ORIGINAL ARTICLE

Development and characterization of liquid and solid self-microemulsifying drug delivery system of Tacrolimus

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Tacrolimus is an immunosuppressant agent for the prevention and treatment of graft rejection in solid organ transplantation patients. Tacrolimus is a poorly water-soluble drug. Its absorption is further limited due to the involvement of an efflux transporter P-glycoprotein and metabolism via cytochrome P450. The objective of present investigation was to develop and characterize a liquid self-microemulsifying drug delivery system (SMEDDS) and a solid SMEDDS by using bioenhancer excipients like Tween 20 and Tween 80, which are known for their inhibiting action on CYP 450 and P-glycoprotein efflux pump. Solubility of Tacrolimus was determined in various vehicles, including oils, surfactants and cosolvents. Pseudoternary phase diagrams were constructed to identify the most efficient self-emulsification region. The optimized formulations were characterized by differential scanning calorimetry (DSC), X-ray diffraction (XRD) and globule size analysis. The optimized liquid SMEDDS formulation contained 20% Phosal 53 MCT as oil, 60% Tween 20 as surfactant and 20% ethanol as cosolvent. Liquid SMEDDS was converted to solid SMEDDS by using Aerosil-200 and Florite-RE as inert solid adsorbents. The optimized liquid and solid SMEDDS showed higher drug release than the marketed capsule and pure API powder. For optimized liquid SMEDDS and solid SMEDDS, the globule sizes were found to be 140.9 nm and 304.6 nm, respectively. DSC and XRD results of solid SMEDDS confirmed that the drug present in the formulation was in an amorphous state. Shelf-lives for liquid SMEDDS and solid SMEDDS were found to be 1.76 and 2.27 years, respectively. The prepared liquid SMEDDS and solid SMEDDS containing bioenhancer excipients increase the *in vitro* dissolution rate of lipophilic Tacrolimus.

Key words: *Phosal 53MCT, solid SMEDDS, Tacrolimus*

INTRODUCTION

Organ transplantation procedures in patients have increased due to the availability of effective immunosuppressant drugs. Tacrolimus (FK506), an immunosuppressant, is a hydrophobic macrolide obtained from *Streptomyces tsukubaensis*. It is an effective drug and shows better therapeutic results than Cyclosporine in reducing the risk of organ rejections and toxicity.[1] Tacrolimus is a poorly water-soluble drug, with a solubility of 1–2 µg/mL in water.[2,3] It is a substrate for the P-glycoprotein (P-gp) efflux pump and the CYP450 3A4 enzyme system. Bioavailability of Tacrolimus is 20%. Tacrolimus is a BCS class two drug; therefore, dissolution is the rate-limiting step for absorption. Tacrolimus shows a large intra- and interindividual pharmacokinetic variability.[3]

Lipid-based drug delivery systems can increase the dissolution of lipophilic drugs. Self-microemulsifying drug delivery systems (SMEDDS) are used to improve the oral bioavailability of poorly water-soluble drugs by presenting and maintaining the drug in a dissolved state, in small droplets of oil, all over its transit through the gastrointestinal tract. SMEDDS are defined as isotropic mixtures of oil, surfactant, co-solvent and drug that rapidly form o/w microemulsion when exposed to aqueous media under conditions of gentle

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agitation provided by gastrointestinal motion. Recently, SMEDDS formulations were developed for lipophilic drugs like Oridonin, Simvastatin and Curcumin. The results show good absorption profiles when administered as SMEDDS.[4-6]

SMEDDS are thermodynamically stable and produce fine oil globules, which ensure a high dissolution rate. SMEDDS increase bioavailability by increasing the solubility and intestinal wall permeability and reducing the effect of first-pass metabolism and decreasing the food effect.[7-9] Some surfactants such as Phospholipids, Cremophore RH, Tween 80 and Labrasol increase the bioavailability of the absorbed compound by facilitating transcellular and paracellular absorption. These excipients also act as p-glycoprotein and/or CYP450 enzyme inhibitors, decreasing the intestinal efflux and drug biotransformation.[7,9,10]

SMEDDS are almost liquid or semisolid in state because the excipients use to prepare SMEDDS are liquid in state at room temperature. As per the stability concern, solid dosage forms are more stable compared with liquid dosage forms; therefore, in recent years, as an alternative to conventional liquid dosage forms, solid SMEDDS are developed by incorporating SMEDDS into solid inert pharmaceutical excipients. Various solidification techniques are used to prepare solid SMEDDS, such as adsorption to solid carrier, spray drying, melt extrusion, nanoparticle technology, etc. Among these, adsorption to solid carrier is the simplest and most economical technique, which gives a stable, free-flowing solid SMEDDS powder. It can be easily filled in hard gelatin capsules and easily disperses upon GI fluid contact.[11] Generally, the adsorbents used are highly porous in nature and they have the capacity to adsorb high amounts of liquid. Examples of some commonly used adsorbents are Florite RE, Nusilin US2, Aerosil 200, Sylysia 350 and Syloid® 244 FP (porous silicon dioxide). These adsorbents were used by some researchers to successfully develop solid SMEDDS to increase the dissolution of lipophilic drugs like Curcumin and Nitredipin.[6,12]

Several approaches such as oily solution, solid dispersions, complexation with cyclodextrins, liposomes and liquid SMEDDS have been investigated to improve the oral delivery of Tacrolimus. Among these, solid dispersions succeeded in improving its delivery, leading to commercialization (Prograf® Capsules, Fujisawa). However, it requires about 2 h for complete drug release and exhibits 25% bioavailability.[13] Therefore, the objective of the present study was to develop stable liquid SMEDDS and solid SMEDDS of lipophilic immunosuppressant Tacrolimus in order to increase the *in vitro* dissolution by using bioenhancer excipients. Excipients were selected based on solubility, phase diagram and emulsification studies. Prepared batches were characterized by emulsification efficiency, globule size analysis and *in vitro* release profile. Shelf-lives of the optimized formulations were determined. Additionally, solid SMEDDS was characterized by XRD and differential scanning calorimetry (DSC).

MATERIALS AND METHODS

Materials

Tacrolimus was gifted from Alembic Ltd. Pharma, Vadodara, India. The marketed capsule of Tacrolimus (Pangraf) was purchased from a local supplier. Phosal 53 MCT and Phosal 75 SA were kindly gifted from Lipoid, Germany. Cotton seed oil and castor oil were purchased from a local supplier. Florite RE was gifted from Tomita Pharmaceutical Co. Ltd., Tokushima, Japan. Aerosil 200 was purchased from Yarrow Chem., Mumbai, India. Tween 20, Tween 80 and high-performance liquid chromatography (HPLC)-grade Acetonitrile were purchased from RFCL Limited, New Delhi, India.

Methods

Solubility studies

The solubility of Tacrolimus in various vehicles, including oils, surfactants and cosolvents, was determined by the shake flask method.[14] An excess amount of Tacrolimus was added to each vial containing 1 mL of the vehicle. After sealing, the mixture was vortexed using a vortex mixer at a maximum speed for 10 min in order to facilitate proper mixing of Tacrolimus with the vehicle. Mixtures were then shaken in an orbital shaker maintained at room temperature until equilibrium (24 h). Mixtures were centrifuged at 5000 rpm for 5 min, and the resulting supernatant was filtered through a whatman filter paper. The filtrate was quantified by the developed HPLC method.[15] The mobile phase was acetonitrile 100% at a flow rate of 0.9 mL/min. The wavelength used for detection was 210 nm. A calibration curve was constructed from Tacrolimus concentrations ranging between 5 and 250 µg/mL, which yielded a linear correlation ($r^2 = 0.995$).

Emulsification studies

Emulsification studies were conducted to select the best surfactant and cosolvent from a range of cosolvents and surfactants that are used for oral drug delivery. The surfactant and cosolvent were mixed at a fixed ratio of 2:1. The oil to S–Co mixture ratio was 1:3, and the mixture was homogenized with the aid of gentle heat (30–40°C) and vortexed for 2 min in a vortex mixer. 0.2 mL of the mixture was diluted with 200 mL of distilled water with gentle stirring on a magnetic stirrer. The ease of formation of emulsions was noted by noting the time required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their % transmittance was measured at 638.2 nm by a UV-1800 spectrophotometer (Shimadzu, Japan) using distilled water as a blank.[14]

Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams were constructed using the water titration method. The surfactant and cosolvent were mixed in different volume ratios (1:1, 2:1 and 3:1). Oil and S-mixture (S/Co-S) were mixed thoroughly in different volume ratios (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9) in different test tubes. The mixture in each tube was mixed homogeneously

using a vortex mixer until the oily liquid mixture was obtained at room temperature. Water was then added drop-by-drop at 0.05-mL increments using a pipette into each oily mixture. During the titration, samples were stirred vigorously for a sufficient length of time for homogenization and visually monitored against a dark background by illuminating the samples with white light. The concentrations of water at which the solutions became clear were noted down. A pseudoternary phase diagram was prepared using Triplot free version.[4,6]

Preparation of liquid SMEDDS

The formulations were prepared by dissolving the required amount of Tacrolimus in the mixture of oil, surfactant and cosolvent, at 40°C in a water bath. This mixture was mixed by vortexing until a transparent preparation was obtained. Then, a formulation equivalent to 5 mg Tacrolimus was manually filled in a hard gelatin capsule of size "1" [Table 1].

Thermodynamic stability studies

The objective of the thermodynamic stability was to evaluate the effect of temperature variation on the SMEDDS formulations. Tacrolimus SMEDDS were centrifuged at 15,000 rpm for 15 min and the formulations were observed visually for phase separation. The formulations were subjected to freeze–thaw cycles (–5°C for 2 days followed by +40°C for 2 days). The samples were observed visually after the freeze–thaw cycles. Thermodynamically stable formulations were selected for further characterization.

Robustness to dilution

The prepared SMEDDS were diluted with water and 0.1 N HCl up to 250-, 500- and 1000-times. %Transmittance was determined in a UV spectrophotometer at 638.2 nm. The diluted microemulsion was observed at 1 h and 6 h for any sign of phase separation or drug precipitation.[16]

Preparation of solid SMEDDS

Thermodynamically stable liquid SMEDD was converted to solid SMEDDS to get the advantage of the solid dosage form [Table 1]. The prepared liquid formulation of Tacrolimus was added drop-wise on a solid carrier in glass mortar. The mixture was agitated until a uniform, free-flowing powder was obtained. This powder was screened through a 40# sieve. Then, the powder was filled in a "00" size hard gelatin capsule shell.[11]

Drug content

The total amount of the drug in the formulation was analyzed by dissolving the formulation in 10 mL ACN. This solution was vortexed for 10 min in a vortex mixture. The mixture was centrifuged at 5000 rpm for 5 min. Then, the supernatant was filtered through a whatman filter paper. The filtrate was analyzed by HPLC after suitable dilution [Table 1].

In vitro dissolution

Tacrolimus SMEDDS was filled in a capsule shell and the *in vitro* release profile was taken in a USP apparatus 2 at 37 ± 0.5°C, at 100 rpm, in 300 mL of 0.1 N HCl. At predetermined intervals, 3 mL of the medium was sampled and filtered through a whatman filter paper. The resulting solution (1 mL) was mixed with 1 mL of acetonitrile, vortexed for 2 min and centrifuged at 10,000 rpm for 10 min.[17] Then, 20 µL of the supernatant layer was analyzed by HPLC.

Globule size analysis

The globule size, size distribution and zeta potential were analyzed by dynamic light scattering with a globule size apparatus (Malvern Zetasizer version 6.11, United Kingdom). Liquid and solid SMEDDS were diluted 250-times with 0.1 N HCL at 25°C under gentle shaking. After equilibrium, the emulsions were filtered through a whatman filter paper. The filtrates were analyzed by Zeta sizer. A laser beam at 632 nm wavelength was used and light scattering was monitored at 25°C at a 90° angle.[16]

Solid state characterization of solid SMEDDS

DSC analysis

The physical state of Tacrolimus in solid SMEDDS was characterized by the DSC (DSC-60, Shimadzu Corporation, Tokyo, Japan). The samples (about 3.00 mg) were placed in standard aluminum pans and air was used as an atmosphere. All samples were scanned at a temperature ramp speed of 10°C/min, and the heat flow was set from 50 to 200°C.

XRD analysis

X-ray powder scattering measurements were carried out with a D2 Phaser diffractometer at room temperature using monochromatic CuKa-radiation at 34 mA and at 38 kV over a range of 2θ angles from 5° to 60°, with an angular increment of 0.5°/s.[18]

Table 1: Composition of liquid SMEDDS and solid SMEDDS

Ingredient	H1 (mg)	H2 (mg)	H3 (mg)	H4 (mg)	H5 (mg)	H6 (mg)
Tacrolimus	5	5	5	5	5	5
Phosal 53 MCT	90	90	90	90	90	-
Tween 80	327	-	327	-	327	-
Tween 20	-	330	-	330	-	330
Ethanol	78	78	78	78	78	78
Florite RE	-	-	90	90	-	-
Aerosil 200	-	-	-	-	90	90
Drug content	98.98±1.2	101.23±1.6	97.97±1.5	96.99±1.8	97.86±1.9	98.17±2.0

Data are expressed as mean±SD (***n*** = 3)

Determination of the shelf-life of the optimized formulation

Accelerated stability studies were also performed for determination of the shelf-life of the optimized formulations. The SMEDDS formulations were kept at three different temperatures and ambient humidity conditions (30 ± 0.5, 40 ± 0.5 and 50 ± 0.5°C) for 2 months. The samples were withdrawn after specified time intervals (0, 15, 30, 45 and 60 days) and the remaining drug content was measured using the HPLC method. Zero-time samples were used as controls. The order of the reaction was determined. After determination of the order of the reaction, the reaction rate constant (K) for the degradation was measured from the slope of the lines at each elevated temperature using the following equation:

$$\text{Slope} = -K/2.303 \quad (1)$$

The plot of the logarithm of K values at various elevated temperatures against the reciprocal of the absolute temperature was drawn (Arrhenius plot). From the plot, the K value at 25°C was determined and was used to calculate shelf-life by substituting in the following equation:

$$t_{0.9} = 0.1052/K_{25} \quad (2)$$

Where $t_{0.9}$ is the time required for 10% degradation of the drug, and is referred to as the shelf-life.[19]

RESULTS AND DISCUSSION

Solubility studies

The equilibrium solubility of Tacrolimus in various oils, surfactants and cosolvents is shown in Figure 1. From the SMEDDS point of view, solubility in various excipients is an important criteria. In SMEDDS, the drug was soluble in the oil phase and/or was present at the interface. It depended on the hydrophobicity of the drug and the HLB value of the system. Phosal 53 MCT and Phosal 75 SA showed higher solubility compare with groundnut oil and cottonseed oil. Higher solubility in Phosal 53 MCT was addressed by the surfactant-type nature of Phosal 53 MCT. Eventually, phosphatidylcholine (Phosal 53 MCT, 53% phosphatidylcholine in lipid base of medium-chain tri-glycerides [caprylic/capric]), which may be easily digested by physiological lipases in the gastrointestinal tract without losing its solubilization capacity, has been proposed as a potential oil phase in a lipid-based drug delivery system for enhanced dissolution and bioavailability of lutein.[20,21]

Tween 80 shows higher solubility compared with Tween 20. The drug was freely soluble in the cosolvent ethanol. Surfactant and cosolvent were selected based on solubility as well as emulsification studies.

Emulsification studies

Emulsification studies were performed to evaluate the ability of the surfactants and cosolvents to emulsify the oil phase [Table 2]. Phosal 53 MCT was easily emulsified compared with Phosal 75 SA by the Tween 20 and Tween 80 surfactants. This difference in results could be because of the difference in viscosity of the two oils. To increase the miscibility between the surfactant and the oil phases, three different cosolvents were screened. It was found that ethanol was more efficient for producing spontaneous emulsion with higher transparency.

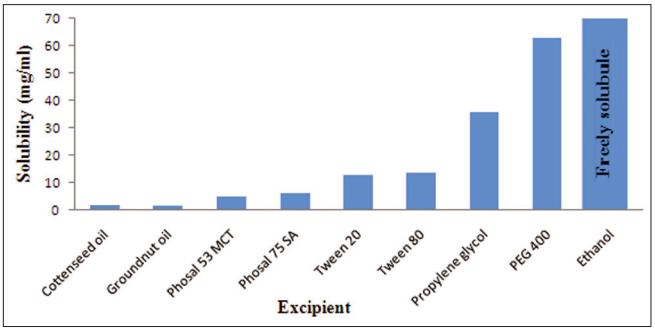

Figure 1: Solubility of Tacrolimus in the different vehicles. Data are expressed as mean of three experiments

Table 2: Emulsification studies for screening of excipients

Oils	Surfactants		DT (s)	% transmittance
	Tween 80	Tween 20		
Phosal 53 MCT	PEG 400	-	35–40	90.45
Phosal 53 MCT	PG	-	30–35	94.14
Phosal 53 MCT	Ethanol	-	20–25	95.48
Phosal 53 MCT	-	PEG 400	35–40	89.3
Phosal 53 MCT	-	PG	33–38	93.08
Phosal 53 MCT	-	Ethanol	25–30	95.67
Phosal 75 SA	PEG 400	-	70–80	84.4
Phosal 75 SA	PG	-	50–60	89.2
Phosal 75 SA	Ethanol	-	50–55	89.9
Phosal 75 SA	-	PEG 400	75–85	84.4
Phosal 75 SA	-	PG	50–60	83.9
Phosal 75 SA	-	Ethanol	50–60	85.9

D.T = dispersion time, co-solvent: PEG 400, PG = propylene glycol, ethanol

Pseudoternary phase diagram

Pseudoternary phase diagrams were constructed to identify the microemulsion regions and to optimize the concentration of the selected vehicles (Phosal 53 MCT, Tween 20, Tween 80 and ethanol) [Figure 2]. For development of a SMEDDS formulation, optimum ratios of excipient concentrations established by means of phase diagram studies provided the area of the monophasic region. It is important to determine this area in order to ensure successful aqueous dilution without "breaking" the microemulsions.[6] Pseudoternary phase diagrams were constructed at three different ratios of surfactant and cosolvent (1:1, 2:1, 3:1). The pseudoternary phase diagram of Tween 20 and Tween 80 exhibits equal microemulsifying regions. Although a slightly higher area of self-emulsification was found for a higher amount of cosolvent (at the ratio 1:1 S:Co-S), 3:1 ratio of S:Co-S was selected for further study to minimize the chances of precipitation, as higher amounts of hydrophilic substances like ethanol in formulation may result in precipitation of drug on dilution with aqueous media.

Thermodynamic stability studies

Thermodynamic stability studies were performed to observe the ability of the formulation to withstand different stress conditions. A stable SMEDDS formulation should not lose its ability of spontaneous emulsification upon dilution. Both liquid formulations were found to be stable in the centrifugation test and in the freeze–thaw cycle. There was no sign of phase separation.

Robustness to dilution

Thermodynamically stable SMEDDS was diluted with different dilution media to observe the effect of pH on microemulsion. Distilled water and 0.1 N HCl were used as different dilution media. The prepared formulations were diluted at different dilution ratios to observe robustness to dilution. The formulations were diluted 250-, 500- and 1000-times. Results of this study confirm that optimized formulations were robust to dilution with different media [Table 3].

In vitro dissolution

The marketed formulation [Figure 3] showed that 71% drug was released within 120 min, whereas the pure API powder showed that only 20% of the drug was released within 120 min. *In vitro* release profile of liquid SMEDDS H1 and H2 showed that 97–98% drug was released within 5 min. This might be due to the quick emulsification properties of SMEDDS and generation of a very small globule size upon dilution with the dissolution media, whereas solid SMEDDS containing Florite RE as a carrier showed 60% drug release within 15 min and formulation containing Aerosil 200 gave only 30–45% drug release within 15 min. Therefore, Florite RE as a carrier was more efficient than Aerosil 200.

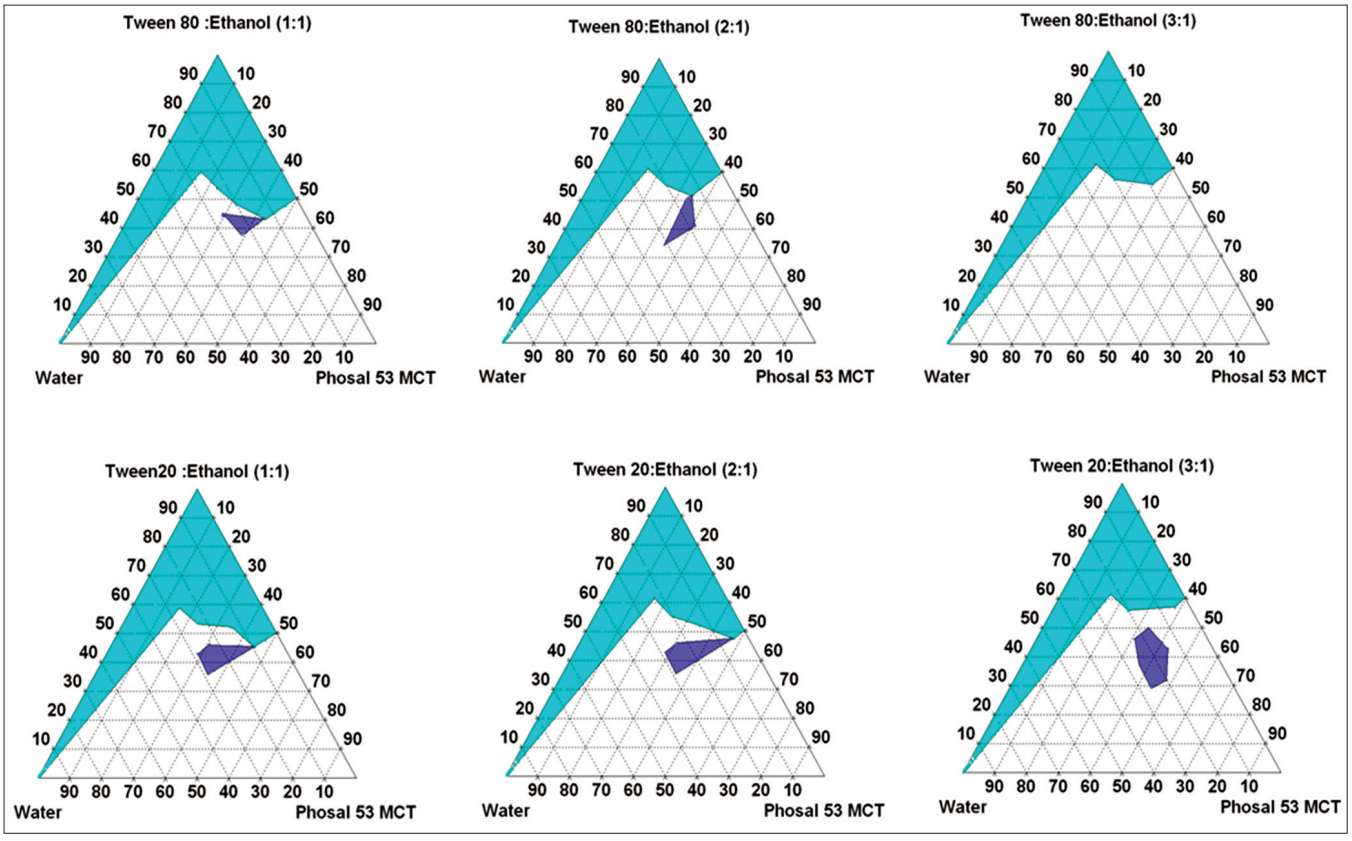

Figure 2: Psuedoternary phase diagrams. Phosal 53 MCT was used as the oil. Aqua color area represents the self-microemulsifying region and blue area represents the gel-like phase

H4 formulation showed a comparatively good dissolution profile than the other solid SMEDDS formulations.

Globule size analysis

For the H2 and H5 formulations, the globule size was found to be 140.9 nm and 304.8 nm, respectively. For liquid SMEDDS (H2), the polydispersibility index was 0.361 and Zeta potential was -3. For solid SMEDDS (H5), the polydispersibility index was 0.523 and Zeta potential was +0.401. Globule size in liquid SMEDDS was small compared with solid SMEDDS, and the formulations had a good polydispersibility index. The Zeta potential in liquid SMEDD was negative due to the negatively charged lipid, whereas in solid SMEDDS it was positive.

Solid state characterization

DSC thermograms are shown in Figure 4. A sharp endothermic peak was observed at a temperature of 129.6°C, which corresponds with the melting temperature of Tacrolimus [Figure 4a]. There was no endothermic peak in the Florite RE thermogram. The physical mixture shows an endothermic peak of Tacrolimus, but this endothermic peak was not found in thermogram of the solid SMEDDS, which confirms the presence of drug in an amorphous form.

The XRD pattern for Tacrolimus powder is shown in Figure 5a. This shows the characteristic sharp peaks at a particular diffraction angle. But, this crystalline pattern was not found in the XRD plot of solid SMEDDS [Figure 5d]. This further confirmed that the drug was present in an amorphous form.

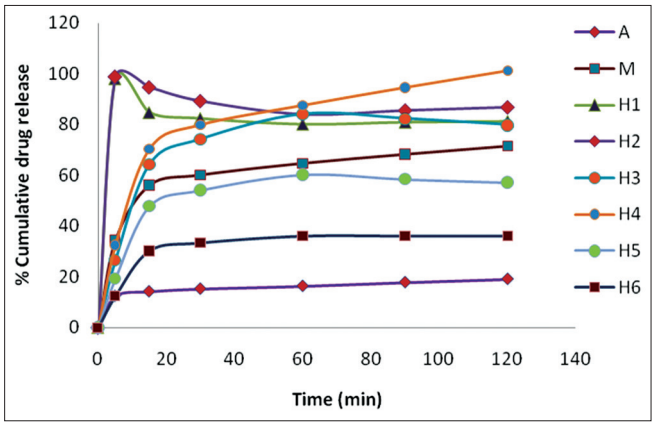

Figure 3: *In vitro* dissolution profile in 0.1 N HCl (A = pure API powder, M = marketed product)

Shelf-life estimation of the optimized formulation

Accelerated stability studies were performed for the determination of the shelf-lives of the SMEDDS formulations H2 and H4. The amount of drug remaining undecomposed in the formulation H2 and H4 at each time interval is shown in Table 4. It can be seen from the table that the concentration of drug remaining undecomposed in the formulation H2 at the end of 60 days was 97.2%, 88.03% and 40% at 30 ± 0.5, 40 ± 0.5 and 50 ± 0.5°C, respectively. The concentration of drug remaining undecomposed in the formulation H4 at the end of 60 days was 97.6%, 88.5% and 32.1% at 30 ± 0.5, 40 ± 0.5 and 50 ± 0.5°C, respectively.

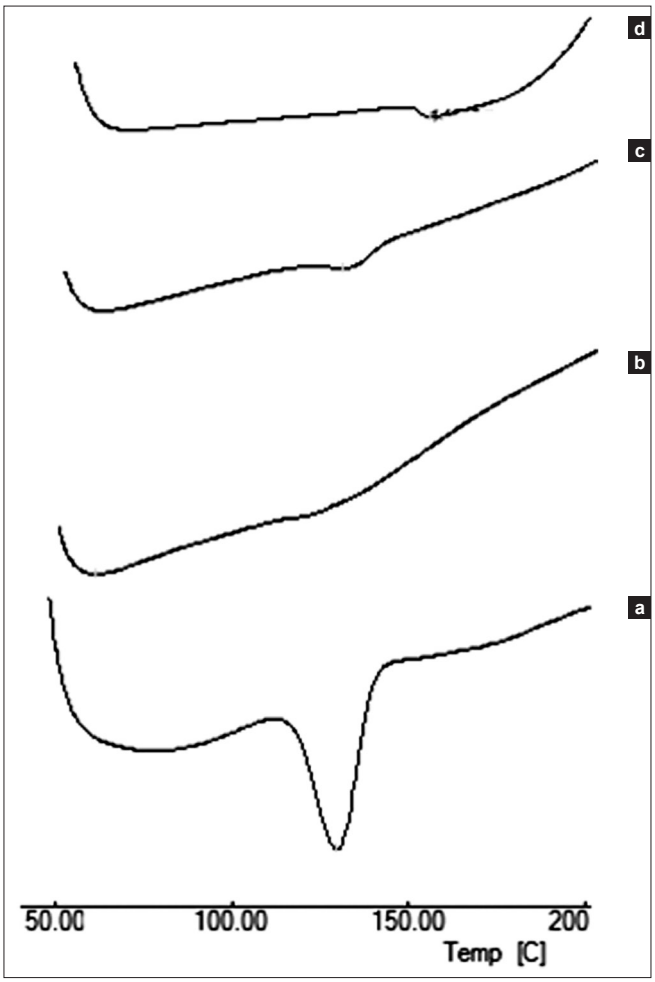

Figure 4: Differential scanning calorimetric thermogram of (a) Tacrolimus powder, (b) Florite RE, (c) physical mixture of Tacrolimus and Florite RE and (d) solid self-microemulsifying drug delivery system of Tacrolimus

Table 3: Precipitation studies

Formulation	Dilution media	% transmittance			Observation (for 250-times dilution)	
		250	500	1000	After 1 h	After 6 h
H1	0.1 N HCl	98.77	99.07	98	-	-
H1	Water	97.1	98.9	98.9	-	-
H2	0.1 N HCl	98.7	98.61	97.56	-	-
H2	Water	98.3	96.6	99.6	-	-

- indicates absence of precipitation

The order of degradation of Tacrolimus in the SMEDDS formulations was found to follow first-order kinetics. The reaction rate constant "K" for the degradation was measured from the slope of the lines at each elevated temperature [Figures 6 and 8]. The plot of the logarithm of the K values for SMEDDS H2 and H4 [Table 5] at each elevated temperature against the reciprocal of the absolute temperature was drawn (Arrhenius plot), as shown in Figures 7 and 9. From the plot, the K value at 25°C was determined and was used to calculate the shelf-life. The shelf-life of SMEDDS H2 at room temperature was calculated to be 1.76 years and for the H4 formulation, the shelf-life was 2.27 years.

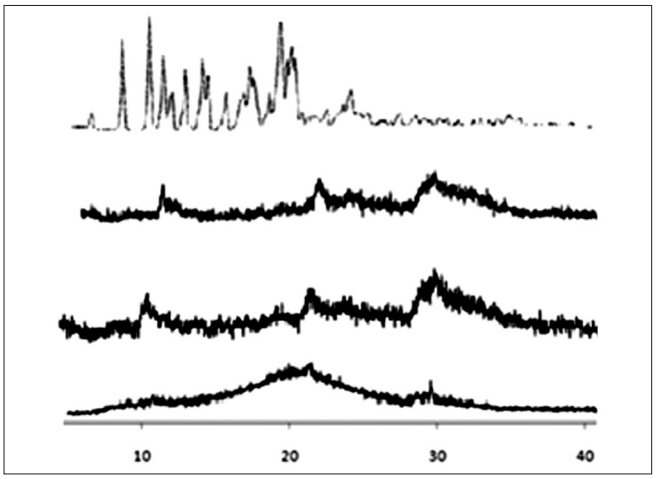

Figure 5: XRD of (a) Tacrolimus powder, (b) Florite RE, (c) physical mixture of Tacrolimus and Florite RE and (d) solid self-microemulsifying drug delivery system of Tacrolimusc

CONCLUSION

Liquid SMEDDS and solid SMEDDS were prepared for immunosuppressant Tacrolimus. Optimized liquid SMEDDS contains 20% Phosal 53 MCT, 60% Tween 20 and 20% ethanol, which showed spontaneous emulsification properties and good thermodynamic stability. Liquid SMEDDS and solid

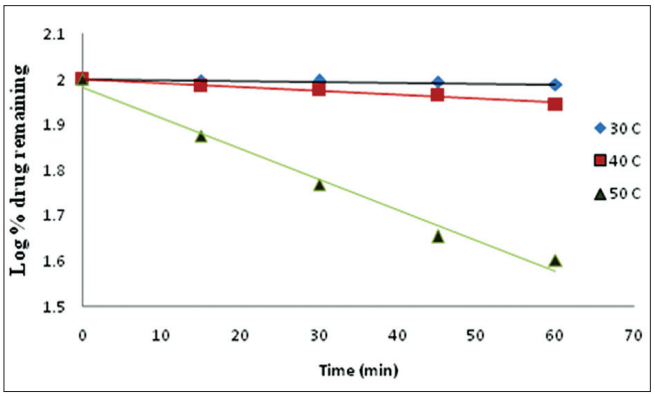

Figure 6: Log percent concentration of drug remaining versus time plot for H2

Table 4: Percent drug remaining in H2 and H4 stored at elevated temperatures (30 ± 0.5, 40 ± 0.5 and 50 ± 0.5°C)

Time (days)	Temperature °C	For liquid (H2)		For solid (H4)	
		% drug remained	Log % drug remaining	% drug remained	Log % drug remaining
0	30±0.5	100	2	100	2
15	30±0.5	99.4	1.997386	99.3	1.996949
30	30±0.5	99.01	1.995679	98.8	1.994757
45	30±0.5	98.51	1.99348	98.2	1.992111
60	30±0.5	97.2	1.987666	97.6	1.98945
0	40±0.5	100	2	100	2
15	40±0.5	96.5	1.984527	97.32	1.988202
30	40±0.5	95.12	1.978272	94.4	1.974972
45	40±0.5	92.04	1.963977	92.34	1.96539
60	40±0.5	88.03	1.944631	88.5	1.946943
0	50±0.5	100	2	100	2
15	50±0.5	75	1.875061	70.21	1.846399
30	50±0.5	58.57	1.767675	50.03	1.699231
45	50±0.5	45	1.653213	39.81	1.599992
60	50±0.5	40	1.60206	32.1	1.506505

Table 5: Degradation rate constant for Tacrolimus in H2 (liquid SMEDDS) and H4 (solid SMEDDS)

Temp °C	Temp °k	1/T*1000 k-1	For liquid H2			For solid H4		
			Slope	$k*10^{-4}$	Log k	Slope	$k*10^{-4}$	Log k
30	303	3.30033	–0.00020	4.734099	–3.3247	–0.00018	4.04951	–3.39259735
40	313	3.194888	–0.00092	21.25258	–2.6725	–0.00088	20.36494	–2.69111684
50	323	3.095975	–0.00663	152.7426	–1.8160	–0.008	184.24	–1.73461608
25	298	3.355705		1.6513	–3.7814		1.28	–3.89114339

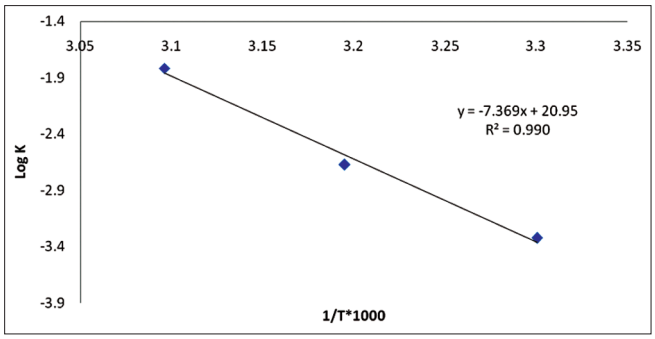

Figure 7: Arrhenius plot for liquid self-microemulsifying drug delivery system H2

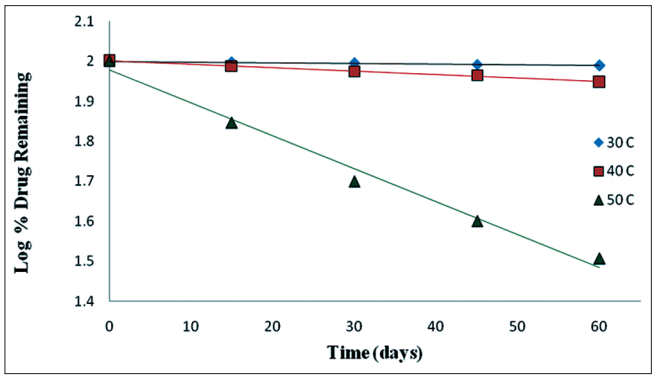

Figure 8: Log percent concentration of drug remaining versus time plot for H4

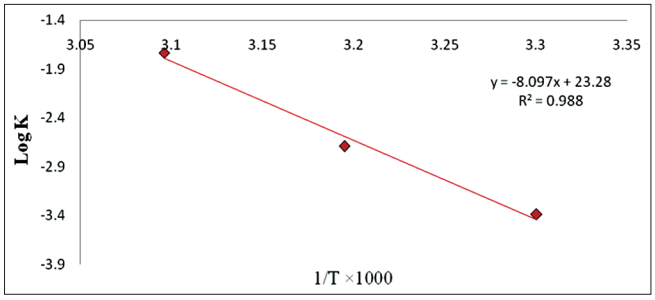

Figure 9: Arrhenius plot for solid self-microemulsifying drug delivery system H4

SMEDDS showed a better *in vitro* drug release profile compared with pure API and the marketed product. XRD and DSC results proved that the drug was present in an amorphous state in solid SMEDDS. The present study confirmed that the new self-microemulsifying systems containing bioenhancer excipients are promising strategies for enhancing the dissolution rate of Tacrolimus.

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