Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 44 / Number 2 / June 2024 / pp. 108-123

The Impact of Co-crystal Formation on the Stability of Camylofin Dihydrochloride Immediate Release Tablets

Sanika KOLE[1]
ORCID: 0000-0003-3705-1656
Rutuja VİNCHURKAR[1],
ORCID: 0000-0001-8941-5672
Ashwini GAWADE[1],
ORCID: 0000-0003-4171-0319
Ashwin KUCHEKAR[1]*
ORCID: 0000-0003-0496-6059

[1]School of Health Sciences and Technology, Dr. Vishwanath Karad, MIT, WPU, Kothrud, Pune-411038, Maharashtra, India

Corresponding author:
Ashwin KUCHEKAR
School of Health Sciences and Technology, Dr. Vishwanath Karad, MIT, WPU, Kothrud, Pune-411038, Maharashtra, India
Email: ashwinkuchekar@gmail.com
Tel: +91-9420627436

Received date : 24.07.2023
Accepted date : 27.05.2024

DOI: 10.52794/hujpharm.1331991

ABSTRACT

The objective of this study was to select an appropriate co-former and investigate its impact on the formation of co-crystals involving Camylofin dihydrochloride and Fumaric acid. To determine co-former, a molecular docking study was conducted, and among the compounds evaluated, fumaric acid exhibited the highest number of hydrogen bonds formed with Camylofin dihydrochloride and demonstrated a favorable Glide score of -5.21 kcal/mol. The kneading method was employed after optimizing the molar ratio of Camylofin dihydrochloride to Fumaric acid, which was found to be 1:1, 1:2, and 1:3. The resulting Camylofin dihydrochloride co-crystals underwent various analytical techniques, including Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy, Powder X-ray Diffraction, and Differential Scanning Calorimetry. The Camylofin dihydrochloride-Fumaric acid co-crystals, immediate-release tablets were formulated. A result of *ex-vivo* study revealed that Camylofin dihydrochloride-Fumaric acid co-crystals and their immediate release tablets were more potent than plain Camylofin dihydrochloride, with the immediate release tablets being the most potent of all. Stability analysis demonstrated that the final batch F5 remained stable under accelerated ambient stability conditions (40°C±2°C, 75% RH±5%RH) and accelerated stability conditions (25°C±2°C,62% RH±5%RH). The co-crystal technology utilized in this study successfully improved the stability of Camylofin dihydrochloride without altering its chemical composition.

Keywords: Camylofin dihydrochloride, Co-crystals, *Ex-vivo* study, Immediate release tablets, Stability Drug release,

1. Introduction

India, as well as other nations in Latin America and Africa, has access to the anti-spasmodic medication Camylofin dihydrochloride (CAM 2HCl), which is used to alleviate abdominal colic and hasten labor. It is a part of the class of drugs called gastrointestinal sedatives, anticholinergics, and spasmolytics. One of the most effective antispasmodics is CAM 2HCl, which acts directly on the smooth muscle-like papaverine while also having a modest anticholinergic effect similar to atropine [1]. It prevents the activity of the phosphodiesterase enzyme, which raises the level of cyclic AMP and relaxes smooth muscle [2,3].

CAM 2HCl is a non-steroidal anti-inflammatory drug described as 3-methylbutyl 2-[2-(diethyl amino) ethyl amino]-2-phenylacetate dihydrochloride [4]. The molecular weight is 393.4 g/mol and its chemical formula is $C_{19}H_{32}N_2O_2.2HCl$. (Figure 1) depicts its structure. It is a pH-dependent, white crystalline powder having a melting point of 170 °C to 180 °C. CAM 2HCl is a highly moisture-sensitive as well as light-sensitive drug. It is 0.067 mg/mL soluble in water and soluble at acidic pH. It is mainly absorbed in the stomach and has a narrow window for absorption [5].

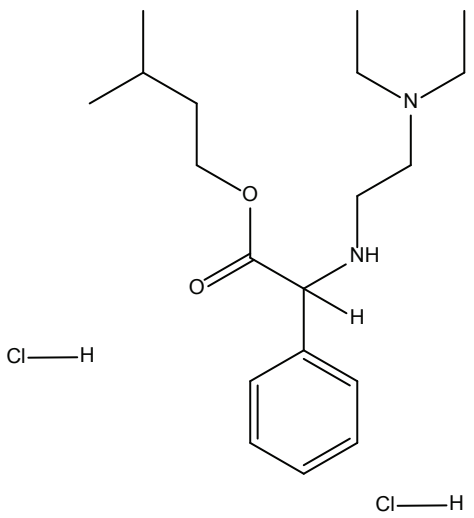


Figure 1. Structure of Camylofin dihydrochloride.

Based on the literature survey CAM 2HCl degrades under different stress conditions like oxidation, acid and base hydrolysis, and photolytic and thermal degradation [6,7]. So, to overcome the problem of degradation and to make the CAM 2HCl more stable, different novel technologies have to be applied. Co-crystal development is a novel technology. Developing co-crystals of CAM 2HCl will enhance its stability, solubility, bioavailability, and dissolution rate. Conversion of co-crystals to solid dosage will help to develop an immediate-release drug delivery system. Active ingredients and co-formers are combined in a compound called a co-crystal in a stoichiometric ratio (1:1, 1:2, 1:3). These co-crystals are joined together by robust at room temperature non-covalent interfaces like π-π packaging, Van Der Waals forces, and hydrogen bonds. A grounding molecule is a co-crystal former. The most crucial element in good co-crystal development is still recognizing and choosing the right co-former [8,9].

2. Materials and Methods

2.1 Materials

CAM 2HCl as a gift sample was acquired from Khandelwal Laboratories (Mumbai, India). Fumaric acid was taken from Analab Fine Chemicals. Additional chemicals Starch, Talc, have been purchased from JRS Pharma, and Magnesium stearate, Microcrystalline cellulose Analab Fine Chemicals (Vadodara, Gujarat).

2.2 Methods

Molecular docking and Selection of co-formers

Molecular docking aims to use computational techniques to anticipate the geometry of the ligand-receptor complex. The two stages required to attain docking are sampling ligand conformations in the receptor-ligand active site and grading those conformations by a scoring function. The experimental binding mode must ideally be reproducible by sampling methods, and it is also given the best score among all created conformations [10,11].

Five co-formers were chosen for the co-crystal preparation according to the literature. A molecular docking study using AutoDock V4.2.6 software [12,13] was performed on selected five co-formers. The examination of intricate receptor-ligand inter-

ISSN: 2458 - 8806

actions, involving the conformational states of both ligands and the formation of hydrogen bonds, was performed through the utilization of Pymol software, UCSF Chimera, and Accelrys Discovery Studio Visualizer software. Further application of the docking score of selected co-formers having the benefit of displaying multidimensional data devoid of statistical analysis was assessed using a radar chart.

Development of Camylofin dihydrochloride-fumaric acid co-crystals by kneading method

The kneading technique is widely used for co-crystal formation. This is an easy and simple method and less time-consuming. The kneading method is one of the solid-assisted grinding methods that involves grinding of API and co-former in an optimal ratio to form co-crystals [14].

Utilizing the kneading approach, Camylofin dihydrochloride and Fumaric acid (CAM 2HCl, FA) co-crystals were formed. Various co-formers in an ideal molar ratio screened the development of CAM 2HCl-FA co-crystals (1:1, 1:2, and 1:3). A blend of the 1:3 ratio of CAM 2HCl and FA was kneaded in a mortar and pestle for five minutes at high speed as shown in (Figure 2) [15]. Formed co-crystals were stored at accelerated ambient, intermediate, and accelerated stability conditions as per ICH recommendations [16].

2.3 Characterization of Camylofin dihydrochloride-fumaric acid co-crystals

The characterization of co-crystals encompasses the analysis of their structural and physical properties by using Fourier transform-infrared spectroscopy (FTIR), Powder X-ray diffraction (PXRD), Scanning Electron Microscopy (SEM), Differential scanning calorimetry (DSC).

Fourier transform-infrared spectroscopy (FTIR)

From 4000 cm^{-1} to 1000 cm^{-1}, FT-IR spectra were recorded on a Precisa XM60 spectrophotometer (Precisa XM60). The DTGS KBr detector was used to gather and examine CAM 2HCl, and CAM 2HCl-FA co-crystals [17].

Powder X-ray diffraction (PXRD)

CAM 2HCl, CAM 2HCl-FA co-crystals powder X-ray diffraction (PXRD) patterns were obtained utilizing a silicon sample holder and an Ultima IV system operating at room temperature. Each specimen was mounted on a motorized goniometer head that allowed it to spin while data was being collected, and the instrument was fitted with a fine-focus X-ray tube. At a 40.0 kV voltage and a 40.0 mA current, samples were irradiated with Cu-K (α) radiations that had been Ni-filtered. Over a 2°/min diffraction angle, the scanning rate varied from 3º to 50º. After obtaining the values for 2Θ, a graph was drawn [18].

Scanning Electron Microscopy (SEM)

To evaluate the surface appearance and structure of CAM 2HCl, co-crystals of CAM 2HCl-FA (JEOL FESEM FEI Nova NanoSEM 450) Scanning Electron Microscopy was used. Samples of CAM 2HCl-FA co-crystals were given a thin film of gold-palladium by a sputter-coated unit, mounted on double-faced adhesive tape, and the surface topography was studied. At 50X, 100X, and 1000X, three different resolutions of images were acquired [19].

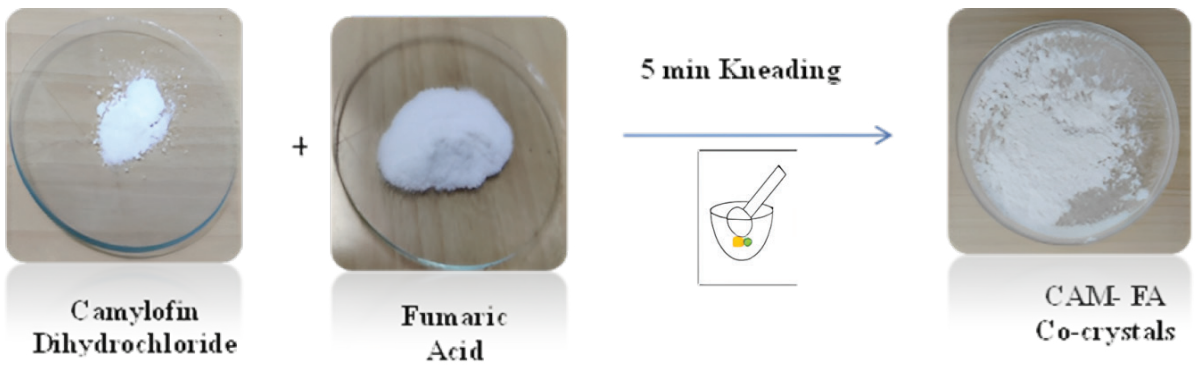


Figure 2. CAM 2HCl-FA Co-crystals by Kneading Method.

Differential scanning calorimetry (DSC)

For the thermal evaluation of the CAM 2HCl and CAM 2HCl-FA co-crystals samples, a differential calorimeter scanning (DSC7020 thermal analysis system HITACHI) system was employed. 1.0 mg of powder samples were loaded in aluminum uncovered crucibles and heated to 400°C at a rate of 10°C/min [20].

Precompression studies for Preliminary batches of immediate-release tablets of CAM 2HCl-FA co-crystal

The pre-compression parameters like bulk density, tapped density, Carr's index, Hausner's ratio, and angle of repose were studied to check the tabletability properties of CAM 2HCl- FA co-crystals immediate release (IR) tablets [21].

Bulk Density (ρB)

A measuring cylinder and the constant mass approach are utilized to determine bulk density. The mass-to-volume ratio of an untouched powder sample of 10g, considering the interparticulate void volume's contribution, is known as the bulk density of a powder [22]. It's measured in g/ml.

Formula: Bulk density (ρB) = M/Vo (1)

Where, M = mass of the powder (weight in g), Vo = Void volume (Untapped Volume in ml).

Tapped density (ρT)

By taping the 100 times measuring cylinder of powder 10 g until the measurement does not alter, the tapped volume is calculated [22]. It is stated in g/ml.

Formula: Tapped density (ρT) = M/Vf (2)

Where, M = mass of the powder (weight in g), Vf= Tapped Volume (bulk volume after tapped in ml)

Hausner's ratio

Hausner's ratio is an auxiliary statistic for forecasting powder flow [22].

Formula: Hausner's ratio = Tapped density (ρT)/ Bulk density (ρB) (3)

The following formula is also used to calculate Hausner's ratio.

Hausner's ratio = Vo/Vf (4)

Compressibility index (Carr's index)

The compressibility index, often known as Carr's index, is a metric used to infer the powder's flow characteristics. Carr's index is determined by measuring the initial volume (V_o) and final volume (V_f) after fully taping a powder sample in a measuring cylinder [22].

Formula: Compressibility index (CI) = $(V_o–V_f)/V_o$ X 100 (5)

As a supplement, the compressibility index can be determined by using the measured data for tapped density (ρT) and bulk density (ρB).

Compressibility index = 100 x {(ρT-ρB)/ρT} (6)

Angle of repose

The three-dimensional angle is called the angle of repose that a cone-shaped pile of material created by various techniques assumes (relative to the horizontal base). The process uses a fixed height. In the fixed funnel, the graph paper was spread out on a horizontal, level surface, and a funnel was used to attach its end at a predetermined height (2 cm) from above. The mixture of 10 g powder was gently poured through the funnel (size 100mm) until the top of the conical pile barely touched the funnel's tip [23]. Where r is the radius of the conical pile's base.

Formula: Angle of repose (θ) = $\tan^{-1}$ (h/r) (5)

Where, h = powder pile's height, r = radius of pile

2.4. Development and Preliminary batches of immediate-release tablets of Camylofin CAM 2HCl-FA

The 10 preliminary batches (F1 to F10) were taken for the preparation of CAM-FA co-crystals immediate release tablets. Pre-compression parameters like bulk and tapped density, Hausner's ratio, Carr's index, and angle of repose were used for the evaluation of the final batch selection. By adjusting the super disintegrant, filler, glidant, and binder concentrations, preliminary batches were made. Table 1 below lists the preliminary batches mixture. Starch as a disintegrant in 9% concentration made a major impact on evaluation parameters. So, the F5 batch was further selected for the preparation of tablets by direct compression method [24].

ISSN: 2458 - 8806

Table 1. Preliminary batches for immediate-release tablets of CAM 2HCl – FA co-crystals.

Batches	CAM-FA (%)	MCC (%)	Starch (%)	Talc (%)	Magnesium Stearate (%)
F1	16	60	15	6	3
F2	16	59.5	15	6	3.5
F3	16	58.5	15	6	4.5
F4	16	64	5	10	5
F5	16	60	9	10	5
F6	16	59	10	10	5
F7	16	49	20	10	5
F8	16	58	15	6	5
F9	16	56	15	8	5
F10	16	50	19	10	5

(*F1-F10: It is assigned code for the formulation of preliminary batches)

2.5. Direct Compression Method for Preparation of CAM 2HCl-FA co-crystals Immediate Release Tablets

Microcrystalline Cellulose as filler was combined with precisely weighed co-crystals of CAM 2HCl-FA. This combination was then prepared for direct compression by adding variable amounts of Starch as disintegrant and binder, Talc as a glidant, and Magnesium stearate as a lubricant as other excipients [25]. The co-crystal was weighed accurately and combined with the other components in a mortar and pestle for 15 minutes and then compressed the blend with a 6 mm punch tablet compression mini press DII machine by Lab India. Direct compression method for preparation of IR Tablets as shown in (Figure 3) [26].

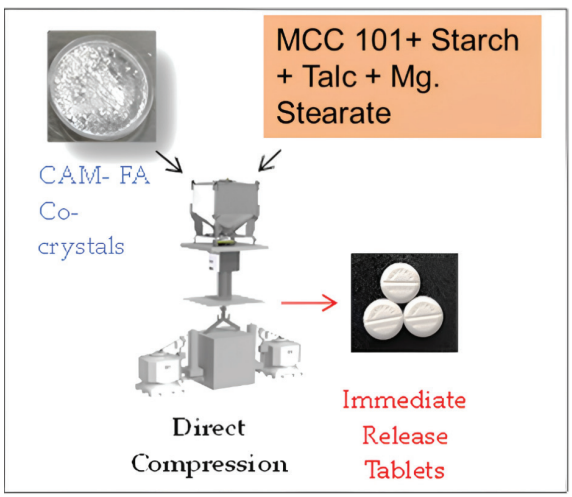


Figure 3. CAM 2HCl-FA co-crystals IR tablets by Direct Compression Method.

2.6. Evaluation of post-compression parameters

Thickness and weight variation

Evaluation of thickness and weight variation was carried out by selecting twenty tablets randomly from the formulation. By using a digital Vernier caliper, the thickness of the tablets was measured. The thickness of the tablet was assessed [27] Results are displayed as mean and standard deviation (SD). An electronic balance was used to calculate the average weight. Next, for the weight variations, individual weights were analyzed with the average weight [28].

$$PD = [(W\ avg - W\ initial)/ (W\ avg)] \times 100 \quad (6)$$

Where, PD = Percentage deviation, Wavg = Tablet's Average weight, W initial = Tablet's individual weight.

Hardness

By a Monsanto hardness tester, hardness was calculated as the amount of force needed to shatter the tablet. A batch of twenty randomly chosen tablets is tested for hardness. The Cam 2HCl-FA co-crystals IR tablet was positioned vertically while supporting the anvil of the hardness tester. The pointer was then set at the scale's position of zero by turning the screw in a forward manner. The screw was then turned until the tester broke the tablet. The tablet breaking indicates the hardness of the material. Tablet hardness is expressed in kg/cm^2 [29].

Friability test

A friability tester was used to measure the friability of CAM 2HCl-FA co-crystals IR tablets (Roche Friability Test Apparatus). The drum contained twenty tablets that had been carefully weighed [30]. Withdraw the tablets after 4 minutes of drum rotation (100 turns at 25 rpm). The tablets should be thoroughly cleaned and precisely weighed. For the majority of products, a mean maximum weight loss from the three samples of not more than 1.0 percent is regarded as acceptable [31]

Percent friability = (Initial weight-final weight)/Initial weight x 100 (7)

In-vitro disintegration time

With the aid of a disintegration apparatus (Lab India), a disintegration test is conducted. Each of the six tubes in the basket contained a single dose unit. The temperature is kept at 37°±2 °C and the immersion fluid is distilled water. The device was run until each unit dosage emerged from the basket. The number of seconds it took for a tablet to completely disintegrate without a residue still in the device was measured as the mean SD [32].

Drug content

Six tablets were weighed and crushed in a mortar and pestle to get a powder for testing the drug content. Using a UV spectrophotometer, a powder containing 25 mg of CAM 2HCl was weighed, diluted in 100 ml of methanol, and then tested for drug concentration at 259 nm [6][27].

In-vitro dissolution study

Utilizing a USP II dissolution test apparatus (Paddle Apparatus), *in vitro* dissolution experiments for CAM 2HCl and CAM 2HCl-FA co-crystals IR tablets were performed. Applying a dissolution apparatus with 900 ml of phosphate buffer acidic pH 1.2 at a temperature of 37°C, the dissolution study was conducted. The paddles were initiated at the present rate as soon as the tablets were dropped into the medium (50 rpm). 5 ml of the sample is withheld over the indicated time (5, 15, 30, 45, and 60 min). At 259 nm, those samples are examined utilizing a UV spectrophotometer. To determine drug release, several calculations are made. The plotted and tested drug release data had zero order kinetic study [33, 34].

Stability studies

For the stability study, CAM 2HCl, CAM 2HCl-FA co-crystals, and CAM 2HCl-FA co-crystals IR tablets were packed in an amber-colored bottle and again covered with silver foil in three different stability conditions in a triple stability chamber (Thermolab). The sample was subjected to stability conditions at 40°C± 2%C/ 75% RH±5%RH (accelerated ambient zone), 30°C±2°C/ 65% RH±5% RH (intermediate zone), and 25°C±2°C/ 62% RH±5%RH (accelerated zone) then evaluated for 3, 6, 9 and 12 months [35]. They were packaged in HDPE pouches and kept in a stability chamber for the period specified by the ICH recommendations for expedited research [36,37].

Dose-Response Curve of Acetylcholine alone and in the Presence of Different Concentrations of CAM 2HCl, CAM 2HCl-FA co-crystals, and CAM 2HCl-FA co-crystals IR tablets

The concentration-response relationships of CAM 2HCl, CAM 2HCl-FA co-crystals, and CAM 2HCl-FA co-crystals IR tablets in chicken ileum were evaluated using an isolated organ bath test as a pharmacological screening technique [38]. An inner organ bath was filled with Tyrode solution, the oxygen supply was constant, and the temperature was maintained. The desired magnification value was adjusted. A piece of the ileum about 2-3 cm long was taken, and the mesentery was removed. Connective tissue adhered with blunt forceps. One end of the tissue

ISSN: 2458 - 8806

was tied to the holder (the aeration tube) and another to the isotonic frontal writing lever without closing the lumen. The tissue in an organ bath containing Tyrode solution was mounted at 37 ± 0.5℃ and bubbled with oxygen. A tension of 0.5 g was placed on the lever, allowing the tissue to stabilize for 30 minutes. During this period, the PSS was changed every 10 minutes. Once the tissue stabilized, a graded dose of Acetylcholine (10µg/ml) was added to obtain a contractile response [39]. The solution responses of Acetylcholine, CAM 2HCl, CAM 2HCl co-crystals, and CAM 2HCl co-crystals IR tablets were recorded until the ceiling effect was observed.

3. Results and Discussion

Molecular Docking Study

CAM 2HCl consists of one aromatic ring, an amine group, and an ester group. CAM 2HCl has one hydrogen bond donor as well as four hydrogen bond acceptors due to ester and amine groups and significant conformational stability; so, the formation of co-crystals with co-formers can be possible. FA as a co-former was selected for CAM 2HCl-FA co-crystal formation based on interaction type, hydrogen bonding, compatibility, and docking score, as shown in (Figure 4).

Camylofin dihydrochloride-Fumaric Acid

The ideal co-former was chosen using the glide (docking) score and hydrogen bonding parameters. The strongest interaction between CAM 2HCl and co-formers can be seen at the lowest Glide score value. Higher binding affinity is aided by the highest hydrogen bond formation. Table 2 below provides a summary of the information. The application of a Radar chart to evaluate the docking score of selected co-formers is represented in (Figure 5). FA showed the maximum amount of hydrogen bonds formed with CAM 2HCl and the lowest Glide score of -5.21 kcal/mol [15]. FA had the lowest docking score when compared visually using Radar charts and molecular modeling. The interaction between the API and co-former is defined by van der Waals forces and electrostatic energy. Therefore, the FA Co-former was chosen for further investigation based on the number of hydrogen bonds and lowest glide score [12].

By kneading method and FA as a co-former; CAM 2HCl-FA co-crystals in the ratio of 1:3 were prepared as they showed good flowability and more stability. By comparing the pure drug with co-crystals, a preliminary evaluation of co-crystal production was carried out.

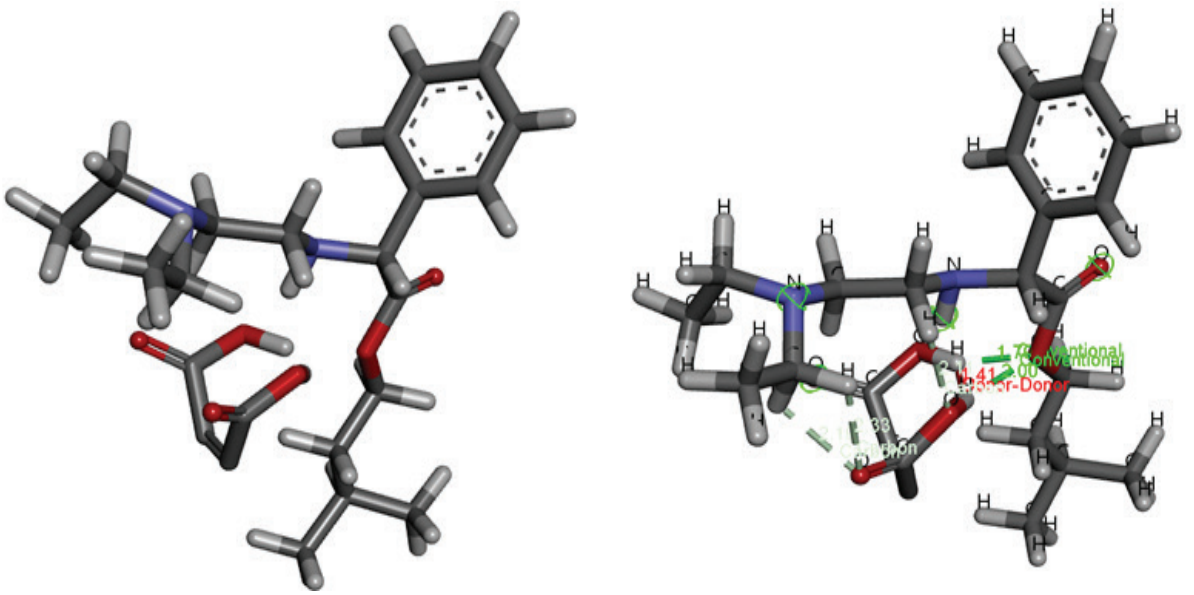
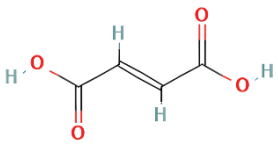
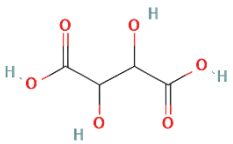
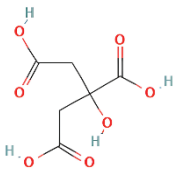
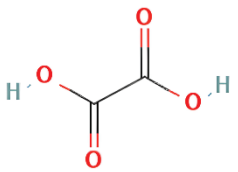
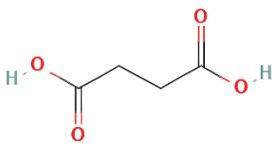


Figure 4. Molecular docking of CAM 2HCl – FA - 3D model of the interactions and the type of interaction.

Table 2. Molecular modeling of co-formers for the synthesis of co-crystal.

Sr. No.	Name	Structure	Glide Score (kcal/mol)	Hydrogen bonding	Distance between atoms
01	Fumaric Acid		-5.21	OH (Carbon) OH (Conventional) Hydrogen bonding	2.11 Å 1.70 Å 1.41 Å
02	Tartaric Acid		-0.56	OH (Conventional) Hydrogen bonding	1.82 Å 1.48 Å
03	Citric Acid		+1.14	OH (Conventional)	2.85 Å
04	Oxalic Acid		-4.67	OH (Conventional) OH (Carbon)	1.81 Å 2.25 Å
05	Succinic Acid		-5.15	OH (Carbon) OH (Conventional) Hydrogen bonding	1.99 Å 1.68 Å 1.37 Å

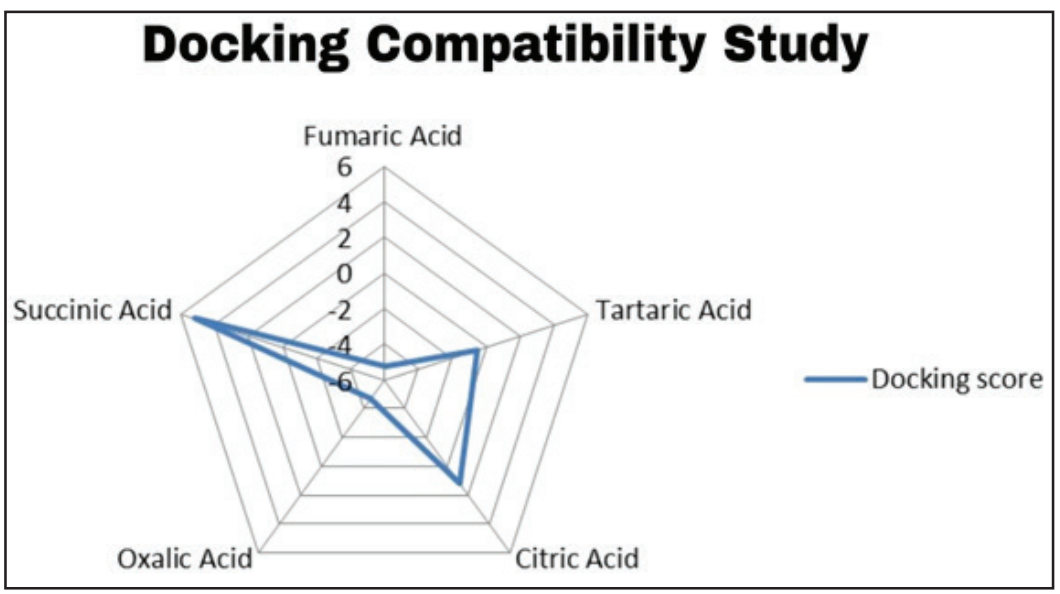


Figure 5. Application of Radar chart to evaluate the Docking Score of Selected Co-formers.

ISSN: 2458 - 8806

Fourier transform-infrared spectroscopy (FT-IR)

To identify and characterize, the IR spectrum of the drug was recorded and displayed in (Figure 6). The primary bands were recognized, and modifications related to those bands were noted. Pure CAM 2HCl's IR spectra reveal the existence of the recognizable peaks, which were measured at 1650 cm^{-1} for the carbonyl group of carboxylic acid i.e. c=o stretching, 3439 cm^{-1} for the formed primary amine i.e. NH stretching, 3350 cm^{-1} for formed secondary amine NH stretching and 1650 cm^{-1} NH bending. The spectrum and the data published were in close conformity. The FTIR revealed peaks in the CAM 2HCl and CAM 2HCl-FA co-crystals had shifted and changed in intensity. By reducing the intensity of the OH peak at 1050 cm^{-1} OH bending and 3590 cm^{-1} OH stretching, hydrogen bonding in the co-crystals was discovered. Hydrogen is implicated in hydrogen bonding as evidenced by a reduction in the frequency of the NH stretching at 3349 cm^{-1} and bending at 1646 cm^{-1}. The amount of hydrogen bonding can be calculated from the frequency reduction and the relative band widening. Reduced frequency is a function of the hydrogen bond's degree and strength. The production of fresh hydrogen has been demonstrated by significant changes in the region of the covalent link between the amine (N-H) stretch and C-C [36].

Powder X-ray diffraction (PXRD)

CAM 2HCl's X-ray diffractograms in (Figure 7) revealed characteristics peaks at 2Ɵ values of 3.8 °, 9.6 °, 11.2 °, 18.4 °, 20.6 ° with peak intensities 12450, 5380, 4744, 4288, 4538 suggesting the drug's crystalline structure. The co-crystals of CAM 2HCl-FA exhibit strong and intense peaks at 5827, 1885, 8987, 10454, and 6735 at 2Ɵ values of 3.6 °, 8.9 °, 22.87 °, 28.6 °, 29.62 ° indicating the existence of the highest crystallinity. Alteration in crystal habit or formation of amorphous shape may be the cause of decreased intensities and the shifting of fewer peaks. Tablets containing CAM 2HCl-FA co-crystals may dissolve more quickly than tablets containing pure medication due to reduced crystalline properties [41].

Scanning Electron Microscopy (SEM)

SEM images of CAM 2HCl resulting at 50 and 1000 magnifications are represented in (Figure 8). The SEM images revealed that the structural morphology of the pure drug is smooth surfaces. SEM pictures of CAM 2HCl-FA co-crystals at 100 and 1000 magnifications revealed the morphological distinctions between the pure materials and their co-crystallization product are visible in the SEM images, which suggests that a novel compound was formed by layer-by-layer deposition. CAM 2HCl-FA co-crystals were observed as amorphous blocked particles with irregular shapes and edges having rough surfaces [42].

Differential Scanning Calorimetry (DSC)

CAM 2HCl showed a sharp endothermic peak at 175.8°C, as shown in (Figure 9A). For CAM 2HCl-FA co-crystals, a small endothermic peak around 133°C was observed, while a sharp endothermic peak is present at 241.7°C and 248.6°C, accredited to the melting point of the compound, as shown in (Figure 9B). An endothermic peak at 48°C, 224.9°C, and 245.6°C represented the melting point of CAM 2HCl-FA co-crystals IR tablets, as shown in (Figure 9C). It indicates the formation of a new phase in DSC thermograms, which altered melting behavior and supports an assumption of CAM 2HCl-FA co-crystal formation, as shown in (Figure 9) [38].

Development and preliminary batches of Immediate release tablets of Camylofin Dihydrochloride- Fumaric acid co-crystals

Ten preliminary batches of immediate-release tablets of CAM 2HCl-FA co-crystal were prepared to select the concentration of starch as disintegrants and binder, MCC as filler, magnesium stearate as a lubricant, and talc as a glidant; the trial batches (F1 to F10) were assessed, and the final selected F5 batch was evaluated for the post-compression parameters and dissolution study.

Preformulation Studies

The results for precompression and post-compression parameters are accessible below in Table 3. The F5 batch containing 9% of each disintegrant and binder showed excellent results. The bulk and flow properties were better for the F5 batch as compared to others. All the batches exhibited acceptable weight variation, hardness, friability, and thickness.

Post-compression studies

The parameters of the final F5 formulation were assessed as hardness, friability, thickness, weight vari-

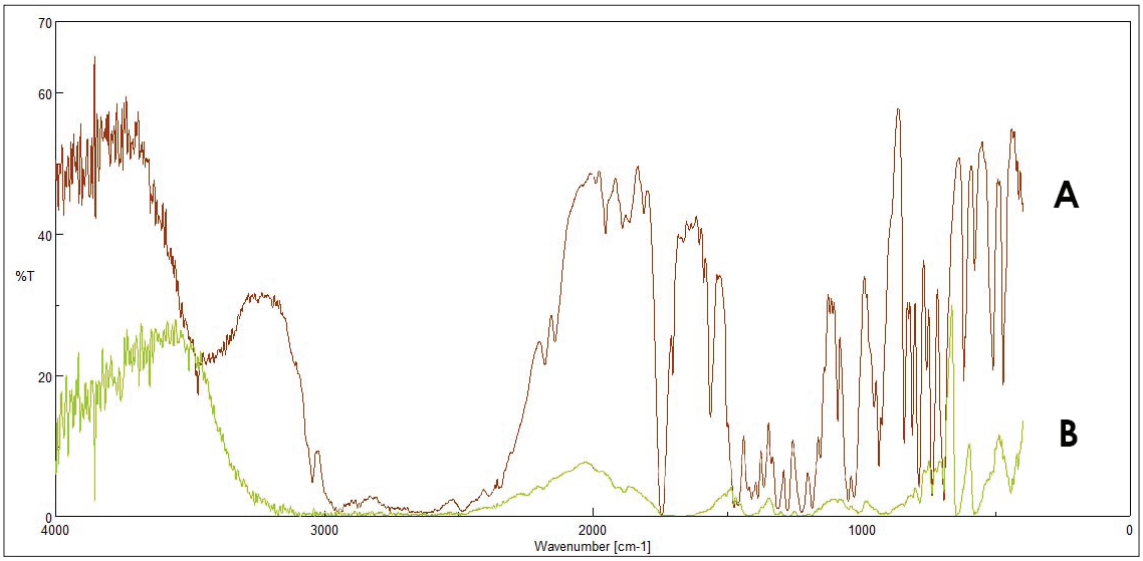


Figure 6. (A) Fourier Transform-Infrared Spectroscopy of CAM 2HCl (B) Fourier Transform-Infrared Spectroscopy Overlay of CAM 2HCl, CAM 2HCl-FA Co-crystals.

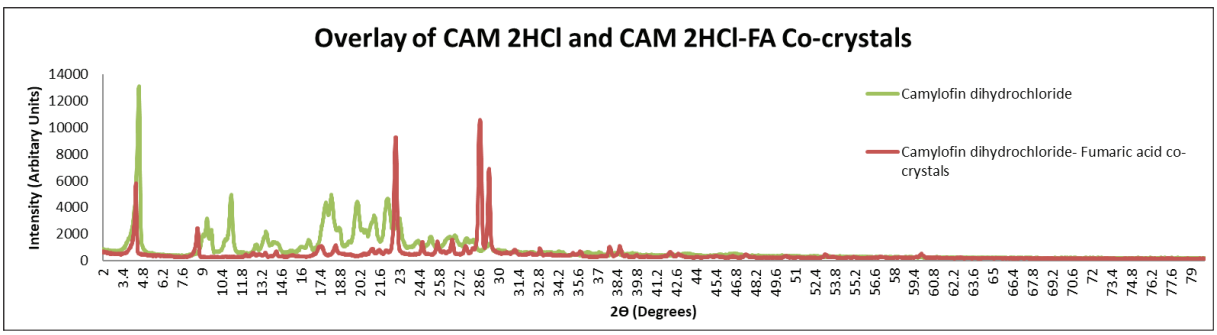


Figure 7. Powder X-ray Diffraction Overlay of CAM 2HCl, CAM 2HCl-FA co-crystals.

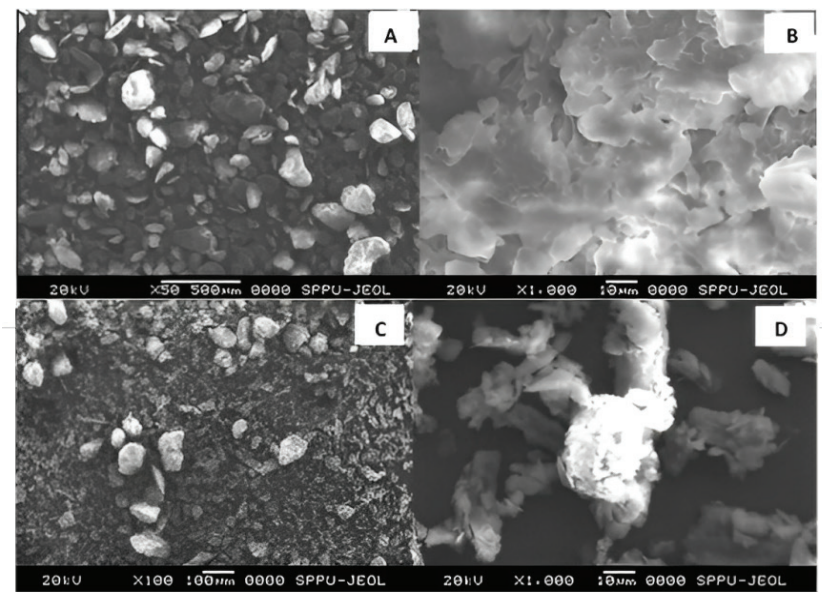


Figure 8. Scanning Electron Microscopy of CAM 2HCL at magnification (A)50, (B)1000 and CAM 2HCl-FA co-crystals (C)100, (D)1000.

ISSN: 2458 - 8806

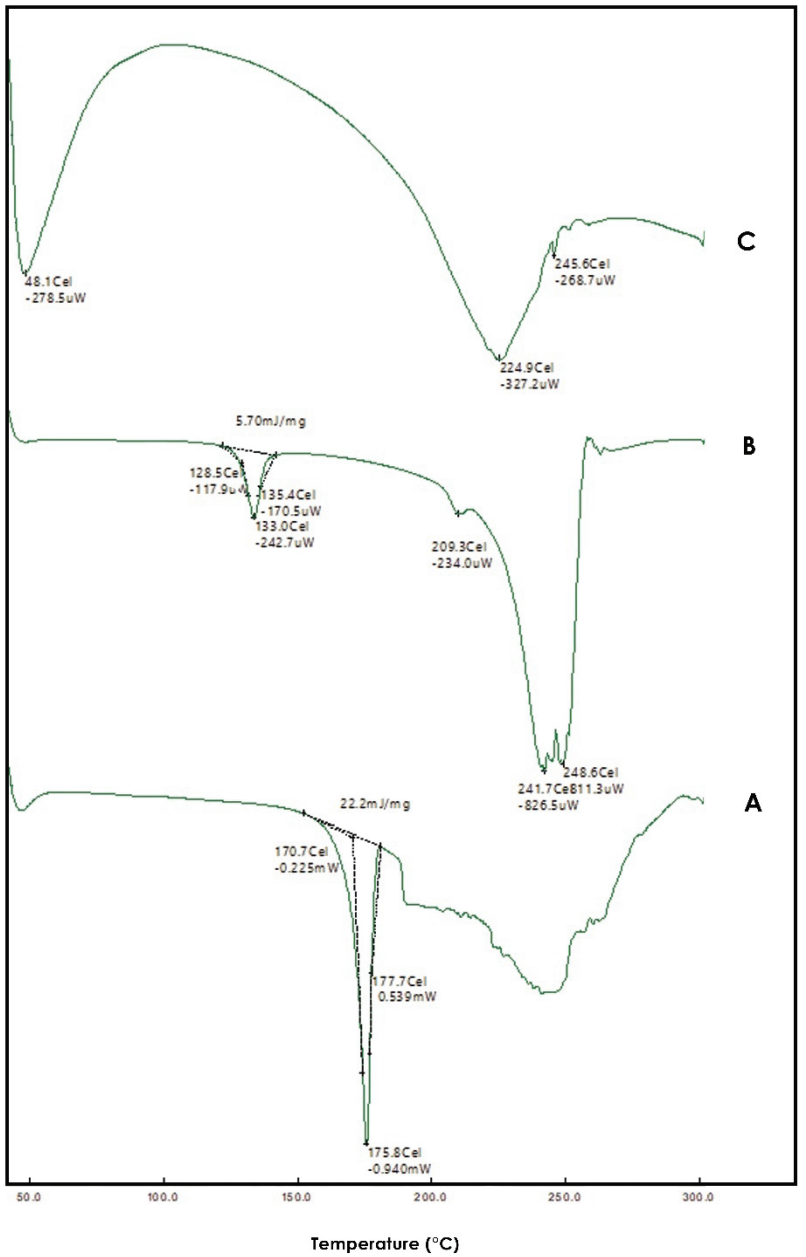


Figure 9. Differential Scanning Calorimetry thermograms (A) CAM 2HCl (B) CAM 2HCl-FA co-crystals (C) CAM 2HCl-FA co-crystals IR tablets.

ation, *in-vitro* disintegrating time, drug content, and *in-vitro* dissolution rate.

The hardness of final tablet formulations (F5) was found to be 8.28 Kg/cm^2 which was reasonably good for a 2.8 mm tablet. The Friability of final tablet formulations (F5) was 0.03% which was found to be satisfactory within the limit that is not more than 1.0%. The thickness of the final tablet formulation (F5) was found to be 2.8 mm. Weight variation of the final tablet formulation (F5) was found to be 2.8% which is satisfactory because as per IP weight, variation for 250 mg tablets is ±5%.

The final tablet formulation (F5) was shown to disintegrate *in vitro* in 3.03 minutes. The disintegration time of tablets is the most crucial element that needs to be adjusted in the creation of immediate-release tablets. On the *in-vitro* disintegration of CAM 2HCl-FA disintegrating tablet formulations, the impact of

Table 3. Pre-compression parameters of CAM 2HCl-FA Co-crystal IR Tablets.

Formulation Code	The angle of repose (deg)	Bulk density (g/cc)	Tapped Density (g/cc)	% Compressibility	Hausner's Ratio
F1	46.3	0.59	1	41	1.6
F2	46.3	0.42	0.57	26	1.35
F3	41.02	0.41	0.54	24	0.31
F4	40.03	0.5	0.58	13.7	1.16
F5	24.7	0.5	0.58	13.7	1.16
F6	38.65	0.43	0.58	25.86	1.34
F7	46	0.52	0.71	36.53	1.3
F8	37.9	0.46	0.57	19.2	1.23
F9	37.9	0.47	0.58	18.9	1.23
F10	39	0.5	0.6	16.6	1.2

(*F1-F10: It is assigned code for the formulation of preliminary batches)

filler (Microcrystalline cellulose) and (Starch) as both a binder and disintegrant (9%), respectively, were assessed. The final formulation (F5) batch was deemed the most optimal formulation for stability experiments since it had the lowest disintegration time of 3.03 min. The drug content of the CAM 2HCl co-crystals immediate-release tablets (F5) batch was found to be 83.96 %. This indicates a high level of consistency in the formulation process, ensuring that each tablet delivers a substantial amount of the active pharmaceutical ingredient. [44].

Evaluation of in vitro dissolution

The purpose of the study was to assess developed preparations that behaved during *in vitro* dissolution. The dissolution profile of CAM 2HCl indicates that the API has a good dissolution rate in acidic pH, with (32.18%) dissolved at 30 minutes, compared to (29.18%) for CAM 2HCl-FA co-crystals IR tablets. After 45 minutes, (57.21%) of CAM 2HCl had dissolved, whereas (62.77%) of CAM 2HCl-FA co-crystals IR tablets had dissolved, demonstrating that the co-crystals IR tablets have a better dissolution rate in acidic pH. (Figure 10) shows the drug release 60 minutes after it was considered. The greatest drug release was seen in the F5 batch (95.5%). Lower binder and disintegrant concentrations influence it. The F5 batch with 9% starch showed the highest dissolving and shortest disintegration time based on all evaluation criteria [28].

Stability Studies

The optimized formulation F5 was subjected to a stability study following ICH guidelines. Parameters such as hardness, friability, thickness, weight variation, and disintegration time were evaluated.

CAM 2HCl, formulated CAM 2HCl-FA co-crystals, and CAM 2HCl-FA co-crystals IR tablets were packed in three various packing circumstances and exposed to expedited ambient, intermediate, and accelerated refrigerated stability conditions for 3, 6, 9, and 12 months, as shown in Table 4. Moisture content was observed in CAM 2HCl at stability conditions at (40°C±2°C, 75% RH±5%RH) and (30°C±2°C, 65% RH±5%RH). CAM 2HCl was found to be more stable at (25°C±2°C, 62±5% RH). The stability of the formulated CAM 2HCl-FA co-crystals was found to be stable at (40°C± 2%C, 75% RH±5%RH) and (25°C±2°C, 62±5% RH) conditions. As a result, co-crystal stability was improved over that of pure drugs. The optimized formulation of CAM 2HCl-FA co-crystals immediate release tablets (F5 batch) was found to be stable at (40°C± 2°C, 75% RH±5%RH) and (25°C±2°C, 62±5% RH) conditions. This illustrates how co-crystals might increase the stability of drugs [45].

ISSN: 2458 - 8806

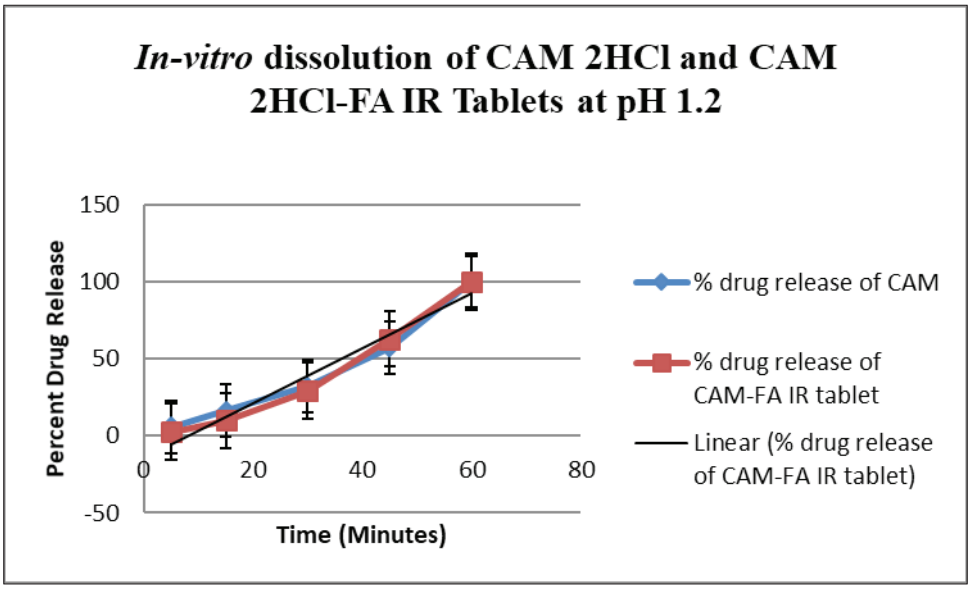


Figure 10. *In-vitro* Dissolution Profile for CAM 2HCl and CAM 2HCl-FA Co-crystals IR Tablets at pH 1.2.

Table 4. Stability studies of CAM 2HCl, CAM 2HCl-FA Co-crystals, CAM 2HCl-FA Co-crystals IR Tablet.

Formulation parameter	25±2°/62±5% RH	40±2°/75±5% RH	30±2°/65±5% RH
Hardness (kg/cm^2)	8.28	8.11	7.40
Friability (%)	0.03	0.05	0.08
Tablet Thickness (mm)	2.8	2.8	3.4
Weight variation (%)	2.8	2.9	3.8
Disintegration time(sec)	3.02	3.01	2.92

Effect of CAM 2HCl, CAM 2HCl Co-crystals, and CAM 2HCl Co-crystals IR tablets on ex-vivo study using Chicken ileum

Acetylcholine induces contractions via activating muscarinic M3 receptors on ileum smooth muscle cells. In this study, plain CAM 2HCl, CAM 2HCl-FA co-crystals, and CAM 2HCl-FA co-crystals IR tablet showed concentration-dependent inhibition of the contraction induced by acetylcholine, which was attributed to the blockade of M3 receptors of smooth muscles of chicken ileum. The two formulations, CAM 2HCl-FA co-crystals, and CAM 2HCl-FA co-crystals IR tablets were found to be more potent than plain CAM 2HCl and CAM 2HCl-FA co-crystals IR tablets were found to be most potent amongst all of the drugs. These results indicated the increased solubility, bioavailability, and pharmacodynamic activity of CAM 2HCl-FA co-crystals and CAM 2HCl-FA co-crystals IR tablet as compared to plain CAM 2HCl.

4. Conclusions

The stability, solubility, and dissolution rate were improved by successfully preparing, screening, and optimizing the CAM 2HCl-FA co-crystals and CAM 2HCl-FA co-crystals IR tablets. CAM 2HCl-FA co-crystals and CAM 2HCl-FA co-crystals IR tablet were successfully developed in the current experiment. The CAM 2HCl needed to be stabilized which was the study's main obstacle. CAM 2HCl has low solubility in water and a pH-dependent solubility that is high in acidic media. CAM 2HCl's physicochemical and pharmacokinetic properties were enhanced by CAM 2HCl-FA Co-crystals. This type of deliv-

ery system will aid in enhancing the drug content, disintegration, and absorption of CAM 2HCl, which will improve its *ex-vivo* antispasmodic activity as well as create a future platform for targeted drug delivery systems. This would improve effectiveness and boost patient compliance. Additionally, it is anticipated that the suggested investigation would be economical. Future research will focus on scale-up issues and evaluation methods to improve the status of co-crystals in the pharmaceutical and intellectual contexts.

Acknowledgments

This study was supported by the School of Health Sciences and Technology, Dr. Vishwanath Karad, MIT, WPU, Kothrud, Pune-411038, Maharashtra, India. The author would like to thank Dr. Ashwin Kuchekar and Dr. Ashwini Gawade for their constant support and guidance.

Conflict of interest

The author/editor has no conflicts of interest, financial or otherwise, to declare.

Statement of Contribution of Researchers

Literature searches, designed and performed the study, wrote the first draft of the manuscript- S.K.; Review, editing, and supervision- A. K.; statistical analysis- A.G., R.V. All authors read and approved the final manuscript.

References

1. Palshetkar N, Purandare A, Mehta H, et al. Effectiveness and Safety of Camylofin in Augmentation of Labor: A Systematic Review and Meta-Analysis. *IJOGR*. 2020; 70:425–439. https://doi.org/10.1007/s13224-020-01343-3
2. Mayadeo N, Gangadhar A, Das S. Camylofin in the management of prolonged labor: a review of evidence. *Int J Reprod Contraception, Obstet Gynecol*. 2017; 6:776. https://doi.org/10.18203/2320-1770.ijrcog20170545
3. Sarbhjit K, Bajwa SK, Parmjit K, et al. To compare the effect of camylofin dihydrochloride (Anafortin) with combination of valethamate bromide (epidosin) and hyoscine butyl-n-bormide (buscopan) on cervical dilation. *J Clin Diagnostic Res*. 2013; 7:1897–1899. https://doi.org/10.7860/JCDR/2013/6231.3345
4. Singh RKR, Rathnam M V., Singh SJ, et al. Determination of Camylofin Dihydrochloride and Nimesulide in Pharmaceutical Preparation by Gas Chromatography. *Am J Anal Chem*. 2011; 2:944-952 https://doi.org/10.4236/ajac.2011.28110
5. Kokilambigai KS, Lakshmi KS. Camylofin dihydrocloride - A review of analytical methods. *Int J Pharm and Pharm Sci*. 2014; 6:36–37
6. Singh RKR, Rathnam M V., Singh SJ, et al. Stability Indicating Method for Simultaneous RP HPLC Determination of Camylofin Dihydrochloride and Nimesulide in Pharmaceutical Preparations. *ISRN Anal Chem*. 2012. https://doi.org/10.5402/2012/586415
7. Singh RR, Rathnam M V., Singh SJ, et al. A stability indicating GC-FID method for camylofin dihydrochloride and diclofenac potassium in pharmaceutical preparation. *Int J Pharm Pharm Sci*. 2012; 4(1):317-324
8. Guo M, Sun X, Chen J, et al. Pharmaceutical cocrystals: A review of preparations, physicochemical properties and applications. *Acta Pharm Sin B*. 2021; 11:2537–2564. https://doi.org/ 10.1016/j.apsb.2021.03.030
9. Chauhan V, Mardia R, Patel M, et al. Technical and Formulation Aspects of Pharmaceutical Co-Crystallization: A Systematic Review. *ChemistrySelect*. 2022; 37:e202202588. https://doi.org/10.1002/slct.202202588
10. Shete AS, Shah V V., Bhosale PA, et al. Fenofibrate-Nicotinamide Cocrystals: Molecular Docking Studies and Evaluation in Tablet Dosage Form. *Indian J Pharm Sci*. 2022; 84(3):560-568. https://doi.org/10.36468/pharmaceutical-sciences.950
11. Mangesh B, Sumedh P, Sumedh M, et al. Scientific Coformer Screening, Preparation and Evaluation of Fenofibrate Tartaric Acid Cocrystal. *J Drug Deliv Ther*. 2019; 9:406–410. https://doi.org/10.22270/jddt.v9i4.3199
12. Dhibar M, Chakraborty S, Basak S, et al. Critical Analysis and Optimization of Stoichiometric Ratio of Drug-Coformer on Cocrystal Design: Molecular Docking, In Vitro and In Vivo Assessment. *Pharmaceuticals*. 2023; 16(2): 284. https://doi.org/10.3390/ph16020284
13. Trott oleg, Arthur J. Olson. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *J Comput Chem*. 2010; 31(2):455–461. https://doi.org/10.1002/jcc.21334
14. Kumar Bandaru R, Rout SR, Kenguva G, et al. Recent Advances in Pharmaceutical Cocrystals: From Bench to Market. *Front pharmacol*. 2021;12:780582. https://doi.org/10.3389/fphar.2021.780582
15. Gawade A, Kuchekar A, Boldhane S, et al. Improvement of Physicochemical and Solubility of Dipyridamole by Cocrystallization Technology. *J Drug Deliv Ther*. 2021; 11:43–48. https://doi.org/10.22270/jddt.v11i1-s.4696

ISSN: 2458 - 8806

16. Industry G for. Q1A(R2) Stability Testing of New Drug Substances and Products. Ich

17. Anand R, Nanda A. Formulation and Evaluation of Cocrystals of a Bcs Class Ii Drug Using Glycine As Coformer. Int *J Appl Pharm*. 2022; 14:68–76. https://doi.org/10.22159/ijap.2022v14i6.46090

18. Ouyang J, Xing X, Zhou L, et al. Cocrystal design of vanillin with amide drugs: Crystal structure determination, solubility enhancement, DFT calculation. *Chem Eng Res Des*. 2022; 183:170–180. https://doi.org/10.1016/j.cherd.2022.05.009

19. Kara DD, Rathnanand M. Cocrystals and Drug–Drug Cocrystals of Anticancer Drugs: A Perception towards Screening Techniques, Preparation, and Enhancement of Drug Properties. *Crystals*. 2022; 12(10):1337. https://doi.org/10.3390/cryst12101337

20. Garbacz P, Wesolowski M. Benzodiazepines co-crystals screening using FTIR and Raman spectroscopy supported by differential scanning calorimetry. *Spectrochim Acta - Part A Mol Biomol Spectrosc*. 2020; 234:118242. https://doi.org/10.1016/j.saa.2020.118242

21. Madhuri G, Nagaraju R, Killari KN. Enhancement of the physicochemical properties of poorly soluble lovastatin by co-crystallization techniques - In vivo studies. *Indian J Pharm Sci*. 2020; 82:249–259. https://doi.org/10.36468/pharmaceutical-scien-ces.645

22. USP. Chapter 616 Bulk Density and Tapped Density of Powders. United States Pharmacopeial Conv

23. The United States Pharmacopeial Convention. Basic Methods for Angle of Repose - 1174 - Powder Flow. The United States of Pharmacopoeia

24. Jain S, Bansal M, Sharma A. Formulation And In-Vitro Evaluation Of Cocrystals Of Pantoprazole Sodium For Immediate Release. *Int J Pharm Biol Sci Arch*. 2021; 9(1). https://doi.org/10.32553/ijpba.v9i1.176

25. Dhahir RK, Al-Kotaji MYASAR. Formulation of orally disintegrating tablets of cinnarizine by using direct compression method. *Int J Appl Pharm*. 2019; 11:117–123. https://doi.org/10.22159/ijap.2019v11i1.29599

26. Trivedi HR, Borkar DS, Puranik PK. Experimental design approach for development of cocrystals and immediate release cocrystal tablet of atorvastatin calcium for enhancement of solubility and dissolution. *J Res Pharm*. 2020; 24(5):720-737. https://doi.org/10.35333/jrp.2020.226

27. Al-Dulaimi A, Al-kotaji M, Abachi F. Paracetamol/ naproxen co-crystals; a simple way for improvement of flowability, tableting and dissolution properties. *Iraqi J Pharm*. 2021; 18:1–19. https://doi.org/10.33899/iphr.2021.168798

28. Panzade P, Shendarkar G, Shaikh S, et al. Pharmaceutical Cocrystal of Piroxicam: Design, formulation and evaluation. *Adv Pharm Bull*. 2017; 7:399–408. https://doi.org/10.15171/apb.2017.048

29. Alam K, Shareef H, Bushra R. Formulation development & evaluation of caffeine tablets (200mg) by direct compression. *Int J Drug Dev Res*.2013; 5(3):371-376

30. The United States Pharmacopeial Convention. USP 1216 Tablet Friability. Usp30 - Nf25

31. Osei-Yeboah F, Sun CC. Validation and applications of an expedited tablet friability method. *Int J Pharm*. 2015; 484:146-155. https://doi.org/10.1016/j.ijpharm.2015.02.061

32. Budiman A, Husni P, Shafira, et al. The development of glibenclamide-saccharin cocrystal tablet formulations to increase the dissolution rate of the drug. *Int J Appl Pharm*. 2019; 11:359–364. https://doi.org/10.22159/ijap.2019v11i4.33802

33. Rodríguez-Ruiz C, Montes-Tolentino P, Domínguez-Chávez JG, et al. Tailoring Chlorthalidone Aqueous Solubility by Cocrystallization: Stability and Dissolution Behavior of a Novel Chlorthalidone-Caffeine Cocrystal. *Pharmaceutics*. 2022; 30; 14(2):334. https://doi.org/10.3390/pharmaceutics14020334

34. Marzouk MA, Osman DA, Mohamed OS. In vitro and in vivo evaluation of taste-masked orodispersible tablets of fluoxetine hydrochloride for the treatment of depression. *Drug Dev Ind Pharm*. 2021; 47:645–653. https://doi.org/10.1080/03639045.2021.1908336

35. Tembhare E, Gupta KR, Umekar MJ. An Approach to Drug Stability Studies and Shelf-life Determination. *Arch Curr Res Int*. 2019; 19(1):1-20. https://doi.org/10.9734/acri/2019/v19i130147

36. Vinet L, Zhedanov A. A 'missing' family of classical orthogonal polynomials. *J. Phys. A-Math*. 2011; 44. https://doi.org/10.1088/1751-8113/44/8/085201

37. Al-kazemi R, Al-basarah Y, Nada A. Atorvastatin Cocrystals : Tablet. *Asian J Pharm*. 2020; 14:578–595

38. Undale VR. An isolated chicken ileum: Alternative to laboratory animals for isolated tissue experimentation. *IOSR J Pharm*. 2012; 2(5):39-45. https://doi.org/10.9790/3013-25203945

39. Jain G, Bodakse SH, Mishra S, et al. Development of an ex vivo model for pharmacological experimentation on isolated tissue preparation. *J Adv Pharm Technol Res*. 2012; 3(3):176-81. https://doi.org/10.4103/2231-4040.101013

40. Rahman Z, Agarabi C, Zidan AS, et al. Physico-mechanical and stability evaluation of carbamazepine cocrystal with nicotinamide. *AAPS Pharm Sci Tech*. 2011; 12:693–704. https://doi.org/10.1208/s12249-011-9603-4

41. Zhou Q, Tan Z, Yang D, et al. Improving the solubility of aripiprazole by multicomponent crystallization. *Crystals*. 2021; 11(4):343. https://doi.org/10.3390/cryst11040343

42. Guo C, Zhang Q, Zhu B, et al. Pharmaceutical Cocrystals of Nicorandil with Enhanced Chemical Stability and Sustained Release. *Cryst Growth Des*. 2020; 20:6995–7005. https://doi.org/10.1021/acs.cgd.0c01043

43. Panzade P, Shendarkar G. Superior solubility and dissolution of zaltoprofen via pharmaceutical cocrystals. *Turkish J Pharm Sci*. 2019; 16:310–316. https://doi.org/10.4274/tjps.galenos.2018.15013

44. Khan MS, Vishakante GD, Bathool A. Preparation and evaluation of sodium alginate porous dosage form as carriers for low dosed active pharmaceutical ingredients. *Turkish J Pharm Sci*. 2012; 9:183–198

45. Yang D, Cao J, Jiao L, et al. Solubility and Stability Advantages of a New Cocrystal of Berberine Chloride with Fumaric Acid. *ACS Omega*. 2020; 5(14):8283–8292. https://doi.org/10.1021/acsomega.0c00692

ISSN: 2458 - 8806