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Optimization of sulfobutyl-ether-β-cyclodextrin levels in oral formulations to enhance progesterone bioavailability

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ABSTRACT

Progesterone oral dose regimens are indicated for the treatment of luteal phase deficiency and estrogen dominance. The poor aqueous solubility of progesterone leads to erratic oral absorption, resulting in suboptimal or excessive plasma levels. Developing a formulation to enhance the solubility of progesterone in the gastrointestinal tract would be beneficial to decrease drug absorption variability and increase bioavailability. The solubility of progesterone at 400 mM sulfobutyl-ether-β-cyclodextrin (SBE-β-CD) concentration was ~7000-fold greater than its intrinsic solubility, aided by the formation of SBE-β-CD–progesterone complex. The complex was characterized using differential scanning colorimeter, Fourier-transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy techniques. FTIR and NMR studies of the complex confirm the interaction between functional groups of SBE-β-CD and progesterone to form an inclusion complex. Molecular modeling studies demonstrated progesterone binding poses with four probable SBE-β-CD isomers and these results matched with NMR and FTIR data. The progesterone oral formulations were optimized by increasing the levels of SBE-β-CD in the formulation to prevent the displacement of progesterone from the complex by gastrointestinal contents. The oral bioavailability of progesterone in rats was increased 5-fold when administered with the optimized formulation compared to administration with progesterone API capsules. Studies demonstrated that the optimized formulation prevents precipitation of progesterone in the intestinal tract and increases progesterone oral bioavailability in rats.

Abbreviations: β-CD, β-cyclodextrin; A_L, linear phase solubility curve; API, active pharmaceutical ingredient; AUC, area under the curve; BCS, biopharmaceutical classification system; CE, complexation efficiency; C_{max}, maximum plasma concentration; DMSO-d_6, deuterated dimethyl sulfoxide; DSC, differential scanning calorimetry; F_{abs}, absolute bioavailability; FASSGF, fasted state simulated gastric fluid; FASSIF, fasted state simulated intestinal fluid; FESSGF, fed state simulated gastric fluid; FESSIF, fed state simulated intestinal fluid; FTIR, Fourier transform infrared spectroscopy; GI, gastrointestinal; $^1H/^{13}C$ NMR, proton and carbon nuclear magnetic resonance; HP-β-CD, hydroxy-2-propyl-β-cyclodextrin; HPLC, high performance liquid chromatography; i.p., intraperitoneal; IDR, initial dissolution rate; IS, internal standard; IV, intravenous; $K_{1:1}$, stability constants; LLMOD, large-scale low-mode method; LPD, luteal phase deficiency; M-β-CD, methyl-β-cyclodextrin; MCS, macrocycle conformational sampling; MD, molecular dynamics; MDT, mean dissolution time; MDR, mean dissolution rate; MM-GBSA, molecular mechanics-generalized Born surface area; NCE, new chemical entities; NVT, constant number of particles, volume and temperature; NPT, constant number of particles, pressure and temperature; OPLS, optimized potential for liquid simulations; P_{app}, apparent permeability coefficient; PAMPA, parallel artificial membrane permeability assay; PDB, Protein Data Bank; PRCG, Polak-Ribière conjugate gradient; PO, per os (oral administration); RMSD, root-mean-square deviation; S_0, drug solubility in deionized water; SBE-β-CD, sulfobutyl-ether-β-cyclodextrin aka CAPTISOL®; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; *Sint*, intercept of phase solubility curve; TIP3P, three-site transferrable intermolecular potential; T_{max}, time of peak concentration.

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1. Introduction

Progesterone is a female sex hormone produced by the adrenal gland and corpus luteum (during the luteal phase) (Lignihes, 1999). Luteal phase deficiency (LPD) is a state of deficient progesterone levels in which the patient is unable to maintain and regulate a normal menstrual cycle, pregnancy, and embryogenesis (Mesen and Young, 2016). It is essential to maintain a basal level of 10–20 ng/mL of progesterone to treat LPD and estrogen dominance (Lignihes, 1999; Mesen and Young, 2016). Progesterone is prescribed for assisted reproductive technology cycles (*in vitro* fertilization), to support implantation in early pregnancy, to control anovulatory bleeding, to prevent recurrent pregnancy loss, and for treatment of secondary amenorrhea (Lignihes, 1999).

Progesterone is a BCS class II steroidal drug with a reported aqueous solubility of 11.9 μg/mL and low oral bioavailability (Lahiani-Skiba et al., 2006). Most marketed oral progesterone products are micronized and suspended in a peanut oil base (e.g., Prometrium®) to increase intestinal absorption, and consequently bioavailability and efficacy (Lignihes, 1999; Orange Book). The clinical trials of Prometrium® showed high interpatient and intrapatient variability in C_{max} and $AUC_{(0\text{-}10h)}$, indicating variation in oral absorption resulting in suboptimal or excessive plasma levels of progesterone (Zhi et al., 2010). An alternate approach to enhance the potential efficacy of progesterone with increased oral bioavailability and having low interpatient and intrapatient variability is to develop a formulation to enhance its aqueous solubility.

The aqueous solubility of lipophilic drugs is increased by employing different techniques like particle size reduction (micronization, nanosuspension), solid dispersion, supercritical fluid processing, salt formation, micellar solubilization, solubilizers, hydrotrophy and cyclodextrin inclusion complex preparation (Savjani et al., 2012). In recent years, β-cyclodextrins have been widely used for increasing aqueous solubility of lipophilic drugs (Carrier et al., 2007; Dahan et al., 2010). Cyclodextrins are macrocyclic oligosaccharides which take on a well-defined shape containing a lipophilic cavity and hydrophilic groups on the outer surface. The lipophilic cavity can accommodate a lipophilic drug and because of the solubilizing capability of the hydrophilic groups, the cyclodextrin can significantly enhance the aqueous solubility of otherwise insoluble drugs (Savjani et al., 2012). The bioavailability of cyclodextrin upon oral administration is poor, and highly hydrophilic cyclodextrins have a negligible permeation across the intestinal barrier (Stella and He, 2008; European Medicines Agency, 2014).

Hydroxy-2-propyl-β-cyclodextrins (HP-β-CD) and sulfobutyl-ether-β-cyclodextrins (SBE-β-CD, known as Captisol™) are the cyclodextrin derivatives most extensively evaluated for solubility enhancement of active pharmaceutical ingredients for oral administration. The no observed adverse effect levels (NOAEL) reported for HP-β-CD and SBE-β-CD on oral administration in rats are up to 500 and 3600 mg/kg/day, respectively. Upon oral administration <3% of SBE-β-CD is absorbed and subsequently it is cleared quickly through renal excretion, and hence SBE-β-CD is non-toxic and has a high safety margin (Stella and He, 2008; European Medicines Agency, 2014).

There are previously reported phase solubility studies of progesterone in SBE-β-CD (Lahiani-Skiba et al., 2006) and HP-β-CD (Lahiani-Skiba et al., 2006; Zoppetti et al., 2007), in which the solubility of progesterone was found to be 9.9 and ~7 mg/mL in 100 mM of SBE-β-CD and 60 mM of HP-β-CD, respectively (Lahiani-Skiba et al., 2006). The studies on evaluating the solubility/permeability interplay of progesterone with cyclodextrins have shown that higher concentrations of HP-β-CD in the formulation decrease the permeation of progesterone across Caco2 cell monolayers, in PAMPA, the rat jejunal model (Dahan et al., 2010) and the single-pass intestinal perfusion rat model (Sun et al., 2018). Studies in rat intestine have shown that sodium taurocholate and lecithin displace progesterone from HP-β-CD–progesterone complexes (Sun et al., 2018).

Various *in vivo* (Sun et al., 2018) and *in vitro* studies suggest the optimum concentration of cyclodextrin required to enhance permeability across the intestine or any biological barriers (Zuo et al., 2000; Stappaerts and Augustijns, 2016). Evaluation of the *in vitro* data implies that too little cyclodextrin leads to precipitation of drug and decreases the permeation. By contrast, too much cyclodextrin decreases the free fraction of drug which alters the drug permeation across the barrier. Thus both extremes could lead to a decrease in the permeability and bioavailability (Zuo et al., 2000). The present study is designed to evaluate the effect of excess SBE-β-CD in the formulation to prevent the displacement of progesterone from the SBE-β-CD–progesterone complex by gastrointestinal contents and to increase the oral bioavailability of progesterone.

The SBE-β-CD–progesterone equilibrium complex mixtures were prepared and then characterized by differential scanning calorimetry (DSC), Fourier transforms infrared spectroscopy (FTIR), proton and carbon nuclear magnetic resonance (NMR) spectroscopy and computational methods. The optimal levels of SBE-β-CD required to prevent displacement of progesterone by the contents present in fasted state simulated intestinal fluid (FASSIF) and fed state simulated intestinal fluid (FESSIF) were determined by employing a simple *in vitro* experiment. *Ex vivo* permeation studies were performed to evaluate the permeation of progesterone across rat intestine and the effect of excess SBE-β-CD on the permeation. The oral bioavailability of progesterone, the isolated SBE-β-CD–progesterone complex mixture, and the isolated SBE-β-CD–progesterone complex mixture with excess SBE-β-CD were evaluated in Sprague Dawley rats. *In vitro* dissolution studies were performed to evaluate the potential utility of SBE-β-CD–progesterone complex in humans.

2. Materials and methods

2.1. Chemicals and reagents

Progesterone, pepsin, phosphate-buffered saline, and L-α-phosphatidylcholine (lecithin) were purchased from Sigma Aldrich, Inc., USA. SBE-β-CD (Captisol®) was provided by Ligand Pharmaceuticals, Inc, USA,. Sodium taurocholate was purchased from Alfa Aesar, USA. Euthasol® (pentobarbital sodium and phenytoin sodium) solution was procured from Virbac, USA. Methocel E15 Premium EL (hydroxypropyl methylcellulose) was purchased from the Dow Chemical Company, USA. Brij S20-SO-(MH) (Brij) was a gift sample from Croda Inc, USA. HPLC grade solvents acetonitrile and methanol were procured from Fisher Chemicals, USA. Dibasic potassium phosphate, dibasic sodium phosphate, glacial acetic acid, hydrochloric acid, sodium hydroxide, sodium chloride, sodium acetate, *ortho*-phosphoric acid, and Milli Q water were of research-grade and were used without further purification.

2.2. Phase solubility studies

The solubility enhancement of progesterone by complexation with SBE-β-CD was evaluated by adding excess amounts of progesterone to different concentrations of SBE-β-CD (0, 10, 20, 40, 60, 80, 100, 150, 200, 250, 300, 350 and 400 mM) in Milli-Q® water (Higuchi and Connors method). Samples were kept shaking at 25 °C for 3 days in a bio-shaker at 1500 RPM and at equilibrium, samples were filtered using a Millipore (0.45 μm) syringe filter. The filtrate was analyzed using HPLC to evaluate the saturation solubility of progesterone in SBE-β-CD. Phase solubility study curves were used to calculate complexation efficiency (CE) and stability constants ($K_{1:1}$).

2.3. Characterization of the isolated SBE-β-CD–progesterone equilibrium complex mixture

The complex was prepared by adding excess progesterone (~35 to 40 mg/mL) to 100 mM SBE-β-CD in Milli-Q® water. Samples were kept shaking at 25 °C for 3 days and at equilibrium, samples were filtered

using a Millipore (0.45 μm) syringe filter. The SBE-β-CD–progesterone complex samples were frozen for 24 h at −80 °C and lyophilized using Labconco FreeZone Small Tray Dryers equiped with 4.5 L −84 °C console freeze dryer. The frozen samples were placed in trays at 0 °C and the temperature was ramped up to 24 °C at the rate of 6 °C/h. Then samples were maintained at 24 °C for 48 h. The vacuum presssure was set at 0.01 mBar during the lyophilization process. These lyophilized samples were characterized using DSC, FTIR, and NMR. The complex was also studied by computational methods.

2.3.1. Differential scanning calorimetry

The isolated SBE-β-CD–progesterone complex mixtures were characterized with a DSC 25 Series, TA instrument equipped with Pyris software (Shelton, CT, USA). Approximately 3–3.5 mg of progesterone API, a physical mixture of SBE-β-CD and progesterone, and the lyophilized complex mixture were hermetically sealed in a crimped aluminum pan and heated from 25 °C to ~200 °C, at a heating rate of 10 °C/min to obtain a DSC thermogram.

2.3.2. Fourier transform infrared spectroscopy

The isolated SBE-β-CD–progesterone complex mixtures were characterized by using FTIR. FTIR studies were conducted on an Agilent Technologies Cary 660 (Santa Clara, CA.). The bench was equipped with an ATR (Attenuated Total Reflectance MIRacle™, Pike Technologies, Madison, WI) which was equipped with a single bounce diamond-coated ZnSe (zinc selenide) internal reflection element. FTIR spectroscopic analysis was performed by scanning the samples over the range from 400 to 4000 cm^{-1}.

2.3.3. Nuclear Magnetic Resonance studies

The ^{1}H NMR spectrum of progesterone, the isolated SBE-β-CD–progesterone complex mixture, the physical mixture of SBE-β-CD and progesterone, and pure SBE-β-CD dissolved in DMSO-d_6: D_2O (2:1) were recorded on a Bruker Advance DRX 500 MHz FT NMR instrument at 298 K. The ^{13}C NMR spectra of progesterone and the isolated SBE-β-CD–progesterone complex mixture dissolved in DMSO-d_6 were also obtained. The spectra were processed with software Topspin 3.2.

2.3.4. Molecular modeling

The molecular modeling studies were performed to study the stability of the SBE-β-CD–progesterone complex and to demonstrate the orientation of progesterone binding to four probable isomers of SBE-β-CD. SBE-β-CD (Captisol®) is available as an unspecified mixture of positional or regio isomers with varying degrees of substitution of sulfobutyl ether groups.

The computational study utilized the Maestro 10.5 program from the Schrödinger Software Suite to produce four models of SBE-β-CD isomers. The 3D X-ray structure of β-CD was retrieved from the Protein Data Bank (PDB ID:1JL8) (Yokota et al., 2001), and was modified to produce the four models of cyclodextrin isomers for SBE-β-CD, in an approach similar to that reported by Jain et al. (2011) (Jain et al., 2011), to represent the possible structural arrangements of sulfobutyl ether groups in the SBE-β-CD structure (Supplementary Fig. S1). Isomer 1 was prepared by placing all of its sulfobutyl ether groups on the positions of primary hydroxyl groups (6-OH on the glucose subunits) of β-CD whereas Isomers 2 and 3 were prepared by attaching three sulfobutyl ether groups to primary hydroxyl groups and four to secondary hydroxyl groups (2-OH on the glucose subunits) of β-CD. The final Isomer 4 was constructed by placing one sulfobutyl ether group on a primary hydroxyl group (6-OH), three on secondary 2-OH hydroxyl groups, and three on secondary 3-OH hydroxyl groups (3-OH) on the glucose subunits of β-CD. Isomer 4 is a representative of a general overall distribution found for Captisol (Personal communication. Data on file at CyDex Pharmaceuticals, Inc). The sulfobutyl group was used in its ionized form. The procedures for conformational search and docking were similar to those reported in our previous publication (Mohammed et al., 2016). A conformational search of modified SBE-β-CD (for all four isomers) was done separately using MacroModel's Macrocycle Conformational Sampling (MCS) tool (Schrödinger Release 2016-2), employing the OPLS2005 force field. During the MCS, the large-scale low-mode (LLMOD) method was used with the generalized Born model (GB/SA) solvent treatment. Redundant conformers were eliminated using a root-mean-square deviation (RMSD) cutoff of 0.75 Å. The energy window for saving structures was set to 10 kcal/mol and the enhanced torsional sampling option was used. The resulting conformers were minimized in the gas phase using the Polak-Ribière Conjugate Gradient (PRCG) method with the OPLS2005 force field. Progesterone was sketched in Maestro (Schrödinger Release 2016-2) and energy-minimized using the LigPrep module of Schrödinger (Schrödinger Release 2016-2), using the OPLS2005 force field at pH 7.4. Those conformers within an energy window of 7.5 kcal/mol were selected for docking of progesterone, which was done using Glide (Schrödinger) (Friesner et al., 2004), with Standard Precision and flexible ligand sampling. Docking grids were prepared for each conformer of each SBE-β-CD model in such a manner as to ensure that the whole SBE-β-CD structure was labeled as the active site. The binding free energies were calculated on the representative complex after MD simulation using Prime MM-GBSA (Sherman et al., 2006) considering refinement of polar hydrogens only, applying the variable dielectric generalized Born (VSGB) water model. The 2D structures of SBE-β-CD isomers and progesterone are shown in Supplementary Figs. S1 and S2, respectively.

2.3.5. Molecular dynamics simulations

MD simulations for the best complexes of progesterone and SBE-β-CD were carried out using the Desmond program (Bowers et al., 2006; Shaw, 2017). The best complex of each isomer with progesterone (SBE-β-CD–progesterone complex) was solvated with TIP3P (Mark and Nilsson, 2001) waters. An orthorhombic box was used with dimensions calculated automatically to contain the system by the Desmond software, and with a buffer distance of 10 Å for each dimension. The solvated complex was neutralized by adding $7Na^{+}$ ions, yielding a salt concentration (NaCl) of 0.15 M. The constructed system was simulated with the modified NPT relaxation protocol in Desmond. The protocol involved an initial minimization of the solvent while keeping restraints on the solute, followed by short MD simulations of 12–24 ps in sequential NVT and NPT ensembles with the Langevin thermostat and barostat, respectively. The temperature was maintained at 300 K throughout the production run using the Langevin algorithm and the pressure was isotropically restrained to 1 bar with the Langevin barostat (Quigley and Probert, 2005). The short-range Coulombic interactions were set to a cut-off value of 9.0 Å using the short-range method, while the smooth particle mesh Ewald method was used for handling long-range Coulombic interactions. After completion of the relaxation protocol, the final production run of 5 ns (NPT) with a time-step of 2 fs was carried out on the solvated complex, to avoid any structural artifacts introduced during system buildup and equilibrium steps (Chaurasiya et al., 2019). During the simulation, the trajectories were sampled at an interval of 4.8 ps. The structural stability of the complexes was analyzed based on the RMSD, for which the first frame was used as a reference. The number of intermolecular hydrogen bonds between progesterone and SBE-β-CD was also calculated. Representative complexes were subjected to binding free-energy calculations using Prime MM-GBSA.

2.4. Solubility of progesterone and SBE-β-CD-progesterone complex

The solubility studies were performed in water, fasted state simulated gastric fluid (FASSGF) at pH 1.6, fed state simulated gastric fluid (FESSGF) at pH 5.0, fasted state simulated intestinal fluid (FASSIF) at pH 6.5 and fed state simulated intestinal fluid (FESSIF) at pH 5.8. The above mentioned gastrointestinal simulated fluids were prepared using previously published protocols (Jantratid et al., 2008) and the composition is presented in Supplementary Table SI. The solubility was evaluated by

adding excess progesterone API and 25 mg of isolated SBE-β-CD–progesterone complex mixture in 1 mL of water and simulated gastrointestinal fluids in triplicate. Samples were kept shaking at 37 °C for 8 h at 100 rpm in a Bio-shaker and samples were filtered using a Millipore (0.45 μm) syringe filter. The filtrate was analyzed using HPLC to evaluate the solubility of progesterone.

2.5. Effect of sodium taurocholate on SBE-β-CD–progesterone complex

The effect of sodium taurocholate on the solubility of the isolated SBE-β-CD–progesterone complex mixture was evaluated by adding 25 mg of the lyophilized complex to different concentrations (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40 and 50 mM) of sodium taurocholate in 1 mL of water. Samples were studied in triplicates. The above samples were kept shaking at 37 °C for 8 h at 100 rpm in a Bio-shaker and samples were filtered using a Millipore (0.45 μm) syringe filter. The filtrate was analyzed using HPLC to evaluate the solubility of progesterone in the presence of sodium taurocholate.

2.6. Effect of excess SBE-β-CD on sodium taurocholate displacement of progesterone from SBE-β-CD–progesterone complex

The effect of excess SBE-β-CD on sodium taurocholate displacement of progesterone from the isolated SBE-β-CD–progesterone complex mixture was evaluated by adding 25 mg of the lyophilized complex to different concentrations (0, 5, 10, 20, 30, 40 and 50 mM) of SBE-β-CD in a solution containing 20 mM of sodium taurocholate in 1 mL of water (samples were studied in triplicates). The above samples were kept shaking at 37 °C for 8 h at 100 rpm in a Bio-shaker and samples were filtered using a Millipore (0.45 μm) syringe filter. The filtrate was analyzed using HPLC to evaluate the solubility of progesterone in the presence of excess SBE-β-CD and sodium taurocholate.

2.7. In vitro simulation to evaluate the effect of excess SBE-β-CD on the displacement of progesterone from SBE-β-CD–progesterone complex in FASSIF and FESSIF

The effect of excess SBE-β-CD on the solubility of the SBE-β-CD–progesterone complex in FASSIF and FESSIF was simulated *in vitro*. 25 mg of the lyophilized complex and different concentrations of excess SBE-β-CD were added to 1 mL of FESSIF (2.5, 5, 7.5, 10, 15, and 20 mM of SBE-β-CD) and FASSIF (1, 2, 3, 4, 5, 7.5 and 10 mM of SBE-β-CD), studied in triplicates, respectively. The above samples were kept shaking at 37 °C for 8 h at 100 rpm in a Bio-shaker and samples were filtered using a Millipore (0.45 μm) syringe filter. The filtrate was analyzed using HPLC after appropriate dilution to evaluate quantities of solubilized progesterone in the samples.

2.8. In vitro dissolution studies

The dissolution studies were carried out for progesterone API and the isolated SBE-β-CD–progesterone complex mixture filled in capsules containing the dose equivalent to 100 mg of progesterone. The drug profile was evaluated using a USP dissolution apparatus-I (Hanson SR8, Chatsworth, CA) maintained at 37 ± 0.5 °C and having a shaft rotation speed of 100 rpm. The dissolution test was performed using 900 mL of water, simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8), in triplicates. The samples were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min intervals, and the filtrate was analyzed using HPLC after appropriate dilution. The *in vitro* dissolution profile was used to calculate dissolution parameters including mean dissolution time (MDT), mean dissolution rate (MDR), and initial dissolution rate (IDR) (Manda et al., 2018).

2.9. Stability studies

Lyophilized powder was packed in screwcapped HDPE bottles and liquid equilibrium complex mixture was packed in glass vials in triplicates and stored in a stability chamber under two conditions, at 40 °C and 75% RH as well as at 25 °C and 60% RH. Approximately 25 mg of lyophilized powder was weighed on day 1, day 90 (after 3 months) and day 180 (after 6 months) and each time dissolved in 1 mL of water. These samples were filtered using a Millipore (0.45 μm) syringe filter, and the filtrate was analyzed to calculate the percentage of progesterone solubilized in the water on the 90th and 180th days. The liquid samples were filtered using a Millipore (0.45 μm) syringe filter, on days 1, 90, and 180, and the supernatant was analyzed for progesterone content to determine the percentage of progesterone soluble in water on days 90 and 180. The stability study samples were analyzed using HPLC.

2.10. Animal studies

The animal studies were conducted at the University of Mississippi, School of Pharmacy, as per protocol approved by the Institutional Animal Care and Use Committee. Animal experiments performed complied with National Institutes of Health guidelines for the care and use of Laboratory animals. On arrival, male rats were housed in cages at the Animal care facility in a temperature and humidity controlled room with a 12:12 h light:dark cycle and they were provided free access to food and water for one week before use in the experiments.

2.10.1. Ex vivo rat intestinal permeation studies

Three male rats (225–250 g) fasted overnight the day before the experiment but had free access to water. On the day of the experiment, the rats were removed from the animal care facility and brought to the procedure lab. The rats were euthanized using an intraperitoneal (i.p.) dose of Euthasol® (150 mg/kg body weight). The abdomen was cut open using an incision of 4–5 cm and the proximal small intestine segment was isolated. The isolated small intestine was slowly flushed with phosphate-buffered saline (PBS) at pH 7.4 to remove the intestinal content. The freshly harvested small intestine was cut open to expose the mucosal layer and cleaned by a gentle flow of PBS at pH 7.4 on the mucous surface. Fresh rat intestine was sandwiched between two chambers of a Franz diffusion cell with an active diffusion area of 0.64 cm^2, and the mucosal layer was exposed to the donor chambers. The resistance across rat intestine was measured using a waveform generator to ensure the integrity of the small intestine segment used for the permeation study. Rat intestine with the resistance of $\geq$3 KΩ·cm^2 was used for permeation studies. To the donor compartment, 500 μL of complex dissolved in water (444 ± 6.58 μg), FASSIF (435 ± 7.89 μg), FESSIF (430 ± 50.7 μg), FASSIF (447 ± 7.54 μg with excess 5 mM SBE-β-CD), FESSIF (455 ± 11.3 μg with excess 15 mM SBE-β-CD) and the positive control (456 ± 6.58 μg) of progesterone dissolved in PBS containing 0.5% Brij were added for permeation studies, in triplicates. After filling with the solution for intestinal permeation studies, the donor chamber was sealed with parafilm. The receiver chamber was filled with 5 mL of PBS containing 0.5% Brij, which was stirred at 600 rpm with a 3 mm magnetic stir bar, and the temperature was maintained at 37 °C with a circulating water bath. 200 μL samples were withdrawn from the receiver compartment at different time intervals (0, 0.25, 0.5, 1, 2, 4, 6, and 8 h) and each time an equal volume of fresh receiver media was used to replace what was withdrawn. The above samples were transferred into vials and subjected to HPLC analysis.

2.10.2. In vivo pharmacokinetic studies

The 16 jugular vein cannulated male rats (250–270 g) were used for pharmacokinetic studies. These animals had free access to water and food on the day before the experiment. On the day of the experiment, the rats were removed from the animal care facility and brought to the procedure lab. Animals were randomly divided into four different

groups of 4 animals each (Group I: PO isolated SBE-β-CD–progesterone complex mixture; Group II: PO progesterone API; Group III: PO isolated SBE-β-CD–progesterone complex mixture with excess SBE-β-CD; and Group IV: progesterone IV). Each oral dose was weighed lyophilized complex (equivalent to 20 mg/kg of progesterone dose) and filled in Tropac® Capsule 9el (gelatin). The capsules given to animals of Groups I, II, and III were administered by placing the capsule in the Torpac® capsule gavage needle attached to a dosing syringe. The delivery tube of the dosing syringe was placed in the mouth and advanced along the lower palate as far as the esophagus for administration. After administration of capsules, 1 mL of water was administered to Group I and II animals, and to Group III animals 1 mL of water containing 15 mM of SBE-β-CD was administered. The 2 mg/kg of progesterone dose to Group IV was administered by slow bolus intravenous injection into the tail vein (dosing volume: 2 mL/kg body weight). Approximately 200 μL of blood was drawn into heparin-coated tubes at pre-dose, 0.08, 0.17, 0.33, 0.5, 0.75, 1, 1.5, and 2 h through jugular vein catheter. As per the blood collection protocol from jugular vein catheter, approximately 50 μL of heparin saline solution was injected into the catheter to maintain patency (to prevent clogging of catheter). At every time point, the heparin solution in the catheter was withdrawn and discarded. The blood was withdrawn with a fresh syringe and the catheter was flushed with 100 μL of 0.9% saline (replacement fluid), followed by injection of 50 μL of heparin saline solution to the catheter to maintain patency. Plasma was harvested by centrifuging the blood at 4000 rpm for 5 min and it was stored frozen at −80 ± 10 °C until analysis. After blood collection at 2 h, the animals were euthanized using an intraperitoneal (i.p.) dose of Euthasol® (150 mg/kg body weight).

2.11. HPLC analysis

The HPLC method was developed using a Shimadzu UFLC system, equipped with prominence SPD-M20A (Diode array detector). The chromatographic separation of progesterone and the internal standard (IS) (fenofibrate) was achieved on a Waters XTerra® RP18 5 μm column with dimensions 4.6 mm × 150 mm, which was maintained at ambient room temperature. The binary mobile phase system consisted of methanol:0.1% formic acid in water [80:20 v/v] at a flow rate of 1.2 mL/min. All solubility, *in vitro,* and *ex vivo* samples, were subjected to HPLC analysis without any further extraction procedure.

2.11.1. In vivo sample preparation

A simple protein precipitation method was followed for the extraction of progesterone from *in vivo* study samples. To an aliquot of 50 μL of rat plasma sample, an IS solution (5 μL of fenofibrate 5 μg/mL) was added and the mixture was vortexed, after which 200 μL of acidified acetonitrile was added and the mixture was again vortexed. The sample was centrifuged at 4 °C for 10 min at 14,000 rpm on a Centrifuge 5430R (Eppendorf, Germany) and the supernatant was transferred to a vial for HPLC analysis. The UV detection wavelength was 242 nm. The compound eluted at 3.9 min and the IS eluted at 5.5 min with a total run time of 8 min. The data obtained from the quantification of plasma samples were subjected to non-compartmental analysis and pharmacokinetic parameters were calculated using Pheonix 64® (Certara, Princeton, NJ, USA).

2.12. Statistical analysis

The unpaired student *t*-test was used for statistical comparisons of mean values. The data with *P*-value <0.05 were considered as statistically significant.

3. Results and discussion

Progesterone is a BCS Class II drug (Dahan et al., 2010) and hence its oral absorption is limited due to low aqueous solubility. It is crucial to enhance the solubility/dissolution of the drug to overcome its poor and variable bioavailability issues.

3.1. Phase solubility

The phase solubility curve (Fig. 1) demonstrates a linear solubility increase of progesterone as a function of the increase in the concentration of SBE-β-CD, indicating an A_L type curve (Loftsson, Hreinsdóttir, and Másson, 2005). The aqueous solubility of progesterone increased 1972 ± 77.8 and 7722 ± 180-fold in the presence of 100 and 400 mM of SBE-β-CD, respectively, compared to the intrinsic solubility of progesterone in water (Supplementary Table SII). The $K_{1:1}$ Eq. (1) and CE Eq. (2) of SBE-β-CD–progesterone were calculated based on the parameters of the phase solubility plot,

Stability constant ($K_{1:1}$)

$$K_{1:1} = \frac{m}{S_0(1-m)} \quad (1)$$

Complexation efficiency (CE)

$$CE = \frac{m}{(1-m)} \quad (2)$$

where m is the slope of the curve obtained by plotting the drug solubility versus cyclodextrin concentration, determined by linear regression, and S_0 is the drug solubility in deionized water. The *CE* and $K_{1:1}$ of the SBE-β-CD–progesterone complex were 1.37 ± 0.04 and 43,576 ± 1416 M^{-1}, respectively, considering the intrinsic solubility (0.031 mM or 0.11 mg/mL) for the stability constant calculation. By contrast, the stability constant was determined to be 1477 ± 171 M^{-1}, based on the intercept. This huge variation of stability constant could be due to the non-ideality of water as a solvent. Poorly soluble drugs show a negative intercept deviation, i.e., S_{int} (intercept of phase solubility curve) < S_0, which leads to an overestimation of $K_{1:1}$ when determined from the slope and intercept (Loftsson, Hreinsdóttir, and Másson, 2005). Niels Erik Olesen et al. derived a biopharmaceutical model to assess the effect of cyclodextrin on intestinal absorption and to avoid overdosing of cyclodextrin, which could decrease drug absorption across the intestinal barrier (Erik et al., 2016). This model employs the $K_{1:1}$ constant in its derivation. For poorly soluble drugs, the $K_{1:1}$ value determined from a phase solubility curve is not a true value (Loftsson, Hreinsdóttir, and Másson, 2005). Hence, it is essential to employ an *in vitro* model for determination of the optimum concentration of cyclodextrin in oral formulations.

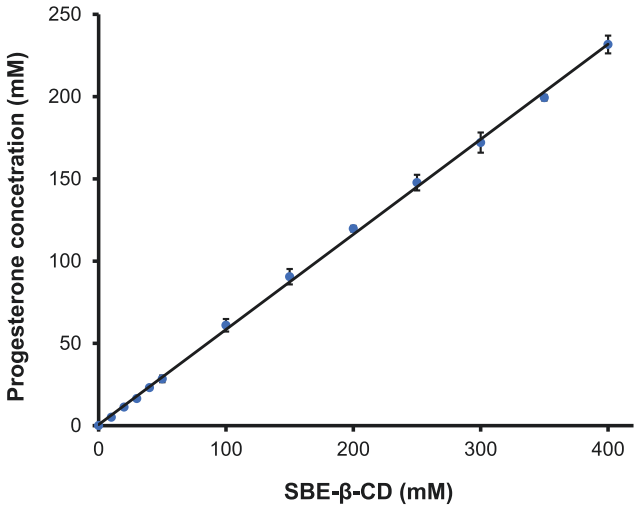

Fig. 1. Phase solubility profile of progesterone in SBE-β-CD (R^2 = 0.9992).

3.2. Characterization of SBE-β-CD–progesterone complex

3.2.1. Differential scanning calorimetry

DSC thermograms of progesterone, SBE-β-CD, physical mixtures of progesterone with SBE-β-CD, and the SBE-β-CD–progesterone complex are represented in Supplementary Fig. S3. SBE-β-CD demonstrates no crystallinity. Progesterone API exhibited a sharp melting endotherm at 132 °C confirming its inherent crystalline property. The endothermic peak (melting peak) of progesterone was reduced in the physical mixture but completely disappeared in the complex, indicating the formation of SBE-β-CD–progesterone complex or conversion of progesterone into an amorphous form in SBE-β-CD solution (Lahiani-Skiba et al., 2006; Jain et al., 2011).

3.2.2. Fourier Transform Infrared (FTIR) spectroscopy

FTIR has been extensively used to analyze the interaction between cyclodextrins and guest molecules in the solid-state. In general, the spectra of inclusion complexes exhibit broadening of bands, indicating the formation of inclusion complexes. The FTIR peaks of progesterone that correspond to the ketone stretch at 1698 (at C3 position) and 1660 (at C20 position) cm^{-1} were broadened in the SBE-β-CD–progesterone complex (Supplementary Fig. S4). This broadening of the ketone stretch bands indicates the formation of inclusion complexes in which a ketone participates in a hydrogen bond between SBE-β-CD and progesterone. The IR bands at 1160–1154 cm^{-1} and 1027–1029 cm^{-1} for SBE-β-CD, the SBE-β-CD–progesterone complex and the physical mixture correspond to the C—O and C—C/C—O stretches in SBE-β-CD. There were no other changes observed in the spectral pattern of SBE-β-CD or progesterone compared to the complex, signifying that there were no other chemical changes involved in the formation of the inclusion complex.

3.2.3. Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance studies

The ^1H NMR spectra of progesterone, SBE-β-CD and its complexes (physical and inclusion complexes) were recorded to gain deeper insight into the interaction of the drug with cyclodextrin.

The changes in the chemical shift patterns of the complex, relative to those for the isolated drug, are indicative of host–guest interaction. Selected proton signals of progesterone and the SBE-β-CD–progesterone complex in DMSO-d_6:D2O are depicted in Supplementary Fig. S5 and summarized in Supplementary Table SIII. The ^{13}C chemical shifts observed for progesterone are depicted in Supplementary Fig. S6 and summarized in Supplementary Table SIV. Due to the structural complexity of SBE-β-CD, it was difficult to assign the protons and carbons of the cyclodextrin itself by ^1H and ^{13}C NMR; therefore, only changes in the spectrum of progesterone in the inclusion complex were monitored. The differences found in the chemical shifts of progesterone in the SBE-β-CD–progesterone complex indicate that Ring A and Ring D of progesterone each can be included within the SBE-β-CD cavity.

3.2.4. Molecular modeling

3.2.4.1. Conformational search. A total of 968, 970, 971, and 669 conformers of SBE-β-CD were generated using the MCS tool within a cutoff window of 10 kcal/mol, for Isomers 1, 2, 3, and 4, respectively. The low energy conformers 10, 8, 15, and 10 for Isomers 1, 2, 3, and 4 were selected respectively, which matched the criteria of having a slightly open cavity (suitable for docking).

3.2.4.2. Docking and molecular dynamics of SBE-β-CD–progesterone complex. The progesterone molecule was docked into the four isomers of SBE-β-CD using the Glide standard precision method implemented in the Schrödinger software with flexible ligand sampling. The docking and scoring of progesterone into the generated grids of each low energy conformer of each isomer resulted in the most favorable SBE-β-CD–progesterone complexes (Supplementary Table SV). The best docking complex of each isomer was subjected to a 5 ns MD simulation to help understand the stability and binding orientation of the complexes.

Since the H4 and H21 protons (located on opposite ends of progesterone) in the ^1H NMR of progesterone (Supplementary Fig. S5 and Table SIII) were downshifted and upshifted, respectively, in the SBE-β-CD–progesterone complex, results were interpreted to show that progesterone forms inclusion complexes with SBE-β-CD in two different orientations, Figs. 2 and 3 (pose A: C3, and pose B: C20 carbonyl facing the hydroxyl of SBE-β-CD). To get more insight into the structure of the inclusion complexes, ^{13}C NMR for SBE-β-CD–progesterone was performed. The chemical shifts of progesterone at C3, C5, C17, C20, and C21 showed changes after the formation of the inclusion complex with SBE-β-CD. These data further supported that progesterone interacts with

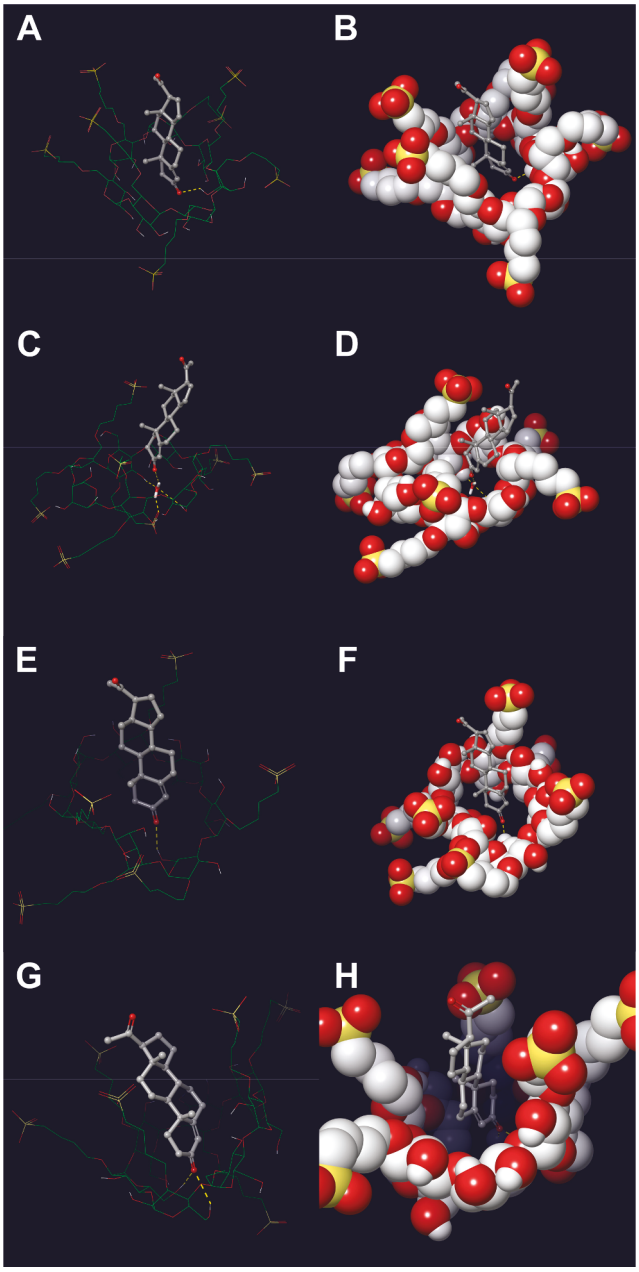

Fig. 2. Binding poses (Pose A) after 5 ns molecular dynamics simulations of representative complexes of progesterone (ball and stick model) with Isomer **1** (A and B); Isomer **2** (C and D); Isomer **3** (E and F) and Isomer **4** (G and H) of SBE-β-CD (line representation, left images; CPK model, right images). Hydrogen bonds are shown in yellow dashes.

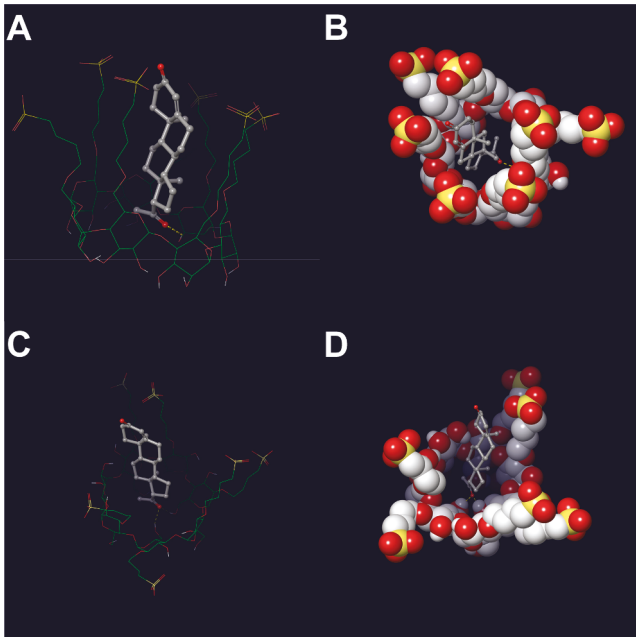

Fig. 3. Binding pose (Pose B) of a representative complex of progesterone (ball and stick model) with Isomer **1** (A and B) and Isomer **4** (C and D) of SBE-β-CD (line representation, left images; CPK model, right images). A hydrogen bond is shown with yellow dashes.

SBE-β-CD in two different orientations. In our MD simulations, both such orientations were explored. In Fig. 2, progesterone was seen to interact with SBE-β-CD on the secondary face in all of the isomers, using the starting point as Pose A, in which the carbonyl at C3 of Ring A formed an H-bond with the hydroxyl of SBE-β-CD. Meanwhile, alternate MD simulations were run starting with the alternative pose (Pose B) of progesterone having the C20 carbonyl of progesterone H-bonded with the hydroxyl of SBE-β-CD for Isomers 1 and 4 (Supplementary Table SV and Fig. 3), and that stable pose was well maintained during the simulation. Pose B for Isomers 2 and 3 were not considered for molecular dynamics simulation, due to very poor docking scores and because the orientation/pose of progesterone was not found to be inside the cavity of SBE- β -CD. The details of the interactions between progesterone and the protein in the different simulations clearly illustrate how the change occurs in the pattern of the NMR signals of the protons (H4 and H21) and carbons (C3, C20) of progesterone (Supplementary Figs. S5 and S6) in the progesterone–SBE-β-CD complex. The binding free energies from the MD simulations (Supplementary Table SV) revealed that there is a more favorable interaction of progesterone with SBE-β-CD when the C3 carbonyl group of Ring A is embedded in the cavity of SBE-β-CD (Pose A). Overall, all four possible isomers of SBE-β-CD showed H-bonds with progesterone at the C3 or the C20 carbonyl group with progesterone partially embedded in the SBE-β-CD cavity, and thus either Ring A or Ring D lies within the SBE-β-CD cavity. This matches well with the experimental (^{1}H NMR and ^{13}C NMR) data (Supplementary Figs. S5 and S6, and Tables SIII and SIV) and helps to confirm the formation of the inclusion complex of progesterone with SBE-β-CD.

The Pose A MD trajectories revealed persistent intermolecular hydrogen bonds between the carbonyl group (at the C3 position) of progesterone and the 2/3-OH of the glucose unit of SBE-β-CD in complexes with Isomers 1, 3 and 4 (Supplementary Figs. S7 and S8). In contrast, in the complex with Isomer 2, only a few instances of hydrogen bonding occurred between the carbonyl group of progesterone and the 2/3-OH of the glucose unit of SBE-β-CD. Interestingly, in Isomer 2 the carbonyl at the C3 position of Ring A formed water-mediated hydrogen bonding instead of direct hydrogen bonding with 2-OH or 3-OH of the glucose unit of SBE-β-CD during the 5 ns simulation (Supplementary Figs. S7 and S8). During the Pose A simulations of all four of the isomers, the carbonyl at the C3 position of Ring A was almost always deeply embedded into SBE-β-CD. Besides, in the Pose B simulations with Isomers 1 and 4 of SBE-β-CD, there were persistent H-bonds during the simulations (Supplementary Fig. S7 and S8). The RMSD analysis revealed that the SBE-β-CD–progesterone complex of all four of the isomers with both orientations was stable during the entire simulation period. The RMSDs computed for the initial structure of SBE-β-CD fluctuated between 2.7 and 4.0 Å (Supplementary Figs. S9 and S10). The RMSD of progesterone in the four isomeric models of SBE-β-CD fluctuated only between 0.4 and 1.2 Å (Supplementary Figs. S9 and S10). Most of the deviations in RMSD were a result of the flexible motion of the sulfobutyl ether arms.

3.3. Solubility of progesterone and SBE-β-CD–progesterone complex

The results in Supplementary Table SVI demonstrate that the solubility of progesterone was decreased compared to its intrinsic solubility in FASSGF, similar to its intrinsic solubility in FESSGF, but increased 2.3-fold and 6.8-fold compared to its intrinsic solubility in FASSIF and FESSIF, respectively.

Lowering the pH and ionic strength of FASSGF and FESSGF did not affect the solubility of the SBE-β-CD–progesterone complex, whereas 75% and 25% of the progesterone from SBE-β-CD–progesterone complex dissolved in FASSIF and FESSIF, respectively. Various studies have reported that bile salts present in intestinal fluid displace drug molecules from cyclodextrin cavities, leading to precipitation of the drug in the intestinal lumen (Stella et al., 1999; Carrier et al., 2007; Stappaerts and Augustijns, 2016).

3.4. Effect of sodium taurocholate on the isolated SBE-β-CD–progesterone complex mixture

The isolated SBE-β-CD–progesterone complex mixture solubility decreased linearly from 4.94 ± 0.30 mM to 1.64 ± 0.04 mM with a linear increase of sodium taurocholate concentration up to 18 mM, which indicates displacement of progesterone from the cavity of SBE-β-CD (Fig. 4). The progesterone displacement was found to saturate beyond a concentration of 18 mM, which could be the result of the formation of sodium taurocholate aggregates (micelles). The size of sodium taurocholate micelles varies from 5.5 to 16.5 Å, depending on intestinal fluid content (Woodford, 1969), whereas the diameter of β-CD cavity is 6 to 6.5 Å, which is too small to accommodate sodium taurocholate micelles (Valle, 2013).

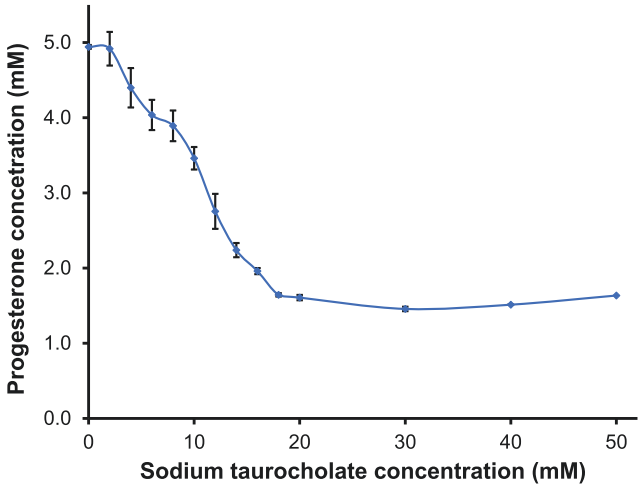

Fig. 4. Progesterone displacement from the inclusion complex by sodium taurocholate.

3.5. Effect of excess SBE-β-CD on sodium taurocholate displacement of progesterone from SBE-β-CD cavity

The results in Fig. 5 demonstrate that the addition of excess SBE-β-CD prevents the precipitation of progesterone and approximately 40 mM of excess SBE-β-CD is required to completely counteract displacement of progesterone from cyclodextrin cavities in water. This indicates that an amount of free SBE-β-CD adequate to form inclusion complexes with bile salts would prevent precipitation or displacement of progesterone from cyclodextrin. The absorption of a drug is increased if the drug is available in the solubilized form at the site of absorption (Savjani et al., 2012). Hence, there is a need to evaluate experimentally the optimum concentration of cyclodextrin required to fully solubilize the drug in the presence of bile salts.

3.6. In vitro simulation to evaluate the effect of excess SBE-β-CD on the displacement of progesterone from the SBE-β-CD–progesterone complex in FASSIF and FESSIF

The results in Fig. 6A and B indicate that an increase in free SBE-β-CD in water, FASSIF, and FESSIF prevents displacement of progesterone from the cyclodextrin cavity. At 5 and 15 mM concentrations of excess SBE-β-CD in FASSIF and FESSIF, respectively, the displacement of progesterone was minimal and further increase in the SBE-β-CD concentration had a slight but negligible effect of increasing progesterone loss from the cyclodextrin cavity. This study demonstrates that the formulation of the SBE-β-CD–progesterone complex administered in the fasted state or fed state should contain 5 mM or 15 mM of free SBE-β-CD, respectively, to prevent precipitation of progesterone in GI fluids.

3.7. Dissolution studies

The dissolution profiles for progesterone and SBE-β-CD–progesterone complex capsules in water, SGF and SIF are shown in Fig. 7. The SBE-β-CD–progesterone complex capsules in all three media have an improvement in dissolution characteristics compared to progesterone API capsules. Greater than 95% of progesterone was dissolved in all three media when testing SBE-β-CD–progesterone complex capsules for 45 min. By contrast, at 120 min, 11.5%, 8.08%, and 16.9% of progesterone was dissolved from progesterone API capsules for the three media. The dissolution parameters of progesterone are given in Supplementary Table SVII. The SBE-β-CD–progesterone complex capsules had the lowest MDT and highest IDR and MDR in SIF compared to SGF or water as dissolution media. Meanwhile, the progesterone API capsules had the highest MDR, MDT and IDR in SIF compared to other dissolution media (water and SGF).The dissolution study results imply that SBE-

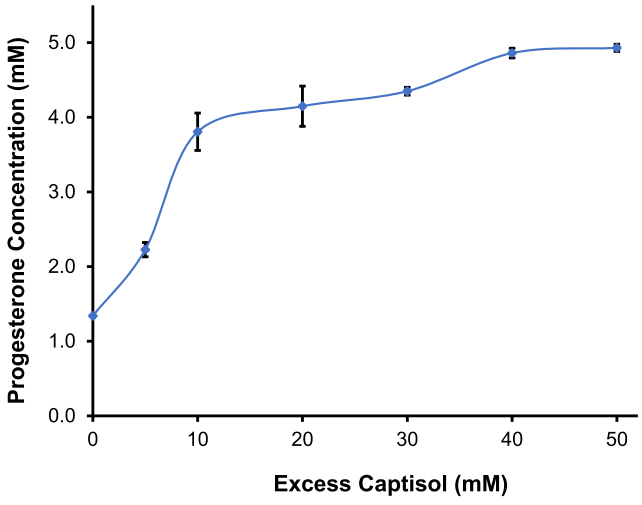

Fig. 5. Effect of excess SBE-β-CD on progesterone displacement from the SBE-β-CD–progesterone complex by sodium taurocholate. Each point represents the mean ± SD of triplicate values.

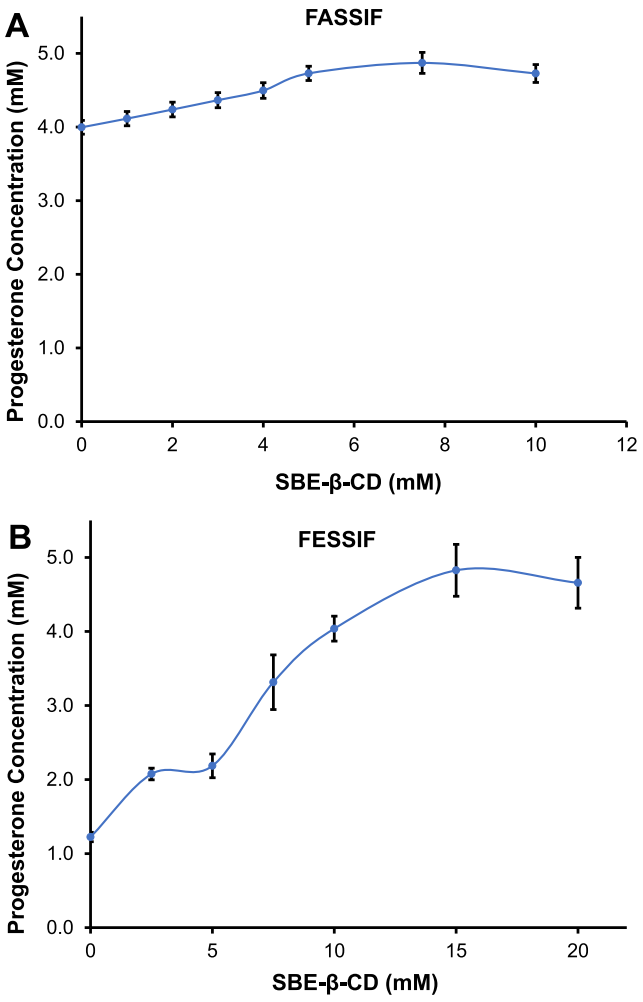

Fig. 6. Excess SBE-β-CD effect in FASSIF (A) and FESSIF (B) on the solubility of the SBE-β-CD–progesterone complex. Each point represents the mean ± SD of triplicate values.

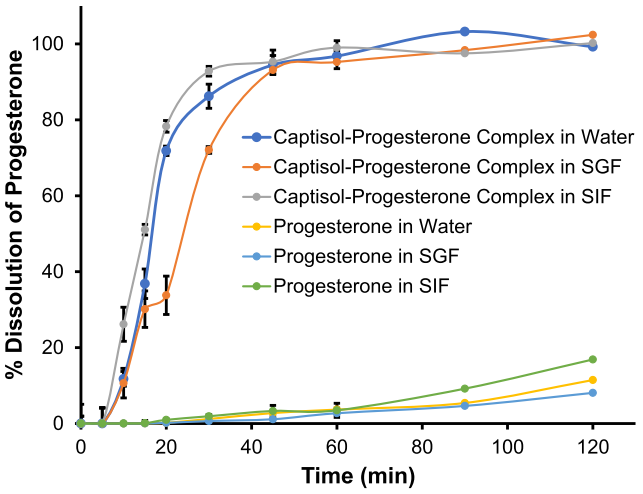

Fig. 7. Dissolution of progesterone and of the SBE-β-CD–progesterone complex in water, simulated gastric fluid (pH 1.2), and simulated intestinal fluid (pH 6.8).

β-CD–progesterone complex capsules would get dissolved in the stomach (greater than95% in SGF at 45 min) before entering the small intestine.

3.8. Stability study

The stability of the SBE-β-CD–progesterone complex in water at 25 °C (60% RH) was ≥98.3 ± 0.71 and 97.3 ± 0.89 for periods of 3 and 6 months, respectively, and at 40 °C (75% RH) the stability was 98.2 ± 0.43 and 97.0 ± 0.92 for periods of 3 and 6 months, respectively. The stability of the lyophilized SBE-β-CD–progesterone complex at 25 °C (60% RH) was 99.0 ± 0.08 and 98.1 ± 0.43 for periods of 3 and 6 months, respectively, and at 40 °C (75% RH) the stability was 99.5 ± 0.23 and 97.7 ± 1.01 for periods of 3 and 6 months, respectively. Supplementary Fig. S11 suggests that the SBE-β-CD–progesterone complex in water or lyophilized form is stable at 25 °C (60% RH) and 40 °C (75% RH), respectively, for periods up to 6 months.

3.9. Ex vivo intestinal permeability study

The bioavailability of cyclodextrin upon oral administration is poor, and highly hydrophilic cyclodextrins have a negligible permeation across the intestinal barrier. Upon oral administration, <3% of SBE-β-CD is absorbed and subsequently it is cleared quickly through renal excretion, and hence it is non-toxic and has a high safety margin (Stella and He, 2008; European Medicines Agency, 2014). Various *in vivo* (Sun et al., 2018) and *in vitro* studies suggest the optimum concentration of cyclodextrin required to enhance permeability across the intestine or any biological barriers (Zuo et al., 2000; Stappaerts and Augustijns, 2016). Evaluation of a few *in vitro* data implies that too little or too much cyclodextrin can decrease the permeability and bioavailability of a drug (Zuo et al., 2000).

The *ex vivo* rat intestinal apparent permeability (Supplementary Eq. (S1)) and permeation study profile results are presented in Fig. 8 and Supplementary Fig. S12. The intestinal apparent permeability coefficient P_{app} (Fig. 8) of progesterone in different groups appeared in the following order: Complex in FASSIF with excess SBE-β-CD > Complex in FASSIF > Complex in FESSIF with excess SBE-β-CD > Complex in water > Progesterone in 0.5% Brij and > Complex in FESSIF. The apparent permeability of progesterone was increased significantly (P-value = 0.0086) in cells loaded with complex in water compared to progesterone in Brij 0.5%. The excess SBE-β-CD used in FASSIF increased by 8% the parent permeability of progesterone compared to for the complex without excess SBE-β-CD in FASSIF. However, the apparent permeability of progesterone was not statistically significantly different (P-value = 0.329) between the complex in FASSIF and the complex with excess SBE-β-CD in FASSIF. The results of Section 3.5 demonstrate this could be attributed to the difference in levels of solubilized progesterone in FASSIF (4.00 ± 0.09) and FASSIF with excess SBE-β-CD (4.73 ± 0.10), a difference of 18%. The apparent permeability of progesterone was increased significantly (P-value = 0.0020) in cells loaded with complex in FESSIF with excess SBE-β-CD compared to complex in FESSIF. The decrease in apparent permeability of FESSIF are a result of progesterone displacement from the complex. The *ex vivo* intestinal permeability study results depict that if SBE-β-CD is used in the formulation, there is increased permeation of progesterone across the intestinal barrier.

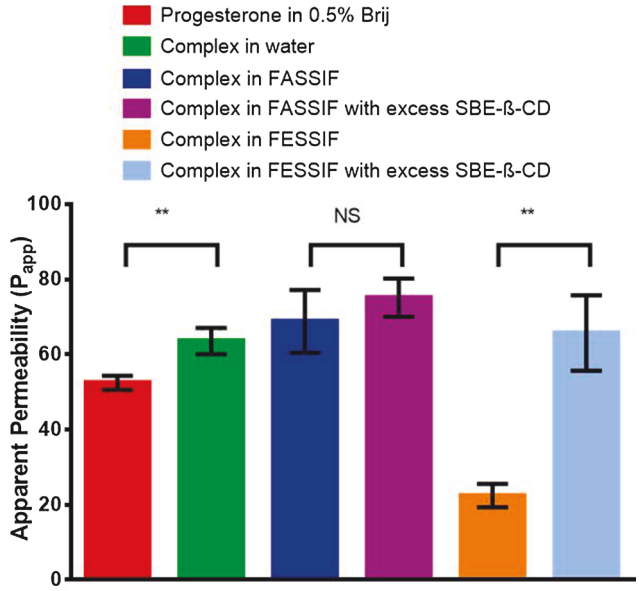

Fig. 8. Apparent intestinal permeability of progesterone: (A) Progesterone in 0.5% Brij (B) Complex in water; (C) Complex in FASSIF; (D) Complex in FASSIF with excess SBE-β-CD; (E) Complex in FESSIF; and (F) Complex in FESSIF with excess SBE-β-CD.

3.10. Rat pharmacokinetic study

The mean plasma concentration versus time profiles of oral progesterone API, oral SBE-β-CD–progesterone complex, and IV progesterone are shown in Fig. 9. It was evident from the plots that the plasma levels of progesterone were higher when it is administered in the form of the SBE-β-CD–progesterone complex as compared with progesterone API. Table 1 shows AUC_{0-1h}, C_{max}, T_{max}, and $\%F_{abs}$ (Supplementary Eq. (S2)) values obtained with oral progesterone API, the oral SBE-β-CD–progesterone complex, or IV progesterone dose. The calculated value for AUC_{0-1h} given in Table I showed that the overall oral bioavailability of progesterone was increased 5-fold when administered via the SBE-β-CD–progesterone complex.

4. Conclusion

The solubility of progesterone in water was enhanced by SBE-β-CD with an A_L type phase solubility curve. Experimental techniques such as DSC, FTIR and ^1H and ^{13}C NMR confirmed the formation of a complex between SBE-β-CD and progesterone. Molecular modeling studies predicted binding poses of progesterone with four isomers of SBE-β-CD to form inclusion complexes. The *in vitro* experiments were employed to determine the amount of SBE-β-CD required to prevent progesterone displacement from SBE-β-CD cavities and this concentration of SBE-β-CD was used in *ex vivo* intestinal permeation studies.. The SBE-

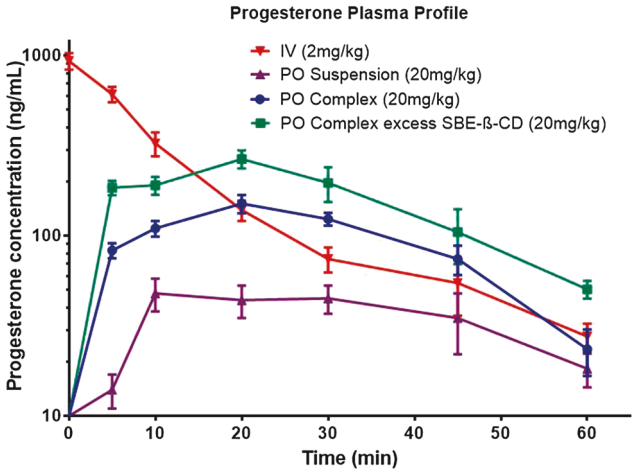

Fig. 9. Plasma profile of progesterone upon oral administration of SBE-β-CD–progesterone complex capsule (20 mg/kg), SBE-β-CD–progesterone complex capsule with excess SBE-β-CD (20 mg/kg), progesterone capsule (20 mg/kg), and IV administered progesterone (2 mg/kg).

Table 1
Pharmacokinetic parameters of progesterone upon oral administration of a SBE-β-CD–progesterone complex capsule (20 mg/kg), a SBE-β-CD–progesterone complex capsule with excess SBE-β-CD (20 mg/kg), a progesterone capsule (20 mg/kg), and IV administered progesterone (2 mg/kg) in rats.

Pharmacokinetic Parameters	Formulation			
	Progesterone capsule	SBE-β-CD – progesterone complex capsule	SBE-β-CD – progesterone complex capsule (Excess SBE-β-CD)	IV progesterone
Dose (mg/kg)	20	20	20	2
$AUC_{0\text{-}1h}$ (ng.h/mL)	34.9 ± 15.3	93.1 ± 5.88	157 ± 22.8	147 ± 15.6
T_{max} (min)	10	20	20	5
C_{max} (ng/mL)	52.6 ± 20.4	151 ± 17.5	267 ± 30.6	611 ± 59.7
% F_{abs}	2.36	6.31	10.7	100

β-CD–progesterone complex with excess SBE-β-CD increased intestinal permeation and oral bioavailability of progesterone. It is of the utmost importance to consider drug displacement by bile salts in the intestine to avoid underestimation of the oral bioavailability of NCE's. Also, it is essential to conduct a simple *in vitro* experiment in early drug discovery to determine the role of bile salts in the displacement of the drug from the cyclodextrin cavity. It can be very helpful to administer additional amounts of free cyclodextrin to prevent precipitation of NCE's in the intestines during preclinical studies. However, the dissolution study results indicate that SBE-β-CD–progesterone complex capsules get dissolved in the stomach before entering the small intestine and the addition of excess SBE-β-CD may not be needed, which would decrease the bulk of the formulation. However, to arrive at the most robust performing formulation, we would recommend evaluating the bioavailability of multiple designs with and without excess cyclodextrin to determine the most advantageous composition of lipophilic drugs.

CRediT authorship contribution statement

Vijay Kumar Shankar: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Supervision. **Anitha Police:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization. **Pankaj Pandey:** Conceptualization, Methodology, Software, Validation, Investigation, Writing - original draft, Visualization. **Zachary G. Cuny:** Methodology, Software, Investigation. **Michael A. Repka:** Resources, Writing - review & editing, Funding acquisition. **Robert J. Doerksen:** Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition. **S. Narasimha Murthy:** Conceptualization, Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2021.120212.

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