RESEARCH ARTICLE

Preparation and *In-vitro* Characterization of Tacrolimus as a Solid Self-microemulsion

Duaa J. Al-Tamimi[1*], Ahmed A. Hussien[2]

[1]*Department of Pharmacy, Al-Rasheed University College, Baghdad, Iraq*
[2]*Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq*

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ABSTRACT

Objective: The present investigation is aimed to improve the dispersibility, dissolution rate, and ultimately the bioavailability of the poorly water-soluble BCS class II drug Tacrolimus by formulating it as self micro emulsifying drug delivery system (SMEDDS).

Materials and Methods: Nine formulas of liquid SMEDDS of the drug were formulated using peceol, labrasol ALF, and transcutol HP as oil, surfactant and co-surfactant, respectively, depending on tacrolimus solubility study in these components. Pseudo ternary phase diagrams were plotted for the identification of an area of micro-emulsification. The prepared systems were characterized for thermodynamic stability, robustness to dilution, self-emulsification time, drug content, globule size, and polydispersity index. The optimized liquid SMEDDS was transformed into powder using Aerosil 200 and Avicel 102 as the adsorbents and characterized for its flow properties.

Results and Discussion: The selected formula (F1) composed of Peceol, Labrasol ALF, Transcutol HP, Aerosil 200, and Avicel 102 powder retained the self micro emulsifying property of the liquid SMEDDS. Differential scanning calorimetry and X-ray powder diffraction studies confirmed the solubilization of the drug in the lipid excipients and the transformation of the drug's crystalline form to the amorphous one in solid-SMEDDS. This was supported by scanning electron microscopy studies. In-vitro dissolution studies revealed the enhanced release of the drug from solid-SMEDDS which reached more than 89% within 10 minutes as compared to pure drug and the marketed formulation, which reached 19% and 80.4% after 1-hour, respectively.

Conclusion: Both liquid and solid SMEDDS showed superior in-vitro drug release and characterization profiles compared with pure powder and the marketed product (Prograf). All of these prove solid SMEDDS to be a promising approach to improving problems associated with poorly soluble drugs' oral delivery.

Keywords: Characterization, Formulation, Microemulsion, SMEDDS.

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INTRODUCTION

The oral drug delivery (ORD) system is commonly regarded as the preferred route for drug administration, especially for patients needing extended therapy due to high patient compliance, among many other benefits.[1] However, it faces many obstacles like the low bioavailability of drugs with low aqueous solubility and the need for drugs to be immune to degradation by gastrointestinal secretions yet readily absorbed upon reaching their specific absorption site. To remove all those hurdles, various novel oral delivery systems have been developed, the most accepted of which is the lipid-based formulations.[2] A popular category of lipid-based formulations is self-emulsifying drug delivery systems (SEDDS). The SEDDS is defined as an isotropic mixture of oil, surfactant, and cosurfactant that make small oil-in-water (o/w) microemulsion when administered in an aqueous environment, two requirements that are readily available in the gastrointestinal tract with its aqueous secretions and continuous peristaltic movements.[3] These two requirements are the spontaneity of this transformation in addition to the large surface area offered by the micro-sized oil droplets facilitates the easy release and absorption of the drug throughout its passage in the gastrointestinal tract.[4]

**Author for Correspondence: duaa.jaafaraltamimi@yahoo.com*

Tacrolimus (FK506) is a macrolide antibiotic that exerts an immunosuppressive effect by selectively inhibiting calcineurin phosphatase. It has long been used as a first-line agent in solid-organ rejection.[5] Oral, intravenous, and topical formulations of tacrolimus are available. However, being a BCS class II drug with low aqueous solubility (1–2 µg/mL), extensive gastrointestinal metabolism and its consequent low absorption, and large intra- and interindividual pharmacokinetic variability have impeded its use as an oral therapeutic agent.[6] The oral bioavailability of the marketed product Prograf produced by (Astellas Pharma Inc., Tokyo, Japan)), a solid dispersion system with cellulose derivatives, was low with high intra- and intersubject variability (average 21%, range 4–89%).[7] Hence, this study aimed to formulate and evaluate tacrolimus as a SMEDDS to enhance its dissolution and, consequently, improve bioavailability and stability for better oral delivery.

MATERIALS AND METHODS

Materials

The tacrolimus supplied from Hyper-Chem LTD CO (China). Labrasol ALF, Transcutol HP, Lauroglycol FCC Lauroglycol 90 and Peceol, were donated by Gattefosse Co. (St. Priest, France). Castor oil was provided by now food, (USA). Tween 20 was obtained from SCRC (China). Tween 80 from Pure chemistry (Germany). Aerosil 200, Avicel pH102, Cremophor EL, Cremophor RH were supplied by Hyper-Chem LTD CO (China). PEG 200 was earned from BDH limited Poole (England). PEG 400 from SCRC (China). Methanol and Propylene glycol were bought from Sigma-Aldrich (Germany). Deionized water was purchased from JT Baker (Netherlands). The Prograf®, hard capsule form supplied by the Astellas Korea Pharm. Co. (South Korea).

Methods

Solubility Studies: Shake flask method was used by introducing an excess amount of tacrolimus into 2 mL of each vehicle in glass vials separately. The mixtures were mixed well using a vortex mixer for 10 minutes. The obtained mixtures were shaken for 48 h in a water bath shaker to reach equilibrium, then centrifuged (3000/20) rpm/min. The supernatants then filtered through a membrane filter (0.45 µm, Millipore filter).[8] Methanol was used as a solvent to dilute the filtered solutions, and drug concentrations were analyzed using a UV-Visible spectrophotometer at λ_{max} 213 nm.

Pseudo Ternary Phase Diagram Construction

the oil surfactant/cosurfactant (Smix) and water Pseudoternary phase diagrams were acquired using the water titration method; each represents a side of the triangle.[9] Surfactant and cosurfactant have been mixed in ratios of 1:1, 2:1, 3:1. Oil and specific Smix ratios were mixed thoroughly in various weight ratios (1:9 to 9:1), so those maximum ratios were covered to delineate each phase's boundaries precisely.[10] Upon forming transparent and homogeneous mixtures of oil/Smix, each mixture was titrated with water under moderate stirring, then equilibrated and assessed visually. When the system became turbid, titration was stopped, and the percentage of oil, surfactant, and cosurfactant was calculated for determining microemulsion region boundaries which corresponding to the selected value of oil and S_{mix} ratio. Phase diagrams were plotted using CHEMIX ternary plot software.[11]

Preparation of Tacrolimus Loaded SMEDDS

A series formulation of tacrolimus was prepared, with varying ratios of excipients, by dissolving Tacrolimus in Peceol oil then adding Labrasol ALF and Transcutol HP in a screw-capped glass. The ingredients were mixed by gentle stirring and heated in a water bath at 40–50°C for 30 minutes until a homogenous isotropic mixture was obtained. The formulations of SMEDDS were stored at room temperature until further use.

Evaluation and Characterization of Tacrolimus Loaded SMEDDS

Thermodynamic Stability Studies

The physical stability of the SMEDDS formulation is essential since poor stability could cause precipitation of the drug in an excipient matrix, phase separation of excipients, in addition to low bioavailability and therapeutic efficacy.[12] All prepared formulations were subjected to different thermodynamic stability tests, where the physical appearances of the formulae were visually observed at the end of each stage.[13]

The cycle of Heating-cooling (H/C cycle)

It subjected to 6 cycles ranged 4 and 45°C with storage at each temperature for 48 hours. The formulae that did not show any phase separations, creaming, or cracking were subjected to a centrifugation test.

Centrifugation test

It centrifuged at (3500/30) rpm/min and observed. Stable formulations were taken to the freeze-thaw cycle.[13]

The cycle of Freeze-thaw

the freeze-thaw cycles implemented for three-cycle ranged -21 and +25 °C with storage at each temperature 48 hours was done. Formulations that passed these thermodynamic stress tests were selected for further studies.

Robustness to Dilution and Effect of pH

This test was done to simulate in vivo dilution behavior by diluting each formula for 50, 100, 250, and 1000 times with deionized water, 0.1 N HCl, and phosphate buffer (pH 6.8). These systems were stored at ambient temperature for 24 hours. Then visually observed for changes.[14]

Dispersibility Test and Self-emulsification Time

The USP dissolution apparatus II was used to assess the efficiency of SMEDDS formulations. About 1 mL of SMEDDS formulation was mixed to 500 mL of deionized water at 37 ± 0.5°C to simulate body temperature with a stirring speed of 50 rpm to ensure complete homogeneity.[15] The formations were assessed visually using the following grading system:[16]

Grade A	Through 1-minutes forming an emulsion with a clear/ bluish appearance rapidly

Grade B	Through 1-minutes making a slightly less clear emulsion, with bluish-white appearance rapidly
Grade C	Within 2 minutes formed Fine milky emulsion.
Grade D	More than 2 minutes an emulsion have a dull, greyish-white, and slightly oily appearance forming in slow to emulsify.
Grade E	Formula clarified either poor or minimal emulsification with large oil globules present on the surface.

Analysis of Droplet Size and Polydispersibility Index (PDI) Determination

One mL of each stable SMEDDS formula was mixed with 100 mL dH2O in a beaker with stirring by magnetic stirrer at 37°C to form a fine emulsion. The resultant microemulsion was analyzed by dynamic light scattering with particle size apparatus.[17]

Drug Content Determination

The formulations were dissolved with 100 mL methanol in a volumetric flask and mixed well. The extracted solution was suitably filtered, diluted and the absorbance was detected at 213.[18]

***In vitro* Drug Release Studies**

The in vitro drug release profiles of SMEDDS formulations and the pure drug was performed using USP dissolution apparatus-II. The dissolution medium was 0.1N HCl (300 mL), at 37 ± 0.5°C and 100 rpm, using the dialysis bag technique (Molecular cut off 12000 Da).[19] Each SMEDDS was placed in a dialysis bag, and Aliquots (5 mL) were withdrawn at regular time intervals of 10, 20, 30, 40, 50, and 60 minutes. The withdrawn samples were replaced by equal volumes of fresh dissolution media (0.1N HCl) to maintain the volume constant. The amount of dissolved drug was obtained at 213 nm using UV–Vis Spectrophotometer.[20]

Preparation of Tacrolimus Loaded S-SMEDDS

The optimal liquid SMEDDS formulae, according to the characterization tests, were converted to solid dosage form using the adsorption process; by mixing them with various adsorbents at a fixed ratio of 1:1. After each addition, the mixture was homogenized by trituration using mortar and pestle to confirm uniform distribution and to obtain a free-flowing powder. Each prepared mixture was kept dry in an oven for 48 hours at 40°C. After drying, the resultant powder was sieved through a sieve (pore size 150 µm) to break down any agglomerates then, the powder was tested for its flow properties before filling into hard gelatin capsules as a solid self-emulsifying capsule.[21]

Tacrolimus loaded S- SMEDDS characterization

S-SMEDDS Micromeritic properties

The angle of repose (θ): The funnel method detected the angle of repose of S-SMEDDS; the height (h) of the funnel was adjusted above a horizontal plane and the powder formulations were poured through the funnel onto the surface. The diameter (D) of the powder cone was measured and the angle of repose calculated using the following formula: $\tan \theta^\circ = 2h/D$. [22]

Bulk Density (BD): A quantity of 2 g of S-SMEDDS powder was introduced into a 10 mL measuring cylinder, and the volume was determined. Bulk density was calculated using the following equation:

Bulk density (BD) = weight of powder blend/poured volume of the powder blend

Tapped Density: Tapped density was calculated using the equation:

Tapped density (TD) = weight of powder blend/tapped volume of the powder blend

Carr's Compressibility Index: The compressibility of the S-SMEDDS formulations was determined by Carr's compressibility index using the following equation:[23]

Carr′s index = tapped density − bulk density/tapped density× 100

Hausner Ratio: Hausner ratio gives an idea about the flow characters of powder particles and was measured using the following equation:

Hausner's ratio = tapped density/bulk density

Drug Content Estimation

S-SMEDDS were dissolved in 100 mL of methanol and mixed thoroughly then suitably diluted, and the absorbance was measured at 213 nm using UV–Vis spectrophotometer.[24]

***In vitro* Drug Release Study**

Using USP type II dissolution apparatus, the dissolution studies were performed in a dissolution medium containing 300 mL of pH 1.2 with a rotation speed of 100 rpm. Five mL aliquot of the dissolution medium was withdrawn at intervals of 3, 5, 10, 20, 30, 40, 50, and 60 minutes. The volume withdrawn was replaced with fresh solvent (0.1 N HCl) following each drawing.[25] The concentration of tacrolimus was analyzed using a UV spectrophotometer at 213 nm. Similarly, the dissolution profiles of the pure drug were also analyzed.

Statistical Analysis

The in-vitro dissolution studies results were statistically evaluated using similarity factor (f2); its results range between 0 and 100, with 100 indicating identical profiles and 0 indicating dissimilarity. This method is more acceptable to compare dissolution profiles when more than three or four dissolution time points are available.[26]

Evaluation of Selected Optimum Tacrolimus Solid Self Micro Emulsion

According to the evaluation and characterization tests, the best formula was selected that include angle of repose, Hausner's ratio, Carr's index, drug content, and in vitro drug release study.

Morphological Analysis

Scanning transmission electron microscopy analysis was done to examine particle size, crystal morphology, magnetic domains, and surface defects in pure tacrolimus and optimum S-SMEDDS.[27]

X-ray Diffraction (XRD) Analysis

The X-ray powder scattering measurements of pure drug, solid carriers, 1:1 mixture of tacrolimus with solid components of the optimum solid formula, and that of the optimum S-SMEDDS powder were carried-out using an XRD, at a continuous scan range of 10–90° with a current of 30 mA and an operating voltage of 40 (KV).

Differential Scanning Calorimetry (DSC) Analysis

The DSC analysis was used to qualitatively assess the physicochemical status of tacrolimus in the S-SMEDDS formula along with its compatibility problems, thermotropic properties, and thermal behavior. Two mg of each sample was heated in a sealed aluminum pan in the DSC instrument, and all samples were scanned at a temperature ramp speed of 10°C/min, covering a temperature up to 300°C.[28]

Fourier Transform Infrared Spectroscopy (FTIR)

This study was done to determine the compatibility and interactions between the components of the formulations. Infra-red spectra of tacrolimus, solid carriers, and the selected S-SMEDDS formula were recorded. The scanning range of wave number was 400–4000 cm^{-1}.[24]

RESULTS AND DISCUSSION

Solubility Studies

Solubility studies are crucial to achieving optimal SMEDDS drug loading and physicochemical characteristics.[29] In this study, Peceol, Labrasol ALF, and Transcutol HP showed the best solubilization among the studied oils, surfactants, and cosurfactants, respectively, as shown in Figure 1 and were therefore chosen for further investigations.

Construction of Pseudoternary Phase Diagram

The effect of dilution on the system is crucial when designing SMEDDS since they will be mixed with body fluids after administration, which may be caused by the precipitation of drug according to the solvent capacity loss.[30] Thus, the digram of the pseudo ternary phase was created to detect the self-microemulsifying region and determine the suitable concentration of excipients in the liquid SMEDDS formulations to ensure successful aqueous dilution without breaking the microemulsions.[31]

In the pseudoternary phase plot, the shaded area represents the microemulsion area, whereas the unshaded area represents the emulsion area, with a higher emulsion efficacy corresponding to a larger microemulsion area. The microemulsion region's size was compared and observed to be higher at surfactant: the cosurfactant ratio of 2:1 and 3:1 than that of 1:1, as shown in Figure 2.

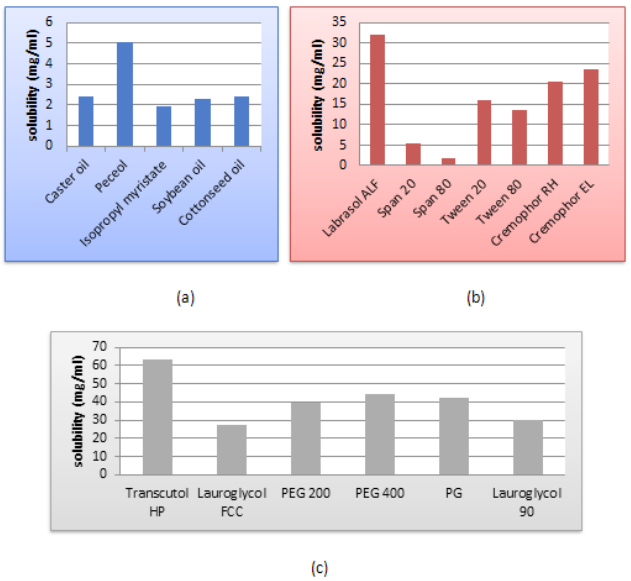

Figure 1: Solubility studies of tacrolimus (a) in various oils; (b) in various surfactants; (c) in various cosurfactants.

Preparation of Tacrolimus loaded SMEDDS

Different liquid SMEDDS batches were prepared with the composition (Table 1), which were then characterized and evaluated for various factors.

Evaluation and Characterization of Tacrolimus Loaded SMEDDS

Thermodynamic Stability Studies

All SMEDDS formulations passed the thermodynamic stability tests with no detectable signs of phase or drug precipitation, cloudiness, separation or flocculation. These results suggest their ability to withstand storage in extreme conditions and ensure the reconstituted microemulsion's stability.

Robustness to Dilution and Effect of pH

All the resulting emulsions remained transparent, clear and exhibited no phase separation or drug precipitation after 24 hours. of dilution. These results may be attributed to the high solubilizing properties of the excipients. The composition and pH of the aqueous phase did not affect the emulsions' properties because nonionic surfactants are less affected by pH and ionic strength changes than ionic surfactants.[32] All of which indicates that these formulations were stable, robust to high dilution and variations in pH.

Dispersibility Test and Self-emulsification Time

All the prepared SMEDDS formulae were self-emulsified in less than 1-minute, forming clear transparent grade A emulsions, except F7, F8, F9, which formed grade B emulsions as shown in Table 1. The time of self-emulsification was

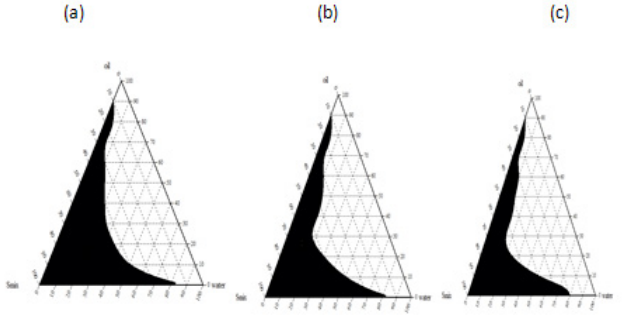

Figure 2: Pseudo-ternary phase diagram of Smix (a) [3:1]; (b) [2:1]; (c) [1:1]

Table 1: Compositions and characterization of tacrolimus liquid SMEDDS

F	*Peceol*	*Labrasol ALF*	*Transcutol HP*	*Emulsification time(sec)*	*Particle size (nm)*	*PDI*	*Drug content %*
1	10	67.5	22.5	24	60.8	0.005	99.48
2	10	60	30	27	61.2	0.005	99.12
3	10	45	45	30	61.5	0.005	98.68
4	20	60	20	36	62.7	0.189	98.92
5	20	53.3	26.7	35	65.5	0.005	98.34
6	20	40	40	44	69	0.005	98.55
7	30	52.5	17.5	54	76.9	0.005	98.86
8	30	46.7	23.3	58	78	0.244	98.65
9	30	35	35	56	81.3	0.005	98.07

inversely related to the proportion of the excipients in the formulation, i.e., as the concentration of surfactant increased, the spontaneity of emulsification increased, and the time of self-emulsification decreased. This may be due to Labrasol ALF's capacity to reduce the interfacial tension between oil and aqueous phases and facilitate dispersion and formation of o\w emulsion.[33]

Droplet Size Analysis and Polydispersibility Index (PDI) Determination

Droplet size measurement is a crucial step in evaluating SMEDDS formulations that determine the absorption and bioavailability of the drug. While globule size distribution (polydispersity index) is a parameter of uniformity; where values between 0-1 indicate good uniformity and homogeneity of particles after dilution, with consequent uniformity and stability of the prepared formulations.[13] In this study, All the prepared SMEDDS formulations had a PDI less than 0.25 as shown in Table 1, and mean particle size in the range of 60.8 to 81.3 nm, indicating their efficiency as SMEDDS.

The optimal ratio to produce a microemulsion with the smallest droplet size was found to be 10% oil: 90% surfactant/cosurfactant. Additionally, formulations with less oil percentage had comparatively smaller droplet size, probably due to the increased surfactant/cosurfactants ratio (Smix), with more surfactants/cosurfactant available for absorption oil/water interface, providing low interfacial tension and a more stable emulsion.[34]

Drug Content Determination

The drug content of all the prepared SMEDDS formulae was more than 98%, as demonstrated in Table 1. These results meet the USP requirement of a range between 90–110% and indicate a uniform dispersion with no tacrolimus precipitation.[19]

In-vitro Drug Release Studies

Conventional dissolution tests of SMEDDS can provide a measure of dispersibility in the dissolution medium but have a limited ability to mimic real-time in vivo dissolution.[35] To evaluate the actual drug release of formulations, the proportion of drug dissolved in the aqueous medium should be separated from that associated with the emulsion.[36] Hence, the dialysis bag membrane method was employed to ensure that only the

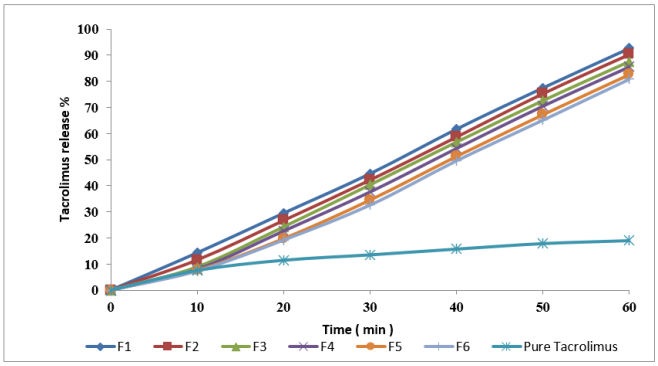

Figure 3: *In vitro* release profiles of Tacrolimus SMEDDS formulae compared with pure tacrolimus.

dissolved drug molecules were permeated out, creating an actual drug release pattern from SMEDDS. The dialysis bags used had a petite pore size, with a molecular weight cutoff of 12000 Da, to ensure a large surface area subjected to the dissolution medium.[37] They were washed with deionized water to remove the preservatives and then soaked in a dissolution medium (0.1 N HCl) overnight to attain equilibration state.[38] No surfactants or other components were added to the dissolution media to ensure accurate discrimination between different formulations' drug release profiles.[39]

The *in-vitro* release profiles of formulae (F1-F6) were evaluated in 0.1 N HCL throughout 1-hour, together with those of pure tacrolimus (Figure 3). The SMEDDS formulae revealed a constantly superior release rate compared to that of the pure drug (19%). The highest release rate (92.66%) as shown by F1 followed by F2 (90.35%). While F6 had the lowest release rate (80.9%). This enhancement in the in-vitro release rate and extent could be attributed to the spontaneous and quick emulsification properties of SMEDDS and the generation of a fine globule size with a high surfactant mixture concentration upon dilution.[40]

Preparation of Tacrolimus Loaded S-SMEDDS

Both F 1 and F 2 were chosen as the best Tacrolimus liquid SMEDDS formulae according to the evaluation as mentioned earlier studies' results, and therefore these formulae were used. They were converted to solid SMEDDS by adsorbing on a different set of solid adsorbent carrier mixtures (F1: Avicel pH 102 plus Aerosil 200, F2: Avicel pH 101 plus Aerosil

Table 2: Results of angle of repose, Hausner's ratio and Carr's index of Tacrolimus S-SMEDDS

Formula	*The angle of repose (degree)*	*Hausner's ratio*	*Carr's index*	*Result*
F-1S (Avicel PH 102)	26.94	1.09	8.33	Excellent
F-2S (Avicel PH 101)	33.55	1.14	12.5	Good

Table 3: Powder flowing properties

No.	*Carr's index*	*Hausner's ratio*	*The angle of repose (degrees)*	*Type of flow*
1	1-10	1.00–1.11	(25–30)	Excellent
2	11-15	1.12–1.18	(31–35)	Good
3	16–20	1.19–1.25	(36–40)	Fair
4	21-25	1.26–1.34	(41–45)	Passable
5	26–31	1.35–1.45	(46–55)	Poor
6	32-37	1.46–1.59	(56–65)	Very poor
7	> 38	> 1.60	Over 66	Very, very poor

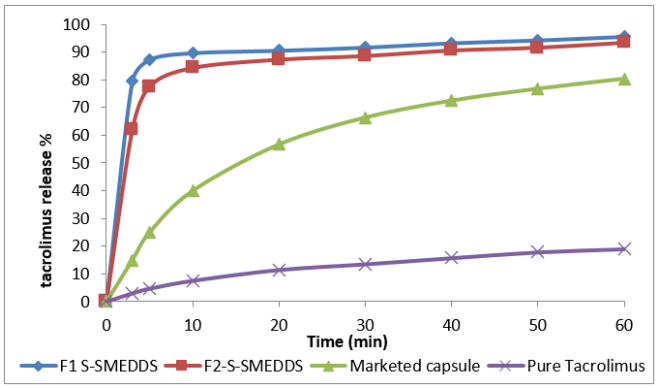

Figure 4: *In vitro* release profiles of Tacrolimus S-SMEDDS formulae (F1 and F2) compared with pure tacrolimus and marketed product.

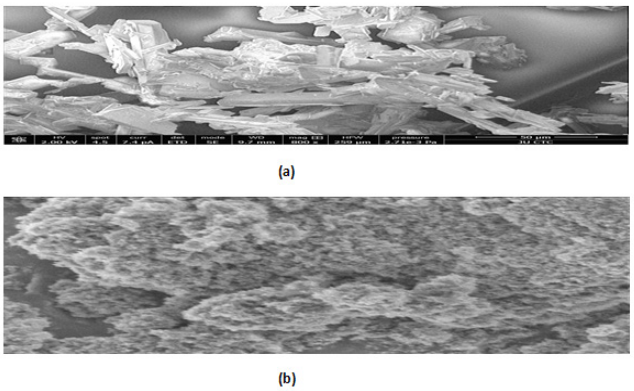

Figure 5: STEM photograph of (a) Tacrolimus; (b) S-SMEDDS F1

200), producing white and fluffy powders with a porous structure.

Characterization of Tacrolimus Loaded S-SMEDDS

Micromeritic Properties of S-SMEDDS

The angle of repose, Hausner's ratio, and Carr's index of Tacrolimus S-SMEDDS formulations are illustrated in Table 2. These tests show improved flowability and compressing properties that may be due to the good sphericity of particles and were found to be within the good to an excellent range of the USP powder flowing properties as illustrated in Table 3.[22]

Drug Content Estimation

Drug content results of the prepared Tacrolimus S- SMEDDS were within the accepted USP requirement range of 90–110% and were almost identical to those found in the liquid form. [19] The drug loading efficiency was 99.11 for formula F1 and 98.68 for formula F2, indicating a successful entrapment of the drug without precipitation or degradation.

In-vitro Drug Release Studies

Dissolution profiles of optimized hard gelatin capsules filled with Tacrolimus S-SMEDDS in 0.1 N HCl medium at 37°C are presented in Figure 4. They show that within one hour of the in-vitro release study, only 19 % and 80.4% of tacrolimus was dissolved from the pure drug and marketed capsules, respectively. Whereas the proportion of tacrolimus dissolved and released from S-SMEDDS within 10 minutes reached 89.7% and 84.3 for formula F1 and F2, respectively.

Statistical Analysis of Dissolution Data

All the prepared liquid and solid SMEDDS formulations had dissimilar release profiles relative to pure Tacrolimus powder (f2 <50). They resulted in the spontaneous formation of a microemulsion with a small droplet size, which allowed a faster release rate than that of pure drug powder.

Evaluation of Selected Optimum Tacrolimus Solid Self Micro Emulsion

The S-SMEDDS-1 was selected as the optimum capsule, consisting of 10% of w/w Peceol oil, 67.5% w/w of Labrasol ALF and 22.5 % w/w of Transcutol HP, 137.35 mg of Avicel pH102, and 137.35 mg of Aerosil 200; based on its excellent flowing properties, optimum drug content, and fast in-vitro cumulative drug release.

Morphological Analysis

The morphological properties of Tacrolimus dry powder and F1 SMEDDS formulation were determined by scanning transmission electron microscopy (STEM), as shown in

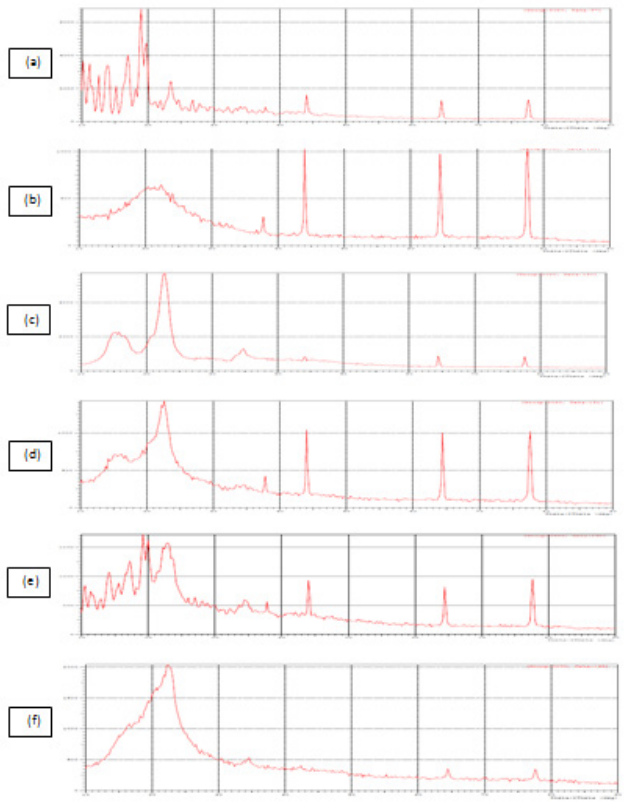

Figure 6: The X-ray diffractograms of (a) pure Tacrolimus powder, (b) Aerosil 200, (c) Avicel 102, (d) solid carriers at 1:1 ratio, (e) physical mixture of Tacrolimus: Avicel: Aerosil 200, and (f) SMEDDS formula (F1)

Figure 5. The Tacrolimus powder [Figure 5a] shows a smooth-surfaced crystalline powder. While in the solid SMEDDS formulation [Figure 5b] the liquid SMEDDS was absorbed or coated inside the pores of solid carriers, resulting in an amorphous structure.

X-ray Diffraction (XRD) Analysis

The X-RD profiles of tacrolimus, solid adsorbents, physical mixture, and solid- SMEDDS formula (F1) are illustrated in Figure 6. The X-ray diffractogram of pure Tacrolimus powder showed characteristic sharp, intense peaks since the drug is in the crystalline form. Similarly, physical mixture diffractogram revealed all the characteristic peaks but with a lower intensity. While the solid-SMEDDS diffractogram had diffuse broader peaks suggesting that the drug was present in a solubilized state in the lipid excipients and transformed from crystalline to amorphous state.

Differential Scanning Calorimetry (DSC)

A sharp endothermic peak is observed in the thermogram of pure tacrolimus at 134.43°C, corresponding to its melting point, and inferring the presence of the crystalline form of the drug as shown in Figure 7a. Conversely, this endothermic peak was broader in the thermogram of the S-SMEDDS formulae (F1), as shown in Figure 7b, indicating the solubilization of the drug in the lipid excipients, and its transformation from the crystalline to the amorphous form.

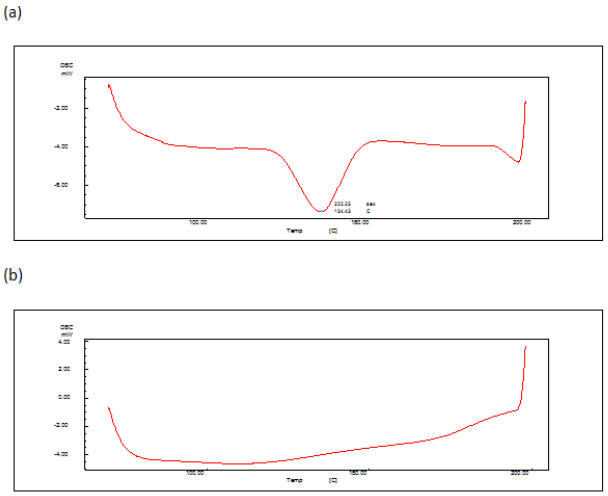

Figure 7: DSC thermograms of (a) pure Tacrolimus; (b) Tacrolimus S-SMEDDS formula (F1)

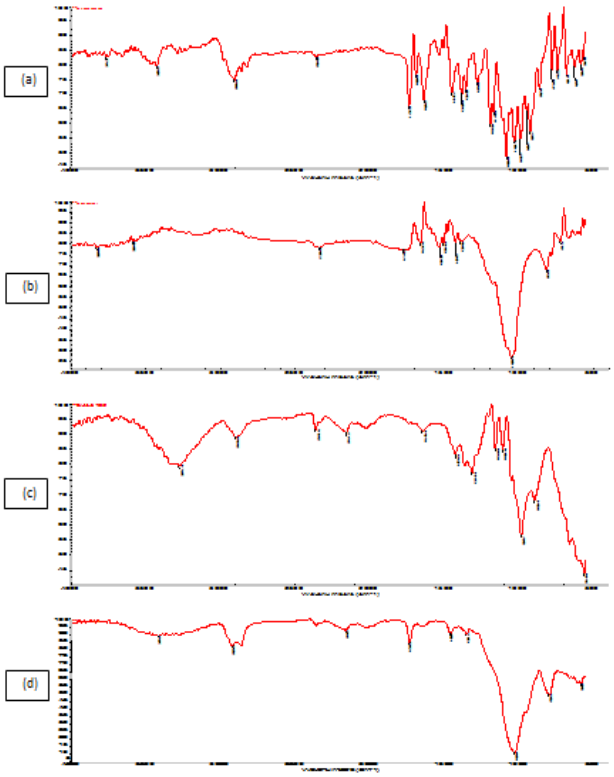

Figure 8: FTIR spectra of (a) pure Tacrolimus; (b) Aerosil 200; (c) Avicel 102; (d) Tacrolimus S-SMEDDS formula (F1).

Fourier Transformed Infrared Spectroscopy (FTIR)

The FTIR spectra of Tacrolimus, Aerosil 200, Avicel 102 and S-SMEDDS formulae (F1) are presented in figure 8. The IR spectrum of Tacrolimus powder revealed a characteristic peak of OH stretching vibration at 3426.2, C-H stretching of CH3 at 2906, the characteristic sharp peak of C = O (ester) stretching vibrations at 1737.9, and C = O (ketone) stretching at 1688.9, C = C stretching vibration at 1636.2, C–O stretching vibration at 1192.9, C-H bending for CH3 at 1383.9 cm−1, O-H bending at 1355.8 cm^{-1}. All the characteristic peaks of tacrolimus were

present, indicating the purity of the drug.[25] and the absence of interfering peaks indicate no undesirable chemical interaction between tacrolimus and the excipients used in this study. And no change in the peaks of pure Tacrolimus spectrum, indicating that all the functional groups of tacrolimus were maintained in the selected S-SMEDDS formula (F1).

CONCLUSIONS

An optimized liquid SMEDDS containing 10% Peceol, 67.5% Labrasol ALF, and 22.5% Transcutol HP was formulated and further developed into solid SMEDDS using Avicel 102 and Aerosil 200 as the solid carriers. The prepared liquid SMEDDS showed good thermodynamic stability and a globule size in the micrometric range. While the solid SMEDDS preserved the self-emulsification performance of the liquid SMEDDS, and its characterization test results (STEM, X-RD, DSC, and FTIR) proved the drug's presence in an amorphous state. The new liquid and solid SMEDDS showed superior in-vitro drug release profiles compared with pure powder and the marketed product (Prograf), confirming the bio-enhancer qualities of the used excipients and potentially increasing the absorption and oral bioavailability of tacrolimus. All these prove S-SMEDDS to be a promising approach to improving problems associated with poorly soluble drugs' oral delivery.

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