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Preparation and evaluation of cefuroxime axetil gastro-retentive floating drug delivery system via hot melt extrusion technology

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Abstract

Cefuroxime Axetil (CA) is a poorly soluble, broad spectrum antibiotic which undergoes enzymatic degradation in gastrointestinal tract. The objective of the present study was to develop lipid-based gastro-retentive floating drug delivery systems containing CA using hot-melt extrusion (HME) to improve absorption. Selected formulations of CA and lipids were extruded using a twin screw hot-melt extruder. Milled extrudates were characterized for dissolution, floating strength, and micromeritic properties. Solid-state characterization was performed using differential scanning calorimetry (DSC), scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and hot-stage microscopy. *In vitro* characterization demonstrated that the formulations exhibited a sustained drug release profile for 12 h. All formulations showed desired floating and flow properties. Solid-state characterization revealed no phase separation and no chemical interactions between the drug and excipients. Based on *in vitro* study results, an optimized formulation (F8) was further evaluated for *in vivo* performance. Oral bioavailability ($C_{\max}$ and AUC_{0-24h}) of F8 was significantly higher than that of pure CA. This study describes the use of lipid-based gastro-retentive floating drug delivery systems to achieve desired sustained release profile for more complete dissolution which could potentially reduce enzymatic degradation. This study also highlights the effectiveness of HME technology to improve dissolution and bioavailability.

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5. DECLARATION OF INTEREST

All the authors report no conflicts of interest.

Keywords

Cefuroxime axetil; Sustained release; Floating drug delivery systems; Pharmacokinetic study; Lipids; Enzymatic degradation

1. INTRODUCTION

Cefuroxime Axetil (CA) is an acetoxyethyl ester 'prodrug' of cefuroxime, a broad-spectrum cephalosporin antibiotic. Cefuroxime was the first commercially available second-generation oral antibiotic to be widely used in therapy (Adams et al., 1985; Brambilla et al., 1992; Fabre et al., 1994). It exerts broad-spectrum antibacterial activity against methicillin-sensitive staphylococci, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella (Branhamella) catarrhalis*, and group A β -hemolytic streptococci (Perry and Brogden, 1996). It also shows broad activity against some β -lactamase gram-positive respiratory pathogens. The primary mechanisms of action of cefuroxime are inhibition of transpeptidation and peptidoglycan layer synthesis of the bacterial cell wall (Fisher et al., 2005; Gordon et al., 2000).

Since cefuroxime is not well absorbed when administered orally, it is mainly administered parenterally in a salt form (Dhumal et al., 2008). Due to its short elimination half-life (1.2–1.6 h), the necessity for frequent dosing restricts patient compliance (Dhumal et al., 2006; Sommers et al., 1984). Addition of a 1-acetoxyethyl ester group to the cefuroxime molecule results in a prodrug form (CA), that enhances its oral absorption due to increased lipophilicity of the molecule ($\log P \sim 0.55$). CA also exhibits gastric stability due to its higher pK_a value (Oszczapowicz et al., 1995). However, upon oral administration, CA is mainly 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 (Figure 1A) (Sommers et al., 1984). Although the 1-acetoxyethyl ester group of CA increases lipophilicity of cefuroxime, de-esterification due to esterase enzymes prior to absorption in the intestinal fluids leads to low permeation across the intestinal mucosa (Barrett et al., 1997; Harding et al., 1984; Leder and Carson, 1997). CA is poorly soluble, and exhibits greater bioavailability in the presence of lipid-rich foods due to lower specificity of esterase enzymes toward CA in the intestine (Finn et al., 1987; Williams and Harding, 1984).

Amorphous solid dispersion has been widely used and is a promising approach for solubility enhancement of poorly water-soluble drugs (Taylor and Zhang, 2016). The higher internal energy of amorphous materials compared to crystalline materials results in greater thermodynamic properties and a subsequent increase in solubility and bioavailability (Huttenrauch, 1978). As previously reported, absorption of CA is higher in the proximal region of the intestine than other parts of the GI tract (Rouge et al., 1996). Therefore, it is important to temporally control dissolution of CA to facilitate absorption prior to passing through the small intestine. A gastro-retentive drug delivery system can provide better control over complete and timed dissolution of active pharmaceutical ingredients (APIs) (Baumgartner et al., 2000; Deshpande et al., 1997). Moreover, due to continuous transport of

released drug molecules toward the intestines, supersaturation in the microenvironment of the dosage form is never achieved, which further prevents the ability of amorphous drug molecules to undergo recrystallization in solution. Gradual release of these drug molecules from their dosage forms ensures their availability at the site of absorption (Streubel et al., 2006).

Carriers such as glyceride lipids and polyethylene glycol – lipid conjugates have been used in self-emulsifying and self-microemulsifying drug delivery systems to improve the dissolution of poorly water-soluble drugs (Charkoftaki et al., 2011; Shimpi et al., 2005). In addition, lipid-based carriers can facilitate permeation of intact CA molecules across the intestinal mucosa by potentially decreasing the enzymatic activity and preventing degradation of the prodrug CA prior to its absorption (Figure 1B) (Zaro, 2014). Glyceride-conjugated lipids are available with varying degrees of hydrophobicity and melt at low temperatures to aid in thermal processing, making them ideal carriers for controlled drug delivery (Ainaoui and Vergnaud, 1998). Siripuram et al. demonstrated the sustained release properties of Gelucire® 43/01 (Siripuram et al., 2010).

Based on the chemical properties of the prodrug CA, a lipid-based gastro-retentive drug delivery system may allow for greater temporal control of drug release to potentially minimize the enzymatic degradation and maximize its absorption. Gastro-retentive drug delivery systems can be categorized as floating, mucoadhesive, high density, and expandable. Floating drug delivery systems have been extensively studied. Gastro-retentive floating drug delivery systems can be used to deliver drug molecules locally over prolonged periods of time, resulting in improved bioavailability and therapeutic efficacy (Deshpande et al., 1996). Although floating drug delivery systems can be affected by regular gastric emptying, stomach movements, meals, and posture, potentially resulting in variable absorption, they do not adversely affect stomach movements or function. Hence, floating drug delivery provides the safest approach to achieve maximal bioavailability (Fell, 1996).

HME is a solvent-free technique for preparation of amorphous solid dispersions (Repka et al., 2008). HME is also an easily scalable, continuous, and efficient process specifically beneficial for processing of APIs such as CA which exhibit poor flow properties and mixing issues during conventional processing (Repka et al., 2007).

In the present study, a novel lipid-based gastro-retentive floating drug delivery system containing CA was prepared using HME technology. Gelucire® 43/01, a hard-fat mixture of glycerides and PEG esters was chosen as the floating lipid due to its highly hydrophobic nature and low melting point, which aids in the extrusion process. Kolliphor® TPGS and Gelucire® 44/14 were chosen based on their amphiphilic non-ionic surfactant-like properties and they both melt below 45 °C, which is an added advantage for extrusion processing. Optimized mixtures of hydrophobic lipids and surfactants, in combination can help the drug molecules to dissolve in a more controlled manner. The prepared drug delivery system has the potential to improve absorption and bioavailability of CA through sustained release of CA from the lipid matrix and by potentially preventing enzymatic degradation due to competitive lipolysis of lipids caused by esterase enzymes.

2. MATERIALS AND METHODS

2.1. Materials

CA was purchased from Corned Chemicals Ltd. (Vadodara, Gujarat, India). Cefuroxime was purchased from AK Scientific Inc. (CA, USA). 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).

2.2. Solubility studies

UV-Visible spectrophotometry (GENESYS 6 UV- Vis spectrophotometer, Thermo Scientific, Madison WI, US) was used to estimate the concentration of CA at a wavelength of 278 nm (United States Pharmacopeial Convention, 2000). The standard curve was linear across the range of 0.8 to 16 µg/mL ($R^2 = 0.999$).

pH-dependent saturation solubility measurements of CA were performed by adding a known excess amount of CA to 1 mL of 0.1 N HCl (pH 1.2), 0.1 M phosphate buffer (pH 6.8), or water. Samples were agitated using a mechanical shaker at 50 rpm (37 ± 0.5 °C) for 24 h. Samples were centrifuged at 10,000 rpm for 5 min and the supernatant was filtered, then diluted prior to UV-Vis spectrophotometric analysis at 278 nm. Phase solubility studies were performed for Kolliphor® TPGS and Gelucire® 44/14 at concentrations of 2–10% w/v in water by adding a known excess amount of CA. Samples were agitated in a water bath (37 ± 0.5 °C) for 48 h, then centrifuged at 10,000 rpm for 5 min. The supernatant was diluted and analyzed by UV-Vis spectrophotometry (Mader and Higuchi, 1970).

2.3. Differential scanning calorimetry (DSC)

DSC was used to assess the polymorphic and thermal properties of the API and excipients. Pure components, physical mixtures, and formulations were investigated using a TA Instruments DSC equipped with TRIOS software. Samples were prepared by weighing 5–8 mg of each sample into non-hermetic aluminum pans. The pans were crimped and placed onto the DSC system.

All samples except the pure API were stabilized by equilibrating at 10 °C followed by heating from 10 °C to 100 °C at a ramp rate of 10 °C/min with 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 using the obtained thermograms.

2.4. Extrusion processing

Gelucire® 43/01, Kolliphor® TPGS and Gelucire® 44/14 were milled separately with dry ice in a grinder. Powdered dry ice was used to harden these waxy materials to aid the milling process. Milled samples were passed through a USP #40 mesh sieve after complete evaporation of dry ice from the mixture. CA was sieved separately through a USP #25 mesh

to eliminate lumps and aggregates. Fifty-gram mixtures of each formulation were prepared by physically mixing to obtain 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 with a chiller and a feeder. A standard screw configuration was used for this study (Figure 2). The temperatures of zones 2 and 3 were kept at 20 °C while feeding was done through zone 3. Zones 7 and 8 were set at 40 °C, and the remaining zones were kept at 50 °C. Screw speed and feed rate were maintained at 200 rpm and 3 g/min, respectively.

To obtain thermal equilibrium prior to the extrusion process, the system was kept at pre-defined temperatures for 10 min. After discarding the initial 5 g, extrudates were collected while maintaining the system at a steady state. The obtained extrudates were milled using a comminuting Fitz mill (Fitzpatrick, Model “L1A”, Elmhurst, IL, USA) and sieved through a mesh. The milled granules were stored in a desiccator at 20–25 °C for further analysis and processing.

2.5. Assay and drug content uniformity

The milled granules were accurately weighed, extracted in methylene chloride, then diluted and analyzed by UV-Visible spectrophotometry. The extrudates were evaluated for content uniformity by selecting five different samples. Samples were powdered and dissolved in methylene chloride, sonicated for 10 min, then centrifuged. The supernatant was diluted and analyzed for CA content using a UV-Visible spectrophotometer (Shimpi et al., 2004).

2.6. *In vitro* drug release

Size 00 hard gelatin capsules were filled with floating granules equivalent to 125 mg of CA. Dissolution was performed in 900 mL of pH 1.2 simulated gastric fluid (SGF, enzyme-free) using a USP dissolution apparatus II (Hanson SR8™; Hanson Research) maintained at 37 ± 0.5 °C with a paddle rotation speed of 50 rpm. At predetermined time intervals, 2 mL of dissolution medium was removed with replacement of an equal volume of fresh dissolution medium. Samples were filtered through 0.2-µm PTFE membrane filters (Whatman, Inc., Haverhill, MA, USA), diluted with dissolution medium, and analyzed using a UV-Visible spectrophotometer (Bomma and Veerabrahma, 2013; United States Pharmacopeial Convention, 2000).

2.7. Density of floating granules

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

$$D_{tap} = \frac{m}{V} \quad \text{Equation (1)}$$

The true density of the floating granules was evaluated using a gas pycnometer (AccuPyc II 1340, Micromeritics, Norcross GA, USA) with nitrogen gas. Measurements were repeated in triplicate and the data were processed using AccuPyc II software. Carr's Index and Hausner's ratio were also calculated per the standard procedure (Wells and Aulton, 2007).

2.8. Floating strength determination

Timmermans and Moës first reported the 'resultant weight' measurement method to determine the floating strength of a substance in a liquid medium (Timmermans and Moës, 1990). A resultant weight measurement apparatus was assembled, which measured 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 balance. In a glass beaker, 500 mg of floating granules in capsules were stirred at 50 rpm in 200 mL of 0.1 N HCl medium 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 height where all granules were submerged under the cone. After the cone was steady, the difference between initial and final values was recorded to calculate the specific floating force of the granules. The beaker was kept stirring between measurements (Figure 3).

2.9. *In vitro* floating ability

The *in vitro* floating ability of the granules was assessed using a USP II apparatus in 900 mL of 0.1 N HCl. Stir speed was maintained at 50 rpm and temperature was maintained at 37 ± 0.5 °C. The granules were placed into the medium and floating lag times and total floating times were measured visually (Iannuccelli et al., 1998).

2.10. Drug-excipient compatibility

Infrared spectra of pure CA, excipients, and extruded granules were collected using a 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.

2.11. 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 thermal behavior and homogeneity. The samples were heated to 200 °C at a ramp rate of 10 ± 0.1 °C/min and observed visually.

2.12. Scanning electron microscopy (SEM)

Samples were placed on aluminum stubs held with a black carbon adhesive film and coated using by a Hummer® 6.2 sputtering system (Anatech Ltd., Battlecreek MI, USA) in a high-vacuum evaporator. The surface topography of each sample was analyzed using a scanning

electron microscope operated across an accelerating voltage range of 1.0 kV to 5.0 kV (JEOL JSM-5600; JEOL, Inc., Peabody MA, USA).

2.13. X-ray powder diffraction (XRPD)

XRPD 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. Samples were dispersed on a low background Si sample holder and compacted gently with the back of a metal spatula. Scans ran from 5° to 40° 2θ with a 0.05 step size at 3 seconds per step.

2.14. High performance liquid chromatography (HPLC) analysis

A simple and precise reversed phase HPLC - UV system (Waters Corporation, Milford, MA) was utilized for analysis of cefuroxime in plasma. A 250 mm x 4.6 mm internal diameter (ID) Waters XBridge™ C18 chromatographic column with 5 μ m particle size was used for separation. The mobile phase consisted of potassium phosphate monobasic buffer (0.01 M) and methanol at a 55:45 (v/v) ratio. The mobile phase was maintained at a constant flow of 1 mL/min, and samples were analyzed using a photodiode array (PDA) detector at a wavelength of 278 nm (Dhumal et al., 2006). The retention time of cefuroxime was 4.2 min. The method was linear across the concentration range of 10 to 2000 ng/mL of cefuroxime with a correlation coefficient of 0.999.

To plot the standard curve in rat plasma, blood was collected from the jugular vein of rats through a cannula into heparinized microcentrifuge tubes. Samples were centrifuged for 10 min at 8,000 rpm at 4° C and the supernatant (plasma) was collected for further analysis. Plasma (80 μ L) was mixed with 20 μ L of previously prepared drug solutions in methanol and water at an 80 : 20 ratio (concentrations 10 – 2000 ng/mL). After 2 min of vortex mixing, 4 mL of ethyl acetate was added, and samples were centrifuged at 3,000 rpm for 5 min. The supernatant layer was isolated and dried overnight at room temperature to completely evaporate ethyl acetate. The dried samples were diluted with 1 mL of 80 : 20 (methanol and water) and injection volume of 20 μ L was injected into HPLC system for analysis (Dhumal et al., 2009). The standard curve in rat plasma was linear across the range of 10–2000 ng/mL.

2.15. *In vivo* pharmacokinetic study

Twelve male Sprague-Dawley jugular vein-cannulated rats weighing 250 ± 20 g were used in this study after an acclimatization period of one week. All rat experiments were conducted per an Institutional Animal Care and Use Committee (IACUC) protocol at the University of Mississippi (Protocol number: 16–017).

Animals were divided into three groups with four animals in each group (optimized formulation, pure CA, and the marketed formulation). A dose of 45 mg/kg body weight was administered by oral gavage. Blood (250 μ L) was withdrawn from the jugular veins of the rats and transferred into heparinized microcentrifuge tubes at 0.5, 1, 2, 4, 8, 10, 12, and 24 h after dosing. After collection, plasma was isolated for further analysis. All plasma samples were treated in the same manner as samples previously prepared for plotting the calibration

curve. Samples were injected onto an HPLC system for analysis. Pharmacokinetic parameters of cefuroxime such as area under curve (AUC), peak plasma concentration (C_{max}), and peak plasma concentration time (T_{max}) were calculated using Phoenix® WinNonlin™ 6.4 (Certara®, Princeton, NJ, USA) software. Statistical analyses were performed using an unpaired Student's t-test. A p-value less than 0.05 was considered statistically significant (Dhumal et al., 2009).

3. RESULTS AND DISCUSSION

3.1. Solubility

A solubility study demonstrated that CA exhibited higher ($p < 0.05$) solubility (0.84 ± 0.03 mg/mL) in pH 1.2 (0.1 N) HCl medium than in water (0.32 ± 0.03 mg/mL). Solubility was higher (2.49 ± 0.14 mg/mL) in pH 6.8 phosphate buffered saline. Higher solubility in gastric pH medium than that in water suggested that drug release would be enhanced in the gastric environment. CA showed a nearly 3-fold higher phase solubility in Kolliphor® TPGS (6.87 ± 0.12 mg/mL) than in Gelucire® 44/14 (2.17 ± 0.01 mg/mL) in 10% w/v aqueous solutions. CA showed a linear increase in phase solubility as amounts of both lipids increased. Kolliphor® TPGS and Gelucire® 44/14 are amphiphilic surfactants used to enhance water penetration into the core of the drug-lipid matrix to promote drug solubilization. Based on solubility studies, both Kolliphor® TPGS and Gelucire® 44/14 were chosen for further evaluation.

3.2. Thermal properties

Pure CA and all excipients were assessed for their respective melting temperatures and crystallinity. Pure CA had an endothermic peak near 75–80 °C, corresponding to its glass transition temperature (T_g), confirming its amorphous nature. Melting points (T_m) of Gelucire® 43/01, Gelucire® 44/14, and Kolliphor® TPGS were observed to be below 45 °C (Table 1).

3.3. Experimental parameters

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

Finally, rapid exposure to a temperature of 50 °C in mixing zones, higher than the melting temperatures of lipids (which ranged between 37°C – 45 °C), followed by rapid cooling at room temperature, resulted in solidified extrudates which maintained their structures for prolonged periods. CA was observed to be sticking to the barrel and mixing zones when extrusions were carried out at temperatures above T_g of CA (>78 °C). The highly sticky nature of the pure drug above its glass transition temperature resulted in phase separation of the drug and lipids. Therefore, the temperature of the barrel should not be too close to the T_g , as solid CA tends to have better flowability than its melted form. It is also advisable to keep the operational temperatures of the barrel and mixing zones just above the melting points of the lipids to obtain highly uniform extrudates.

3.4. Preparation of floating granules

In this study, Gelucire® 43/01, Kolliphor® TPGS and Gelucire® 44/14 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 slow release of the drug embedded in the lipid matrix. The hydrophobic nature of Gelucire® 43/01 pellets allowed them to float in 0.1 N HCl for more than 24 h, making these pellets the right choice to confer floating properties to the formulation.

Eight ratios of drug, hydrophobic lipid, and surfactants were extruded and further characterized (Table 2). All extrudates showed good milling properties when milled with dry ice and formed granules of uniform size and shape upon cryomilling. Granules were able to float in SGF for up to 12 h and sustained drug release from the lipid matrices over a period of 12 h. The average drug content of all the formulations was 500.6 ± 27.1 mg which confirmed that the drug was uniformly mixed with the lipids during the extrusion processing. This was further supported by content uniformity results, which showed a drug content of $99 \pm 1.79\%$. The Acceptance Value (AV) for uniformity was calculated using equation (2) as per USP <905>, where M and $\bar{X}$ denote reference and mean value of contents respectively, which are equal in this case. Whereas k is an acceptability constant and s is the standard deviation, which were calculated to be 2.4 and 1.8 respectively. The equation yielded a satisfactory AV value of 4.3 for content uniformity of all formulations.

$$AV = |M - \bar{X}| + ks \quad \text{Equation (2)}$$

3.5. Micromeritic properties of floating granules

All formulations were evaluated for true, bulk, and tap densities. Carr's Indices and Hausner's ratios were calculated to estimate the flowability of the floating granules during processing. Micromeritic properties of CA showed that flowability was not within the recommended range for the extrusion process, but all formulations showed good flowability. Carr's indices were between 6–12% and Hausner's ratios were below 1.18 for all formulations, which indicated good flow properties of the granules (Figure 4). Addition of Gelucire® 43/01 significantly lowered granule density, imparting good flow properties, whereas pure drug formulated with hydrophilic lipids resulted into high density granules with average flow properties.

Surface characterization of the granules was performed using scanning electron microscopy (SEM). Non-porous and consistent smooth surfaces indicated the presence of lipids on the surfaces of the granules. Although granules were irregularly shaped, the presence of lipids on the surface suggests that the drug was uniformly mixed within the lipid carriers (Figure 5). Therefore, we concluded that the presence of both hydrophobic and hydrophilic lipids during the HME processing determined the distribution of CA within the extrudates.

3.6. *In vitro* drug release

Pure CA showed only 42% drug release after 12 h in SGF. CA drug particles formed a stiff clustered mass after being placed in the medium, which may have been due to the presence of gelation and aggregation caused by cohesion between drug molecules. Formulations extruded with only Gelucire® 43/01 significantly slowed drug release ($p < 0.05$). This may have been due to low water permeation into the hydrophobic lipid matrix, which resulted in approximately 20% drug release in 12 h (Figure 6). Incremental addition of release enhancers improved drug release proportional to the amount of release enhancer included. However, release was higher in formulations that included Kolliphor® TPGS than those that included Gelucire® 44/14. This was supported by the higher solubilization capacity of Kolliphor® TPGS, as confirmed in the phase solubility study. Formulations that included Kolliphor® TPGS or Gelucire® 44/14 exhibited greater release with a lesser drug load (30%) than with a greater (40%) drug load ($p < 0.05$) (Figure 6). This was attributed to higher hydrophilic lipid to drug ratio, which contributed to more complete dissolution of CA.

3.7. Floating strength of the granules

Floating strength of the granules was an important parameter to evaluate performance of granules and to optimize the formulation. Many factors can affect floating strength of formulations including gastric emptying time and pH of the stomach. Buoyancy kinetics of granules could be significantly altered in response to gastric churning movements and can affect the desired performance of the drug delivery system (Van Den Abeele et al., 2017). Floating strength should be high enough to be robust to these potential issues and allow for consistent performance of the delivery system. To address these issues, drug delivery systems involving both floating and muco-adhesive properties have been studied (Vo et al., 2017).

Granules of all formulations evaluated in this study floated immediately without any observed lag time in pH 1.2 SGF (without enzymes). Granules of most of the formulations floated for more than 8 h.

The specific floating strength of the granules did not vary significantly between formulations but followed specific patterns and decreased over time. The initial floating force ranged from 695 $\mu\text{N/g}$ to 717 $\mu\text{N/g}$ and decreased to 630 $\mu\text{N/g}$ to 668 $\mu\text{N/g}$ over a period of 12 h (Figure 7). The low floating force at time zero was likely due to added weight of the capsule shell, and as the capsule shell dissolved increased floating force was observed after one hour. Higher amounts of Gelucire® 43/01 contributed to better floating properties over a prolonged period, whereas drug loading and amount of surfactant inversely affected floating ability. Decreased floating strength over 12 h can be explained by water penetration into the

lipid matrix, making the matrix less buoyant than the initial formulation. Based on specific floating strength values and *in vitro* drug release study, the formulation F8 was optimized for further studies, which could potentially float in the gastric fluid with higher floating strength and release the drug for prolonged period.

3.8. Characterization of polymorphic forms and phase separation

3.8.1. DSC—Pure CA exhibited a phase transition band at around 78 °C, which corresponded to its glass transition temperature (T_g), signifying that the API was amorphous (Figure 8). The DSC thermograms for Gelucire® 43/01, Gelucire® 44/14, and Kolliphor® TPGS showed endothermic peaks at their corresponding melting points (Figure 8). Gelucire® 44/14 exhibited two endothermic peaks associated with structural and polymorphic variability because it is a multicomponent lipid system. This was attributed to the complex polymorphic profile of glyceride lipids (Otun et al., 2015). However, the thermogram of extruded granules showed endothermic peaks corresponding to polyglycolized glycerides, but no evidence of a glass transition (T_g) representative of CA. These changes in enthalpy were due to homogenous extrudates and the absence of multiple glass transition temperatures, which confirmed that there was no phase separation between CA and the lipid excipients. Therefore, DSC analysis was indicative of the presence of a homogenous distribution of CA in the lipid matrix.

3.8.2. XRPD Analysis—XRPD analysis was performed to characterize polymorphs of unprocessed and processed CA in each formulation. The broad and diffuse diffraction peak of pure CA indicated its amorphous nature. The diffraction patterns of optimized formulation F8 and the physical mixture showed high intensity characteristic peaks which were attributed to Kolliphor® TPGS, which is crystalline (Figure 9).

However, peak intensity was reduced in the diffractogram of formulation F8, confirming homogeneity of the extruded formulation. The large peaks in the diffractogram of the physical mixture were due to the pure crystalline form of Kolliphor® TPGS. Therefore, the chosen screw design provided high shear energy, sufficient 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.

3.8.3. Hot-stage polarized microscopy—Homogeneity of CA was assessed using polarized light hot-stage microscopy. At room temperature, in the presence of polarized light, the irregularly shaped amorphous form of CA was clearly observed. Conversion of amorphous CA to its glassy form was observed as the temperature of the stage increased.

Optimized extrudates did not show any significant phase separation, even after reaching the melting points of both included lipids, indicating that CA was homogeneously distributed in the lipid matrix (Figure 10).

3.9. Chemical interactions between the API and excipients (FTIR)

The objective behind studying the FTIR spectra of CA and excipients was to investigate any possible interactions between the drug and lipid carriers. CA showed two absorption bands

corresponding to carbonyl groups at 1678 cm^{-1} and 1680 cm^{-1} , which were assigned to amide and carbonyl group stretching (Figure 11).

For CA, the peak at 1760 cm^{-1} was assigned to for carbonyl group stretching in the acetate, and the absorption bands for NH and NH_2 complex were seen from 3260 cm^{-1} to 3480 cm^{-1} . All major peaks assigned to carbonyl stretching vibrations were present at lower intensity in formulation F8 as compared to the pure CA. This was indicative of a weak interaction between CA and the excipients. It is possible that the C=O group of CA formed hydrogen bonds with -OH groups of Gelucire lipids, which agreed with results from a previous study (Shimpi et al., 2005).

3.10. *In vivo* pharmacokinetic study

The T_{max} of cefuroxime for groups administered pure CA and the marketed formulation was less than 2 h, as demonstrated by more rapid release than the group administered optimized floating formulation (F8), which had a T_{max} of 8 h, indicating sustained drug release from the floating granules. C_{max} of cefuroxime was significantly higher ($p<0.05$) for the optimized formulation (F8) than that of pure CA and the marketed formulation, indicating enhanced absorption of the drug from the optimized formulation (Figure 12).

The $\text{AUC}_{0-24\text{h}}$ of the optimized formulation F8 (15816 ng h/mL) was significantly higher ($p<0.05$) than that of pure CA (3184 ng h/mL) and the marketed formulation (8728 ng h/mL), indicating a 4-fold increase in bioavailability of cefuroxime compared to pure CA (Table 3). In addition, the higher half-life $t_{1/2}$ ($\sim 23\text{ h}$) of formulation F8 suggested sustained release of the drug from the optimized floating granules. Thus our *in vivo* pharmacokinetic study demonstrated that the optimized floating lipid granules potentially enhanced the bioavailability of cefuroxime.

4. CONCLUSION

Lipid-based gastro-retentive floating granules loaded with amorphous cefuroxime axetil were successfully prepared using HME technology. The floating formulations were well-characterized, and effects of various factors on properties of the granules were investigated. The granules had excellent flow properties and floated for more than 8 h in SGF (without enzymes). The granules also provided for sustained drug release for up to 12 h, which will ensure controlled dissolution and absorption of the drug in the proximal part of the intestine. These results demonstrated improved processability of cefuroxime axetil in the presence of lipids using HME. HME allowed for preparation of a drug delivery system with desired characteristics that will allow for improved absorption of CA. Pharmacokinetic studies of floating granules, pure CA, and the marketed formulation in rats revealed that optimized formulation F8 is a promising formulation to improve oral bioavailability of cefuroxime through more complete dissolution of CA, longer residence time in the stomach, and potentially decreased enzymatic degradation due to presence of lipids. This lipid drug delivery system prepared via HME significantly improved performance of the drug *in vitro* and *in vivo*.

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(A)

(B)

Figure 1.
(A) Enzymatic degradation of cefuroxime axetil to cefuroxime (B) Schematic of lipid-based floating drug delivery system containing cefuroxime axetil

Figure 2.
Standard screw configuration used to prepare the extrudates

Figure 3. Experimental assembly for measurement of floating strength of the granules (A) Before and (B) After immersing in 0.1 N HCl

Figure 4.

(A) True density, (B) Carr's indices, and (C) Hausner's ratios for formulations and pure drug (n=3), *P<0.05, F8 compared to other formulations ***P<0.05, Pure drug compared to other formulations

Figure 5.
Scanning electron microscope images of F1, F2, F5, and F8 formulations

In vitro drug release in SGF (pH 1.2)

Figure 6.
Drug release profiles of cefuroxime axetil from floating granules in pH 1.2 simulated gastric fluid (without enzymes) (n=3)

Figure 7.
Specific floating strength profiles of each formulation (n=3)

Figure 8.
Differential scanning calorimetry thermograms of CA, lipids, and the optimized formulation

Figure 9.
X-Ray Powder Diffraction patterns for pure CA, physical mixture, and optimized formulation F8

Figure 10.

Hot stage micrographs of (A) Pure CA at room temperature; (B) Pure CA above its glass transition temperature; (C) Extrudates at 50 °C

Figure 11.

FTIR Spectra of CA, Gelucire® 43/01, Kolliphor® TPGS and Formulation F8

Figure 12.
Log of plasma cefuroxime concentration vs time curve of pure CA, marketed formulation, and formulation F8

Table 1.

Thermal properties of Cefuroxime Axetil and formulation components

Cefuroxime Axetil (CA)	$T_g = 77.4\text{ }^{\circ}\text{C}$
Gelucire® 43/01	$T_m = 42.5\text{ }^{\circ}\text{C}$
Gelucire® 44/14	$T_m = 42.7\text{ }^{\circ}\text{C}$
Kolliphor® TPGS	$T_m = 37\text{ }^{\circ}\text{C}$

Table 2.

Experimental formulation compositions of floating granules

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 3.

Pharmacokinetic parameters of pure CA, marketed formulation, and optimized formulation F8 after a single oral dose of 20 mg/kg to rats

Parameter	Pure CA	Marketed Formulation	Optimized Formulation F8
C_{max} (ng/mL)	480.6±45.7	1227.3±293.8 ^{*,#}	2382±108.9 ^{*,#}
T_{max} (h)	2	2	8 ^{*,#}
AUC_{0-24h} (ng h/mL)	3184±120.4	8728±1252.2 ^{*,#}	15816±1556 ^{*,#}
t_{1/2} (h)	6±0.8	10.1±1.4 [*]	22.7±8.4 [*]

Each value represents the mean ± S.D. (n = 4).

^{*} P < 0.05, compared to pure CA;

[#] P < 0.05, compared to marketed formulation.