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Development and Evaluation of Extended Release Tacrolimus Tablets by Melt Granulation in Combination with Magnesium Alumino Metasilicate

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

To increase the effectiveness of tacrolimus, the present study sought to develop extended-release tablets. It is used to treat mild to severe atopic dermatitis as well as to prevent organ rejection after organ transplantation. Tacrolimus, on the other hand, has a poor bioavailability and a short half-life. The current research aims to create extended-release tacrolimus tablets by melting and granulating utilizing lipid and adsorbent materials to ensure excellent flow and a hypromellose matrix system to regulate release for 12 hr. Tacrolimus granules were prepared by melting with glyceryl behenate in rapid mixer granulator in the presence of polyethylene glycol followed by magnesium alumino metasilicate for adsorption purposes. Adsorbed granules were milled, sifted, and mixed with extra granular material such as hypromellose, lactose monohydrate and magnesium stearate. Granules were compressed to tablets using oval-shaped plain tooling. *In vitro* dissolution profile of solid dispersion and extended release tablets were investigated. Release profile of tablets and solid dispersion were improved by melt granulation due to the polymorphic form conversion of drug substance from crystalline to amorphous.Release kinetics of tacrolimus extended release tablets were fitted with zero order release with R^2 value of 0.964, which designate constant release for 12 hr. Therefore, the present findings from the study revealed that the formulation was successfully prepared and showed extended release pattern over 12 hr.

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Keywords: *Tacrolimus; extended release tablets; melt granulation; magnesium alumino metasilicate; adsorbent material.*

ABBREVIATIONS

BCS : Biopharmaceutical Classification System
MR : Modified Release
IR : Immediate Release
CMC : Chemical, manufacturing and Control
PEG : Polyethylene Glycol
SEM : Scanning Electron Microscopy
DSC : Differential Scanning Calorimetry
XRPD : X ray Powder Diffraction
HPLC :High Performance Liquid Chromatography
USP : United State Pharmacopeia;
RPM : Rotation per Minute
HPC : Hydroxy Propyl Cellulose
SLS : Sodium Lauryl Sulfate
PPM : Parts per Million
mg : Milligram; ml: Milli Liter
mm : Milli Meter
g : Gram
cps : Centopoise

1. INTRODUCTION

Tacrolimus comes under macrolide antibiotic category and is immunosuppressant agent used in organ transplantation [1]. Being BCS (Biopharmaceutical classification system) class II drug and with NTI category (narrow therapeutic index), close monitoring of dose is required to avoid any side effects and patient safety due to inter and intra subject variability [2]. As an area of research, biopharmaceutics examines a wide range of variables, from formulation to absorption to metabolism to excretion, to ensure the availability of a medication or drug product [3]. So, biopharmaceutical assessments are concerned with understanding the particular relationships between physiologic, biological/pathologic, and pharmacological factors and optimizing drug delivery [4]. As a result, biopharmaceutical assessment is more critical for modified oral release (MR) dose forms than for immediate release (IR) [5]. Medication products must be created with quality in mind, and chemical, manufacturing, and control (CMC) sectors should adopt control measures to assure the quality and performance of oral (MR) drug products [6]. Optimization of oral MR products' performance and quality characteristics is essential for safety and effectiveness among transplant patients, Tacrolimus is still a top choice. Since the extended-release formulation provides bioequivalent drug exposure, effectiveness and safety to the standard-release formulation, patients can be converted from twice-daily to once-daily regimen [7].

As an immunosuppressive medication, tacrolimus is widely utilised in kidney transplantation. Tacrolimus is a white to off-white powder with the chemical formula $C_{44}H_{69}NO_{12}$ and 804 g/mol molecular weight [8]. Tacrolimus is used as an anti-inflammatory medication. It belongs to the category of macrolide antibiotic FK506 or Fujimycin family [9]. Anti-immunosuppressive properties are one of its primary functions. Intercellular FKBP-12, which has a similar structure to cyclophilin, is the target of Tacrolimus's binding. Later FKBP-12 complexes limit calcineurin-phosphatase activity, which is necessary for T-cell activation. Primary antibody responses to T cell-dependent antigens are reduced, but not secondary antibody responses, natural killer cell activity, or IL-2-stimulated cell proliferation [10]. The digestive tract has variable and imperfect absorption. Its bioavailability varies from 10 to 60 percent after oral dosing, with peak blood concentrations reaching between 1 and 2 hr after oral ingestion [11]. The cytochrome P450 enzyme system in the liver and the intestinal wall metabolizes it to different metabolites. Some of the active metabolites have been identified. In transplantation of kidneys, lungs, pancreas, and liver, tacrolimus decreases the immunological response. As a result of therapy, nephrotoxicity, neurotoxicity, headache and other adverse effects have been documented [12].

Hence to achieve sustained release and improve oral bioavailability with reduced subject variability, tacrolimus extended release tablets have been produced for many years [13]. Envarsus XR was developed in US as a once-daily dosage form with improved pharmacokinetic performance and bioavailability [14]. Tacrolimus was first approved under brand Prograf as Tacrolimus immediate-release capsules. Solid dispersion technology has been used in Prograf® capsules, whereas in Envarsus XR tablets melt dose technology has been used. It has been reported that melt granulation using lipid with surfactant improves drug absorption and water solubility by changing physical form of drug substance from crystalline to amorphous [15]. Glyceryl behenate (Compritol®ATO888), with a melting temperature of 74°C, is an

excellent alternative for melting granulation. Lipophilic-based drug granules require adsorbent to avoid any issues in processing like milling of granules and flow of blend during compression. Hence a novel excipient (Magnesium alumino metasilicate; brand name Neusilin) with good adsorbent capacity is used. Neusilin® is a synthetic, amorphous form of magnesium aluminometasilicate [16]. It is a multifunctional excipient that can be used in direct compression and wet granulation of solid dosage forms. It possesses a large surface and high oil adsorbent capacity. Neusilin helps in compression and is very much stable in heat [17].

In the present study, tacrolimus was first dissolved in hot-melted lipid-based carrier. Melted material was absorbed by magnesium alumino metasilicate to transfer liquid into solid powder for process feasibility. The tacrolimus loaded material was then compressed into tablets with hydrogel matrix material (extended release polymer) and other pharmaceutical excipients. This study is aimed to provide a novel, feasible and scalable method to obtain stable tacrolimus extended release tablets using various polymers, such as polyethylene glycol (PEG) it is a non ionic surfactant and is hydrophilic. It improved the dissolution profile of tacrolimus once added in dispersion. Another polymer used in the present work is magnesium alumino metasilicate; it is amorphous in nature and has high melting point and good oil adsorption capacity. It adsorbs tacrolimus dispersion and imparts good flow of blend. In the present study adsorption of tacrolimus dispersion was done with the two adsorbents (Neusillin US2, silicon dioxide colloidal). Hence once-daily dose extended release tablets of Tacrolimus were developed with the novel approach.

2. MATERIALS AND METHODS

2.1 Materials

Tacrolimus was obtained as a gift sample from Biocon (Bangalore, India), Glyceryl behenate (Compritol 888 ATO) received from Gattefosse, Magnesium Alumino metasilicate (Neusillin US 2) received from Fuji chemical, Polyethylene glycol (PEG 6000) received from Dow, Kollidone SR received from BASF, hypromellose received from colorcon, ethyl cellulose (ethocel) received from Dow, Lactose monohydrate (Flowlac 100) received from Meggle and magnesium stearate received from Crest cellulose.

2.2 Preparation of Tacrolimus Dispersion by Melt Granulation

The tacrolimus granules were prepared by melt granulation (Fig. 1) in a rapid mixer granulator(Bectochem, Loedige Process Technology Pvt. Ltd) equipped with a hot water circulation facility.[18] The raw material glyceryl behenate (compritol 888ATO) and polyethylene glycol 6000 were added into a rapid mixture granulator and heated up to 85°C till the materials were melted. Tacrolimus was added into a rapid mixer granulator and continue mixing till a uniform molten mixture was obtained.

2.3 Adsorption of Tacrolimus Solid Dispersion on Magnesium Alumino Meta Silicate

To melted tacrolimus dispersion, magnesium alumino metasilicate was added in the rapid mixer granulator in the rotating condition of impeller and chopper, [19] further molten dispersion was slowly cooled by passing cold water. Granules were milled through Quadro co-mill fitted with a 40G screen. Milled granules were passed through #30 mesh (ASTM 600 µm). Various formulation of melt granulation is listed in Table 1.

2.4 Evaluation of Tacrolimus Dispersion (Adsorbed on Magnesium Alumino Metasilicate)

A flow property of tacrolimus dispersion was evaluated by measuring Carr's compressibility index and Hausner ratio. To determine Carr's compressibility index, the powder was filled in a measuring cylinder and tapped for a fixed duration of time. The volume of both before tapping and after tapping was measured and Carr's index was calculated. The relationship between bulk and tapped density indicates flow properties of the powder, the more the difference between bulk density and tapped density, the poorer the blend for flow. A valueless than 1.25 shows good flow of powder.

2.5 Scanning Electron Microscopy (SEM) of Tacrolimus Dispersion

Scanning electron microscopy is an ideal choice for the examination of the surface morphology of the particles [20]. Both magnesium alumino metasilicate and tacrolimus dispersion adsorbed on magnesium alumino metasilicate was

examined by scanning electron microscope (JEOL JSM 6360A microscope) operating at 10 kV. The samples were mounted on a metal stub with double-sided tape and coated with platinum in an inert atmosphere. The samples were analyzed at 200X magnification.

2.6 Differential Scanning Calorimetry

DSC (Differential scanning calorimeter, model DSC Q10 V24.11) was used to detect crystallinity in tacrolimus dispersion [21]. Approximately 2 mg of samples were taken in an aluminum pan and heated at a 20°C/min from a temperature of 0°C to 150°C under a nitrogen gas purge. The DSC patterns of both tacrolimus drug substance and tacrolimus dispersion adsorbed on magnesium alumino metasilicate were measured [22].

2.7 Stability of Tacrolimus Solid Dispersion (Adsorbed on Magnesium Alumino Metasilicate)

Tacrolimus dispersion was stored at accelerated condition (40°C/75%RH) for up to 4 weeks in an open petri dish. The stability of tacrolimus dispersion was checked in terms of retention of the amorphous nature of the drug substance by DSC [23].

2.8 X-ray Powder Diffraction (XRPD)

Crystallinity properties of tacrolimus, [24] and tacrolimus solid dispersions adsorbed on magnesium alumino metasilicate were measured using an X-ray diffractometer (PANalytical, Almelo, the Netherlands) with a copper anode with radiation at 40kV and 30mA.

2.9 Tablet Compression

Extra granular material controlled release polymer and lactose monohydrate were sifted through #30 mesh sieve and pre-lubricated with granules prepared by melt granulation technology for 15 min in a double cone blender. Blended granules were lubricated with magnesium stearate (pre sifted through #60 mesh) for 5 min in a double cone blender. The lubricated granules were compressed (using compression machine Cadmach, 16 station single rotary) in tablets using oval-shaped plain tooling. Formulation details of extended-release tacrolimus tablets are provided in Table 2.

2.10 Evaluation of Tablets

The extended-release tacrolimus tablets were evaluated for weight variation, friability, hardness, thickness and assay (presented in Table 3). The hardness of the tablets was checked by the hardness tester apparatus (Pharmatron). Friability test was performed using Electrolab EF-2 friability test apparatus. The thickness of the tablets was determined by using a vernier Caliper. Weight variation test was performed by using an electronic balance (Sartorius electronic balance, Model BSA 4235, range: 1 mg to 420 gm). The average weight of the tablet was determined. Percentage weight variation for each tablet was calculated from the average weight.

2.11 Drug Content Assay

To prepared a standard solution 75 mg of tacrolimus standard was transferred to a 25 mL volumetric flask, dissolved in acetonitrile and diluted to make up the volume with water in a 7:3 v/v, and mixed thoroughly [25]. Sample solution was prepared by transferring about 75 mg of sample into a 25 mL clean and dry volumetric flask, dissolve and dilute to volume with diluents (acetonitrile and water in the ratio 7:3 v/v respectively) and mix well. Sample solution was injected into the high-performance liquid chromatography [Model: Waters 2695, column Luna C18 (2), 3µm (150 mm X 4.6 mm)] injection volume 20µL, with a flow rate 1.5 ml/minute. The obtained results were recorded, and area responses, open ring and 19 epimer of tacrolimus were calculated for assay.

2.12 pH Solubility Profile of Tacrolimus

A 5 mL amber-tinted glass vial was filled with 3 mL of the appropriate media and different buffers from pH1.2 to pH7.2. Each vial was then sealed with a stopper once the extra medication had been added. An orbital shaking water bath was used to place these glass vials. At 50 rpm at a temperature of 37°C, the shaking was carried out for 48 hr. The HPLC technique was used to analyze the filtered test materials.

2.13 Forced Degradation Studies in Tacrolimus

Stress testing was carried out to analyze the nature of the drug material in terms of degradation (reduction in drug content) and rise

in contaminants. In the present study, four types of stress tests have been carried out. Heat treatment (0.6 g of a sample heated to 100-105°C for 24 hr in oven), acid treatment (0.6 g of sample dissolved in 50 ml with 0.5N hydrochloric acid samples heated at 60°C for 24 hr), alkali Treatment(0.6 g of sample dissolved in 50 mL with 0.05N sodium hydroxide samples heated at 60°C for 24 hr), and oxidation (0.6 g of sample dissolved in 50 ml with 5% hydrogen peroxide samples heated at 60°C for 24 hr).

2.14 *In-vitro* Dissolution Studies

Dissolution of Tacrolimus extended release tablets was done by using USP type II paddle apparatus at 100 rpm in 0.005 % HPC in water with 0.50% SLS. The pH was adjusted to pH 4.5 [26]. The temperature of dissolution apparatus was maintained at 37 ± 0.5°C. The samples were withdrawn at a regular interval (0.5, 1, 2.5, 4.5, 6.5, 8.5 and 12 h). The withdrawn samples were replaced with equal medium to maintain sink condition. The samples were analyzed using a high-performance liquid chromatography system using a C8 column with a flow rate of 1.8mL/min and run time is 25 min.

2.15 Related Substances

Standard solution was prepared by transferring 50 mg of tacrolimus into a 200 ml clean and dry volumetric flask, dissolve and dilute to make up the volume with acetonitrile and mix well. Dilute 3 mL of this solution to 100 mL in volumetric flask [27]. Filter the solution through 0.45 micron filter (Tacrolimus concentration is about at 7.5 ppm). Sample solution was prepared by transfer tablet powder equivalent to 15 mg of tacrolimus into a 25 mL clean and dry volumetric flask, add 10 mL of acetonitrile and mix well. Filter the solution through 0.45µ filter (Tacrolimus concentration is about 1500 ppm). The placebo sample solution was prepared by transfer tablet powder about 15 mg into a 25 mL clean and dry volumetric flask, and adds 10 ml of acetonitrile and mix well. Filter the solution through 0.45µ filter. Examine the blank peak and placebo chromatogram for any extraneous peak. Disregard any unknown peak in the chromatogram with an area smaller than tacrolimus peak in the sensitivity solution (0.1%). As tacrolimus 8 epimer and tacrolimus 8 propyl analogue impurities were eluting closely perform tangential integration if necessary. The data were analysed using HPLC, and the flow rate was maintained at 1.5mL/min.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Tacrolimus Dispersion

A total of six batches were prepared to evaluate the dispersion and check the physical properties and release of drug and improve the drug's solubility and dissolution. Tacrolimus dispersion was prepared without using magnesium alumino metasilicate (D-3) shows poor blend flow (shown in Fig. 2), difficulty to mill the granules, whereas other batches (D-1, D-2, D-4, D-5 and D-6) with magnesium alumino metasilicates ease to process. Physical properties of tacrolimus dispersion were tabulated in Table 4.

As shown in Fig. 3, the dissolution rate of plain tacrolimus was very low, the release was observed 40% after 12 hr. Tacrolimus' lipophilic nature makes it insoluble in water, resulting in a slow release. Solubility of tacrolimus was increased by melting the drug in the presence of lipid and polyethylene glycol. Solid dispersion prepared with glyceryl behenate (lipid material) and without polyethylene glycol (surfactant) showed less and incomplete release profile after 12 hr. Whereas tacrolimus dispersion prepared in polyethylene glycol (surfactant) showed complete release profile after 12 hr (Fig. 4).

Table 1. Composition of solid dispersion of tacrolimus extended release tablets by melt granulation

Formulation code	Tacrolimus (mg)	Glyceryl behenate (mg)	Polyethylene glycol (mg)	Magnesium alumino metasilicate (mg)	Colloidal silicon dioxide (mg)
D-1	4	180	12	60	-
D-2	4	180	12	100	-
D-3	4	180	12	-	-
D-4	4	180	-	60	-
D-5	4	90	12	60	-
D-6	4	180	12	-	60

Table 2. Composition of tacrolimus extended release tablets 4 mg

	mg/tablet					
Ingredients	**F-1**	**F-2**	**F-3**	**F-4**	**F-5**	**F-6**
Tacrolimus dispersion [a]	256	256	256	256	256	256
Kollidon SR	-	-	80	-	-	-
Ethyl cellulose 100 cps	-	80	-	-	-	-
Hypromellose K15M	80	-	-	-	-	-
Hypromellose K4M	-	-	-	40	80	120
Lactose monohydrate	62	62	62	102	62	22
Magnesium stearate	2	2	2	2	2	2

[a] *solid dispersion containing glyceryl behenate, polyethylene glycol and magnesium alumino metasilicate in 12:1:5*

Table 3. Evaluation of extended release tacrolimus tablets 4 mg

	Evaluation of granules			Evaluation of tablets			
Run	**Bulk density (g/ml)**	**Tapped density (g/ml)**	**Compressibility index (%)**	**Hardness (kp)**	**Friability (%)**	**Thickness (mm)**	**Weight variation (mg)**
F-1	0.502	0.6	19.52	9.5±0.15	0.12 ±0.15	5.20 ± 0.15	400 ±0.15
F-2	0.488	0.608	19.74	10.1±0.08	0.14 ±0.12	5.18 ± 0.08	399 ±0.24
F-3	0.48	0.594	19.19	9.8±0.10	0.10 ±0.13	5.25 ± 0.14	399 ±1.11
F-4	0.522	0.61	14.43	11.1±0.12	0.09 ±0.16	5.33 ± 0.15	401 ±1.54
F-5	0.482	0.59	18.31	10.2 ±0.07	0.11 ±0.13	5.22 ± 0.09	398 ±1.34
F-6	0.512	0.625	18.08	9.2.1±0.12	0.13 ±0.16	5.32 ± 0.15	401 ±2.53

Table 4. Evaluation of tacrolimus dispersion

Batch	**Bulk density (g/ml)**	**Tapped density (g/ml)**	**Compressibility index (%)**	**Hausner ratio**	**Remarks**
D-1	0.482	0.586	17.74	1.22	Fair flow
D-2	0.451	0.568	20.59	1.26	Passable
D-3					Difficult to mill the granules, unable to process
D-4	0.498	0.606	17.82	1.21	Fair flow
D-5	0.501	0.6	16.5	1.2	Fair flow
D-6	0.402	0.56	28.21	1.39	Poor flow

Table 5. Release kinetics (Regression Value: R^2) of tacrolimus extended release tablets

Formulation	**Higuchi**	**Zero order**	**First order**
F-1	0.713	0.869	0.958
F-2	0.751	0.985	0.967
F-3	0.757	0.99	0.981
F-4	0.825	0.966	0.99
F-5	0.754	0.742	0.977
F-6	0.725	0.863	0.964

Table 6. Stability data of tacrolimus extended release tablets (F-5)

Tests	**Initial**	**Accelerated condition (40°C/75% RH); 6 months**	**Long term condition (25°C/60% RH); 6 months**
Assay (%)	99.6	99	99.5
Impurities (%)			
Tacrolimus 8 epimer	5	42	0.12
Any Unknown impurity	0	11	0.06
Total impurities	5	53	0.18

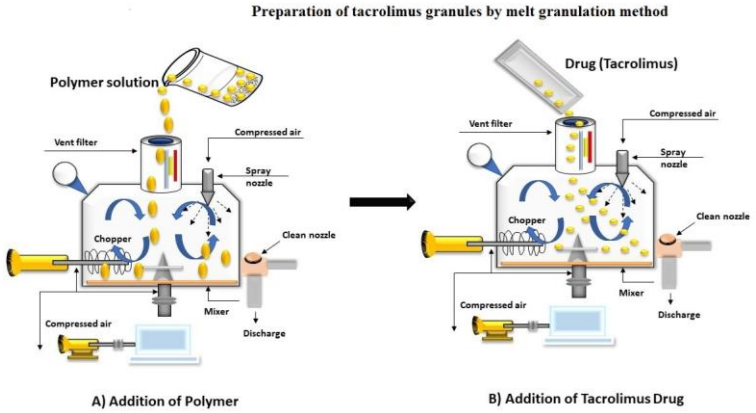

Fig. 1. Preparation of tacrolimus granules by wet granulation method

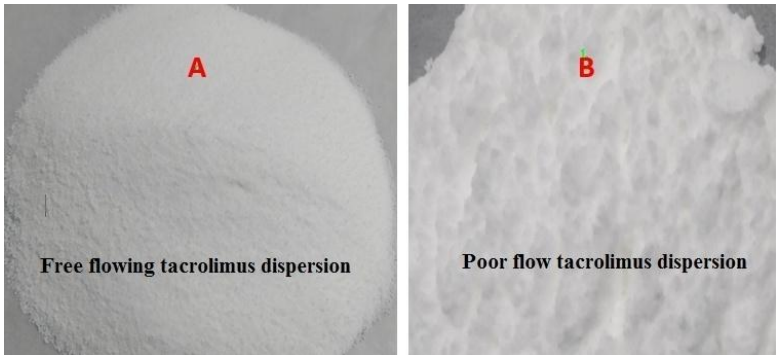

Fig. 2. Flow properties of tacrolimus dispersion blend

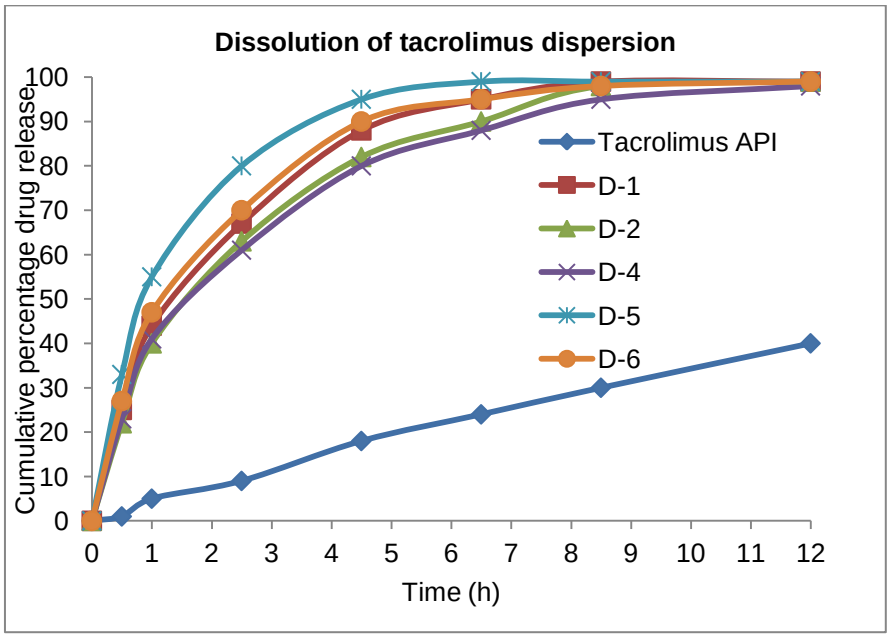

Fig. 3. Dissolution profile of tacrolimus dispersion: D-1 (Glyceryl behenate: PEG: magnesium alumino metasilicate 180:12:60 mg); D-2 (Glyceryl behenate: PEG: magnesium alumino metasilicate 180:12:100 mg); D-4 (Glyceryl behenate: PEG: magnesium alumino metasilicate 180:0:60 mg); D-5 (Glyceryl behenate: PEG: magnesium alumino metasilicate 90:12:60 mg); D-6 (Glyceryl behenate: PEG: colloidal silicon dioxide 180:12:60 mg)

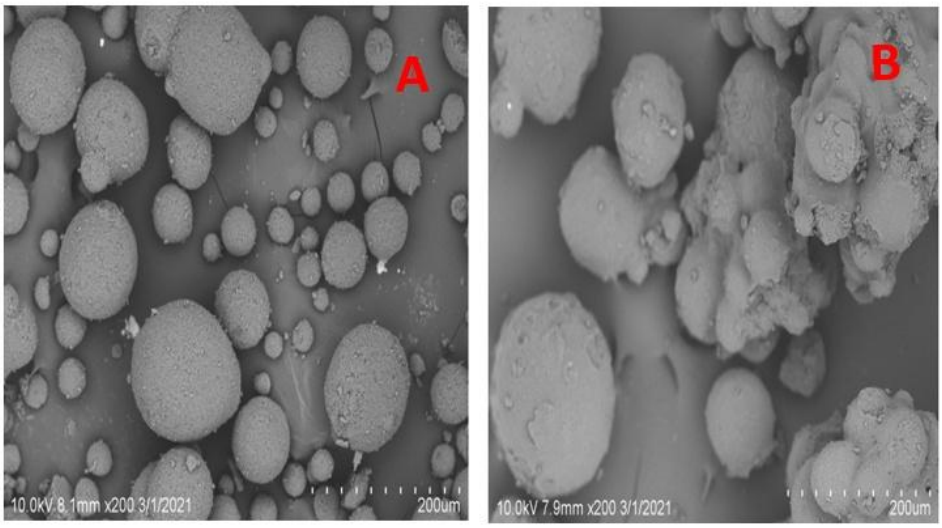

Fig. 4. SEM (scanning electron microscopy) of (a) magnesium alumino metasilicate and (b) tacrolimus dispersion

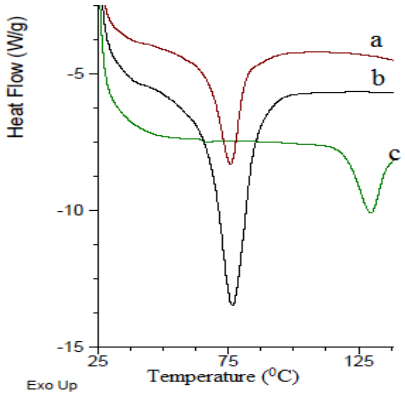

Fig. 5. DSC thermogram of (a) tacrolimus dispersion initial, (b) tacrolimus dispersion stored at open petri-dish at 40°C/75%RH for 4 weeks and (c) tacrolimus drug substance

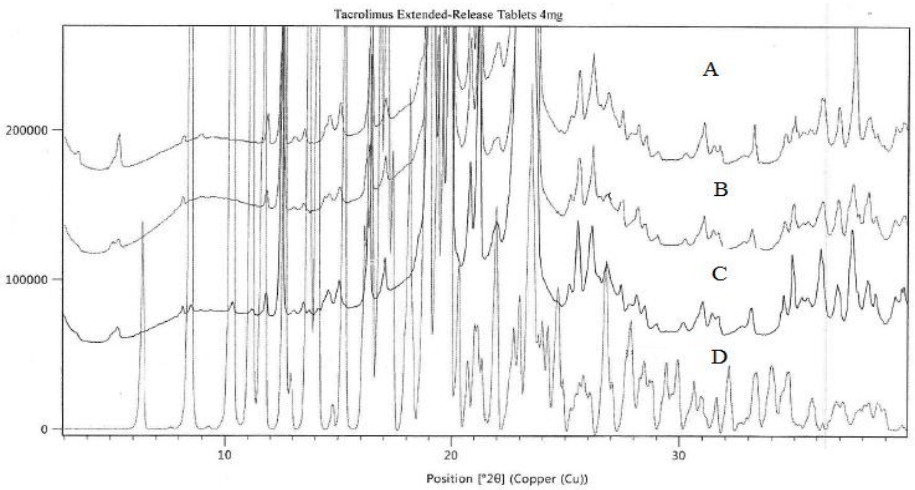

Fig. 6. XRD thermogram of (A) Placebo, (B) tacrolimus extended release tablets initial, (C) Tacrolimus extended release tablets 6 months 40°C/75%RH and (D) tacrolimus drug substance

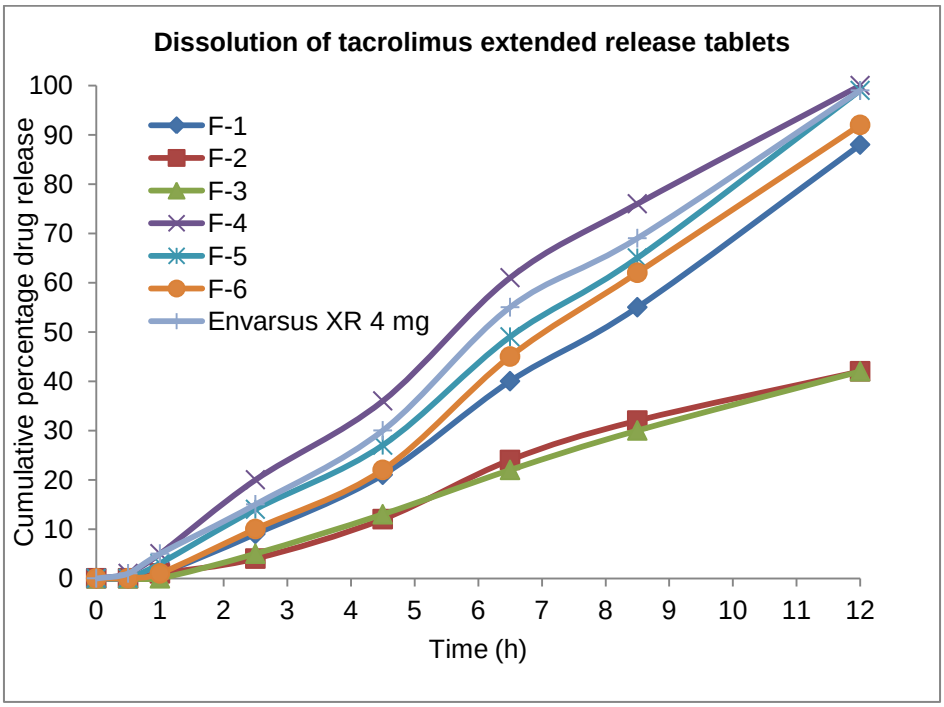

Fig. 7. Dissolution profile of various formulations of tacrolimus extended release tablets and Envarsus XR tablets

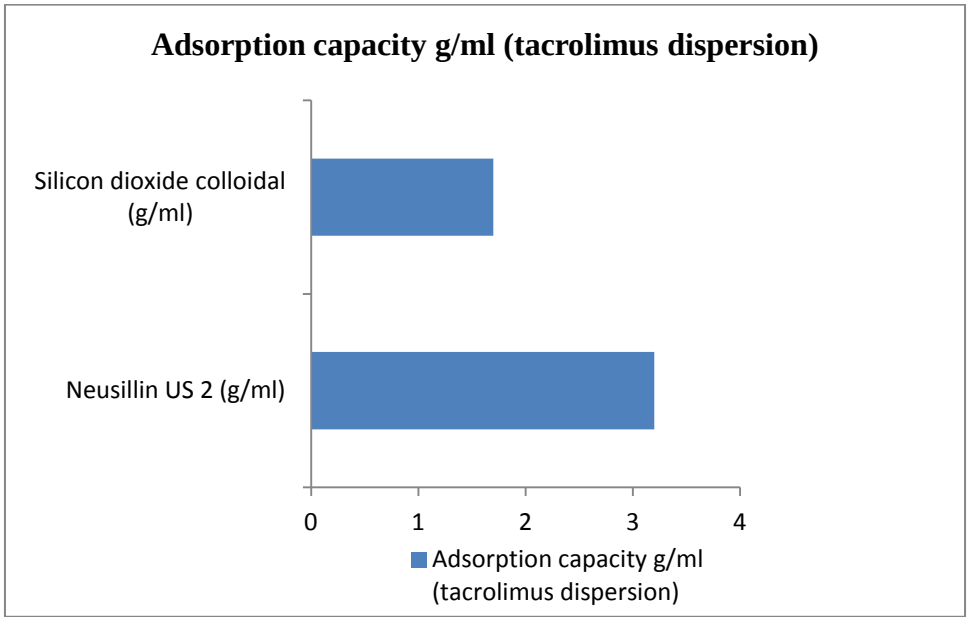

Fig. 8. Adsorption capacity of colloidal silicon dioxide and NeusillinUS2

The melting point of both glyceryl behenate (approx. 70°C) and polyethylene glycol (approx. 65°C) are significantly less show uniform dispersion with the tacrolimus API during heating. Polyethylene glycol is a non ionic surfactant and is hydrophilic in nature. It improved the dissolution profile of tacrolimus once added in dispersion. Magnesium alumino metasilicate is amorphous in nature and has high melting point and good oil adsorption capacity. It adsorbs tacrolimus dispersion and imparts good flow of blend. In the present study, adsorption of tacrolimus dispersion was done with the two adsorbents

(Neusillin US2, silicon dioxide colloidal). Fig. 5 depicts the adsorption capacity. Oil adsorption capacity of Magnesium alumino metasilicate is about 3.2 g/mL, whereas Oil adsorption capacity of silicon dioxide colloidal is 1.7 g/mL. Based on the oil adsorption capacity, magnesium alumino metasilicate was used for further study.

3.2 Scanning Electron Microscopy (SEM) Studies

In tacrolimus dispersion, Neusillin US2 (magnesium alumino metasilicate) has been used as an adsorbent. Neusillin is amorphous in nature and having higher surface area, and is an ideal choice as a solid carrier. SEM micrographs of Neusillin US2 and tacrolimus dispersion containing Neusillin US2 are shown in Fig. 6a and 6b. In Fig. 6a, larger spherical particles were observed with a smooth surface. Particles are very regular in shape provides a good flow of granules.

3.3 Differential Scanning Calorimetry (DSC) Studies

DSC peaks of crystalline tacrolimus and the solid dispersions of tacrolimus are shown in Fig. 7. The melting peak of tacrolimus was about 130.0°C. Solid dispersion of tacrolimus does not show any melting peak indicates absence of crystalline form. This could be due to the conversion of polymorphic form through crystalline to amorphous.

3.4 X Ray Powder Diffraction (XRPD) Studies

Both the pure tacrolimus medication and its extended release tablet counterparts were evaluated for their XRPD patterns. In contrast, the XRPD pattern of tacrolimus dispersion reveals crystallinity peaks. The polymorphic form of tacrolimus, illustrated in Fig. 8, has changed from crystalline to amorphous.

3.5 pH Solubility Studies

pH-dependent solubility of Tacrolimus was found at pH 4.5, but at strongly acidic and basic pH levels, solubility was extremely low. Considering the solubility nature of drug molecule, pH 4.5 identified as a more preferable media for dissolution studies. Tacrolimus' saturation solubility is shown in Table 5.

3.6 Forced Degradation Results

Based on the forced degradation studies at different stress conditions, it was observed that tacrolimus shows less degradation in heat, whereas in basic and acidic conditions, more degradation was observed. Table 6 summarizes the results of impurities generated during forced degradation studies.

Table 7. pH solubility profile of tacrolimus

S. No	Solvent	Solubility (mg/ml)
1	Hydroxypropyl Cellulose Solution (1 in 20,000 in 0.1N HCl) pH adjusted to 1.2	0.04
2	Hydroxypropyl Cellulose Solution (1 in 20,000 in 0.1N HCl) pH adjusted to 2.0	0.07
3	Hydroxypropyl Cellulose Solution (1 in 20,000 in acetate buffer) pH adjusted to 4.5	0.1
4	Hydroxypropyl Cellulose Solution (1 in 20,000 in phosphate buffer) pH adjusted to 6.8	0.05
5	Hydroxypropyl Cellulose Solution (1 in 20,000 in phosphate buffer) pH adjusted to 7.2	0.04

Table 8. Forced degradation study of tacrolimus API

Particulars	Heat treatment		Acid treatment	Alkali treatment	Oxidation
Impurities (%)	**Initial**	**24 hr**	**24 hr**	**24 hr**	**24 hr**
Tacrolimus 8 propyl analog	0	0	0.13	0	0
Tacrolimus 8 epimer	0	0.05	5.3	0.05	0.06
Tacrolimus 19 epimer	4.5	4.6	0.38	Not detected (Drug degraded completely)	4.2
Ascomycin	0.05	0.06	0.42	0.04	0.04
Desmethyl Tacrolimus	0.04	0.1	0.04	0.05	0.06
Maximum unknown	0	0	35	9.5	15

3.7 Evaluation of Tacrolimus Extended Release Tablets

Similarly compacted tablets were found, hypromellose batch showed higher weight fluctuation due to the mix's reduced flow (Presented in Table 3).

3.8 Dissolution Studies

There has been a study on the dissolving characteristics of tacrolimus extended release tablets (F1 to F6). The components listed in Table 2 were used in the study. The magnesium alumino metasilicate was used as adsorbent. Ethyl cellulose, hypromellose and kollidone SR were used as a controlled release polymer. Lactose was used as a diluent and magnesium stearate was used as a lubricant. Dissolution of tablets containing tacrolimus solid dispersions with different controlled release polymer was given in Fig. 3. The formulation containing Kollidon SR (F-3) and ethyl cellulose (F-2) showed less and incomplete dissolution profile. About 42% drug release was observed after 12 hr. Due to more hydrphobicity and dense matrix system, dissolution medium cannot enter the matrix system and shows more minor drug release. Formulation with hypromellose K15M CR (F-1)shows slightly higher release profile (88%) than formulation F-2 and F-3, but still rate of release was observed less as compare with the reference product (Enavarsus XR 4 mg).Hence lower viscosity grade of hypromellose was tried in the formulation (F-4, F-5 and F-6) with different ratio. Thus, a complete release profile was observed due to the lower viscosity of the polymer. Among all the prepared formulations, F-5 (containing 80 mg of Hypromellose K4M showed a comparable release profile with the reference. Similarity factor (f2) was used to compare dissolution profiles of optimized formulation with the reference.

3.9 Drug Release Kinetics

The release kinetics of tacrolimus extended release tablets were evaluated by various models such as zero order, first order, and Higuchi model. The dissolution results (from the values of 1 to 12 hr release) of all extended release formulations were fitted into mathematical models (zero-order, first-order and Higuchi model) to know the best-fitted model for release. The releases kinetic for formulations are presented in Table 7. Considering the highest regression coefficient analysis (r^2) the best-fit model for the formulations was zero order kinetics. When the graph plotted according to a zero order equation, all the formulations F-1, F-2, F-3, F-4, F-5 and F-6 showed a good linearity, with regression analysis values 0.958, 0.967, 0.981, 0.990, 0.977 and 0.964 respectively. Release of the insoluble drug from a extended release matrix tablet containing alone or in combination of hydrophobic and hydrophilic matrix polymers provides zero order kinetics. Diffusion in combination with erosion could be the reason for drug release for this kind of system.

3.10 Stability Studies

3.10.1 Stability evaluation

The formulations (F-5) showing comparable release profile with the reference (Envarsus 4 mg), was subjected for stability study with the ICH guidelines up to 6 months (accelerated condition; 40°C/75% RH and longer term condition; 25°C/60% RH) for assay and related substances. Results were provided in Table 8. It was observed that formulation was stable and no rise in impurity was observed.

4. CONCLUSION

Tacrolimus is a calcineurin inhibitor used to manage mild to severe atopic dermatitis and avoid organ transplant rejection. Using melt granulation, we created 12 hr prolonged release granules with acceptable flow characteristics and compressibility parameters. Up to six months of granule stability can be achieved by adding an adsorbent during melt granulation. Neither any polymorphic form conversion was observed on storage, also no degradation was observed, and dissolution profile remains same on stability up to 6 months accelerated condition. Hence magnesium alumino metasilicate based granules of tacrolimus with hydrophilic polymer provides an extended release profile, ideal for patient safety and enhance product quality.

SUMMARY

In the current study, extended release tablets of tacrolimus were prepared using melt granulation method. Magnesium alumino metasilicate was used as an adsorbent along with the hypromellose to control the drug release over a period of 12 hr. It was observed from the stability study that drug product is stable at accelerated

condition up to 6 months with respect to both polymorphic form conversion and impurities.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tönshoff B. Immunosuppressants in organ transplantation. In Pediatric Pharmacotherapy. Springer, Cham. 2019;441-69.
2. Yoshida T, Kurimoto I, Yoshihara K, Umejima H, Ito N, Watanabe S, Sako K, Kikuchi A. Aminoalkyl methacrylate copolymers for improving the solubility of tacrolimus. I: Evaluation of solid dispersion formulations. International journal of pharmaceutics. 2012;428(1-2):18-24.
3. Al-Tamimi DJ, Hussien AA. Preparation and *In-vitro* characterization of tacrolimus as a solid self-microemulsion. IJDDT. 2021;11(1):70-78.
4. Nouri Z, Hajialyani M, Izadi Z, Bahramsoltani R, Farzaei MH, Abdollahi M. Nanophytomedicines for the prevention of metabolic syndrome: a pharmacological and biopharmaceutical review. Frontiers in Bioengineering and Biotechnology. 2020;8:425.
5. Qiu Y, Lee PI. Rational design of oral modified-release drug delivery systems. In developing solid oral dosage forms. Academic Press. 2017;519-54.
6. Gujral G, Kapoor D, Jaimini M. An updated review on modified release tablets. Journal of Drug Delivery and Therapeutics. 2018;8(4):5-9.
7. Perry SL, McClements DJ. Recent advances in encapsulation, protection, and oral delivery of bioactive proteins and peptides using colloidal systems. Molecules. 2020;25(5):1161.
8. Al-Tamimi DJ, Hussien AA. Formulation and characterization of self-microemulsifying drug delivery system of tacrolimus. Iraqi Journal of Pharmaceutical Sciences. 2021;30(1):91-100.
9. Ordóñez-Robles M, Santos-Beneit F, Martín JF. Unraveling nutritional regulation of tacrolimus biosynthesis in Streptomyces tsukubaensis through omic approaches. Antibiotics. 2018;7(2):39.
10. Saffari TM, Bedar M, Zuidam JM, Shin AY, Baan CC, Hesselink DA, Hundepool CA. Exploring the neuroregenerative potential of tacrolimus. Expert review of clinical pharmacology. 2019;12(11):1047-57.
11. Chen L, Prasad GR. CYP3A5 polymorphisms in renal transplant recipients: Influence on tacrolimus treatment. Pharmacogenomics and personalized medicine. 2018;11:23.
12. Enokiya T, Nishikawa K, Hamada Y, Ikemura K, Sugimura Y, Okuda M. Temporary decrease in tacrolimus clearance in cytochrome P450 3A5 non-expressors early after living donor kidney transplantation: Effect of interleukin 6–induced suppression of the cytochrome P450 3A gene. Basic and Clinical Pharmacology and Toxicology. 2021;128(3):525-33.
13. Xu H, Liu L, Li X, Ma J, Liu R, Wang S. Extended tacrolimus release via the combination of lipid-based solid dispersion and HPMC hydrogel matrix tablets. Asian journal of pharmaceutical sciences. 2019;14(4):445-54.
14. Torabi J, Konicki A, Rocca JP, Ajaimy M, Campbell A, Azzi Y, Pynadath C, Liriano-Ward L, Akalin E, Kinkhabwala M, Graham JA. The use of LCP-Tacrolimus (Envarsus XR) in simultaneous pancreas and kidney (SPK) transplant recipients. The American Journal of Surgery. 2020;219(4):583-6.
15. Seth P. Forster, erin dippold and tiffany chiang. Twin-Screw Melt Granulation for Oral Solid Pharmaceutical Products. Pharmaceutics. 2021;13:665.
16. Cho CH, Min JH, Hwang KM, Park ES. Development of sustained-release microparticles containing tamsulosin HCl

for orally disintegrating tablet using melt-adsorption method. Drug delivery and translational research. 2018;8(3):552-64.
17. Chernetska S, Beley N, Demchuk M. The method of random balance for studying the influence of excipients quantities on technological parameters of tablets based on Origanum vulgare L. dry extract. Dry Extract (March 31, 2021). EUREKA: Health Sciences. 2021;73-81.
18. Krstic M, Djuris J, Petrovic O, Lazarevic N, Cvijic S, Ibric S. Application of the melt granulation technique in development of lipid matrix tablets with immediate release of carbamazepine. Journal of Drug Delivery Science and Technology. 2017; 39:467-74.
19. Uegaki Y, Hirai N, Takatani-Nakase T, Takahashi K. Developmen t of controlled-release solid dispersion granules containing a poorly watersoluble drug, porous calcium silicate, and the water-soluble polymer polyvinylpyrrolidone. J PharmaceuPharmacol. 2018;6(1):7.
20. Akhtar K, Khan SA, Khan SB, Asiri AM. Scanning electron microscopy: Principle and applications in nanomaterials characterization. In Handbook of materials characterization Springer, Cham. 2018;113-45.
21. Xiao Wu, Don Hayes, Jr, Joseph B Zwischenberger, Robert J Kuhn and Heidi M Mansour. Design and physicochemical characterization of advanced spray-dried tacrolimus multifunctional particles for inhalation. Drug Des DevelTher. 2013; 7:59–72.
22. Michael H. Chiu and Elmar J. Prenner. Differential scanning calorimetry: An invaluable tool for a detailed thermodynamic characterization of macromolecules and their interactions. J Pharm Bioallied Sci. 2011;3(1): 39–59.
23. Ponnammal P, Kanaujia P, Yani Y, Ng WK, Tan RB. Orally disintegrating tablets containing melt extruded amorphous solid dispersion of tacrolimus for dissolution enhancement. Pharmaceutics. 2018; 10(1):35.
24. Sadul SK, Perumal A, Somanath T. Development and validation of method for the estimation of tacrolimus crystalline API in tacrolimus extended release capsules 0.5 mg, 1mg and 5mg by power x-ray diffractometer (PXRD). Research Journal of Pharmacy and Technology. 2020; 13(11):5375-81.
25. Perez JD, Aragão DS, Ronchi FA, Febba AC, Rosso CF, Junior HT, de Abreu Pestana JO, Casarini DE. Simultaneous determination of everolimus, sirolimus, tacrolimus, and cyclosporine-a by mass spectrometry. In Transplantation proceedings. 2020;52(5):1402-08.
26. Yi H, Liu F, Zhang G, Cheng Z. Evaluation of a modified flow-through method for predictive dissolution and *In vitro/In vivo* correlations of immediate release and extended release formulations. Journal of Nanomaterials. 2021;28:2021.
27. Patrizia Ferraboschi, Diego Colombo, Maria De Mieri and ParideGrisenti. Evaluation, synthesis and characterization of tacrolimus impurities. The Journal of Antibiotics. 2012;65:349–54.

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