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FORMULATION AND *IN-VITRO* EVALUATION OF TACROLIMUS HYDROGEL

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ABSTRACT
The aim of the present work was to formulate and evaluate formulation of Tacrolimus as oral hydrogel to improve the efficacy & bioavailability of Tacrolimus. Tacrolimus gel was prepared with Gellan gum and Carbopol 934P as gelling agents with polyethylene glycol as a penetration enhancer. The formulations were examined for pH, spreadability, consistency, viscosity, homogeneity, drug content and stability. In vitro drug release was evaluated using Franz diffusion cell. The viscosity of all formulation follows a pseudo-plastic flow behavior. The pH of formulation Lies in between 6.3to 7.2. The formulation F2 and F5 showed good released pattern. Tacrolimus is used in the treatment of eczema, in particular atopic dermatitis. It suppresses inflammation in a similar way to steroids, and is equally as effective as a mid-potency steroid. An important advantage of tacrolimus is that, unlike steroids, it does not cause skin thinning (atrophy), or other steroid related side effects.

KEYWORDS: Tacrolimus, Gellan gum, antifungal, Rheology, *in- vitro*; Diffusion.

INTRODUCTION
Hydrogels, cross-linked 3D networks of hydrophilic polymer chains, are capable of holding large Amounts of water due to their hydrophilic structure.[1] Thus, the hydrogel networks can extensively swelling water media. Since water is the greatest component of the human body, a hydrogel, which can absorb large quantities of water, is considered to have great potential when applied for biomedical purposes. Recently, wide investigation has been going on into the feasibility of applying hydrogels in fields including tissue engineering, drug delivery, self-healing materials, biosensors, and hemostasis bandages. Compared with other types of biomaterials, hydrogels have the advantages of increased biocompatibility, tunable biodegradability, properly mechanical strength, porous structure, and so on. However, due to the Low mechanical strength and fragile nature of the hydrogels, the feasibility of applying hydrogels is still limited. Thus, novel hydrogels with strong and more stable properties are still needed and remain an important direction for research. As expected, naturally formed hydrogels are gradually replaced by synthetic hydrogels to achieve longer service life, high capacity of water absorption, and high gel strength. Fortunately, with various developed synthetic strategies, hydrogels with defined network structures, desirable chemical compositions, and mechanical strength can be designed. Hydrogels can be prepared from completely artificial components and show remarkable stability even under severe conditions such as high temperature or a very acidic or basic environment. Additionally, by modifying the polymer chains with stimuli-responsive functional groups, the hydrogel properties can be switched by stimuli including heat, light, magnetic fields, chemical agents, and pH. This form of tacrolimus is used on the skin to treat a skin condition called eczema (atopic dermatitis) in patients who have not responded well to (or should not use) other eczema medications Eczema is an allergic-type condition that causes red, irritated, and itchy skin This drug works by weakening the skin's defense (immune) system, thereby decreasing the allergic reaction and relieving the eczema. Tacrolimus belongs to a class of drugs known as topical calcineurin inhibitors (TCIs).

MATERIAL AND METHOD
Material
Tacrolimus was obtained as kind gift sample from Glaxo smith line Pharma Pvt. Ltd.Mumbai., Carbopol 934 was purchased from Vishal-Chem, Mumbai, Gellan gum was purchased from Research-Lab Fine Chem Industries, Mumbai. All other materials used of analytical grades.

Methods
Preparation of Tacrolimus hydrogel
Weight quantity of carbopol and gellan gum were measured and allowed to swell in a beaker for 24hrs.Accurately weight amount of drug solubilized in PEG was then added to soaked polymer solution with stirring; then the solution was neutralized by using Triethanolamine, Glycerin as a moistening agent and

ascorbic acid with methyl paraben as preservative to then formulation.[4]

Table No. 1: Formulation chart of Carbopol and Gellan Gum Batches.

Ingredient	F1	F2	F3	F4	F5	F6	F7
Tacrolimus(mg)	100	100	100	100	100	100	100
Carbopol (mg)	1000	1000	1000	--	---	---	500
Gellan gum (mg)	--	--	--	1000	1000	1000	500
Ascorbic Acid (mg)	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Glcerine (mg)	0.5	1	1.5	--	--	--	1
Polyethylene glycol (ml)	--	---	---	0.5	1	1.5	0.5
Methyl paraben (mg)	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Triethanolamine(ml)	q.s.	q.s.	q.s.	--	--	--	q.s.
Water(ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

EVALUATION OF TOPICAL PREPARATION

The factors to be evaluated for semisolid at various stages of its development are described below. These must be within prescribed specification, and all must remain so over the stated lifetime for the product.

Organoleptic properties

Colour, odour, and appearance may get changed on storage. The change in properties indicates decomposition, so it is necessary to check the organoleptic properties of gel formulation.[5]

pH

The pH of gel is measured, using a pH meter, which was calibrated before each use with standard buffer solution. The electrode was inserting into the sample prior to taking the reading at room temperature. The determinations were carried out in triplicate and the average of three reading is recorded.[6]

Spredability

The spreadability of the gel was determined using the following technique; spreadability was measured on the basis of 'Slip' and 'Drag' characteristics of gels. A ground glass slide was fixed on this block. Anexcess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 Kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. With the help of string attached to the hook and the time (inseconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability

Calculation of spreadability (S) is as follows;

$$S = M \times L / T$$

Where:

S, is the spreadabliy of gel formations

M, is the weight (g) tied on the upper plate,

L, is the length (cm) of the glass plates, and

T, is the time taken for plate to slide the entire length.

Drug content (%)

Accurately weight amount of gel was mixed with methanol and allowed to stand for 24 hrs and filter the solution and making the appropriate concentration is analysed spectrophotometrically to determine percentage drug content.[7]

Viscosity

Time dependent rheological behavior of semisolids may also signal physical or chemical change. The tools for rheological assessments are cone and plate research viscometer, which, in principle, precisely quantify viscosity, or simply utilitarian rheometers, that include extrusion rheometers, penetrometers and Brookfeild viscometers with spindle helically through a semisolid. Increase or decreases in viscosity by any of these measuring tools indicate changes in the structural elements of the formulation. Substantial irreversible rheological changes are a sign of poor physical stability.[8, 9]

Rheological properties

The Rheological property is determined by Brook-field viscometer. hydrogel system containing polymers in various ratio and combinations such as gellan gum and carbopol was prepared and evaluated viscosity in order to identify the composition suitable for hydrogel systems. Many experiments were conducted by varying the concentration of these polymers in order to identify the optimum concentration required for the gel. The hydrogel system containing gellan gum and carbopol are improving the rheology. Rheological properties of the delivery system may be achieved by the addition of viscosity enhancing polymers such as gellan gum and pH. It also helped the gels for its adhesion. All the formulations were behaving as shear thinning systems.[10]

Gelling capacity

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of simulated salivary fluid (pH 6.8) freshly prepared and equilibrated at 37ºC and visually assessing the gel formation and nothing the time taken for the gelation formed and the time taken for the gel formed dissolve. Weight and rate of formation of gel with respect to time. Average of three reading; - No

gelation; + Gel after few minutes, dissolved rapidly; ++ Gelation immediately, remains for few hours; +++ Gelation immediately, remains for extended period.[11]

Extrudability

It is useful empirical test to the measure the force required to extrude the materials from a tube. Since the packing of have a gained a considerable importance in delivery of desired quantity of gel from collapsible tube, therefore measurement of extrudability becomes an important criteria for oral gel. Evaluating gel formulation for extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5cm ribbon of gel in 10 seconds. More quantity extruded better was extrudability. The measurement of extrudability of each formulation was in triplicate and the average values are presented. The extrudability was than calculated by using the following formula:

Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in cm2)

***In-vitro* permeation study**

The cellophane membrane was mounted between the compartments of the Frantz diffusion cell with stratum corneum facing the donor compartment. Reservoir compartment was filled with 10 ml artificial saliva of pH 7.2. Franz diffusion cell with cellophane mounted between compartments. The study was carried out at 37° ± 1°C and speed was adjusted untill the vortex touches the membrane and it carried out for 20mins with the 1 ml of sample was withdrawn from reservoir compartment at 2 min interval and absorbance was measured spectrophotometrically at 230 nm. Each time the reservoir compartment was replenished with the diffusion medium to maintain constant volume.[12]

Accelerated stability study of gel formulation

It is responsibility of manufacturers to see that the medicine reaches the consumer in an active form. Stability of medicinal product may be define as the capability of particular formulation in a specific container to remain with its physical, chemical, microbial, therapeutic and toxicological specification,i.e. stability of drug is ability to resist deterioration. 90% of labelled potency is generally recognized as the minimum acceptable potency level. So stability study was carried out for three months at 40º C/ 75% RH. 14.

RESULT AND DISCUSSION

Evaluation and characterization of Gel

Appearance

The gel formulations were observed for their visual appearance, odour, colour, texture and feel upon application such as grittiness, uniformity, and stickiness of formulation. Result shown in table No. 2.

Table No. 2: physical Evaluation of Gel formulations.

Formulation	Colour	Odour	Consistency	Appearance	Grittiness	Stikiness
F1	White	Characteristic	Good	Smooth	No	None
F2	White	Characteristic	Good	Smooth	No	None
F3	White	Characteristic	Good	Smooth	No	None
F4	White	Characteristic	Good	Smooth	No	None
F5	White	Characteristic	Good	Smooth	No	None
F6	White	Characteristic	Good	Smooth	No	None
F7	White	Characteristic	Good	Smooth	No	None

The values of spreadability indicate that all gels are easily spreadable by applying just a small amountof shear. Result of homogeneity all formulation of Tacrolimus hydrogel posses the homogeneous in nature. pH measurement is very important because the pH of the formulation should not deviate from oral pH The pH of prepared Tacrolimus gel was measured using a pH meter. Result of Gelling capacity are shown in the Table no. 3 grades were allotted (+ + +Excellent, + + Good, + fair)All the gel formulation were also evaluated for spreadability test it was found in the range 53 to 71 mm.the highest and good spreadability of gel F2 gel formulation is 71 mm. and lowest Spreadability value is F6 formulation is 54 mm.

Table No.3: Spreadability, pH, Homogeneity, Extrudability, Drug content of Gel formulations.

Formulation	Spreadability (mm)	Homogeneity	pH	Drug content	Extrudability (g)	Gelling Capcity
F1	68	Homogeneous	6.9	99.92±0.23	165	++
F2	71	Homogeneous	6.9	99.91±0.62	149	++
F3	70	Homogeneous	6.9	98.99±0.51	150	+++
F4	60	Homogeneous	6.9	99.89±0.12	129	++
F5	63	Homogeneous	7.1	99.90±0.27	158	++
F6	54	Homogeneous	7.1	99.91±0.18	162	+++
F7	67	Homogeneous	7.2	99.93±0.48	160	++

Drug content was determined by titrimetric method which was found within the limits. The viscosity of formulated gel was determined. The viscosity determination was carried out by Brook-field viscometer.

Table No. 4: Viscosity of gel (cps).

RPM	F1	F2	F3	F4	F5	F6	F7
1	7498	24220	19121	7499	23450	18121	10987
2	3842	11151	12641	3937	11051	13647	3897
5	2630	7438	4258	2598	7338	4357	2678
10	1507	3637	2354	1577	3530	2458	1687
20	1057	1186	1856	1134	1167	1899	1184
30	653	906	1120	657	950	1124	797
60	409	419	468	450	461	470	580

The viscosity of all formulation follows a pseudoplastic flow behavior. The material flows as soon as shear stress is applied; the slope of the curve gradually decreases with increasing rate of shear. The viscosity was derived from the slope which is found to decrease as the shear rate is increased.

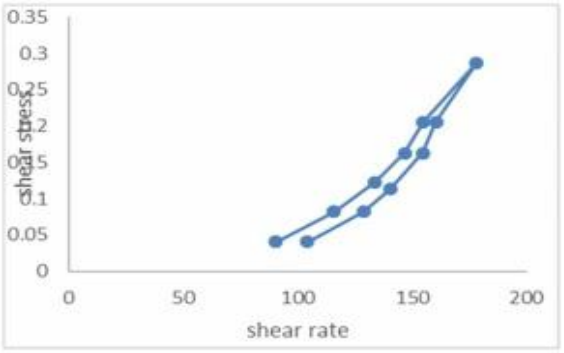

Fig.No.1: Rheogram of F1.

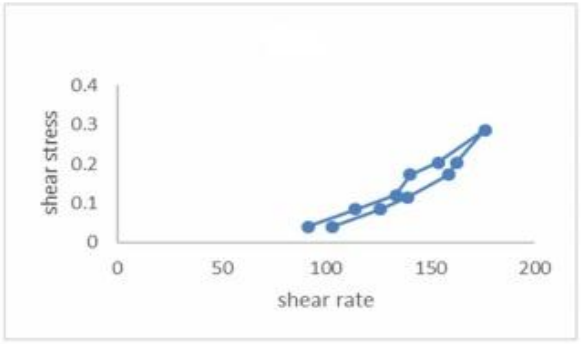

Fig.No.2: Rheogram of F2.

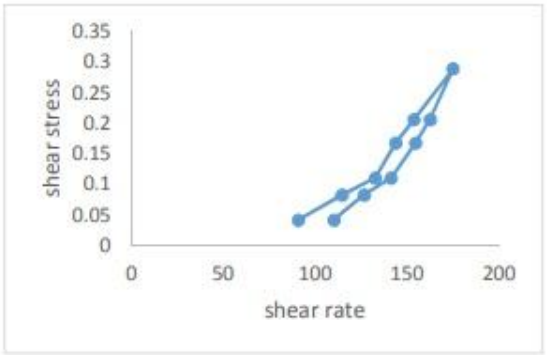

Fig.No.3: Rheogram of F3

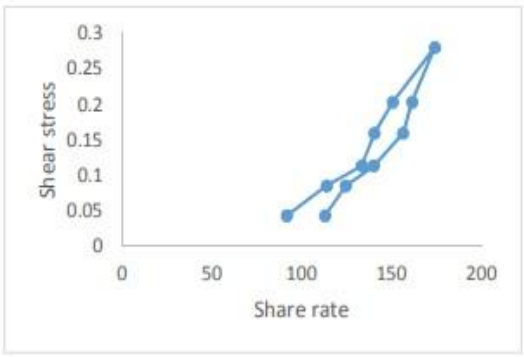

Fig.No.4: Rheogram of F4

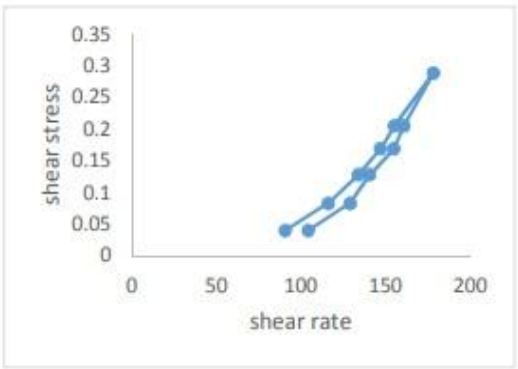

Fig.No.5: Rheogram of F5

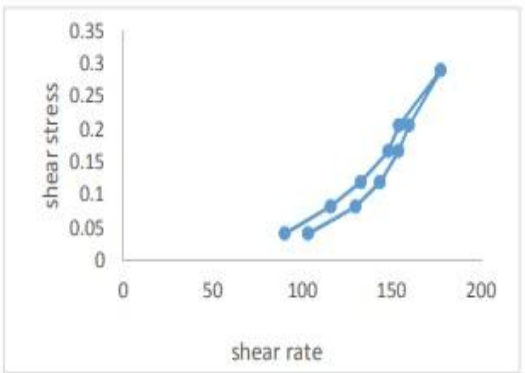

Fig.No.6: Rheogram of F6

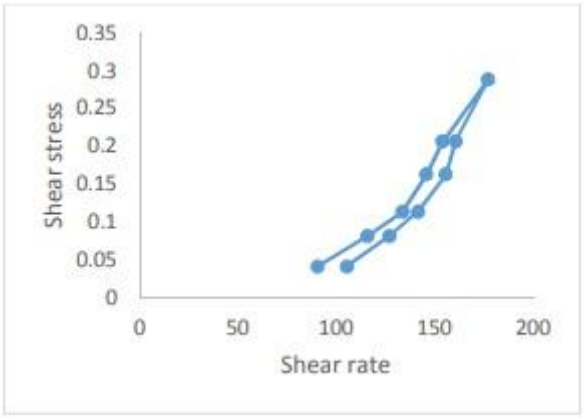

Fig.No.7: Rheogram of F7.

In-Vitro diffusion study

The In-vitro diffusion study was taken by using Franz diffusion cell which shows Cumulative % drug release of Tacrolimus gel formulation was F1-91.84±0.62, F2-94.99±0.16, F3-92.84±0.37, F4-93.62±0.27, F5-95.23±0.39, F6-92.84±0.40 And F7- 92.23±0.12 The formulation F2 and F5 showed good released pattern.

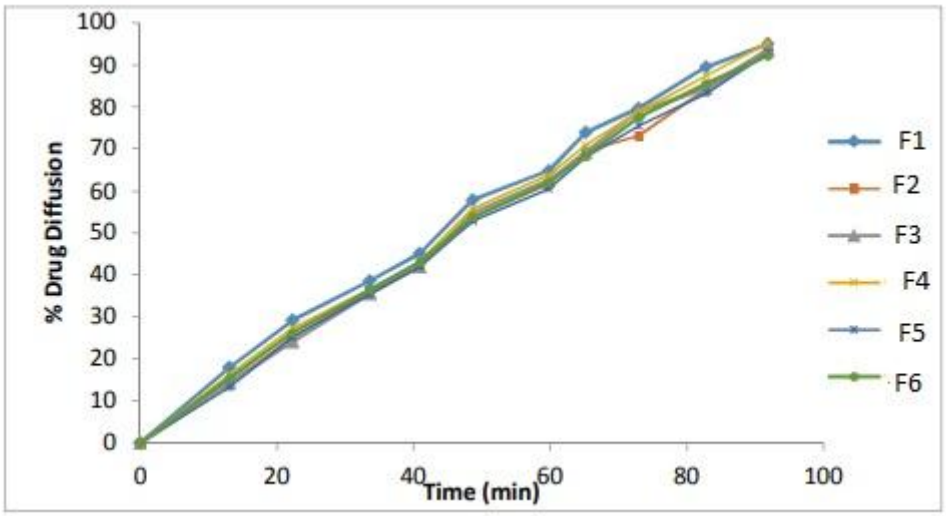

Figure No. 8: In-vitro release of Gel.

Accelerated Stability Testing

The formulation was kept for stability studies there was no significance change observed in physical parameter i.e. (appearance colour change and grittiness) at 40º C±2 / 75%± RH. There was negligible difference in the drug content observed after stability study suggested that all the formulations are stable under the given conditions for 60 days.

Table No. 5: Result of accelerated stability testing.

Days	0	15	30	45	60
Colour	No Change	No Change	No Change	No Change	No Change
Drug Content	99.93 ± 0.48	99.80 ± 0.51	99.23 ± 0.16	98.93 ± 0.16	98.13 ± 0.13
%Drug Release	92.23 ± 0.12	92.13 ± 0.72	91.80 ± 0.52	91.63 ± 0.62	91.23 ± 0.42
Spreadability (mm)	65	66	64	63	62

CONCLUSION

As per the results of the present study showed that the permeation rate of formulation F2 and F5 was enhanced without any significant change in the pH, Viscosity, Spreadability and drug content and Gelling capacity. The F1 and F7 formulation are show the good drug content Tacrolimus hydrogel. It was more worthwhile to evaluate the formulation at the clinical level as a further study.

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