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Graphical Abstract

Highlights:

- CBD-SNEDDS formulations were developed using a digestion-resistant surfactant.
- Digestion of the CBD-SNEDDS formulations resulted in minimal drug precipitation.
- CBD-SNEDDS improved the *in vivo* exposure to CBD relative to a simple oil formulation.
- CBD-SNEDDS were also compared *in vivo* with a sesame oil-based formulation of CBD.

Abstract

The number of lipophilic drug candidates in pharmaceutical discovery pipelines has increased in recent years. These drugs often possess physicochemical properties that result in poor oral bioavailability, and their clinical potential may be limited without adequate formulation strategies. Cannabidiol (CBD) is an excellent example of a highly lipophilic compound with poor oral bioavailability, due to low water solubility and extensive first-pass metabolism. An approach that may overcome these limitations is formulation of the drug in self-nanoemulsifying drug delivery systems (SNEDDS). Herein, CBD-SNEDDS formulations were prepared and evaluated *in vitro*. Promising formulations (F2, F4) were administered to healthy female Sprague-Dawley rats via oral gavage (20 mg/kg CBD). Resulting pharmacokinetic parameters of CBD were compared to those following administration of CBD in two oil-based formulations: a medium-chain triglyceride oil vehicle (MCT-CBD), and a sesame oil-based formulation similar in composition to an FDA-approved formulation of CBD, Epidiolex® (SO-CBD). Compared to MCT-CBD, administration of the SNEDDS formulations led to more rapid absorption of CBD (median T_{max} values: 0.5 h (F2), 1 h (F4), 6 h (MCT-CBD)). Administration of F2 and F4 formulations also improved the

systemic exposure to CBD by 2.2 and 2.8-fold compared to MCT-CBD; however, no improvement was found compared to SO-CBD.

Keywords
oral delivery, lipophilic drugs, self-nanoemulsifying drug delivery systems, bioavailability, pharmacokinetics

Abbreviations

ACN	acetonitrile
AUC	area under the curve
CBD	Cannabidiol
DAD	diode-array detector
DI	deionized
DLS	dynamic light scattering
FaSSGF	fasted state simulated gastric fluid
FaSSIF	fasted state simulated intestinal fluid
GI	gastrointestinal
HPLC	high performance liquid chromatography
HPLC-MS	high performance liquid chromatography-mass spectrometry
MCT	medium chain triglyceride
PBS	phosphate buffered saline
PDI	polydispersity index
PEG	polyethylene glycol
PNL	ProNanoLipospheres
PVDF	polyvinylidene fluoride
SNEDDS	Self-nanoemulsifying drug delivery system
SO-CBD	sesame oil formulation of CBD
THC	Δ^9-tetrahydrocannabinol

Introduction

The oral bioavailability of lipophilic compounds is typically limited due to their low water solubility (Mu et al., 2013). In general, only drugs that are molecularly dissolved will be available for absorption, hence the dissolution of the drug in the gastrointestinal (GI) fluids must precede the absorption process (B.

Shekhawat and B. Pokharkar, 2017). Recent advances in drug discovery efforts, such as high throughput screening, selects for molecular entities with high ligand affinity, and this is often at the expense of physicochemical properties such as solubility and dissolution rate. Moreover, the oral route of administration is one of the most preferred by patients due to convenience and ease of administration (Eek et al., 2016; Stewart et al., 2016). Among the 53 new drugs and biological products that were approved by the U.S. Food and Drug Administration (FDA) in 2020, 24 products were intended for oral use, accounting for approximately 45% of the newly approved drugs (U.S. Food and Drug Administration, 2020a). Therefore, despite the challenges of delivering lipophilic drugs orally, the demand for such products drives research and development in this space.

Cannabidiol (CBD) is a prime example of a highly lipophilic compound that is difficult to deliver orally (Figure 1). CBD has been approved by the FDA as an oral solution (Epidiolex®) for the treatment of seizures associated with Lennox-Gastaut and Dravet syndromes, as well as tuberous sclerosis complex (U.S. Food and Drug Administration, 2020b, 2018). There are also several oral formulations of CBD that are currently reported to be, or have been, evaluated in the clinic for other indications (Cardiol Therapeutics, 2021; Mitelpunkt et al., 2019). However, oral delivery of CBD remains challenging due to its low oral bioavailability. Due to its low water solubility and high lipophilicity, CBD may be considered a Class II compound in the Biopharmaceutics Classification System (Perucca and Bialer, 2020). In addition to its poor water solubility, the oral bioavailability of CBD is further limited by extensive first-pass metabolism (Millar et al., 2018; Perucca and Bialer, 2020). A study in dogs has reported that 74% of CBD is metabolized in the liver following intravenous administration of CBD (Samara et al., 1988). As a result, the absolute oral bioavailability of CBD in dogs was found to range between 13% to 19%, following the administration of CBD in gelatin capsules (Samara et al., 1988).

The challenges associated with the formulation of CBD echo many of the issues that are present in the development of oral pharmaceutical drugs. Poor pharmacokinetic properties (e.g., low oral bioavailability, lack of systemic exposure to the drug) are reported to be one of the factors that leads to attrition of drug candidates (Basavaraj and Betageri, 2014; Waring et al., 2015). This may be a result of various issues, such as poor water solubility and significant first-pass effects. However, some reports have suggested that lipophilic compounds are becoming more common in early drug development (Leeson, 2016; Waring et al., 2015). For example, Leeson and St-Gallay (2011) analyzed the physicochemical properties of patented compounds from 18 companies between 2000 to 2010, and found the average calculated log P values to be higher compared to marketed oral drugs from previous years (Leeson, 2016). This finding has been attributed to prioritization of binding affinity to drug targets during drug optimization, as well as the involvement of intracellular drug targets that require more lipophilic ligands (Bergström et al., 2016; Leeson, 2016). This selection for lipophilic drugs necessitates advanced formulation steps to be conducted during early drug development. In many cases, an appropriate formulation strategy can enhance the oral delivery of such challenging drug candidates.

Various formulation strategies have been developed to address the solubility issues of lipophilic compounds, such as amorphous solid dispersions, multicomponent crystal systems and solid lipid nanoparticles (Kalepu and Nekkanti, 2015). One promising formulation strategy for CBD and other highly

lipophilic drugs is to use lipid-based drug delivery systems, such as self-nanoemulsifying drug delivery systems (SNEDDS). SNEDDS are defined as isotropic mixtures of oil, surfactant and cosurfactant/cosolvent, and these formulations can be easily administered as a preconcentrate in a gel capsule (Gursoy and Benita, 2004). Upon oral administration, the encapsulated SNEDDS will disperse into oil-in-water nanoemulsions in the GI fluids, a process that is facilitated by gastric movements (Porter et al., 2008; Vithani et al., 2019). Self-emulsifying platforms have been successfully employed in the past to improve the oral bioavailability of several lipophilic drugs, such as cyclosporine, ritonavir and saquinavir (Neoral®, Norvir® and Fortovase®, respectively) (Gursoy and Benita, 2004; Mundada et al., 2016). In general, SNEDDS have been proposed to enhance the availability of lipophilic drugs to absorption, due to the solubilization of the drug in nanoemulsions that disperse in the GI fluids (Atsmon et al., 2018a; Cherniakov et al., 2017b). Additionally, SNEDDS have also shown potential to be transported through the lymphatic system and bypass first-pass metabolism (Sun et al., 2011), hence this formulation strategy may also be beneficial to drug candidates that undergo significant first-pass metabolism. However, despite the advantages associated with using SNEDDS as an oral formulation strategy, these lipid-based delivery platforms also have their limitations. For example, they are susceptible to digestion in the GI tract, which can result in drug precipitation and limited absorption (Cuiné et al., 2008; Feeney et al., 2014). One potential approach to overcome such limitations is to consider digestion-resistant components in SNEDDS formulations.

The aim of the current study was to develop SNEDDS formulations of CBD that rapidly disperse in biorelevant media using a digestion-resistant surfactant. These newly developed formulations were characterized *in vitro*, and the most promising formulations were evaluated *in vivo* to obtain the pharmacokinetic parameters for CBD following oral administration in healthy Sprague-Dawley rats. The results were compared to two oil-based formulations of CBD: a medium chain triglyceride (MCT) oil formulation (MCT-CBD), and a sesame oil-based formulation similar in composition to Epidiolex (SO-CBD).

1. Materials and Methods

Materials

Brij® O10 (polyoxyethylene (10) oleyl ether), sesame oil, olive oil, Tween® 80 (polyoxyethylene sorbitan monooleate), ethyl 3-methyl-3-phenylglycidate, maleic acid and lipase from porcine pancreas (Type II) were purchased from Sigma Aldrich (Oakville, ON, Canada). Light mineral oil NF/FCC was obtained from Fisher Scientific (Waltham, MA, USA). Captex® 355 EP/NF (MCT oil) was a gift from Abitec Corporation (Janesville, WI, USA). Labrafac™ CC (MCT oil) was a gift from Gattefossé (Saint-Priest, France). FaSSIF/FeSSIF/FaSSGF and FaSSIF-V2 powders were purchased from Biorelevant.com Ltd. Calcium chloride dihydrate, propylene glycol, sodium chloride and sodium hydroxide pellets were purchased from BioShop Canada Inc. (Burlington, ON, Canada). Hydrochloric acid (6 M) and sodium hydroxide (10 M) were obtained from VWR Chemicals (Radnor, PA, USA). CBD isolate (98%) was obtained from Toronto Research Chemicals.

Preparation of crystalline CBD

Crystalline CBD was prepared via recrystallization of crude CBD resin in *n*-pentane. A saturated solution (i.e., at 22.5°C) of CBD resin in *n*-pentane was prepared at a concentration of 0.6 g/g (i.e., g of CBD resin per g of *n*-pentane) in a 20 mL glass vial wrapped in aluminum foil. For each experiment, vials contained approximately 6 - 10 g of *n*-pentane and a magnetic stir bar. These saturated solutions were then filtered using 20 mL syringes and syringe filters (0.2 µm PTFE, 25 mm) into fresh vials containing new magnetic stir bars. The filtered saturated solutions were then stirred (150 rpm) at 4°C for 1 h. Following 1 h, supersaturated solutions were seeded with ~20 mg of CBD isolate (Toronto Research Chemicals, 98%). These seeded, supersaturated CBD solutions were then stirred (150 rpm) at 4°C for a total of 24 h. Following this, the resulting crystals were removed from the solutions via vacuum filtration using a Buchner flask and Buchner funnel fitted with filter paper. CBD crystals were then washed with approximately 10 mL of cold *n*-pentane (cooled to -20°C), transferred to a clean vial capped with a rubber septum, and dried under nitrogen gas for approximately 2 h to remove any residual solvent.

HPLC method

Quantification of CBD was performed on an Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) coupled with a diode-array detector (DAD). Samples were injected in 5 µL volumes and separated using a Brownlee SPP C18 column (150 x 3.0 mm, 2.7 µm, Perkin Elmer) that was maintained at 30°C. Isocratic elution was performed with 70% v/v ACN (A) and 30% v/v 5 mM ammonium phosphate buffer containing 0.1% v/v formic acid (B). The mobile phase was delivered at a flow rate of 1 mL/min. CBD was detected at a wavelength of 228 nm. The calibration curves were linear over a range of 1-100 µg/mL with a correlation coefficient of 0.998 ± 0.002. The inter-day variations were less than 10% for all examined concentrations (5, 20 and 100 µg/mL). The method was specific, and no interfering peaks were observed.

Solubility of CBD in excipients

The solubility of CBD was evaluated in the following excipients: sesame oil, olive oil, Labrafac CC, Captex 355, mineral oil, Tween 80, Brij O10, and propylene glycol. An excess of CBD and a fixed amount of excipient were added to a glass vial containing a magnetic stir bar. This mixture was stirred at 100 rpm for 24 h at 37°C. The samples were then centrifuged at 14,000 rpm, and 37°C, for 10 min. Following centrifugation, 20 mg of supernatant was weighed into a clean glass vial and mixed with 20 mL of ACN. This mixture was then vortexed for 30 seconds and sonicated for 5 min. Following this, the samples were diluted appropriately, and analyzed via HPLC.

The extraction efficiency of CBD from the excipients was determined as follows: CBD was added to each excipient at 2% w/w, and the samples were treated as described above. Concentrations obtained from the HPLC analysis were compared against theoretical concentration values, and the extraction efficiency was calculated using Equation (1):

$$\text{Extraction efficiency} = \text{actual concentration} / \text{theoretical concentration} \times 100\% \quad (1)$$

Extraction efficiency values are reported in Table S2. All obtained concentrations from the solubility study were adjusted according to the extraction efficiency using Equation (2):

$$\text{Adjusted concentration} = \text{obtained concentration} / \text{extraction efficiency} \quad (2)$$

Establishment of a pseudo-ternary phase diagram

The pseudo-ternary phase diagram was constructed using a previously published procedure (Cuiné et al., 2007). Mixtures of Brij O10, Captex 355, and propylene glycol were prepared in various compositions. The mixtures were vortexed and incubated at 37°C overnight, followed by visual assessment for homogeneity. Mixtures that displayed phase separation after the incubation period were not evaluated further. The homogeneous mixtures were then dispersed in phosphate buffered saline (PBS) (0.5% w/v) stirred at 100 rpm and 37°C for 1 h. Following dispersion, the solution was first assessed visually, and the sizes of the resulting droplets were measured using dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments Ltd, UK) at 37°C. The pseudo-ternary phase diagram was plotted using OriginPro Learning Edition.

Preparation of CBD-loaded SNEDDS

CBD-loaded SNEDDS (CBD-SNEDDS) were prepared in two steps. First, bulk mixtures of SNEDDS were prepared by mixing the various excipients until a clear homogeneous solution was obtained. SNEDDS loaded with CBD were then prepared by dissolving CBD crystals in the drug-free SNEDDS to a concentration of 10% w/w. These mixtures were stirred at 500 rpm and 37°C to dissolve the CBD and the resulting solutions were cooled to room temperature prior to use.

Preparation of a sesame oil-based CBD formulation

SO-CBD was prepared according to the composition listed in a patent (Guy et al., 2018). In brief, 3.95 g of ethanol, 25 mg of sucralose and 10 mg of ethyl 3-methyl-3-phenylglycidate (strawberry flavoring) were added to a 50 mL volumetric flask. Sesame oil was then added to produce a 50 mL solution. Aliquots of the oil solution were taken and loaded with 10% w/w CBD to prepare the SO-CBD formulation.

Preparation of fasted state simulated gastric fluids and fasted state simulated intestinal fluids

Fasted state simulated gastric fluid (FaSSGF) and fasted state simulated intestinal fluid (FaSSIF) were prepared following protocols provided by Biorelevant.com Ltd. Briefly, for FaSSGF, a solution with a pH of 1.6 that consisted of sodium chloride (34.22 mM) and hydrochloric acid (25 mM) was prepared in deionized (DI) water. The pH was measured and adjusted to 1.6 with 6M hydrochloric acid when necessary. The FaSSGF powder was then dissolved in the buffer at a concentration of 0.0596 mg/mL, as per the protocols provided by the supplier. For FaSSIF, a buffer (pH 6.5) consisting of sodium hydroxide

pellets (34.8 mM), maleic acid (19.12 mM), and sodium chloride (68.62 mM) was prepared in DI water. Adjustment to pH 6.5 was achieved with 10 M sodium hydroxide or 6 M hydrochloric acid when necessary. Calcium chloride dihydrate was added to the buffer to achieve a Ca^{2+} concentration of 1.4 mM. The FaSSIF-V2 powder was then dissolved in the buffer (1.79 mg/mL) and the media was allowed to stand for at least 1 h before use, as per the protocols provided by the supplier.

Characterization of the droplet size of the CBD-SNEDDS formulations

The FaSSGF and FaSSIF were pre-warmed to 37°C, and 50 mg of the CBD-SNEDDS formulations were dispersed in 10 mL of the media. This mixture was then stirred (100 rpm) at this temperature for 1 h prior to evaluation of droplet size. The resulting size and polydispersity index (PDI) of the droplets were then measured via DLS at 37°C. Droplet sizes and PDI were analyzed using a parametric paired t-test.

In vitro dispersion study

CBD-SNEDDS (1.25 g) formulations were added to 50 mL of pre-warmed FaSSGF or FaSSIF at 37°C with stirring at 100 rpm. At 2, 5, 10, 20, 30, 45, 60, 90, and 120 min, 800 µL of the dispersion media was removed and replenished with an equal volume of fresh media. The sample was filtered (0.45 µm polyvinylidene fluoride (PVDF) syringe filter) and diluted with 1:2 chloroform/methanol prior to HPLC analysis.

To determine the extraction efficiency, the CBD-SNEDDS were dispersed in FaSSGF and FaSSIF at a lower concentration of CBD in the biorelevant media (0.5 mg/mL). Additionally, the same formulations were prepared with a lower drug loading level (3% w/w CBD) and dispersed in FaSSGF and FaSSIF at a CBD concentration of 2.5 mg/mL. All formulations were placed under the same conditions and were treated as described above, and the efficiency of the extraction was calculated using Equation (1). Extraction efficiency values are reported in Table S3. The concentrations obtained from the dispersion study were adjusted according to the extraction efficiency using Equation (2).

The initial dispersion rate for each formulation was estimated from the slope of the line between 0 to 2 min in the concentration-time plot, using Equation (3):

$$\text{Dispersion rate} = \frac{dC}{dt} \approx \frac{\text{Concentration (at 2 min)} - \text{Concentration (at 0 min)}}{\text{2 min} - \text{0 min}} \qquad (3)$$

Area under the curve (AUC) values were obtained using the linear trapezoidal method in Equation (4), where *C* represents concentration, *t* represents time and *n* represents the number of time points:

$$AUC_{0-t} = \sum_{i=1}^{n-1} \frac{(C_{i+1}+C_i)}{2}(t_{i+1} - t_i) \qquad (4)$$

In vitro digestion study

Following the aforementioned dispersion study, an *in vitro* digestion study was conducted on selected formulations. This digestion study was adapted from previously published methods (Cuiné et al., 2008;

Sassene et al., 2014). To initiate digestion, 40 mL of the dispersion media containing SNEDDS was added to a clean vial containing 667 mg of pancreatic lipase. The media was stirred at 100 rpm and 37°C throughout the digestion study. At 5, 15, 30, 60, 90, and 120 min, 800 µL of the media was removed and immediately filtered with a 0.45 µm PVDF syringe filter, followed by a dilution in 1:2 chloroform/methanol. The digestion vessel was replenished with fresh digestion media (i.e., FaSSIF containing pancreatic lipase) at each time point. All samples were analyzed via HPLC. AUC values were calculated as above (Equation (4)).

In vivo studies

Animal studies were approved by the Animal Care Committee at the University of Toronto. Female Sprague-Dawley rats aged 35 - 55 days were purchased from Charles River and were housed in the Division of Comparative Medicine. All animals were allowed to acclimatize for a week prior to the study. The study was performed in the fed state (i.e., animals had free access to water and food throughout the study).

An initial pilot study was conducted as follows: animals received MCT-CBD (containing 6 mg/mL CBD in MCT oil) via oral gavage, at a CBD dose of 19.3 mg/kg (n = 4). Serial blood samples were collected from the lateral saphenous vein at 1, 2, 4, 6, 8, and 10 h post-administration, using Microvette® CB 300 LH (Sarstedt AG & Co, Germany). The animals were sacrificed through cardiac puncture at 24 h, and blood was collected.

A second study was conducted to assess the pharmacokinetics of CBD following administration in select CBD-SNEDDS and the SO-CBD formulations. The animals (n = 5/group) received either one of two formulations of CBD-SNEDDS, or the SO-CBD formulation. All animals were administered the respective formulations via oral gavage at a CBD dose of 20 mg/kg. Serial blood samples were collected from the lateral saphenous vein at 0.5, 1, 2, 4, 6, 8, and 10 h post-administration into heparinized tubes. At 12 h after administration, the rats were sacrificed through cardiac puncture, and blood was collected.

Plasma was separated by centrifugation at 2000 × g at room temperature for 5 min and stored at -80°C until further processing.

CBD extraction from plasma

CBD was extracted from plasma using a method adapted from Paudel *et al* (2010). Briefly, 20 µL of plasma was added to 200 µL of ACN/ethyl acetate (50% v/v). The samples were then vortexed for 30 seconds and centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant in each sample was transferred to a clean vial. These samples were concentrated via evaporation under nitrogen at 37°C using a Glas-Col ZipVap evaporator (Terre-Haute, IN, USA) followed by reconstitution in 100 µL of ACN. These reconstituted samples were then sonicated for 5 min and analyzed using HPLC-mass spectrometry (HPLC-MS).

Blank Sprague Dawley rat plasma was obtained from Innovative Research, Inc (Michigan, USA). The plasma was spiked with CBD analytical standards to final concentrations of 1 and 0.5 µg/mL. The plasma was vortexed, and CBD was extracted following the procedure above. The extraction efficiency was calculated using Equation (2) and was found to be 63.5 ± 8.4%.

HPLC-MS method

HPLC-MS analysis was performed using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of an InfinityLab Poroshell 120 EC-C18 column (150 x 2.1 mm, 1.9 µm, Agilent Technologies, Santa Clara, CA, USA) and a TSQ Endura™ Triple Quadrupole MS (Thermo Fisher Scientific, Mississauga, ON). The column oven was kept at 40°C. The mobile phase consisted of 25 mM ammonium acetate pH 4.75 (A) and ACN with 0.1% (v/v) formic acid (B). A gradient elution was programmed to commence with 68% B post-injection of sample followed by a gradual increase of B to 81.6% over 15 min. The B phase was then decreased back to 68% over 1 min. The mobile phase was maintained at 32% A and 68% B for 7 min prior to the next injection. The flow rate was 0.7 mL/min throughout the run and 20 µL of sample was injected. The injector needle was washed with methanol after each sample. Instrument control and data analysis were performed using Thermo Scientific Xcalibur software (version 4.1.50). Calibration curves were linear over the range of 5 - 500 ng/mL (r^2 = 0.9997 ± 0.0004, coefficient of variation < 25%). The lowest concentration of standard was 5 ng/mL. Additional details are described in Tables S4 and S5.

Determination of pharmacokinetic parameters

Plasma concentrations from the *in vivo* study were adjusted according to the extraction efficiency using Equation (2). Noncompartmental pharmacokinetic analysis was performed on individual animal data. The terminal elimination rate constant (k_{el}) was estimated using the regression slope of the log-linear terminal elimination phase, and the elimination half-life ($t_{1/2}$) was calculated using Equation (5):

$$t_{1/2} = \ln(2) / k_{el} \quad (5)$$

Maximum plasma concentration (C_{max}) and the time at which it was achieved (T_{max}) were determined for each individual animal. The mean and median values were reported for C_{max} and T_{max} respectively. The area under the plasma concentration-time curve (AUC_{0-t}) was calculated using the linear trapezoidal method (Equation 4) from 0 to the last measured plasma concentration (C_{last}). $AUC_{0-\infty}$ was calculated by adding C_{last}/k_{el} to AUC_{0-t}, and $AUC_{0-t(last)}/AUC_{0-\infty}$ values were determined (where t_{last} = 12 h for F4 and SO-CBD administration). Relative oral bioavailability values of CBD compared to MCT-CBD and SO-CBD were estimated as ratios of mean AUC values between 0 to 4 h, 0 to 10 h, 0 to 12 h and 0 to ∞.

Statistical analysis

Values are expressed as mean ± standard deviation, unless stated otherwise. Statistical significance between three or more groups were examined using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test, unless stated otherwise. Comparisons between two groups were performed using unpaired t-test with Welch's corrections. P-values below 0.05 were considered

statistically significant. All statistical analyses were performed on GraphPad Prism 7.04 (GraphPad Software, La Jolla, CA).

2. Results

Selection of excipients

The solubility of CBD in various excipients was evaluated to guide the selection of components for a SNEDDS formulation (Table 1). The solubility of CBD in the following excipients: Brij O10, Tween 80, propylene glycol, Captex 355 and Labrafac CC was found to be > 50% w/w. The solubility of CBD in the remaining excipients investigated was found to be < 50% w/w and followed the order: sesame oil > olive oil > mineral oil. Based on these results, Brij O10, Captex 355 and propylene glycol were selected (as the surfactant, oil carrier and co-solvent, respectively) to develop a SNEDDS formulation and construct a pseudo-ternary phase diagram.

Construction of phase diagram and selection of lead formulations

A pseudo-ternary phase diagram was established by evaluating the miscibility of the excipients at 37°C and the resulting droplet sizes of the drug-free SNEDDS after dispersion in PBS (Figure 2). At 20% w/w Brij O10, phase separation was observed in most formulations, and these compositions were not evaluated further. All other compositions remained miscible and homogeneous upon visual inspection at 37°C. The miscible compositions were dispersed in PBS and evaluated via visual assessment and droplet size measurements. The individual results from the visual assessment, droplet sizes and PDI have been summarized in Table S6. Several trends can be observed from the droplet sizes in Figure 2. Most of the compositions with 10% w/w propylene glycol formed droplet sizes near or above 250 nm. Within a fixed composition of Brij O10, decreasing the proportion of oil (thus increasing the proportion of co-solvent) appeared to decrease the resulting droplet size. The smallest droplet sizes (< 50 nm) were observed at 10% and 20% w/w Captex 355. Four lead formulations containing 20% w/w Captex 355 were selected for further optimization, to minimize surfactant content.

Droplet size characterization of CBD-SNEDDS in biorelevant media

The droplet sizes of the drug-free formulations in biorelevant media (FaSSGF and FaSSIF) were equivalent to their sizes in PBS. The lead formulations (F1-F4) were then prepared according to Table 2 with 10% w/w CBD (i.e., CBD-SNEDDS). The lead CBD-SNEDDS formulations (F1-F4) were evaluated in terms of resulting droplet sizes following dispersion in FaSSGF and FaSSIF (Table 3). The droplet sizes of F1, F2, F3 and F4 formulations in FaSSGF were found to be 626 ± 44, 175 ± 44, 71 ± 12, and 48 ± 15 nm, respectively. In FaSSIF, the droplet sizes were found to be 674 ± 57, 192 ± 43, 72 ± 7, and 41 ± 6 nm for the F1, F2, F3 and F4 formulations, respectively. There were no significant differences in the average droplet size of each CBD-SNEDDS formulation in FaSSGF compared to FaSSIF. However, droplet sizes did appear to increase with increasing surfactant concentration (i.e., decreasing co-solvent concentration). The F1 formulation was excluded from further studies due to its large droplet size.

In vitro dispersion studies

Dispersion studies were carried out on F2, F3 and F4 formulations in FaSSGF and FaSSIF (Figure 3). All formulations were found to disperse within 5 min of addition to either media. The initial dispersion rates for F2, F3 and F4 formulations in FaSSGF were found to be 1.10 ± 0.08, 1.15 ± 0.04 and 1.22 ± 0.05 $mg{\cdot}mL^{-1}min^{-1}$, respectively (Table 3). Thus, in FaSSGF, the dispersion rates were not found to be significantly different between the three lead formulations. The initial dispersion rates for F2, F3 and F4 formulations in FaSSIF were found to be 1.09 ± 0.04, 1.32 ± 0.1 and 1.33 ± 0.01 $mg{\cdot}mL^{-1}min^{-1}$, respectively. The dispersion rate for the F2 formulation was significantly lower compared to F3 and F4 formulations in FaSSIF. To determine differences in the solubilization of CBD, the CBD-SNEDDS were compared in terms of their AUC, and no significant difference was found. No drug precipitation was observed throughout the dispersion studies.

In vitro digestion studies

Immediately after the dispersion studies in FaSSIF, each dispersed CBD-SNEDDS formulation was exposed to porcine pancreatin to evaluate their susceptibility to digestion under *in vitro* conditions. Some drug precipitation was observed for all formulations, but approximately 80% of the initial concentration of CBD remained solubilized by the SNEDDS during the digestion study (Figure 4). There was no significant difference in the degree of solubilization of CBD for F2, F3 or F4 formulations as measured by this assay (Table 3). The MCT-CBD and SO-CBD formulations were also subjected to the same *in vitro* dispersion and digestion conditions to evaluate the solubilization of CBD. However, these oil formulations did not disperse in the biorelevant media, and hence there was no solubilization of CBD observed in the media throughout the study.

In vivo study

The CBD-SNEDDS formulations with the largest droplet size (F2) and smallest droplet size (F4) were selected as treatment groups for the *in vivo* studies. Oral administration of CBD in the CBD-SNEDDS formulations (F2 and F4) and the oil-based formulations (SO-CBD and MCT-CBD) in rats resulted in the pharmacokinetics profiles depicted in Figure 5. The pharmacokinetics parameters were estimated and reported in Table 4. There were no significant differences between the C_{max} values between groups. However, administration of CBD in the SO-CBD formulation resulted in the highest C_{max} (629 ± 118 ng/mL), while the lowest C_{max} was found in the group administered MCT-CBD (128 ± 60 ng/mL). The median T_{max} value for F4 administration (1 h) is similar to SO-CBD (1 h), while the median T_{max} for F2 administration (0.5 h) is shorter compared to SO-CBD. However, the group administered the MCT-CBD formulation required a longer time to reach C_{max} (6 h). Further details on T_{max} can be found in Figure S1. Plasma concentrations were measured up to 12 or 24 h post-dose. However, for some animals, estimation of $t_{1/2}$ was difficult and unreliable due to potential error in the identification of the terminal elimination phase. For those where the slope could be reliably estimated (F4 and SO-CBD: n=3), the $t_{1/2}$ values of CBD were found to be 7.3 ± 2.3 h and 7.7 ± 2.5 h for the groups administered F4 and SO-CBD,

respectively. For the animals administered the F2 formulation, the terminal elimination phase could not be accurately identified as double peaks were observed in the plasma concentration-time curves in several animals (n=3). Additionally, the $t_{1/2}$ of CBD in the animals administered the MCT-CBD formulation could not be obtained due to an insufficient number of datapoints in the elimination phase.

The AUC values of CBD were first calculated for 0 to 4 h to compare partial AUCs for the early time points. The animals that received F2 and F4 formulations were found to have AUC values of 539 ± 74 ng·h/mL and 718 ± 336 ng·h/mL, respectively, during the first 4 h. These values were significantly lower in comparison to the value obtained for the SO-CBD formulation (1497 ± 332 ng·h/mL). However, the $AUC_{0\text{-}4h}$ values of CBD from the CBD-SNEDDS administration were significantly greater than those found for MCT-CBD administration (80 ± 45 ng·h/mL). Ratios of the mean $AUC_{0\text{-}4h}$ values show that the exposure to CBD from F2 and F4 administration were 6.7 and 9.0-fold greater compared to MCT-CBD (Table S7). However, SO-CBD administration resulted in the greatest exposure to CBD within the first 4 h.

The AUC values of CBD for the treatment groups were also compared against the oil-based formulations MCT-CBD and SO-CBD from 0 to 10 h and 0 to 12 h, respectively (Table 4). Between 0 to 12 h, the AUC values were 1104 ± 56 ng·h/mL and 1321 ± 434 ng·h/mL for the F2 and F4 formulations, respectively. Meanwhile, the AUC value for CBD following administration in the SO-CBD formulation was 2235 ± 317 ng·h/mL. The $AUC_{0\text{-}12h}$ values for both treatment groups were significantly lower ($p<0.05$) in comparison to the SO-CBD administration. Ratios of the $AUC_{0\text{-}12h}$ values of CBD relative to SO-CBD were 0.5 and 0.6 for F2 and F4 formulations respectively (Table S7). From 0 to 10 h, the AUC values for CBD in the treatment groups were significantly greater compared to the MCT-CBD administration ($p<0.05$). Administration of the F2 and F4 formulations led to $AUC_{0\text{-}10h}$ values of 978 ± 89 ng·h/mL and 1246 ± 468 ng·h/mL, while the $AUC_{0\text{-}10h}$ value obtained from the administration of the MCT-CBD formulation was 445 ± 191 ng·h/mL. The systemic exposure to CBD following administration of F2 and F4 formulations was increased by 2.2 and 2.8-fold, respectively, compared to the MCT-CBD administration (Table S7).

Extrapolations of the AUC values to infinity were performed for animals where k_{el} could be estimated (F4 and SO-CBD: n=3). The obtained $AUC_{0\text{-}\infty}$ values for CBD were 1949 ± 759 ng·h/mL and 2766 ± 260 ng·h/mL following the administration of F4 and SO-CBD, respectively. There was no significant difference in the $AUC_{0\text{-}\infty}$ values for CBD when administered in the F4 formulation compared to SO-CBD. The oral bioavailability value for CBD relative to SO-CBD was 0.7 following the administration of F4 (Table S7).

For the animals where $AUC_{0\text{-}\infty}$ could be calculated (F4 and SO-CBD: n=3), $AUC_{0\text{-}t(last)}/AUC_{0\text{-}\infty}$ values were obtained. These values suggest that approximately 76 ± 3% of the total exposure to CBD was achieved within 12 h following the administration of F4 (Table S7). In comparison, administration of the SO-CBD formulation led to 81 ± 9% exposure to CBD within 12 h.

3. Discussion

Like many lipophilic drugs, the oral bioavailability of CBD is known to be poor due to its low water solubility, limited absorption and extensive first-pass metabolism (Perucca and Bialer, 2020; Taylor et al.,

2018). Without adequate formulation, it may be difficult to achieve the desired therapeutic response via oral delivery of CBD. Various advanced formulation strategies have been explored in the past to improve the oral bioavailability of poorly water-soluble drug candidates. SNEDDS formulations have previously demonstrated the potential to enhance the oral bioavailability of various lipophilic drugs (Alqahtani et al., 2013; Holm et al., 2003; Hong et al., 2006; Knaub et al., 2019; Lee et al., 2015), and several self-emulsifying formulations (e.g., Neoral®, Norvir® and Fortovase®) have reached the market (Gursoy and Benita, 2004). Among the advanced formulations that were developed for oral delivery of CBD, SNEDDS platforms also appear to be one of the most common (Atsmon et al., 2018a, 2018b; Cherniakov et al., 2017b; Knaub et al., 2019; Koch et al., 2020; Mitelpunkt et al., 2019; Nakano et al., 2019). In the current study, SNEDDS formulations were developed for the oral delivery of CBD, and these formulations were compared to MCT-CBD and SO-CBD in healthy Sprague-Dawley rats.

Previously developed SNEDDS formulations of CBD have shown some positive results in animal studies and clinical trials. These formulations include Pro-Nano Lipospheres (PNL) (Cherniakov et al., 2017b, 2017a) as well as an optimized version of this PNL formulation, known as PTL401 (Phytotech Therapeutics) (Atsmon et al., 2018a), VESIsorb® CBD-SNEDDS formulation (VESIfact AG) (Knaub et al., 2019), and nanoemulsions of CBD (CBD-NE) (Nakano et al., 2019). Both the PTL401 formulation and the previous version (PNL) are composed of Tween 20, Span 80, lecithin, Cremophor RH 40, ethyl lactate and tricaprin (Atsmon et al., 2018a; Cherniakov et al., 2017a). The PTL401 formulation contains 1.1% w/w CBD and 1.2% w/w Δ^9-tetrahydrocannabinol (THC), which is similar in drug content to the commercially available sublingual formulation of CBD and THC, Sativex®. Clinical studies have shown that the oral administration of PTL 401 (10 mg CBD and 10.8 mg THC) resulted in a 1.3 and 1.2-fold increase in AUC values (i.e., $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$) for CBD and THC, compared to the sublingual administration of Sativex (Atsmon et al., 2018a). In another study, a CBD-SNEDDS formulation developed from the proprietary VESIsorb technology was evaluated in a clinical study in comparison to an MCT oil formulation of CBD (Knaub et al., 2019). In this study, the authors reported two AUC values (i.e., $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}24h}$) and, following oral administration of their formulation (25 mg CBD), 2.8 and 1.7-fold increases in $AUC_{0\text{-}8}$ and $AUC_{0\text{-}24}$ were reported in comparison to the MCT-CBD formulation. Finally, the CBD-NE formulation consisted of vitamin E, ethanol, Tween 20 and water, with CBD loaded at a concentration of 30 mg/mL (Nakano et al., 2019). Both CBD-NE and PTL 401 formulations formed emulsion droplets that were similar in size (~30 nm). Studies in rats showed that the AUC values of CBD (i.e., $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}\infty}$) increased 1.3 and 1.7-fold, respectively, after administration of the CBD-NE formulation, compared to an olive oil formulation of CBD. However, the difference in AUC values was not found to be statistically significant (p-values > 0.05). Generally, these SNEDDS formulations have been shown to improve the relative bioavailability of CBD compared to oral administration of an oil formulation of CBD or sublingual administration of Sativex. However, to date, these formulations have not been compared to a sesame oil-based formulation with compositions similar to Epidiolex.

In this study, CBD-SNEDDS formulations were prepared with a higher concentration of CBD (10% w/w) compared to the previous studies (Atsmon et al., 2018a; Cherniakov et al., 2017a; Nakano et al., 2019). This concentration of CBD was chosen in order to compare the performance of the CBD-SNEDDS formulations to SO-CBD. Based on the solubility of CBD in Table 1, the excipients in which CBD has the

greatest solubility were selected to ensure that the desired concentration of CBD was achieved in solution with the minimum amount of excipients (i.e., a high drug to material ratio). For the oil carrier, as Captex 355 and Labrafac CC exhibited similar solubility, Captex 355 was selected for the formulation based on availability. Among the lead candidate formulations selected in this study (F1-F4), the droplet size and PDI values obtained for the F4 formulation (Table 3) were the most similar in size to other SNEDDS formulations previously reported (PTL401, PNL and CBD-NE) (Atsmon et al., 2018a; Cherniakov et al., 2017b; Nakano et al., 2019).

The droplet sizes of SNEDDS formulations have been suggested to influence the absorption of encapsulated drugs (Atsmon et al., 2018a; Sun et al., 2011). Following oral administration, drug entry into the systemic circulation is typically known to involve the movement of the drug into the portal vein. However, there are studies which show that drug transport through the lymphatic system is also possible, and this provides an alternative pathway for drug compounds to enter the systemic circulation while avoiding first-pass metabolism (Brocks and Davies, 2018; Franco et al., 2020). Lymphatic transport has been suggested to be one of the potential drug transport pathways for other oral drug delivery systems, and particle sizes have been proposed to play a role in this absorption pathway (Schudel et al., 2019; Zhang et al., 2021). For example, lipid nanoparticles with particle sizes less than 500 nm have been hypothesized to be absorbed through the lymphatic system (Li et al., 2009; Patel and Patel, 2021). There is also some evidence to suggest that droplet sizes of lipid-based delivery systems such as SNEDDS may similarly influence lymphatic absorption. One study has suggested that smaller droplet sizes of SNEDDS may increase intestinal absorption and may also be transported through the lymphatic system (Sun et al., 2011). This was proposed to occur through uptake by M cells via pinocytosis. Three of the lead formulations in this study (F2, F3, and F4) formed droplet sizes that were well within the proposed size range for potential transportation through the lymphatics, in addition to absorption via the portal route.

Digestion studies of SNEDDS formulations have been shown to lead to drug precipitation in *in vitro* digestion models, thus resulting in a decreased potential for the formulations to enhance the oral bioavailability of the drug (Anby et al., 2012; Cuiné et al., 2008; Feeney et al., 2014). For the first time, a CBD-SNEDDS formulation has been developed using an ether-based surfactant (Brij O10). These ether-based surfactants have been used in other SNEDDS formulations (Bibi et al., 2017; Feeney et al., 2014), but to date they have not been used to formulate CBD. In addition, ether-based surfactants have also been shown to be resistant to digestion (Cuiné et al., 2008). Cuiné *et al.* (2008) have shown that SNEDDS formulations containing digestion-resistant surfactants may be less susceptible to drug precipitation during *in vitro* digestion conditions. In another study, certain surfactants were proposed to sterically inhibit lipase enzyme access to the oil component of the SNEDDS formulations, and the formulations composed of these surfactants were also found to have minimal drug precipitation during *in vitro* digestion studies (Feeney et al., 2014). In both studies, SNEDDS formulations that were less susceptible to drug precipitation during *in vitro* digestion were found to result in greater AUC values of the drugs *in vivo* (Cuiné et al., 2008; Feeney et al., 2014). However, digestion has also been suggested to be beneficial to certain types of formulations (i.e., surfactants only). In one study, greater *in vivo* exposure was observed following the oral administration of surfactant-only formulations that were more prone to digestion, compared to the administration of surfactant formulations that were less susceptible to

digestion (Koehl et al., 2020). The authors suggested that the increased absorption noted in their study could potentially be due to the different nature of the colloidal systems (i.e., surfactant only micelles versus oil and surfactant emulsions).

The CBD-SNEDDS formulations in this study were developed with a digestion-resistant surfactant in an attempt to minimize drug precipitation that may occur due to digestion. Results from the digestion studies suggest that the lead formulations (F2, F3 and F4) are largely resistant to digestion, as only a limited amount of drug precipitation was observed during the forced digestion study (Figure 4). By comparison, no precipitation was noted in the dispersion studies, indicating good colloidal stability of these emulsion-based formulations in biorelevant media (Figure 3). Our CBD-SNEDDS appear to be slightly more susceptible to digestion and drug precipitation, compared to other SNEDDS formulations in the literature that were reported to be digestion-resistant (Cuiné et al., 2008). However, the drug precipitation observed in our CBD-SNEDDS was still considerably less than that reported for digestible formulations (Cuiné et al., 2008; Feeney et al., 2014). Although Brij O10 has been shown to be digestion-resistant, many oils used in SNEDDS formulations are themselves susceptible to digestion, including the MCT oil used here (Kaukonen et al., 2004). In some cases where Brij O10 was combined with a digestible oil carrier, this surfactant was shown to offer little protection to the oil carrier from digestion (Feeney et al., 2014). Feeney *et al.* (2014) reported a relationship between the extent of digestion and the molecular weight of the polyethylene glycol (PEG) moiety present in the head groups of certain surfactants. The relatively low molecular weight of PEG present in the surfactant head groups of Brij O10 (441 g/mol) was found to be outside of the optimal range necessary to prevent the digestion of the SNEDDS formulations. Their study demonstrated that there was a parabolic relationship between the molecular weight of the PEG head groups and the extent of digestion. For the ether-based surfactants screened, a molecular weight of around 800 g/mol of PEG in the head groups led to complete inhibition of digestion. The mechanism for this inhibition was suggested to be steric hindrance by the PEG head groups, which prevented the adsorption of lipase onto the oil and water interface, thereby inhibiting the digestion of the formulations (Feeney et al., 2014). Here, the results of our digestion studies suggest that it may be necessary to use surfactants with higher molecular weight of PEG to prevent drug precipitation from the CBD-SNEDDS formulations.

Our CBD-SNEDDS formulations were evaluated *in vivo* and compared to two oil-based formulations, MCT-CBD and SO-CBD. To our knowledge, this was the first *in vivo* study that compared a SNEDDS formulation of CBD against a sesame oil-based formulation that is similar in composition to Epidiolex . It should be noted that the oil carrier in Epidiolex (sesame oil, long chain triglycerides) differs from the oil phase used in our SNEDDS formulations (medium chain triglycerides). The sesame oil-based formulation was included in our study to compare the pharmacokinetics of CBD when administered in the SNEDDS formulations to a formulation similar in composition to Epidiolex. The concentration-time curves in Figure 5 suggest that CBD is absorbed quickly, and the concentration of CBD declines rapidly following administration in the SNEDDS and SO-CBD formulations. The median T_{max} values of CBD-SNEDDS are within 1 h, while the T_{max} for MCT-CBD is substantially greater (6 h) (Table 4). Additionally, the $AUC_{0\text{-}4h}$ values show that there is greater exposure to CBD from the CBD-SNEDDS, compared to MCT-CBD in the first 4 h post-administration. These data suggest that the absorption of CBD for CBD-SNEDDS was faster

compared to MCT-CBD, which may be due to the improved solubilization of CBD in the GI fluids. Interestingly, the absorption of CBD from one CBD-SNEDDS formulation (F2) was found to be faster compared to the SO-CBD formulation. However, administration of the SO-CBD formulation was found to result in a greater $AUC_{0\text{-}4h}$ in comparison to administration of CBD in the SNEDDS formulations. This suggests that the extent of CBD absorption following administration in the SO-CBD formulation is greater than that achieved with the SNEDDS formulations.

Similarly, the $AUC_{0\text{-}12h}$ values suggest that the systemic exposure to CBD from our CBD-SNEDDS formulations were lower in comparison to that from the SO-CBD formulation. However, in comparison to administration of the MCT-CBD formulation, the $AUC_{0\text{-}10h}$ values showed that administration of the SNEDDS formulations resulted in significantly greater exposure to CBD, suggesting that the F2 and F4 formulations improved the bioavailability of CBD by 2.2 and 2.8-fold relative to MCT-CBD (Table S7). This enhancement in oral bioavailability has been reported for other SNEDDS formulations of CBD, and the improvement was attributed to the increased solubilization of CBD in the SNEDDS, as well as the increased potential to access the inter-villous space due to the small droplet size (Cherniakov et al., 2017b; Knaub et al., 2019; Nakano et al., 2019). Additionally, the increase in oral bioavailability of our formulations may, to some extent, be due to absorption through the intestinal lymphatic system, as some SNEDDS have also shown potential to be transported through the lymphatic system and improve oral bioavailability (Arya et al., 2017; Holm et al., 2003; Sun et al., 2011). The absorption mechanism of our CBD-SNEDDS is currently not known, but in some rats, the pharmacokinetic profiles for CBD following oral administration of the F2, F4 and SO-CBD formulations showed a secondary peak in the plasma concentration of CBD. This double peak phenomenon may be attributed to a combination of distinct mechanisms including, but not limited, to enterohepatic recycling and intestinal lymphatic absorption (Brocks and Davies, 2018). Interestingly, THC and its metabolites have been observed to undergo enterohepatic recycling – a phenomenon where compounds are secreted into the bile and reabsorbed in the intestine, resulting in multiple absorption phases in the pharmacokinetic profiles of the compounds (Fabritius et al., 2012; Garrett and Hunt, 1977; Roberts et al., 2012). In such cases, the half-life of the compounds may be prolonged, and oral bioavailability may also be affected (Roberts et al., 2012). While enterohepatic recycling of CBD may be possible, further investigation is needed to determine the cause of these double peaks. Additionally, the absorption mechanism of CBD in our SNEDDS formulations remains to be investigated. Overall, the results imply an improvement in the extent of CBD absorption.

Finally, the C_{max} values suggest that all formulations achieved similar maximum concentrations of CBD in the blood. The k_{el} could not be accurately determined in some of the animals due to the lack of points in the terminal elimination phase as well as the presence of extra peaks in the concentration-time curves, which led to difficulties in characterizing the terminal elimination phase. Consequently, $t_{1/2}$ and the AUC values to infinity ($AUC_{0\text{-}\infty}$) could not be estimated for those animals. For the animals where k_{el} could be calculated, there was no significant difference in the $t_{1/2}$ values between the groups. The ratio of $AUC_{0\text{-}\infty}$ values suggests that the oral bioavailability of CBD following the administration of F4 was lower compared to SO-CBD (relative bioavailability = 0.7). However, the variability in these values is high, and since k_{el} could not be calculated for some animals, future work may be needed to confirm the data.

Conclusions

In summary, our results suggest that the oral bioavailability of lipophilic compounds such as CBD can be improved using advanced formulations. Administration of the CBD-SNEDDS led to more rapid absorption of CBD compared to MCT-CBD. While the bioavailability of CBD following the administration of CBD-SNEDDS formulations was not improved compared to the SO-CBD formulation, the CBD-SNEDDS formulations significantly enhanced the bioavailability of CBD relative to MCT-CBD from 0 to 10 h post-administration. Additionally, one CBD-SNEDDS formulation (F2) appeared to improve the rate of absorption (T_{max}) compared to SO-CBD. The absorption mechanism for the encapsulated drugs delivered in digestion-resistant SNEDDS formulations is not well understood, and further work is needed to elucidate the potential absorption pathways. This warrants further investigation and additional optimization of our SNEDDS formulation may result in improvements in the delivery of CBD and other lipophilic drug candidates. In addition, this study also highlights the disconnect that currently exists between *in vitro* testing (i.e., dispersion and digestion assays) and *in vivo* performance of oral micellar-based formulations. More consideration is needed to select better *in vitro* assays, especially for lipid-based drug delivery systems, in order to bridge this knowledge gap and better model *in vivo* absorption. Overall, these limitations demonstrate the complexity and challenges that are associated with developing adequate oral delivery strategies for lipophilic drugs.

Author contributions

L.Y.K designed and performed all experiments, analyzed and interpreted the experimental data, and wrote the first draft of the manuscript. P.B. prepared the crystalline CBD for experimental use, assisted in the animal studies, and provided guidance in the experimental design and data analysis. F.S. contributed to the analysis and interpretation of the pharmacokinetics data. J.C.E. contributed to the execution of the animal studies and provided the data for the pilot animal study. M.D. developed the HPLC-MS method and operated the HPLC-MS to obtain data for the pilot animal study. L.A. assisted in the animal studies. M.R. contributed to the modification of the HPLC-MS method and the acquisition of data from the HPLC-MS for the second animal study. D.D. critically reviewed and provided guidance on the analysis of the pharmacokinetics data. C.A. provided oversight for the experimental studies and edited the manuscript. L.Y.K., P.B. and F.S. finalized the manuscript with critical feedback from C.A. All authors edited, reviewed, and provided input to the final manuscript. The final version of the manuscript was approved by all authors.

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Declaration of interests

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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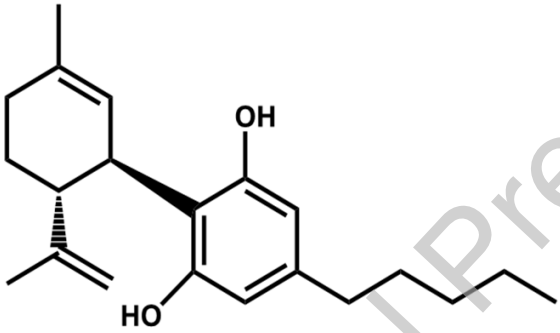

Molecular weight (g/mol)	314.5
Calculated logP	6.1
Calculated water solubility (µg/mL)	12.6
Hydrogen bond donor count	2
Hydrogen bond acceptor count	2
Topological polar surface area (Å²)	40.5

Figure 1. Molecular structure of CBD and its physicochemical properties (DrugBank, 2021; National Center for Biotechnology Information, 2019).

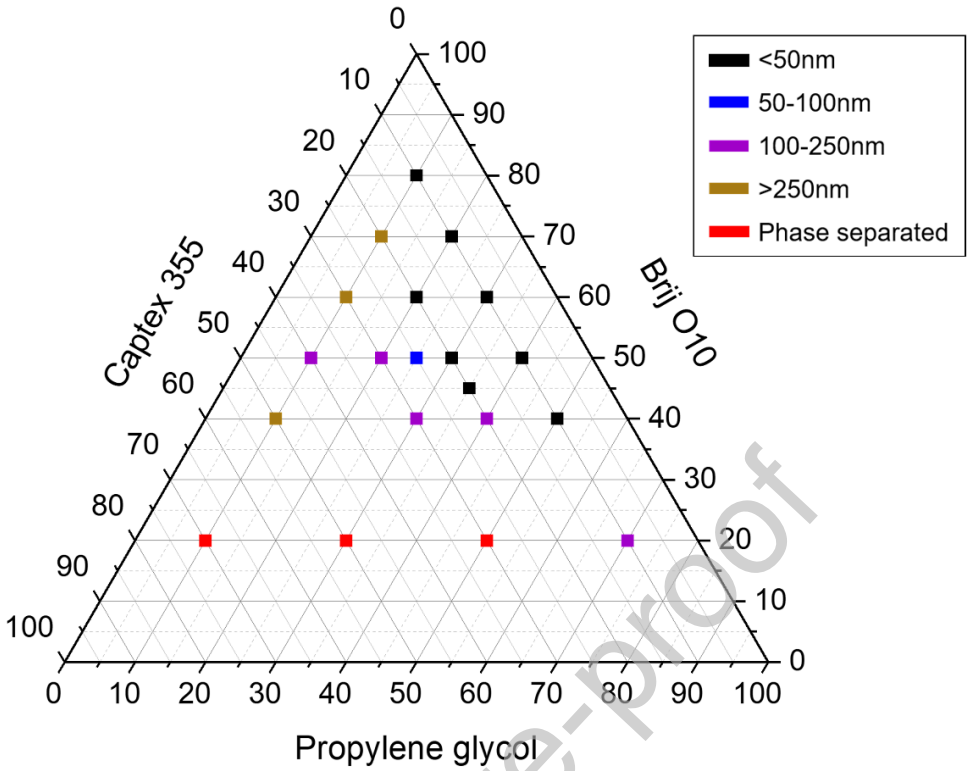

Figure 2. Pseudo-ternary phase diagram of drug-free SNEDDS formulations composed of Brij O10, Captex 355, and propylene glycol (n=3). Points in red indicate the compositions that phase separated overnight during the incubation at 37°C. For those that were dispersed in PBS, droplet size ranges are indicated as shown in the legend.

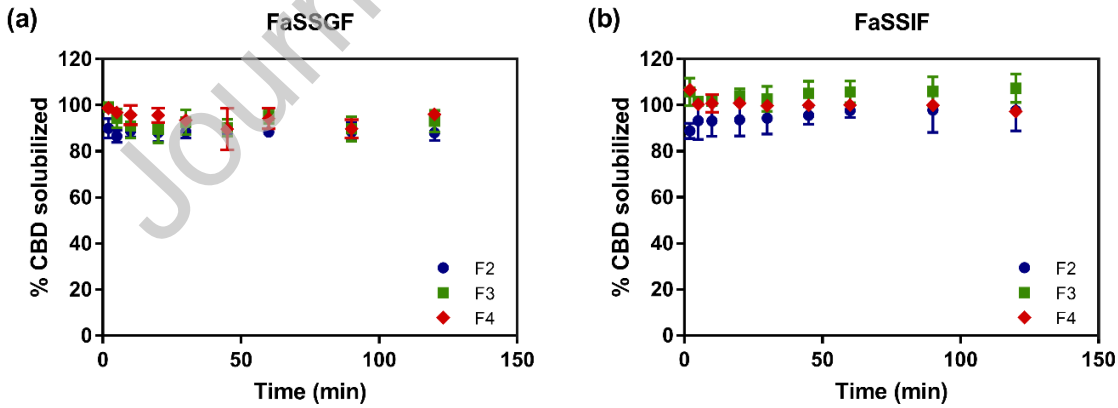

Figure 3. Dispersion profiles of the SNEDDS loaded with 10% w/w CBD in a) FaSSGF and b) FaSSIF at 37°C, with stirring at 100 rpm (n≥3, error bar = standard deviation of the mean).

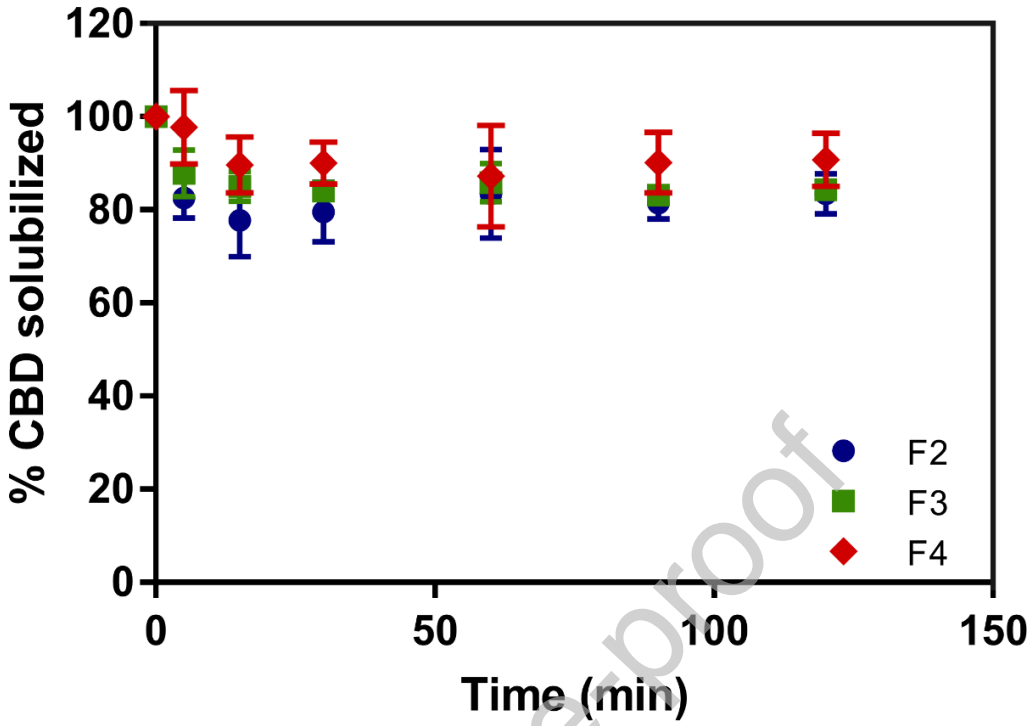

Figure 4. Solubilization of CBD during digestion of the CBD-SNEDDS formulations in FaSSIF with porcine pancreatic lipases (n=3, error bar = standard deviation of the mean).

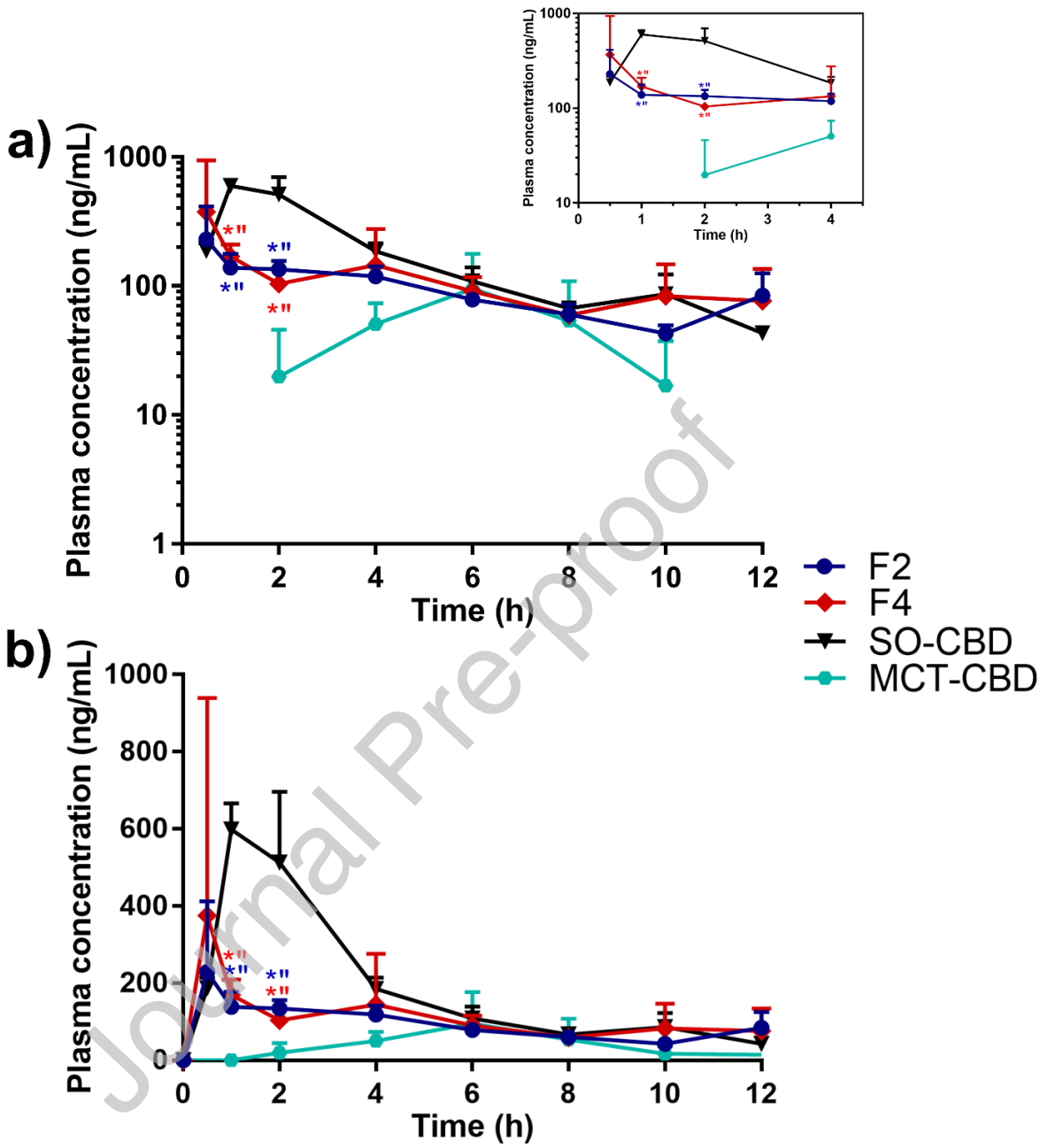

Figure 5. Mean plasma concentration versus time curves for CBD administered in the SNEDDS formulations in comparison to MCT-CBD and SO-CBD CBD (**a**-semi-log axes and **b**-linear axes) following a single oral dose of CBD normalized to 20 mg/kg (n=4-5 per group, error bar = standard deviation of the mean). Statistical differences in the mean plasma concentration values at individual time points are indicated as follows: *: $p<0.05$ compared to SO-CBD, ": $p<0.05$ compared to MCT-CBD. The lowest detectable concentration was 5 ng/mL. For MCT-CBD, no CBD was detected in the plasma samples

analyzed at 24 h. Inset figure shows the concentration versus time curves during the first 4 h following oral administration of the formulations.

Table 1. Saturated solubility of CBD in individual excipients at 37°C after stirring at 100 rpm for 24 h (n≥3).

Oils	**Saturated solubility (%w/w)**
Sesame oil	37.9 ± 0.9
Olive oil	36.9 ± 0.4
Labrafac^{TM} CC	>50
Captex 355	>50
Mineral oil	4.1 ± 0.3
Surfactants/co-solvents	**Saturated solubility (%w/w)**
Tween 80	>50
Brij O10	>50
Propylene glycol	>50

Table 2. Compositions of the lead formulations F1-F4 and SO-CBD formulation with 10% w/w CBD.

Formulation	**F1**	**F2**	**F3**	**F4**	**SO-CBD**
Brij O10 (% w/w)	54	45	40.5	36	-
Captex 355 (% w/w)	18	18	18	18	-
Propylene glycol (% w/w)	18	27	31.5	36	-
Sesame oil (% w/w)	-	-	-	-	82
Ethanol (% w/w)	-	-	-	-	8
Sucralose (% w/w)	-	-	-	-	0.05
Ethyl-3-methyl-3-	-	-	-	-	0.02

phenylglycidate (% w/w)

Table 3. Parameters of the dispersed CBD-SNEDDS loaded with 10% w/w CBD in FaSSGF and FaSSIF at 37°C, with stirring at 100 rpm (n≥3).

	FaSSGF				**FaSSIF**			
	Size (nm)	**PDI**	**Initial dispersion rate ($mg \cdot mL^{-1}min^{-1}$)**	**Dispersion AUC (min·mg/mL)**	**Size (nm)**	**PDI**	**Initial dispersion rate ($mg \cdot mL^{-1}min^{-1}$)**	**I A (**
F1	626 ± 44	0.48 ± 0.03	-	-	674 ± 57	0.57 ± 0.04	-	-
F2	175 ± 44	0.29 ± 0.03	1.10 ± 0.08	256 ± 10	192 ± 43	0.26 ± 0.01	1.09 ± 0.04	2
F3	71 ± 12	0.39 ± 0.06	1.15 ± 0.04	254 ± 10	72 ± 7	0.43 ± 0.03	1.32 ± 0.1	1
F4	48 ± 15	0.21 ± 0.1	1.22 ± 0.05	273 ± 9	41 ± 6	0.22 ± 0.07	1.33 ± 0.01	2

Table 4. Pharmacokinetic parameters obtained following oral administration of CBD-SNEDDS formulations (F2, F4) vs oil-based formulations (SO-CBD and MCT-CBD) in rats (n ≥ 3). Ratios of $AUC_{0\text{-}t(last)}/AUC_{0\text{-}\infty}$ were calculated with t_{last} = 12 h for F4 and SO-CBD. All values are presented as mean ± standard deviation, unless stated otherwise.

Group	**C_{max} (ng/mL)**	**Median T_{max} (h) [Range]**	**k_{el} (1/h)**	**$t_{1/2}$ (h)**	**$AUC_{0\text{-}4h}$ (ng·h/mL)**	**$AUC_{0\text{-}10h}$ (ng·h/mL)**	**$AUC_{0\text{-}12h}$ (ng·h/mL)**	**AU (ng·h**

F2	243 ± 174	0.5 [0.5-1]	-	-	539 ± 74*†	978 ± 89†	1104 ± 56*	-
F4	454 ± 522	1 [0.5-4]	0.10 ± 0.03	7.3 ± 2.3	718 ± 336*†	1246 ± 468†	1321 ± 434*	1949 ±
SO-CBD	629 ± 118	1 [1-2]	0.10 ± 0.03	7.7 ± 2.5	1497 ± 332	-	2235 ± 317	2766 ±
MCT-CBD	128 ± 60	6 [4-8]	-	-	80 ± 45	445 ± 191	-	-

*$p < 0.05$ compared to SO-CBD.

†$p < 0.05$ compared to MCT-CBD.

Statistical significance between SO-CBD and MCT-CBD was not considered.

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