Cubosomes for Enhancing Intestinal Absorption of Fexofenadine Hydrochloride: In situ and in vivo Investigation

Amal A Sultan, Nourhan F El Nashar, Shimaam M Ashmawy, Gamal M El Maghraby

Department of Pharmaceutical Technology, College of Pharmacy, University of Tanta, Tanta, Egypt

Correspondence: Amal A Sultan, Department of Pharmaceutical Technology, College of Pharmacy, University of Tanta, Tanta, Egypt, Tel +20403336007, Fax +20403335466, Email amal.sultan@pharm.tanta.edu.eg

Purpose: The aim of this work was to probe cubosomes for enhanced intestinal absorption and oral bioavailability of poorly absorbable fexofenadine HCl (FEX-HCl).

Materials and Methods: Two cubosomal systems were fabricated utilizing glyceryl mono-oleate, a lyotropic mono lamellar lipid as oil phase and poloxamer407 as stabilizer at weight ratios of 8:2 and 7:3. The morphology of cubosomes was researched using transmission electron microscopy (TEM) and particle size was measured using photon correlation spectroscopy. FEX-HCl release was monitored in vitro. The effect of cubosomal encapsulation on intestinal absorption was assessed using in situ rabbit intestinal perfusion technique. Carrageenan induced rat paw edema model was utilized to monitor in vivo anti-inflammatory effect before and after cubosomal encapsulation.

Results: TEM revealed the existence of spherical and polygonal nanostructures arranged in honeycomb organization. Size measurement reflected nanoparticles with reduced size at higher poloxamer concentration. Release studies revealed liberation of FEX-HCl from cubosomes based on Higuchi kinetics model. The intestinal permeability data indicated incomplete absorption of FEX-HCl from simple aqueous solution with P-glycoprotein efflux contributing to this poor intestinal absorption. Incorporation of FEX-HCl in cubosomes enhanced membrane transport parameters. The intestinal absorption did not correlate with drug release suggesting that drug release is not the rate limiting with possible intact cubosomal transport. Cubosomal encapsulation of FEX-HCl significantly enhanced its in vivo anti-inflammatory efficacy compared to the aqueous FEX-HCl dispersion.

Conclusion: Cubosomes are promising novel carriers for enhancing intestinal absorption of FEX-HCl. Intact FEX-HCl-cubosomal absorption is possible via trans-lymphatic pathway but this requires further investigations.

Keywords: fexofenadine, cubosomes, release kinetics, in situ perfusion, correlation

Introduction

Oral medicament administration is the most preferred for drug delivery regarding its convenience and patient compliance. However, innate physicochemical properties of newly developed active pharmaceutical ingredients (APIs) may hamper the feasibility of development of a suitable oral delivery system. Oral delivery can be complicated by poor drug dissolution and/or poor permeability through the gastrointestinal membrane. This process is governed by the biopharmaceutical classification system. Biopharmaceutical class III APIs express compromised oral bioavailability owing to their hydrophilic nature with subsequent limited biological membranes permeability. FEX-HCl is an FDA approved anti-histaminic medicament for allergic rhinitis and chronic idiopathic urticaria management. It is considered to be more advantageous second-generation histamine H1 receptor antagonist concerning its selective, non-anticholinergic, non-sedating and safer effect. Unfortunately, FEX-HCl belongs to BCS class III drugs and exhibits diminished oral bioavailability (33%) with the P- glycoprotein (P-gp) efflux being implicated as a key parameter in limiting its intestinal permeability. There are many evolving approaches for enhancement of FEX-HCl membrane permeability and oral bioavailability. These include preparation of FEX-HCl/cyclodextrin inclusion complex, phospholipid complexation. Using the solid dispersion approach, membrane permeability...
modulators such as gelucire 44/14, tocopheryl polyethylene glycol 1000 succinate and poloxamer188 were adopted to improve the intestinal permeability of FEX-HCl.1,9 Lipid based systems were also tried for augmented intestinal absorption of FEX-HCl. These systems were fabricated microemulsion.8

Cubosomes which are developed by self-assembly of amphiphilic lipid like peceol (glycerol mono-oleate) or phytantriol in presence of amphiphilic stabilizer like poloxamer can provide promising tool for enhanced oral bioavailability of drugs. Utilization of lyotropic non-lamellar lipid was reported to create liquid crystalline nanostructures through cubic phase assembly. These nanoarchitectures forms cubosomes upon dispersion.12,13 Cubosomes have the capacity to carry hydrophilic, lipophilic and amphiphilic drug molecules. Cubosomes showed promising potential in delivering active pharmaceutical ingredients via different routes of administration including oral, ocular and transdermal routes.14–16 However, the mechanism of enhanced oral bioavailability from cubosomal formulation requires verification. Accordingly, the objective of this work was to evaluate cubosomes for oral delivery of FEX-HCl hydrochloride. The scope was extended to correlate the in situ intestinal absorption with the in vitro release data to characterize possible mechanisms for enhanced oral drug availability after cubosomal administration.

**Materials and Methods**

**Materials**
FEX-HCl was procured from Amoun for Pharmaceutical Industries, Cairo, Egypt. Peceol (glyceryl mono-oleate) was obtained as a gift sample from Gattefosse, Saint Priest Cedex, France. Poloxamer407 was donated by Sigma for Pharmaceutical Industries, Quesna, Egypt. Ethanol, sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate and o-phosphoric acid were obtained from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.
Preparation of the Tested Cubosomes Formulation

Cubosomes are nanostructured systems self-assembled from amphiphilic lipids in water with the aid of suitable stabilizers.\textsuperscript{14,15,17} Glyceryl mono-oleate (Peceol), a lyotropic mono lamellar lipid was selected as the lipid phase with Poloxamer407 being utilized as stabilizing polymer.\textsuperscript{18,19} The composition of the selected cubosomal formulations included peceol and Poloxamer407 at a weight ratio of either 8:2 or 7:3, respectively (Table 1). FEX-HCl-loaded cubosomes were prepared through controlled hydration of peceol/poloxamer407 mixture using homogenization technique. This technique was previously utilized by other investigators for cubosomes preparation.\textsuperscript{20,21} Briefly, peceol and poloxamer407 were mixed and heated on water bath at 60°C till complete melting. FEX-HCl was added to the melt with continuous stirring before gradual addition of water heated to similar temperature and agitation for 30 minutes by overhead homogenizer (Virtis-s23, USA) to give a crude homogenous dispersion. Cubosomal dispersion was equilibrated for an overnight at room temperature before bath sonication for 15 minutes (Elmasonic S23, Germany).

Transmission Electron Microscopy (TEM)

Morphological analysis of the liquid crystalline cubosomes applied TEM analysis using transmission electron microscope (JEOL-JSM-1400 PLUS, Tokyo, Japan). This involved loading the tested cubosomal dispersions on copper grid prior to staining with uranyl acetate for 5 minutes and lead citrate for 2 minutes, subsequently. TEM photomicrographs were captured for the air dried stained cubosomes samples.

Particle Size Analysis

The prepared cubosomal dispersions were suitably diluted with double filtered water and sonicated for few minutes to create uniform clumps-free dispersions. The mean particle size and polydispersity index (PDI) were assessed utilizing dynamic light scattering technique (DLS). This employed a Nano-ZS Zetasizer (Malvern Instruments Ltd., Worcestershire, UK). Particle size analysis was performed at 25°C with light scattering at an angle of 90°. The computed data were presented as the mean particle size (Z-average) ± the standard deviation (SD).

Assay of Drug

Analysis of in vitro release samples employed UV spectrophotometry but quantification of FEX-HCl in the in situ intestinal perfusion samples was accomplished using high pressure liquid chromatography (HPLC).

Spectrophotometric FEX-HCl analysis was conducted at 218 nm using UV-visible spectrophotometer (Thermo Fisher Scientific, USA). Standard drug solutions at concentrations of 10, 12, 15, 18 and 20 μg/mL were prepared in 0.001N HCl, phosphate buffered saline (pH 6.8) and phosphate buffered saline (pH 7.4). The absorbance of each was recorded and used to construct the calibration graphs that were utilized for subsequent quantification of FEX-HCL in the release studies at the corresponding pH value.

Chromatographic drug analysis was accomplished employing HPLC system (Agilent Technologies 1260 infinity, DE, Germany). The system is supplied with a variable wavelength UV detector (VWD 1260) and an automated sampling system (TCC 1260). This computer aided system-control utilizes Agilent Open LAB ChemStation software. Isocratic drug separation was attained employing a reversed phase column (ODS, 15 cm x 4.6 mm) with an average particle size of 5 μm (GL Sciences Inc., Tokyo, Japan). Chromatographic analysis of FEX-HCl was accomplished following the methodology established by Oliveira and coworkers with slight modification.\textsuperscript{22} The mobile phase comprised a mixture of 0.01 M potassium di-hydrogen phosphate buffer containing 0.1% v/v of triethylamine (pH adjusted to 3.2 with o-phosphoric acid), acetonitrile and methanol at a ratio of 50:30:20 v/v/v, respectively. The samples injection volume was

| Table 1 The Composition of Tested Cubosomes Formulations |
|--------------------------------------------------------|
| Formula | Peceol (mg) | Poloxamer407 (mg) | Water (mL) | FEX-HCl (mg) |
|---------|-------------|------------------|------------|--------------|
| F1      | 1600        | 400              | 50         | 317          |
| F2      | 1400        | 600              | 50         | 317          |
programmed to be 30 μL with the mobile phase being allowed to flow at a rate of 1.2 mL/min and the column effluent was monitored at 218 nm.

**In vitro Release Studies**

FEX-HCl release from its prepared cubosomal formulations was monitored employing dialysis method. This used dialysis membrane (cellulose tubing, cut off 14000 Da, Sigma-Aldrich, St. Louis, MO), which was formerly soaked in water for 24 hours to ensure complete swelling and constant pore width throughout the whole experiment.23 The dialysis sacs were loaded with a known volume (2 mL) of the tested cubosomes dispersions and were incubated at 37°C in 100 mL of 0.001N HCl which was utilized as release medium. The dialysate was subjected to gentle stirring intermittently with 5 mL samples being collected at predetermined time points (1, 2, 3, 4, 6, and 8 h). The release medium was replenished after each sample with equivalent volume of fresh medium preheated to 37°C to maintain sink condition. Furthermore, F2 cubosomes were dialyzed against 100 mL phosphate buffered saline (PBS) adjusted to pH values of 6.8 or 7.4. Samples were collected at 10 minutes intervals for 2 hours with fresh release medium of equivalent volume being reloaded after each sample. This was conducted to mimic the in vivo physiological pH of the perfused intestinal segment which allow subsequent correlation analysis to be conducted between in vitro release and in situ membrane permeability data.24 The amounts of FEX-HCl in collected samples were analyzed spectrophotometrically and the cumulative amount released was expressed as percentage of the total amount in the dialysis sac. Release profiles were constructed via plotting percentage cumulative amount released as a function of corresponding time points. The release efficiency values were computed for the developed release profiles from the area under plot at time t relative to that calculated supposing 100% drug release at all time points.25 The release experiments were conducted in triplicates.

**In situ Intestinal Perfusion Studies**

FEX-HCl intestinal membrane transport was assessed from its aqueous solution and F2 cubosomes formulation using in situ rabbit intestinal perfusion technique. Twelve albino rabbits with an average weight of 2kg were employed in this study. The study protocol and animal manipulations were performed according to the Ethical Committee approval of College of Pharmacy, University of Tanta (approval number, 181218). This protocol conforms with the National Institute of Health guide for the care and use of laboratory animals. Drug absorption from four intestinal segments was investigated. The intestinal segments employed in the study were duodenum, jejunum, ileum and colon. The surgical procedures and segment preparation were achieved according to well-established methodology.26–28

Briefly, the rabbits under study were fasted overnight with free water supply. On the day of the experiment, the rabbit was injected by chlorpromazine HCl (2 mg/kg) as a muscle relaxant then anesthetized with ketamine HCl (50 mg/kg) in two sequential doses separated by 15 minutes. The anaesthetized rabbit was placed in a supine position lying on a heating pad. A midline abdominal longitudinal incision with an average length of 7 cm was made after shaving of hair and cleansing of the abdominal area.

The intestinal segments under study were exposed and their ends were tied off with surgical threads. The desired intestinal length of each isolated segment was adjusted and then cannulated. A three-way stopcock cannula was utilized to cannulate the proximal end receiving the perfusion solution, while L-shaped cannula was employed to cannulate the distal end for sample collection. This isolates the segment under study and eliminates the effect of food while maintaining tissue viability.

The measured intestinal lengths were adjusted at 15, 30, 30, and 10 cm for duodenum, jejunum, ileum, and ascending colon, respectively. Warm saline was utilized in order to cleanse the cannulated intestinal segments before assembling the selected segments in a multi-S-pattern to assure steady flow rate. The segments under study were kept moist and warm by repeated application of 37 °C normal saline to a gauze cover of the isolated segments.26

FEX-HCl was included in the control perfusion solution in phosphate buffered saline and cubosomal dispersion at a concentration of 22.5μg/mL. This concentration was estimated regarding the maximum daily dose of FEX-HCl and the average daily fluid input into gastrointestinal tract.27 The pH values of the prepared perfusion solutions were adjusted to 6.6 for perfusion into the duodenum, 6.8 for perfusion through the colon and to 7.4 for perfusion through jejunum and ileum segments. The temperature of the prepared solutions was adjusted to 37°C prior to perfusion. The pump flow rate
was set at 0.27 mL/min for two hours which was kept constant utilizing a Harvard-22 perfusion pump (Harvard Apparatus, Millis, MA, USA). Perfusate samples outflowing from the exposed segments were collected from the L-shaped cannula every 10 minutes for 2 hours. The volume of each sample was accurately measured before samples centrifugation for 5 minutes to precipitate any mucus debris. The samples collected during the second hour were analyzed for drug content employing the developed HPLC assay method to monitor drug absorption at steady state. Finally, the animal was sacrificed, and the isolated segments were excised for determining their actual lengths which were used for data analysis.

Analysis of Intestinal Perfusion Data
Data obtained from the in situ technique are used for determination of the net water flux and the membrane permeability parameters of the investigated drug. The water flux was determined by calculating the difference between the expected and the actual perfusate volumes. The calculated permeation parameters have been utilized to study the mechanism of drug transport throughout the gastrointestinal tract. The complete methodology for calculating the membrane transport parameters are documented in literature reviews. The following sections will summarize the employed methodology for data analysis.

Absorptive Clearance
The remaining amount of FEX-HCl in each sample (C_{out}) was determined according to the corrected concentration of the drug in the collected sample regarding the net water flux. The ratio between the corrected FEX-HCl concentration exiting the selected segment (C_{out}) and the amount of the drug flowing into the segment (C_{in}) was utilized to calculate the fraction of FEX-HCl remaining in each sample after perfusion. The remaining fraction at the steady state \{(C_{out}/C_{in})_{ss}\} was determined taking the mean of the remaining fractions in the collected samples of the second hour of perfusion. This was used to determine the permeability area product (Pe.A) which is known as the absorptive clearance determined in mL/min utilizing the following equation:

\[
\text{Pe.A} = -Q \times \ln\left\{\frac{C_{\text{out}}}{C_{\text{in}}}\right\}_{ss}
\]

Where \(\text{Pe}\) is a symbol for the apparent permeability coefficient (cm/min), \(A\) represents the effective surface area (cm\(^2\)) and \(Q\) denotes the average flow rate of the perfusate through the investigated segment (mL/min).

The fraction absorbed was calculated according to the following equation at the steady state:

\[
\text{Fa} = 1 - \left\{\frac{C_{\text{out}}}{C_{\text{in}}}\right\}_{ss} = 1 - \exp^{-(\text{Pe.A}/Q)}
\]

The anatomical reserve length (ARL) parameter is related to the intestinal drug absorption which is described as the intestinal length remaining after complete absorption of the drug. The ARL was determined according to the following equation:

\[
\text{ARL} = (L^*) - (l^*)
\]

Where \(L^*\) is the maximum anatomical length of the selected intestinal segment ready for absorption (cm) and \(l^*\) is the length of the intestinal segment required for complete drug absorption (cm).

In theory, the luminal drug concentration cannot be reduced to zero at the intestinal length \((l^*)\) due to the nature of logarithmic function. Consequently, an extremely small percent of the remaining solute in the intestinal lumen is thought to be an indicator for complete drug absorption. This percent is assumed to be 5% at it is substituted in Equation (1). Accordingly, the length required for 95% absorption of the drug \((L_{95\%})\) was determined utilizing following equation:

\[
0.05 = \exp^{-(\text{Pe.A}.l^*)/Q}
\]

Where Pe.A is the absorptive clearance normalized to intestinal length and \(l^*\) is \(L_{95\%}\) for the drug under study.2,26

Solvent Drag Effect on the Intestinal Absorptive Clearance
The mechanism of the drug transport through the intestinal membranes was investigated by studying the influence of water flux on the drug absorption. This was performed by plotting the absorptive clearance normalized to intestinal...
length (Pe.A/l) versus the net water flux per unit intestinal length (Jw/l) which is calculated from the difference between the predictable sample volume in a certain time interval (Qin) and the actual perfusate volume attained from the intestinal segment at the same time interval (Qout). This is stated by the next equation:

$$J_w = Q_{in} - Q_{out}$$ (5)

The amount of drug absorbed per unit time is reliant on the participation of two pathways of drug absorption that are the diffusive transcellular and the convective paracellular processes. Accordingly, the rate of drug absorption (Js) which is calculated in (µg/min) is determined from the following equation:

$$J_s = K_s (C - C_p) + \Theta_s J_w C$$ (6)

The diffusive process is represented by the part of equation \([K_s (C-C_p)]\) at which \(K_s\) is the drug diffusive permeability coefficient, \(C\) is the drug concentration in the intestinal lumen and \(C_p\) is the plasma drug concentration, while the convective process is represented by \((\Theta_s J_w C)\), at which \(\Theta_s\) is the sieving coefficient of the given drug. From Equation (6) we can make an approximation to Equation (7) during the steady state, considering the blood sink condition.

$$J_{ss} = \frac{D A K_p}{\Delta x (C_{ss})} + \Theta_s J_w C_{ss}$$ (7)

Where \(J_{ss}\) is the drug flux at the steady state (µg/min), \(D\) is the diffusion coefficient of the drug, \(K_p\) denotes the drug (octanol/water) partition coefficient, \(A\) symbolizes the effective surface area of drug absorption, \(\Delta x\) represents the path length and \(C_{ss}\) is the length averaged solute concentration in the gastrointestinal lumen at the steady state (µg/mL). As a result, rearrangement of the equation (7) gives:

$$\frac{J_{ss}}{C_{ss}} = \frac{D A K_p}{\Delta x} + \Theta_s J_w$$ (8)

The term \(J_{ss}/C_{ss}\) is the overall absorptive clearance of the given drug (mL/min) that is achieved by different permeation pathways, and it is calculated as the permeability surface area product Pe.A. Plotting the absorptive clearance versus the net water flux both normalized to the intestinal length yields a line with a slope representing a measure for the sieving coefficient (\(\Theta_s\)) and the intercept with the y-axis gives a measure for the transcellular diffusive contribution to the overall absorptive clearance.28

### In vivo Evaluation of Anti-Inflammatory Effect

The anti-inflammatory activity of cubosomes incorporated FEX-HCl was investigated employing carrageenan induced paw edema method.32 The protocol of study and animal manipulation procedures were approved by the Ethical Committee, College of Pharmacy, Tanta University (Approval number 181218). The study employed 24 male Wistar albino rats with an average weight of 200±20 g. Appropriate housing of the utilized animals was allowed for three days preceding the experiment for adaptation to lab conditions with free access to standard pellet diet and water being given. According to the experimental design, the rats were divided into three groups and fasted overnight prior to experiment with free access to water. The tested cubosomal formulation (F2) was orally administered to the first animal group and an aqueous dispersion of unprocessed FEX-HCl in distilled water (6.35 mg/mL) was administered to the second group (positive control group) utilizing a feeding syringe with the last group receiving no medication (negative control group). The orally administered volume was adjusted to deliver a dose equivalent to 15.87 mg/kg which was estimated relying on the FDA dose conversion tables.33 Acute inflammation induction involved intraplantar injection of 100 µL carrageenan solution in distilled water (1% w/v). Each rat was anaesthetized by ether inhalation and injected into the sub plantar region at midline of the right hind paw 15 minutes after oral FEX-HCl administration. Baseline paw volume measurement at zero time was performed immediately before carrageenan intraplantar injection using a Vernier caliper. Paw edema volume at predetermined post injection time intervals (0.25, 0.5, 1, 2, 3, 4, 5 and 6 h) was measured and % edema volume was computed using the following equation:

$$\text{Edema volume}(\%) = \left[\frac{(P_t - P_0)}{P_0}\right] \times 100$$

Where, \(P_t\) represents the paw volume at time t, and \(P_0\) is the paw volume at time zero.
The edema formation curve was constructed by plotting the recorded % edema volume values as a function of time. Area under the curve (AUC) represents the total edema formation and was calculated to assess the efficacy of the tested cubosomal formulation relative to control groups.\textsuperscript{35,36}

\textbf{Statistical Analysis}

Kruskal–Wallis test was applied to assess statistical data significance (P<0.05). Individual variation between tested formulations was explored utilizing post-hoc Tukey’s multiple comparison. These were performed using SPSS 23.

\textbf{Results}

\textbf{Transmission Electron Microscopy (TEM)}

\textbf{Figure 1} shows representative transmission electron micrographs of cubosomes prepared using peceol with poloxamer407 at weight ratios of 8:2 and 7:3. The dispersion employed homogenization of 1 gram of each mixture in 25mL of water. This provides final poloxamer concentration of 0.8\% and 1.2\% w/v, respectively. For cubosomes incorporating lower poloxamer concentration (F1), TEM micrographs revealed spherical nanostructures free from any aggregates (\textbf{Figure 1A–C}). Increasing poloxamer concentration in F2 cubosomes resulted in creation of spherical and polygonal structures arranged in honeycomb organization (\textbf{Figure 1D–F}). This morphological discrepancy of the micro-graphed cubosomes was reported by previous investigators who prepared cubosomes using lyotropic lipid with poloxamer.\textsuperscript{16} The mean particle size values for the captured nanostructures were calculated to be 106±37 nm and 98±52 nm for cubosomes containing lower and higher poloxamer concentrations, respectively.

\textbf{Particle Size Analysis}

\textbf{Figure 2} shows the particle size distribution of the tested cubosomes. The recorded average particle size values were 162.1 nm (SD= 46.8) and 112.2 nm (SD= 7) for F1 and F2 cubosomes, respectively. This correlates to the recorded particle size rank measured from TEM micrographs. Moreover, the computed polydispersity index value for F1 formulation was 0.202 (SD = 0.016) with the value recorded for F2 formulation being increased to 0.317 (SD = 0.015) reflecting higher discrepancy for the fabricated F2 cubosomes. This finding is supported by the morphological investigations which showed the development of mixed nano-architectures.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image1}
\caption{Transmission electron micrographs of F1 (A–C) and F2 (D–F). Formulation details are in Table 1.}
\end{figure}
Assay of Fexofenadine HCl

The UV spectrophotometric analysis was successfully utilized for FEX-HCl quantification in drug release studies employing 0.001N HCl, PBS (pH 6.8) and PBS (pH 7.4) as release media. The calibration graphs were constructed in the corresponding release media and were linear in the range of 10–20 μg/mL.

The HPLC method eluted FEX-HCl after 4.84 and 4.78 minutes after injecting FEX-HCl solutions in phosphate buffered saline, pH 6.8 and 7.4, respectively. The standard calibration graphs were linear in the concentration range of 0.5–20 μg/mL for drug solutions prepared in both pH values. The equations of the standard curve were Y = 51.976X −1.6612 and Y = 52.439X +2.3633 at pH values of 6.8 and 7.4, respectively with a correlation coefficient of 0.9996 and 0.9998, respectively.

In vitro Release Studies

The release rate of FEX-HCl from fabricated cubosomes formulations was monitored using 0.001N HCl as release medium. The recorded release profiles for F1 and F2 cubosomes are shown in Figure 3A. These profiles revealed sustained drug release from both cubosomal formulations with almost 98.8% and 97.5% of the loaded drug being released after 8 hours from F1 and F2 formulations, respectively. This was further evidenced from the computed overall release efficiency values which were 60.3% ± 1.5% for F1 cubosomes and 58.6%± 1.2% for F2 cubosomes. With respect to the FEX-HCl release rate constant, there was no significant difference between F1 and F2 cubosomes.

FEX-HCl release from F2 cubosomes was monitored at pH values of 6.8 and 7.4 for 2 hours at 37°C simulating the experimental conditions of in situ perfusion studies. This permits subsequent correlation analysis between the released amounts of FEX-HCl and in situ intestinal drug absorption at the same time points. Figure 3B presents the release profiles constructed for F2 cubosomes at pH values of 6.8 and 7.4 relative to that recorded using 0.001N HCl as release medium.
medium (pH 3). The computed 2 hours release efficiency values were 20.0%, 11.8% and 12.8% at pH values of 3, 6.8 and 7.4, respectively. The results revealed significantly reduced release efficiency at higher pH values compared to that recorded in 0.001N HCl (P < 0.05).

The release kinetics of FEX-HCl from cubosomes were estimated by fitting the data to zero, first, and Higuchi release kinetics models. Linear regression was conducted for the data after fitting to different models and the correlation coefficient ($R^2$) was calculated for each model. The calculated $R^2$ values are presented in Table 2. The fitting reflected that the cubosomes liberated FEX-HCl by Higuchi release kinetics suggesting matrix diffusion-based release.

In situ Intestinal Perfusion of Fexofenadine HCl

The primary objective of this research was to investigate the efficacy of cubosomes for intestinal absorption enhancement. The in situ intestinal membrane transport parameters of FEX-HCl are presented in Table 3. Perfusion of simple aqueous solution of FEX-HCl showed incomplete absorption of FEX-HCl from the tested intestinal segments. The incomplete absorption was noticed from the membrane transport parameters which reflected negative values for the ARL which were calculated to be $-308.2$, $-930.7$, and $-977$ and $-217.4$ cm in cases of the duodenum, jejunum and ileum and colon, respectively. The calculated $L_{95\%}$ values were $328.3$, $1050.7$, $1037$, $232.4$ for duodenum, jejunum and ileum and colon, respectively.

### Table 2: Correlation Coefficient ($R^2$) Values Obtained from Linear Regression Analysis of FEX-HCl Release Data Fitted to Different Release Models

| Formula | Release Medium | Zero Order  | 1st Order | Higuchi  |
|---------|----------------|------------|-----------|----------|
| F1      | 0.001N HCl     | 0.949 (0.004) | 0.939 (0.023) | 0.980 (0.003) |
| F1      | 0.001N HCl     | 0.966 (0.007) | 0.896 (0.06) | 0.973 (0.006) |
| F2      | PBS, pH 6.8    | 0.978 (0.003) | 0.980(0.06) | 0.984 (0.006) |
| F2      | PBS, pH 7.4    | 0.970 (0.026) | 0.966 (0.023) | 0.975 (0.003) |

**Notes:** PBS is phosphate buffered saline. Drug release from F2 was monitored at different pH values to allow correlation with in situ intestinal absorption. Values between brackets are SD (n=3).
Table 3 Membrane Transport Parameters of FEX-HCl After Perfusion Through Different Segments of Rabbit Intestine in the Form of Simple Aqueous Solution or F2 Cubosomal Dispersion

| Parameters | Duodenum | Jejunum | Ileum | Colon |
|------------|----------|---------|-------|-------|
| PeA/L (mL/min.cm) | 0.0036 (0.0012) | 0.0009 (0.0000) | 0.0012 (0.0003) | 0.0051 (0.0014) |
| Rout/Rin | 0.8029 (0.0656) | 0.9027 (0.0047) | 0.8586 (0.0393) | 0.8052 (0.0604) |
| L95% (cm) | 328.2 (94.95) | 1050.7 (211.02) | 1037 (419.10) | 232.4 (54.14) |
| %Fa/L (%cm⁻¹) | 1.32 (0.4549) | 0.3250 (0.0279) | 0.4711 (0.1311) | 1.874 (0.5311) |
| JW/L (mL/min.cm) | 0.0031 (0.0011) | 0.0010 (0.0004) | 0.0023 (0.0005) | 0.0046 (0.0017) |
| ARL (cm) | −308.2 (94.95) | −930.7 (211.02) | −977.0 (419.10) | −217.4 (54.14) |

Incorporation of FEX-HCl into cubosomes increased the intestinal absorption of the drug compared with the corresponding aqueous solution. The absorptive clearance normalized to segment length was increased by 1.25, 4.78, 2.25 and 2.22-fold after perfusion of FEX-HCl cubosomes in case of duodenum, jejunum, ileum and colon, respectively. This increase in the absorptive clearance was associated with noticeable increase in the contribution of the transcellular pathway in drug absorption from all segments. This was estimated from the absorptive clearance versus length-normalized water flux plots constructed for FEX-HCl loaded cubosomes (Figure 5). For example, the % transcellular absorption was increased from zero to 64.41% in case of ileum after perfusion of cubosomal dispersion (Table 4).
Correlation analysis of the intestinal absorption data to the released amounts of FEX-HCl at the same time points, determined under the same experimental conditions was conducted. This employed bivariate Pearson’s correlation. The computed results reflected no in vitro-in situ correlation which was shown from the recorded non-significant Pearson’s correlation coefficient “r” values (P > 0.05). The computed “r” values were 0.749, 0.127, −0.270, −0.257 for the correlation between drug release and % fraction absorbed from duodenum, jejunum, ileum and colon, respectively (P > 0.05).

Evaluation of Anti-Inflammatory Effect
The anti-inflammatory effect of FEX-HCl was monitored using carrageenan provoked paw edema technique. Figure 6 shows the increase in paw volume due to edema formation as a function of time after administration of FEX-HCl in the form of aqueous suspension or cubosomes. The edema formation was monitored with reference to that induced in rats administering plain water (negative control). Injection of carrageenan to the control rats resulted progressive swelling to
reach maximum volume (peak edema) 3 hours after carrageenan injection. The edema started to resolve slowly after the maximum but remained above 60% increase in the paw volume after 6 hours. The area under the edema formation curve was 407.27% hour for the negative control group. Administration of a single dose FEX-HCl simple suspension resulted in marginal reduction (non-significant) in the edema volume compared to negative control group. The AUC was non-significantly reduced (P > 0.05) to reach 355.25% hour. Administration of FEX-HCl cubosomal dispersion resulted in significant reduction in edema formation compared with the negative control group or that treated with simple suspension. The AUC was significantly reduced (P < 0.05) to reach 157.61% hour after administration of cubosomes (Figure 6).

**Discussion**

Two FEX-HCl loaded cubosomal formulations were constructed. These formulations, F1 and F2 incorporated peceol and poloxamer407 at weight ratios of 8:2 and 7:3, respectively. Morphological evaluation of F1 cubosomes using TEM revealed spherical nanostructure with F2 cubosomes presenting spherical and polygonal nanoparticles. Organization of cubosomes as honeycomb architecture was revealed for F2 cubosomes (Figure 1F). This complies with the literature describing honeycomb structural organization for cubosomes. The tested poloxamer407 concentrations were in the acceptable range which was previously reported. The recorded morphology complies with the published data which suggested development of spherical and polygonal cubosomes. The recorded size values for F1 and F2 formulations were comparable. Particle size distribution data correlate to that recorded employing TEM. The computed PDI value for F1 cubosomes revealed homogenous nanostructured particles. Higher PDI value was shown for F2 formulation supporting the morphological discrepancy recorded using TEM analysis. These results conform with the published work on similar formulations with size reduction being recorded at higher poloxamer concentrations.

The UV spectrophotometric analysis was sensitive, selective and accurate enough to successfully quantify the amounts of FEX-HCl released in vitro using 0.001N HCl, PBS (pH 6.8) and PBS (pH 7.4) as release media.

The developed HPLC method was successfully employed to determine the concentration of FEX-HCl in the perfusate which was obtained after in situ intestinal perfusion study. The selection of HPLC was based on its ability to selectively detect and quantify FEX-HCl without interference from endogenous materials which may be eluted in the perfusate samples.

Previous investigations highlighted that the maximum concentration of poloxamer407 that can impart stabilization and size reduction of cubosomal systems is 1.5% w/v, above this concentration no further size reduction can take place. Accordingly, formulation containing poloxamer407 with peceol at a weight ratio of 3:7 (F2 cubosomes) was selected for in situ and in vivo evaluation.

In vitro release studies of FEX-HCl encapsulated cubosomes revealed sustained drug release over 8 hours. This pattern was recorded for both F1 and F2 cubosomes. Release sustainability of cubosomes encapsulated drugs was previously reported by other investigators.

| Table 4 | The Percentage of FEX-HCl Absorbed via Transcellular and Paracellular Pathways from Its Aqueous Solution or F2 Cubosomal Dispersion |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
| **Aqueous Solution** |                                                                                                                                  |
| Absorption Pathway | Duodenum | Jejunum | Ileum | Colon |
| Transcellular (%) | 35.79 | 57.51 | 0 | 31.23 |
| Paracellular (%) | 64.21 | 42.49 | 100 | 68.77 |
| **Cubosomes** |                                                                                                                                  |
| Absorption Pathway | Duodenum | Jejunum | Ileum | Colon |
| Transcellular (%) | 46.32 | 75.05 | 64.41 | 55.78 |
| Paracellular (%) | 53.68 | 24.95 | 35.59 | 44.22 |

https://doi.org/10.2147/IJN.S370235

DovePress
International Journal of Nanomedicine 2022:17

Sultan et al

DovePress

Powered by TCPDF (www.tcpdf.org)
FEX-HCl release from cubosomes formulation selected for in situ intestinal permeability investigation was monitored at pH values 6.8 and 7.4 for 2 hours. This allows for investigation of the effect of drug release on intestinal absorption. FEX-HCl release from F2 cubosomes revealed pH dependent release pattern. This was evidenced from the significantly reduced release efficiency values at pH values 6.8 and 7.4 compared to that recorded using 0.001N HCl as release medium. This can be explained taking into consideration the reported pH dependent solubility of FEX-HCl. Reduced FEX-HCl solubility at the higher pH values increases its partitioning into cubosomal lipid bilayer with subsequent reduction of release efficiency.

The release kinetics of FEX-HCl from cubosomal formulations followed Higuchi model suggesting matrix diffusion-based release. This may be ascribed to the possible cubosomal lamellar structure. Similar release kinetics were recorded by other investigators from cubosomes. Similar release rate was recorded from the tested cubosomal formulations employing 0.001N HCl as release medium. This is acceptable taking into consideration the composition of both formulations which differs only in relative proportions of peceol to poloxamer407. Both formulations were physically

Figure 5 Absorptive clearance of fexofenadine HCl from its F2 cubosomal dispersion as a function of water flux in different intestinal segments: (A) duodenum, (B) jejunum, (C) ileum and (D) ascending colon.
similar with particles of comparable size and it will be acceptable to have similar release pattern taking into consideration
the recorded release kinetics which depended on the architecture of cubosomes.

In situ rabbit intestinal diffusion technique was selected to monitor the efficacy of cubosomes for intestinal absorption
enhancement. This selection depended on the reported advantages of such model. This strategy eliminates the effect of
complicating factors such as food, drug dissolution and the variability of stomach residence time. In addition, the model
allows preservation of the tissue viability with the rabbit intestine providing additional advantage of good correlation
with human intestinal structure. Such strategy provides chance for deduction of a lot of information about the intestinal
absorption, its pathways and limiting factors.\textsuperscript{26,27,31,43,44} Perfusion of simple aqueous solution of FEX-HCl showed
incomplete drug absorption from the tested intestinal segments. The recorded incomplete absorption of FEX-HCl from
simple aqueous solution correlates with previous literature reports which categorized FEX-HCl as class III drug due to its
poor permeation through biological membranes.\textsuperscript{1,9,11} The recorded ARL values reflect site dependent absorption with
poor absorption from the distal parts of the small intestine. The regional difference suggests possible contribution for the
P-gp efflux transporter in limited intestinal absorption of FEX-HCl. This suggestion is based on the regional expression
of P-gp in the intestine which is expected to be more abundant in the ileum followed by the jejunum.\textsuperscript{28,45,46} The
contribution of P-gp efflux to the limited intestinal absorption of FEX-HCl has been highlighted in literature reports.\textsuperscript{1,9,11}

The correlation between water flux and absorptive clearance reflected dependence of the absorptive clearance on the
water flux suggesting a role of paracellular pathway in the overall absorptive clearance of FEX-HCl. The existence of
100% paracellular absorption in the ileum is surprising taking into consideration its relatively large surface compared
with the colon but this may be attributed to the effect of P-gp efflux transporters which minimize the transcellular
absorption. These transporters are expressed to greater extent in the ileum.\textsuperscript{28} Incorporation of FEX-HCl into cubosomes
increased the intestinal absorption of the drug compared with the corresponding aqueous solution. Interestingly, the
increase in the absorptive clearance was associated with noticeable increase in the contribution of the transcellular
pathway in drug absorption from all segments. These findings suggest that drug loaded cubosomes permeate mainly via
transcellular route. The suggested permeation pathways of cubosomal FEX-HCl is schematically illustrated in Figure 7.
Another supposition depends on the ability of cubosomal components to fluidize the intestinal membrane with subse-
quent enhancement of membrane permeability. Enhanced trans-lymphatic transport is another possibility for augmented
intestinal absorption from cubosomes. The later pathway may provide the benefit of bypassed hepatic metabolism. The

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{The edema formation profile after induction with carrageenan and administration of fexofenadine HCl as cubosomes (F2) or aqueous suspension. Details of formulation are presented in Table 1. All the values are expressed as the mean ± S.E.M (n=7).}
\end{figure}
mechanisms of enhanced intestinal absorption from cubosomes were highlighted and similar suggestions were hypothesized.\textsuperscript{17,47} The effect of cubosomal components on intestinal absorption is also documented in literature reports. Peceol which is the principle component of cubosomes was shown to increase intestinal membrane permeability via inhibition of P-gp drug efflux and/or augmenting trans-lymphatic transport of drugs.\textsuperscript{48} The P-gp inhibitory effect was indicated from in vitro studies employing Caco-2 cell culture which proved down-regulation of P-gp expression in presence of peceol.\textsuperscript{49,50} Moreover, poloxamer407 which is the second component of cubosomes is believed to impart membrane permeabilization in addition to its capacity to inhibit the CYP 450 enzymes. The later can reduce presystemic metabolism which can be shown in vivo.\textsuperscript{51}

The anti-inflammatory effect of FEX-HCl cubosomes was researched to verify the recorded enhanced in situ intestinal absorption using carrageenan provoked paw edema technique. Carrageenan induced edema formation was monitored after administration of plain water (negative control), FEX-HCl simple aqueous dispersion or cubosomal formulation. The negative control rats produced typical edema formation curve which was similar to that recorded by other researchers who employed the same.\textsuperscript{32,34,52,53} Administration of a single dose FEX-HCl simple suspension was not effective. The recorded non-significant effect of FEX-HCl aqueous dispersion correlates with the recorded in situ intestinal perfusion data which reflected its incomplete absorption from the GIT. This implies poor bioavailability after oral administration which has been shown by other investigators who classified FEX-HCl as poorly permeable drug.\textsuperscript{1,9} Poor permeability of FEX-HCl was also assisted by P-gp efflux which has been reflected in our in situ intestinal perfusion studies and highlighted in literature reports.\textsuperscript{1,9,11} The recorded enhancement in the anti-inflammatory effect of FEX-HCl after cubosomal administration correlates with the recorded increase in the intestinal permeability from cubosomes. This can suggest that cubosomal dispersion is able to enhance drug absorption of poorly permeable drugs. Alternative mechanisms have been suggested for

\textbf{Figure 7} Schematic illustration of the probable intestinal absorptive pathways of fexofenadine HCl encapsulated cubosomes.
enhanced oral bioavailability from cubosomes. Being colloidal dispersion, cubosomes can traverse the intestine via lymphatic pathway bypassing the negative effects of pre-systemic disposition\textsuperscript{17,27,47} The effect of colloidal nature of cubosomes is further magnified by the nature of the primary components. For example, peceol is believed to inhibit P-gp efflux and augment trans-lymphatic transport of drugs\textsuperscript{48–50} Poloxamer can also contribute by permeabilization of intestinal membrane with additional inhibitory effect on CYP 450 enzymes\textsuperscript{51} These factors combine to explain the recorded increase in the in vivo efficacy of FEX-HCl after administration in cubosomal formulation.

To understand further the mechanism of enhanced intestinal absorption of FEX-HCl from cubosomes, the intestinal absorption data were correlated to the amount of drug released at the same time points, determined under the same experimental conditions. The computed Pearson’s correlation coefficient values were non-significant in all intestinal segments with negative correlation being noticed in ileum and colon segments. The results indicated that drug release is not the rate limiting factor in enhanced intestinal absorption of FEX-HCl from cubosomes. This is logic taking into consideration the poor permeability nature of FEX-HCl. These results suggest that the recorded enhancement can be mainly due to permeation of drug loaded cubosomes probably via trans-lymphatic pathway. However, this requires further investigations.

**Conclusion**

Cubosomes comprising glyceryl mono-oleate with poloxamer407 were successfully prepared as nanostructures with combined spherical and polygonal morphology. Fexofenadine HCl (FEX-HCl) undergoes site-dependent absorptive clearance with the permeability ranking as colon < duodenum < jejunum < ileum, suggesting its liability for intestinal efflux. Cubosomal encapsulation of FEX-HCl increased its intestinal permeability compared with the corresponding aqueous solution. The ability of cubosomes to enhance the intestinal permeability depends on their components capacity to inhibit P-gp efflux transporters and fluidize intestinal membrane. Drug absorption from cubosomes favored the transcellular pathway. In vivo investigation of anti-inflammatory effect of FEX-HCl revealed cubosomal superiority over the aqueous drug dispersion. The study thus introduced cubosomes as novel carrier for enhanced oral absorption of hydrophilic poorly permeable drugs.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**

1. Eedara BB, Veerareddy PR, Jukanti R, Bandari S. Improved oral bioavailability of FEX-HCl hydrochloride using lipid surfactants: ex vivo, in situ and in vivo studies. *Drug Dev Ind Pharm.* 2014;40(8):1030–1043. doi:10.3109/03639045.2013.801984
2. Sultan AA, El-Gizawy SA, Osman MA, El Maghraby GM. Self dispersing mixed micelles forming systems for enhanced dissolution and intestinal permeability of hydrochlorothiazide. *Colloids Surf B Biointerfaces.* 2017;149:206–216. doi:10.1016/j.colsurf.b.2016.10.028
3. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm Res.* 1995;12(3):413–420. doi:10.1023/A:1016212804288
4. Breier AR, Paim CS, Steppe M, Schapoval EES. Development and validation of dissolution tests for FEX-HCl hydrochloride capsules and coated tablets. *J Pharm Pharm Sci.* 2005;8(2):289–298.
5. Axelrod D, Bielory L. FEX-HCl hydrochloride in the treatment of allergic disease: a review. *J Asthma Allergy.* 2008;1:19–29. doi:10.2147/jaa.s3092
6. Qiang F, Shin HJ, Lee BJ, Han HK. Enhanced systemic exposure of FEX-HCl via the intranasal administration of chitosan-coated liposome. *Int J Pharm.* 2012;430(1–2):161–166. doi:10.1016/j.ijpharm.2012.04.007
7. Piao HM, Balakrishnan P, Cho HJ, et al. Preparation and evaluation of FEX-HCl microemulsions for intranasal delivery. *Int J Pharm.* 2010;395(1–2):309–316. doi:10.1016/j.ijpharm.2010.05.041
8. Gundogdu E, Gonzalez Alvarez I, Karasulu E. Improvement of effect of water-in-oil microemulsion as an oral delivery system for FEX-HCl: in vitro and in vivo studies. *Int J Nanomedicine.* 2011;6:1631–1640. doi:10.2147/IJN.S22673
9. Eedara BB, Nyavanandi D, Narala S, Veerareddy PR, Bandari S. Improved dissolution rate and intestinal absorption of FEX-HCl hydrochloride by the preparation of solid dispersions: in vitro and in situ evaluation. *Pharmaceutics.* 2021;13(3):310. doi:10.3390/pharmaceutics13030310
10. Sapkal NP, Kilor VA, Shewale BD, Bhusari KP, Daud AS. Study of the complexation behaviour of FEX-HCl with β-cyclodextrin. *Indian J Pharm Sci.* 2010;72(3):318–323. doi:10.4103/0250-474X.70477
11. Mandeepa KS, Samala SK, Roy S, Sangamwara AT. Successful oral delivery of FEX-HCl hydrochloride by improving permeability via phospholipid complexation. *Eur J Pharm Sci.* 2020;149. doi:10.1016/j.ejps.2020.105338
12. Angelova A, Drechsler M, Garamus VM, Angelov B. pep lipid cubosomes and vesicles compartmentalized by micelles from self assembly of multiple neuroprotective building blocks including a large peptide hormone PACAP-DHA. *Chem Nano Ma.* 2019;5:1381–1389.
13. Rakotoarisoa M, Angelov B, Espinoza S, et al. Composition-switchable liquid crystalline nanostructures as green formulations of curcumin and fish oil. ACS Sustainable Chem Eng. 2021;9(44):14821–14835. doi:10.1021/acssuschemeng.c104706
14. Peng X, Zhou Y, Han K, et al. Characterization of cubosomes as a targeted and sustained transdermal delivery system for capsaicin. Drug Des Devel Ther. 2019;5:4209–4218. doi:10.2147/DDDT.S6370
15. Gaballa SA, El Garhy OH, Abdellaker H. Cubosomes: composition, preparation, and drug delivery applications. J Adv Biomed Pharm Sci. 2020;3(1):1–9.
16. Mathews PD, Mertins O, Angelov B, Angelova A. Cubosomal lipid nanoassemblies with PH-sensitive shells created by biopolymer complexes: a synchrotron SAXS study. J Colloid Interface Sci. 2022;607(1):440–450. doi:10.1016/j.jcis.2021.08.187
17. Vaghmar A, Mu H. Recent advances in drug delivery applications of cubosomes, hexosomes, and solid lipid nanoparticles. Acta Pharm Sin B. 2021;11(4):871–885. doi:10.1016/j.apsb.2021.02.013
18. Lai J, Chen J, Lu Y, et al. Glyceryl monooleate/poloxamer 407 cubic nanoparticles as oral drug delivery systems: i. In vitro evaluation and enhanced oral bioavailability of the water-soluble drug simvastatin. AAPS Pharm Sci Tech. 2009;10(3):960–966. doi:10.1208/s12249-009-9292-4
19. Faria AR, Silvestre OF, Maibohm C, Adão RMR, Silva BFB, Nieder JB. Cubosome nanoparticles for enhanced delivery of mitochondria anticancer drug elesclomol and therapeutic monitoring via sub-cellular NAD(P)H multi-photon fluorescence lifetime imaging. Nano Res. 2019;12:991–998. doi:10.1007/s12274-018-2231-5
20. Salas S, Mahmoud AA, Kamel AO. Etodolac transdermal cubosomes for the treatment of rheumatoid arthritis: ex vivo permeation and in vivo pharmacokinetic studies. Drug Deliv. 2017;24(1):84–856. doi:10.1080/10717544.2017.1362539
21. Yang Z, Peng X, Tan Y, et al. Optimization of the preparation process for an oral phytantriol-based amphoterinic B cubosomes. Nanomaterial. 2011;4(1):1–10.
22. Oliveira DC, Weigh A, Rolim CMB. Simple and reliable HPLC analysis of FEX-HCl hydrochloride in tablets and its application to dissolution studies. Pharmazie. 2007;62(2):96–100.
23. Fayad ND, Goda AE, Essa EA, El Maghraby GM. Chitosan-encapsulated niosomes for enhanced oral delivery of atorvastatin. J Drug Deliv Sci Technol. 2021;66. doi:10.1016/j.jddst.2021.102845
24. Sultan AA, El-Gizawy SA, Osman MA, El Maghraby GM. Pecosomes for oral delivery of glibenclamide: in vitro in situ correlation. J Drug Deliv Sci Technol. 2017;41:303–309. doi:10.1016/j.jddst.2017.08.003
25. Khan KA. The concept of dissolution efficiency. J Pharm Pharmacol. 1975;27:48–49. doi:10.1111/j.2042-7158.1975.tb09378.x
26. Osman MA, El Maghraby GM, Hedaya MA. Intestinal absorption and presystemic disposition of sildenafil citrate in the rabbit: evidence for site-dependent absorptive clearance. Biochip Drug Dispos. 2006;27(2):93–102. doi:10.1002/bdd.487
27. Sultan AA, El-Gizawy SA, Osman MA, El Maghraby GM. Colloidal carriers for extended absorption window of furosemide. J Pharm Pharmacol. 2016;68(3):324–332. doi:10.1111/jphp.12516
28. Ashmawy SM, El-Gizawy SA, El Maghraby GM, Osman MA. Regional difference in intestinal drug absorption as a measure for the potential effect of P-glycoprotein efflux transporters. J Pharm Pharmacol. 2019;71(3):362–370. doi:10.1111/jphp.13036
29. Komiyi I, Park J, Kamani A, Ho N, Higuchi W. Quantitative mechanistic studies in simultaneous fluid flow and intestinal absorption using steroids as model solutes. Int J Pharm. 1980;4(3):249–262. doi:10.1016/0378-5173(80)90140-4
30. Amidon G, Ho N, French A, Higuchi W. Predicted absorption rates with Simultaneous bulk fluid flow in the intestinal tract. J Theor Biol. 1981;89(2):195–210. doi:10.1016/0022-5193(81)90306-4
31. Riad LE, Sawchuk RJ. Absorptive clearance of carbamazepin and selected metabolites in rabbit intestine. Pharm Res. 1991;8(8):1050–1055. doi:10.1007/BF02057505
32. Winter CA, Rescue EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544–547. doi:10.3181/00379727-111-27849
33. Center for Drug Evaluation and Research (CDER). Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. US Food and Drug Administration; 2005.
34. Alshaikh RA, Essa EA, El Maghraby GM. Eutexia for enhanced dissolution rate and anti-inflammatory activity of nonsteroidal anti-inflammatory agents: a melting point cofactor modulator. Int J Pharm. 2019;56:395–405. doi:10.1016/j.ijpharm.2019.04.024
35. Al-Haboubi HA, Zeitlin JJ. Re-appraisal of the role of histamine in carrageenan induced paw oedema. Eur J Pharmaceut. 1983;88(2–3):169–176. doi:10.1016/0021-9799(83)90003-1
36. Cong HH, Khaziaikmetova VN, Zigashina LE. Rat paw oedema modeling and NSAIDs: timing of effects. Int J Risk Saf Med. 2015;27(Suppl 1):76–77. doi:10.3233/JRS201569
37. Wei Y, Zhang J, Zheng Y, et al. Cubosomes with surface cross-linked chitosan exhibit sustained release and bioavailability enhancement for vinpocetine. RSC Adv. 2019;9(11):6287–6298. doi:10.1039/C8RA10302J
38. Ahirrao M, Shrotriya S. In vitro and in vivo evaluation of cubosomal in situ nasal gel containing resveratrol for brain targeting. Drug Dev Ind Pharm. 2017;43(10):1–26. doi:10.1080/03639045.2017.1338721
39. Nasr M, Ghorab MK, Abdelazem A. In vitro and in vivo evaluation of cubosomes containing 5-fluorouracil for liver targeting. Acta Pharmacol Sinica B. 2015;5(1):79–88. doi:10.1016/j.apsb.2014.12.001
40. Hosny KM. Nanosized cubosomal thermogelling dispersion loaded with saquinavir mesylate to improve its bioavailability: preparation, optimization, in vitro, and in vivo evaluation. Int J Nanomedicine. 2020;15:5113–5129. doi:10.2147/IJN.S261855
41. Rosa LS, Marinho FM, Braga SL, Souza J. Equilibrium solubility study to determine fexofenadine hydrochloride BCS class and challenges in establishing conditions for dissolution profiles applied to suspension. Braz J Pharm Sci. 2020;56:1–10. doi:10.1590/s1759-97902020000217737
42. Boyd BJ. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. Int J Pharm. 2003;260:239–247. doi:10.1016/S0378-5173(02)00262-X
43. Loehe CA, Kingham J, Baker J. Small intestine permeability in animal and man. Gut. 1973;14(9):683–688. doi:10.1136/gut.14.9.683
44. Maceda T, Takenaka H, Yamahira Y, Nomuchi T. Use of rabbits for GI drug absorption studies. J Pharm Sci. 1977;66(1):69–73. doi:10.1002/jps.2600660117
45. Mouly S, Paine MF. P-glycoprotein increases from proximal to distal regions of human small intestine. Pharm Res. 2003;20(10):1595–1599. doi:10.1023/A:1026183200740
46. Stephens RH, Tanianis-Hughes J, Higgs NB, Humphrey M, Warhurst G. Region-dependent modulation of intestinal permeability by drug efflux transporters: in vitro studies in mdr1a(-/-) mouse intestine. *J Pharmacol Exp Ther.* 2002;303(3):1095–1101. doi:10.1124/jpet.102.041236

47. Pan X, Han K, Peng X, et al. Nanostructured cubosomes as advanced drug delivery system. *Curr Pharm Des.* 2013;19(35):6290–6297. doi:10.2174/1381612811319350006

48. Risovic V, Sachs-Barrable K, Boyd M, Wasan KM. Potential mechanisms by which Pceol increases the gastrointestinal absorption of amphotericin B. *Drug Dev Ind Pharm.* 2004;30(7):767–774. doi:10.1081/DDC-120039793

49. Sachs-Barrable K, Thamboo A, Lee SD, Wasan KM. Lipid excipients Pceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells. *J Pharm Pharm Sci.* 2007;10(3):319–331.

50. Bansal T, Akhtar N, Iagge M, Khar RK, Talegaonkar S. Novel formulation approaches for optimising delivery of anticancer drugs based on P-glycoprotein modulation. *Drug Discov Today.* 2009;14(21–22):1067–1074. doi:10.1016/j.drudis.2009.07.010

51. El Maghraby GM, Alomrani AH. Synergistic enhancement of itraconazole dissolution by ternary system formation with pluronic f68 and hydroxypropyl methylcellulose. *Sci Pharm.* 2009;77(2):401–417. doi:10.3797/scipharm.0901-08

52. Ustundag Okur N, Apaydin S, Karabay Yavasoglu NU, Yavasoglu A, Karasulu HY. Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations. *Int J Pharm.* 2011;416(1):136–144. doi:10.1016/j.ijpharm.2011.06.026

53. Lee YY, Saba E, Irfan M, et al. The anti-inflammatory and anti-nociceptive effects of Korean black ginseng. *Phytomedicine.* 2018;54:169–181. doi:10.1016/j.phymed.2018.09.186