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Formulation and Evaluation of Tacrolimus Transdermal Gel

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ABSTRACT

The present investigation is concerned with formulation and evaluation of Transdermal gels of Tacrolimus, anti-psoriasis drug, to circumvent the first pass effect and to improve its bioavailability with reduction in dosing frequency and dose related side effects. Twelve formulations were developed with varying concentrations of polymers like Carbopol 934P, HPMCK4M and Sodium CMC. The gels were tested for clarity, Homogeneity, Spreadability, Extrudability, Viscosity, surface pH, drug Content uniformity, in-vitro drug diffusion study and ex-vivo permeation study using rat abdominal skin. FTIR studies showed no evidence on interactions between drug, polymers and excipients. The best in-vitro drug release profile was achieved with the formulation F4 containing 0.5 mg of exhibited 6 hr drug release i.e. 98.68 % with desired therapeutic concentration which contains the drug and Carbopol 934p in the ratio of 1:2. The surface pH, drug content and viscosity of the formulation F4 was found to be 6.27, 101.3% and 3, 10,000cps respectively. The drug permeation from formulation F4 was slow and steady and 0.89gm of tacrolimus could permeate through the rat abdominal skin membrane with a flux of 0.071 gm hr-1 cm-2. The in-vitro release kinetics studies reveal that all formulations fit well with zero order kinetics followed by non-Fickian diffusion mechanism.

Keywords: Transdermal gel, Viscosity, In-vitro drug release, In-vitro drug release kinetics study, Ex-vivo permeation study

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INTRODUCTION

The Trans dermal drug delivery systems are self-contained, discrete dosage forms which when applied to intact skin deliver the drug through the skin at a controlled rate to the systemic circulation. At Present, the most common form of delivery of drugs is the oral route. While this has the notable advantages of easy administration, it also has significant drawbacks namely poor bioavailability due to hepatic metabolism (first pass) and the tendency to produce rapid blood level spikes leading to a need for high and/or frequent dosing, which can be both cost prohibitive and involvement. To overcome these difficulties there is a need for the development of new drug delivery system; which will improve the therapeutic efficacy and safety of drugs by more precise (i.e. site specific), spatial and temporal.[1-3]

Placement with in the body thereby reducing both size and number of doses. One of the methods most often utilized has been Transdermal delivery. This delivery transport therapeutic substance through The skin for systemic effect. The success of Tran's dermal delivery depends on the ability of the drug to permeate the skin in sufficient quantities to achieve its desired therapeutic effects. The skin is very effective as a selective penetration barrier. Percutaneous absorption involves the passage of the drug molecule from the skin surface into the stratum corneum under the influence of a concentration gradient and its subsequent diffusion through the stratum corneum and underlying epidermis through the dermis and into the blood circulation. The skin behaves as a passive barrier to the penetrating molecule. The stratum corneum provides the greatest resistance to penetration and it is the rate-limiting step in percutaneous absorption. Gels are transparent to opaque semisolids containing high ratio of solvent to gelling agent merge or entangle to form a three-dimensional colloidal network structure. This network limits fluid flow by entrapment and immobilization of the solvent molecules. The network structure is also responsible for a gel resistance to deformation and therefore, its visco-elastic properties. Gels tend to be smooth, elegant, non-greasy and produce cooling effect and utilize better drug release as compared to other semisolid formulation. Gels have better potential as a

vehicle to administered drug topically in comparison to ointment, because they are non-sticky requires low energy during the formulation are stable and have aesthetic value [4-6].

Tacrolimus (FK 506) is an effective and well-tolerated primary immunosuppressant drug used in solid organ transplantation. Although its mode of action is similar to that of cyclosporine, its molecular weight is lower and its potency in inhibiting T-cell activation is 10 to 100 times greater. Moreover, topically applied tacrolimus appears to penetrate the skin sufficiently to affect local immunosuppressant. Molecular Weight is 296.149; Protein binding More than 99%, Metabolism by Hepatic Excretion of the unchanged drug in urine and faeces is negligible.

Generally the formulations of Tacrolimus commercially available are in oral and ointment form. More recently, a topical gel formulation will be introduced specifically for the treatment of localized painful and inflammatory condition, such as soft tissue musculoskeletal disorders and osteoarthritis. So the present study, formulation and evaluation of Tacrolimus transdermal gel will attempt to increase the efficacy of the drug at the site of action.

MATERIALS AND METHODS

Materials:

Tacrolimus was a gift sample from Yacht Parma, Hyderabad. Carbopol 934P and Sodium CMC were purchased from S.D. Fine chem. Ltd, Mumbai. HPMCK4M was purchased from Yarrow chemicals ltd, Mumbai. All other reagents used were of analytical grade.

Preformulation studies: [7-9]

Characterization of Tacrolimus:

Description

The sample of tacrolimus was analyzed for its nature, colour and taste.

Melting Point

The melting point was determined by using these are tube apparatus method.

Drug Excipientcompatibility studies:

The drug polymer and polymer-polymer interaction was studied by the FTIR spectrometer using Shimadzu 8400-S, Japan. Two percent (w/w) of the sample with respect to a potassium bromide disc was mixed with dry KBr. The mixture was grind into a fine powder using an agate mortar and then compressed into a KBr disc in a hydraulic press at a pressure of 1000psi. Each KBr disc was scanned 16times at 2 mm/sec at a resolution of 4 cm^{-1} using cosine apodization. The characteristic peaks we rerecorded.

Preparation of Transdermal Gels

1% w/w Tacrolimus Transdermal gels were prepared by using different Concentrations of polymers such as Carbopol 934P, HPMCK4M and Sodium CMC. The formulation data for the preparation of Diclofenac sodium Transdermal gels using Carbopol 934P, HPMCK4M and Sodium CMC in different ratio's is shown in [Tables 01]

Procedure:

Accurately weighed amount of Polymers (Carbopol 934P, HPMC K4M and Sodium CMC) in four different ratios was placed in known amount of distilled water (Twelve different formulations were prepared using varying concentrations of Carbopol 934P, HPMC K4M and Sodium CMC). After complete dispersion, the polymer solution was kept in dark for 24 hours for complete swelling. Accurately weighed amount of Tacrolimus was dissolved in a specified quantity of suitable solvent. The drug solution was added slowly to the aqueous dispersion of polymer with the help of high speed stirrer (500 rpm) taking precaution that air did not entrap. Finally, the remaining ingredients were added to obtain a homogeneous dispersion of gel.

Evaluation of Gels

About twelve formulations i.e. F1 to F12 were conducted. Gels were evaluated for their clarity, pH, viscosity, Spreadability, Extrudability, skin irritation test, percentage drug content, *in-vitro* diffusion studies, *in-vitro* drug release kinetic study, *ex-vivo* permeation studies using rat abdominal skin and stability studies by using standard procedure. All studies were carried out in triplicate and average values were reported.

Clarity

Clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows: turbid +; clear ++; very clear (glassy) +++. The results are shown in [Table 02].

Homogeneity:

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates. The results are shown in [Table 02].

Consistency

The estimation of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fixed distance of 10cm in other way that it should fall down on the centre of the glass cup was filled with the gel. The penetration by the cone was accurately measured from the surface of the gel to the tip of the cone inside of the gel. The distance traveled by cone in the period was noted down after 10sec. The results are shown in [Table02]

Spreadability:

It was determined by wooden block and glass slide apparatus. For the determination of Spreadability, excess of sample was applied in between two glass slides and then was compressed to uniform thickness. The weight (50gm) was added to pan. The time required to separate the two slides i.e., the time in which upper glass slide moves over the lower plates was taken as a measure of Spreadability(S).

It is calculated by using the formula: S = M .L / T

Where M = wt. tied to upper slide L = length of glass slides

T = time taken to separate the slides The results are shown in [Table 02].

Extrudability

Extrudability test was carried out by using Pfizer hardness tester. 15gm of gel was filled in collapsible aluminium tube. The plunger was adjusted to hold the tube properly the pressure of $1kg/cm^2$ was applied for 30 sec. The quantity of the gel extruded was weighed. The procedure was repeated at three equidistance places of the tube. The test was carried out in triplets.The results are shown in [Table 02].

Surface pH [8]

2.5 gm of gel was accurately weighed and dispersed in 25ml of distilled water. The pH of the dispersion was determined by using digital pH meter. The results are shown in [Table 03].

Viscosity [8]

Viscosity was determined by using Brookfield viscometer. Viscosity measurements were carried out at room temperature (25- 27°C) using a Brookfield viscometer (Model RVTDV II, Brookfield Engineering Laboratories, Inc, Stoughton, MA). The results are shown in [Table03].

Drug content [8]

A specified quantity (100mg) of developed gel and marketed gel were taken and dissolved in 100ml of phosphate buffer of pH 6.8. The volumetric flask containing gel solution was shaken for the period 2hr on mechanical shaker in order to get absolute solubility of drug. This solution was filtered and estimated spectrophotometrically at 294.0nm using phosphate buffer (pH 6.8) as blank. The results are shown in [Table03].

Drug content was measured at 294nm against their blank. The results are shown in [Fig. 07-10].

Drug release kinetic studies

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data was fitted into zero-order, first order, Higuchi and Korsmeyer Pappas release model, to study the drug release from the dosage form. The results are shown in [Table04].

Zero order release rate kinetics:-

To study the zero-order release kinetics the release rate data are fitted to the following equation.

$$F=K_0t$$

Where 'F' is the drug release, 'K' is the release rate constant and 't' is the release time. The plot of % drug release versus time is linear.

First-order release rate kinetics:-

The release rate data are fitted to the following equation.

Log (100-F) = kt

A plot of log % drug release versus time is linear.

Higuchi release model:-

To study the Higuchi release kinetics, the release data were fitted to the following equation.

$$F = kt^{1/2}$$

Where 'k' is the Higuchi constant.

In Higuchi model, a plot of % drug release versus square root of time is linear.

Korsmeyer-Pappas release model:-

The release rate data were fitted to the following equation.

$M_t/M_\infty = kt^n$

Where, M_t/M_∞ is the fraction of drug released, 'K' is the release constant, 't' is the release time's' is diffusion exponent. If n = 0.89, the release is zero order. If n = 0.45 the release is best explained by Fickian diffusion, and if0.45 < n < 0.89 then the release is through anomalous diffusion or non Fickian diffusion (Swellable & Cylindrical Matrix). In this model, a plot of log (M_t/M_∞) versus log (time) is linear.

The drug release data of optimized tablet were fitted to Zero-order, First-order, Higuchi and Korsmeyer- Pappas model to study the kinetics of drug release.

Ex vivo permeation studies [9] Tissue Isolation

Rats weighing 135-160 gm were used to obtain freshly excised full thickness skin. Animal was sacrificed by spinal dislocation. Hairs from abdominal regions was removed by means of surgical and razor taking care not to damage the epidermal surface, Subcutaneous fats was removed carefully without damaging to the skin.

In vitro drug permeation through rat abdominal skin membrane

In vitro permeation of transdermal gel was studied through the rat abdominal skin membrane. The skin membrane was mounted between the donor and receptor compartment of the standard Franz diffusion cell with a diffusion area of2.1 cm^2 and the acceptor compartment volume of 21ml.The two chambers were tied with the help of springs so that the skin membrane did not move from its place. The phosphate buffer pH 6.8 in the acceptor compartment was continuously stirred at 600rpm using a magnetic stirrer. The entire setup was placed over a magnetic stirrer and the temperature was maintainedat37°±0.5°Cbyplacingthediffusioncellin a water bath. The selected gel (F4) containing 1mg of Tacrolimus was placed into the donor compartment. The amount of drug permeated through the membrane was determined by removing aliquots from the receptor compartment and by replacing the same volume of buffer. The amount of Tacrolimus in the diffusion samples was estimated by the HPLC method the flux (J) through the membrane was calculated by using the equation.

J = dQ / A dt

Where J is flux (mg $h^{-1}cm^{-2}$);

dQ/dt is the slope obtained from the steady-state portion of the curve and A is the area of diffusion (cm^2)

HPLC analysis:

Instrument: youngling instrument Software: Autochro 3000+ Column: C18, 5µm

Lambda max: 294nm

Temp: 35°C

Injection volume: 20µl

Time -10min

Mobile phase: Methanol: Phosphate buffer (4:1 ratio)

PH: adjusted to 2 with HCl

The results are shown in [Table 05] and [Fig 11]

Skin Irritation Test

The hair on the dorsal side of Wister albino rat was removed by clipping 1 day before the experiment. The rabbitsweredividedinto3groups.Group1servedas control; group 2 received optimized formulation; group 3 received 0.8% v/v aqueous solution of formalin as a standard

irritant. Finally, the application sites were graded according to visual scoring scale.

Stability studies

The optimized formulation F4 was subjected to a stability testing for the period of three months as per ICH norms at a temperature of 25˚±2°C with relative humidity RH= 60±5% and 40º ± 2°C with relative humidity RH= 75±5%. The optimized formulation F4 was analyzed for the changes in appearance, pH, percentage of drug content and *in-vitro* diffusion study by procedure stated earlier. The results are shown in [Table 06].

RESULTS AND DISCUSSIONS

The objective of the present study was to formulate Transdermal gels of Tacrolimus. Total twelve different Tacrolimus transdermal gels with different polymer ratios were prepared. In order to select the optimized formulation, various evaluation parameters were checked and subjected to *in-vitro* diffusion study and their release kinetic study were observed. The optimized formulation was further studied for *ex-vivo* permeation using rat abdominal skin.

Preformulation studies Characterization of Tacrolimus:

The following tests were performed according to British Pharmacopoeia.

Description: A white or almost white powder Solubility: Methanol and Ethanol

Melting Point: 296.149˚C

From these tests it was confirmed that the sample complies with the monograph.

Compatibility studies

The incompatibility between the drug and excipients. The were studied by FTIR spectroscopy. The results indicate that there was no chemical incompatibility between drug and excipients. used in the formulation. The results are shown in [Fig 01-05].

Evaluation of Transdermal gels: Clarity:

Carbopol 934P gels were found to be sparkling and transparent, HPMC K4M gels were found to be translucent. All gels were free from presence of particles. The results are shown in [Table 02].

Homogeneity:

All developed gels (F1-F12) showed sgood homogeneity with absence of lumps. The developed preparations were much clear and transparent. The results are shown in [Table02].

Spreadability:

The value of Spreadability indicates that the gel is easily spreadable by small amount of shear. In formulations F1 to F4, Spreadability of Carbopol 934P gel was in the range 18.75- 27.39 g.cm/sec. In formulations F5 to F8, Spreadability of HPMCK4M gel was in the range 20.06- 24.27 g.cm/sec. In formulations F9 to F12, Spreadability of Na CMC was in the range of 19.07- 24.57 g.cm/sec, indicating Spreadability of Carbopol 934P containing Diclofenac sodium gel i.e. F4 was good i.e. 27.39 g. cm/sec as compared to HPMC K4M gel and Na CMC gel. The results are shown in [Table02].

Extrudability:

The extrusion of the gel from the tube is an important during its application and in patient acceptance. Gels with high consistency may not extrude from tube whereas, low viscous gels may flow quickly, and hence suitable consistency is required in order to extrude the gel from the tube. Extrudability of Carbopol 934P gel i.e. F4 formulation was found to be Excellent when compared to other formulations. The results are shown below in [Table02].

Surface pH:

The pH value of all developed formulations of Carbopol gels (F1-F4) were in the range of 5.71- 6.27, HPMC gels (F5-F8) were in the range of 6.45- 6.82 and Na CMC gels (F9-F12) were in the range of 5.65- 6.91 which is well within the limits of skin pH i.e. 5.6-7.5. Hence, it was concluded that all the formulations could not produce any local irritation to the skin. The results are shown in [Table03].

Viscosity Measurement:

The Viscosity of the formulations i.e. F1-F4 containing drug and Carbopol 934P were in the range of 1, 92,000-3,10,000 cps, whereas the formulations i.e. F5-F8 containing drug and HPMC K4M were in the range of 1,36,000 – 1,47,000 cps, whereas formulations i.e. F9- F12 containing drug and Sodium CMC were in the range of 1,52,000- 1,80,000 cps. From the results it was found that the formulation F1 showed maximum viscosity i.e. 3,20,000 cps and formulation F8 showed minimum viscosity i.e. 1,36,000cps.The results are shown in[Table03].

Drug Content:

The percentage drug content of all prepared gel formulations i.e. F1 to F12 were found to be in the range of 97.21±0.18 to 101.46±0.26%. The percentage drug content of formulations was found to be within the I.P limits. Hence methods adopted for gels formulations were found suitable. The results are shown in [Table 03].

In-vitro drug diffusion studies:

In-vitro drug release study of different gel formulations i.e. F1 to F12 were carried out through dialysis sac (cellophane membrane) and are plotted. The percentage drug release for the formulations containing drug and Carbopol 934P i.e. F1 to F4 were found to be in the range of 82.88% to 98.68% in 6 hours. Among these formulations, formulation F4 containing drug and Carbopol 934P in the ratio1:2 showed high percentage of drug release i.e. 98.68%in 6 hours. The results indicate that increase in the concentration of Carbopol 934p, increases the drug release.

The percentage drug release for the formulations containing drug and HPMC K4M i.e. F5-F8 were in the range of 79.59 – 87.72% in 6 hours. Among these, formulation F8 containing drug and HPMCK4M in the ratio 1:4 showed highest percentage of drug release i.e. 87.72% in 6 hours. From the above it was observed that increase in the concentration of HPMC K4M, increases the drug release. The percentage drug release for the formulations containing drug and Na CMC i.e. F9-F12 were found to be in the range of 83.77 – 90.38% in 6 hours. Among these, the formulation F10 containing drug and Na- CMC in the ratio 1:1.5 showed highest percentage drug release of drug release i.e. 90.38% in 6 hours. From the above it was observed that increase in the concentration of Na CMC increases the drug release. The comparison of *in-vitro* drug release studies were conducted for the formulations F4, F8 and F10. The results are shown in [Fig 07-10].

From the above result it is observed that the formulation F4 containing drug and Carbopol 934P in the ratio 1:2 showed highest percentage drug release i.e. 98.68% in 6 hours.

Drug release kinetics:

In-vitro drug release data of F1 to F12 were fitted to zero order, first order, and Higuchi and Korsmeyer-Pappas equations to ascertain the pattern of drug release. The results are shown in [Table 04]. In-vitro drug release data for all the formulations F1 to F12 were subjected to release kinetic study according to Zero order, First order, Higuchi and Korsemeyer-Peppas equation to ascertain the mechanism of drug release. Among the zero-order and first-order, the R2 values were found to be higher in zero-order. So, all the formulations followed zero-order kinetics. But in case of mechanism of drug release, between Higuchi and Korsemeyer-Peppas equation, the R2 value were found to be higher in Korsemeyer-Peppas equation and release exponent "n" value less than 1 i.e. (n > 0.5). This indicates that all the formulations followed non- Fickian diffusion. Hence it was concluded that all the formulations followed zero-order drug release with non-Fickian diffusion.

Ex-vivo permeation studies:

It was concluded that the formulation F4 containing drug, Carbopol 934P in the ratio 1:2, showed good Spreadability, Extrudability and in-vitro drug release. On the basis of above results formulation F4 was studied for ex-vivo permeation using rat abdominal skin. The optimized formulation was analyzed by HPLC method at 285nm for 6hrs release through rat abdominal skin. The flux was calculated.

The results of drug permeation from optimized formulation through the rat abdominal skin revealed that Tacrolimus was released from the optimized formulation and permeate through the rat abdominal membrane and could possibly permeate through the human abdominal membrane. The drug permeation from F4 was slow and steady and 0.89gm of Tacrolimus could permeate through the skin membrane with a flux of 0.071 gm hr-1 cm-2. The results are shown in [Table 05].

Skin irritation test:

Based on in-vitro diffusion study formulation F4 containing drug and Carbopol 934P in the ratio 1:2 was optimized. Further, Skin irritation test was performed with optimized formulation F4 in white rabbits divided in 3 groups. It was found that the gel F4 causes no irritation or Erythema.

Stability Studies:

Accelerated stability studies was conducted in best formulation F4, according to ICH guidelines i.e. 25˚±2˚C/60±5%RH for first 30 days and 40˚±2˚C/75±5%RH up to 90 days. The results indicate that there was no so much change in appearance, pH, drug content and in-vitro drug release studies. The results are shown in [Table 06].

Table 1: Formula for the preparation of Tacrolimus Transdermal gels using Carbopol 934P, HPMCK4M and Sodium CMC.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Tacrolimus (gm)	1	1	1	1	1	1	1	1	1	1	1	1
Carbopol 934P (gm)	0.5	1	1.5	2	-	-	-	-	-	-	-	-
HPMC K4M (gm)	-	-	-	-	2.5	3	3.5	4	-	-	-	-
Sodium CMC	-	-	-	-	-	-	-	-	1	1.5	2	2.5
Triethanolamine (ml)	0.4	0.6	0.8	1.0	-	-	-	-	-	-	-	-
Alcohol (ml)	20	20	20	20	-	-	-	-	-	-	-	--
Propylene glycol (ml)	10	10	10	10	30	30	30	30	30	30	30	30
PEG 400 (ml)	-	-	-	-	7	7	7	7	7	7	7	7
Distilled water (ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

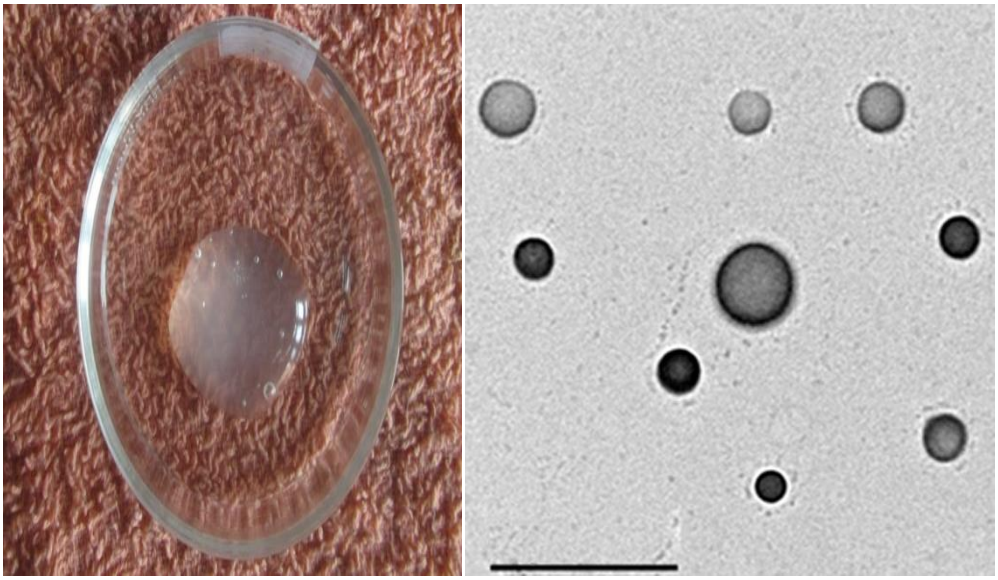


Figure 1& 2: transmission electron microscopy (TEM) images of the prepared Tacrolimusgel

Table 2: Clarity, Homogeneity, Spreadability, Extrudability Parameters

ɔrmulation Code	Clarity	Homogeneity	Spreadability	Extrudability
F1	+	Satisfactory	18.75	+
F2	++	Good	19.85	++
F3	++	Good	22.55	++
F4	+++	Excellent	27.39	+++
F5	++	Good	20.06	+
F6	++	Good	21.08	++
F7	++	Good	23.54	++
F8	++	Good	24.27	+ +
F9	++	Good	19.07	++
F10	+++	Excellent	21.81	+++
F11	++	Good	24.57	++
F12	++	Good	23.25	++

Table 3: pH, Viscosity and Drug Content (%)

Formulation code	pH	Viscosity (cps)	Drug Content (%)
F1	5.71±0.05	3,20,000	98.53±0.21
F2	5.79±0.15	1,92,000	97.21±0.18
F3	6.12±0.02	2,40,000	98.92±0.27
F4	6.27±0.03	3,10,000	101.3±0.22
F5	6.64±0.02	1,44,000	101.46±0.26
F6	6.45±0.07	1,47,000	98.92±0.25
F7	6.82±0.05	1,38,000	98.82±0.31
F8	6.60±0.04	1,36,000	99.95±0.18
F9	6.91±0.02	1,52,000	97.94±0.33
F10	6.73±0.09	1,60,000	98.08±0.40
F11	5.98±0.12	1,70,000	97.24±0.38
F12	5.65±0.14	1,80,000	99.13±0.19

Table 4: Drug release kinetics of all the formulations (F1 - F12)

Formulation code	Zero order	First order	Korsmeyer-Pappas		Higuchi
	R^2	R^2	R^2	N	R^2
F1	0.989	0.899	0.996	0.783	0.955
F2	0.990	0.871	0.997	0.780	0.955
F3	0.989	0.870	0.990	0.7765	0.951
F4	0.990	0.932	0.997	0.784	0.953
F5	0.990	0.922	0.993	0.788	0.951
F6	0.990	0.908	0.995	0.789	0.952
F7	0.984	0.969	0.944	0.784	0.927
F8	0.987	0.963	0.972	0.809	0.939
F9	0.989	0.927	0.983	0.787	0.942
F10	0.982	0.977	0.980	0.774	0.952
F11	0.987	0.967	0.972	0.789	0.940
F12	0.985	0.970	0.960	0.793	0.936

Table 5: *Ex-vivo* drug permeation of optimized Formulation F4

Time (h)	Cumulative drug permeated (gm)
0	0
1	0.17
2	0.31
3	0.49
4	0.62
5	0.77
6	0.89

Table 6: Stability studies of formulation F4

Formulation	Days	Temperature and Relative Humidity	Appearance	pH	Drug content	In-vitro drug release
F4	0	25˚±2˚C/60±5% RH	Clear	6.27	101.3	98.68
F4	15	25˚±2˚C/60±5% RH	Clear	6.25	101.1	98.60
F4	30	25˚±2˚C/60±5% RH	Clear	6.20	99.8	98.50
F4	60	40˚±2˚C/75±5% RH	Clear	6.18	99.5	98.35
F4	90	40˚±2˚C/75±5% RH	Clear	6.15	99.2	98.20

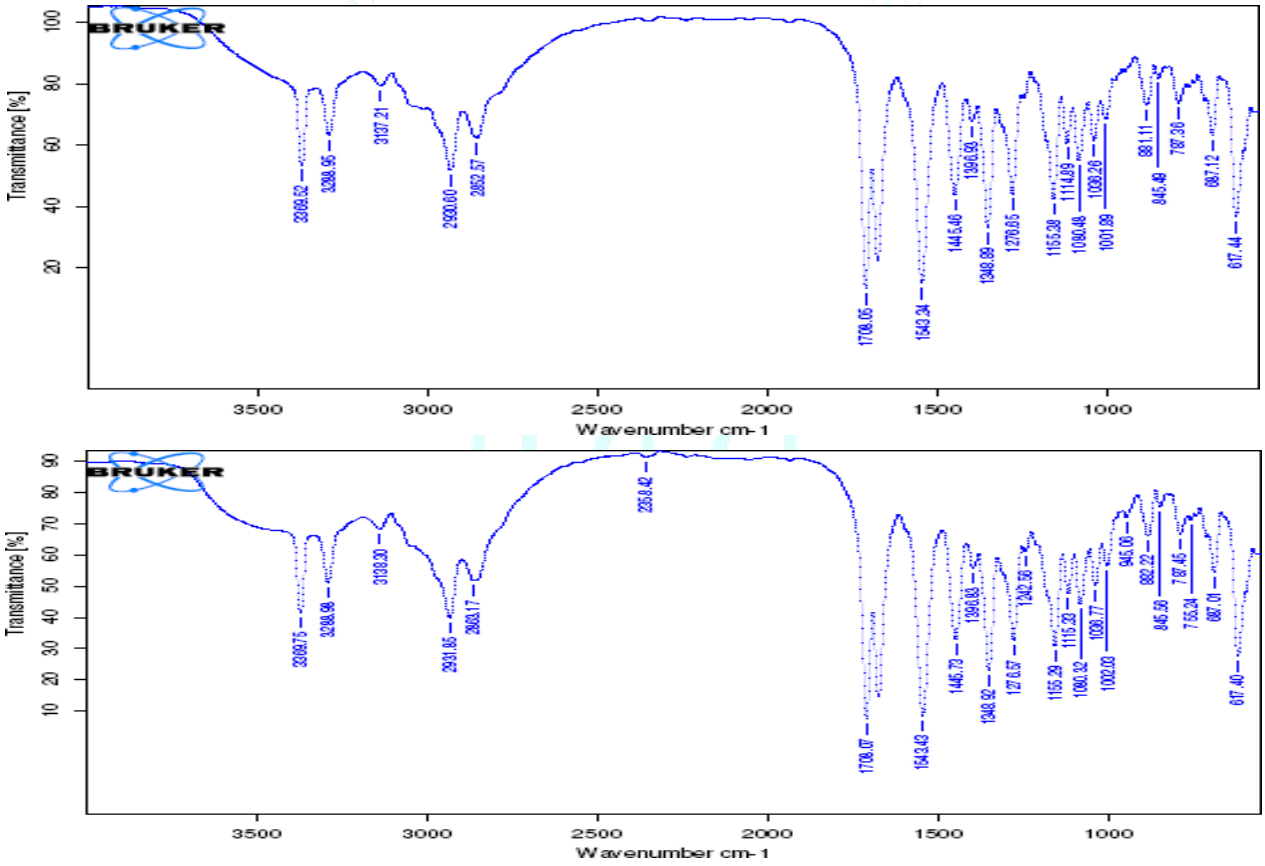


Figure 3&4: FTIR Spectra

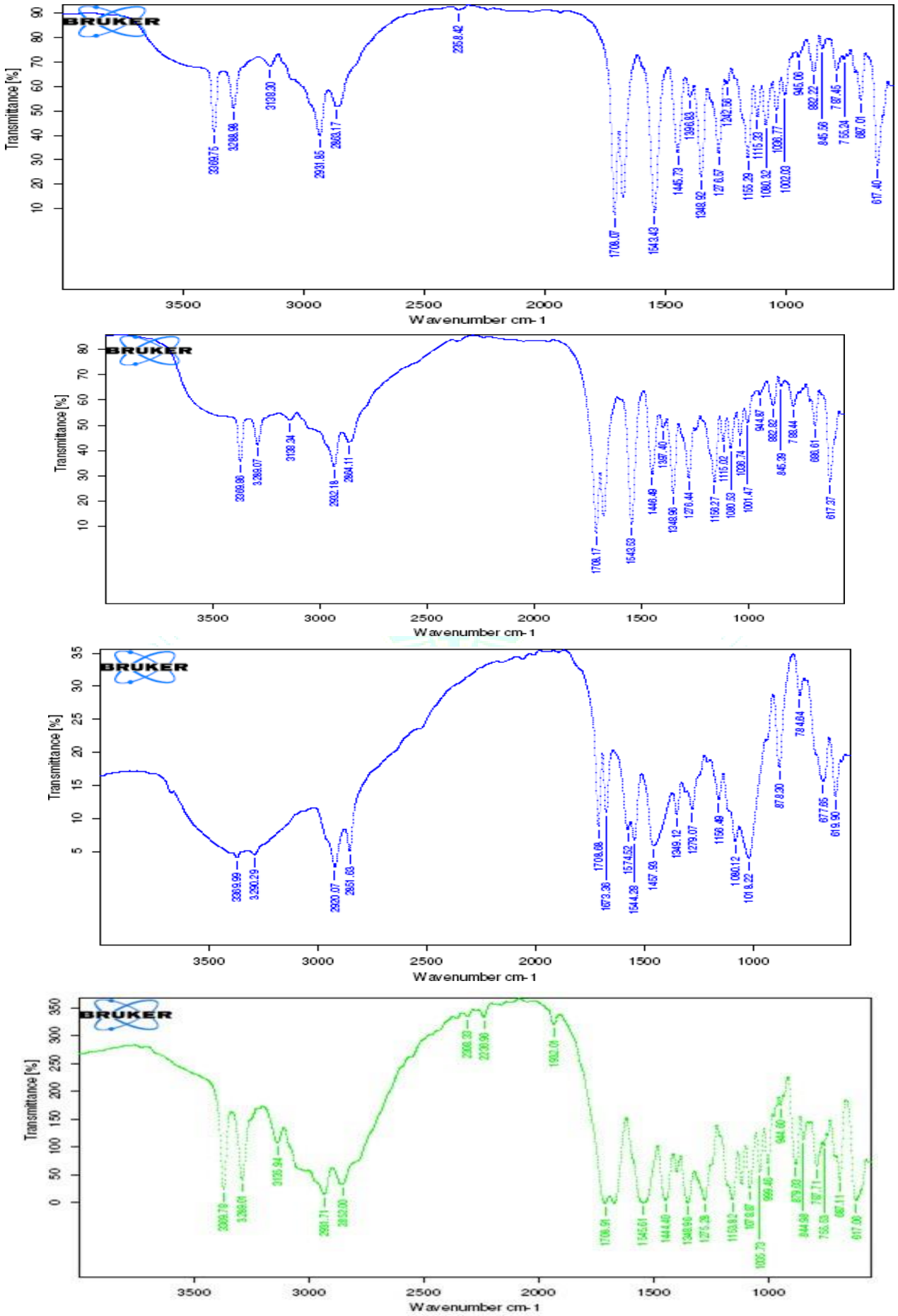
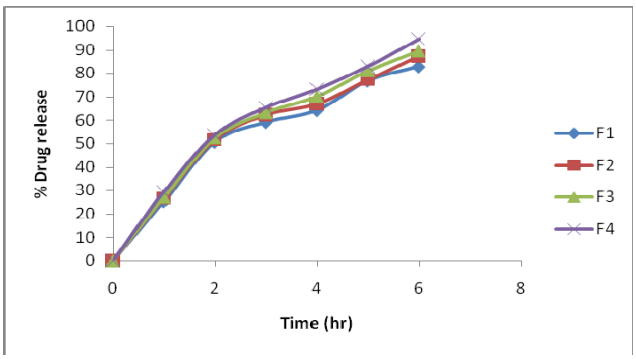
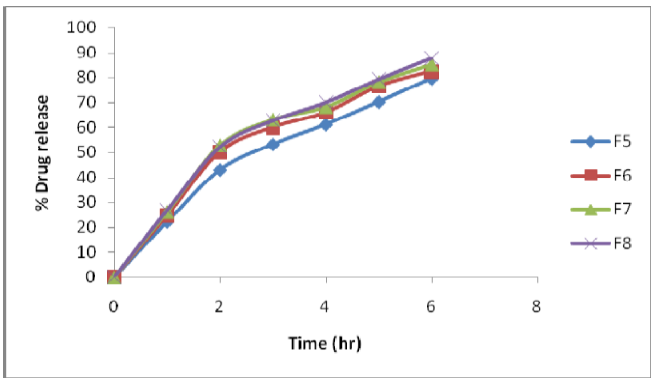
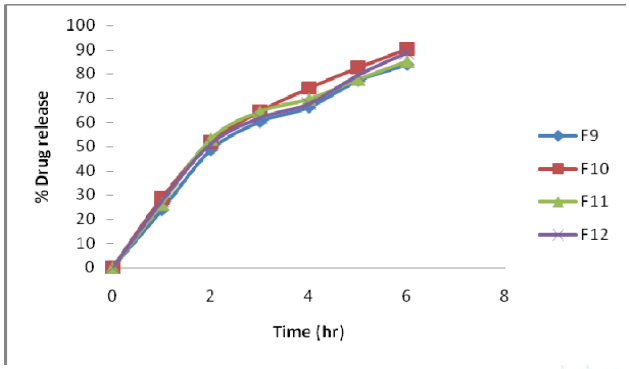
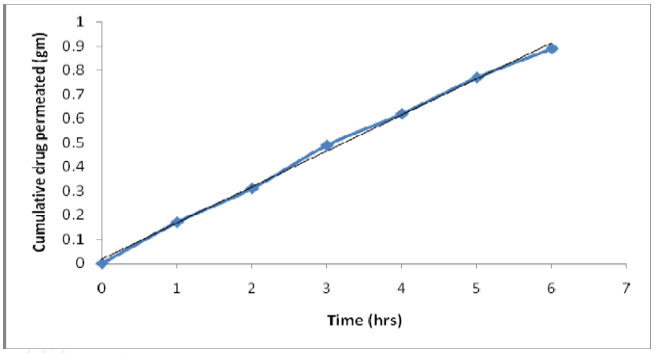


Figure 5 to 8 : FTIR Spectra









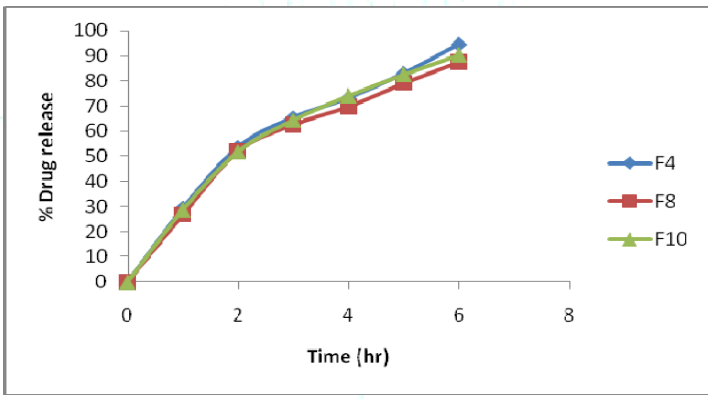


Figure 9 to 13: Drug release kinetics of all the formulations (F1 - F12)

CONCLUSION

It was observed that Carbopol 934P gel containing Tacrolimus in 1:2 ratios (F4) produced better Spreadability and consistency as compared to other formulations. The developed F4 gel showed good homogeneity, suitable pH, no skin irritation and good stability. The maximum percentage of drug release was found to be 98.68% in 6 hours in formulation F4. The drug permeation from optimized formulation i.e. F4 was slow and steady and 0.89 gm of Diclofenac sodium could permeated through rat abdominal skin membrane with a flux 0.071 gm hr^{-1} cm^{-2} and could possibly permeate through human abdominal membrane. The Carbopol 934P forms water washable gel because of its water solubility and has wider prospects to be used as a topical drug delivery system.

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