Technological research article

Solid self-emulsifying dosage forms for carrying Amphotericin B: A preformulation study

Francisco Alexandrino-Jr[1a,*], Michelle A. Sarcinelli[1b], Gabriel Barcellos[1,2c], Leny A. H. Nascimento[1d], Thalita M. Silva[1,2e], Kattya G. H. Silva[3f], Livia D. Prado[1g], Helvécio V. A. Rocha[1,2h] & Beatriz F. C. Patricio[4i]

[1] Laboratório de Micro e Nanotecnologia (LMN), VPPIS, Fundação de Oswaldo Cruz (Fiocruz), 21040361, Rio de Janeiro, RJ, Brazil

[2] Programa de Pós-graduação em Pesquisa Translacional em Fármacos e Medicamentos, Farmanguinhos, Fiocruz, Rio de Janeiro, RJ, Brazil

[3] Faculdade de Farmácia, Universidade Federal do Rio de Janeiro (UFRJ), 21941902, Rio de Janeiro, RJ, Brazil

[4] Instituto Biomédico, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), 20211005, Rio de Janeiro, RJ, Brazil. beatriz.patricio@unirio.br

*Corresponding author E-mail: alexandrino_jr@yahoo.com.br or francisco.alexandrino@fiocruz.br

ORCID IDs and E-mail addresses:

[a] https://orcid.org/0000-0002-1173-9164 alexandrino_jr@yahoo.com.br
[b] https://orcid.org/0000-0002-2364-9270 malvaressarcinelli@gmail.com
[c] https://orcid.org/0000-0002-3060-1355 briel08@gmail.com
[d] https://orcid.org/0000-0003-4703-6361 leny.anhenrique@gmail.com
[e] https://orcid.org/0000-0002-5691-9900 liviaderis@gmail.com
[f] https://orcid.org/0000-0001-7685-3120 thalitamartins.far@gmail.com
[g] https://orcid.org/0000-0002-8750-1726 holanda.gyselle@gmail.com
[h] https://orcid.org/0000-0002-9624-6405 helvecio.rocha@fiocruz.br
[i] https://orcid.org/0000-0002-2477-9798 beatriz.patricio@unirio.br

Received: August 1, 2024
Corrected: January 31, 2025
Accepted: February 6, 2025

https://doi.org/10.15446/rcciquifa.v54n1.119557

SUMMARY

Introduction: Amphotericin B (AmB) is a macrolide derived from *Streptomyces nodosus* used to treat fungal infections and leishmaniasis. Its main clinical limitation is associated with severe nephrotoxicity after iv administration. **Aim:** Therefore, this study aimed to develop and characterize AmB-loaded solid self-emulsifying drug delivery systems (SEDDS) for oral administration. **Methodology:** to reach this goal, first an extensive solubility test was performed on 45 excipients, followed by a pseudo-ternary phase diagram to understand the phase behavior after mixing the chosen excipients with intestinal bio-relevant media. Thereafter, solid SEDDS were produced either by filling hard capsules with SEDDS (LFC) or by adsorbing them onto solid excipients to formulate tablets and granules. **Results:** Phosal 50 PG presented the highest AmB solubility, and based on the phase behavior, two formulations with different oil-surfactant proportions were selected. The nitrogen adsorption method indicated that solid excipients incorporated the SEDDS, and no significant difference between these two formulations was

found regarding droplet size, PDI, and AmB concentration. **Conclusions:** the LFC revealed to be the most promising dosage form, not only carrying the highest drug load, but also displaying an average size assigned as the ideal range for intestinal absorption. Therefore, supporting further *in vivo* studies to evaluate its therapeutic potential.

***Keywords*:** Leishmaniasis; Fungal infections; Solubility; Solid SEDDS; Biorelevant media.

RESUMEN

Formulaciones autoemulsionantes sólidas conteniendo Anfotericina B: Un estudio de preformulación

Introducción: La Anfotericina B (AmB) es un macrólido derivado de *Streptomyces nodosus* utilizado para tratar infecciones fúngicas y leishmaniasis. Su principal limitación clínica está asociada con una nefrotoxicidad severa después de la administración intravenosa. **Objetivo:** Por lo tanto, este estudio tuvo como objetivo desarrollar y caracterizar sistemas de administración de medicamentos autoemulsionantes sólidos (SEDDS) cargados con AmB para administración oral. **Metodología:** Para alcanzar este objetivo, primero se realizó una prueba de solubilidad extensa en 45 excipientes, seguida de un diagrama de fase pseudo-ternaria para entender el comportamiento de fase después de mezclar los excipientes elegidos con medios biorelevantes intestinales. Posteriormente, los SEDDS sólidos fueran desarrojados sea llenando cápsulas duras con SEDDS (LFC) o incorporandolos en excipientes sólidos para formular comprimidos y gránulos. **Resultados:** Phosal 50 PG presentó la mayor solubilidad de AmB y, basado en el comportamiento de fase, se seleccionaron dos formulaciones con diferentes proporciones de aceite y surfactante. El método de adsorción de nitrógeno indicó que los excipientes sólidos incorporaron los SEDDS, y no se encontró una diferencia significativa entre estas dos formulaciones en cuanto al tamaño de las gotas, PDI y concentración de AmB. **Conclusiones:** El LFC demostró ser la forma farmacéutica prometedora, no solo llevando la mayor carga de medicamento, sino también mostrando un tamaño promedio como el rango ideal para la absorción intestinal. Por lo tanto, corroborando la realización de estudios *in vivo* para evaluar su potencial terapéutico.

***Palabras clave*:** Leishmaniasis; Infecciones fúngicas; Solubilidad; SEDDS sólidos; Medios biorelevantes.

RESUMO

Formulações sólidas autoemulsionantes contendo anfotericina B: um estudo de pré-formulação

Introdução: A Anfotericina B (AmB) é um macrolídeo, derivado de *Streptomyces nodosus,* usado para tratar infecções fúngicas e leishmaniose. Sua principal limitação clínica está associada à nefrotoxicidade severa após a administração intravenosa. **Objetivo:** Portanto, este estudo teve como objetivo desenvolver e caracterizar sistemas autoemulsionantes sólidos (SEDDS) contendo AmB para administração oral. **Metodologia:** Primeiramente foi realizado um extenso teste de solubilidade em 45 excipientes, seguido por um diagrama de fase pseudo-ternário para entender o comportamento de fase após a mistura dos excipientes escolhidos com meios biorelevantes intestinais. Posteriormente, os SEDDS sólidos foram produzidos preenchendo cápsulas duras com SEDDS (CL) ou adsorvendo-os em excipientes sólidos para formular comprimidos e grânulos. **Resultados:** a maior solubilidade da AmB foi encontrada no Phosal 50 PG. Com base no comportamento de fase, duas formulações com diferentes proporções de óleo-surfactante foram selecionadas. O método de adsorção de nitrogênio indicou que os excipientes sólidos incorporaram os SEDDS, e não foi encontrada diferença significativa entre essas duas formulações em relação ao tamanho das gotículas, PDI e concentração de AmB. **Conclusões:** as CL revelaram ser a forma farmacêutica mais promissora, não apenas carregando a maior quantidade de AmB, mas também exibindo um tamanho médio considerado como a faixa ideal para a absorção

intestinal. Portanto, corroborando com a realização de estudos *in vivo* para avaliar seu potencial terapêutico.

***Palavras-chave*:** Leishmaniose; Infecções fúngicas; Solubilidade; SEDDS sólidos; Meios biorelevantes.

1. INTRODUCTION

Amphotericin B (AmB) is a polyene macrolide derived from the actinomycete *Streptomyces nodosus.* Due to its attractive characteristics for clinical use, *e.g*., potent antifungal activity, a relatively broad spectrum of action, and low resistance rate, this molecule is currently one of the main therapeutic agents to treat life-threatening fungal infections and leishmaniasis [1, 2]. Moreover, it is also present in the Essential Medicines List (EML) of the World Health Organization (WHO). Therefore, been considered an essential item to the promotion of primary health care [3].

In contrast, numerous studies have ascribed AmB to significant toxic side effects, with nephrotoxicity being one of the most severe [4]. Such an injurious effect is, in part, related to a direct toxic effect of AmB over the renal tubular cells [5]. Moreover, it is noteworthy to mention that AmB physicochemical properties have not only been assigned as a cause to its aggregation state in an aqueous medium, which has been associated with its *in vivo* toxicity [6], but also, as the main hindrance to its use through the oral route [7], *i.e*., the simultaneous amphiphilic and amphoteric character, due to the polyene chain and the polyol; and the presence of a free carboxyl group and an amino group respectively, confers to AmB low aqueous solubility [7] and low oral permeability [8] (it violates 3 of the 5 Lipinski's rule [7, 9]), characterizing it as class IV in the Biopharmaceutical Classification System [10, 11]. These factors promote low oral bioavailability, limiting its clinical use to intravenous administration [12].

A strategy to overcome such drawbacks and provide an outpatient treatment would be developing an oral formulation with features that surpass these troublesome physicochemical properties of AmB. Several drug delivery systems have diligently been developed to reach this goal, among them lipid systems [2, 7, 13] such as self-emulsifying drug delivery systems (SEDDS), which consist of a mixture of oil, surfactant, and co-surfactant, able to spontaneously form oil-in-water (o/w) emulsions when dispersed in the aqueous media under mild homogenization [14]. These systems are extensively applied to solubilize poorly water-soluble drugs and increase their oral bioavailability due to the enhanced permeation caused by surfactant presence [15, 16]. Usually, the approach applied to obtain SEDDS involves solubilization of drugs in oil and their blending with suitable solubilizing agents [7]. The use of SEDDS as a liquid dosage form potentially displays limitations regarding the dosage inaccuracy, high production costs, as well as drug instability [17]. That is one of the main reasons the currently commercialized systems consist of soft or hard capsules filled with the lipid formulation, allowing the administration as unit doses [18]. However, this approach presents significant limitations related to the development of a capsule liquid-filled dosage form, namely, problematic filling with high viscous lipid mixtures, which increases the cost of the process [19], interaction between the filling material and the capsule shell [20], therefore, restricting its concentration into the formulation and potentially decreasing the formulation efficiency. Moreover, most pharmaceutical companies do not own *in loco* facility to manufacture such capsules. Therefore, requiring outsource to the development and manufacturing activities, which might be accounted for an inconvenient business strategy [19, 20].

To avoid such drawbacks and enable a simplified manufacturing process using more cost-effective and conventional equipment, several approaches have been reported, mostly by solidifying the lipid-based system and convert it into a powder, which can be used to fill hard gelatin capsules or to produce tables, *e.g*. (I) adsorption of the lipid formulation into solid carriers; (II) spray drying using hydrophilic or hydrophobic carriers (III) extrusion; (IV) microencapsulation using ionotropic gelation technique; (V) wet granulation; (VI) melt granulation, *etc*. [17, 18, 21].

Since these strategies are still under investigation, the role of these solidification processes on the formulation performance remains uncertain, even though it is evident they may impact not only the self-emulsification behavior but also exhibit relevant drug-excipient and excipient-excipient interactions. Therefore, the lack of understanding of these physicochemical interactions can impact properties such as manufacturability, stability, and bioavailability of a drug product [22].

The scenario mentioned above makes the development of solid SEDDS challenging in the pre-clinical phase, not just during the process of selecting the appropriate excipients but also establishing the effect of their properties on final formulation features. Therefore, the current study aimed to develop distinct solid SEEDS dosage forms as a potential AmB carrier for oral administration and perform a thorough characterization. The AmB solubility was evaluated in 45 different excipients, the self-emulsifying process was conducted in a biorelevant medium, and the drug-excipient interaction was conducted throughout the gas adsorption method.

2. MATERIALS AND METHODS

2.1. Materials

Amphotericin B was obtained from Hangzhou Dayangchem (China) (purity 89.6 %). Phosal 50 PG (PPG), Phosal 50 SA+, and Phosal 53 MCT were obtained from Lipoid. Kolliphor HS 15, Kolliphor RH40, Kolliphor EL were kindly donated by BASF. Capmul MCM EP, Capmul MCM C8, Capmul GMO – 50, Capyrol 90, Captex 300, Captex 355, Captex 100 were given by Abitec. Transcutol HP (purity > 99.9 %), Plurol oleique, Labrasol, Labrafac Liphophile WL 1349, Peceol, Labrafac, Labrafil M 1944 CS, Lauroglycol 90, Labrafac LG were kindly supplied by Gattefossé. Crodamol GMCC-SS, Super Refined® Polysorbate 80, Glycerox 767, Super Refined® Castor oil, Super Refined® Arlasolve (purity 98.5%), Super Refined® Oleic acid (purity 86.7%), Super Refined® PEG 300, Super Refined® PEG 400 were kindly donated by Croda. Imwitor 742, Imwitor 308 (GMC), Imwitor 988, Miglyol® 812 N, Softigen 767 were provided by IOI Oleo GmBH. Dhaytan L20, Isopropyl palmitate, Dhaykol 6040 were purchased from Dhaymers. Dichloromethane (DCM) analytical grade (purity ≥ 99.9 %), colloidal silicon dioxide (SiO2), microcrystalline cellulose 102 (MCC), dibasic calcium phosphate ($CaHPO_4$) (purity 98.0 - 105.0 %), Span 80 (SM) (purity > 60.0 %), Poloxamer 188 (80.2% of oxyethylene moieties), Isopropyl myristate (purity > 97.5 %), Sesame oil, and all other chemicals solvent were obtained from Sigma-Aldrich and Merck. Dimethyl sulfoxide (DMSO, purity 99.7 %) analytical grade was obtained by Proquimios, Neusilin US2 type I-A (Neusilin U2) were donated by Fuji Chemical Industry. The biorelevant media Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) were acquired from Biorelevant.com.

2.2. Methods

2.2.1. Development of Self-emulsifying drug delivery system

2.2.1.1. Solubility Studies

The solubility of the AmB in 45 different excipients (oils, surfactants, and co-surfactants) was evaluated using the shake flask method [23]. Briefly, an excess of AmB was added to 3 g of each excipient, with further homogenization (250 rpm, 25 ± 0.1 °C) for 48 h in an incubator shaker (KS 4000 IC, IKA, Germany). Afterward, the mixtures were placed in a centrifuge (Megafuge 16, Thermo Fisher Scientific, USA), for 30 min at 14,500 x g. This process was repeated until no pellet of AmB was observed. Subsequently, the drug was quantified in the supernatant as described in section *Quantification of AmB in the oils and lipid mixtures (2.2.2.1.)*. Each experiment was carried out five times.

2.2.1.2. Pseudo-ternary phase diagram

To evaluate not only the effect of the oil-surfactant proportion but also the media composition in the self-emulsifying process, pseudo-ternary phase diagrams were formulated containing as oil phase a ratio of excipients that exhibited high AmB solubility. For this purpose, the assay was performed using the titration method [24]. Briefly, the oil phase was comprised of PPG:GMC (1:1 w/w), and SM was used as a surfactant. The oil-surfactant ratios ranged from 99:1 to 70:30, an excess of AmB was added to these mixtures, magnetically stirred for 5 minutes, and homogenized in an incubation shaker for 24 h (250 rpm, 25 °C). Afterward, the excess of AmB was withdrawn by multiple spin cycles as described for the solubility studies. Subsequently, the mixtures (2.5 g) were weighed, and the respective aqueous medium (ranging from 10% w/w to 90% w/w) was added dropwise. Thereafter, the samples were placed in an incubator shaker to further homogenization (150 rpm, 30 min, 37 °C). To better simulate the physiological conditions, this assay was performed with intestinal biorelevant media (FaSSIF or FeSSIF) as aqueous medium.

After each homogenization cycle, each system was visually evaluated and classified as emulsion (white milked aspect), microemulsion (limpid and translucid aspect), and phase separation (heterogeneous system) [25].

2.2.1.3. Formulation of self-emulsifying drug delivery systems

Based on the phase behavior exhibited by different oil-surfactant mixtures in FaSSIF medium, two SEDDS formulations (Table 1) were chosen for further characterization, *viz*, AmB solubilization capacity, droplet size, and polydispersity index (PDI). The lipid mixture was prepared according to the methodology described in section *Development of Self-emulsifying drug delivery system (2.2.1.)*. The self-emulsifying process was carried out in FaSSIF (oil phase:media ratio 1:99, 150 rpm, 37.0 °C ± 0.1 °C). After predetermined time intervals (1.5 h and 3.5 h) the samples were characterized as described in the appropriate section.

Table 1. Composition of self-emulsifying drug delivery systems (SEDDS)

Excipient	Formulation (%, w/w)	
	F-70:30	F-80:20
Phosal 50 PG (PPG)	35	40
Imwitor 308 (GMC)	35	40
Span 80 (SM)	30	20

Note: the formulation ID highlights the oil:surfactant proportion, *i.e.*, F-70:30 and F-80:20 have 70:30 and 80:20 oil:surfactant proportion respectively.

2.2.2. Characterization

2.2.2.1. Quantification of AmB in the oils and lipid mixtures

The amount of AmB solubilized was measured by UV-Vis spectroscopy. First, the samples were diluted in a mixture of DCM:DMSO (8:2, v/v). Thereafter, scanning was performed in a range of 300-450 nm, with a stepwise of 0.1 nm, in a medium velocity. Afterward, the first derivative of the spectrum was calculated, and the absorbance values in the valley at 417 nm were applied in the standard curve previously validated with AmB in DCM:DMSO (8:2, v/v). The derivative spectrophotometry is based on the transformation of a typical spectrum by derivation into a derivative spectrum. This technique increases the number of absorption bands, which allows a satisfactory individualization of the constituents, and eliminates wide bands [26]. Such an approach was successfully applied to remove the interference caused by excipients that exhibited absorption at the same wavelength as AmB, therefore, allowing its adequate quantification.

2.2.2.2. Determination of hydrodynamic droplet size and polydispersity index

The hydrodynamic diameter of the SEDDS was determined by dynamic light scattering (DLS, Zetasizer 3000, Malvern Instruments Ltd, United Kingdom) using a photon correlation spectrometer model equipped with a laser beam at 632 nm wavelength. The samples were evaluated at 37 °C, and the scattering angle was settled to 90°. The described methodology was applied to characterize the samples prepared in sections *Formulation of self-emulsifying drug delivery systems (2.2.1.3.)* and *Development of solid self-emulsifying dosage forms (2.2.3.)*. The results are expressed as an overall mean of three measurements, each one with 15 scans.

2.2.3. Development of solid self-emulsifying dosage forms

To evaluate how the self-emulsifying performance would be affected when the lipid formulations were present within distinguish drug dosage forms, three different formulations were prepared, namely, hard gelatin capsule filled with the lipid formulation (LC-F-70:30 or LC-F-80:20), hard gelatin capsule filled with granules containing the lipid formulation (GC- F-70:30 or GC-F-80:20) and tablets prepared from these granules (T-F70:30 or T-F-80:20).

To obtain the capsule filled with the lipid formulations, translucid hard gelatin capsules (size 00, ~0.95 mL) were filled with 0.65 mL of the lipid formulations (F-70:30 or F-80:20). To produce the granules, the lipid formulations (1 g) were poured into a container, followed by the gradual addition of the solid adsorbent material (SiO_2 and Neusilin U2 (1:1)). After each addition, the mixture was homogenized to achieve a uniform distribution of the lipid material. Once obtained a free-flowing solid powder (oil phase-adsorbent ratio 64:36), the material was subsequently passed through a sieve N° 20 (pore size 850 μm) to obtain standardized granules. Subsequently, the hard gelatin capsules were filled with 0.35 g of these granules. Moreover, to formulate the tablets, the granules were mixed with a powder mixture of MCC – and $CaHPO_4$

(1:1), keeping a granule-powder mixture ratio of 25:75. The mixture was compacted with flat-faced punches using a mechanical press (CE03, International Crystal Laboratories, USA) with constant pressure (1,390 ± 11 psi) for 30 seconds producing discs with a surface area of 0.5 cm^2.

2.2.4. Solid self-emulsifying dosage forms characterization

2.2.4.1. Specific surface area measurement of the granules

Nitrogen absorption-desorption isotherms were obtained using a surface area and pore size analyzer (NOVA e-4200, Quantachrome Instruments, USA). First, the granules containing the lipid formulation and the granules' raw materials were weighed until fill 2/3 of the total volume of the bulb. Afterward, the degassing phase was carried out under vacuum for 15 h at 50 °C. It is noteworthy to mention that a previous evaluation was performed by TGA to guarantee that no sample degradation occurs at this temperature. The adsorption isotherms were collected with 21 points with the relative pressure (*P*/*Po*) ranging from 0.01 to 0.99, while the desorption isotherms had 18 points from 0.98 to 0.05. All analyses were conducted using nitrogen as the adsorptive at 77 K. To calculate the surface area (SBET), the Brunauer-Emmett-Teller (BET) equation [27] was applied using 13 points between a *P*/*Po* ranging from 0.01 to 0.035. Moreover, only measurements with a correlation coefficient (R^2) higher than 0.995 were considered for this evaluation, and the BET C-values were calculated to evaluate changes in the interaction of the nitrogen with the surface of the sample [28].

2.2.4.2. Evaluation of the self-emulsifying performance

The self-emulsifying evaluation was carried out in an incubator shaker, where each dosage form was added to 250 mL of FaSSIF and homogenized (150 rpm, 37.0 °C ± 0.1 °C) for 3 h. Thereafter, each system was withdrawn (3 mL), filtered with a 45 mm membrane, and characterized regarding their droplet size and PDI, as described in section *Determination of hydrodynamic droplet size and polydispersity index (2.2.2.2.)*. Each experiment was performed three times.

2.2.5. Statistical analysis

Statistical analysis was carried out using the GraphPad Prism 6.01 software (GraphPad Software Inc, USA). The results were exhibited as the mean of at least three individual experiments ± standard deviation of the mean. To establish the difference between the means, one or two-way analysis of variance (ANOVA) were performed with *post-hoc* of Tukey with $a < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Solubility

The dissolution process is one of the limiting steps to the oral absorption of BCS class IV drugs, such as AmB [12]. Therefore, to avoid this hindrance, the drug should be available as a molecular dispersion into the lipid formulation [29]. For this purpose, a solubility assay of the AmB was performed in 45 different excipients. The results revealed that the evaluated excipients displayed a considerable variability in their ability to solubilize AmB, of which 15 exhibited an AmB solubility lower than 0.001 mg/mL (Table 2). On the other hand, the PPG exhibited a remarkable AmB solubilization capacity (1.237 mg/mL), with values more than three-fold higher than the other excipients.

Even though part of these data agreed with the ones previously reported [24], others revealed divergent solubility values. Probable sources of variation could be the settled temperature, the period of homogenization, as well as the G-force applied during the centrifugation process. The selection of the excipients used to formulate the SEDDS considered not only the AmB solubility capacity but also the role of the excipient into the formulation (*e.g.,* oil, surfactant, or co-solvent), and the ease of the mixture to self-emulsifying, which was evaluated in previous experiments. Therefore, the selected oil phase was GMC:PPG (1:1), and SM as the surfactant.

Table 2. Amphotericin B solubility in lipid excipients at 25.0 ± 0.1 °C

Excipient	**Solubility (mg/mL)**
Capmul® MCM C8	0.083 ± 0.007
Capmul® MCM EP	0.142 ± 0.007
Capmul® GMO-50	0.035 ± 0.004
Crodamol® GMCC-SS	0.175 ± 0.007
Dhaytan® L 20	0.075 ± 0.004
Dhaytan® S 80	0.005 ± 0.000
Inwitor® 308	**0.205 ± 0.053**
Inwitor® 742	0.131 ± 0.021
Inwitor® 988	0.069 ± 0.004
Kolliphor® EL	0.020 ± 0.001
Kolliphor® RH 40	0.027 ± 0.001
Kollisolv® PEG 300	0.223 ± 0.011
Kollisolv® PEG 400	0.108 ± 0.008
Labrasol®	0.019 ± 0.001
Maisine® CC	0.006 ± 0.001
Peceol®	0.003 ± 0.000
Phosal® 50 PG	**1.237 ± 0.107**
Phosal® 50 SA+	0.110 ± 0.003
Phosal® 53 MCT	0.077 ± 0.004
Plurol® Oleique	0.022 ± 0.003
Softigen® 767	0.008 ± 0.001
Span® 80	**0.013 ± 0.001**
Super Refined® Castor Oil	0.022 ± 0.003
Super Refined® PEG 300	0.470 ± 0.133
Super Refined® PEG 400	0.339 ± 0.000
Super Refined® Polysorbate 80	0.070 ± 0.016
Transcutol® HP	0.083 ± 0.002
Tween® 20	0.003 ± 0.000
Tween® 40	0.004 ± 0.000
Tween® 80	0.004 ± 0.000
Capryol® 90	Not measurable
Captex® 100	Not measurable
Captex® 300	Not measurable
Captex® 355	Not measurable
Dhaykol® 6040	Not measurable
Isopropyl Myristate	Not measurable
Isopropyl Palmitate	Not measurable
Labrafac® Lipophile WL 1349	Not measurable

Labrafac® PG	Not measurable
Labrafil® M 1944 CS	Not measurable
Lauroglycol® 90	Not measurable
Miglyol® 812 N	Not measurable
Sesame oil	Not measurable
Super Refined® Arlasolve	Not measurable
Super Refined® Oleic Acid	Not measurable

3.2. Characterization of the SEDDS phase behavior

A pseudo-ternary phase diagram is a graphical approach that displays the phase behavior of the oil-water interface after adding an aqueous medium to a surfactant-oil mixture. Usually, the initial characterization of the obtained systems is by visual inspection, in which emulsions show milky appearance and microemulsions are optically translucent [30]. Despite being a qualitative and subjective method, the visual inspection indicates the dispersiveness of the mixture and provides a reliable forecast of the excipient proportions expected to exhibit an anomalous behavior in distinct assays [31].

Based on this approach, it was possible to infer that the mixtures formulated with SM, dispersed in FaSSIF, displayed a large microemulsion region (Figure 1A) corresponding to approximately 70% of the total area evaluated, followed by 26% of phase separation and approximately 4% of emulsion region. On the other hand, when the FeSSIF medium was added to the oily mixture, the microemulsion (~65%) and the phase separation (~9%) region decreased, whereas the emulsion region increased about 6.5 times (26%) (Figure 1B). These results may seem unexpected since the FeSSIF medium presents a higher concentration of surfactant than the FaSSIF one. However, the salinity content held by FeSSIF is about three times higher than FaSSIF (~1.8 g of NaCl /100 mL and 0.62 g of NaCl /100 mL, respectively) [32]. It has been reported that salinity is a parameter that plays a role in the phase behavior, as its increment enables the transition Winsor I → III → II [33, 34]. These results agree with the literature since SEDDS produced with FeSSIF were more polydisperse due to the higher mixed micelles concentration [33, 35]. Knowing that the oil phase (HLB ~ 6.25) is a 1:1 mixture of GMC (HLB ~ 6) [36] and PPG (HLB = 6.49, reported by Lipoid), it is possible to infer that the effectiveness of the interfacial stabilization provided by SM relies on its partitioning at the interface since it displays a hydrophobic feature (HLB = 4.3) [36].

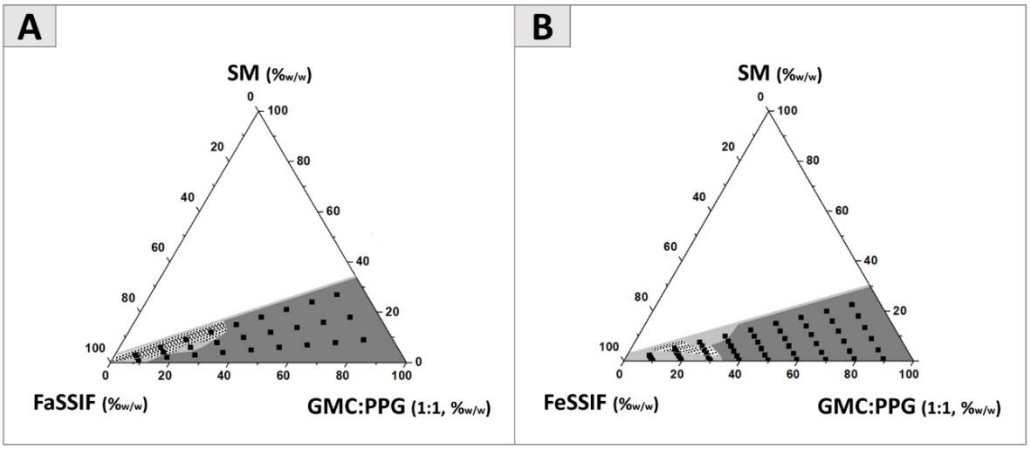

Figure 1. Pseudo-ternary phase diagram using Imwitor 308 (GMC) and Phosal 50 PG (PPG) (ratio 1:1) as oil phase; Span 80 (SM) as the surfactant; and Fasted State Simulated Intestinal Fluid (FaSSIF) (A) or

Fed State Simulated Intestinal Fluid (FeSSIF) as aqueous phase (B). Key: (■) experimental data. The dark gray area corresponds to microemulsion; the light gray area describes the emulsion region; the dashed one is the phase separation area.

3.3. Self-emulsifying performance of lipid systems

Once established the boundaries of the relevant region in the pseudo-ternary phase diagram and relying on the requirements of (I) high AmB solubilization, (II) low amount of surfactant, and (III) appropriate amount of aqueous medium to simulate physiological conditions, two formulations were chosen (Table 1) and classified as type II according to the Lipid Formulation Classification System [37]. These SEDDS were characterized regarding their homogeneity, AmB solubility, polydispersity index, and hydrodynamic droplet size. This last feature has been assigned to provide adequate intestinal absorption when formulations display droplet sizes ranging from 100 nm to 300 nm [38].

Based on the droplet size displayed in Figure 2, it is possible to infer that, regardless of the formulation evaluated, the systems revealed a size distribution behavior that over time tends to achieve equilibrium as a bimodal distribution. An experimental data that supports this idea is the one exhibited by F-70:30, in which the droplet population ranging from 3 μm to 5 μm pointed out in 1:30 h is no longer detected after 3:30 h, which also explains the decrease in the PDI values (Figure 2A).

The bimodal population of the samples ranged from 50 nm to 100 nm and from 350 nm to 600 nm (Figure 2B). This distribution pattern explains the observed PDI values ranging from 0.3 to 0.5 (Figure 2A). On the other hand, the medium used to perform the emulsification process (FaSSIF) displayed a unimodal population (50 nm to 100 nm) (Figure 2B). Even though these data might suggest that the presence of this population in the distribution pattern displayed by the samples may be attributed to the medium, it is not possible to draw a reliable conclusion, using only DLS data, that the droplet size of the samples should be only addressed to the population ranging from 350 nm to 600 nm. Therefore, the Z-average (the overall mean) seems to be the appropriate approach.

Based on this perspective, no significant difference was found regarding the hydrodynamic diameter and the polydispersity index of the evaluated systems (p-value = 0.25, Figure 2A). Moreover, no difference in the AmB solubilization capacity was noticed, as formulations F-80:20 and F-70:30 displayed a solubilization of 0.678 ± 0.024 mg/mL and 0.623 ± 0.067 mg/mL, respectively. Revealing that the chosen formulations exhibited the following required features: appropriate dispersion in biorelevant medium, low surfactant concentration, and high AmB solubilization.

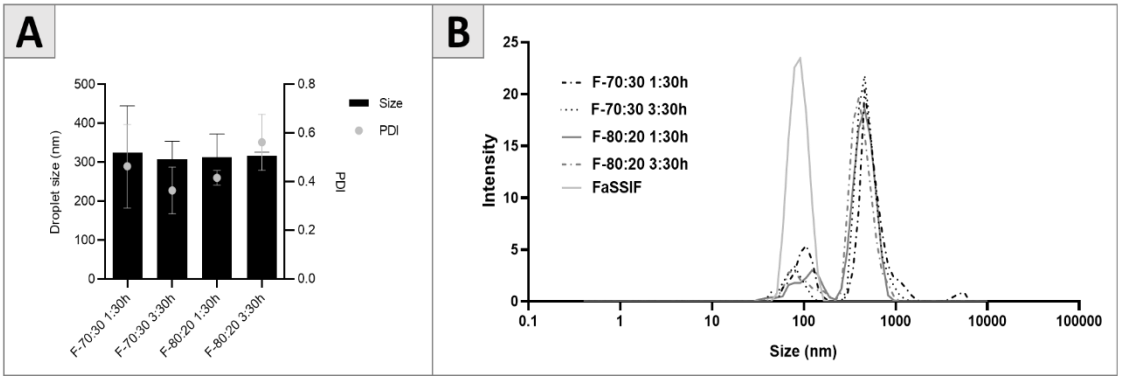

Figure 2. (A) Average droplet size and polydisperse index (PDI) of SEDDS formulations F-70:30 and F-80:20 at 1:30 h and 3:30 h. The results are expressed by mean ± SD. (B) Distribution of the droplet size produced by the formulations F-70:30 and F-80:20 at 1:30 h and 3:30 h and FaSSIF

3.4. Characterization of the solid dosage forms

3.4.1. Specific Surface area

To calculate the surface area (SBET) by gas adsorption it is commonly applied the BET equation to the isotherm. The suitability of the analysis is evaluated by the correlation coefficient (R^2) and the BET C-value, with the former corresponding to the linearity of the isotherm in the BET region, and the latter the interaction of the nitrogen with the sample. As depicted in Table 3, this mathematical approach displayed a good fitting with the experimental data (> 0.999) [28].

The C-value parameter in the BET equation not only expresses the extent of coverage of the adsorbate on the adsorbent but also the affinity between them. All samples analyzed presented a C-value higher than 2, which confirms that the BET equation can be used to calculate the surface area (Table 2) [28]. The granules containing the lipid formulation exhibited lower values of BET C-values when compared to the solid raw excipients, indicating that the presence of the lipid formulation affected the interaction between the sample (adsorbent) and the nitrogen (adsorbate). Furthermore, as reported by Lapham *et al*. BET C-values as the ones obtained in this study are closer to 10, which makes the distinction between isotherm II and III a challenging task [28].

The SBET values calculated for Neusilin U2 and colloidal SiO2 agree with those previously reported in the literature [38]. Also, as expected, the Neusilin U2:colloidal SiO_2 mixture (1:1) exhibited intermediate surface area values compared with its isolated constituents (Table 3). Besides, the Neusilin U2 had a higher surface area. When the SEDDS were incorporate into it, the obtained powder exhibited poor flowability. To solve this problem colloidal SiO_2 was added, and all the granules were prepared with Neusilin U2:colloidal SiO_2 mixture (1:1).

When compared to the one exhibited by the colloidal silicon alone, the large surface area of the mixture suggests that the mixture can be a better reservoir for the pre-emulsion SEDDS [39]. The decrease in the SBET values and the total pore volume of the granules, when compared to the Neusilin U2:colloidal SiO_2 mixture (1:1), indicates that the adsorption of the pre-emulsion SEDDS where effective, with nearly 96% of the total pore volume and the specific surface area filled by the lipid formulation (Table 3).

Table 3. Specific surface area (SBET), BET correlation coefficient (R^2), BET C-value, and total pore volume of solid components of granules and sample granules.

Sample	S_{BET} (m^2/g)	R^2	BET C-value	Total pore volume (mL/g)

Neusilin U2	272.604	0.9998	181.676	0.8479
SiO_2	150.062	0.9997	37.430	0.2650
SiO_2:Neusilin U2 (1:1)	212.166	0.9997	32.715	0.7195
F-70:30 granules	3.068	0.9996	13.924	0.0211
F-80:20 granules	10.792	0.9989	10.022	0.0409

Note: the formulation ID highlights the oil:surfactant proportion, i.e., granules of F-70:30 and F-80:20 were prepared with self-emulsifying drug delivery systems (SEDDS) having 70:30 and 80:20 oil:surfactant proportion respectively. Neusilin U2 and SiO2 denotes the amorphous form of magnesium aluminometasilicate and colloidal silicon dioxide respectively.

3.4.2. Self-emulsifying performance of solid dosage forms

Regarding the emulsification behavior exhibited by different drug dosage forms in FaSSIF, the data revealed that the addition of the liquid lipid formulations to the capsules did not affect the emulsification process in terms of average droplet size (p-value = 0.421, Table 4 and Figure 3A). The comparison of the average droplet size and PDI values revealed that the composition of the lipid formulation did not affect the average droplet size displayed by the system, *i.e.*, there was no significant difference in the droplet size and PDI values exhibited by the same dosage form manufactured with different lipid formulations ($p > 0.05$). On the other hand, different dosage forms displayed significant differences among each other ($p < 0.05$) with average droplet size values revealing the following pattern: capsule filled with liquid lipid formulation (LC) > capsule filled with granules (GC) = tablet (T) (Table 4 and Figure 3). This pattern highlights that the hydrodynamic diameter displayed by the droplets from granules and tablets significantly decreased compared to the ones from LC (respectively p-value < 0.014 and p-value < 0.012, Table 4). In contrast, there was no significant difference between granules and tablets regardless of the SEDDS formulation (p-value > 0.994). As stated by Joyce *et al*., the adsorption of liquid SEDDS into solid carriers can significantly affect the interfacial surface area of the lipid [40]. These results corroborate with this hypothesis, suggesting that the enhancement in the self-emulsifying performance was caused by the increment of the surface area when the SEDDS were added to the adsorbent material, favoring the SEDDS-FaSSIF interaction.

Table 4. Average droplet size (nm) and PDI of the capsules filled with liquid lipid formulation (LC), capsules filled with granulated SEDDS (GC), and tablets (T) with the SEDDS formulations F-70:30 and F-80:20.

Formulation	**Droplet size (nm)(±SD)**	**PDI(±SD)**
LC-F-70:30	382.8 (± 98.76)	0.57 (± 0.12)
LC-F- 80:20	327.9 (± 31.15)	0.44 (± 0.03)
GC-F-70:30	136.0 (± 40.93)	0.30 (± 0.06)
GC-F-80:20	156.4 (± 37.12)	0.33 (± 0.08)
T-F-70:30	153.7 (± 21.70)	0.36 (± 0.02)
T-F-80:20	134.3 (± 36.11)	0.31 (± 0.08)
FaSSIF	85.8 (± 3.40)	0.01 (± 0.01)

Note: the formulation ID highlights the oil:surfactant proportion, i.e., F-70:30 and F-80:20 have 70:30 and 80:20 oil:surfactant proportion respectively.

It is noteworthy to mention the average droplet size exhibited by the dosage forms is in a range assigned as ideal for intestinal absorption [41]. Therefore, highlighting the potential of these systems as carriers for oral administration. However, the amount of SEDDS carried by the

solid raw material is lower in granules and tablets than in the LC. From this point of view, it is possible to conclude that granules and tablets are suitable for carrying drugs that require low oral doses. Which does not apply to AmB. For this reason and considering that the LC carried *ca.* 0.42 mg/dose, while granules and tablets exhibited respectively *ca.* 65% and 93% less drug, it is possible to infer that LC is the most appropriate system to be used in an AmB oral dosage regimen.

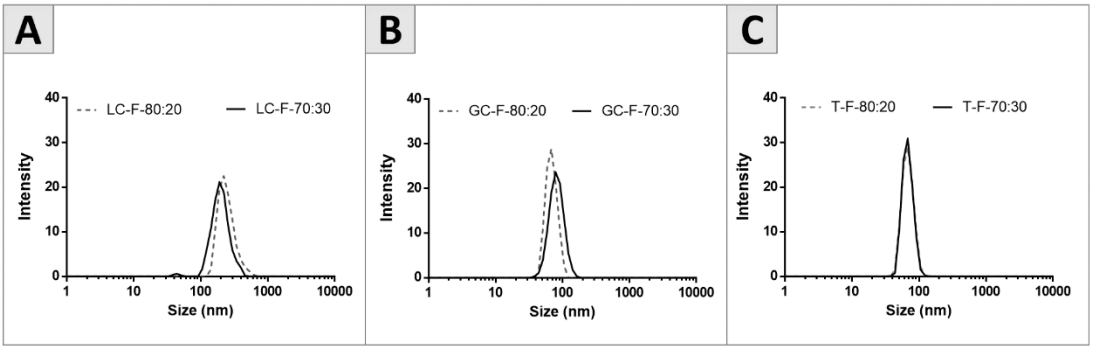

Figure 3. Distribution droplet size measured from the different dosage forms loaded with SEDDS F-70:30 or F-80:20, 3 h after agitation, and FaSSIF. (A) Liquid-filled capsules. (B) Capsules filled with granules. (C) Tablet.

4. CONCLUSIONS

The results revealed that the 45 different excipients evaluated exhibited a heterogeneous AmB solubilization capacity, with PPG exhibiting the maximum one, more than three-fold higher than the other excipients. The phase behavior of the chosen mixture demonstrated through the pseudo-ternary phase diagram that the emulsification process was enhanced in FaSSIF medium when compared with FeSSIF. Most likely due to the higher salinity content displayed by the last one, which might trigger the transition Winsor I → III → II when compared with FaSSIF. This observation suggested that the fasted state may be the appropriate condition when performing *in vivo* studies. The chosen lipid formulations (F-80:20 and F-70:30) displayed a similar behavior with a bimodal population (from 50 nm to 100 nm and from 350 nm to 600 nm) and no significant difference regarding the hydrodynamic diameter, the polydispersity index, as well as the AmB solubilization capacity. The values of pore volume and surface area of the granules suggest both formulations were successfully incorporated in the mixture of colloidal silicon dioxide and Neusilin U2 (1:1). Once added to dosage forms, these formulations displayed a monomodal distribution. However, its lipid load was 13.5 times lower than the one used to manufacture the LFC. Even though the AmB dose carried by the tablets does not seems to be appropriated to its clinical use (*e.g.*, the treatment of leishmaniasis), this technology can be applied to drugs that require lower oral doses (*i.e.*, drug with high potency). Thus, the LFC is apparently the appropriate drug dosage form, displaying the highest lipidic load among all the evaluated formulations, and exhibiting an average droplet size in an ideal range for intestinal absorption. Therefore, highlighting the potential of this system as an AmB carrier for oral administration.

ACKNOWLEDGEMENTS

This research was funded by the Oswaldo Cruz Foundation (FIOCRUZ), The Brazilian Development Bank (BNDES) [grant number Funtec 0473/2015], Carlos Chagas Filho Research Support Foundation of Rio de Janeiro (FAPERJ) [grant E-26/202.951/2018 and E-26/200.210/2023-Bolsa], and the Brazilian National Council for Scientific and Technological Development (CNPq) [grant PIBITI/2019/2020]. Moreover, the authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, F.A.Jr, M.A.S., H.V.A.R., B.F.C.P.; methodology, F.A.Jr, M.A.S.,G.B., L.A.H.N., T.M.S., H.V.A.R., B.F.C.P.; validation, F.A.Jr, G.B., L.A.H.N.; investigation, F.A.Jr, M.A.S.,G.B., L.A.H.N., T.M.S., B.F.C.P.; writing—original draft preparation, F.A.Jr, M.A.S., B.F.C.P.; review and editing, K.G.H.S., L.D.P., H.V.A.R; supervision, K.G.H.S., L.D.P., H.V.A.R, B.F.C.P.; project administration, H.V.A.R, B.F.C.P.; funding acquisition, H.V.A.R. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

Authors have no conflict of interest.

REFERENCES

1. F. Ibrahim, O. Sivak, E.K. Wasan, K. Bartlett & K.M. Wasan. Efficacy of an oral and tropically stable lipid-based formulation of Amphotericin B (iCo-010) in an experimental mouse model of systemic candidiasis. *Lipids in Health and Disease*, **12**, 158 (2013) Doi: https://doi.org/10.1186/1476-511X-12-158
2. A. Lemke, A.F. Kiderlen & O. Kayser. Amphotericin B. *Applied Microbiology and Biotechnology*, **68**, 151 (2005). Doi: https://doi.org/10.1007/s00253-005-1955-9
3. WHO. *World Health Organization Model List of Essential Medicines - 22nd Edition*. World Health Organization, Geneva, 2021; 66 p. URL: https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2021.02
4. D.W. Bates, L. Su, D.T. Yu, G.M. Chertow, D.L. Seger, D.R.J. Gomes, E.J. Dasbach & R. Platt. Mortality and costs of acute renal failure associated with Amphotericin B therapy. *Clinical Infectious Diseases*, **32**(5), 686–693 (2001) Doi: https://doi.org/10.1086/319211
5. R.A. Zager, C.R. Bredl & B.A. Schimpf. Direct amphotericin B-mediated tubular toxicity: Assessments of selected cytoprotective agents. *Kidney International*, **41**, 588–1594 (1992) Doi: https://doi.org/10.1038/ki.1992.229
6. J. Barwicz, S. Christian & I. Gruda. Effects of the aggregation state of amphotericin B on its toxicity to mice. *Antimicrobial Agents and Chemotherapy*, **36**(10), 2310–2315 (1992) Doi: https://doi.org/10.1128/AAC.36.10.2310
7. D.R. Serrano & A. Lalatsa. Oral amphotericin B: The journey from bench to market. *Journal of Drug Delivery Science and Technology*, **42**, 75-83 (2017) Doi: https://doi.org/10.1016/j.jddst.2017.04.017
8. S. Chakraborty, D. Shukla, B. Mishra & S. Singh. Lipid – An emerging platform for oral delivery of drugs with poor bioavailability. *European Journal of Pharmaceutics and Biopharmaceutics*, **73**(1), 1–15 (2009). Doi: https://doi.org/10.1016/j.ejpb.2009.06.001
9. C.A. Lipinski, F. Lombardo, B.W. Dominy & P.J. Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, **46**(1-3), 3–26 (2001). Doi: https://doi.org/10.1016/S0169-409X(00)00129-0

10. G.L. Amidon, H. Lennernäs, V.P. Shah & J.R. Crison. A theoretical basis for a Biopharmaceutic Drug Classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharmaceutical Research*, **12**, 413–420 (1995). Doi: https://doi.org/10.1023/A:1016212804288
11. C.-Y. Wu & L.Z Benet. Predicting drug disposition via application of BCS: Transport/Absorption/Elimination interplay and development of a Biopharmaceutics Drug Disposition Classification System. *Pharmaceutical Research*, **22**, 11–23 (2005). Doi: https://doi.org/10.1007/s11095-004-9004-4
12. R. Ghadi & N. Dand. BCS class IV drugs: Highly notorious candidates for formulation development. *Journal of Controlled Release*, **248**, 71–95 (2017). Doi: https://doi.org/10.1016/j.jconrel.2017.01.014
13. B. Rivnay, J. Wakim, K. Avery, P. Petrochenko, J.H. Myung, D. Kozak, S. Yoon, N. Landrau & A. Nivorozhkin. Critical process parameters in manufacturing of liposomal formulations of amphotericin B. *International Journal of Pharmaceutics*, **565**, 447-457 (2019). Doi: https://doi.org/10.1016/j.ijpharm.2019.04.052
14. C.W. Pouton. Self-emulsifying drug delivery systems: assessment of the efficiency of emulsification. *International Journal of Pharmaceutics*, **27**(2-3), 335–348 (1985). Doi: https://doi.org/10.1016/0378-5173(85)90081-X
15. J. Liu, C. Hirschberg, M. Fanø, H. Mu & A. Müllertz. Evaluation of self-emulsifying drug delivery systems for oral insulin delivery using an in vitro model simulating the intestinal proteolysis. *European Journal of Pharmaceutical Sciences*, **147**, 105272 (2020). Doi: https://doi.org/10.1016/j.ejps.2020.105272
16. A. Patel, P. Shelat & A. Lalwani. Development and optimization of solid self-nanoemulsifying drug delivery system (S-SNEDDS) using Scheffe's design for improvement of oral bioavailability of nelfinavir mesylate. *Drug Delivery and Translational Research*, **4**, 171–186 (2014). Doi: https://doi.org/10.1007/s13346-014-0191-1
17. B. Tang, G. Cheng, J.-C. Gu & C.-H. Xu. Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms. *Drug Discovery Today*, **13**(13-14), 606–612 (2008). Doi: https://doi.org/10.1016/j.drudis.2008.04.006
18. J. Mandić, A. Zvonar Pobirk, F. Vrečer & M. Gašperlin. Overview of solidification techniques for self-emulsifying drug delivery systems from industrial perspective. *International Journal of Pharmaceutics*, **533**(2), 335–345 (2017) DOI: https://doi.org/10.1016/j.ijpharm.2017.05.036
19. Y. Choudhari, U. Reddy, F. Monsuur, T. Pauly, H. Hoefer & W. McCarthy. Comparative evaluation of porous silica based carriers for lipids and liquid drug formulations. *Open Material Sciences*, **1**, 61–74 (2014). Doi: https://doi.org/10.2478/mesbi-2014-0004
20. A. Tan, S. Rao & C.A. Prestidge. Transforming lipid-based oral drug delivery systems into solid dosage forms: An overview of solid carriers, physicochemical properties, and biopharmaceutical performance. *Pharmaceutical Research*, **30**, 2993–3017 (2013). Doi: https://doi.org/10.1007/s11095-013-1107-3
21. A. Seo, P. Holm, H.G. Kristensen, T. Schæfer. The preparation of agglomerates containing solid dispersions of diazepam by melt agglomeration in a high shear mixer. *International Journal of Pharmaceutics*, **259**(1-2), 161–171 (2003). Doi: https://doi.org/10.1016/S0378-5173(03)00228-X
22. A.S. Narang, D. Desai & S. Badawy. Physicochemical interactions in solid dosage forms. *Pharmaceutical Research*, **29**, 2635–2638 (2012). Doi: https://doi.org/10.1007/s11095-012-0867-5
23. M.M. Gade & P.J. Hurkadale. Formulation and evaluation of self-emulsifying orlistat tablet to enhance drug release and *in vivo* performance: factorial design approach. *Drug Delivery and Translational Research*, **6**, 276–288 (2016). Doi: https://doi.org/10.1007/s13346-016-0289-8
24. C. Salerno, S. Gorzalczany, A. Arechavala, S.L. Scioscia, A.M. Carlucci & C. Bregni. Novel gel-like microemulsion for topical delivery of Amphotericin B. *Revista Colombiana de Ciencias Químico-Farmacéuticas*, **44**(3), 359–381 (2015). Doi: https://doi.org/10.15446/rcciquifa.v44n3.56285
25. S.G. Gumaste, B.O.S. Freire & A.T.M. Serajuddin. Development of solid SEDDS, VI: Effect of precoating of Neusilin® US2 with PVP on drug release from adsorbed self-emulsifying lipid-based

formulations. *European Journal of Pharmaceutical Sciences*, **110**, 124–133 (2017). Doi: https://doi.org/10.1016/j.ejps.2017.02.022

26. E.N. Vaikosen, S.J. Bunu, J.N. Oraeluno & D. Friday. Comparative application of derivative spectrophotometric and HPLC techniques for the simultaneous determination of lamivudine and tenofovir disoproxil fumarate in fixed-dose combined drugs. *Future Journal of Pharmaceutical Sciences*, **9**, 21 (2023). Doi: https://doi.org/10.1186/s43094-023-00471-7
27. S. Brunauer, P.H. Emmett & E. Teller. Adsorption of gases in multimolecular layers. *Journal of the American Chemical Society*, **60**(2), 309–319 (1938). Doi: https://doi.org/10.1021/ja01269a023
28. D.P. Lapham & J.L. Lapham. Gas adsorption on commercial magnesium stearate: Effects of degassing conditions on nitrogen BET surface area and isotherm characteristics. *International Journal of Pharmaceutics*, **530**(1-2), 364–376 (2017). Doi: https://doi.org/10.1016/j.ijpharm.2017.08.003
29. K. AboulFotouh, A.A. Allam, M. El-Badry & A.M. El-Sayed. Role of self-emulsifying drug delivery systems in optimizing the oral delivery of hydrophilic macromolecules and reducing interindividual variability. *Colloids and Surfaces B: Biointerfaces*, **167**, 82–92 (2018). Doi: https://doi.org/10.1016/j.colsurfb.2018.03.034
30. E.S. Mahdi, M. Sakeena, M. Abdulkarim, G. Abdullah, M.Z. Abdul-Sattar & A. Noor. Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. *Drug Design, Development and Therapy*, **5**, 311–323 (2011). Doi: https://doi.org/10.2147/dddt.s15698
31. D.Q.M. Craig, S.A. Barker, D. Banning & S.W. Booth. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. *International Journal of Pharmaceutics*, **114**(1), 103–110 (1995). Doi: https://doi.org/10.1016/0378-5173(94)00222-Q
32. S. Klein. The use of biorelevant dissolution media to forecast the in vivo performance of a drug. *AAPS Journal*, **12**, 397–406 (2010). Doi: https://doi.org/10.1208/s12248-010-9203-3
33. S. Ahmed & K.A. Elraies. Microemulsion in enhanced oil recovery. In: Selcan Karakuş (editor). *Science and Technology Behind Nanoemulsions*. InTechOpen, London, 2018. Doi: https://doi.org/10.5772/intechopen.75778
34. M. Sanchez-Dominguez, C. Aubery & C. Solans. New trends on the synthesis of inorganic nanoparticles using microemulsions as confined reaction media. In: A. Hashim (editor). *Smart Nanoparticles Technology*. InTechOpen, London, 2012. Doi: https://doi.org/10.5772/33010
35. E. Kontogiannidou, T. Meikopoulos, C. Virgiliou, N. Bouropoulos, H. Gika, I.S. Vizirianakis, A. Müllertz & D.G. Fatouros. Towards the development of Self-Nano-Emulsifying Drug Delivery Systems (SNEDDS) containing trimethyl chitosan for the oral delivery of amphotericin B: *In vitro* assessment and cytocompatibility studies. *Journal of Drug Delivery Science and Technology*, **56**(Part A), 101524 (2020). Doi: https://doi.org/10.1016/j.jddst.2020.101524
36. I. Nardin & S. Köllner. Successful development of oral SEDDS: Screening of excipients from the industrial point of view. *Advanced Drug Delivery Reviews*, **142**, 128–140 (2019). Doi: https://doi.org/10.1016/j.addr.2018.10.014
37. C.W. Pouton. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. *European Journal of Pharmaceutical Sciences*, **29**(3-4), 278–287 (2006). Doi: https://doi.org/10.1016/j.ejps.2006.04.016
38. Z. Ujhelyi, M. Vecsernyés, P. Fehér, D. Kósa, P. Arany, D. Nemes, D. Sinka, G. Vasvári, F. Fenyvesi, J. Váradi & I. Bácskay. Physico-chemical characterization of self-emulsifying drug delivery systems. *Drug Discovery Today: Technologies*, **27**, 81–86 (2018). Doi: https://doi.org/10.1016/j.ddtec.2018.06.005
39. G. Quan, Q. Wu, X. Zhang, Z. Zhan, C. Zhou, B. Chen, Z. Zhang, G. Li, X. Pan & C. Wu. Enhancing *in vitro* dissolution and in vivo bioavailability of fenofibrate by solid self-emulsifying matrix combined with SBA-15 mesoporous silica. *Colloids and Surfaces B: Biointerfaces*, **141**, 476–482 (2016). Doi: https://doi.org/10.1016/j.colsurfb.2016.02.013

40. P. Joyce, T.J. Dening, T.R. Meola, H.B. Schultz, R. Holm, N. Thomas & C.A. Prestidge. Solidification to improve the biopharmaceutical performance of SEDDS: Opportunities and challenges. *Advanced Drug Delivery Reviews*, **142**, 102–117 (2019). Doi: https://doi.org/10.1016/j.addr.2018.11.006
41. E. Franceschinis, A.C. Santomaso, L. Benda, B. Perissutti, D. Voinovich & N. Realdon. Influence of process variables on the properties of simvastatin self-emulsifying granules obtained through high shear wet granulation. *Powder Technology*, **274**, 173–179 (2015). Doi: https://doi.org/10.1016/j.powtec.2015.01.026

HOW TO CITE THIS ARTICLE

F.A.-Jr, M.A. Sarcinelli, G. Barcellos, L.A.H. Nascimento, T.M. Silva, KG.H. Silva, L.D. Prado, H.V.A. Rocha & B.F.C. Patricio. Solid Self-Emulsifying Dosage Forms for carrying Amphotericin B: A preformulation study. *Rev. Colomb. Cienc. Quim. Farm.*, **54**(1), 231–214 (2025). Doi: https://doi.org/10.15446/rcciquifa.v54n1.119557