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Formulation and Characterization of Self-Microemulsifying Drug Delivery System of Tacrolimus

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Abstract

The present investigation aimed to formulate a liquid self-microemulsifying drug delivery system (SMEDDS) of tacrolimus to enhance its oral bioavailability by improving its dispersibility and dissolution rate. Four liquid SMEDDS were prepared using Maisine® CC as oil phase, Labrasol® ALF as surfactant and Transcutol® HP as co-surfactant based on the solubility studies of tacrolimus in these components. The phase behavior of the components and the area of microemulsion were evaluated using pseudoternary phase diagrams. The formulations were also assessed for thermodynamic stability, robustness to dilution, self-emulsification time, drug content, globule size and polydispersity index. The prepared SMEDDS formulations exhibited improved drug release compared to the pure drug powder. From this study, it was concluded that using a composition of 10% Maisine® CC, 67.5% Labrasol® ALF and 22.5% Transcutol® HP produced a liquid SMEDDS with good thermodynamic stability and enhanced *in-vitro* drug release profiles compared with pure tacrolimus powder. All which supports the use of self-micro emulsifying drug delivery systems as a promising approach to improve dispersibility, dissolution and stability of poorly soluble drugs like tacrolimus.

Keywords: SMEDDS, Microemulsion, Tacrolimus.

تحضير وتوصيف نظام توصيل دواء ذاتي الاستحلاب للتاكروليمس

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الخلاصة

الهدف من الدراسة الحالية هو تحضير نظام إيصال دواء مايكروي سائل ذاتي الاستحلاب لعقار التاكروليمس لتعزيز توافره الحيوي عن طريق تحسين معدل التشتت والذوبان. تم تحضير أربع مركبات سائلة باستخدام ميسين ولابراسول وترانسكتول كزيت وسرفكتانت وكوسيرفكتانت بالتعاقب وتم تعيين المستحلب المايكروي باستخدام مخطط ثلاثي الحالة. كما تم تقييم الثبات الديناميكي الحراري، ومتانة التخفيف، ووقت الاستحلاب الذاتي، ومحتوى الدواء، وحجم الجزيئات، ومؤشر التشتت المتعدد لجميع المركبات. وجد أن معدل تحرير العقار من المركبات المصنعة كان أفضل مقارنة بمسحوق الدواء النقي مما يدل على إمكانية استخدام نظام إيصال الأدوية ذاتي الاستحلاب المايكروي كتقنية واعده لتحسين ذوبان الأدوية قليلة الذوبانية كالتاكروليمس وزيادة استقرارها.

الكلمات المفتاحية: نظام دوائي ذاتي الاستحلاب، مستحلب مايكروي، تاكروليمس.

Introduction

Tacrolimus (FK506) is a selective calcineurin phosphatase inhibitor macrolide. It is the first-line agent used for preventing organ rejection in solid organ transplant patients. [(1)] Oral dosage forms such as capsules, tablets and granules for oral suspension are available of the drug and are preferred by patients due to their convenient administration. [(2)] However, being a biopharmaceutics classification system (BCS) class II drug, tacrolimus is poorly soluble in gastric pH leading to low gastrointestinal (GI) absorption and high inter- and intraindividual variations in pharmacokinetic parameters [(3)]. Its chemical formula is $C_{44}H_{69}NO_{12,}$ and its molecular weight is 822 Da , Log P is 3.3. The average absolute bioavailability of tacrolimus was found to be about 21%, with a wide range of 4% to 89% [(4,5)].

All which necessitated the development of an enhanced oral delivery system to overcome the low solubility of tacrolimus [(4,5)]. Self-microemulsifying drug delivery systems (SMEDDS) are lipid-based formulations containing an isotropic mixture of oil, surfactant and cosurfactant that forms small oil-in-water (o/w) microemulsion upon contact with the aqueous medium of GI secretions under the mild agitation of peristaltic activity[(6)] . The spontaneous transformation of SMEDDS along with the micro size of particles offer faster drug release profiles and a larger surface area for absorption, all leading to increased bioavailability; meaning that a smaller dose might be used to achieve the therapeutic effect with a reduction of GI irritation and other common side effects leading to improved patient compliance and overall therapeutic outcome [(7)] .

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This study aimed to formulate a tacrolimus-loaded liquid SMEDDS with an enhanced dissolution and consequently its oral bioavailability compared to the pure drug powder.

Materials and Methods

Materials

Tacrolimus was purchased from Hyper-Chem LTD CO (China). Labrasol® ALF, Transcutol® HP, lauroglycol FCC lauroglycol 90, peceol and Maisine® CC 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 was obtained from Pure Chemistry (Germany). Cremophor EL, cremophor RH were purchased from Hyper-Chem LTD CO (China). PEG 200 was received 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 J.T. Baker (Netherlands). All other chemicals were of analytical grade and used as supplied.

Methods

Differential scanning calorimetry (DSC) analysis

The process was carried out by weighing two milligram of the pure drug, sealing it in an aluminum pan and then placing in DSC instrument. The sample was heated at temperature up to 300 °C and at a rate of 10 °C/min, using nitrogen as blank gas.

Solubility studies

Tacrolimus was added in excess amounts to glass vials containing 2mL of each vehicle separately including oils, surfactants and co-surfactants, the components were thoroughly mixed for 10 minutes with a vortex mixer then shaken using a water bath shaker for 48 hours at 25±1°C followed by centrifugation at 3000 rpm for 20 minutes to separate the undissolved drug. Aliquots from the supernatants were then filtered by a 0.45 µm millipore filter. [8] The resulting solution was then diluted with 100 mL methanol and analyzed using UV-visible spectrophotometer at λ_{max} 213 nm to measure its drug concentration. The analysis was done according the calculated λ_{max} and calibration curve.[9]

Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams of oil, surfactant/cosurfactant (Smix), and water were constructed using the aqueous titration method. [10] The surfactant to cosurfactant mixtures were prepared in ratios of (1:1, 2:1, 3:1, 4:1) then mixed with oil in various weight ratios from 1:9 to 9:1 in multiple glass vials. [11] The resulting homogeneous mixtures of oil/Smix were titrated with water using magnetic stirrer with 50 rpm and at room temperature. After each addition of water, the mixture was checked for phase clarity. The turbidity of different solutions samples would indicate the formation of a coarse emulsion. Whereas a clear isotropic solution sample would indicate the formation of a microemulsion. The formation of microemulsion regions was checked visually for turbidity–transparency–turbidity. When the system became turbid, titration was stopped and the percentage of oil, S_{mix}, and water in 100 % w/w mixture were calculated and was utilized for the determination of the microemulsion region boundaries corresponding to the chosen value of oil and Smix ratio. The percentages of oil, surfactants to cosurfactants ratios and water in each system were determined and plotted on triangular coordinated using CHEMIX ternary plot software. [12]

Preparation of tacrolimus-loaded SMEDDS

Tacrolimus (0.5mg) was dissolved in Maisine® CC oil with different ratios of Labrasol® ALF and Transcutol® HP in separate screw-capped glasses vials. They were stirred gently by vortex mixer (50 rpm). The resulting homogenous mixtures were then stored at room temperature. The concentration was 2mg/ml.The composition of tacrolimus liquid SMEDDS formulations are illustrated in Table 1.

Table 1. Composition of tacrolimus liquid self-microemulsifying drug delivery systems (%w/w)

Formula	Smix ratio	Oil: Smix ratio	Maisine® CC %	Labrasol® ALF %	Transcutol® HP %
1	1:1	1:9	10	45	45
2	2:1	1:9	10	60	30
3	3:1	1:9	10	67.5	22.5
4	4:1	1:9	10	72	18

Characterization and evaluation of tacrolimus-loadedSMEDDS

Thermodynamic stability studies

Assessing the physical stability of SMEDDS is essential to prevent drug precipitation and excipients phase separation and ensure a good bioavailability and therapeutic drug profile upon administration. [13] Therefore, the prepared formulas were serially assessed by various thermodynamic stability tests with thorough visual observation for any changes in physical appearance [14] .

Centrifugation test

The formulations underwent centrifugation at 3500 rpm for 30 minutes under observation. Stable formulations were then subjected to the freeze-thaw cycle.

Heating-cooling cycle (H/C cycle)

Six heating-cooling cycles were performed in which the formulas were placed for 48 hours in alternating temperatures of 4 and 45 °C. The formulas that exhibited no phase separations, creaming or cracking were subjected to centrifugation test.

Freeze-thaw cycle

The formulas were stored for 48 hours at alternating temperatures of -21 and +25 °C for three consecutive cycles.

Robustness to dilution and effect of pH

The formulations that passed all the thermodynamic studies were diluted with deionized water and 0.1 N HCl for 50, 100, 250 and 1000 times to simulate *in-vivo* conditions. The formulations were stored at room temperature for 24 hours then observed visually for any changes. [15]

Dispersibility test and self-emulsification time

The efficiency of SMEDDS formulations was assessed using the USP dissolution apparatus II. One mL of each formulation was mixed with 500 mL of deionized water at 37 ± 0.5 ºC under stirring speed of 50 rpm.[16] The resulting solutions were visually evaluated using the grading system shown in Table 2. [17]

Table 2. Grading system of in-vitro performance of self-microemulsifying drug delivery system (dispersibility and self-microemulsification time).

Grade	Time required for microemulsion formation	Appearance
A	Rapidly forming emulsion (within 1 min).	Having a clear or bluish appearance
B	Rapidly forming, (within 1 min).	Slightly less clear emulsion, having a bluish-white appearance
C	Formed within 2 min.	Fine milky emulsion
D	Slow to emulsify (longer than 2 min).	Dull, greyish-white emulsion having a slightly oily appearance
E	Slow to emulsify (longer than 2 min).	Formula exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Droplet size analysis and polydispersity index (PDI) determination

Fine microemulsions were formed by diluting each stable SMEDDS formula with deionized water to 100 times under stirring with a magnetic stirrer at 37 °C. Dynamic light scattering method was used to analyze the particle size using particle size analyzer apparatus (Brookhaven, USA) of the resultant microemulsions and the PDI was accordingly calculated.[18]

Drug content determination

Each formula was dissolved in 100 mL methanol in a volumetric flask and thoroughly mixed. After appropriate filtration and dilution, UV–visible spectrophotometer was used to measure drug absorbance [19].

In-vitro drug release studies

Using the USP dissolution apparatus-II, the *in-vitro* release profiles of all prepared formulations along with pure drug were obtained. The dissolution medium consisted of 0.1N HCl at 37±0.5 °C and 75 rpm. [20] Each tacrolimus-loaded SMEDDS formula was placed in a dialysis bag (molecular weight cutoff of 12000 Da), and a regular withdrawal of 5 mL aliquots at 10, 20, 30, 40, 50 and 60 minutes was done. Equal volumes of fresh dissolution media (0.1N HCl) were added to replace the withdrawn samples in order to maintain the volume constant and keep sink condition. The amount of drug dissolved was measured using UV–visible spectrophotometer according to the calibration curve [21].

Statistical analysis

The results of the experiments were presented as a mean of triplicate samples± standard deviation (± SD). The *in-vitro* dissolution studies results were statistically evaluated using the similarity factor (f2) equation. The results of the f_2 test range between 0 and 100. Two dissolution profiles are considered similar when the f_2 value is ≥ 50. This method is more acceptable to compare dissolution profile when more than three or four dissolution time points are available[22].

$$f_2 = 50 \times \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}|R_t - T_t|^2\right]^{-0.5} \times 100\right\}$$

Results and Discussion

Differential scanning calorimetry (DSC)

The DSC was performed to determine the crystalline state of the drug and to provide specific information about the physicochemical status of tacrolimus. In addition, to evaluate the thermotropic properties and thermal behavior of tacrolimus.

The DSC thermogram of pure tacrolimus shows a sharp endothermic at 134.43 °C, corresponding to its melting point, which lies within the melting point readings of that reported in the references, which are from 126 °C to 135 °C [1], and inferring the presence of the crystalline form of the drug as shown in Figure 1.

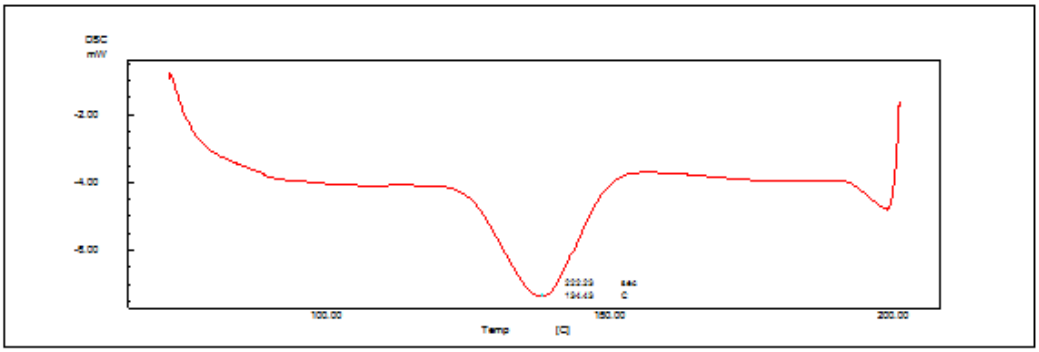

Figure 1. DSC thermograms of pure tacrolimus

Solubility studies

Assessing the extent of the solubility of tacrolimus in the different microemulsion components is an essential step in formulating SMEDDS, as it greatly affects the physicochemical characteristics and drug loading capabilities of the SMEDDS formulations. [24] In this study, the highest solubility of tacrolimus was observed in Maisine® CC as oil phase, Labrasol® ALF as surfactant and transcutol as cosurfactant. These components were consequently chosen for further assessment. The analysis was done according the calculated λ_{max} and calibration curve as illustrated below in Figures 2 and 3. The solubility studies are shown in Figure 4.

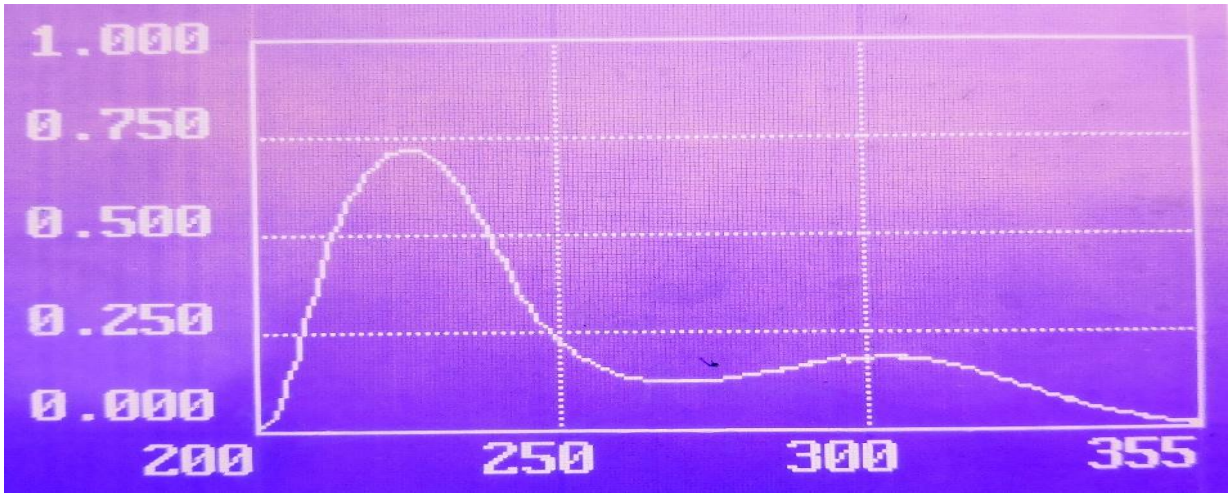

Figure 2. Tacrolimus UV-spectrum in methanol.

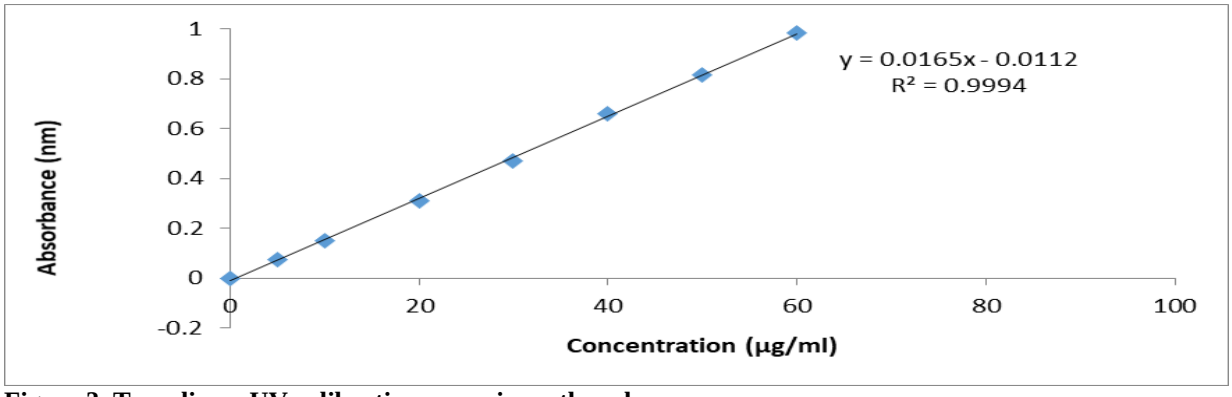

Figure 3. Tacrolimus UV calibration curve in methanol

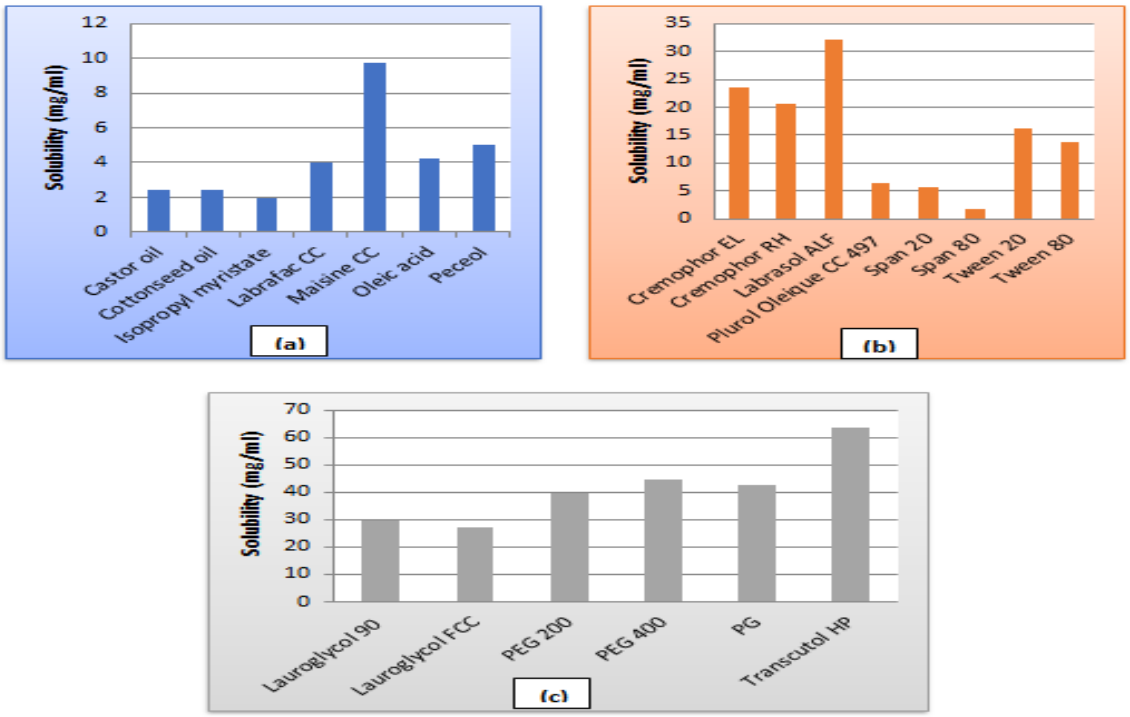

Figure 4. Solubility studies of tacrolimus (a) in various oils; (b) in various surfactants; (c) in various cosurfactants.

Construction of pseudoternary phase diagram

Pseudoternary phase diagrams were utilized to determine the optimal components concentration needed to create stable SMEDDS formulas that could withstand the aqueous dilution effects of the gastrointestinal system without losing solvent and microemulsifying capacity. [25] [26]

The pseudoternanary phase diagrams of the various formulas with different Smix ratios are illustrated in Figure 5. The comparison of these phase diagrams shows that a higher concentration of labrasol corresponded with a larger microemulsions area indicating a higher emulsifying efficacy.

This could be attributed to the fact that the surfactants stabilizes the oil-water interface and its concentration increased at the interface upon decreasing the oily content in the ternary system. Thus, small size of the generated emulsions.[27]

In addition, the reports indicate that a high HLB value of surfactant facilitates the formation of a stable microemulsion. [28]

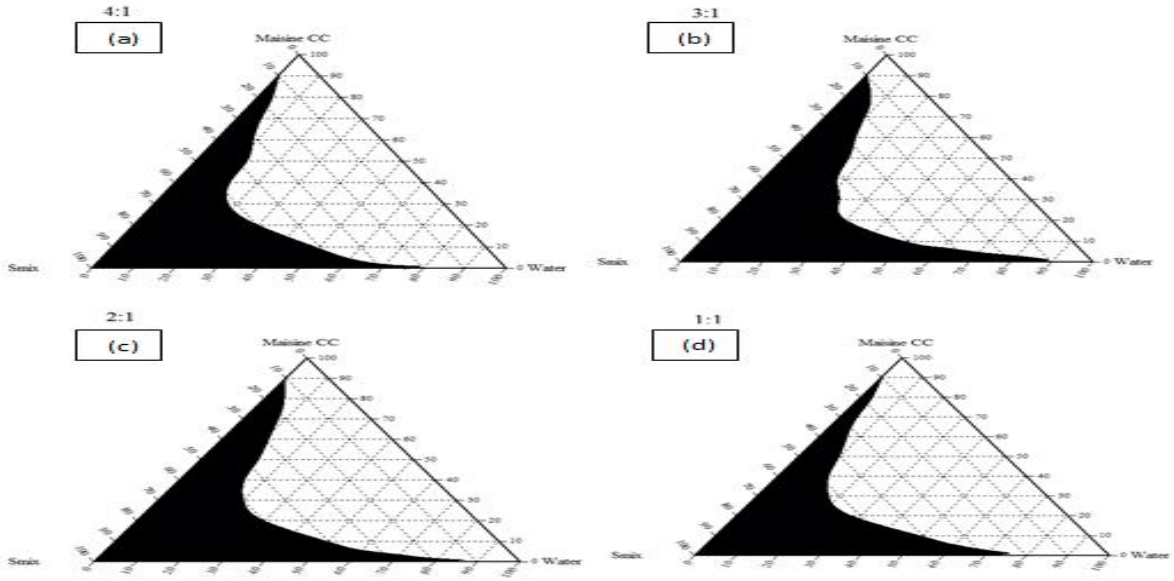

Figure 5. Pseudoternary phase diagram of Smix (a) (4:1); (b) (3:1); (c) (2:1); (d) (1:1)

Preparation of tacrolimus-loaded SMEDDS

Different concentrations of surfactant and cosurfactant were added to Maisine® CC oil in a fixed oil: Smix ratio of 1:9 as shown in table 1.

In all formulations, while simultaneously increasing and decreasing the concentrations of oil, surfactant and co-surfactant, respectively, Smix was held at fixed ratios.

The prepared formulas demonstrated a clear, homogeneous appearance with no change in the phase behavior or drug precipitation upon storage before characterization.

Characterization and evaluation of tacrolimus-loaded SMEDDS

Thermodynamic stability studies

All the prepared SMEDDS formulations passed the centrifugation, heating-cooling cycles and freeze-thawing cycles tests as shown in Table 3, which indicates their thermodynamic stability under extreme conditions.

Table 3. Thermodynamic stability studies of tacrolimus liquid self-micro emulsifying drug delivery systems

Formula	Centrifugation test	Heating-cooling cycles test	Freeze-thawing cycles test
F1	Pass	Pass	Pass
F2	Pass	Pass	Pass
F3	Pass	Pass	Pass
F4	Pass	Pass	Pass

Robustness to dilution and effect of pH

The formulations showed excellent robustness to dilution and pH effect, as no drug or phase separation was observed in any of the prepared emulsions after 24 hours of dilution in 0.1N HCl and deionized water, as illustrated in table 4. These results reveal the high solubilizing properties of the SMEDDS components and their resilience to changes in pH and ionic strength, probably due to their non-ionic nature. [29]

The previous researches concurred with these findings, Vincent Jannin et al. who established a binary phase diagrams database for the development of self-emulsifying lipid-based formulations found that water-soluble surfactant labrasol ALF can be associated with up to 30% of peceol oils and form a miscible mixture and this is an appropriate combination of excipients which able to dissolve the drug and form stable formulations. [30]

Table 4. Robustness to dilution of various tacrolimus liquid self-microemulsifying drug delivery systems

Formula	Phase separation		Drug precipitation	
	0.1N HCl	Deionized water	0.1N HCl	Deionized water
F1	pass	pass	pass	pass
F2	pass	pass	pass	pass
F3	pass	pass	pass	pass
F4	pass	pass	pass	pass

Dispersibility test and self-emulsification time

All the prepared formulas spontaneously produced clear grade A microemulsions in less than one minute as shown in Table 5. Higher Labrasol® ALF concentration was associated with reduced self-emulsification time. This may be attributed to its ability to enhance dispersion and emulsion formation by reducing the interfacial tension between the oil and aqueous phase. [31]

Table 5. Dispersibility and self-micro emulsification time of tacrolimus liquid self-microemulsifying drug delivery systems

Formula	Grade	Emulsification time (sec)	Formula	Grade	Emulsification time (sec)
F1	A	23 ± 1.12	**F3**	A	16 ± 1.72
F2	A	20 ± 1.96	**F4**	A	15 ± 2.14

Droplet size analysis and polydispersity index (PDI) determination

Assessing droplet size and PDI values is crucial in evaluating SMEDDS formulas as they directly affect the absorption of the drug and its uniformity after dilution [14]. In this study, all prepared formulations had acceptable droplet size measurements and PDI values closer to zero indicating good homogeneity and uniformity, as shown in Table 6 and presented in Figure 6.

Similar results obtained from Juno Yoo et al. who studied the effect of different properties

(labrasol to transcutol concentration is one of these factors) on self-emulsifying drug delivery system and found that when transcutol concentration increased to up to 35%, the droplet size will be increased.[32]

Table 6. Droplet size measurement and polydispersity index (PDI) of tacrolimus liquid self-microemulsifying drug delivery systems

Formula	Particle size ± SD (nm)	Polydispersity index
F1	44.3 ± 1.073	0.005
F2	32.3 ± 1.073	0.005
F3	29.5 ± 1.073	0.005
F4	34.2 ± 1.141	0.018

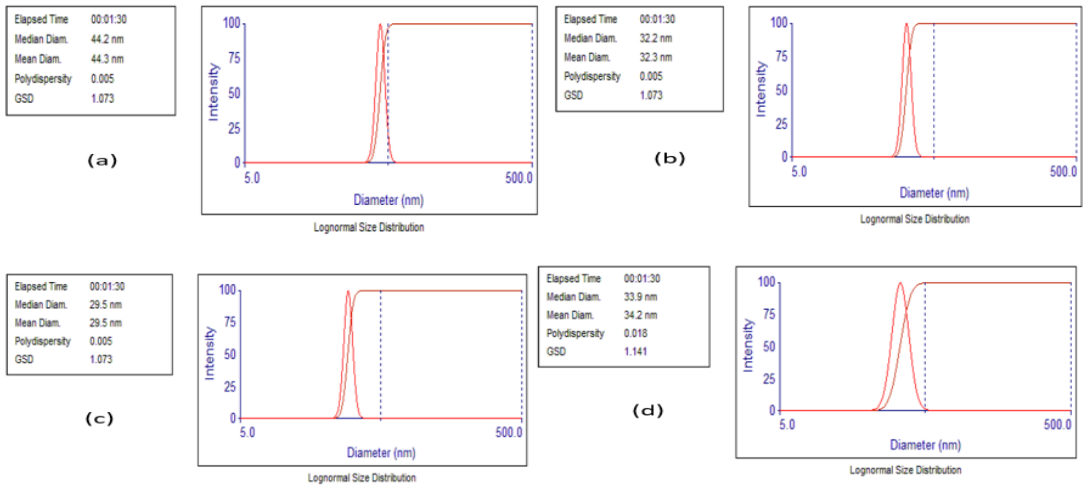

Figure 6. Droplet size and polydispersity index of of tacrolimus liquid self-microemulsifying drug delivery systems (a) F1; (b) F2; (c) F3; (d) F4.

Drug content determination

The drug content in all the prepared formulations exceeded 98% and was within the USP-recommended range of 85%-115%. as shown in Table 7. These results indicate the uniform dispersion of tacrolimus within SMEDDS.[20]

Table 7. The drug content percent of tacrolimus liquid self-microemulsifying drug delivery system (mean ±SD) n=3

Formula	Drug content %	Formula	Drug content %
F1	98.84 ± 0.175	F3	99.76 ± 0.081
F2	99.53 ± 0.122	F4	98.44 ± 0.093

In-vitro drug release studies

While conventional dissolution tests are useful in assessing dispersibility of SMEDDS in the dissolution media, they are inadequate in simulating in-vivo dissolution and evaluating actual drug release profiles as they do not distinguish between the proportions of drug dissolved and those associated with the emulsion. [33]

In order 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 [34].

For this purpose, the dialysis bag method is utilized to permeate the dissolved drug only and enable a more accurate estimation of drug release from the SMEDDS formulations. In this study, a dialysis bag with a very small pore size (molecular weight cutoff of 12000 Da) was used to ensure a large surface area of particles subjected to the dissolution medium. [35] It was soaked overnight in 0.1 N HCl dissolution medium to reach equilibrium. [36]

The calibration curve of tacrolimus in 0.1 N HCL shown in Figure 7.The *in-vitro* release profiles of the prepared formulas along with that of the pure drug were assessed in 0.1 N HCl over one hour as shown in Figure 8. All the prepared liquid SMEDDS formulations had dissimilar release profiles relative to the pure drug (f2 <50). Formulation F3 showed the highest release rate (98.71%) followed by F2 (97.33%) while the release rate of the pure drug (19%) was the lowest among all tested formulations. The noticeable increase in the *in=vitro* drug release profiles could be explained by the rapid self-emulsification properties of SMEDDS and their ability to generate

microemulsions with fine droplet size upon dilution. [37]

Furthermore, it could be observed from the figures that the particle size of the produced emulsion greatly affects the drug release rate, which could be explained by the droplet size-dependent release of tacrolimus from F3 formulations [38], suggesting that formulations with smaller particles possess a higher release rate and vice versa, which explains why formulations F3 has the highest release [39,40].

The *in-vitro* release rate and extent enhancement could be attributed to the SMEDDS fast spontaneous emulsification properties and the production of a small globule size with a high surfactant concentration [37].

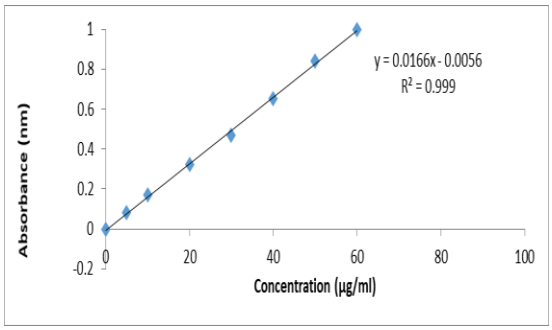

Figure 7. Tacrolimus UV calibration curve in 0.1 N HCL.

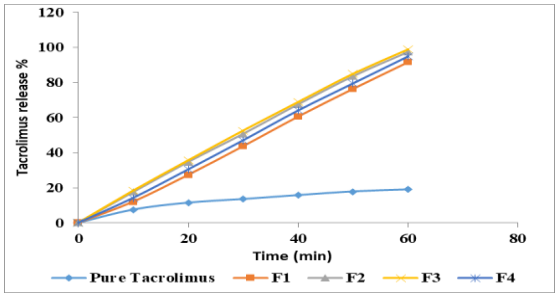

Figure 8. *In-vitro* release profiles of tacrolimus-loaded SMEDDS formulae compared with pure tacrolimus.

Conclusions

From this study, it is concluded that the liquid SMEDDS containing 10% Maisine® CC, 67.5% Labrasol® ALF and 22.5% transcutol showed good thermodynamic stability and a globule size in the nanoometric range. The new liquid SMEDDS showed enhanced *in-vitro* drug release profiles compared with pure tacrolimus powder, which confirms the enhancing characteristics of the SMEDDS components and provide a potential for higher absorption and bioavailability.

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