

## PREPARATION, EVALUATION AND COMPARISON OF LIPID BASED DRUG DELIVERY SYSTEMS OF TACROLIMUS

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### ABSTRACT

**Objective:** To prepare and evaluate lipid based drug delivery systems of Tacrolimus.

**Methods:** Lipid based solid dispersion (SD) and self microemulsifying drug delivery systems (SMEDDS) of Tacrolimus are prepared and evaluated. Firstly, solid dispersions, liquid and solid self microemulsifying drug delivery systems of Tacrolimus were prepared. Prepared formulations were evaluated by dissolution test. The optimised formulation of Tacrolimus was subjected for invitro metabolism and intestinal permeation studies for comparison with marketed preparation (Pangraf 5 mg) and pure drug powder.

**Results:** Among four different formulations, the liquid SMEDDS showed significantly higher dissolution rate. This optimised formulation also showed lower rate of drug metabolism and higher intestinal permeation rate compared to marketed preparation and pure drug powder.

**Conclusion:** In this study, we have successfully prepared liquid SMEDDS which showed significantly higher dissolution rate, intestinal permeability and lower rate of drug metabolism.

**Keywords:** Tacrolimus, Solid dispersion, Self emulsifying drug delivery systems.

### INTRODUCTION

The oral bioavailability of Tacrolimus is poor and exhibits high intra and inter-subject variability (4 to 89%, average 25%) in liver and kidney transplant recipients and in patients with renal impairment. Various factors have been reported for low and variable oral bioavailability of Tacrolimus, including low solubility, high but site dependent permeability (highest in jejunum intermediate in ileum and lowest in colon), extensive first pass metabolism by CYP-450 3A4 in gut and liver, P-gp (p glycoprotein) mediated drug efflux, influence of food intake and concomitant medication. It is reported that CYP3A5 genetic polymorphisms may be associated with the pharmacokinetic variation of Tacrolimus in specific group of people [1].

Lipid based solid dispersion can be prepared by using excipient like Gelucire. Gelucire excipients have been used in the formulation of semi-solid dispersions. They are solid waxy materials that are amphiphilic in character and identified by two values: their melting points and their hydrophilic-lipophilic balance (HLB) values. Gelucires are saturated polyglycolized glycerides consisting of mono-, di-, and triglycerides and of mono- and di-fatty acid esters of polyethylene glycol. The nature and proportion of each component are specific to a given grade of Gelucire. Gelucire 44/14 is a semi-solid excipient from this group, while Gelucire 50/13 is a solid pellet. Melting point of Gelucire 44/14 and Gelucire 50/13 is 44 and 50 °C respectively [2-4].

SMEDDS formulation contains oil (synthetic or natural) with hydrophilic or lipophilic surfactants and co-surfactant. This mixture is isotropic in nature and emulsifies when exposed to GI media under mild agitation and forms oil in water emulsion. Based on the type of surfactants used and the presence or absence of oil they are classified in four different types. Among these, type-III are known to form SMEDDS that is self-micro emulsifying oil in water emulsion having globule size less than 200 nm in most cases. Lipid-based formulations specially SMEDDS improve as well as normalize drug absorption, which is particularly beneficial for low therapeutic index drug like Tacrolimus. These formulations enhance absorption by a number of mechanisms like improving drug solubility and maintaining drug in solution state throughout GI tract, inhibition of P-glycoprotein-mediated drug efflux and pre-absorptive metabolism by gut membrane-bound cytochrome enzymes, the promotion of lymphatic transport, which delivers drug directly to the systemic

circulation. Although avoiding hepatic first-pass metabolism, and also by increasing GI membrane permeability [5-7].

### MATERIALS AND METHODS

Tacrolimus was gifted from Alembic Ltd. Pharma Vadodara, India. Gelucires 44/14 and Gelucire 50/13 were kindly gifted from Gattefosse, India. High-performance liquid chromatography (HPLC) grade Acetonitrile was purchased from RFCL limited, New Delhi, India. The Marketed Capsule of Tacrolimus (Pangraf-Panacea) was purchased from a local supplier.

#### Reversed phase high performance liquid chromatography (RP-HPLC)

Quantification of drug was carried out using reversed phase HPLC (Young Lin 9160 photodiode array detector, Seoul, South Korea). The mobile phase used was 100 % acetonitrile. The effluent was monitored with PDA detector at 213.0 nm and total run time is 15 min. Retention time was 5 min at a flow rate of 0.9 mL/ min. 20µL prepared solution (all standard solution were prepared in 100 % acetonitrile) were injected into HPLC containing column LiChrospher 100 RP-18 (5 µm), 4/120 mm. A calibration curve was constructed from Tacrolimus concentrations ranging between 5 and 250 µg/mL, which yielded a linear correlation ( $r^2= 0.9978$ ) [8].

#### Preparation of Tacrolimus/Gelucires solid dispersions

Solid dispersions were prepared as 1:7 (drug/carrier) ratios by a fusion method. Gelucire 44/14 and Gelucire 50/13 were melted at  $50 \pm 5$  °C for 3 min in a porcelain dish on a water bath under stirring, followed by the addition of Tacrolimus powder to the molten carrier and stirring for an additional 5 min until a homogenous dispersion was obtained. Lactose monohydrate was added to the molten mass during cooling (ratio of Gelucire lactose was 1:5 for Gelucire 44/14 and 1:3 for Gelucire 50/13). The prepared solid dispersion were collected, sieved through 40 mesh sieve, and stored at room temperature until subsequent analysis [9]. Detailed method of preparation, evaluation and characterization of Tacrolimus solid dispersions was published in our previous article [9].

#### Preparation of liquid SMEDDS

The formulations were prepared by dissolving the required amount of Tacrolimus in the mixture of oil (Lauroglycol FCC- 0.05mL), surfactant (Cremophor RH- 0.3mL) and co-surfactant (PEG 400-

0.15mL), 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 (0.5 mL mixture of oil, surfactant and co-surfactant) Tacrolimus was manually filled in a hard gelatin capsule of size "1". This liquid formulation was converted in solid SMEDDS by adding 90 mg of Florite RE [10]. Detailed optimisation process of Tacrolimus SMEDDS was published in our previous article [10].

### Comparative dissolution study of Tacrolimus formulations

Prepared solid dispersion and liquid and solid SMEDDS were filled in capsule shell and in vitro release profile was taken in a USP (united state pharmacopoeia) apparatus 2 at  $37 \pm 0.5$  °C, at 75 RPM in 300 mL 0.1N HCl. At pre-determined intervals, 3 mL of the medium was sampled and filtered through the Whatman filter paper. Then, 20 µL of the filtrate was analyzed by HPLC method.

### Animals handling

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, Ahmadabad as per guidelines of Committee for Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Protocol number is IPS/PCOL/PhD 11-12/2003, Item number 23. Healthy wistar albino rats of either sex (250-300 gm weight) were selected for the study. Animals were maintained at  $18 \pm 2$  °C and kept in well ventilated animal house under natural photoperiodic condition in polypropylene cages with free access to food and water. During the period of experiment the animals were fed with the Amrut rat diet supplied by Pranav agro industry ltd. Animals were acclimatized for one week before starting the experiment.

### Metabolism study

#### Preparation of rat liver microsomes

Wister rats weighing 220–270 g were procured from animal facility of the Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India. The care and the use of these animals were in accordance with the guidelines of the CPCSEA (committee for the purpose of control and supervision of experiments on animals). Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) (protocol no. IPS/PCOL/PhD 11-12/2003, Item number 23). The rats were sacrificed by using deep dose of ether. Livers from fed, male wister rats (10 g) are removed, perused with 0.9 % sodium chloride, and homogenized in 0.25 M sucrose; the 10 gm of liver homogenate is then differentially centrifuged as shown in fig. 1. Cooling centrifuge was used during whole procedure and all remaining work was carried out on cooling water bath to maintain temperature 5-10 °C [10].

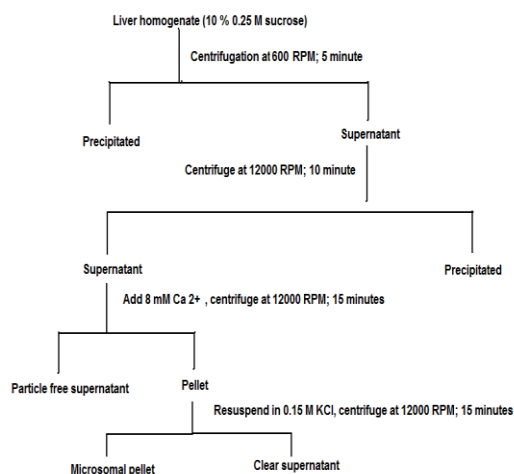


Fig. 1: Microsomal pellet extraction [Reproduced from reference 10].

### Metabolism of Tacrolimus by rat liver microsomes

The metabolism of Tacrolimus was assayed by measuring the reduction of Tacrolimus in incubation system [11]. Briefly, the incubation system, with a total volume of 2mL, contained 100mM potassium phosphate buffer (pH 7.4), rat liver microsomes (final concentration 0.2 mg/mL), and Tacrolimus alone, marketed preparation of Tacrolimus or Tacrolimus containing optimized formulation (final concentration 50 µg/mL). The reaction was started by addition of 0.2mL of 10mM NADPH to the system. The mixture was incubated for 15 min at 37 °C; then, 2mL of ice-cold diethyl ether was added to the reaction mixture to stop the reaction. Mixtures were filtered through whatman filter paper, diluted with acetonitril and centrifuged at 15000 RPM for 10 minutes before it was injected in HPLC system. Tacrolimus was determined by HPLC method. Peak area at drug's retention time (5 minute) was considered to calculate intact drug concentration.

### Intestinal permeability study

The rats were sacrificed by using deep dose of ether and the intestine was excised and flushed through several times with NaCl solution (0.9%, w/v) at room temperature and cut in to small sacs of 10 cm. The intestine was gently everted over a glass rod and filled with incubation solution and ligated on both ends. Each sac was placed in beaker (Krebs henseleit bicarbonate buffer- 50 mL) containing Tacrolimus powder suspension/optimized formulation (micro-emulsion) /marketed preparation (dissolved in incubation system) at concentration 50 µg/mL. The sacs were incubated at 37°C in an orbital shaker (60 cycles/min). After 1 and 2 h, sacs were removed, washed four times in 0.9% NaCl solution and blotted dry. The sacs were cut open and the serosal fluid drained into small tubes. Each sac was weighed before and after serosal fluid collection to calculate accurately the volume of medium inside the sac [12]. The Tacrolimus concentration transported through the intestinal membrane was measured using HPLC technique.

## RESULTS AND DISCUSSION

### Comparative dissolution study of Tacrolimus formulations

To find out the optimized formulation of Tacrolimus, we have compared dissolution profile of solid dispersions, liquid SMEDDS and solid SMEDDS and performed statistical analysis using t-test as well as  $f_2$ -test. As indicated in the fig.2, it was found that Gelucire 50/13 solid dispersion has superior dissolution profile as compared to Gelucire 44/14 solid dispersion. At 30 minutes, % cumulative drug release was 30% and 40 % for Gelucire 44/14 solid dispersion and Gelucire 50/13 solid dispersion respectively. Approximate 45 % and 55 % drug released at the end of 120 minutes from Gelucire 44/14 solid dispersion and Gelucire 50/13 solid dispersion respectively. Hence for comparison between solid dispersion and SMEDDS formulations Gelucire 50/13 solid dispersion was selected. As seen in fig.2 liquid SMEDDS showed significantly higher rate and extent of dissolution when compared to solid SMEDDS.  $f_2$  values were given below. At 30 minutes % cumulative drug release was around 100% and 75 % for liquid SMEDDS and solid SMEDDS respectively. Solid SMEDDS showed approximately 75 % drug release at 120 minutes. Hence liquid P1 SMEDDS was selected for further ex-vivo studies.

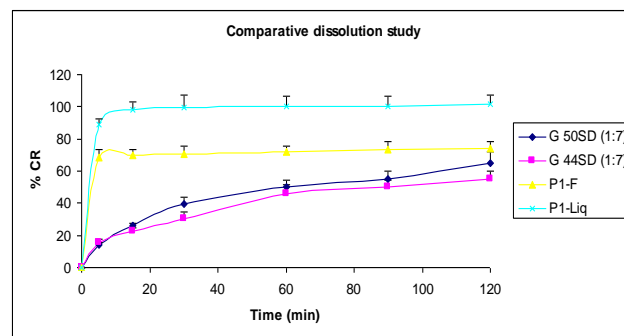


Fig. 2: Comparative dissolution study of Tacrolimus formulations.

(G 50SD= gelucire 50/13 solid dispersion; G 44SD= gelucire 44/14 solid dispersion; P1-Liq= liquid SMEDDS; P1-F= solid SMEDDS; % CR- % cumulative drug released;  $f_2$  value between liquid SMEDDS and solid SMEDDS was 25 and  $f_2$  value between liquid SMEDDS and solid dispersion was 13 )

### Metabolism study

From the dissolution study liquid SMEDDS was selected for further ex vivo evaluations. To compare the ability of formulation excipients to prevent the metabolism of drug, optimized formulation, marketed preparation and plain drug powder were incubated with enzyme solution as described in methods section. At the end of 15 minutes incubation time the metabolism of Tacrolimus was decreased in optimised formulation and in marketed preparation (Pangraf) compared to plain drug powder (fig.3). At the end of incubation period, almost 15%, 75% and 20% drug was detected in plain Tacrolimus powder, optimized formulation and marketed preparation respectively. In optimized formulation excipients might have prevented metabolism of Tacrolimus almost 5 times compared to plain drug and 3 times compared to marketed preparation. These could be explained by presence of bio enhancer excipients like surfactants and co-surfactants. Cremophor RH and PEG 400 are known for their ability to inhibit cytochrome P450 enzyme system which is responsible for metabolism of Tacrolimus. As optimized formulation contains 90 % of Cremophor RH and PEG 400 these might have protected drug from cytochrome P450 enzyme system [13].

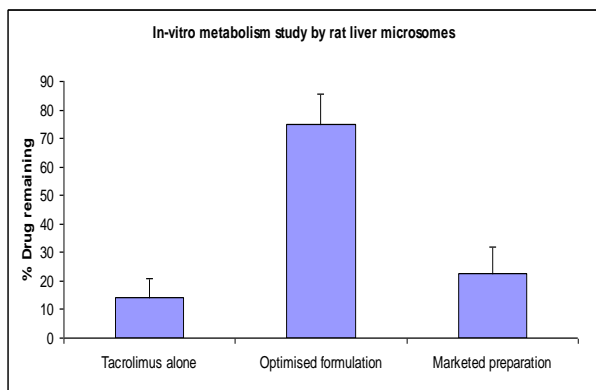


Fig. 3: Comparative metabolism study (n=6)

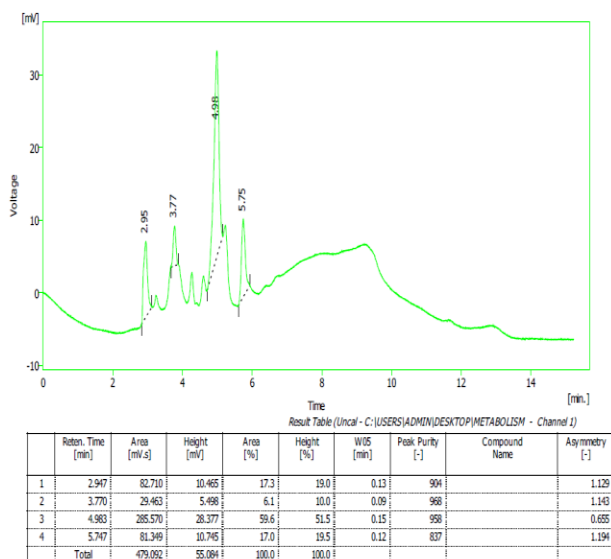


Fig. 4: HPLC spectra of metabolism study

Fig. 4 showed the representative HPLC spectra of metabolism study. The peak at 4.98 minute was considered as intact Tacrolimus peak. Remaining peaks were not considered for calculation of amount of Tacrolimus after incubation period.

### Intestinal permeability study

A Tacrolimus-loaded liquid SMEDDS established up to 40% and 65 % drug permeation in 60 minutes and 120 minutes respectively, whereas marketed preparation and drug powder formulations exhibited a maximum of 40 % and 15 % permeability at 120 minutes respectively (fig.5). Optimized formulation showed around 6 fold and 1.8 fold higher permeability than plain drug powder and marketed preparation. Higher permeation of optimized formulation can be explained by nano size of emulsion formed by liquid SMEDDS, (emulsification results were published in our previous study [10]). Further enhancement may be attributed to the Pgp inhibitory effect of the surfactants, oil and/ or the membrane perturbation effect of the surfactants [14]. Similar observation was reported in 2011 by Ming et al, where authors reported that Gancyclovir permeability was significantly improved by excipients like PEG 400, Tween 80, Cremophor EL35 and Pluronic F68. Authors concluded that enhancements of intestinal absorption of Gancyclovir by these excipients are probably due to inhibition of P-gp-mediated drug efflux [15].

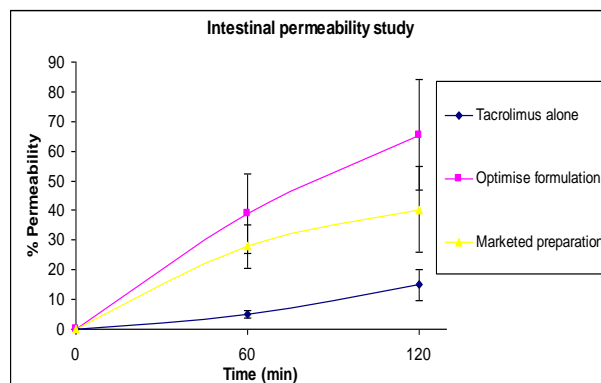


Fig. 5: Comparative intestinal permeability study (n=6)

### CONCLUSION

Compared to solid dispersion of and solid SMEDDS, liquid SMEDDS of Tacrolimus showed significantly higher dissolution rate. We have successfully prepared new liquid SMEDDS of Tacrolimus which showed higher dissolution and higher intestinal permeability than marketed product. Moreover this liquid SMEDDS protected drug from metabolism by CYP450 enzyme systems. Presence of surfactant and co surfactant might have played a important role in enhancing intestinal permeability and reducing drug metabolism. All of these factors can have potential to enhance the oral bioavailability of Tacrolimus, as not only poor solubility of Tacrolimus but also poor permeability and higher metabolism of CYP 450 were responsible factors for low and variable oral bioavailability of Tacrolimus.

**Conflict of Interest:** None declared.

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