Formulation Consideration and skin retention-permeation study of insitu nanogel containing dimethylfumarate for treatment of psoriasis.

ABSTRACT:

Aims/Objective: to develop and evaluate an insitu nanogel formulation containing dimethylfumarate for targeted topical delivery therapy of psoriasis.

Study Design: 3^2 full factorial design

Place and Duration of Study: Department of Pharmaceutics, Parul Institute of Pharmacy and Research, Parul University, Vadodara, between 2016 to 2019.

Methodology: Nanogel were formulated by chemical cross linked gel method using Polyvinyl alcohol and Hyaluronic acid (1:5) ratio using Glutaraldehyde (GA) (25 %w/v) and Hydrochloric acid (HCl) (6%v/v) as a crosslinking agent and catalyst. Dimethylfumarate loaded nanogel were clear and showed physicochemical parameters desired for topical delivery and stability.

Results: The Permeation profile of dimethylfumarate through rat skin from selected nanogel formulation exhibited highest skin uptake. The Micoscopic observations indicated that the optimized nanogel had n significant effect on the microscopic structure of the sin and epithelial cells appered mostly unchanged. The surface epithelium lining and the granular cellular structure of the skin were totally intact. The developed Nanogel may be a potential drug delivery vehicle for targeted topical delivery of dimethylfumarate in the treatment of psoriasis.

Conclusion: As per drug retention study the highest amount of drug retained on the skin and lowest amount of drug permeate to the skin. Hence it was observed that there was no significant correlation between skin retention and skin permeation study.

Keywords: *Dimethylfumarate, Psoriasis, Skin retention and Permeation study, Topical delivery.*

1. INTRODUCTION

1.1 Psoriasis:

Skin inflammation may be a most typical drawback in medicine. they are available in several forms, from occasional rashes among skin itch and redness, to chronic conditions like eczema, roscea, dermatitis and skin disorder.

Skin inflammation may be characterised by 2 types:

1) Acute inflammation: that comes from exposure to UV radiation, Contact with chemical irritants, radiation and allergens
2) Chronic inflammation results from a sustained immune cell mediate inflammatory response among skin itself. This inflammation is long lasting and might cause important and heavy tissue destruction.

The process of skin inflammation is complicated and continues to be not utterly understood. once the skin is exposed to a “triggering” stimulant, like UV radiation, associate pain (e.g. soaps or fragrances), or to allergens, the cells within the skin manufacture a range of inflammatory “hormones” known as cytokines and chemokines. These “inflammatory messengers” bind to specific receptors heading in the right direction cells and stimulate the assembly of further inflammatory sign “hormones”. a number of this cause dilation whereas others activate nerve cells. Still alternative cytokines cause immune cells to go away the blood and migrate into the skin wherever they then manufacture additional inflammatory hormones, likewise as enzymes, free radicals, and chemicals that harm the skin. the tip results of the initial triggering event is that the amplification of an oversized inflammatory response that, whereas designed to assist the skin fight infection from incursive microorganism, really causes hefty harm to the skin [1].

Psoriasis may be a long-lived disease characterised by patches of abnormal skin. These skin patches area unit usually red, itchy, and scaly. they will vary in severity from tiny and localized to complete body coverage. Injury to the skin will trigger psoriatic skin changes at that spot, that is understood as Koebner development.

There area unit 5 main forms of psoriasis: plaque, guttate, inverse, pustular, and erythrodermic.

Plaque skin disorder, conjointly called skin disorder vulgaris, makes up concerning ninetieth of cases. It usually presents with red patches with white scales on high. Areas of the body most typically affected area unit the rear of the forearms, shins, round the belly button, and therefore the scalp.

Guttate skin disorder has drop-shaped lesions.

Pustular skin disorder presents with tiny non-infectious pus-filled blisters.

Inverse skin disorder forms red patches in skin folds. Erythrodermic skin disorder happens once the rash becomes terribly widespread, and might develop from any of the opposite varieties. Fingernails and toenails area unit affected in the general public at some purpose in time. this could embody pits within the nails or changes in nail color.

Psoriasis is usually thought to be a inherited disease that is triggered by environmental factors. In twin studies, twins area unit 3 times additional seemingly to each be affected compared to non-identical twins; this implies that genetic factors incline to skin disorder. Symptoms usually worsen throughout winter and with bound medications like beta blockers or NSAIDs. Infections and psychological stress may additionally play a job. Skin disorder isn't contagious. The underlying mechanism involves the system reacting to skin cells. Identification is often supported the signs and symptoms.

Psoriasis is treated by Topical agent, radiation, Systematic agent.

Disadvantages of that treatment:

When mistreatment topical agent like calciferol with steroids. because of calciferol mistreatment long run with steroids will occur haptic sensation, rubor, sunburn, poikiloderma , its caused irritation by rubbing or applying by any agent.

Phototherapy mistreatment UV Radiation that cause broken desoxyribonucleic acid. because of PUVA treatment will occur birth defects and liver harm.

Progressive multifocal brain disorder happens because of general agents.

Surgery

Limited proof suggests removal of the tonsils might profit individuals with chronic plaque skin disorder, guttate skin disorder, and palmoplantar pustulosis[2].

Alternative medical aid

Uncontrolled studies have advised that people with skin disorder or rheumatism might get pleasure from a diet supplemented with animal oil wealthy in omega-3 fatty acid (EPA) and docosahexaenoic acid (DHA). Conflicting proof exists indicating that there could also be associate accrued incidence of skin disorder in individuals with disorder. Psoriatic sickness severity shriveled once three months of a protein free diet in patients with anti-gliadin antibodies.

1.2 Dimethylfumarate: Dimethyl fumarate is associate immunological disorder agent. Dimethyl fumarate degradation to its active substance monomethyl fumarate (MMF) then MMF up-regulates the Nuclear issue (erythroid-derived a pair of)-like 2 (Nrf2) pathway that's activated in response to aerobic stress. phase III clinical trial clinical trials found that DMF (BG-12) with success reduced relapse rate and accrued time to progression of incapacity in sclerosis (trade name Tecfidera). DMF is believed to possess immunomodulatory properties while not important immunological disorder.[3]

Disadvantages:

Dimethyl fumarate is obtainable in Oral capsules forms

- more time needed for drug absorption.
- Nausea
- Vomiting
- Abdominal Pain
- Diarrhea.

Hyaluronic acid (HA) is one among the foremost vital topical carriers for the localized delivery of medication to the skin and conjointly performs as a drug delivery agent for ophthalmic, nasal, pulmonary, duct and topical routes of administration [4]. HA acts as mucoadhesive, holding to the drug at specific website of action/absorption. It can also modify the in vivo release/absorption rate of the therapeutic agent, once applied locally, to localize delivery of drug to the cuticle [5].

Poly(vinyl alcohol) (PVA) may be a water soluble perishable artificial compound with smart biocompatibility, and it will with chemicals cross-linked to make hydrogels helpful in pharmaceutical formulations [6].

The in place nanogel primarily based topical spray formulation will deliver the drug speedily on inflamed website while not irritation and might win higher drug defense potency. [7,8,9].

In situ nanogel is extremely potent, biocompatible, perishable aside from topical formulations with low toxicity. It conjointly sprays through fast swelling property that converts in to gel formation through higher drug loading capability to extend binding drug with compound, will increase drug therapeutic efficaciousness, sustain unleash of drug in vivo, reduces facet effects and improves patient compliance than alternative formulations [10].

In situ nanogel relies topical spray unleashes medication speedily within the sort of nano sized droplets on cuticle layer that regenerate answer into gel type and release drug. an instantaneous and fast dispersion of an

answer of a vigorous agent over as an oversized portion as potential of the skin layer, that absorbs the active. during this means, an oversized space would be reached, thereby fast absorption of the active.

Towards of this, A Nanogel is nanoparticle as composed to a colloidal gel – a cross connected deliquescent compound network. It may be most frequently composed of artificial polymers or biopolymers that area unit with chemicals or physically cross connected and area unit typically within the 10 to many nanometres in diameter. wish to like of this hydrogels, the pores in nanogels may be stuffed with tiny molecules or macromolecules and their properties, like swelling, degradation and chemical practicality, may be controlled [11]. The formulation of in place nanogel is outlined as sol to gel transition at the factual of administration within which the formation will happens because of lyophilized nanogel become gel by sorb fluid at skin disorder site.[12]

As it has been initiated and ready by chemical cross connected gels by mistreatment mucopolysaccharide and polyvinyl alcohol, in presence of chemical cross linker (glutaraldehyde)[13,14]

Chemical cross connected in place nanogel converts into lyophilized powder. This lyophilized powder hundreds topical spray directly on the inflamed skin that provides dispersion to gel formation through contact of skin disorder skin and drug unleash to start out on broken stratum layer.

In advantage purpose of read, the topical spray may be a safe and effective treatment and use to regulate itch with allergic eczema. It conjointly releases medication speedily within the sort of lyophilized powder on cuticle layer and gets regenerate into gel upon application. an instantaneous and fast dispersion of a lyophilized powder of the active over as giant portion as potential of the skin layer, that absorbs the active. during this means, an oversized space would be lined, thereby fast absorption of the active [15].

The objective of gift investigation is to develop and characterize in place nanogel primarily based topical spray for the treatment of skin disorder. The investigation in the main centered on development of in place nanogel dose type that may simply administer through topical spray for the treatment of skin disorder.

1.3 3^2 Full Factorial design:

To optimize the batches, we proceeded by experimental design, which consists in the arrangement of experiments in the design space in such a way that gives reliable and consistent result with minimum number of experiments. It relies on a well-established statistical tool such as factorial design; this technique was effective method of indicating the relative significance of a number of variables and their interactions.

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses. The number of experiments required for the studies is dependent on the number of independent variables selected. The response is measured for each trial.

$$\mathbf{Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_{12}X_1X_2+\beta_{11}X^2+\beta_{22}X^2+\varepsilon}$$

Where, Y is the dependent variable,

β_0 = is the arithmetic means response of the nine run and

β_i = is the estimated coefficient for the factor X_i.

The main effects (X1 and X2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X1X2) showed how the response changes when two factors are simultaneously changed.

A 3^2 randomized full factorial design was utilized in the present study. Two factors were evaluated, each at three levels, to further carry out nine experimental trials as all possible combinations.

2. MATERIALS AND METHOD

2.1 Materials:

Dimethyl fumarate (DMF) pharmaceutical grade were kindly supplied as gift sample by adventus laboratories pvt. Ltd. Makarpura, Vadodara. Polyvinyl alcohol (PVA) were purchased from chemdyes corporation, Vadodara, hyaluronic acid (HA) were purchased from sigma Aldrich. Glutaraldehyde as a chemical cross linker was purchased from sigma Aldrich. Ethanol was purchased from Sisco Research Lab. Ltd, Mumbai, India. Menthol was purchased from aatur chemicals, Vadodara. PVP K30, PEG 6000, propylene glycol was purchased from sigma Aldrich, Vadodara. Mannitol was purchased from sigma Aldrich, Vadodara.

2.2 Methodology:

2.2.0 Method of Preparation of *in situ* nanogel:

Dimethyl fumarate containing *In situ* Nanogel was prepared by chemical cross linked gel method.

Dimethyl fumarate *insitu* nanogel was prepared by the following steps using chemical crosslinked gel method. In the process 120 mg dimethylfumarate (dose defined as per Tecfidera tablet) was dissolved in 10 ml distilled water. Hyaluronic acid (0.5%w/v, 1%w/v, 1.5%w/v) was dissolved in distilled water at room temperature for 1 hr on magnetic Stirrer and Polyvinyl alcohol (PVA) (Different concentration like 2.5% w/v, 5%w/v, 7.5%w/v) were dissolved in distilled water at 50ºC on magnetic stirrer for 12 hour (1 hour for dissolve and further 11hr to make homogeneous solution). 1ml of DMF Solution was added in 5ml of Hyaluronic acid solution and 3ml PVA Solution was mixed and the mixed solution was stirred at room temperature for 2 hrs. PVA was cross linked in the presence of HA, using 1ml Glutaraldehyde (GA) (25 %w/v) and 0.2 ml Hydrochloric acid (HCl) (6%w/v) as a crosslinking agent and catalyst.

2.2.1 Viscosity measurement:

This is an important parameter for the in situ nanogel. Viscosity was measured using Brookfield viscometer employing Spindle (T-shaped spindle) rotated at 5 rpm. Calculate Viscosity of Insitu nanogel dispersion were determined with Brookfield viscometer.

2.2.2 Particle size measurement and zeta potential:

Particle size and zeta potential were measure by using Malvern Zetasizer. Switch on the instrument and allow it to stabilize for 30 minutes. Clean the disposable zeta cuvette. Fill the sample in cuvette and load the sample in the instrument. Open new file in software and create new SOP for sample analysis. Start the measurement for sample. Check the quality of measurement and interpret the data. The results of zeta potential and particle size are shown in **figure 4.28, 4.29 respectively**.

2.2.3 Gelling Time:

Insitu nanogel gelling time was measured by using stopwatch. adding of Glutaraldehyde as chemical crosslinker in HA-PVA polymer solution with drop of Hydrochloric acid as catalyst. After addition of glutaraldehyde gelling time was measured for insitu nanogel.

2.2.4 In vitro skin permeation study on rat skin:

Pretreated skin of rat will used in the Franz diffusion cell experiment. The receptor compartment contained 100 ml of phosphate buffer pH 5.5 One gram of the test formulation or reference will applied to the skin over an area of 1.131 cm^2 and placed across the donor compartment. The donor cell will expose to ambient temperature and covered with parafilm to prevent evaporation. The temperature of the diffusion medium will maintained at 37 ± 1°C while the buffer solution was stirred continuously with magnetic stirrer at 500 rpm. Samples (1 ml each) were withdrawn from the release medium at 20, 40, 60 min and replaced with an equal volume of fresh buffer solution to maintain sink conditions. The samples were analyzed by HPLC as per below method for Dimetyl fumarate[16].

Table 1. HPLC Method References of dimethyl fumarate:

Name	DMF
ReferenceMethod	DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUSESTIMATION OF DIMETHYL FUMARATE AND ONDANSETRON
Column	C18 column (250×4.6 mm, 5 µm)
MobilePhase	Methanol: Acetonitrile: PhosphateBuffer with pH 5.5 (50:20:30 v/v/v)
WavelengthUsed	239nm (As reported in Reference Method)

Table 2. Chemical Details used for HPLC method of dimethyl fumarate:

Sr. No	Name of Chemical/Reagent	Grade	Make
1	Methanol & Acetonitrile	HPLC	*Merck Specialties Pvt Ltd*, Mumbai
2	Water	HPLC	*Milli – Q Water Purification System*
3	Potassium dihydrogen phosphate (KH_2PO_4) & Dipotassium hydrogen Phosphate (K_2HPO_4)	AR	Spectrochem
4	Tri-ethyl amine	AR	Merck

2.2.5 Drug retention & Drug permeation analysis Using Reference Method of DMF, Initial trial for Standard Solution was taken with following chromatographic conditions as reported in the Ref. Method:

- **Column:**

Column: Lichrospher 100, C18 (250X4.6 mm, 5µ).

- **Flow rate:**

1.0 mL/min.

- **Wavelength:**

239 nm

- **Mobile Phase:**

Methanol: Acetonitrile: Phosphate Buffer with pH 5.5 (50:20:30 v/v/v).

- **Selection of Sample Concentration:**

Label claim of DMF is 120mg in Proposed Dosage Formulation, so concentrations were decided as 120µg/mL for DMF.

2.2.5.1 Mobile Phase Preparation:

Volume of 500 mL HPLC grade Methanol, Volume of 200mL Acetonitrile was mixed with 300mL phosphate buffer, prepared by dissolving 13.61 gm of potassium dihydrogen phosphate (Solution I) and 35.81gm of disodium hydrogen phosphate (Solution II) in 1000 mLof Millipore water and then mix 96.4mL of (Solution I) and 3.6mL of (Solution II), filtered with 0.45µ filter paper and sonicated for 10 mins. Mobile phase was used as diluent. Diluent Preparation: Mobile phase is used as diluent.

2.2.5.2 Diluent:

Mobile Phase was used as a Diluent.

2.2.5.3 Standard Injection:

2.2.5.3.1 Standard Injection was injected in above chromatographic conditions.

Initially DMF was analyzed so as to check the Reference Method.

Once good peak of DMF was observed, Samples were injected to analyses Drug Retention and Drug Permeation.

2.2.5.3.2 Preparation of DMF standard solutions: (120µg/mL)

2.2.5.3.2.1 Preparation of Stock Solution:

Accurately 12 mg of DMF was weighed into a clean and dry 50mL volumetric flask separately dissolved with sufficient volume of diluent. The final volume was made up to 50mL with diluent to get the concentration of 1200µg/mL for DMF.

2.2.5.3.2.2 Preparation of working standard solution of DMF:

5 mL of standard stock solution was pipetted out into 10mL volumetric flask and further diluted withdiluent to 10mL to get concentration of 120µg/mL for DMF.

This solution was injected and chromatogram was recorded as below. Stock and Final solutions were stored at 2-8°C conditions for further use in next trials.

2.2.8 Histopathological study:

Skin retention study was performed in order to analyse the content of the drug in the skin. At the end of the in vitro skin permeation study, the skin samples were washed with water and methanol on both sides and carefully dried. Then a defined amount of methanol was added to each piece of skin. The samples were vortexes for 10 min in order to extract its drug content and stirred overnight. [17].

3. RESULTS AND DISCUSSIONS

3.1 Viscosity:

Viscosity was measured using Brookfield viscometer employing Spindle (T-shaped spindle) rotated at 5 rpm. Calculated Viscosity was found for 7 centipoise insitu nanogel.

Discussion:

Viscosity is main characteristic in preparation of *Insitu* nanogel formulation. Viscosity shown that gel behaviour.

3.2 Particle size measurement:

Particle size was measured by Malvern zetasizer. Results of particle size of optimized batch B_{13} are shown in **Table 3 and Figure 1.**

Table 3. Results of evaluation of optimized batch:

Batch	Particle size (nm)	PDI Mean±SD
B_5	290 nm ±1.2 nm	0.213 ±0.15

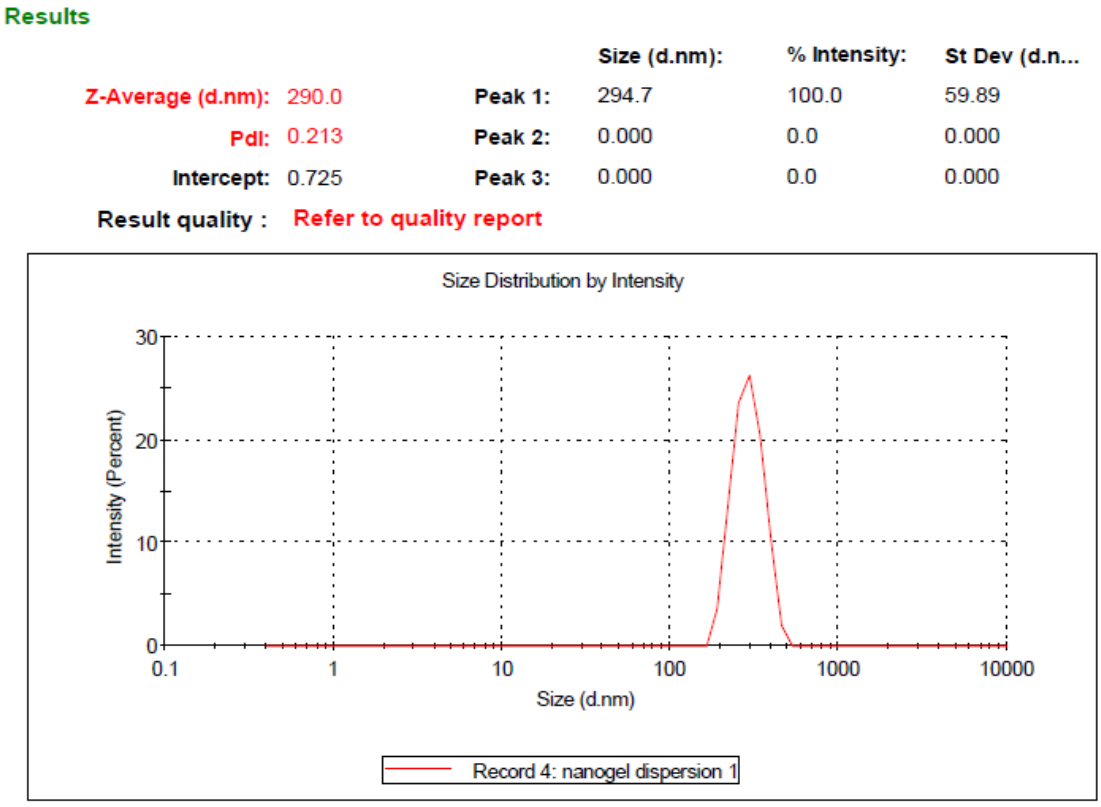

Fig. 1. Particle size of optimized batch B_5

Discussion:

Particle size of the nanogel dispersion was measured by malvern zeta sizer. Optimized batch particle size was found 290 nm ±1.2 nm.

The lower particle size suitable for fast penetration at affected area**.**

3.3 Zeta potential measurement:

Surface charge measurement was done using Malvern zetasizer. The zeta potential of a particle is the overall charge that the particle acquires in a particular medium. It is a physical property which is exhibited by any particle in dispersion. The zeta potential of optimized batch was found to be -1.81 mV.

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-1.81	Peak 1:	7.81	73.9	9.30
Zeta Deviation (mV):	21.5	Peak 2:	-20.8	10.1	4.57
Conductivity (mS/cm):	3.10	Peak 3:	-31.6	6.7	3.54
Result quality :	See result quality report				

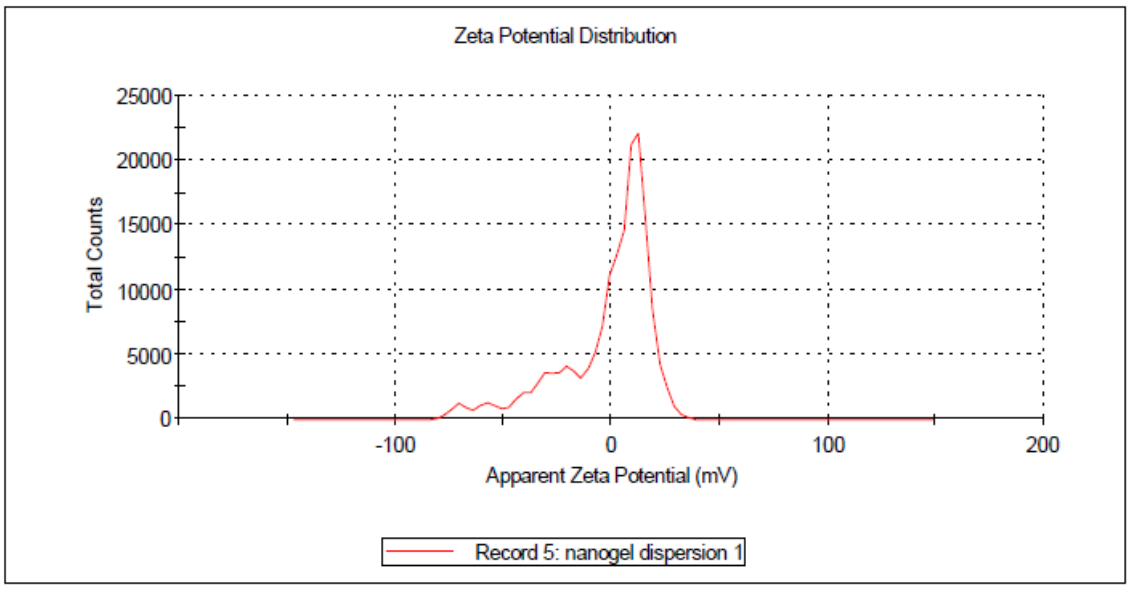

Fig. 2. Zeta potential of optimized batch B_{13}

Discussion:

Surface charge measurement was done using zetasizer. The zeta potential of a particle is the overall charge that the particle acquires in a particular medium. It is a physical property which is exhibited by any particle in dispersion. The zeta potential of optimized batch was found to be -1.81±1.56 mV. PDI is a measure of distribution of the particles in the sample.

Based on result of zeta potential, it was concluded the nanogel is not stable, so, it was lyophilized using mannitol with ratio of the suitable batch..

3.4 Gelling time:

Insitu nanogel gelling time was measured by using Stopwatch. Adding of Glutaraldehyde as chemical cross linker in HA-PVA Polymer solution with drop of Hydrochloric acid as catalyst. After addition of glutaraldehyde gelling time was measured for *insitu* nanogel.

Gelling time was measured for batch B5: **7 mins**

Discussion:

Dimethyl fumarate insitu nanogel gelling time was observed less other than batches.

3.5 Skin retention study and skin permeation study on rat skin

Skin retention study and skin permeation study were performed in order to analyse the content of the drug in the skin and its permeation through skin respectively.

At the end of the in vitro skin permeation study, the skin samples were washed with water and methanol on both sides and carefully dried. Then a defined amount of methanol was added to each piece of skin. The samples were vortexes for 10 min in order to extract its drug content and stirred overnight.

The samples were analysed by HPLC method after centrifugation.

3.5.1 Drug retention & Drug permeation analysis Using Reference Method of DMF, Initial trial for Standard Solution was taken with following chromatographic conditions as reported in the Ref. Method:

Standard solution injection:

Standard solution injection was injected and chromatogram was recorded as below in Figure 1. Stock and Final solutions were stored at 2-8°C conditions for further use in next trials.

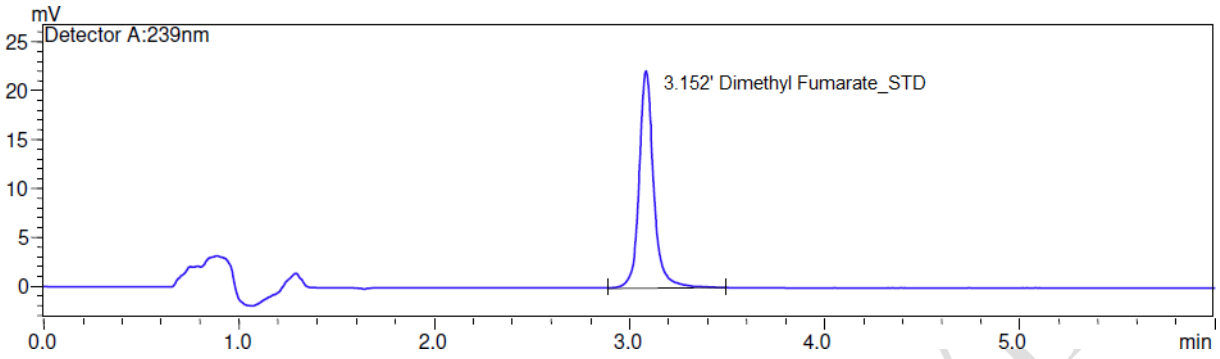

Fig.3. Chromatogram of DMF Standard

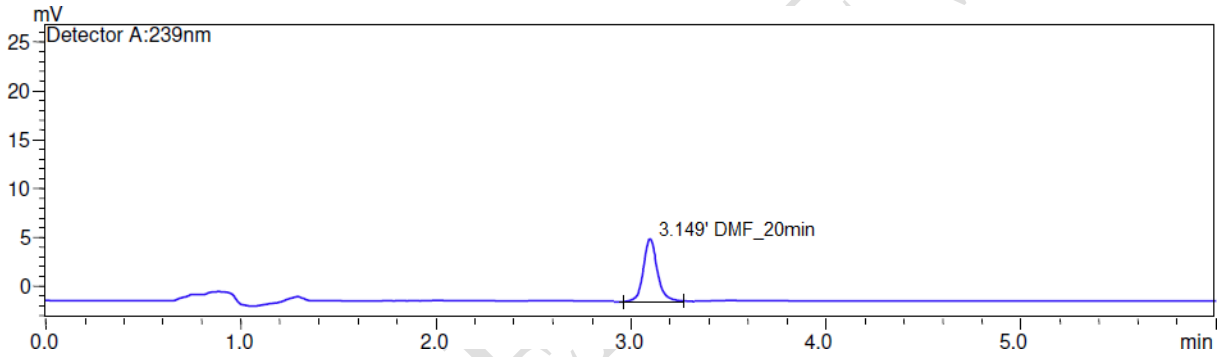

Fig. 4: Chromatogram of Blank (Diluent)

Table 4. Peak Table of dimethyl fumarate Standard

No.	Peak Name	Retention Time	Area	Tailing Factor	Theoretical Plates	Resolution
1	Dimethyl Fumarate_STD	3.152	225986	1.0	15785	-

Observation:

It was observed that the peak shape of DMF was found symmetrical and sharp in shape.Above method was finalized to analyze the Retention and Permeation samples of the formulation.

Sample Injections:

Samples (1 ml each) were withdrawn from the release medium at 20, 40, 60 min and replaced with an equal volume of fresh buffer solution to maintain sink conditions. The samples were analyzed by HPLC as per below method for Dimetyl fumarate

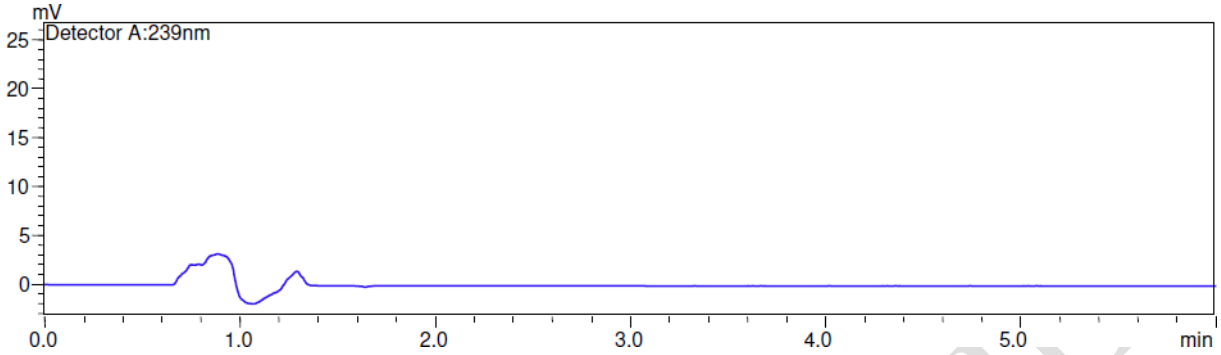

Fig. 4. Chromatogram of retention of 20 min Sample

Table 5. Peak Table of dimethyl fumarate of 20 min drug retention

No.	Peak Name	Retention Time	Area	Tailing Factor	Theoretical Plates	Resolution
1	DMF_20min	3.149	35031	1.1	14985	-

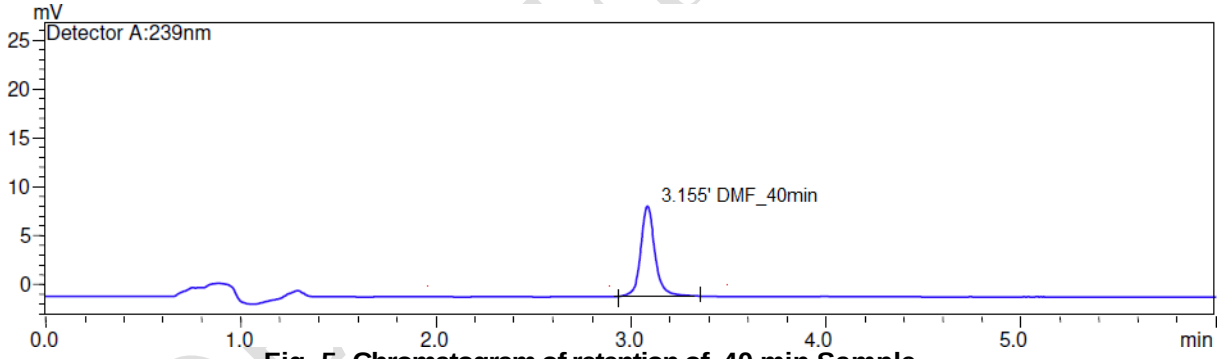

Fig. 5. Chromatogram of retention of 40 min Sample

Table 6. Peak Table of dimethyl fumarate of 40 min drug retention

No.	Peak Name	Retention Time	Area	Tailing Factor	Theoretical Plates	Resolution
1	DMF_40min	3.155	79339	1.0	15145	-

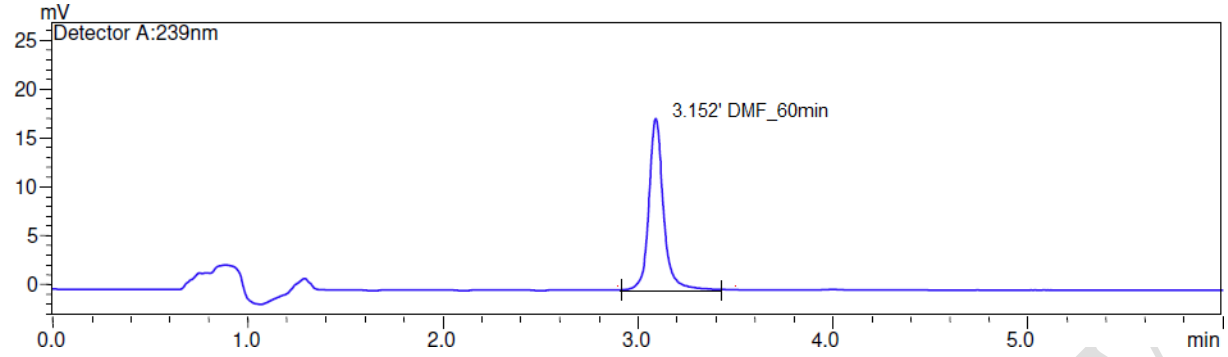

Fig. 6. Chromatogram of retention of 60 min Sample

Table 7. Peak Table of dimethyl fumarate of 60 min drug retention

No.	Peak Name	Retention Time	Area	Tailing Factor	Theoretical Plates	Resolution
1	DMF_60min	3.152	162604	1.0	15425	-

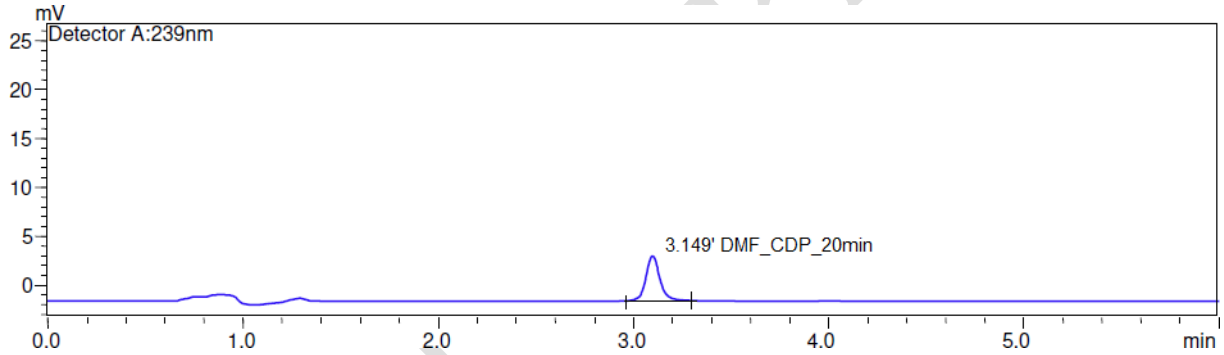

Fig.7. Chromatogram of Permeation 20 min Sample

Table 8. Peak Table of dimethyl fumarate of 20 min drug Permeation

No.	Peak Name	Retention Time	Area	TailingFactor	Theoretical Plates	Resolution
1	DMF__20min	3.149	23565	1.0	14852	-

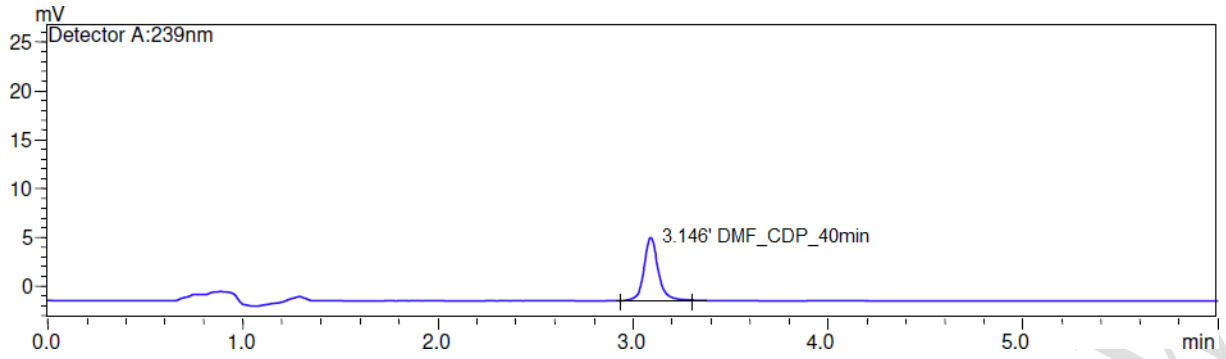

Fig.8. Chromatogram of Permeation 40 min Sample

Table 9. Peak Table of dimethyl fumarate of 40 min drug Permeation

No.	Peak Name	Retention Time	Area	Tailing Factor	Theoretical Plates	Resolution
1	DMF_40min	3.146	35324	1.0	14986	-

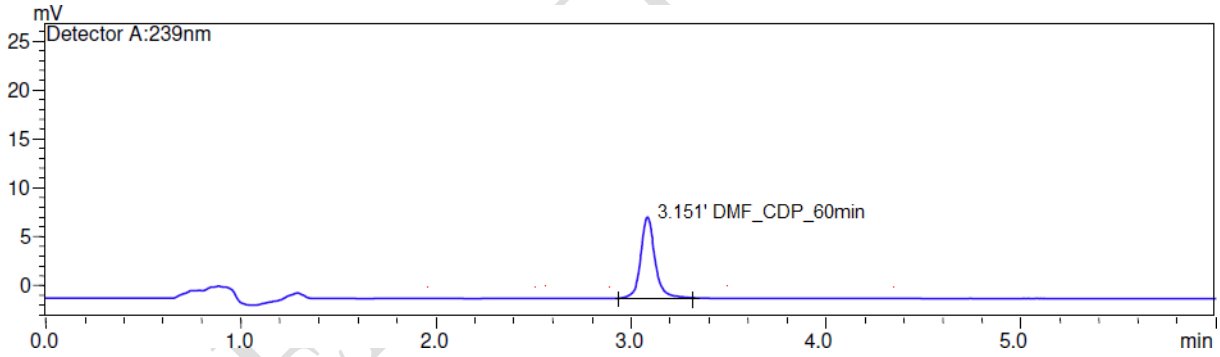

Fig. 9. Chromatogram of Permeation 60 min Sample

Table 10. Peak Table of dimethyl fumarate of 60 min drug Permeation

No.	Peak Name	Retention Time	Area	Tailing Factor	Theoretical Plates	Resolution
1	DMF__60min	3.151	59159	1.1	14785	-

Discussion:

As per drug permeation and retention study determined by HPLC which says that the highest amount of drug retained on the skin and lowest amount of drug permeate to the skin. Hence it was observed that there was no significant correlation between skin retention and skin permeation study.

Table 11. System Suitability / Repeatability of DMF:

System Suitability / Repeatability:						
ComponentName	**InjectionNo.**	**Retention time**	**Area**	**TailingFactor**	**Theoretical plates**	**Resolution**
DMF	1	3.152	225986	1.0	15785	-
	2	3.142	225468	1.1	15658	-
	3	3.153	225132	1.0	15782	-
	4	3.158	224986	1.0	15495	-
	5	3.149	226124	1.0	15698	-
	6	3.155	226589	1.1	15268	-
		Mean	**225714**			
		SD	622.4			
		%RSD	**0.3**			

Acceptance Criteria: %RSD of six replicate injections should be not more than 2.0.

Table 12. Percent CDR of DMF nanogel drug dispersion from Skin: (Retention)

Time in min	Percent Drug CDR
20	15.52 %
40	35.15 %
60	72.04 %

Table 13. Percent CDP of DMF nanogel drug dispersion from Skin: (Permeation)

Time in min	Percent Drug CDP
20	10.44 %
40	15.65 %
60	26.21 %

Table 14. Drug Retention results:

Sample	Drug content in formulation	Drug retained on the skin	Drug permeate to the skin
DMF Loaded *in situ* lyophilized nanogel	120 mg in 10 ml	72.04% in 60 mins	26.21%

Discussion:

Based on Table 12 and 13 data observed that the DMF loaded *in situ* lyophilized nanogel formulation concluded that the drug retention observed.

Drug retained on the skin 72.04% in 1 hr. Hence it protects localized effect on site of action (psoriatic skin). DMF could help to explain the antipsoriatic activity and suggest the benefits of further characterization of their pharmacological potential for the treatment of psoriasis. DMF inhibits the proliferation of actively growing endothelial cells.

The immunosuppressive drug like dimethyl fumarate and cyclosporine are a novel class of compounds sharing a macrolide-like structure and potent immunosuppressive activity. These both drugs have effective in dermatological disease like psoriasis.

3.5 Histopathological study:

The mice skin was mounted on Franz diffusion cell. The optimized drug loaded *in situ* nanogel was applied on psoriasis skin which is from in in vivo study and the effects were compared against control. A piece of fresh excised untreated skin sample was used as control. The skin was fixed in 10 % neutral formalin for 24 h and then cut vertically against the surface at the central region (4 mm width). Each section was dehydrated using ethanol and then embedded in paraffin wax. Tissues were divided into small pieces and stained with haematoxylin and eosin. The sections were observed under 100 x magnifications and photographed.

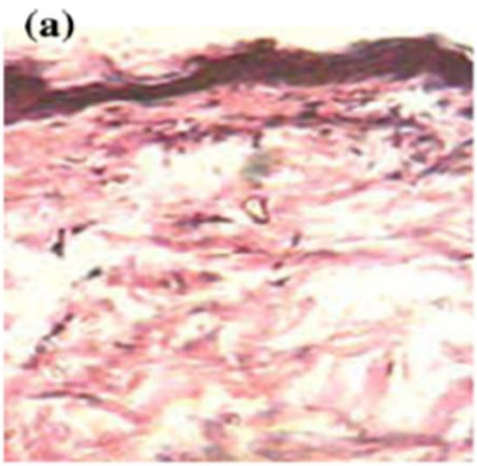

Fig. 9.Negative Control Sample skin

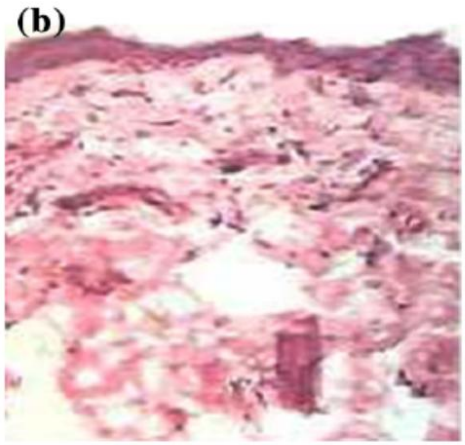

Fig. 10. DMF *in situ* nanogel treated Skin

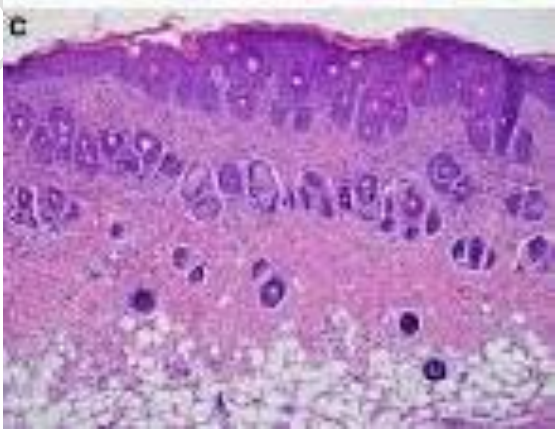

Fig.11. Imiquimod applied skin

Discussion:

The mice skin is a multilayered organ with many histological layers.The histology of excised mice skin in control (in fig. a) and Psoriasis skin treated with optimized drug loaded insitu nanogel indicate that the optimized nanogel has no significant effect on the microscopic structure of the skin (in fig. b). The surface epithelium lining and the granular cellular structure of the skin were totally intact. No major changes in the ultra structure of skin morphology could be seen and the epithelial cells appeared mostly unchanged.

CONCLUSION:

Based on formulation and evaluation parameters it was observed that hyaluronic acid and polyvinyl alcohol drug containing nanogel based topical spray has good effect on psoriasis skin than other dosage form. Hyaluronic acid is water soluble polymer which provide a potential strategy for improving retention of drugs on the skin. Polyvinylalcohol polymer helps to get better gelling property.

In histopathological study, several reports have presented that imiquimod activates immune cells via a toll-like receptor to induce psoriasis-likes inflammation. After ten days of its application on the shaved back of mice, typical symptoms of psoriasis were manifested including erythema, scaling and thickening. Treatment was started from the eleventh day of the study. Initially, PASI score was 2, which decreased to 0.3, 1 and 1.3 for DMF *in situ* nanogel after the completion of treatment. However, it persisted in the positive control group. Interestingly, there was assumption of hair growth in the DMF *in situ* nanogel treated animals, which suggest the recovery of hair follicles and regeneration of normal skin.

ETHICAL APPROVAL:

CPCSEA 921/PO/ReBi/S/05/CPCSEA PIPH 03/19

CERTIFICATE

This is to certify that the project title "***Insitu*nanogel based topical spray for treatment of psoriasis**" has been approved by the IAEC.

(Dr. AbhayDharamsi) 20/07/19

Chairman/ Member-Secretary of IAEC

(Dr. B.Suresh) 20.7.2019

CPCSEA Main nominee

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. 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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