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PREPARATION AND EVALUATION OF CEFUROXIME AXETIL GASTRO-RETENTIVE

FLOATING DRUG DELIVERY SYSTEM FOR IMPROVED DELIVERY VIA HOT-MELT

EXTRUSION TECHNOLOGY

A Thesis

Submitted to the Graduate School at The University of Mississippi

In partial fulfillment of the requirements for the degree of

Master of Science in Pharmaceutical Sciences

By

Rahul Madhukar Lalge

May 2018

ABSTRACT

Purpose: The objective of the present study was to develop lipid-based gastro-retentive floating drug delivery system of amorphous Cefuroxime Axetil (CA) to minimize the enzymatic degradation in the gastro intestinal tract utilizing hot-melt extrusion technology for improved absorption.

Methods: Preliminary studies were performed to select the appropriate lipids. Selected ratios of CA and lipids were extruded using a twin screw hot-melt extruder. Extrudates obtained were milled to obtain floating granules and were further evaluated for drug content, floating strength and micromeritic properties. In vitro drug release studies were performed in 900 mL of simulated gastric fluid (without enzyme) of pH 1.2. The formulations were also studied for their polymorphic nature. Differential scanning calorimetry (DSC) and hot-stage microscopy were used to assess the homogeneity of the formulations and to detect if there is any phase separation during processing and storage. Fourier transform infrared (FTIR) spectroscopy analysis was further used to assess any drug-excipients interactions.

Results: Solubility studies revealed that CA was highly soluble in Kolliphor® TPGS and Gelucire® 44/14, and solubility increased linearly as the concentration increased. All the extruded granules showed floating lag time of less than 5 seconds and floated for more than 12 h simulated gastric fluid. Optimized formulation was able to give a sustained drug release profile for 12 hours. Micromeritic properties of optimized formulation showed good flow properties compared to the pure drug. Surface characterization revealed that granules had a smooth surface indicating the

presence of lipids on the surface. DSC and hot-stage microscopy studies confirmed that there was no phase separation between CA and the excipients. The FTIR studies showed that there was no major interaction between the CA and excipients studied.

Conclusion: Lipid-based gastro retentive floating drug delivery systems were prepared and showed desired sustained release profiles, which will ensure more complete dissolution of CA with potential improved absorption due to reduced enzymatic degradation and longer residence time in the stomach.

Keywords: Cefuroxime axetil, sustained release, floating drug delivery systems, lipids, enzymatic degradation

DEDICATION

This thesis is dedicated to my parents Madhukar and Surekha Lalge, and to my sisters Reshma

and Rohini.

LIST OF ABBREVIATIONS

API- Active Pharmaceutical Ingredient

CA- Cefuroxime Axetil

DSC- Differential Scanning Calorimetry

USP/NF- United States Pharmacopeia National Formulary

UV- Ultraviolet

PXRD- Powder X-Ray Diffraction

SEM- Scanning Electron Microscopy

FTIR- Fourier Transform Infrared

SGF- Simulated Gastric Fluid

ACKNOWLEDGEMENT

I want to thank Shweta for always being there for me. You were always there to celebrate good times, to share happiness and sorrow. Your crazy antics have always put a smile on my face. I would like to thank my friends, ATM (Adwait, Tarul and Mittal) for making this journey wonderful. Cheers to the fun times and to our friendship too !!! I will always feel fortunate to have friends like you.

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CHAPTER 1

PREPARATION AND EVALUATION OF CEFUROXIME AXETIL GASTRO-RETENTIVE
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1. INTRODUCTION

Cefuroxime axetil (CA) is a broad-spectrum cephalosporin antibiotic, which is an acetoxy ethyl ester prodrug of cefuroxime. Cefuroxime was the first commercially available oral antibiotic of the second-generation antibiotics [1]. It has a broad spectrum antibacterial activity against methicillin-sensitive staphylococci, *Streptococcus pneumoniae*, *Haemophilus influeza*, *Moraxella (Branhamella) catarrhalis* and group A β-Pheamolytic streptococci [2]. It also shows a broad activity on some β-lactamase gram positive respiratory pathogens. Mechanism of action mainly includes inhibition of transpeptidation and peptidoglycan layer synthesis of bacterial cell wall [3, 4].

Since the cefuroxime is not well absorbed when administered orally, it is mainly given by parenteral route in the salt form [5]. With the short elimination half-life (1.2- 1.6 hours) of the drug, frequent dosing restricts the patient compliance [6,7]. The addition of 1-acetoxy ethyl ester group to the cefuroxime molecule and converting it into a prodrug form enhances its oral absorption. CA also exhibits higher lipid solubility and gastric stability [8]. However, upon oral administration of the prodrug, it mainly gets absorbed in the proximal region of the GI

tract and undergoes rapid hydrolysis to form cefuroxime in the presence of non-specific esterase enzymes in the intestinal mucosa and blood [9]. Even though 1-acetoxy ethyl ester group in CA, improves the lipophilicity of the cefuroxime, de-esterification prior to absorption in the intestinal fluids leads to low permeation across the intestinal mucosa. CA imparts low solubility and found to have improved bioavailability in presence of lipid-rich food due to decreased specificity of esterase enzymes towards the CA in the intestine [10].
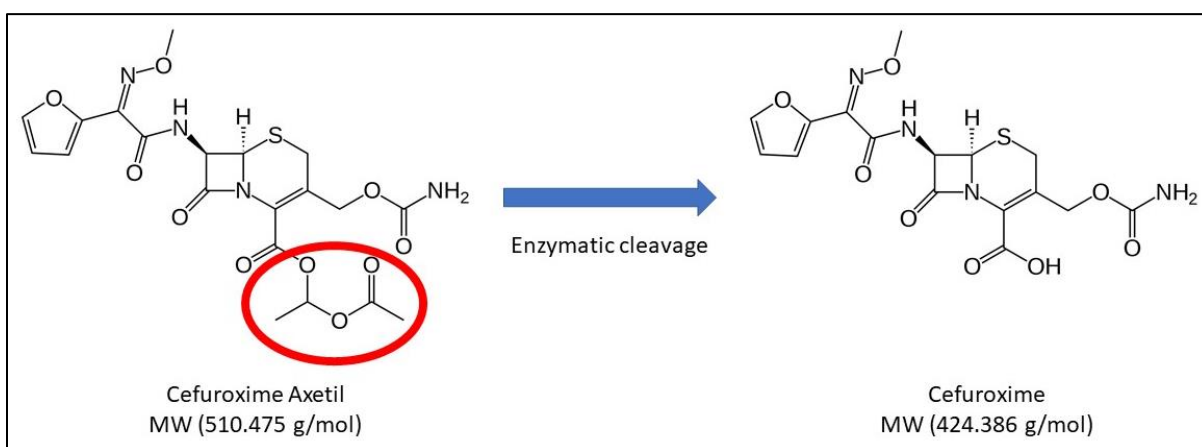
Figure 1. Conversion of prodrug CA to non-absorbable Cefuroxime

Amorphous solid dispersion has been widely used and is a promising approach for the solubility enhancement of poorly water-soluble drugs [11]. The higher internal energy of amorphous materials as compared to their crystalline state exhibits greater thermodynamic properties, resulting into higher solubilization capacity and therefore the enhanced bioavailability [12]. As previously reported, absorption of CA is found to be higher in the proximal part of the intestine compared to other parts of GI tract [13]. Therefore, it is important to have more dissolved form of CA in a time-controlled manner to facilitate its absorption

before it passes through the small intestine. A gastro-retentive drug delivery system can provide a better control over the more complete and timed dissolution of the active pharmaceutical ingredients (APIs) [14, 15]. Moreover, due to the continuous transportation of the released drug molecules towards the intestines, supersaturation in the microenvironment of the dosage form is never achieved which further prevents the ability of the amorphous form of drug molecules to undergo recrystallization in the solution state. The gradual release of the drug molecules from dosage form will ensure their availability at the site of absorption [16].

The lipid-based carriers such as glyceride conjugates and polyethylene glycol derivatives have been used for improving the solubility of poorly water-soluble drugs [17, 18]. In addition to the solubility enhancement, presence of lipid-based carriers can facilitate the permeation of intact CA molecules across the intestinal mucosa by decreasing the enzymatic activity and preventing the degradation of prodrug CA prior to its absorption [19]. Glyceride conjugated lipids are available with a range of hydrophilic lipophilic balance, which also melt at low temperatures for the ease of thermal processing, making them ideal carriers for the controlled drug delivery [20]. Siripuram et al. has reported the sustained release properties of Gelucire 43/01 [21].

Considering the above parameters for prodrug CA, a lipid-based gastroretentive drug delivery system can be potentially used to tailor the drug release profiles in timed manner to ensure minimum enzymatic degradation and maximum absorption. Gastroretentive drug delivery systems are categorized in several forms including floating, mucoadhesive, high density and expandable systems out of which the floating drug delivery systems have been extensively studied. Gastroretentive floating drug delivery system can be used to deliver the drug molecules locally for the prolonged period and therefore can improve the bioavailability

and therapeutic efficacy [22]. Although the floating drug delivery systems can be affected by regular gastric emptying, stomach movements, meals, posture etc. and can giver variable absorption, they do not affect the stomach movements and functioning adversely. Hence, floating drug delivery provides with a safest approach to achieve the maximal bioavailability [23].

Hot melt extrusion is widely known as a solvent-free technique for the preparation of amorphous solid dispersion. Hot-melt extrusion gives an advantage over the conventional techniques by way of molecularly dispersed solid dispersions without the use of any organic solvents [24]. Hot-melt extrusion is also an easily scalable, continuous, and efficient process specifically for the APIs like cefuroxime axetil which have poor flow properties and mixing issues during the conventional processing [25].

In the present study, a lipid-based gastro-retentive floating drug delivery system of CA was prepared by using hot-melt extrusion technology. The prepared drug delivery system can potentially improve the absorption and bioavailability by a sustained release of CA from lipid matrix for prolonged period of time and by preventing the enzymatic degradation due to competitive lipolysis of lipids caused by esterase enzymes.

2. MATERIALS

CA was purchased from Comed Chemicals Ltd (Vadodara, Gujarat, India). Kolliphor® TPGS USP/NF was kindly gifted by BASF Corporation (Florham Park, NJ, USA). Lipids, Gelucire® 43/01 pellets USP/NF and Gelucire® 44/14 USP /NF, were generous gift samples

provided by Gattefosse Corporation (Saint-Priest, Cedex, France). Other analytical grade solvents and chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA).

3. METHODS

3.1.   UV- Visible Spectrophotometric Analysis

UV- Visible spectrophotometric method was utilized (GENESYS 6 UV- Vis spectrophotometer, Thermo Scientific, Madison WI, US) for estimation of CA throughout analysis at a wavelength of 278 nm [26]. The standard curve was linear over the range of 0.8 to 16 µg/mL with R^2 equal to 0.999.

3.2.   Solubility Studies

pH dependent saturation solubility measurements of CA were carried out by adding a known excess amount in 1 ml of each 0.1N HCl (pH 1.2), phosphate buffer (pH 6.8) and water. Samples were agitated using a mechanical shaker at 50 rpm (37 ± 0.5 °C) for 24 h. Samples were centrifuged at 10000 rpm for 5 minutes and supernatant was filtered and then diluted suitably to analyze using UV-vis spectrophotometer at a wavelength of 278 nm. Phase solubility studies were also performed for Kolliphor® TPGS and Gelucire® 44/14 of concentration 2-10 % w/v in water by adding the known excess amount of CA. Samples were subjected to agitation in water bath (37 ± 0.5 °C) for 48 h and then centrifuged at 10000 rpm for 5 minutes to separate the supernatant and further diluted and analyzed using UV-Visible spectrophotometry [27].

3.3. Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) was used to assess the polymorphic and thermal properties of the API and excipients. The pure components, their physical mixtures and formulations were investigated using a TA Instruments DSC equipped with TRIOS software. Samples were prepared by crimping aluminum pans (non-hermetic pan) for the sample weights of 5- 8 mg and were placed into the DSC system.

All the samples except the pure API, were then stabilized by equilibrating at a temperature of 10 °C followed by heating from 10 °C to 100 °C at a ramp rate of 10 °C/min under an inert nitrogen cell purge flow of 50 mL/min. Pure API was equilibrated at a temperature of 20 °C followed by heating from 20 °C to 150 °C at a ramp rate of 20 °C/min. All the thermal events were analyzed from obtained thermograms.

3.4. Extrusion Processing

Initially, Kolliphor® TPGS, Gelucire® 43/01 and Gelucire® 44/14 were milled in grinder and were passed through a USP #40 mesh sieve. Dry ice was used to harden these waxy materials for the ease of milling process. CA was separately sieved through a USP #25 mesh to avoid any lumps and aggregates. 30 g mixtures for each formulation were prepared by simply physically mixing to get the homogeneous mixtures.

The system used to obtain lipid extrudates was composed of a twin-screw extruder (Process 11™, Thermo Fisher Scientific, Odessa, TX, USA), without a die insert and was provided with a chiller and a feeder. A standard screw configuration (Figure 1) was used for the study. The temperature of Zone 2 and 3 were kept at 20° C while feeding was done

through Zone 3. Zone 7 and 8 were set at 40° C, keeping rest of the zones at 50 °C. Screw speed and feeding rate was set at a constant 200 rpm and 3 g/min, respectively.

To obtain thermal equilibrium prior to the actual extrusion processing, system was kept at pre-defined temperatures for 10 minutes. After discarding the initial 5 g the extrudates samples were collected while maintaining the system at the steady state. Obtained extrudates were milled by using a comminuting Fitz mill (Fitzpatrick, Model "L1A", Elmhurst, IL, USA) and sieved through mesh. The milled granules were stored in foil lined polyethylene bags in desiccator at 20- 25 °C for further analysis and processing.
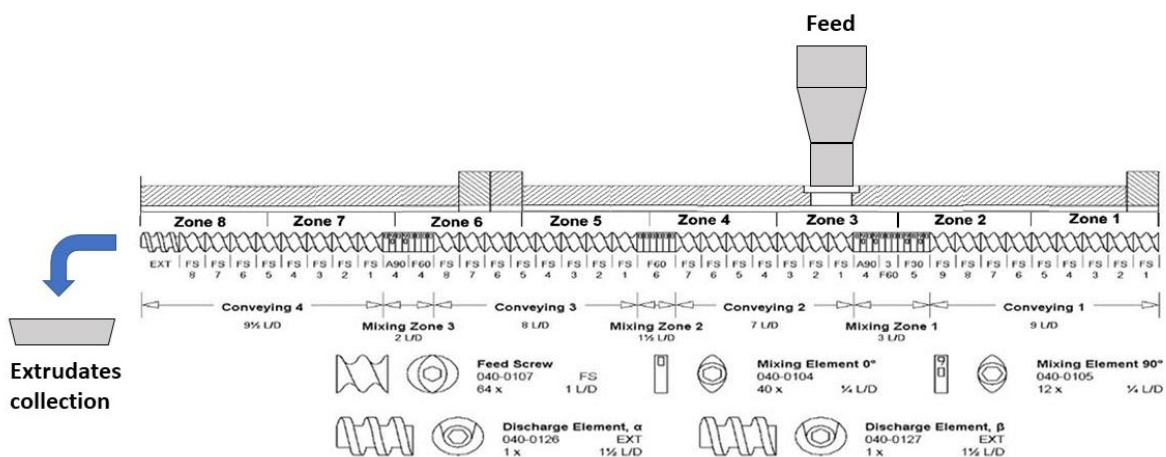
Figure 2. Standard screw configuration used to prepare the extrudates

3.5. Assay and Drug Content Uniformity

The milled granules were accurately weighed and were dissolved in methylene chloride and were subsequently analyzed by UV- Visible spectrophotometry. The extrudates were also evaluated for content uniformity. Briefly, from milled extrudates, 5

different samples were randomly selected and powdered using mortar and pestle. After weighing, powders were weighed and dissolved in methylene chloride to determine CA content using UV- Visible spectrophotometer.

3.6. In Vitro Drug Release

Hard gelatin capsules of size 00 were filled with floating granules equivalent to 125 mg of CA. Dissolution was performed in 900 mL of simulated gastric fluid (enzyme free) of pH 1.2 using USP dissolution apparatus II (Hanson SR8™; Hanson Research) maintained at a temperature of 37 ± 0.5 °C with a paddle rotation speed of 50 rpm. At pre-determined time intervals, a volume of 2 mL of dissolution media was removed and a same volume of fresh dissolution media was added. The samples were filtered through 0.2 µm, 13 mm PTFE membrane filters (Whatman, Inc., Haverhill, MA, USA) and diluted suitably with dissolution media to analyze using UV-vis spectrophotometer with above stated method [26, 28].

3.7. Density of Floating Granules

To determine the tap density, approximately 1-2.5 g of each formulation was accurately weighed (m) and transferred to 10 mL cylinder. Tap density was determined by tapping the filled cylinder carefully by hand approximately 50 times until the constant volume is achieved (v). The equation used to calculate the tap density was:

$$D_{Tap} = m/v$$

The true density of the floating granules was evaluated by a gas pycnometer (AccuPyc II 1340, Micromeritics, Norcross GA, USA) using nitrogen gas. The

measurements were repeated two times and the data was processed using AccuPyc II software. Carr's Index and Hausner's ratio were also calculated as per the standard procedure [29].

3.8. Floating Strength Determination

Timmermans and Moes first reported the 'resultant weight' measuring method to determine the floating strength of the substance in a liquid media [30]. A resultant weight measuring apparatus was assembled, which measures the difference between the resultant weights corresponding to the floating force based on the lever principle. Through a lever, a cone suspended string was attached to the counterpoise weight which was placed on a sensitive electronic weighing balance. In a glass beaker, 500 mg of floating granules filled in capsules were stirred at 50 rpm in a 200 mL of 0.1 N HCl media maintained at 37 ± 0.5 °C. At predetermined time intervals, the beaker was carefully placed into the assembly on a holder ensuring that, it was raised to a certain height, where all the granules are submerged under the cone. After the cone was steady, the difference between initial and final values those displayed on the electronic balance was noted down to calculate the specific floating force of the granules. The beaker was kept for stirring until the next measurement.

3.9. In Vitro Floating Ability

The in vitro floating ability of the granules was assessed using an USP II apparatus in 900 mL of 0.1 N HCl, maintained at a stirring speed of 50 rpm and temperature 37 ± 0.5

°C. The granules were placed into the medium and, floating lag times were measured visually [31].

3.10. Drug-excipient Compatibility

The infrared spectra of pure drug CA, excipients and extruded granules were collected using a bench top Fourier Transform Infrared (FTIR) spectrometer (Agilent Technologies, Cary 660; Agilent, Santa Clara CA, USA) fitted with a MIRacle ATR sampling accessory (Pike Technologies, Madison WI, USA). The bench top ATR was equipped with a single bounce diamond-coated ZnSe internal reflection element.

3.11. Hot-stage Polarized Microscopy

An optical microscope (Agilent Cary 620 IR; Agilent, Santa Clara CA, USA) was equipped with an electronically controlled hot-stage (T95 LinkPad and FTIR 600; Linkam, Tadworth, UK). Photographs were collected without using crossed polarizers to determine the thermal behavior and homogeneity. The samples were heated to 200 °C at a ramp rate of 10 ± 0.1 °C/min and were visually observed for the analysis.

3.12. Scanning Electron Microscopy

Samples were placed on an aluminum stubs held with a black carbon adhesive film and were coated using by a Hummer® 6.2 sputtering system (Anatech Ltd., Battlecreek MI, USA) in a high-vacuum evaporator machine. The surface topography of each sample was analyzed by a scanning electron microscope operating at an accelerating voltage from 1.0 kV to 5.0 kV (JEOL JSM-5600; JEOL, Inc., Peabody MA, USA).

3.13.   Powder X-Ray Diffraction

Powder X-Ray Diffraction (PXRD) was performed using a Bruker D8- Advance (Bruker, Billerica MA, USA) with a Cu-source and θ-2θ diffractometer equipped with a Lynx-eye Position Sensitive Detector. The generator was subjected to a voltage of 40 kV and a current of 30 mA. The samples were dispersed on a low background Si sample holder and compacted gently with the back of a metal spatula. The scan ran from 5° to 40° 2θ with a 0.05 step size at 3 seconds per step.

4. RESULTS AND DISCUSSION

4.1.   Solubility

Solubility study demonstrated that the CA exhibited higher ($p<0.05$) solubility (0.84 ± 0.03 mg/mL) in 0.1N HCl media of pH 1.2 than water (0.32 ± 0.03 mg/mL). Solubility was found even higher (2.49 ± 0.14 mg/mL) in Phosphate buffer saline of pH 6.8. Higher solubility in the gastric pH media as compared to water will facilitate the drug release in gastric environment. CA showed almost 3-fold higher phase solubility in Kolliphor® TPGS (6.87 ± 0.12 mg/mL) than Gelucire® 44/14 (2.17 ± 0.01 mg/mL) in 10% w/v aqueous solutions with a linear increase in phase solubility as amounts of both the lipids were increased ($p<0.05$).

4.2.   Thermal Properties

Pure CA and all excipients were assessed for their respective melting temperatures and crystalline nature. Pure CA found to have an endothermic peak near 75-80 °C

corresponding to its glass transition temperature (T_g), confirming its amorphous nature. Melting points of Gelucire® 43/01, Gelucire® 44/14 and Kolliphor® TPGS were confirmed at around 43 °C, 44 °C and 40 °C respectively.

4.3. Experimental Parameters

A preliminary study was performed to check the feasibility of the extrusion processing of lipids to optimize extrusion conditions. Standard screw configuration was finalized on Process 11™, an 11 mm extruder from Thermo Fisher Scientific (Odessa, TX, USA) to achieve the complete dispersive mixing of CA and excipients due to large mixing zones of the screw configuration. Critical process parameters including barrel temperature, feeding zone and screw speed were assessed. At high temperatures and higher L/D ratio thereby longer residence time, extrudates were coming out in a clear liquid state mostly in phase-separated form without traces of the powdered API. Hence, the simultaneous selection of the feeding zone and screw speed played an important role to get the desired extrudates from the extrusion processing. Therefore, feeding was done through Zone 3 to reduce the residence time and temperatures of mixing zones were set to 50 °C keeping the feeding zone at 20 °C and discharge zones at 40 °C to get uniform extrudates. Screw speed was also optimized at 200 rpm to decrease the mean residence time of the extrusion processing. Torque of the system was stabilized between 3- 5% during the entire process.

Finally, the rapid exposure to the temperature higher than the melting temperatures of lipids in the mixing zones and rapid cooling below their melting point resulted into solidified extrudates which maintained their structures for prolonged times. At temperatures above the glass transition temperature of the API, it was observed that the

molten CA was stuck to the barrel and mixing zones. Highly sticky nature of the pure drug above its glass transition temperature resulted into phase-separation of the drug and lipids. Therefore, the temperature of the barrel should not be high enough near the melting point of the API as CA in solid state tends to have better flowability than its molten form. It is also advisable to keep the operational temperatures of barrel and mixing zones just above the melting point of the lipids to get the highly uniform extrudates.

4.4.   Preparation of Floating Granules

In this study, Gelucire® 43/01, Gelucire® 44/14 and Kolliphor® TPGS were used to disperse the drug molecules in a lipid-based matrix in a stable amorphous form. Gelucire® 43/01, a hydrophobic lipid was able to retard the release of the drug embedded in the lipid matrix. The hydrophobic nature of Gelucire® 43/01 pellets were able to float in the 0.1 N HCl for more than 24 h, making them a right choice to give the floating properties to the formulation. Gelucire® 44/14 and Kolliphor® TPGS are amphiphilic surfactants and were used to enhance the water penetration deep inside the core of the drug-lipid matrix and to get the drug solubilized. Based on the extrudability and solubility studies, both Gelucire® 44/14 and Kolliphor® TPGS were chosen for further evaluation.

Formulation	Drug loading	Gelucire® 43/01	Gelucire® 44/14	Kolliphor® TPGS
F1	60%	40%	-	-
F2	50%	50%		
F3	50%	47.5%	-	2.5%
F4	50%	45%	-	5%

F5	40%	40%	20%	-
F6	40%	40%	-	20%
F7	30%	50%	20%	-
F8	30%	50%	-	20%

Table 1. Experimental formulation compositions of floating granules

In total 8 ratios of drug, hydrophobic and hydrophilic lipids as shown in Table 1, were extruded, and further characterized. All extrudates showed good milling properties when milled with dry ice and were able form granules of uniform size and shape upon cryomilling. Granules were able to float in simulated gastric fluid up to 12 hours and were able to control the drug release from lipid matrices over the period of 12 hours. The drug content of the formulations ranged between 94.25% and 106.35% of the calculated values, which confirmed that the drug was uniformly mixed with the lipids during the extrusion processing. This was supported with the content uniformity study which also showed the drug content of 97.41% to 101.94%.

4.5. Micromeritic Properties of Floating Granules

All the formulations were evaluated for true, bulk and tap densities. Carr's Indices and Hausner's ratios were also calculated to estimate the flowability of the floating granules during the processing. Micromeritic properties of the CA showed that flowability was not within the recommended range for the processing but all the formulations showed good flowability as shown in Table 2. For all the formulations, Carr's indices were within

6-12 % and Hausner's ratios were below 1.18, indicative of the good flow properties of the granules. Addition of Gelucire® 43/01 contributed to significant lowering in density of granules and imparting good flow properties whereas amount of pure drug and hydrophilic lipids resulted into high dense granules with average flow properties.

Formulation	True Density (g/cm³)[a]	Bulk Density (g/cm³)[b]	Tap Density (g/cm³)[b]	Carr's Index (%)	Hausner's Ratio
CA	-	0.6584 ±0.0170	0.9009 ±0.0270	26.90 ±1.68	1.37 ±0.03
F1	1.1405 ±0.0005	0.4352 ±0.0165	0.4742 ±0.0186	8.22 ±0.19	1.090 ±0.002
F2	1.1136 ±0.0001	0.4433 ±0.0420	0.4918 ±0.0471	9.84 ±0.28	1.109 ±0.003
F3	1.1657 ±0.0005	0.4355 ±0.0139	0.4815 ±0.0213	9.46 ±4.65	1.106 ±0.057
F4	1.1773 ±0.0009	0.3875 ±0.0169	0.4369 ±0.0163	11.24 ±4.72	1.129 ±0.062
F5	1.1588 ±0.0001	0.4486 ±0.0303	0.4900 ±0.0361	8.41 ±1.68	1.092 ±0.020
F6	1.1282 ±0.0001	0.4886 ±0.0396	0.5415 ±0.0378	9.81 ±1.93	1.109 ±0.024
F7	1.1374 ±0.0007	0.4019 ±0.0177	0.4458 ±0.0266	9.79 ±2.29	1.109 ±0.028
F8	1.0968 ±0.0006	0.4450 ±0.0236	0.4742 ±0.0312	6.11 ±1.39	1.065 ±0.016

Mean ± SD, [a] n=2, [b] n=3

Table 2. Micromeritic properties of floating granules

The surface characterization of the granules was done using scanning electron microscopy (SEM) as shown in the Figure 3. The non-porous and consistent smooth surface of the granules indicated the presence of lipids on the surface. Although granules

were irregularly shaped, presence of lipids on the surface signifies that the drug was uniformly mixed within the lipid carriers. Therefore, it can be concluded that presence of both hydrophobic and hydrophilic lipids in the microenvironment of carriers, tailored the diffusion of the drug in presence of the media.
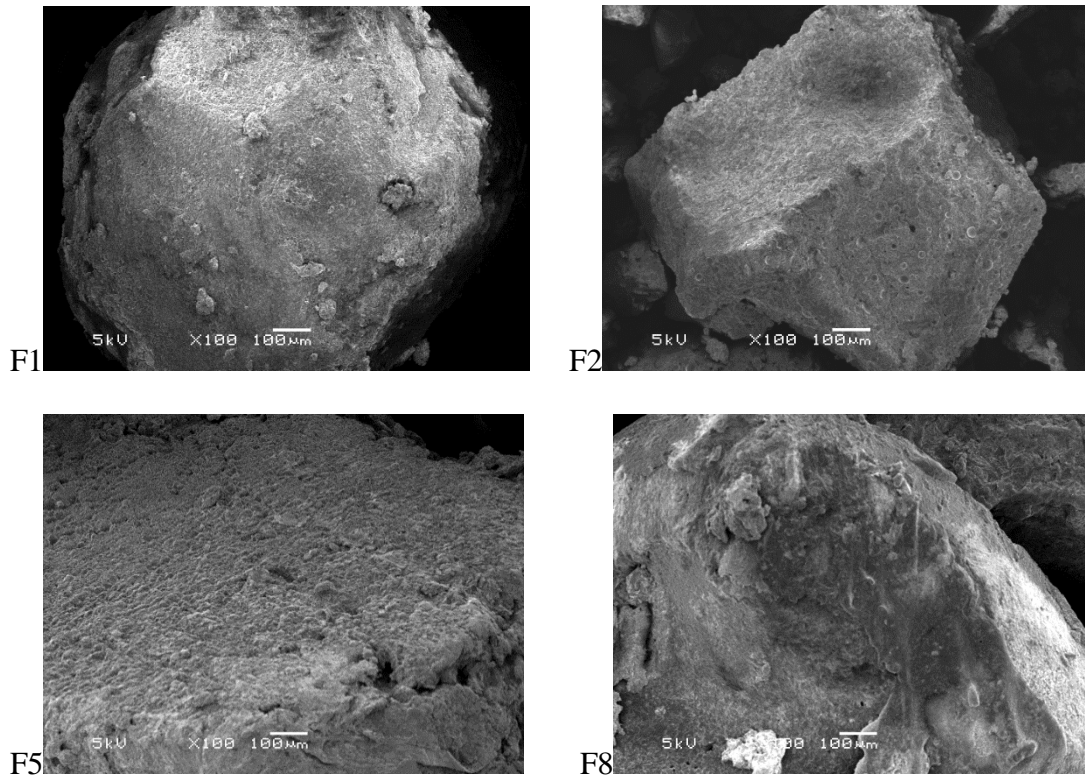

Figure 3. Scanning Electron Microscope images of F1, F2, F5 and F8 formulations

4.6. In Vitro Drug Release

In vitro drug release profiles of different formulations are shown in the Figure 4. Pure CA showed only 42% drug release after 12 hours in SGF. The drug particulates formed a stiff mass in cluster form after encountering the media, which could be due to the

gelation and aggregates caused by cohesive nature of the drug molecules. Formulations extruded with only Gelucire® 43/01 significantly retarded the drug release (p<0.05). This could be due to lesser water permeation inside the hydrophobic lipid matrix which allowed the total drug release of around 20% in 12 hours. Addition of release enhancers improved the drug release substantially in proportional manner. However, the release was found to be higher in presence of Kolliphor® TPGS than with Gelucire® 44/14. This can be supported by the higher solubilization capacity of Kolliphor® TPGS as confirmed in phase solubility study. With both the lipids, Kolliphor® TPGS and Gelucire® 44/14, lesser drug load (30%) formulations showed higher release as compared to 40% drug load (p<0.05). This was attributed to the higher hydrophilic lipid to drug ratio, which helped to impart more complete dissolution of the CA.
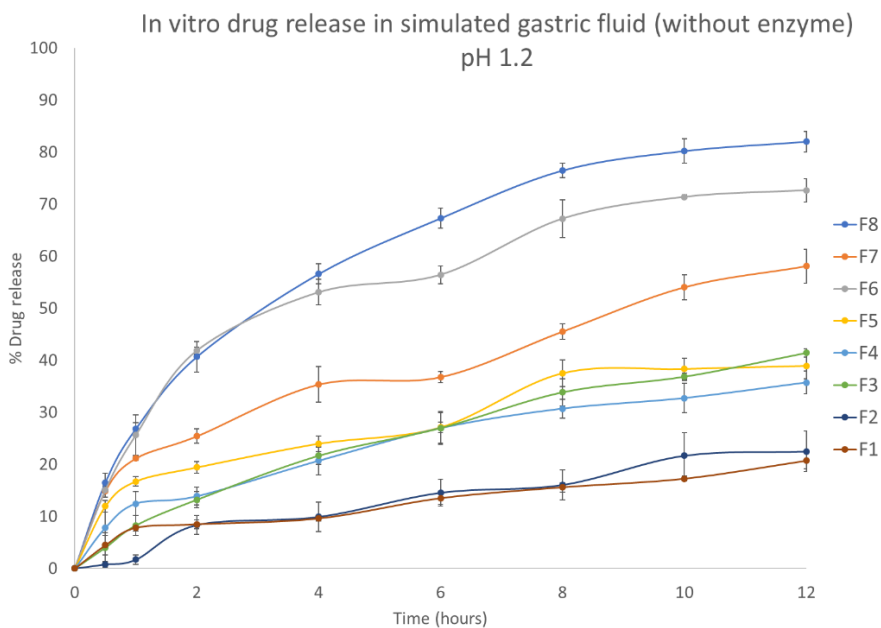

Figure 4. Drug release profiles of CA from the floating granules in SGF (without enzyme) of pH 1.2 (n=3)

4.7. Floating Strength of the Granules

Measuring the floating strength of granules was an important parameter to evaluate the performance of granules and to optimize the formulation. Many factors can affect the floating strength of the formulations including gastric emptying time and pH of the stomach. Buoyancy kinetics of granules could be significantly altered due to gastric churning movements and can affect the desired performance of the drug delivery system [32]. The floating strength should be high enough to encounter these hurdles and give the desired outputs. To approach this issue, drug delivery systems involving both floating and muco-adhesive properties have been studied by Vo et al [33].

The granules of all the formulations floated immediately without any observed lag time in the simulated gastric fluid of pH 1.2 (without enzyme). Most of the granules floated for more than 8 hours for all formulations (Figure 5).
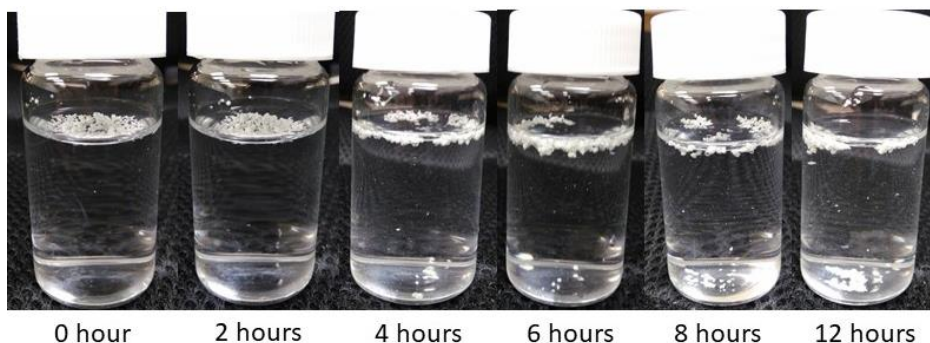

Figure 5. Photographs of granules floating in simulated gastric fluid of pH 1.2 at 37±0.5°C in water bath

The specific floating strength of the granules did not vary much but showed a specific pattern and decreased over the time. The initial floating force ranged from 695 µN/g to 717 µN/g and decreased to the values from 630 µN/g to 668 µN/g over the period of 12 hours (Figure 6). The low floating force at zeroth time point can be presumably resulted due to added weight of the capsule shell with granules, and as the capsule shell dissolves completely, increase in the floating force can be observed after one hour. Higher amount of Gelucire® 43/01 contributed to the better floating properties for prolonged period, whereas drug loading and amount of hydrophilic lipids inversely affected floating ability with lower specific floating strength granules. Decreased floating strength values at the end of 12 hours can be explained by process of water penetration inside the lipid matrix and making the matrix less buoyant compared to the initial formulation.
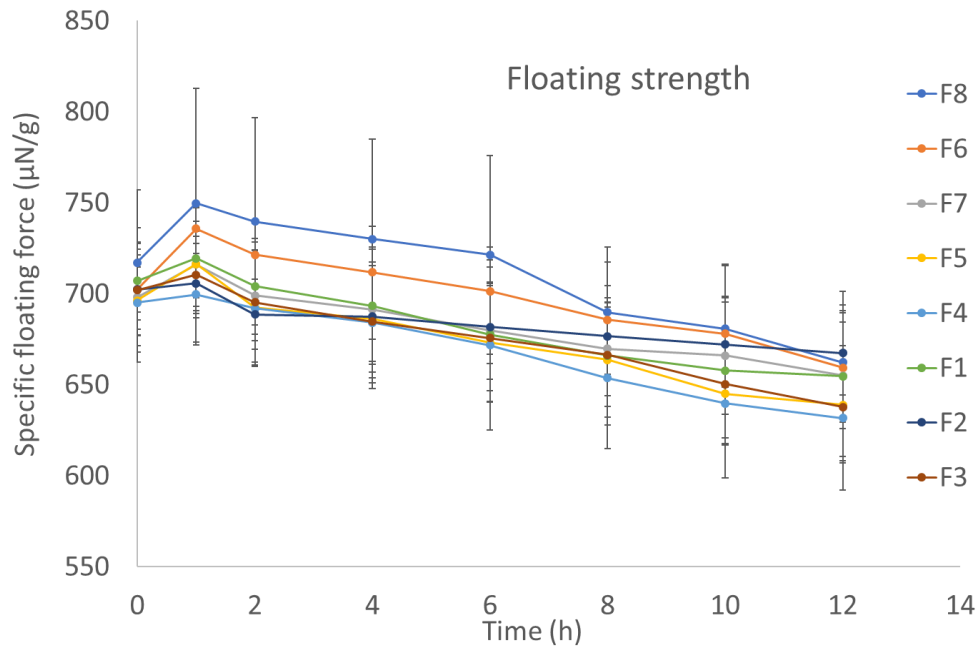

Figure 6. The specific floating strength profiles of formulations (n=3)

4.8. Characterization of Polymorphic Forms and Phase Separation

4.8.1. Differential Scanning Calorimetry

DSC thermograms of pure CA, excipients, physical mixture, and optimized formulations are shown in the Figure 7. Pure CA exhibited a phase transition band at around 78 °C, which corresponds to its glass transition temperature (T_g), signifying an amorphous nature of the API. The DSC thermograms for Gelucire® 43/01, Gelucire® 44/14 and Kolliphor® TPGS showed endothermic peaks at their corresponding melting points. Gelucire® 44/14 exhibited two endothermic peaks associated with the structural and polymorphic variability due to multicomponent lipid system. This was attributed to the complex polymorphic profile of glyceride lipids [34]. However, the thermogram of extruded granules showed endothermic peaks corresponding to polyglycolized glycerides but no evidence of glass transition temperature (T_g) of CA.

It can be concluded that, the change in the enthalpy is due to the homogenous extrudates and absence of multiple glass transition temperatures confirm that there is no phase separation between CA and the lipid excipients. Therefore, DSC analysis is an indicative of the presence of a homogenous distribution of CA in the lipid matrix.
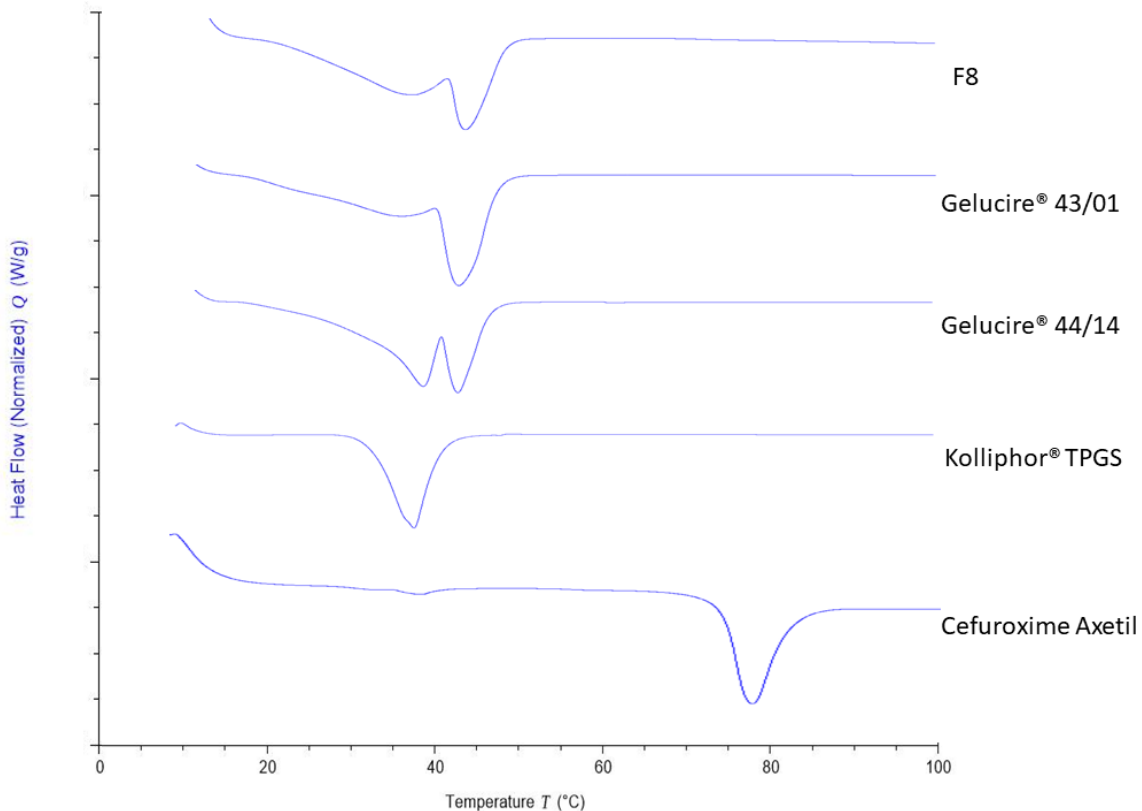
Figure 7. Differential Scanning Calorimetry thermograms of CA, lipids, and optimized

formulation

4.8.2. PXRD Analysis

PXRD analysis was further done to characterize the polymorphs of the unprocessed and processed CA in the formulations. The PXRD patterns of pure drug, physical mixture and optimized formulation are shown in the Figure 8. The broad and diffused diffraction peaks of pure CA indicated its amorphous nature. Whereas the diffraction patterns of both optimized formulation F8 and physical mixture showed high intensity characteristic peaks which can be attributed to Kolliphor® TPGS, which is crystalline in nature.
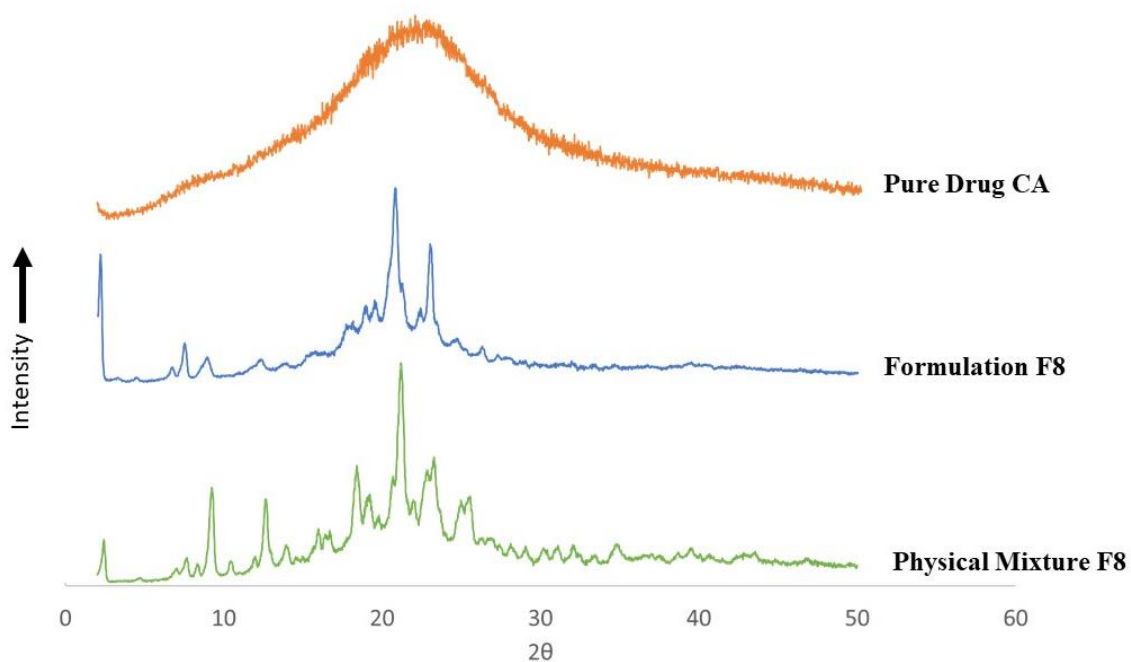
Figure 8. Powder X-Ray Diffraction patterns for pure CA, physical mixture, and

optimized formulation F8

However, the peak intensity was reduced in case diffractogram of the formulation F8, confirming the homogeneity of the extruded formulation. The large peaks in the diffractogram of the physical mixture can be seen due to the pure crystalline form of the Kolliphor® TPGS. Therefore, the chosen screw design provided high shear energy, sufficient enough to prepare a solid dispersion of CA. Hence, it was concluded that high shear mixing at ambient temperature imparts significant mixing of both API and excipients.

4.8.3. Hot-stage Polarized Microscopy

Homogeneity of CA was also assessed using polarized-light hot-stage microscopy. At room temperature, in presence of polarized light an indefinite shaped amorphous form of CA could be clearly seen (Figure 9A). Conversion of amorphous CA to its glassy form could be seen, as the temperature of the stage was raised (Figure 9B).
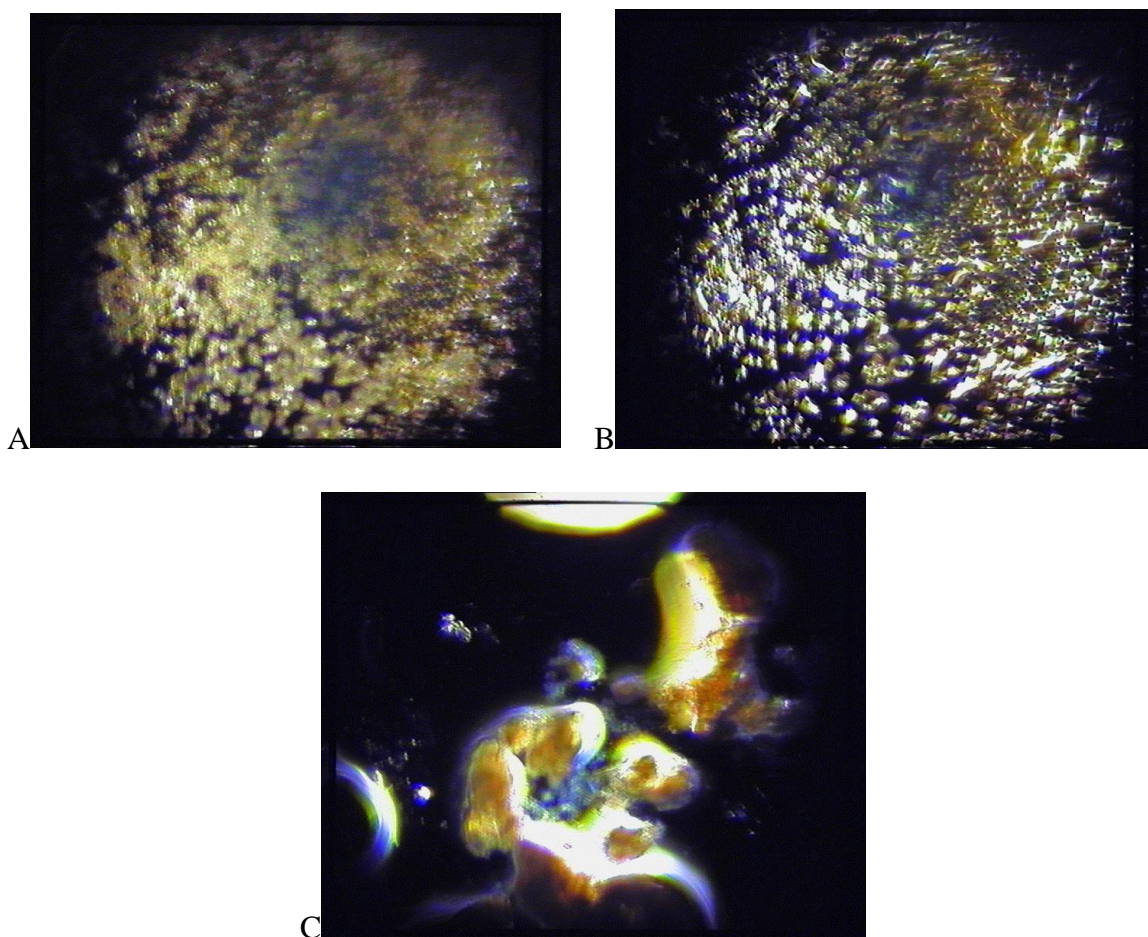

Figure 9. A-Pure CA at room temperature B- Pure CA above its glass transition temperature C-Extrudates at 50°C

Optimized extrudates did not show any significant phase separation of drug (Figure 9C), even after reaching the melting points of both the lipids, indicating that the CA was suspended in lipid matrix in homogenous manner.

4.9.   API- excipients Chemical Interactions (FTIR)

The FTIR spectra of Cefuroxime Axetil, Gelucire® 43/01 and Kolliphor® TPGS are given in Figure 10. The objective behind studying the FTIR spectra of CA and excipients was to investigate any possible interactions between drug and lipid carriers. CA showed two absorption bands corresponding to carbonyl groups at 1678 cm^{-1} and 1680 cm^{-1} assigned to amide and carbonyl group stretching.
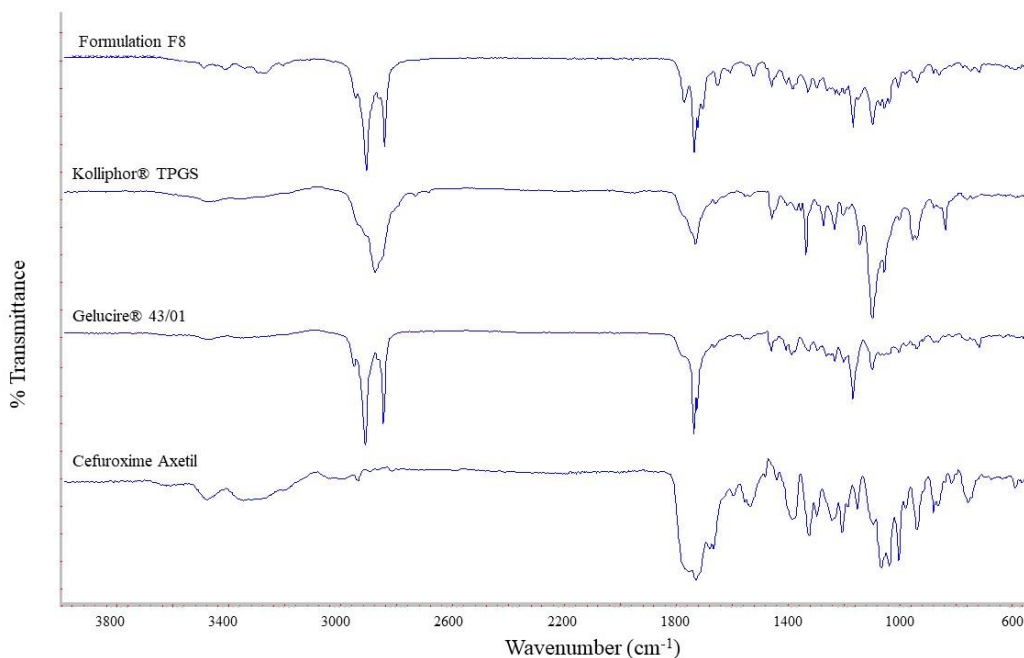

Figure 10. FTIR Spectra of CA, Gelucire® 43/01 and % Kolliphor® TPGS

The peak at 1760 cm^{-1} is characterized for carbonyl group stretching in the vinyl ester group, and the absorption bands for NH and NH$_2$ complex were seen from 3260 cm^{-1} to 3480 cm^{-1}. All major peaks of carbonyl stretching vibrations were present with less intensity. This is an indicative of presence of an interaction between CA and excipients. Possibly the C=O group of CA must have formed hydrogen bonding with -OH groups of gelucire lipids, which was also supported by the data by Shimpi et al [17].

5. CONCLUSION

Lipid-based gastro-retentive floating granules loaded with amorphous cefuroxime axetil were successfully prepared using hot-melt extrusion technology. The floating formulations were well characterized, and effects of various factors on the properties of granules were investigated. The granules had excellent flowing and floating properties, which were able to float more than 8 hours in the simulated gastric fluid. The granules were also able to give a sustained drug release up to 12 hours which will ensure more controlled dissolution and absorption of the drug in the proximal part of the intestine. The results demonstrated that lipids can be processed in a hot melt extruder to prepare the drug delivery system with desired characteristic that will ensure the improved absorption of CA. In conclusion, this drug delivery system has the potential to reduce the enzymatic degradation and to improve the bioavailability of the prodrug CA substantially.

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