumbai, India). Acetonitrile (HPLC) grade, methanol, trimethyl amine (Scharlau Chemie SA, Barcelona, Spain), sodium dihydrogen phosphate (Koch-Light Laboratories, Colnbrook Bucks, UK), magnesium stearate, and talc and were all of pharmaceutical grade and used as received.

Determination of ACF solubility in different bases

Quantitative solubility of ACF in various solid lipid bases like Cr, Pr, GMS, PEG 4000, PEG 6000, and Poloxamer 188 was verified as mentioned by S. Galal *et al.* 2004 [17]. Briefly, 10 mg of ACF was placed in different glass beakers. Twenty milligrams of each base were accurately weighed and carefully added to ACF to obtain a series of mixtures with different drug/bases ratios. Drug/base mixtures were kept in a hot air oven adjusted at 60o C for 6 h, 5 gm of each of the mixtures was then dissolved in 20 ml of absolute methanol centrifuged for 15 min at 1500 RPM and the amount of ACF in the supernatant was detected by HPLC method according to the method mentioned by M. Y. Momin, *et al.*, 2006 [18].

Formulation of SLP and compressing into a compact tablet

A certain weight of bases selected based on the solubility test was taken into glass beakers and heated to 60oC on a thermo-controlled water bath. The required amount of ACF was added to molten bases and stirred using a magnetic stirrer continuously for 30 min to ensure homogenous distribution. The mass was then cooled to room temperature and passed through 125 µm sieves and mixed with sodium docusate, magnesium stearate, and, talc (table 1) [19]. The mixtures were then compressed into compact tablets using a single punch compression machine (Cadmach, Ahmedabad, India) fit with 11 mm concave punches. Compression force was adjusted to produce a 6-9 kg/cm2 tablet-crushing strength [20].

Table 1: Composition of Aceclofenac tablets formulations

Formula code	ACF	PEG 6000	Pr	Poloxamer 188	Cr	PEG 4000	Coating solution		
							Eudragit RS100 RS-100 (0.5% w/v)	Eudragit RS100 RS-100 (1% w/v)	Eudragit RS100 RS-100 (2% w/v)
F1	100	200	-	-	-	-	-	-	-
F2	100	-	200	-	-	-	-	-	-
F3	100	-	-	200	-	-	-	-	-
F4	100	-	-	-	200	-	-	-	-
F5	100	-	-	-	-	200	-	-	-
F6	100	200	-	-	-	-	✓	-	-
F7	100	-	200	-	-	-	✓	-	-
F8	100	-	-	200	-	-	✓	-	-
F9	100	-	-	-	200	-	✓	-	-
F10	100	-	-	-	-	200	✓	-	-
F11	100	200	-	-	-	-	-	✓	-
F12	100	-	200	-	-	-	-	✓	-
F13	100	-	-	200	-	-	-	✓	-
F14	100	-	-	-	200	-	-	✓	-
F15	100	-	-	-	-	200	-	✓	-
F16	100	200		-	-	-	-	-	✓
F17	100	-	200	-	-	-	-	-	✓
F18	100	-	-	200	-	-	-	-	✓
F19	100	-	-	-	200	-	-	-	✓
F20	100	-	-	-	-	200	-	-	✓

ACF: Aceclofenac, all ingredients weight were in mg

Coating of ACF-tablets

The ACF compressed tablets were further coated with Eudragit RS100 RS-100 solution at different concentrations (0.5, 1, and 2 % w/v). The coating solution of Eudragit RS100 RS-100 was prepared by dissolving the required weights of Eudragit RS100 RS-100 powder in a mixture of Isopropyl alcohol/acetone in a ratio of 1:1. The coating of the tablets was completed by immersion the tablet in the coating solution based on dip coating technique [21].

Determination of ACF content in tablets

ACF content in each tablet was identified by crushing three tablets from each batch separately and 20 ml of methanol was added to 200 mg of each of the crushed tablets; the mixture was centrifuged for 30 min at 1500 rpm, 5 ml of the supernatant was then withdrawn, and the amount of ACF was detected at 270 nm using UV spectrophotometer after adequate dilution [22].

***In vitro* evaluation of physicochemical characteristics of SLP of ACF**

Evaluation of the pre-compression parameters of SLP of ACF was conducted, including particle size distribution, angle of repose, carr's index, and Hausner's ratio [23].

Determination of particle size distribution

The particle size distribution of particles was identified using the sieve analysis method. Twenty grams of ACF-SLP were weighed and placed on the first screen and shaken for a certain time (15 min). Each fraction is then removed from the screen and weighed. The particle size distribution was determined as follows: [24]

$$\text{dave} = \sum \text{nd} / \sum \text{n}$$

Where d_{ave} is the average diameter of particles, n is the percent of each fraction retained on each sieve and d is the mean size of the sieve opening. The experiment was done in triplicate.

Angle of repose

The angle of repose of ACF-SLP was identified by the funnel method. An accurate weight of ACF-SLP were passed through the funnel. The height of the funnel was adjusted at 2 cm in such a way that the funnel tip just touched the top of the poured granules. The diameter made by pouring the particles through the cone was measured and the angle of repose (θ) was calculated using the following equation: [25].

$$\tan\theta = H/r$$

Where θ: is the angle of repose, H: is height (= 2 cm), and r = radius of the circle made by poured particles.

Compressibility index

Bulk density is measured as the ratio of the total volume of powder to the bulk volume. It was measured by adding certain weights of the

powder into a graduating measuring cylinder and the volume was noted. The particles were then tapped to find the tapped volume; the compressibility index was then calculated according to the following equation: [26]

$$CI = (Dt - Db) * 100$$

Where: Dt refers to the tapped density of the powder and Db refer to the bulk density of the particles.

Hausner ratio

The Hausner ratio is a number that identifies the flowability of the powder. It is measured by the following formula:

$$Hr = Dt/Db$$

Where: Dt is the tapped density, and Db is the bulk density. Hr greater than 1.25 is an indicator of poor flowability according to USP [27].

Evaluation of physicochemical characteristics of immediate and sustained released tablets of ACF

Compressed tablets were characterized for moisture uptake, weight variation, diameter, hardness, thickness, friability.

Moisture uptake studies for ACF-tablets

Moisture uptake studies were accomplished on formulations. The formulations were weighed and stored in 75% relative humidity (RH) at room temperature (25±2 °C). RH of 75% was maintained by keeping a saturated solution of sodium chloride in the base of closed desiccators. The tablets were weighed every day for seven days, and the percentage (%) moisture uptake by different tablets was determined on the weight base [28].

Determination of ACF release from immediate and sustained release tablets

The release of ACF from both immediate and sustained release tablets was measured using USP dissolution test apparatus II (paddle type). The temperature of the media was maintained at 37 °C±0.5 °C with a paddle speed 50 rpm. The dissolution media was 900 ml of 0.1N HCl dissolution medium for 2 h, the media pH was then shifted to the alkaline range (6.8 pH) after 2 h with phosphate buffer. Samples with the volume of five milliliter were withdrawn at various intervals 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h and filtered through Millipore filters (0.45 mm). An equal volume of pre-warmed fresh dissolution medium was immediately added. After appropriate dilutions, the amount of ACF in samples was analyzed spectrophotometrically (UV-1601; Shimadzu, Kyoto, Japan) at 270 nm [29]. All dissolution runs were performed in triplicate.

Kinetic study of *in vitro* release data

Four kinetic models were used to analyze the *in vitro* release data. These models were: [30]

Zero order: $Q_t = Q_{0+}k_0\ t$,

First order: $\text{Log}\ Q_t = \log Q_o\text{-}kt/2.303$

Higuchi model: $Q_t = k\ t^{1/2}$

Korsmeyer-Peppas model: $Q_t/Q_\infty = kt^n$

where Q_t is the amount of drug released at time t, Q_∞ is the total amount of drug released after infinite time, Q_t/Q_∞ is the fraction of drug released at time t, k is the kinetic constant, and n is the release exponent, which depends on the release mechanism and it is thus used to characterize it either Fickian diffusion (n<0.5), anomalous diffusion (non-Fickian transport) where (0.5<n<1) or zero-order release (n =1). To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time.

***In vivo* pharmacokinetic study of optimal formula in rabbits**

The study of *in vivo* pharmacokinetic parameters of the selected immediate and sustained release formulations in comparison to commercial tablet was conducted in accordance with the ethical guidelines for examinations in laboratory animals and was approved by the Research Ethics Committee, Qassim University, (number [PI/1535]). The animals were provided from the animal house-Qassim university. The process and care of the rabbits were in a good agreement with guidelines for animal use in laboratory researches. Six rabbits weighing between 3.5 and 4.5 kg were used and distributed into three groups randomly, and the study was conceded based on crossover experimental pattern in three periods with an in-between one-week washout period for removing the effect of the prior dose before the next administration. All rabbits fasted overnight; no food was permitted until 2 h after dosing. Water was accessible ad libitum thru the study period. During each phase, rabbits in each group received by the oral intubation either F1 (immediate-release tablet), or F11 (sustain-release tablet), or weighted dose of market tablet. Five-milliliter blood samples were withdrawn from the rabbit ear vein into heparinized tubes at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dose [31]. Plasma samples was separated by centrifugation at 4000 rpm for 15 min, and samples were freezed at-20 °C until further analysis. The plasma concentration was assayed using HPLC method.

Chromatographic system for quantitative analysis of ACF in plasma of rabbits

ACF concentrations in plasma were identified by the HPLC method reported by Prashant Musmade, 2007 [32]. The method was followed for determining the concentration of ACF in plasma using Ibuprofen as an internal standard. The chromatographic analysis was carried out using a reversed-phase C_{18} column (250 mm, 4.6 mm i.d., 5 m particle size) using an isocratic mobile phase (methanol/trimethylamine 60:40%, v/v) that buffered at pH 7.0 and flow rate 1.0 ml $min^{-1}$and ACF concentration in effluent was detected at wavelength 278 nm using UV detector. Before the analysis, the mobile phase media was filtered through filters (0.45 µm Millipore) and the system was equilibrated with the mobile phase before injection. The experiment was accompanied at room temperature. All data were analyzed using (Lynx TMV 4.1 software, Waters Corp.). The method was validated for linearity, precision, accuracy, selectivity, and stability briefly before the start of the study.

Analysis of ACF concentrations in plasma samples

Briefly, 200 µl plasma samples were mixed with 20 µl of Ibuprofen "internal standard" in a 2 ml Eppendorf tube. The mixture was then vortexed (Paramix II; Julabo, Seelbach, Germany) for one minute, and 800 µl of acetonitrile was added and the mixture was vortexed again for one minute and centrifuged at 15,000 rpm for twenty minutes. The supernatant was taken into a clean tube and evaporated until complete dryness. About 200 µL of mobile phase was added to the residue, vortexed for 1 minute, and 20 µL was injected into the HPLC system [33].

Calculation of ACF parameters and statistical analysis of data

Pharmacokinetic parameters were evaluated from the plasma concentrations time curve. Concentration of ACF in plasma samples are presented as the mean±SE. The peak of plasma concentration (C_{max}), the time to achieve the peak (t_{max}). The extent of absorption ($AUC_{0\text{-}t}$) was measured using linear trapezoidal rules. The relative bioavailability (F) was deliberate using the following equation with the commercial product as a reference.

$$F = AUC_{test}/AUC_{ref} \times 100$$

Statistical evaluation of the results

The *in vivo* experiment was constructed to evaluate the difference between the pharmacokinetic parameters obtained after oral administration of each of the three dosage forms to each group of rabbits in a crossover model. All statistical data were evaluated using IBM SPSS Statistics 20 (Armonk, NY, USA) by means of one-way ANOVA with extended "LSD post hoc" test for determination of pharmacokinetic parameters, and P value ≤0.05 was considered significant.

***In vitro*/*in vivo* correlation**

In an attempt to find the type of relationship between ACF plasma concentration and the concentration of the drug released from the selected formula (F11) in the dissolution medium. Plasma

concentrations of a fraction of the drug absorbed were plotted against a fraction of the drug released *in vitro* at the same time of 0.5, 1, 2, 3, 4, 5, 6, and 8 h. Exploring a relationship between the *in vivo* absorption and *in vitro* drug release from a dosage form [34].

RESULTS AND DISCUSSION

Solubility study

Formulations of ACF as SLP by dispersing the drug in each base at higher temperatures and forming a matrix of ACF dispersed in a molten base, which is then sieved through a sieve screen to form SLP of ACF. Bases able to dissolve the drug usually help to improve its dissolution rate by increasing solubility and wettability. In addition, increasing the ability of the base to trap any fine precipitates of the drug on the molten mass may also improve the solubility and stability of the formula. A solubility study was hence accomplished to check the different solubilizing ability of various bases and their capability to avoid precipitation and recrystallization upon cooling. The solubility of ACF in different bases is shown in fig. 2. ACF showed different solubility in different bases; the highest solubility was in PEG 6000 (80.5 µg/ml) followed by the solubility in Poloxamer 188 (63.8 µg/ml), followed by Cr (60.5 µg/ml), PEG 4000, Pr, and finally GMS where ACF showed the least solubility. The order could be explained based on melting point and HLB value that the lower melting point and higher HLB values will help to increase drug miscibility and dispersibility in the carrier base. GMS was the poorest solubilizer for ACF because of its low HLB value [35]. Based on the study results, PEG 6000, Poloxamer 188, Cr, PEG 4000, and Pr were selected for preparing immediate-release ACF formulations, followed by coating the compressed immediate-release tablets with 0.5 mg, 1 mg, and 2 mg w/v of eduragit 100 SR solution to form sustained release formulations.

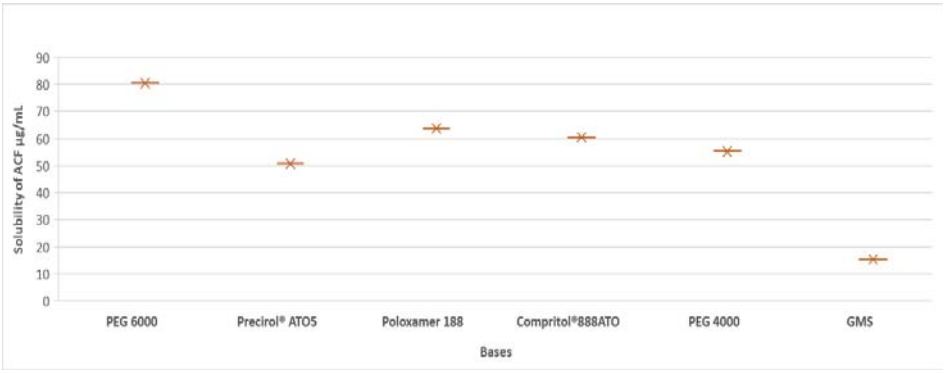

Fig. 2: Quantitative solubility of ACF in different bases

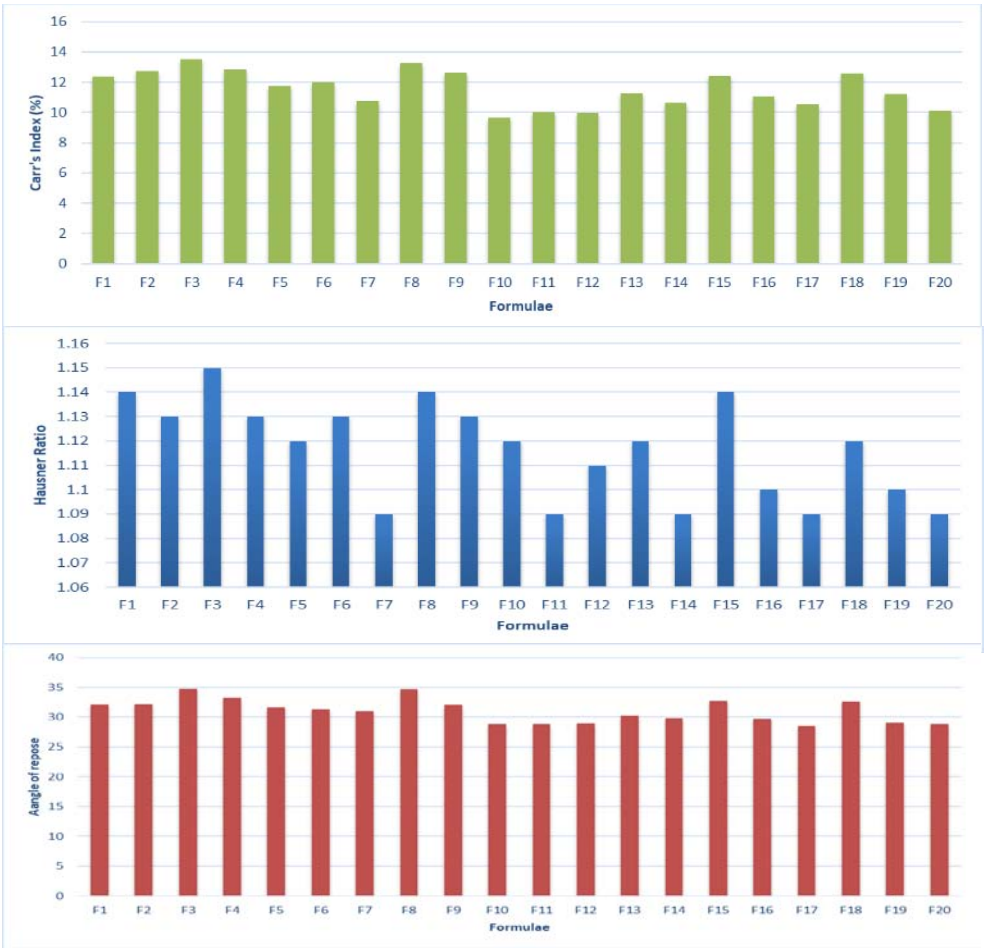

Fig. 3: Flowing properties of ACF-SLP formulations (n=3±SD)

Flow characterization and particle size distribution of ACF-SLP

All ACF-SLP formulations were free-flowing. The angle of repose (Θ) of the powder mixture for all formulations ranged between 26-29o, indicating good flow properties. Hausner's ratios values and compressibility indices ranged from 1.23 to 1.27 and 9.11% to 10.98%, respectively. The flow properties results were acceptable for all formulations mixtures; fig. 3. The particle size of the SLP of ACF was affected by polymer type. The average particle size of SLP noticeably increased with PEG 4000 and PEG 6000 and ranged between 405 to 520 µm (fig. 4). Concerning the effect of matrix polymer on the size of SLP, polymers could be arranged as follows: PEG 6000>PEG 4000>Cr>Pr>Poloxmer. This could be related to differences in the structure, molecular weight, and viscosity of the polymers, which results in solutions with different viscosities [36].

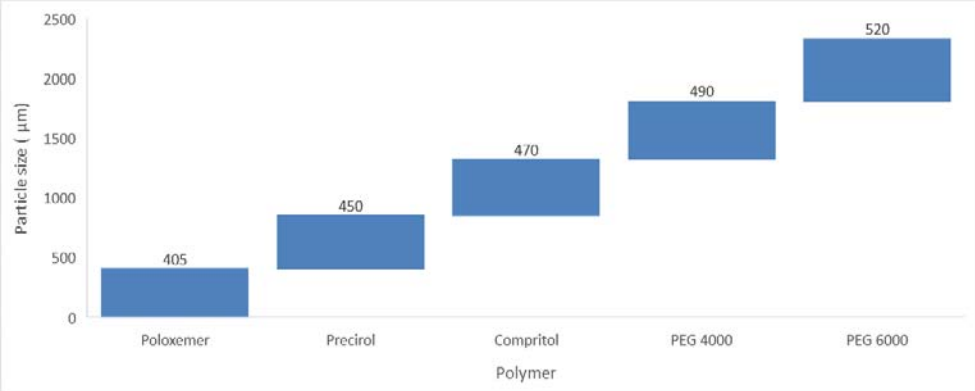

Fig. 4: Particle size distribution of SLP of ACF ((n=3±SD)

Physicochemical characteristics of ACF immediate release and sustained release tablets

ACF immediate tablets were white in color, round in shape, with concave sides, with smooth surfaces without cracks or pettings. The mean diameter was 11.0±0.0 mm, while the thickness ranged between 3.0 and 3.1 mm. The hardness of the tablets was in the range of 7.86±0.68 and 8.46±0.41 kg/cm2, indicating sufficient strength of tablets to withstand abrasion. The percentage loss upon the friability test was less than 1% for all formulations, which indicates satisfactory mechanical resistance. All formulations lay within the pharmacopoeial range of±5% for weight variation. The percentage of drug content ranged between 97.5±1.5% and 100.3±0.86%, which satisfies the pharmacopeial requirements. Coated formulations were buff in color, rounded shape, with smooth surfaces; the mean diameter and thickness ranged between 11.2-11.5 mm, and 3.3-3.6 mm, respectively. The friability test showed a non-significant loss. All formulations passed the weight variation test and the drug content was above 98.2±1.6% (table 2).

Table 2: Average weight, thickness, diameter, hardness, friability, and content uniformity of ACF-tablets

Formula code	Average weight (mg)	Average thickness (mm)	Average diameter (mm)	Average hardness (Kg/cm²)	Average friability (%)	Content uniformity (%)
F1	375±3.34	3.0±1.32	11	8.25±0.57	0.29±0.12	100.41±1.25
F2	375±3.25	3.0±1.30	11	8.16±0.62	0.31±0.24	99.62±2.24
F3	375±4.25	3.1±1.34	11	8.05±0.82	0.15±0.11	98.60±0.95
F4	375±3.85	3.0±3.01	11	8.23±0.26	0.35±0.21	99.6±1.24
F5	375±4.34	3.1±1.04	11	8.00±0.35	0.24±0.42	101.24±1.02
F6	386±4.28	3.3±1.14	11.2	7.95±0.54	0.40±0.33	100.54±1.25
F7	385±5.65	3.3±1.21	11.2	8.24±0.68	0.36±0.41	99.68±0.95
F8	385±3.24	3.3±1.33	11.2	8.43±0.46	0.28±0.32	101.2±1.23
F9	385±3.64	3.3±1.01	11.2	8.24±0.52	0.31±0.11	100.23±1.45
F10	385±4.78	3.3±2.03	11.2	8.10±0.25	0.42±0.05	99.5±2.35
F11	405±3.65	3.4±2.23	11.3	8.32±0.74	0.35±0.21	101.5±1.35
F12	404±4.24	3.4±1.89	11.3	8.45±0.22	0.42±0.15	100.7±1.85
F13	405±4.85	3.4±2.05	11.3	8.34±0.32	0.36±0.20	99.9±2.14
F14	404±3.24	3.5±1.62	11.3	8.46±0.41	0.22±0.21	100.54±1.8
F15	404±3.25	3.4±2.04	11.3	7.86±0.68	0.34±0.16	101.3±2.03
F16	411±3.67	3.6±2.13	11.4	8.31±0.48	0.41±0.23	99.84±1.25
F17	412±2.97	3.6±1.55	11.5	8.22±0.33	0.53±0.12	102.3±0.56
F18	411±3.45	3.6±2.21	11.5	8.35±0.16	0.40±0.11	101.8±1.31
F19	411±2.63	3.6±1.89	11.5	8.06±0.54	0.24±0.20	100.24±1.54
F20	410±3.25	3.6±1.85	11.5	8.13±0.08	0.45±0.31	99.8±2.10

*mean±SE, n=3

Moisture uptake of ACF tables

ACF tablet formulations must be stable at different environmental conditions as termed by International Conference on Harmonisation (ICH) guidelines [37]. Moisture uptake studies for all ACF tablet formulations at 75% RH were accompanied to have a perception of the stability of ACF formulations. The immediate-release tablet formulations picked up moisture ranging between 0.4% and 0.7%. While moisture uptake by the sustained-release ACF formulations ranged between 0.2% and 0.5%. The ACF formulations showed accepted stability at 75% RH as the percentage of moisture uptake was less than 2% as identified by the guidelines.

***In vitro* dissolution study of immediate release tablets of ACF**

The study aimed to improve the dissolution and consequently the release of ACF from compressing SLP of ACF in immediate-release tablet form that can release more than 85% of the drug within 2 h in various dissolution media simulators to the gastrointestinal tract pH. This principle was certain to avoid dissolution rate problems that are usually detected for class II drugs like ACF. Dissolution of unformulated drugs and commercial tablets (100 mg) was performed. Release studies showed that ACF "weakly acidic drug" shows low dissolution in acidic pH, which increased in more alkaline media (fig. 5) [38]. Not more than 19.4% of the total amount of ACF in the un-formulated sample was released within 2 h in 0.1 N HCl. The release pattern from the marketed product showed also a poor release in acidic pH followed by a faster release in basic media as compared to acidic media. This difference in the release can be explained based on the pH-dependent solubility of ACF. Dissolution data for various SLP formulations of ACF are presented in fig. 5. Among all the polymers studied, PEG 6000 showed a higher dissolution rate that about 49.5%±3.5 was released within 2 h, and about 95%±2.5 was released within 4 h. The following rank order can express the dissolution rate from different matrices: PEG 6000>PEG 4000>Poloxamer 188>Cr>Pr. Results revealed that increasing the HLB of the polymer reflected an increase in the dissolution rate of ACF in all matrices [39]. However, this increase in dissolution was not significant for Cr and Pr-based formulations. A two-fold increase in the initial release was observed in F5 when compared to F1, while the time taken for the release of all drug amounts remained unchanged. The results could be interpreted based on the increase in the diffusion pathway of the drug from more lipophilic bases. The drug release rate from all matrices was meaningfully more in acidic pH when compared to an unformulated and marketed product which expresses the improvement in wettability and emulsification of weakly acidic drug (ACF) based on the amphiphilic character of the tested bases.

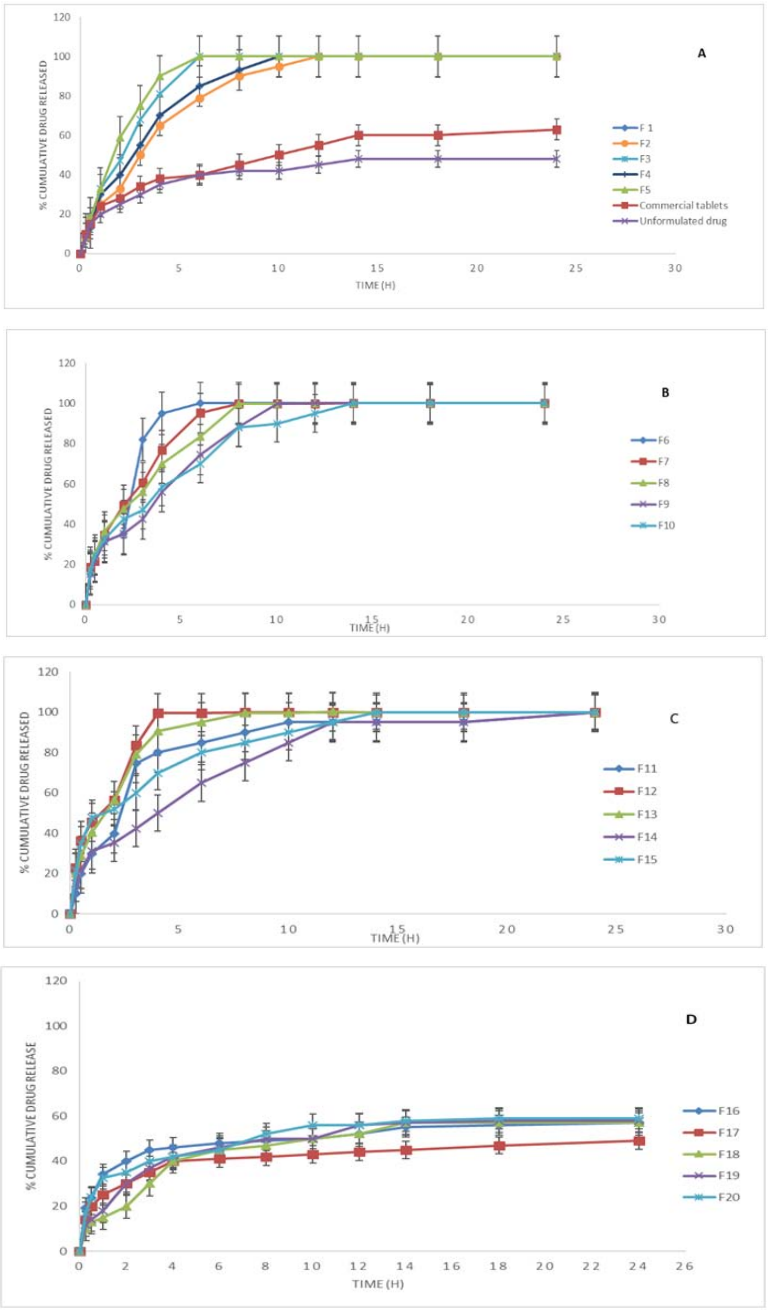

Fig. 5: *In vitro* release data of immediate releae tablets of ACF (A), sustained release tablets coated with 0.5% of Eudragit RS100 solution (B), sustained release tablets coated with 1% of Eudragit RS100 solution (C), sustained release tablets coated with 2% of Eudragit RS100 solution (D), mean±SD, n=3

In vitro dissolution rate of sustained release coated tablets of ACF

Compressed tablets of ACF-SLP were coated using three concentrations of lipophilic Eudragit RS100 (0.5, 1, 2% W/V) to obtain sustained release formulations of ACF that aimed to sustain the drug release for 24 h. Complete drug release within 8-10 h occurred in F6, F7, F8, F9, and F10 formulations, whereas only 45-57% of ACF was released within 24 h for formulations F16, F17, F18, F19, and F20 (fig. 5). This difference is probably due to the concentration of coated material that increases the strength of the gel around the tablet matrix, which significantly decreases the release rate of ACF since the drug should diffuse through the gel barrier into the bulk media. A direct relationship was observed between Eudragit RS100 concentration and duration of drug release and greater lipophilic characters, decreasing the drug release into the dissolution media [40, 41]. To optimal coating concentration to achieve the desired release profile, Eudragit RS100 1%w/v that 85-99% of the drug was released within 24 h. Formulations F11-F15 gave an accepted sustained release profile within 24 h. A higher concentration of Eudragit RS100 in F16-F20 leads to an incomplete release of ACF that high concentration of lipophilic polymer hinders the release to a great extent that not more than 60.1±2.5% of the drug was released by the end of 24 h. These observations are in agreement with those described by Sanchez-Lafuente *et al.*, who found that Eudragit RS100 RSPM with high concentrations led to a significant delay in drug release [42].

Kinetic study of release data

In order to study the release kinetics of ACF from the immediate and sustained tablets, the *in vitro* release results of all formulations were fitted to zero order, first order, Higuchi, and Korsmeyer–Peppas model (table 3). The selection of the most appropriate model was depending on the best fit shown by the coefficient of determination (R2). The best fit with the highest determination coefficient (R2) for immediate-release tablets (F1-F5) was shown with the Higuchi model, while sustained tablets showed the highest values of R2 in the Peppas equation. The n values for F6-F15 indicate a non-Fickian diffusion mechanism that drug release was by both diffusion and matrix erosion mechanism. Whereas the n value of F16-F20 was less than 0.5 exposed a Fickian release mechanism of ACF from the tablet that the release rate of ACF from these tablets is controlled by gradual diffusion polymer layers [43].

Table 3: Kinetic analysis of release data of ACF from tablets according to different kinetic models

Formula	Zero order $Q_t = Q_{0+}k_0 t$		First-order $Q = Q_o e^{-kt}$		Higuchi $Q_t = k t^{1/2}$		Peppas $Q_t/Q_\infty = kt^n$	
	R^2	K (%min^{-1})	R^2	K (min^{-1})	R^2	K (%min^{-1})	R^2	N
F1	0.951	0.166	0.848	0.002	0.97	4.126	0.96	0.573
F2	0.974	0.174	0.871	0.001	0.74	3.958	0.948	0.388
F3	0.965	0.151	0.822	0.001	0.99	3.789	0.984	0.471
F4	0.951	0.166	0.848	0.002	0.97	4.126	0.96	0.573
F5	0.946	0.17	0.8	0.001	0.99	4.289	0.988	0.433
F6	0.954	0.142	0.85	0.003	0.98	4.28	0.991	0.558
F7	0.965	0.151	0.822	0.001	0.89	3.789	0.990	0.571
F8	0.88	0.178	0.905	0.002	0.96	5.378	0.974	0.598
F9	0.974	0.174	0.871	0.001	0.97	3.958	0.98	0.588
F10	0.88	0.178	0.905	0.002	0.96	5.378	0.97	0.598
F11	0.82	0.147	0.21	0.001	0.87	4.25	0.98	0.525
F12	0.895	0.085	0.755	0.001	0.97	2.178	0.98	0.542
F13	0.931	0.11	0.754	0.001	0.98	2.918	0.99	0.521
F14	0.934	0.174	0.702	0.001	0.96	4.407	0.97	0.533
F15	0.945	0.174	0.743	0.001	0.97	4.39	0.98	0.522
F16	0.965	0.126	0.816	0.001	0.97	3.162	0.98	0.461
F17	0.957	0.249	0.792	0.002	0.97	5.463	0.98	0.421
F18	0.923	0.198	0.765	0.001	0.97	5.015	0.99	0.475
F19	0.936	0.195	0.77	0.002	0.97	4.985	0.98	0.430
F20	0.92	0.089	0.76	0.001	0.97	2.277	0.98	0.490

Pharmacokinetic study of selected formulation of ACF immediate and sustained release tablets

A pharmacokinetic study of the optimized ACF immediate-release tablets (F1) and sustained-release tablets (F11) was compared with marketed ACF tablets done in rabbits in three phases after oral administration. The concentration vs time profiles of F1, F11, and commercial tablets are shown in fig. 6, and other pharmacokinetic parameters are presented in table 4. Results exposed that after oral administration of F1, F11, and commercial tablets to rabbits, the drug appeared in blood samples after 0.33±0.11 h, 0.41±0.20 h, and 0.50±0.14 h, respectively. The mean maximum drug concentration (Cmax) of F1 and F11 was 2.10±0.33 and 1.98±0.29 µg/ml, respectively, which was significantly higher than that of the commercial product 1.25±0.25 µg/ml. The time to achieve the peak concentration (tmax) was almost the same and a statistically non-significant difference (P>0.05) was observed among the three samples. The AUC0–24 value was 30.19±7.16, 54.07±6.87, and 72.82±9.62 (µg·h ml-1) for the commercial tablets, F1, and F11 respectively. The results showed that F11 has the longest rate and extent of drug absorption, whereas the commercial tablet has the lowest rate and extent of absorption. The relative bioavailability of F1 and F11 were 179.01% and 241.2%, respectively. The significant increase in the bioavailability of F11 may be interpreted as the increase in the solubility of ACF in SLP in addition to the coating layer of Eudragit RS100 polymer that form a dense layer surrounding the core matrix, which increases the ability of the tablet to control the release of the drug from the core; also polymer concentration (1%) showed the optimal viscosity and shell thickness with convenient diffusion length that could control the penetration of the outer dissolution medium, so the drug release was prolonged over longer time. ANOVA test was applied to AUC0–∞ and Cmax data as shown in table 5 [44, 45]. The results showed that the total amount of the drug and the total number of administrations per day will be decreased, which is in good accordance with recent WHO recommendations to use the least amount of chemicals; the clinical studies verified that fewer side effects and more safety could be predicted with this performance.

In vitro/*in vivo* correlation

Exploring the relationship between the *in vivo* absorption and *in vitro* release from a controlled-release dosage form is important for the dosage form development process. Furthermore, it is fundamental to develop a reproducible and predictable *in vitro* dissolution test to be used for the optimization of the dosage form. A diversity of factors affects the *in vivo* dissolution process, including physicochemical feature of the drug and physiological features in the gastrointestinal tract, such as intestinal motility and fluid secretion [46]. In this study, the relationship between the *in vitro* dissolution and the *in vivo* pharmacokinetic was observed by plotting the

fraction of drug dissolved (FD) after 0.5, 1, 2, 3, 4, 5, 6, and 8 h and the fraction absorbed data (FA) calculated at the same time post-dosing (fig. 7). The linear regression analysis showed that a statistically significant relationship (R2=0.9864) existed between the FD and FA for the sustained matrix tablets and was best described by the following equation: y=0.9667x-0.0267. The slope and intercept were close to 1, indicating that the *in vivo* fraction absorbed could be predicted from *in vitro* dissolution data [47]. Recently it has been proposed that a 1:1 (level A) relationship between *in vitro* dissolution data and *in vivo* absorption data is the most desirable type of correlation for sustained-release dosage forms, as reported by Guan J, *et al.*, 2010 [48].

Table 4: Mean pharmacokinetic parameters of ACF after oral administration of commercial tablets, F1, and F11 to rabbits

Parameters	Formulations		
	Commercial tablets	F1	F11
$AUC_{0\text{-}24h}$ (µg. h. ml^{-1})	30.19±7.16	54.07±6.87	72.82±9.62
$AUC_{0\text{-}\alpha}$ (µg. h. ml-1)	33.41±5.82	57.49±7.70	76.08±8.03
C_{max} (µg ml^{-1})	1.25±0.25	1.98±0.29	2.10±0.33
T_{max} (h)	0.50±0.14	0.33±0.11	0.41±0.20
F		179.01	241.2

*mean±SE, n=6

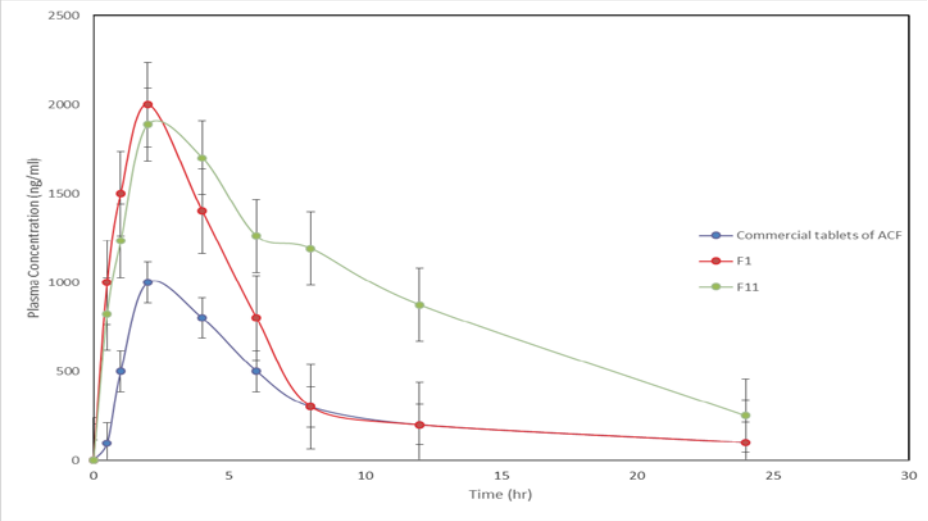

Fig. 6: Pharmacokinetic parameters of F1, F11, and commercial tablets of ACF, mean±SE, n=6

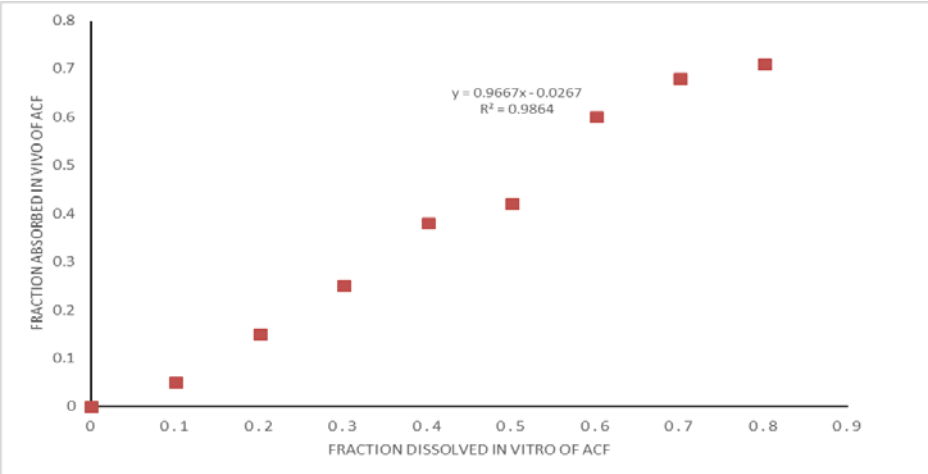

Fig. 7: Relationship between the ACF fraction dissolved *in vitro* and the fraction absorbed *in vivo* for F6

CONCLUSION

The study results showed the capabilities of solid lipid particles formulations based on different polymers to enhance the solubility of ACF. Immediate-release tablets with improved dissolution rate were well formulated using PEG6000, which was suggestively better than unformulated drug and commercial tablets. Sustained-release ACF tablets coated with Eudragit RS100 (1% w/v) are able to control the drug release for a longer period of time with peppas kinetics. These commercially achievable formulations based on the ease of operations and low cost of polymers will help in designing successful oral dosage forms for many drugs belonging to class II.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest and no financial fund was supported for this article.

REFERENCES

1. Mudie DM, Amidon GL, Amidon GE. Physiological parameters for oral delivery and *in vitro* testing. Mol Pharm. 2010;7(5):1388-405. doi: 10.1021/mp100149j, PMID 20822152.
2. Desai NS, Galatage ST, Swapnil S. Harale, Suresh G Killedar, Ravindra B Kumbhar, Arehalli S Manjappa. Design and characterization of aceclofenac bio nanocomposite using natural solubilizes. IJPSR. 2021;12(12):6510-21. doi: 10.13040/IJPSR.0975-8232.
3. Prakash G, Chandra SA, Sandhya P, Bidur C, Samir D. Pharmacopoeial comparison of in-process and finished product quality control test for pharmaceutical tablets. GSC Biol and Pharm Sci. 2020;11(3):155-65. doi: 10.30574/gscbps.2020.11.3.0174.
4. Kumar S, Gupta A, Mishra C, Singh S. Synthesis, characterization and performance evaluation of aceclofenac-urea cocrystals. Indian J Pharm Sci. 2020;82(5):881-90. doi: 10.36468/pharmaceutical-sciences.717.
5. Sagare RD, Bolmal UB. Formulation and evaluation of aceclofenac matrix tablets using solid dispersed products. IJPSR. 2022;13(3);1231-40. doi: 10.13040/IJPSR.0975-8232.
6. Gaber DA, Radwan MA, Alzughaibi DA, Alail JA, Aljumah RS, Aloqla RM. Formulation and evaluation of piroxicam nanosponge for improved internal solubility and analgesic activity. Drug Deliv. 2023;30(1):2174208. doi: 10.1080/10717544.2023.2174208, PMID 36744372.
7. Patel HM, Patel UB, Shah C, Bhavesh Akbari. Formulation and development of nanosuspension as an alternative approach for solubility and dissolution enhancement of aceclofenac. International Journal of Advances in Pharmaceutics. 2018;07(05):33-47. : https://doi.org/10.7439/ijap
8. Gaber DA, Alhawas HS, Alfadhel FA, Abdoun SA, Alsubaiyel AM, Alsawi RM. Mini-tablets versus nanoparticles for controlling the release of amoxicillin: *in vitro*/*in vivo* study. Drug Des Devel Ther. 2020;14:5405-18. doi: 10.2147/DDDT.S285522. PMID 33324038.
9. Shankar B, Jalapathi P, Saikrishna B, Perugu S, Manga V. Synthesis, anti-microbial activity, cytotoxicity of some novel substituted (5-(3-(1H-benzo[d]imidazol-2-yl)-4-hydroxybenzyl)benzofuran-2-yl)(phenyl)methanone analogs. Chem Cent J. 2018;12(1):1. doi: 10.1186/s13065-017-0364-3. PMID 29318401.
10. El Nabarawi MA, Teaima MH, Abd El-Monem RA, El Nabarawy NA, Gaber DA. Formulation, release characteristics, and bioavailability study of gastroretentive floating matrix tablet and floating raft system of mebeverine HCl. Drug Des Devel Ther. 2017;11:1081-93. doi: 10.2147/DDDT.S131936, PMID 28435220.
11. Santosh R, Shambhavi M, Kusuma B. Design, optimization and evaluation of aceclofenac fast dissolving tablets employing starch valerate–a novel super disintegrate. Int J Appl Pharm. 2021;13(4):168-76.
12. Barakat NS, Elbagory IM, Almurshedi AS. Formulation, release characteristics and bioavailability study of oral monolithic matrix tablets containing carbamazepine. AAPS PharmSciTech. 2008;9(3):931-8. doi: 10.1208/s12249-008-9108-y, PMID 18686038.
13. Hadi M, Babu V, Pal N. Formulation and evaluation of sustained release matrix tablets of glimepiride based on the combination of hydrophilic and hydrophobic polymers. J Appl Pharm Sci. 2012;2:101-7.
14. Sun L, Zhang W, Liu X, Sun J. Preparation and evaluation of sustained-release azithromycin tablets *in vitro* and *in vivo*. Asian J Pharm Sci. 2014;9(3):155-61. doi: 10.1016/j.ajps.2014.03.003.
15. Ghosh S, Barik BB. Formulation and *in vitro* evaluation of once daily sustained release formulation of aceclofenac. Trop J Pharm Res. 2010;9(3):265-73. doi: 10.4314/tjpr.v9i3.56288.
16. Kumar S, Gupta A, Mishra C, Singh S. Synthesis, characterization and performance evaluation of aceclofenac-urea cocrystals. Indian J Pharm Sci. 2020;82(5):881-90. doi: 10.36468/pharmaceutical-sciences.717.
17. Galal S, El Massik MA, Abdallah OY, Daabis NA. Study of *in vitro* release characteristics of carbamazepine extended-release semisolid matrix filled capsules based on gelucires. Drug Dev Ind Pharm. 2004;30(8):817-29. doi: 10.1081/ddc-200030497, PMID 15521328.
18. Wadher S, Momin M, Yeole P, Puranik M. Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form. Indian J Pharm Sci. 2006;68(3):387-9. doi: 10.4103/0250-474X.26672.
19. Fini A, Moyano JR, Gines JM, Perez Martinez JI, Rabasco AM. Diclofenac salts, II. Solid dispersions in PEG6000 and gelucire 50/13. Eur J Pharm Biopharm. 2005;60(1):99-111. doi: 10.1016/j.ejpb.2004.11.005, PMID 15848062.
20. Galal S, El-Massik M, Abdallah O, Daabis N. Formulation of fast release glibenclamide liquid and semi-solid matrix filled capsules. Acta Pharm. 2003;53(1):57-64. PMID 14769253.
21. Manjanna KM, Pramod Kumar TM, Shivakumar B. Effect of manufacturing conditions on physico-chemical characteristics and drug release profiles of aceclofenac sodium microbeads. Drug Invent Today. 2009;1:98-107.
22. Hauss DJ. Oral lipid-based formulations. Adv Drug Deliv Rev. 2007;59(7):667-76. doi: 10.1016/j.addr.2007.05.006, PMID 17618704.
23. Mutalik S, Naha A, Usha AN, Ranjith AK, Musmade P, Manoj K. Preparation, *in vitro*, preclinical and clinical evaluations of once daily sustained release tablets of aceclofenac. Arch Pharm Res. 2007;30(2):222-34. doi: 10.1007/BF02977698, PMID 17366745.
24. Kumardarapu B, Sundaramoorthy K, Vetrichelvan T. Formulation and *in vitro* evaluation of gastroretentive floating microspheres of ranitidine hydrochloride. RJPBS. 2011;2:789-801.
25. Chordiya M, Gangurde H, Borkar V. Technologies, optimization and analytical parameters in gastroretentive drug delivery systems. Current Science 2017;112(5):94653. doi: 10.18520/cs/v112/i05/946-953.
26. Gu X, Fediuk DJ, Simons FE, Simons KJ. Evaluation and comparison of five matrix excipients for the controlled release of acrivastine and pseudoephedrine. Drug Dev Ind Pharm. 2004;30(10):1009-17. doi: 10.1081/ddc-200040237, PMID 15595567.
27. Ghosal K, Rajabalaya R, Chakraborty S, Nanda A. Formulation and characterization of both hydrophilic and hydrophobic HPMC-based hydrogels containing diclofenac potassium. Lat Am J Pharm. 2010;29:1137-43.
28. US FDA guidance for industry. Waiver of *in vivo* bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a biopharmaceutics classification system. Rockville, MD: Center for Drug Evaluation and Research (CDER); 2000.
29. Soni T, Nagda C, Gandhi T, Chotai NP. Development of a discriminating method for dissolution of aceclofenac marketed formulations. Dissolution Technol. 2008;15(2):31-5. doi: 10.14227/DT150208P31.
30. Sharma PR, Lewis SA. Design and *in vitro*/*in vivo* evaluation of extended release matrix tablets of nateglinide. J Young Pharm. 2013;5(4):167-72. doi: 10.1016/j.jyp.2013.11.003.
31. Yin L, Qin C, Chen K, Zhu C, Cao H, Zhou J. Gastro-floating tablets of cephalexin: preparation and *in vitro*/*in vivo* evaluation. Int J Pharm. 2013;452(1-2):241-8. doi: 10.1016/j.ijpharm.2013.05.011, PMID 23680730.
32. Musmade P, Subramanian G, Srinivasan KK. High-performance liquid chromatography and pharmacokinetics of aceclofenac in rats. Anal Chim Acta. 2007;585(1):103-9. doi: 10.1016/j.aca.2006.11.080, PMID 17386653.
33. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/ elimination interplay and

development of a biopharmaceutics drug disposition classification system. Pharm Res. 2005;22(1):11-23. doi: 10.1007/s11095-004-9004-4, PMID 15771225.

34. Yuksel N, Karataş A, Ozkan Y, Savaşer A, Ozkan SA, Baykara T. Enhanced bioavailability of piroxicam using gelucire 44/14 and labrasol: *in vitro* and *in vivo* evaluation. Eur J Pharm Biopharm. 2003;56(3):453-9. doi: 10.1016/s0939-6411(03)00142-5, PMID 14602190.
35. Vidyadhara S, Rao P, Prasad J. Formulation and evaluation of propranol hydrochloride oral controlled release matrix tablets. Ind J Pharm Sci. 2004;66(2):188-92.
36. Maderuelo C, Zarzuelo A, Lanao JM. Critical factors in the release of drugs from sustained release hydrophilic matrices. J Control Release. 2011;154(1):2-19. doi: 10.1016/j.jconrel.2011.04.002, PMID 21497624.
37. FDA guidance for industry. Supac-MR–modified release solid oral dosage forms: scale-up and post-approval changes. Rockville, MD: Center for Drug Evaluation and Research (CDER); 1997.
38. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;13(2):123-33. doi: 10.1016/s0928-0987(01)00095-1, PMID 11297896.
39. Venkateswara B, Reddy K, Naga S. Formulation and evaluation of mebeverine hydrochloride sustained release tablets. Asian J Pharm Res. 2015;5(1):58-66.
40. Sood A, Panchagnula R. Design of controlled release delivery systems using a modified pharmacokinetic approach: a case study for drugs having a short elimination half-life and a narrow therapeutic index. Int J Pharm. 2003;261(1-2):27-41. doi: 10.1016/s0378-5173(03)00267-9. PMID 12878393.
41. Sinha S, Ali M, Baboota S, Ahuja A, Kumar A, Ali J. Solid dispersion as an approach for bioavailability enhancement of poorly water-soluble drug ritonavir. AAPS PharmSciTech. 2010;11(2):518-27. doi: 10.1208/s12249-010-9404-1, PMID 20238187.
42. Saanchez Lafuente C, Teresa Faucci MT, Fernandez Arevalo M, Alvarez-Fuentes J, Rabasco AM, Mura P. Development of sustained release matrix tablets of didanosine containing methacrylic and ethylcellulose polymers. Int J Pharm. 2002;234(1-2):213-21. doi: 10.1016/s0378-5173(01)00962-0, PMID 11839452.
43. Khadka P, Ro J, Kim H, Kim I, Kim JT, Kim H. Pharmaceutical particle technologies: Aan approach to improve drug solubility, dissolution and bioavailability. Asian J Pharm Sci. 2014;9(6):304-16. doi: 10.1016/j.ajps.2014.05.005.
44. Mani Ganesh M, Mani Vasudevan M, Kaliappan Kamalakannan K, Arthanari Saravana Kumar A, Mari Vinoba M, Swastika Ganguly, Thangavelu Sivakumar. Anti-inflammatory and analgesic effects of pongamia glabra leaf gall extract. Pharmacologyonline. 2008;1:497-512.
45. Al-Saidan SM, Krishnaiah YS, Patro SS, Satyanaryana V. *In vitro* and *in vivo* evaluation of guar gum matrix tablets for oral controlled release of water-soluble diltiazem hydrochloride. AAPS PharmSciTech. 2005;6(1):E14-21. doi: 10.1208/pt060105. PMID 16353958.
46. Longmei W, Ruihua F, Jinhua G, Yanwei X, Guihua H. Generic sustained release tablets of trimetazidine hydrochloride: preparation and *in vitro–in vivo* correlation studies. Asian J of Pharm Pharm Sci. 2016;1:417-26.
47. Guan J, Zhou L, Pan Y, Han H, Xu H, Pan W. A novel gastro-retentive osmotic pump capsule using asymmetric membrane technology: *in vitro* and *in vivo* evaluation. Pharm Res. 2010;27(1):105-14. doi: 10.1007/s11095-009-9984-1, PMID 19859791.