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HEALTH SCIENCES

In Silico studies of novel Sildenafil self-emulsifying drug delivery system absorption improvement for pulmonary arterial hypertension

BARBÁRA A. ABRAHIM-VIEIRA, ALESSANDRA M.T. DE SOUZA, RITA C. BARROS, FLÁVIA A. DO CARMO, LETÍCIA C.L. DE ABREU, RONYSON S.S. MOREIRA, THIAGO S. HONÓRIO, CARLOS R. RODRIGUES, VALERIA P. DE SOUSA & LUCIO M. CABRAL

Abstract: Sildenafil is a potent selective inhibitor of phosphosdiesterase-5 previously used in erectile dysfunction and subsequently approved in 2005 for pulmonary arterial hypertension treatment. Since oral administration of sildenafil shows pharmacokinetic problems with mean absolute bioavailability of 41%, the goal of this work was to develop a novel sildenafil self-emulsifying drug delivery system (SEDDS) for oral absorption improvement and management of dosage. One pharmaceutical solution and four SEDDS containing sildenafil were successfully obtained and SEDDS formed O/W nanoemulsion with droplet size less than 300 nm. The stability studies evidenced that the SEDDS containing 3.3% w/w of sildenafil yielded the best results. The safety of 2-pyrrolidone/isobutanol in oral formulations was assessed in mice and no lethality was achieved in the placebo groups with LD50 of 490 mg/Kg for SEDDS II-3.3, suggesting it as a safe excipient for humans. Therewithal, *in silico* studies using PBPK models provided the pharmacokinetic profile of sildenafil SEDDS. Subsequently, in silico evaluation indicated that the sildenafil SEDDS droplet size influenced its bioavailability, enhancing absorption, assuring a good pharmacokinetic profile. These findings suggest that the formulations developed here presented the potential to enhance drug oral absorption with the possibility to control drug dosage as they are liquid pharmaceutical formulations.

Key words: *In Silico* evaluation, PBPK modeling, self-emulsifying drug delivery systems, Sildenafil.

INTRODUCTION

In 2005, phosphodiesterase-5 (PDE5) was found to be expressed in lung tissue at significant levels (Ghofrani et al. 2003), which led to the approval of sildenafil in 2005 as an orphan drug for the treatment of pulmonary arterial hypertension (PAH) (Raja et al. 2006, Ghofrani et al. 2006, Barnett & Machado 2006, Ramani & Park 2010) . The available treatment for PAH is based on oral administration of tablet (20 mg) or suspension (10 mg/mL) of sildenafil or as an intravenous bolus (10 mg/12.5 mL) (Revatio®) (FDA 2005). This drug is rapidly absorbed after oral administration, although it has a relatively low absolute bioavailability (~40%) (Galiè et al. 2005, Badwan et al. 2001). Its maximum observed plasma concentrations are reached within 30 to 120 minutes (median 60 minutes) of oral dosing and food slowed the rate of absorption, delaying mean t_{max} by approximately 1 h and reducing C_{max} by 29%.

In this context, a new oral formulation becomes relevant and computational approach

is an important strategy to support the process of route of administration and/or new formulations. Physiologically based pharmacokinetic (PBPK) modeling is based on mathematical concepts that combine physiology, population and drug physicochemical characteristics to mechanistically describe the pharmacokinetic behavior of a drug. Given the importance of PBPK modeling and simulation, the number of submissions to regulatory agencies has increased (Shebley et al. 2018, Miller et al. 2019). Since oral administration of sildenafil shows pharmacokinetic disadvantages, many studies with nanoformulations based on nanostructured lipid carriers (NLCs) (Nafee et al. 2018), nanoparticles suspensions (Beck-Broichsitter et al. 2012), solid lipid nanoparticles (SLN) (Paranjpe et al. 2013) have been reported to improve these issues. A promising approach is the use of self-emulsifying drug delivery systems (SEDDS) which have been successfully developed and designed to improve solubility, dissolution, and oral bioavailability of different drugs (Wu et al. 2006, Atef & Belmonte 2008, Lu et al. 2008, Baek et al. 2013, Constantinides 1995, Kommuru et al. 2001, Kang et al. 2004, de Abreu et al. 2018).

Since enhanced bioavailability is achieved with SEDDS formulations for poor water solubility drugs (Balakrishnan et al. 2009, Yi et al. 2008), we described the development of a novel sildenafil SEDDS formulation jointly with PBPK modeling and simulation to support the process of new formulation.

MATERIALS AND METHODS

Reagents and Chemicals

A pharmaceutical grade of sildenafil citrate (Matrix Laboratories Mumbai-India) was used in all formulations produced while a purity grade of sildenafil (Purity Grade Standards, 99.8% content, USA, Lot: 1110-026A1) was used for the validation of all analyses in this study. Sodium metabisulfite, propylene glycol and sodium saccharin were obtained from Vetec (Vetec Chemical, RJ, Brazil), Span® 20, Span® 80 and Tween® 80 were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Isobutanol was purchased from Tedia (Tedia Company Inc, Ohio, USA) and 2-pyrrolidone (Soluphor P®) from BASF (São Paulo, SP, Brazil). Both of these compounds were of pharmaceutical grade. Syringe filters had a pore size of 0.45 µm and cellulose acetate synthetic membranes were purchased from Millipore (Bedford, MA, USA). The water used in this study was obtained using a Milli-Q Gradient A-10 water purification system, Millipore, (Bedford, MA, USA). All other chemicals utilized were either analytical or HPLC grade.

Animals

The animal protocols used in this study were approved by the Ethics Committee on the Animals Use in Research of the Center for Health Sciences/UFRJ under the number FARMÁCIA 015 08/16. Swiss albino mice (20 g ± 2 g) were housed under controlled temperature conditions (25 °C ± 2 °C) and a 12h day/night cycle. Food and water were provided *ad libitum*.

Preparation of SEDDS sildenafil formulations

The SEDDS formulations were prepared adapted from previously studies (Constantinides 1995, Nandi et al. 2003). Sildenafil was incorporated in 2-pyrrolidone as component of the oil phase and isobutanol was used as the cosolvent. In addition, the formulations contained a very small amount of water observed in the surfactants Span®-20 or -80 and Tween®-80 used in this system (*ca*. 1.5 – 2.0 % w/w). Pseudo ternary phase diagrams were constructed to determine the exact concentration range required of each component in the formulation to maintain the

most optimal parameters for the generation of an ideal nanoemulsion *in vivo* when the formulations were administrated (Nornoo et al. 2009, Baek et al. 2013). A mixture of Tween 80 and Span® 20 or Span® 80 surfactant was used at a fixed ratio of 1:1 in the first vertex. The second vertex contained the largest proportion of the oil phase and cosolvent, which was determined ideal when the mass ratio of 2-pyrrolidone and isobutanol was 8:1. The third vertex consisted of water, which was added in 10 - 10 µL increments using an automatic micropipette during the titration process (Nandi et al. 2003, Monteiro et al. 2012). This process was performed to evaluate the maximum amount of water that could be incorporated into the system.

Construction of phase diagram

The pseudo ternary phase diagrams were developed using the following proportions of oil phase/cosolvent:surfactants: 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, and 10:90. Water was titrated into each of these cosolvent with surfactant mixtures and the percentage composition of the component in each ternary system was determined and the observed results were plotted on triangular co-ordinates to construct the phase diagram (Monteiro et al. 2012).

Preparation of sildenafil solution formulations

Sildenafil solutions were also prepared to compare their performance with SEDDS under comparable conditions. The solvent used for this process was 2-pyrrolidone, which was also evaluated for sildenafil solubility. Water, flavoring and preservative agent were also added to the formulation. The solutions were prepared aiming at complete solubilization of 200 mg of drug added to 10 mL of solvent for 24 h in preheated solvent (30 °C). At this point, sodium saccharin and sodium metabisulfite were added with distilled water to obtain the final formulation volume.

Characterization of self-emulsifying drug delivery systems (SEDDS)

The oral SEDDS containing sildenafil was characterized to determine droplet size distribution, refractive index, conductivity, emulsification capacity, drug content and drug release of sildenafil SEDDS. Dynamic light scattering was performed to determine droplet size distribution in the resulting nanoemulsion (after dilution of SEDDS containing sildenafil in buffer solution) on a Horiba LB-550 DLS analyzer (Kyoto, Japan) with the following settings: detection angle of 90°, 100 scans over two minutes per each sample, a refractive index of 1.330, and temperature set to 22 °C, since size characterization is one of the most essential examination through the SEDDS development (Ujhelyi et al. 2018). The refractive index was determined using an Abbe refractometer (Model AR-001, AFAB Enterprises, Eustis, FL). This parameter is important for assessing the stability of nanoemulsions obtained after evaluation of emulsification capacity of the SEDDS since it measures the optical clarity of the systems (Lawrence & Rees 2000). Conductivity was measured using a FE30 FiveEasy Mettler Toledo conductivity meter (Bedfordshire, UK).

Stability Study

Stress stability studies were carried out with formulations in a climatic chamber (Nova Ética 420/CLDTS 300 model, São Paulo, Brazil) at 40 °C ± 2 °C and 75% ± 5% of r.h. (ICH,2003). Samples were collected at the following time points: 0, 15, 30, 60, and 90 days. At each interval, the following properties of the SEDDS were characterized: refractive index, size measurement, conductivity and drug content. In addition, the solution was assessed for variations in pH. Precipitation was

monitored by visual inspection. A sample of each formulation was collected and stored at shelf conditions at 30 °C ± 2 °C and 75% ± 5% R.U., compared to samples stored under stress temperatures and humidity conditions. The sildenafil quantification for drug content and stability studies was performed on HPLC with a Kromasil C18 column (150 mm × 4.6 mm, 5 µm) and a photodiode array detector (Elite Labchrom model, Merck-Hitachi, Darmstadt, Germany). The mobile phase consisted of 0.2 M ammonium acetate (pH 7.0) and acetonitrile (50:50, v/v) that was isocratically delivered at 1.0 mL per minute. The column temperature was maintained at 35 °C and the injection volume was set at 20 µL (Daraghmeh et al. 2001). The amount of sildenafil in the samples was detected using UV absorption (240 nm) and quantified using a five-point standard curve prepared using stock solutions (10, 50, 100, 150 and 250 µg/mL) diluted into the mobile phase. A sildenafil stock solution of 500 µg/mL was prepared in a volumetric flask with the mobile phase solution. The weighed mass of sildenafil citrate used to prepare the solutions was subjected to a correction factor of 1.4.

Emulsification capacity and drug release of sildenafil SEDDS

The formulations of the SEDDS placebo and sildenafil SEDDS were placed into dialysis tubing (33 mm; 1.3 in; Sigma Aldrich) and immersed into flasks that contained either 250 mL simulated gastric fluid (SGF) or simulated intestinal fluid (SIF). While maintaining the temperature at 37 °C ± 0.5 °C, the systems were gentle agitated overnight. At predetermined time intervals, 1.0 mL of each sample was collected and analyzed to determine their content. In addition, the SEDDS systems were evaluated by droplet size and conductivity measurements.

Evaluation of new formulations safety

The safety of 2-pyrrolidone/isobutanol for use as a solvent in the oral administration of drugs was assessed by the lethal dose (LD_{50}). For this test, 300 µL of the placebo solution (SOL) and the placebo SEDDS was used as it corresponded to the final formulation volume required to administer 490 mg/kg of sildenafil to the animal, which is near the LD50 of sildenafil citrate in mouse (500 - 1000 mg/kg) (PFIZER 2008). Swiss albino mice, weighing 20 g ± 2 g, were divided into two groups of five animals each and were administered formulations by gavage. Animals were observed for 7 days, noting the survival. Animals were housed in a climate controlled room with a 12h light/dark cycle. All animal experiments were performed in compliance with the local ethics committee.

Statistical data analysis

Statistical analysis was performed using the one-way analysis of variance test (Tukey's multiple comparisons) with GraphPad Prism® 5.0 software (La Jolla, CA). Linear regression and other statistics were conducted using Statistica 7.

Pharmacokinetic *in silico*

Validation adult PBPK model

PBPK adult model for oral sildenafil citrate was developed using GastroPlus™ (version 9.5, Simulation Plus, Inc., Lancaster, CA, USA). This software provides a built-in detailed whole-body PBPK modeling framework, which incorporates a wide range of anatomical, physiological, and drug disposition parameters and characteristics. This computational model allows the dynamic interplay between the drug, i.e., sildenafil, and the human biological system (Samant et al. 2017). The Advanced Compartmental Absorption and Transit (ACAT) model was used to simulate sildenafil dissolution, absorption, and intestinal

metabolism. The ACAT model combines the compartmental absorption and transit (CAT) model (Yu et al. 1996) with finite dissolution, pH dependence, absorption from stomach or colon, in addition to the seven compartments of the small intestine, carrier-mediated transport, and transporter densities.

The PBPK model was performed to simulate plasma concentration profile after oral sildenafil administration of 20 mg immediate-release drug administration. Physical-chemical properties of sildenafil used as input in the PBPK model were obtained from literature or *in silico* prediction (Table I). All parameters were fixed at default values that represent human fasted physiology (Nichols et al. 2002). Tissue/plasma partition coefficients (Kp's) were calculates using the Lukacova method (Chaudhuri et.al. 2011). The model's performance in adults was evaluated by calculating maximum serum concentration (C_{max}) and area under the plasma drug concentration-time curve (AUC) ratios for observations vs. predictions data.

SEDDS formulation using PBPK model

Once developed and validated the PBPK model of sildenafil tablets for oral administration, it was adjusted to the SEDDS droplet size. Simulations were performed considering the droplet size of 50 µm (software default) and the droplet size obtained from the best formulation by *in vitro* studies (SEDDS 1 with 0.3 µm) were performed. The effects of changing average particle size in bioavailability parameters were obtained from 20 mg of sildenafil. The fold error (FE) of the difference between observed and predicted parameters was calculated according to Equation 1, where Pred and Obs represent the predicted and observed values of the sildenafil (Zhang et al. 2015).

Equation 1 FE= Pred/Obs, if Pred > Obs

FE= -Obs/Pred, if Obs >Pred

Table I. Drug-specific parameters for sildenafil citrate.

Parameter	Value	Reference
Molecular weight (g/mol)[a]	474.576	ChemAxon
logP[a]	2.27ChemAxon	ChemAxon
Ionization constant (pKa)	6.5	(32)
Solubility [mg/mL]	6.965 (pH=3)	(32)
Papp (Caco-2)	7.077 (pH=4)	ChemAxon
Ionization constant (pKa)	2.068 (pH=5)	Program default[b]
Fration unbound (fu)[a]	0.025 (pH=7)	

[a]Chemicalize was used for prediction of molecular weight and logP on March, 2019, https://chemicalize.com/developed by ChemAxon (http://www.chemaxon.com).
[b]GastroPlus ™ (version 9.5, Simulation Plus, Inc., Lancaster, CA, USA).

RESULTS AND DISCUSSION

Sildenafil formulations: characterization and stability studies

In order to evaluate the formulations vehicles, five novel formulations were developed in this study: two SEDDS with different types of surfactant (Formulation I with Span® 20 and Formulation II with Span® 80), and one solution (SOL), which was prepared using the same solvent, 2-pyrrolidone, in order to compare its stability to the SEDDS formulations (Table II). Sildenafil is marketed as the citrate salt and is low bioavailable is due to its low aqueous solubility (4.1 mg/mL in water). Besides its low solubility in water, the solubility of sildenafil citrate in organic solvents was observed only in DMSO, toluene and dimethyl formamide (Pirhayati et al. 2017). As 2-pyrrolidone is a water-miscible and a safety pharmaceutically solubilizer, it is a good option for the preparation of sildenafil SEDDS as a pharmaceutical excipient (Jain & Yalkowsky 2007).

Pseudo ternary phase diagrams were constructed from the different SEDDS compositions (Figure 1) to calculate the expected maximum concentration of water that can be incorporated in the formulations that would produce nanoemulsions *in vivo*. The dark gray region represents the nanoemulsion formation, where the red dot represents formulations I (Figures 1a, b, Figure 2).

As the maximum solubility of sildenafil in both SEDDS formulations was 3.3% (w/w) and these concentrations were used for SEDDS production (I-3.3 and II-3.3). Since the sildenafil concentration 1.3% (w/w) was the optimal concentration of drug for immediately solubilized in 2-pyrrolidone/isobutanol without precipitate after a short time, it was added at SOL-1.3. As a large amount of water was added to this formulation, sodium metabisulfite was also added as a preservative and sodium saccharin was used as a flavoring agent. Additional formulations of each SEDDS with drug concentration of 1.3% (w/w), were also prepared (I-1.3 and II-1.3) to compare with the prepared solutions. Stability studies were conducted on the formulations by maintaining them in a controlled environment of 40 ± 2 °C and 75 ± 5% humidity. At different time points (0, 15, 30, 60 and 90 days), samples were removed to evaluate their physical state. The refractive index of the SEDDS remained constant at 1.47,

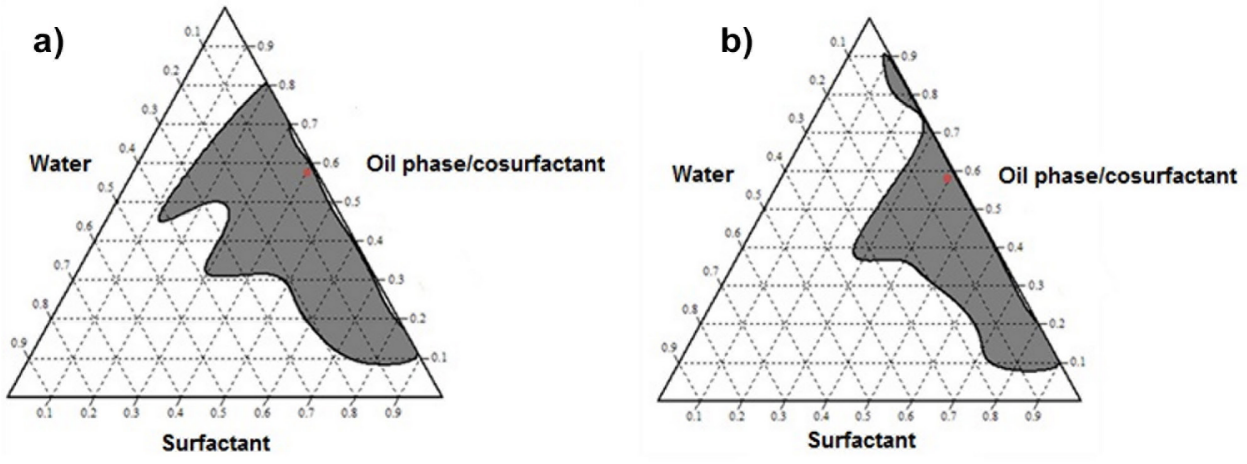

Figure 1. Pseudoternary phase diagrams of self-emulsifying drug delivery systems (SEDDS) formulations I (a) and II (b).

Table II. Self-emulsifying drug delivery systems (SEDDS) I, II and SOL placebo formulations.

Composition	Proportion (%; w/w)		
	I	II	SOL
2-pyrrolidone	52.36	52.36	45.93
Tween® 80	19.65	19.65	--
Span® 20	19.65	--	--
Span® 80	--	19.65	--
Isobutanol	6.58	6.58	--
Saccharin sodium	--	--	0.1
Sodium metabisulfite	--	--	0.1
Water	1.76	1.76	53.87

suggesting the absence of any major changes. In conductivity studies, an increase of SEDDS values was observed after the sildenafil addition, since conductivity for formulations I and II were 4.37 µS/cm ± 0.05 whereas SEDDS formulations containing 1.3% (I-1.3 and I-3.3) and 3.3% of sildenafil (II-1.3 and II-3.3) ranged from 6.33 ± 0.15 to 22.03 ± 0.20 µS/cm. It is important to highlight that SEDDS I-3.3 and II-3.3 showed conductivity higher than SEDDS I-1.3 and II-1.3 in the same times, indicating the sildenafil content influence. Initially, drug content of sildenafil was above 94%. By day 90, all of the formulations demonstrated a decrease in sildenafil concentration. The higher concentration of sildenafil, I-3.3 and II-3.3, appeared to be retained to a higher percentage (~83%) than the 1.3% (w/w) concentration. The measurement of pH of SOL-1.3 presented no significant changes during study determinations, ranging from 5.83 to 5.69 with a sildenafil content of 86.57 ± 0.12 after 90 days (Table III).

Emulsification capacity and drug release of sildenafil SEDDS

In vitro emulsification capacity and drug release of sildenafil SEDDS were evaluated to verify whether SEDDS can form an O/W nanoemulsion under mild conditions and after dilution by aqueous phase. The behavior of the SEDDS formulations I-3.3 and II-3.3 were assessed in SGF and SIF. As conductivity the measurements exceeded 200.00 µS/cm for all the formulations studied, it was an indication that all the nanoemulsions produced were of the O/W type. In all cases, the droplet size distribution obtained was less than 300 nm. These results for the SEDDS are consistent with previous study, since SEDDS typically produce emulsions with droplet size between 100 and 300 nm and also transparent microemulsions, which have a droplet size less than 50 nm (Samant et al. 2017). As the free energy required to form an emulsion is very low, it is suggested that the oil/surfactant/cosurfactant and water phases effectively swell, decreased the oil droplet size and eventually increased the release rate (Avachat & Patel 2015) (Table IV). From the mean droplet size and polydispersity index, the formation of O/W nanoemulsions was confirmed for formulations I-3.3 and II-3.3 in both SGF and SIF.

No retention on sildenafil was observed after 15 min of study, which suggests that this formulation has potential to enhance drug release and absorption. In view of the results obtained, formulation II-3.3 was chosen as the most suitable formulation for further scale up studies.

Table III. Characterization of self-emulsifying drug delivery systems (SEDDS) and SOL formulations after storage.

	Formulation	Days in Storage				
		0	15	30	60	90
Refractive Index	I-1.3	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00	1.46 ± 0.01	1.47 ± 0.00
	I-3.3	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00
	II-1.3	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00
	II-3.3	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00	1.47 ± 0.00
Conductivity (µS/cm)	I-1.3	7.00 ± 0.10	6.53 ± 0.28	6.70 ± 0.10	14.36 ± 0.40	7.43 ± 0.25
	I-3.3	7.53 ± 0.05	7.4 ± 0.17	7.43 ± 0.15	12.60 ± 0.20	10.63 ± 0.35
	II-1.3	6.33 ± 0.15	6.63 ± 0.05	6.90 ± 0.10	8.16 ± 0.15	8.56 ± 0.20
	II-3.3	7.23 ± 0.05	7.63 ± 0.50	7.56 ± 0.15	10.13 ± 0.15	22.03 ± 0.20
Drug Content (%)	I-1.3	96.07 ± 1.64	75.07 ± 1.94	72.55 ± 0.07	62.21 ± 0.33	63.76 ± 0.65
	I-3.3	94.54 ± 0.79	93.39 ± 1.78	86.40 ± 0.67	82.45 ± 0.38	83.77 ± 0.74
	II-1.3	99.59 ± 1.58	86.71 ± 2.80	77.54 ± 0.14	71.34 ± 0.27	70.39 ± 0.56
	II-3.3	94.56 ± 2.13	92.72 ± 2.95	88.45 ± 0.63	83.05 ± 0.30	83.49 ± 1.20
	SOL-1.3	98.98 ± 0.90	95.21 ± 1.80	93.31 ± 1.89	86.37 ± 0.12	86.57 ± 0.12
pH	SOL-1.3	5.80 ± 0.02	5.83 ± 0.02	5.78 ± 0.03	5.80 ± 0.01	5.69 ± 0.01

n = 3, average ± standard deviation.

Table IV. ***In vitro*** **emulsification capacity of sildenafil self-emulsifying drug delivery systems (SEDDS) in simulated gastric fluid (SGF)/simulated intestinal fluid (SIF).**

	SGF	SIF	I-3.3			II-3.3		
			Before release	SGF	SIF	Before release	SGF	SIF
Conductivity (µS/cm)	>200.00	>200.00	7.20 ± 0.10	>200.00	>200.00	7.03 ± 0.15	>200.00	>200.00
Mean droplet size (nm)	N/D	N/D	N/D	224.65 ± 48.29	264.60 ± 24.89	N/D	216.90 ± 21.62	233.16 ± 22.23
PDI	N/D	N/D	N/D	0.47 ± 0.13	0.44 ± 0.05	N/D	0.30 ± 0.02	0.31 ± 0.01

Notes: n = 3, average ± standard deviation; N/D = not determined.

Evaluation of SEDDS formulation safety

Whereas while 2-pyrrolidone has been approved for use in humans, mixing it with isobutanol could lead to some toxicity-grounding profile of the prepared formulations. As both sildenafil and the other constituents of the formulations under study had their toxicological profile already widely studied, the lethality test was chosen as a way to anticipate some kind of toxicity alteration. It has been shown that 2-pyrrolidone has an oral LD50 in rats of 5000 mg/kg (Jain & Yalkowsky 2007). No lethality was achieved in the placebo and II-3.3 SEDDS groups. The LD50 value for II-3.3 SEDDS was 490 mg/Kg. These findings suggest that these vehicles can be considered safe for administration to humans.

In silico pharmacokinetic using PBPK model

Model building and validation results

The model was built with plasma concentration-time profiles of sildenafil after oral administration (20mg) obtained from previous studies (observed data) (Nichols et al. 2002). The validation was carried out by calculating AUC_{0-t} and C_{max} ratios for observed *vs* predicted data, where the prediction for C_{max}, AUC_{0-24h} and $AUC_{0-\infty}$ were within less than a twofold error (Lu et al. 2008) (Table V). This indicates that the model predicted values are in good agreement with the respective observed values for 20 mg of sildenafil.

SEDDS PBPK model results

Once developed and qualified for oral 20 mg tablet sildenafil administration, we expanded the PBPK model to SEDDS oral in nanosuspension administration. The *in silico* pharmacokinetic studies demonstrate an increased bioavailability of the SEDDS formulation 1, measured as an increase in maximum drug concentration (Cmax) and area under the drug concentration-time (AUC) when compared with PBPK model of sildenafil 20mg and with literature data (Table V) (Spence et al. 2008). Similar *in silico* results were already obtained for simvastatin (Ćetković et al. 2018). Moreover, *in vivo* studies of SEDDS containing irbesartan (Patel et al. 2014) and buparvaquone (Smith et al. 2018) corroborate the enhancement of oral bioavailability of these formulations. The notorious improvement of SEDDS formulation is due to the smaller droplets and their polarity which promotes faster drug release into the aqueous phase. It is important to highlight that the droplet size is a crucial factor since the smaller size provides a large interfacial area which determines the enhance of rate and extent of drug release as well as absorption (Shah et al. 1994). This feature was recently reported for butenafine (Bezerra-Souza et al. 2019).

The region absorption data were subsequently analyzed. It was possible to observe the increase of the absorption in duodenum and jejunum in sildenafil SEDDS formulation PBPK model in approximately 28% in comparison to standard droplet size (default) (Figure 2).

We may also infer that the enhanced GI absorption of SEDDS formulation containing sildenafil can be related to a decrease in the

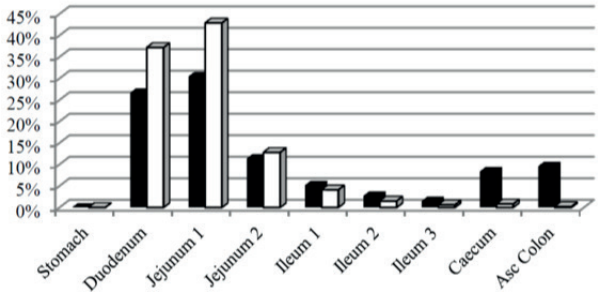

Figure 2. Compartmental absorption profile of PBPK model. Particle size influence of standard particle size (50 µm) in black column and self-emulsifying drug delivery systems (SEDDS) formulation (0.3 µm) in white column.

Table V. ***In vivo*** **observed and simulated oral bioavailability parameters of 20 mg sildenafil in adult.**

Parameters	Observed	Simulated	
Particle size (μm)	-	50 (default)	0.3 (SEDDS 1)
C_{max} (ng/mL)	59.6	62.9	89.77
$AUC_{0-\infty}$ (ngh/mL)	175.5	353.4	367.8

P-gp drug efflux and phase I metabolism by the intestinal cytochrome P450s, significant factors in oral drug bioavailability (Gursoy & Benita 2004).

CONCLUSION

Nanoformulations of SEDDS containing sildenafil were successfully obtained. SEDDS formulations, containing 3.3% w/w sildenafil and 2-pyrrolidone/isobutanol as solvent, with droplet size less than 300 nm, presented potential to enhance drug release and probably assist with absorption. Further, PBPK modeling studies provided a good pharmacokinetic profile of sildenafil SEDDS, highlighting the droplet size influence on its bioavailability and enhancing intestinal absorption. In conclusion, a novel sildenafil SEDDS formulation was developed with reduced droplet size and improved *in silico* pharmacokinetic profile.

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ALESSANDRA M.T. DE SOUZA[1]
https://orcid.org/0000-0001-9986-405X

BARBÁRA A. ABRAHIM-VIEIRA[1]
https://orcid.org/0000-0001-6444-0169

RITA C. BARROS[1]
https://orcid.org/0000-0003-3198-9133

FLÁVIA A. DO CARMO[1]
https://orcid.org/0000-0003-0474-9070

LETÍCIA C.L. DE ABREU[1,2]
https://orcid.org/0000-0002-3616-3667

RONYSON S.S. MOREIRA[1]
https://orcid.org/0000-0001-5911-7471

THIAGO S. HONÓRIO[1]
https://orcid.org/0000-0002-3772-7225

CARLOS R. RODRIGUES[1]
https://orcid.org/0000-0001-8453-7654

VALERIA P. DE SOUSA[1]
https://orcid.org/0000-0003-1589-0846

LUCIO M. CABRAL[1]
https://orcid.org/0000-0002-4550-5729

[1]Universidade Federal do Rio de Janeiro, Departamento de Fármacos e Medicamentos, Faculdade de Farmácia, Av. Carlos Chagas Filho, 373, CCS- bloco Lss, 21941-902 Cidade Universitária, RJ, Brazil

[2]Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro/IFRJ, Direção de Ensino, Av. República do Paraguai, 120 - Vila Sarapui, 25050-100 Duque de Caxias, RJ, Brazil

Correspondence to: **Lucio M. Cabral**
E-mail: lmcabral2@yahoo.com.br

Author contributions

B. Abrahim-Vieira and A.M.T. Souza designed the research and analyzed all in silico studies. R. C. Barros and F.A. Carmo conceived the analytical and in vivo studies. L.C.L. de Abreu, R. S. S. Moreira and V.P. Sousa were responsible for all HPLC analyses. T.S. Honorio collaborated with the PBPK studies. C. R. Rodrigues and L.M. Cabral directed the research.