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FORMULATION AND *IN-VITRO* EVALUATION OF POLYETHYLENE GLYCOL BASED ORAL MUCOADHESIVE GELS OF TACROLIMUS MONOHYDRATE

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Keywords:

Tacrolimus monohydrate, Mucoadhesive drug delivery system, PEG, Gantrez S-97, Carbopol 971P and HPMC K15

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ABSTRACT: Oral Lichen Planus (OLP) is a rare chronic autoimmune mucocutaneous inflammatory disease characterized by painful oral lessions. Immunosuppressive agent Tacrolimus monohydrate is one of the effective therapy options for OLP as it possesses benefits such as greater safety and efficacy at lower doses over currently employed topical corticosteroids. From amongst its topical dosage forms, mucoadhesive oral gels of Tacrolimus monohydrate have advantages such as prolonged residence time, enhancement of absorption and therapeutic efficacy compared to the topical ointments. Considering this, the current work was aimed at formulating oral mucoadhesive gels of Tacrolimus monohydrate using PEG gel bases and mucoadhesive polymers. Firstly, non medicated mucoadhesive gels consisting of PEG gel bases and mucoadhesive polymers were formulated and evaluated for their appearance, feel, viscosity, pH, spreadability, tube extrudability and mucoadhesive strength. The combinations of PEG gel bases having PEG4000+PEG400 in 1:4, PEG 4000+PEG400 in 1:8 and PEG 6000+PEG400 in 1:4 were selected for incorporation of Tacroliimus monohydrate (0.1%) due to their ideal performance in maintaining the requisite gel characteristics at 40^0C and 75% RH after incorporation of the mucoadhesive polymers. These medicated gels were then compared to commercially available smile gel as a reference for viscosity, spreadability, tube extrudability and mucoadhesive strength. It was observed that the medicated gel consisting of Tacroliimus monohydrate (0.1%) incorporated to PEG gel bases (PEG4000+PEG400 in 1:4) having Gantrez S-97 as mucoadhesive polymer exhibited maximum drug release and drug content. This gel batch showed prolonged and better *in-vitro* release compared to the marketed protopic ointment.

INTRODUCTION: Oral Lichen Planus (OLP) is a rare chronic autoimmune mucocutaneous inflammatory disease that may cause bilateral white striations, papules or plaques with or without erythema and ulceration involving buccal mucosa [1, 2].

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These painful oral lessions affect the quality-of-life of the diseased individual. Currently topical corticosteroids, such as Clobetasol Propionate (CP) are administered to control symptoms associated with the disease [3, 4].

However, these agents are associated with side effects such as burning sensations at the site of application as well as increased risk of local oral infections such as *Vericella zoster*, *Herpes simplex* and *Eczema herpeticum*. Immunosuppressive agents like Tacrolimus monohydrate owing to their greater safety and efficacy at lower doses can be great alternative to these currently used topical

steroids [5]. Currently, Tacrolimus monohydrate is available for both local and systemic delivery in the form of various dosage forms such as ointments, capsules, granules for oral suspension and micro pellets respectively. Ointments which are a topical dosage form possess benefits of having a better safety profile for adverse effects as they provide local effect with minimal systemic effects. Owing to this, they are preferred over other oral or liquid dosage forms. However, the limitation associated with topical oral ointments is that they lack the mucoadhesion nature that is needed for prolonged retention of the drug at oral mucosa. This can be achieved by formulating them in the form of mucoadhesive drug delivery system. 'Orabase' which is a fast polymerisable liquid monomer is one such base which has been used successfully previously for formulation of mucoadhesive oral delivery systems for various oral diseases [6].

There are also reports on use of orabase (gelatine, pectin, sodium CMC, plasticized hydrocarbon gel) incorporated with 0.1 % tacrolimus for treatment of oral lichen planus and oral lichen lesions. The mucoadhesion provided by the or abase offers number of advantages such as prolonged residence time at the site of absorption, probable enhancement of absorption and thereby, the therapeutic efficacy, because of high vascularity of oral mucosa, increased drug bioavailability due to avoidance of first pass metabolism, protection of drug from degradation in the acidic environment of GIT, improved patient compliance and ease of drug administration. With these advantages under consideration, the present work was an attempt to develop mucoadhesive oral gel of Tacrolimus monohydrate (0.1% concentration) using PEG bases as an alternative to the currently available or abase gel base. PEG bases are biodegradable hydrogels that offers advantages such as biocompatibility and a mixed hydrogel system can be formulated by incorporating other mucoadhesive polymers to them. Mucoadhesive topical oral Tacrolimus gels have not been formulated previously using these PEG gel bases. This work was thus aimed at formulating tacrolimus oral gels (0.1%) by incorporating hydrophilic gelling polymers such as Gantrez S-97, Carbopol 971P and HPMC K15 to different combinations of PEG bases (having molecular weights 6000, 600, 4000, 400). Sweetening agents such as saccharin sodium and sodium cyclamate were added to the formulations to improve patient compliance. Besides improved patient compliance, the formulation is expected to improve local residence time thereby increasing bioavailability and diminishing side effects. Accordingly, PEG based oral mucoadhesive gels of Tacrolimus monohydrate were formulated using PEG gel bases and mucoadhesive polymers such as Gantrez S-97, Carbopol 971P, HPMC K15 and HPMC K100M. Saccharin sodium and sodium cyclamate were added as sweetening agents. Firstly, non medicated mucoadhesive gels consisting of PEG gel bases and mucoadhesive polymers were formulated and evaluated for their physical characteristics such as appearance and feel, viscosity, pH, spreadability, tube extrudability and mucoadhesive strength.

From amongst these different non medicated formulations, combinations of PEG gel bases having (PEG4000+PEG400 in 1:4, PEG 4000+PEG400 in 1:8 and PEG 6000+PEG400 in 1:4) exhibited ideal performance in maintaining the requisite gel characteristics at 40^0C and 75% RH and were thus selected for incorporation of Tacroliimus monohydrate (0.1%) and one of the three mucoadhesive polymers from Gantrez S-97, Carbopol 971P and HPMC K15 to them. Commercially available oral ulcer gel (smile gel) was used as a reference to compare viscosity, spreadability, tube extrudability and mucoadhesive strength in these PEG based gels of Tacroliimus monohydrate. Besides this, all these PEG based mucoadhesive gel batches of Tacroliimus monohydrate were also compared to marketed oral ointment formulation (protopic ointment) of Tacrolimus monohydrate for their *in-vitro* release performance. It was observed that all the gel batches exhibited prolonged and better *in-vitro* release compared to the marketed oral ointment formulation (protopic ointment) with the gel of Tacroliimus monohydrate containing 0.1% drug incorporated to combination of PEG gel bases (PEG4000+PEG400 in 1:4) with Gantrez S-97 exhibiting maximum drug release and drug content for 45 days.

MATERIALS AND METHODS: Tacrolimus monohydrate was purchased from S.V. Chemicals, Mumbai, India and PEG – 6000, PEG – 600, PEG – 4000, PEG – 400 were purchased from Oxford

Laboratory, Mumbai. Gantrez S-97, Carbopol 971P, HPMC K15, Saccharin sodium, Sodium cyclamate were obtained from Cadila Pharmaceuticals Ltd., Ahmedabad. Benzyl alcohol, Methyl paraben and Propyl paraben were obtained from Merck Specialities Private Ltd, Mumbai.

Study of Interaction between Drug and Excipient:

Fourier Transform Infrared Spectrometry (FTIR): FTIR spectra of pure Tacrolimus monohydrate and its physical mixture with each individual polymer (Carbopol 971P, Gantrez S-97, HPMC K15M, PEG 4000 and PEG 6000) in 1:1 proportion was obtained using Fourier Transform Infrared Spectrometry (FTIR) in order to investigate any possible interactions between the drug and the polymers used. The samples of pure drug and its mixture with the polymers used were prepared using KBr disks compressed under a pressure of 10 Ton/nm^2. The samples were scanned from 4000 to 400cm^{-1}. The change in spectra of the drug compared to its spectra in the presence of polymer was observed which indicates physical interaction of drug molecule with the polymer.

Differential Scanning Colorimetry (DSC): Thermal analysis of pure drug and its mixture with the polymers was carried out using a differential scanning calorimeter (Mettler Toledo DSC 822). For this, firstly the samples were placed in an aluminium sealed pan and preheated to 200°C. After this, the sample was cooled down to room temperature and then reheated from 40° to 450°C at a scanning rate of 10°C/min [9].

Formulation of Mucoadhesive Medicated and Non Medicated Gel Bases: In order to formulate Tacrolimus 0.1% mucoadhesive gels, we systematically approached by firstly formulating a PEG gel base using different types (400, 600, 4000 and 6000) and ratios (1:2, 1:4, 1:6, 1:8) of PEG bases followed by formulation of non medicated mucoadhesive gels using optimized PEG gel batches and incorporation of mucoadhesive polymers to them. Finally, medicated mucoadhesive gels were formed after incorporation of Tacrolimus 0.1% to the optimized non medicated mucoadhesive gels.

Formulation of PEG Gel Bases using Different Types (400, 600, 4000 and 6000) and Ratios (1:2, 1:4, 1:6, 1:8) of PEG Bases: For this, appropriate quantities of different PEG grades were melted separately in 250ml beaker at their respective melting temperatures. The molten masses were cooled to room temperature and the bases were evaluated for viscosity. Subsequently, the mixtures of PEGs grades were also used and characterized for viscosity. The **Table 1** lists the composition of these PEG gel bases and their codes.

TABLE 1: FORMULATION CODES AND COMPOSITIONS OF GEL BASES CONTAINING DIFFERENT TYPES AND RATIOS OF PEGS

Sr. no.	Formulation code	Type of base	Qty.(gms)
1	P1	PEG 400	50
2	P2	PEG 600	50
3	P3	PEG 4000	50
4	P4	PEG 6000	50
5	P1P3G1	PEG 4000 +PEG400(1:2)	50
6	P1P3G2	PEG 4000+PEG400(1:4)	50
7	**P1P3G3**	PEG 4000 +PEG400(1:6)	50
8	P1P3G4	PEG 4000 +PEG400(1:8)	50
9	P2P4G1	PEG 6000 +PEG 600(1:2)	50
10	P2P4G2	PEG 6000 +PEG 600(1:4)	50
11	P2P4G3	PEG 6000 +PEG 600(1:6)	50
12	P2P4G4	PEG 6000 +PEG 600(1:8)	50
13	P2P3G1	PEG 4000+PEG 600(1:2)	50
14	P2P3G2	PEG 4000+PEG 600(1:4)	50
15	P2P3G3	PEG 4000+PEG 600(1:6)	50
16	**P2P3G4**	PEG 4000+PEG 600(1:8)	50
17	P1P4G1	PEG 6000 +PEG 400(1:2)	50
18	**P1P4G2**	PEG 6000 +PEG 400(1:4)	50
19	P1P4G3	PEG 6000 +PEG 400(1:6)	50
20	P1P4G4	PEG 6000 +PEG 400(1:8)	50

Based on the viscosity results, we optimized gel bases with P1P3G3, P2P3G4, P1P4G2 for incorporation of mucoadhesive polymers and all other excipients except the drug in them.

Formulation of Non-Medicated Mucoadhesive Gel Bases: To prepare mucoadhesive gel bases using PEG 400 and PEG 4000 bases, firstly accurately weighed required quantities of mucoadhesive polymers (Carbopol 971P or HPMC K15 or Gantrez S-97) were dispersed with continuous stirring in the appropriate quantity of PEG 400. The dispersion was then heated to 70-75°C and was cooled to about 40-45°C. Following this, preservatives were added to the semisolid mass. Finally, the high viscosity grade PEG 4000 was melted and was added to the semisolid gel mass with continuous stirring until solidification **Table 2**.

TABLE 2: COMPOSITION AND FORMULATION CODE OF MUCOADHESIVE GEL BASES CONTAINING PEG 4000 AND PEG 400 (1:6)

Sr. no.	Ingredients	A1	A2	A3	B1	B2	B3	D1	D2	D3
1	Carbopol 971P	0.5	1.0	2.0	-	-	-	-	-	-
2	HPMCK 15M	-	-	-	3.0	4.0	5.0	-	-	-
3	Gantrez S-97	-	-	-	-	-	-	1.0	2.0	3.0
4	Methyl paraben	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
5	Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6	Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
7	Benzyl alcohol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
8	Saccharin sodium	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
9	Sodium cyclamate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
10	PEG Base P1P3G3	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%

Similar procedure was followed for preparation of mucoadhesive gel bases containing combinations of PEG 4000 with PEG 600, PEG 6000 with PEG 400 and PEG 6000 with PEG 4000 respectively **Table 3, 4** and **5** respectively.

TABLE 3: COMPOSITION AND FORMULATION CODE OF MUCOADHESIVE GEL BASE CONTAINING PEG 6000 AND PEG 600 IN (1:6)

Sr. no.	Ingredients	A1	A2	A3	B1	B2	B3	D1	D2	D3
1	Carbopol 971P	0.5	1.0	2.0	-	-	-	-	-	-
2	HPMC K15M	-	-	-	3.0	4.0	5.0	-	-	-
3	Gantrez S97	-	-	-	-	-	-	1.0	2.0	3.0
4	Methyl paraben	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
5	Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6	Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
7	Benzyl alcohol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
8	Saccharin sodium	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
9	Sodium Cyclamate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
10	PEG Base P2P3G4	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%

TABLE 4: COMPOSITION AND FORMULATION CODE OF MUCOADHESIVE GEL BASE CONTAINING PEG 6000 AND PEG 400 (1:8)

Sr. no.	Ingredient	A1	A2	A3	B1	B2	B3	D1	D2	D3
1	Carbopol 971P	0.5	1.0	2.0	-	-	-	-	-	-
2	HPMCK 15M	-	-	-	3.0	4.0	5.0	-	-	-
3	Gantrez S97	-	-	-	-	-	-	1.0	2.0	3.0
4	Methyl paraben	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
5	Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6	Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
7	Benzyl alcohol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
8	Saccharin sodium	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
9	Sodium Cyclamate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
10	PEG Base P1P4G2	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%

TABLE 5: COMPOSITION AND FORMULATION CODE OF MUCOADHESIVE GEL BASE CONTAINING PEG 600 AND PEG 4000 (1:8)

Sr. no.	Ingredient	A1	A2	A3	B1	B2	B3	D1	D2	D3
1	Carbopol 971P	0.5	1.0	2.0	-	-	-	-	-	-
2	HPMCK 15M	-	-	-	3.0	4.0	5.0	-	-	-
3	Gantrez S97	-	-	-	-	-	-	1.0	2.0	3.0
4	Methyl paraben	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
5	Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6	Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
7	Benzyl alcohol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
8	Saccharin sodium	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
9	Sodium Cyclamate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
10	PEG Base P1P4G2	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%	q.s.to 100%

Evaluation of Oral Mucoadhesive Gel Bases:

Appearance and Feel: All the oral mucoadhesive gel bases were observed visually for appearance, colour and checked for any grittiness.

Viscosity[8]: Viscosities of prepared oral mucoadhesive gel bases were recorded using programmable Brookfield viscometer (RVDV-II Pro). The sufficient quantity of gel mass was filled in the beaker so that quantity is sufficient to dip the spindle (No.91). The rpm of the spindle was adjusted to 2.5-100 rpm. The viscosities (cps) of the gels were recorded at different rpm.

pH [7]: The pH of all oral mucoadhesive gel bases was determined using previously calibrated pH meter.

Spreadability [8]: For this, two glass slides (5x2cm) were placed one above the other. The surface of lower slide was divided into number of squares ($0.25cm^2$) using marker **Fig. 1**.

About 1g of each oral mucoadhesive gel base was placed in central square of lower glass slide. The second slide was placed over it so as to press the gel and spread it over the surface of lower slide.

The comparative spreadability was noted based on the total area of the marked portion on lower slide covered by spreaded mass. The results obtained are average of three determinations.

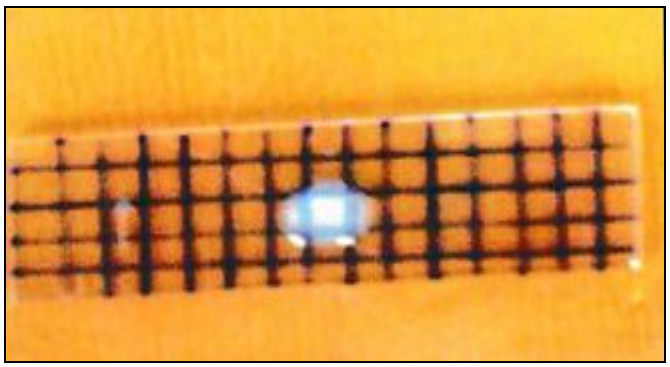

FIG. 1: DEVICE USED FOR DETERMINATION OF SPREADABILITY OF GELS

Tube Extrudability[7]: Extrudability of the prepared oral mucoadhesive gel bases was determined using an extrudability apparatus [9]. A closed collapsible tube containing formulation was pressed firmly at the crimped end **Fig. 2**. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 seconds was determined. The average extrusion pressure in grams was reported.

FIG. 2: DEVICE USED FOR MEASUREMENT OF TUBE EXTRUDABILITY OF GELS

Mucoadhesive Strength [10, 11]: For this a device was fabricated in the laboratory **Fig. 3.**

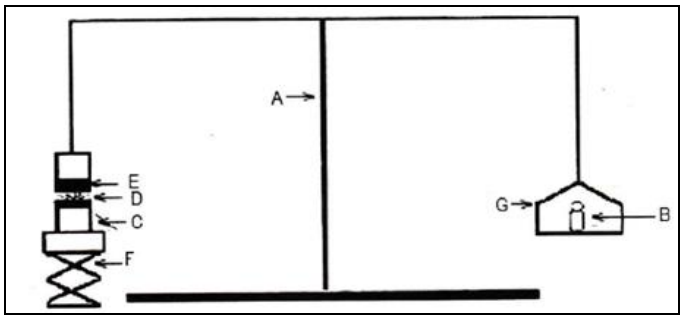

FIG. 3: SET UP USED FOR MEASUREMENT OF MUCOADHESIVE STRENGTH OF GELS

It consisted of vial (C) placed on a height adjustable pan (F) and one vial (E) connected to the balance (A). The height of the vial (C) could be adjusted so that the gels could be placed between the mucosal tissues placed between the two vials. The weights (B) could be placed in the pan (G).

Procedure for Determination of Mucoadhesive Strength: The mucoadhesive potential of each gel formulation was determined by measuring the force required to detach the gel formulations from over the mucosal tissue. A section of oral mucosa was cut using scissor from the oral cavity of goat obtained from the local slaughterhouse, soaked in simulated saliva solution and instantly secured with mucosal side out onto each glass vial (C) using a rubber band and an aluminium cap. The vial was placed on a height-adjustable pan (F). The vials with the oral tissues were stored at 36.5°C for 10 min. Next, one vial (E) with a section of tissue was connected to the balance (A). Gels (D) were applied onto the mucosal tissue on surface of other vial. The weights (B) were kept raised until two vials were attached. Mucoadhesive force in the form of detachment stress (dyne/cm^2), was determined from the minimal weights that could detach two vials. The oral mucosa pieces were changed for each measurement. Detachment stress (dyne/cm^2) = m × g/A where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as (980 cm/s^2) and A is the area of tissue exposed. Measurements were repeated three times for each of the gel preparations, but before each measurement, application of fresh smooth gel was ensured.

Formulation of Medicated Mucoadhesive Gels Containing Tacrolimus Monohydrate (0.1%) using Selected Gel Bases: To prepare medicated mucoadhesive gels, Tacrolimus monohydrate (0.1%) was added to the combination of PEG base and mucoadhesive polymers (Carbopol 971P or HPMC K15 or Gantrez S-97). In order to prepare mucoadhesive gel containing Tacrolimus monohydrate, firstly (0.1%) of Tacrolimus monohydrate was dissolved in small quantity of selected gel base and the solution was dispersed in accurately weighed quantity of mucoadhesive gel bases. The preservatives were added to the semisolid mass and the viscosity adjusted using solid grade PEG. The medicated gels were congealed with continuous stirring. The medicated gels were assigned new formulation codes depending upon the mucoadhesive polymer used as seen in **Table 6** below.

TABLE 6: COMPOSITIONS OF GELS CONTAINING TACROLIMUS MONOHYDRATE (%W/W) AND P1P3G3 AND P2P4G3 AS GEL BASES

Sr. no.	Name of Ingredients	P1P3G3A3	P1P3G3B3	P1P3G3D3	P2P4G3A3	P2P4G3B3	P2P4G3D3
1	Tacrolimus monohydrate	0.1	0.1	0.1	0.1	0.1	0.1
2	Carbopol 971P	1.5	-	-	1.5	-	-
3	HPMCK15M	-	5.0	-	-	5.0	-
4	Gantrez S97	-	-	3.0	-	-	3.0
5	Methyl paraben	0.20	0.20	0.20	0.20	0.20	0.20
6	Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02
7	Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1
8	Benzyl alcohol	1.0	1.0	1.0	1.0	1.0	1.0
9	Saccharin sodium	0.3	0.3	0.3	0.3	0.3	0.3
10	Sodium cyclamate	3.0	3.0	3.0	3.0	3.0	3.0

Evaluation of Medicated Gels Containing Tacrolimus Monohydrate: The prepared mucoadhesive oral gels of Tacrolimus monohydrate was evaluated for following parameters.

Viscosity: For this, 50 gm of medicated gels was taken and the viscosity was determined by following the same procedure as that described for gel bases.

Appearance and Consistency: The medicated gels were observed visually for appearance and checked for air entrapment, grittiness if any.

pH: The pH values of medicated gels were determined by following the same procedure as that described for non medicated gels.

Spreadability: For this, 1 gm of medicated gels was taken and spreadability were determined by following the same procedure described for non medicated gels.

Extrudability: For this, 25 gm of medicated gels was taken and their extrudability was determined by following the same procedure as that described for non medicated gels.

Mucoadhesive Strength: For this, 2 gm of medicated gels was taken and their mucoadhesive strength was determined by following the same procedure as that described for non medicated gels.

Drug Content Assay [12]: The content of Tacrolimus monohydrate in prepared gels was determined by dissolving accurately weighed quantity (10 g) of gel equivalent to 10 mg of drug in 10 ml of acetonitrile.

The final volume was made up to 100 ml and 5 ml of this solution was further diluted to 25 ml with acetonitrile. The absorbances of the solutions were recorded using UV/ visible spectrophotometer at previously recorded λ_{max} of Tacrolimus monohydrate.

Diffusion (*In-vitro*) of Tacrolimus Monohydrate from Oral Mucoadhesive gel [13]: For this, Keshary-Chien diffusion cell was used **Fig. 4.**

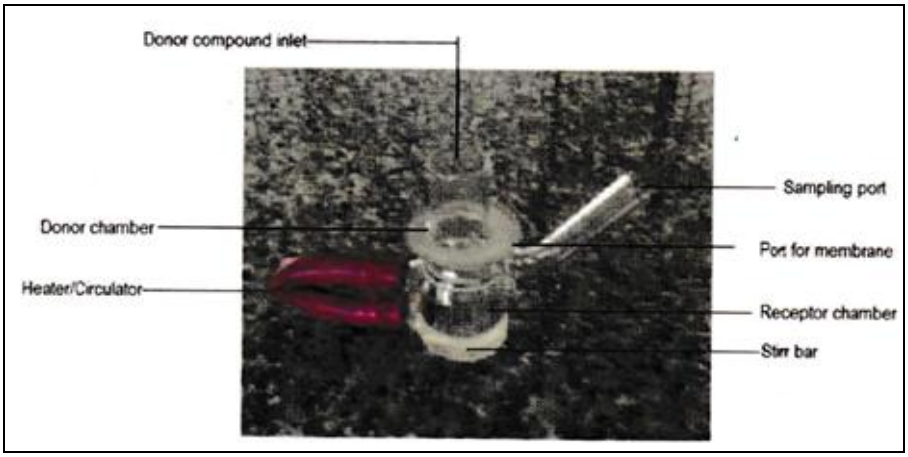

FIG. 4: KISCHERY CHAIN DIFFUSION CELL USED FOR DETERMINATION OF *IN-VITRO* DIFFUSION OF TACROLIMUS MONOHYDRATE

Diffusion Studies using Synthetic Membrane: For this, synthetic membrane was used. It was soaked in simulated saliva solution for 1h before use. The membrane was secured on the top of receptor compartment, 1gm of gel was spreaded evenly over the exposed surface of synthetic membrane affixed to the receptor compartment using a good quality rubber and finally the donor compartment and receptor compartment were fixed using good quality rubber. The entire surface of mucous membrane was in contact with the receptor compartment containing approximately 10ml of simulated saliva solution. The receptor compartment was continuously stirred using a sonicator. The study was carried out for 6 h. The samples were withdrawn (5ml) at predetermined period of time and same volume was replaced with fresh phosphate buffer 6.8. The absorbances of withdrawn samples were measured at previously recorded λ_{max} of Tacrolimus monohydrate.

Diffusion Studies using Biological Membrane: For this, synthetic dialysis membrane was replaced with freshly excised oral mucosal membrane of goat, and the studies were carried out using the same procedure as described for synthetic membrane.

Accelerated Stability Studies: Gel formulations with requisite viscosity, spreadability, adhesion, contents of Tacrolimus monohydrate, release (*in-vitro*) were selected for stability studies. The formulations were stored at ambient conditions and evaluated for appearance, pH, viscosity, spreadability, tube extrudability, adhesion and drug contents and *in vitro* diffusion at the interval of 15 days.

RESULTS AND DISCUSSION:

Drug Excipient Interactions:

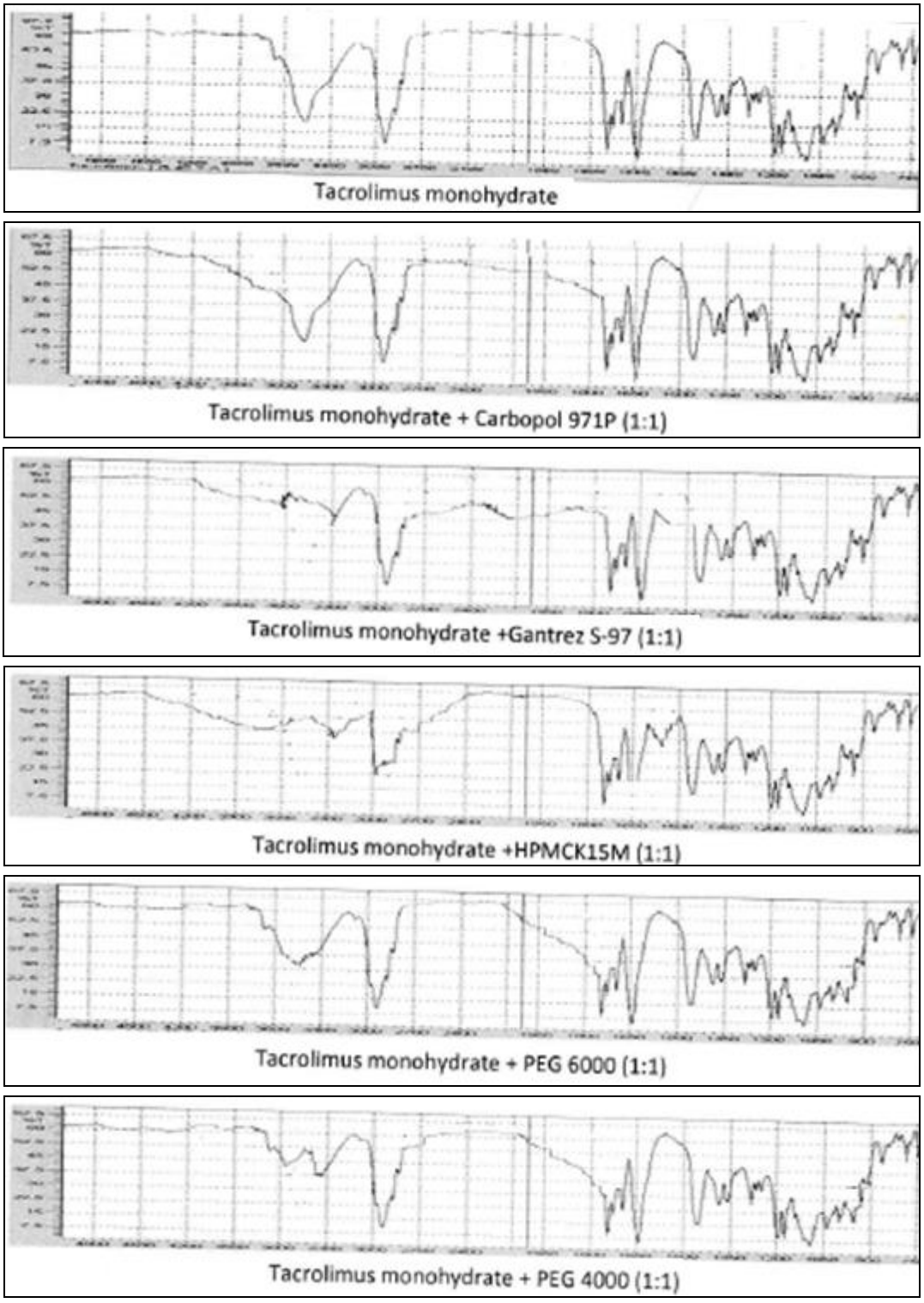

FIG. 5: FTIR SPECTRA OF TACROLIMUS MONOHYDRATE AND ITS PHYSICAL MIXTURES WITH EXCIPIENTS (1:1)

Fourier Transform Infrared Spectroscopy (FTIR): From the obtained FTIR spectrum of pure drug Tacrolimus monohydrate (a) and its physical mixtures with potential excipients at 1:1 (b-d) revealed presence of all major peaks of Tacrolimus monohydrate at the same positions in case of its mixtures with the excipients as those were observed in the spectrum of pure drug as seen in **Fig. 5**. This is suggestive of probable absence of any major interaction of drug with any of the excipients.

Differential Scanning Calorimetry (DSC): DSC thermo grams of Tacrolimus monohydrate retained the same positions of endotherm with Carbopol 971 P, HPMCK15M and Gantrez S-97 as seen in **Fig. 6**. Broadening of peak of Tacrolimus monohydrate in presence of PEG 6000 and PEG 4000 may be due to change in crystalline nature of drug or due to probable dissolution of drug.

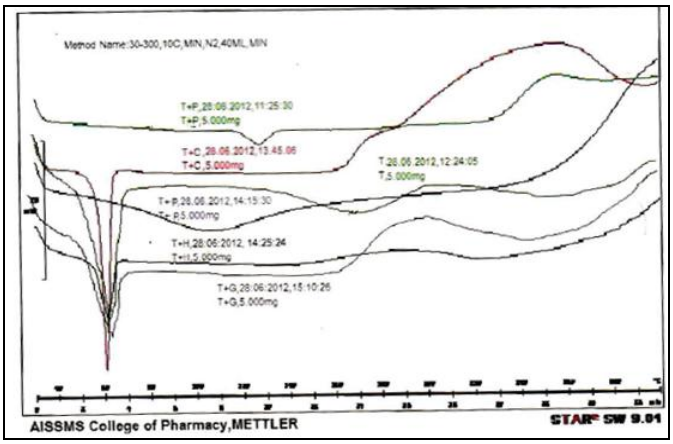

FIG. 6: DSC THERMOGRAM OF TACROLIMUS MONOHYDRATE AND ITS PHYSICAL MIXTURES WITH EXCIPIENTS (1:1)

Evaluation of Medicated and Non-Medicated Mucoadhesive Oral Gel Bases:

Evaluation of Mucoadhesive Oral Gel Bases:

Appearance and Consistency: It was observed that gels containing Carbopol 971P, Gantrez S-97 were off white and smooth non gritty, while those containing HPMC K15M were creamish and smooth non gritty. The appearance of gels remained same irrespective of the concentration of polymers; there was increase in viscosity while the consistency decreased at highest polymer concentration. It is obvious that increasing all the polymers concentration led to a decrease in the flow index. This result was in accordance with what was reported by Sharma *et al* [14] who reported that the consistency depends on the ratio of the solid fraction, which produces the structure, to the liquid fraction and that there was an exponential decrease in the flow index value by increasing the polymer concentration to a constant minimum value ultimately decreasing the consistency of gels. This constant minimum value is reached after the formation of full structured three-dimensional polymer lattices due to increased polymer concentration.

Viscosity: The mucoadhesive gel bases possessed almost similar viscosities to those of marketed gel **Table 8.**

Viscosity is related to the mechanical and physical properties such as spreadability, consistency of the gel which in turn is related to ease of product removal from container, ease of application and product feel on the application site. The apparent viscosity values measured for different gels depicted variation in viscosity for different polymers. This may be attributed to variation in shape and dimensions of crystallites of different polymers and their ordering in the three-dimensional structures within the resulting network where the liquid phase is held by adsorption, capillarity and molecular interaction mechanisms [15]. Besides this, with an increase in concentration of polymers *viz*. Carbopol 971P (0.5-1%), Gantrez S-97 (1-3%) and HPMC K15M (3-5%), the viscosity increased. This may be attributed to the increase in cross linking with increase in concentration of polymers that offers higher resistance to flow. It was observed that the viscosities of selected mucoadhesive oral gel bases were very close to that of marketed gels. Hence, these 12 bases were evaluated further.

TABLE 8: VISCOSITIES (CPS) OF ORAL MUCOADHESIVE GEL BASES AT DIFFERENT RPM

Sr. no.	Batch code	Viscosities (cps) at different rpm					
		2.5 rpm	5 rpm	10 rpm	20 rpm	50 rpm	100 rpm
1	Smile Gel	198640	87430	49650	31360	18530	9580
2	P1P3G3A1	147350	68710	35560	24760	13650	6480
3	P1P3G3A2	168490	79470	37610	26540	15390	8010

4	P1P3G3A3	194760	88510	47980	30890	18770	9470
5	P1P3G3B1	156620	70850	37760	27820	14680	7520
6	P1P3G3B2	175170	82380	40830	29440	17530	8630
7	P1P3G3B3	196210	86480	48990	32640	18990	9480
8	P1P3G3D1	159720	71790	38280	28530	16020	7730
9	P1P3G3D2	180870	87370	41650	28960	17930	8850
10	P1P3G3D3	197930	87590	49850	31460	18130	9520
11	P2P4G3A1	156130	77310	36750	26820	14440	7370
12	P2P4G3A2	177430	79990	38810	27330	16820	8630
13	P2P4G3A3	190320	89730	46670	31650	18990	9550
14	P2P4G3B1	166660	80440	36360	29670	16580	7800
15	P2P4G3B2	184270	83340	40760	29020	17690	8420
16	P2P4G3B3	194400	87280	47650	30870	18780	9330
17	P2P4G3D1	153740	72320	37610	27980	16470	7270
18	P2P4G3D2	188860	86850	43420	28260	17640	8790
19	P2P4G3D3	195560	87240	48790	33210	18650	9670
20	P2P3G4A1	153420	76590	37580	26240	14940	7350
21	P2P3G4A2	176620	79820	37650	27730	16940	8740
22	P2P3G4A3	190870	88970	47720	32420	18970	9750
23	P2P3G4B1	165760	80760	36660	29830	16980	7900
24	P2P3G4B2	185610	83680	40820	29170	17820	8920
25	P2P3G4B3	194400	87280	47650	30870	18780	9330
26	P2P3G4D1	153740	72320	37610	27980	16470	7270
27	P2P3G4D2	188860	86850	43420	28260	17640	8790
28	P2P3G4D3	193480	89940	48590	35120	18650	9680
29	P1P4G2A1	158810	76780	38420	27820	15640	7470
30	P1P4G2A2	186780	80730	39760	29780	16840	8890
31	P1P4G2A3	192010	87670	48620	33410	19230	9880
32	P1P4G2B1	165250	81520	37810	30840	16780	7860
33	P1P4G2B2	187540	84320	41480	29760	17550	8890
34	P1P4G2B3	195600	87880	47690	31470	18780	9440
35	P1P4G2D1	154570	72450	37870	27850	16930	7830
36	P1P4G2D2	187770	87220	41230	27360	18840	8660
37	P1P4G2D3	194540	88960	47850	35350	18870	9790

pH: The pH value of selected oral mucoadhesive gel bases varied between 7.2-7.3. The normal pH range of saliva is 5.5-7.0 and the oral mucoadhesive gel bases possessed the pH nearing to the neutral value. Hence, the bases would cause minimal or no local irritation of oral mucosal sites. Peppas and Buri have demonstrated that some cationic polymers, such as carbopol and those exhibiting hydrophilic nature give superior mucoadhesive properties, especially in a neutral medium [16]. This may be the probable reason for good mucoadhesion of Carbopol 971P and Gantrez S-97 at neutral pH.

TABLE 9: SPREADABILITY AND EXTRUDABILITY ORAL MUCOADHESIVE GEL BASES

Sr. no.	Batch code	Spreadability (cm)	Extrudability	Mucoadhesive strength(dyne/cm^2)
1	Smile Gel	3.0	255±0.2	9554.14
2	P1P3G3A3	3.2	257±0.4	9554.14
3	P1P3G3B3	3.0	258±0.3	3184.71
4	P1P3G3D3	3.2	262±0.4	9554.14
5	P2P4G3A3	3.2	264±0.5	9554.14
6	P2P4G3B3	3.1	256±0.3	6369.42
7	P2P4G3D3	3.2	255±0.6	9554.14
8	P2P3G4A3	3.1	262±0.4	6369.42
9	P2P3G4B3	3.1	258±0.2	3184.71
10	P2P3G4D3	3.2	257±0.4	6369.42
11	P1P4G2A3	3.1	256±0.5	9554.14
12	P1P4G2B3	3.0	255±0.4	6369.42
13	P1P4G2D3	3.2	261±0.2	9554.14

Spreadability and Extrudability of Gel Bases: All oral mucoadhesive gel bases possessed almost similar spreadability and extrudability characteristics **Table 9.** The selected mucoadhesive oral gel bases had acceptable spreadability and extrudability close to that of marketed gel. Hence, they were characterized by mucoadhesive strength.

Mucoadhesive Strength of Oral Mucoadhesive Gel Bases: The selected oral gel bases possessed adhesive strength between 6369.42 dyne/cm^2-9554.14 dyne/cm^2 **Table 9.** At physiological pH the mucus network carries a negative charge (due to the sialic acid and sulphate residues) which may play a role in mucoadhesion. Formation of hydrogen bonds between the hydrophilic functional groups of mucoadhesive polymers and the mucus layer or the mucosal surface is a prerequisite for extensive and longer mucoadhesion. Carbopol971P, cationic in nature and is capable of developing additional molecular attractive forces by electrostatic interactions with negatively charged mucosal surfaces or negatively charged sialic acid groups of the mucus network. The extent of mucoadhesion is dependent on the formation of hydrogen bonds between hydroxyl groups of Gantrez S-97 and HPMC K15M. The increased sites for bond formation may explain the increase in mucoadhesion in case of Gantrez S-97. Likewise electrostatic attractions in case of Carbopol 971P are stronger than that of hydrogen bonds in HPMC K15M but weaker than those of Gantrez S-97. Hence, the adhesive strength was in the order Gantrez S-97>Carbopol 971P>HPMC K15M. Based on characteristics of oral mucoadhesive gels, out of twelve only six bases having following attributes were selected for incorporation of drug Tacrolimus monohydrate (0.1%w/w). They were further compared with two marketed formulations *viz.* Smile gel, Protopic ointment.

1. Appearance and feel- Off white and smooth non gritty
2. Viscosity-190000-195000 cps
3. pH-7.2-7.3
4. Spreadability-3-3.5 cm
5. Extrudability- "+++" (Excellent)
6. Mucoadhesive strength-9554.14dyne/cm^2

Characteristics of Medicated Gels:

Appearance and Consistency: All medicated gels were off white and smooth non gritty.

Viscosity: All medicated gels possessed almost similar viscosities close to that of marketed gels **Table 10.**

pH: All medicated gels were almost neutral.

It was observed that all the gel formulations had pH in the range of 7.0 to 7.5 i.e. neutral in nature, which is within the range of the salivary pH (5.5 to 7.0). Hence, it can be concluded that drug did not alter the pH of gels and the gels may not produce any local irritation to the oral mucosa.

Spreadability and Extrudability: All medicated gels possessed almost similar spreadability and extrudability as that of marketed gel **Table 10.**

Mucoadhesive Strength: All medicated gels possessed excellent mucoadhesive strength almost similar to that of marketed gel **Table 10.**

The mucoadhesive strength of gels increased with increase in concentration of mucoadhesive polymers and the gels containing Carbopol 971P and Gantrez S-97 demonstrated excellent adhesion close to that of marketed gel.

This may be due to enhanced cross linking with increase in concentration of polymers, which resulted in the greater resistance to the detachment of gels from mucous membrane ultimately increasing bioadhesion strength at higher polymer concentration.

TABLE 10: CHARACTERISTICS OF MEDICATED GELS

Sr. no.	Batch code	Viscosity(cps)	Spreadability (cm)	Extrudability	Mucoadhesive strength (dyne/cm^2)	Drug content (%)
1	Smile Gel	198640	3.2	262±0.3	9554.14	
2	Protopic ointment	186750	2.8	257±0.4	9554.14	97.2
3	P1P3G3A3	194830	3.1	263±0.5	9554.14	97.1
4	P1P3G3D3	196970	3.3	258±0.3	9554.14	97.7
5	P2P4G3A3	191220	3.0	255±0.2	9554.14	96.7
6	P2P4G3D3	196570	3.2	262±0.3	9554.14	96.2

7	P1P4G2A3	192120	3.3	258±0.5	9554.14	96.6
8	P1P4G2D3	195350	3.2	254±0.5	9554.14	95.8

Assay: The % content values of Tacrolimus monohydrate of all gel formulations indicated uniform distribution of drug and the contents were compatible with those in marketed ointment product of drug **Table 10.**

Diffusion (*In-vitro*) of Tacrolimus Monohydrate: Through Synthetic Membrane: All medicated gels indicated constant diffusion of Tacrolimus monohydrate over 6 hrs using synthetic membrane. However, the diffusion from gels was faster than marketed ointment due to obvious reasons of difference in viscosity, mucoadhesive strength etc. The gels with composition of Tacrolimus monohydrate were slightly superior to other composition **Fig. 5.**

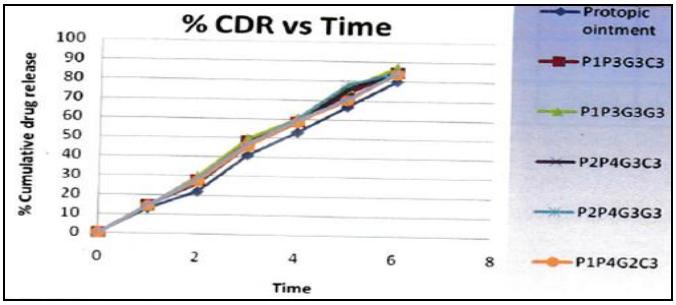

FIG. 7: DIFFUSION (*IN VITRO*) OF TACROLIMUS MONOHYDRATE THROUGH SYNTHETIC MEMBRANE

Through Biological Membrane: All medicated gels indicated constant diffusion of Tacrolimus monohydrate over 6 hrs using biological membrane. However, the diffusion from gels was faster than marketed ointment due to obvious reasons of difference in viscosity, mucoadhesive strength etc. The gels with composition of Tacrolimus monohydrate were slightly superior to other composition **Fig. 6.**

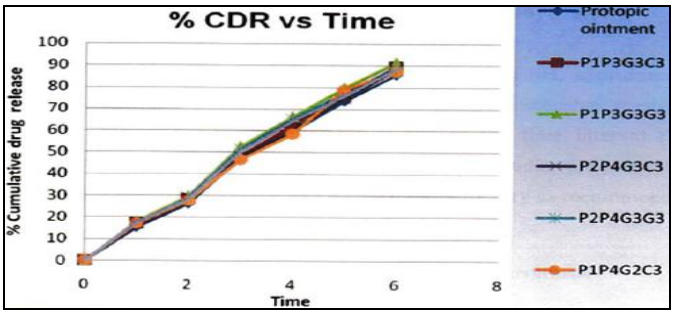

FIG. 8: DIFFUSION (*IN VITRO*) OF TACROLIMUS MONOHYDRATE THROUGH BIOLOGICAL MEMBRANE

It was observed that, increased polymer concentrations were accompanied by a corresponding decrease in the percent of Tacrolimus monohydrate diffused through membrane. The difference in Tacrolimus monohydrate release after 6 h was significant when comparing low and high polymer concentration. This might be due to the fact that at the higher polymer concentrations, the active substance is trapped in polymer chains, and it is structured by its close proximity to those polymer molecules thus increasing the diffusional resistance. Also, the density of chain structure which has been observed in gels microstructure increased at the higher polymer concentration and this limited the movement of the drug [17, 18, 19]. Moreover, as the polymer concentration increased, viscosity increased as well. Thus, decrease in the release could be attributed to increased micro viscosity of the gel by increasing polymer concentration [20, 21].

Besides, the diffusion of Tacrolimus monohydrate through mucous membranes was faster than through the synthetic membrane. The enhanced diffusion through latter may be attributed to the penetration enhancing effect of PEG's used in gels.

Based on evaluation of medicated gel batches, the batch P1P3G3D3 demonstrated maximum drug release and drug content, for 45 days. Hence the samples were stored at ambient conditions and evaluated for appearance, pH, viscosity, spreadability, tube extrudability, adhesion and drug contents and *in-vitro* diffusion at the interval of 15 days. This batch was charged for stability for 45 days.

Stability of Optimized Gel Formulation of Tacrolimus Monohydrate (P1P3G3D3)2: The gel formulation did not indicate gross changes in pH, appearance, consistency, viscosity, spreadability, tube extrudability, mucoadhesive strength, % drug content and cumulative % drug release at any of the time interval. However, more evidence is needed. Hence further studies are needed to be conducted at exaggerated conditions of temperature and humidity as recommended by ICH.

CONCLUSION: The formulated gels were evaluated for various physicochemical and performance characteristics and compared with marketed ointment of Tacrolimus monohydrate. The effect of type of mucoadhesive polymer on viscosity, *in-vitro* release and *in-vitro* dissolution of gel were studied. The selected gel formulation was subjected to stability studies at accelerated conditions of temperature and humidity (40°C and 75% RH) over a period of 45 days.

From the findings of various physical and chemical tests it was found that all gelling polymers and mucoadhesive polymers were compatible with Tacrolimus monohydrate. All the developed oral mucoadhesive gels exhibited prolonged release of drug *in-vitro* for 6 h.

From the three different mucoadhesive polymers used, Gantrez S-97 exhibited optimal characteristics of viscosity, spreadability, mucoadhesive strength when used for formulation of mucoadhesive oral gels. All gel formulations exhibited better *in-vitro* release compared to marketed topical ointment formulation.

In-vitro release studies of Tacrolimus monohydrate gels using biological membrane indicated that diffusion through biological membrane is superior to that through the synthetic membrane. This may be due to probable enhancement of permeation of Tacrolimus monohydrate through biological membrane. The gel of Tacrolimus monohydrate with PEG 4000 and PEG 400 in 1:6 ratio and Gantrez S-97(3%w/w) could maintain the requisite gel characteristics at 40°C and 75% RH.

The *in-vitro* diffusion of Tacrolimus monohydrate demonstrated almost constant diffusion over a period of 6 h. To the best of our knowledge, this work is a first attempt to formulate PEG based mucoadhesive gels and should lay a platform for further formulation research in this area.

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