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. All Plain Dimethylfumarate and 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

Skin inflammation is a most common problem in dermatology. They come in many forms, from occasional rashes accompanied by skin itching and redness, to chronic conditions such as dermatitis, roscea, seborrheic dermatitis and psoriasis.

Skin inflammation can be characterized by two types:

1) Acute inflammation: which comes from exposure to UV radiation, Contact with chemical irritants, ionizing radiation and allergens
2) Chronic inflammation results from a sustained immune cell mediated inflammatory response within skin itself. This inflammation is long lasting and can cause significant and serious tissue destruction.

The process of skin inflammation is complex and is still not completely understood. When the skin is exposed to a “triggering” stimulus, such as UV radiation, an irritant (e.g. soaps or fragrances), or to allergens, the cells in the skin produce a variety of inflammatory “hormones” called cytokines and chemokines. These

“inflammatory messengers” bind to specific receptors on target cells and stimulate the production of additional inflammatory signaling “hormones”. Some of this cause vasodilation while others activate nerve cells. Still other cytokines cause immune cells to leave the blood and migrate into the skin where they then produce more inflammatory hormones, as well as enzymes, free radicals, and chemicals that damage the skin. The end result of the initial triggering event is the amplification of a large inflammatory response that, while designed to help the skin fight infection from invading bacteria, actually causes considerable damage to the skin [1].

Psoriasis is a long-lasting autoimmune disease characterized by patches of abnormal skin. These skin patches are typically red, itchy, and scaly. They may vary in severity from small and localized to complete body coverage. Injury to the skin can trigger psoriatic skin changes at that spot, which is known as Koebner phenomenon.

There are five main types of psoriasis: plaque, guttate, inverse, pustular, and erythrodermic.

Plaque psoriasis, also known as psoriasis vulgaris, makes up about 90% of cases. It typically presents with red patches with white scales on top. Areas of the body most commonly affected are the back of the forearms, shins, around the belly button, and the scalp.

Guttate psoriasis has drop-shaped lesions.

Pustular psoriasis presents with small non-infectious pus-filled blisters.

Inverse psoriasis forms red patches in skin folds. Erythrodermic psoriasis occurs when the rash becomes very widespread, and can develop from any of the other types. Fingernails and toenails are affected in most people at some point in time. This may include pits in the nails or changes in nail colour.

Psoriasis is generally thought to be a genetic disease which is triggered by environmental factors. In twin studies, twins are three times more likely to both be affected compared to non-identical twins; this suggests that genetic factors predispose to psoriasis. Symptoms often worsen during winter and with certain medications such as beta blockers or NSAIDs. Infections and psychological stress may also play a role. Psoriasis is not contagious. The underlying mechanism involves the immune system reacting to skin cells. Diagnosis is typically based on the signs and symptoms.

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

Disadvantages of that treatment:

When using **topical agent** like vitamin D with steroids. Due to Vitamin D using long term with steroids can occur Itchiness, folliculitis, sunburn, poikiloderma , its caused irritation by rubbing or applying by any agent.

Phototherapy using UV Radiation which cause damaged DNA. Due to PUVA treatment can occur birth defects and liver damage.

Progressive multifocal encephalopathy occurs due to **systemic agents.**

Surgery

Limited evidence suggests removal of the tonsils may benefit people with chronic plaque psoriasis, guttate psoriasis, and palmoplantar pustulosis[2].

Alternative therapy

Uncontrolled studies have suggested that individuals with psoriasis or psoriatic arthritis may benefit from a diet supplemented with fish oil rich in eicosapentaenoic acid (EPA) anddocosahexaenoic acid (DHA). Conflicting evidence exists indicating that there may be an increased incidence of psoriasis in people with celiac disease. Psoriatic disease severity decreased after 3 months of a gluten free diet in patients with anti-gliadin antibodies.

Dimethyl fumarate is an immunosuppressive agent. Dimethyl fumarate degradation to its active metabolite monomethyl fumarate (MMF) then MMF up-regulates the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2)

pathway that is activated in response to oxidative stress. Phase III clinical trials found that DMF (BG-12) successfully reduced relapse rate and increased time to progression of disability in multiple sclerosis (trade name **Tecfidera**). DMF is thought to have immunomodulatory properties without significant immunosuppression.[3]

Disadvantages:

Dimethyl fumarate is available in Oral capsules forms

- More time required for drug absorption.
- Nausea
- Vomiting
- Abdominal Pain
- Diarrhoea.

Hyaluronic acid (HA) is one of the most important topical carriers for the localized delivery of drugs to the skin and also perform as a drug delivery agent for ophthalmic, nasal, pulmonary, parenteral and topical routes of administration [4]. HA acts as mucoadhesive, retaining to the drug at specific site of action/absorption. It also can modify the in vivo release/absorption rate of the therapeutic agent, when applied topically, to localize delivery of drug to the epidermis [5].

Poly(vinyl alcohol) (PVA) is a water soluble biodegradable synthetic polymer with good biocompatibility, and it can Chemically cross-linked to form hydrogels useful in pharmaceutical formulations [6].

The *In situ* nanogel based topical spray formulation can deliver the drug rapidly on inflamed site without irritation and can achieve higher drug entrapment efficiency. [7,8,9].

In situ nanogel is highly influential, biocompatible, biodegradable other than topical formulations with low toxicity. It also sprays through rapid swelling property which converts in to gel formation through higher drug loading capacity to increase binding drug with polymer, increases drug therapeutic efficacy, sustain release of drug *in vivo*, reduces side effects and improves patient compliance than other formulations [10].

In situ nanogel is based topical spray releases medicament rapidly in the form of nano sized droplets on epidermis layer which converted solution into gel form and release drug. A direct and rapid dispersion of a solution of an active agent over as a large portion as possible of the skin layer, which absorbs the active agent. In this way, a large area would be reached, thereby accelerating absorption of the active agent.

Towards of this, A **Nanogel** is nanoparticle as composed to a hydrogel – a cross linked hydrophilic polymer network. It can be most often composed of synthetic polymers or biopolymers which are chemically or physically cross linked and are usually in the ten to hundreds of nanometres in diameter. Like to like of this hydrogels, the pores in nanogels can be filled with small molecules or macromolecules and their properties, such as swelling, degradation and chemical functionality, can be controlled [11]. The formulation of *In situ* nanogel is defined as sol to gel transition at the factual site of administration in which the formation can occurs due to lyophilized nanogel become gel by adsorbing fluid at psoriasis site.[12]

As it has been initiated and prepared by chemical cross linked gels by using Hyaluronic acid and polyvinyl alcohol, in presence of chemical cross linker (glutaraldehyde)[13,14]

Chemical cross linked *in situ* nanogel converts into lyophilized powder. This lyophilized powder loads topical spray directly on the inflamed skin which provides dispersion to gel formation through contact of psoriasis skin and drug release to start on damaged epidermal layer.

In advantage point of view, the topical spray is a safe and effective treatment and use to control itching with allergic dermatitis. It also releases medicament rapidly in the form of lyophilized powder on epidermis layer and gets converted into gel upon application. A direct and rapid dispersion of a lyophilized powder of the active agent over as large portion as possible of the skin layer, which absorbs the active agent. In this way, a large area would be covered, thereby accelerating absorption of the active agent [15].

The objective of present investigation is to develop and characterize *in situ* nanogel based topical spray for the treatment of psoriasis. The investigation mainly focused on development of *in situ* nanogel dosage form that can easily administer through topical spray for the treatment of psoriasis.

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:

2.1 IN VITRO SKIN PERMEATION STUDY USING RAT SKIN USING FRANZ DIFFUSION CELL:

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 SIMULTANEOUS ESTIMATION 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

2.1.2 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.

2.1.2.1 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.

2.1.2.2 Diluent:

Mobile Phase was used as a Diluent.

2.1.2.3 Standard Injection:

2.1.2.3.1 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.

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

2.2.3 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.

2.2.4 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.

2.3 Skin retention 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. [55].

3. RESULTS AND DISCUSSIONS

3.1 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.

Table 3 HPLC Method References:

Name	DMF	CYC
Reference Method	DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUSESTIMATION OF DIMETHYL FUMARATE AND ONDANSETRON	DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR THE DETERMINATION OF CYCLOSPORINE A IN NEW BIOADHESIVE NANOPARTICLES FOR ORAL ADMINISTRATION
Column	C18 column (250×4.6 mm, 5 µm)	C18 column (250×4.6 mm, 5 µm)
MobilePhase	Methanol: Acetonitrile: Phosphate Buffer with pH 5.5 (50:20:30 v/v/v)	Water: Acetonitrile in GradientMode
Wavelength Used	239nm (As reported in Reference Method)	205nm (As reported in Reference Method)

Table 4 Chemical Details:

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	KH_2PO_4(Potassium dihydrogen Phosphate) & K_2HPO_4 (Dipotassium Hydrogen Phosphate)	AR	Spectrochem
4	Tri-ethyl amine	AR	Merck

3.2 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 Fomulation, so concentrations were decided as 120µg/mL for DMF.

3.2.1 Mobile Phase Preparation:

Volume of 500 mL HPLC grade Methanol, Volume of 200mL Acetonitrile were 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 mL of Millipore water. The 96.4mL of (Solution I) and 3.6mL of (Solution II) were mixed to each other and filtered using 0.45µ filter paper followed by 10 mins sonication. Mobile phase was used as diluent.

3.2.2 Diluent:

Mobile Phase was used as a Diluent.

3.3.3 Standard Injection:

3.3.3.1 Standard Injection was injected in above chromatographic conditions.

Initially DMF was analyzed to check the Reference Method.

Once peak of DMF was observed as per reference method than samples were injected to analyses Drug Retention and Drug Permeation.

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

3.3.4.1 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.

3.3.4.2 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 in Figure 1. Stock and Final solutions were stored at 2-8°C conditions for further use in next trials.

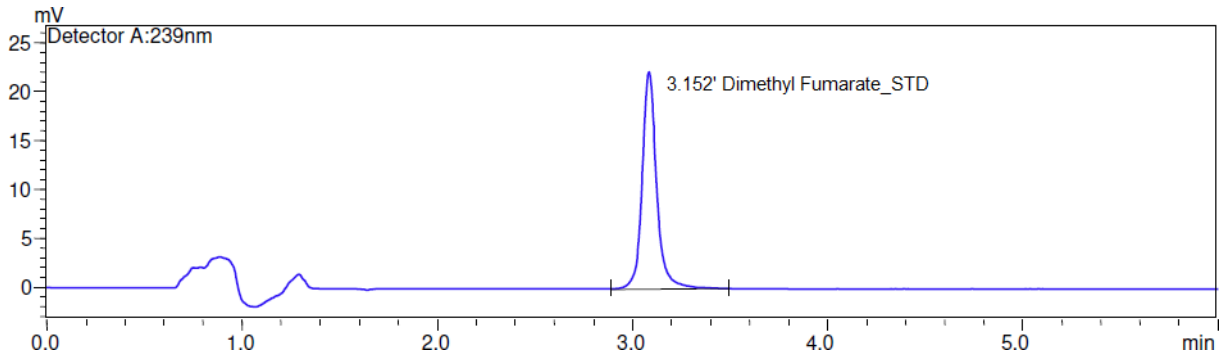

Figure 1: Chromatogram of DMF Standard

Table :5 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:

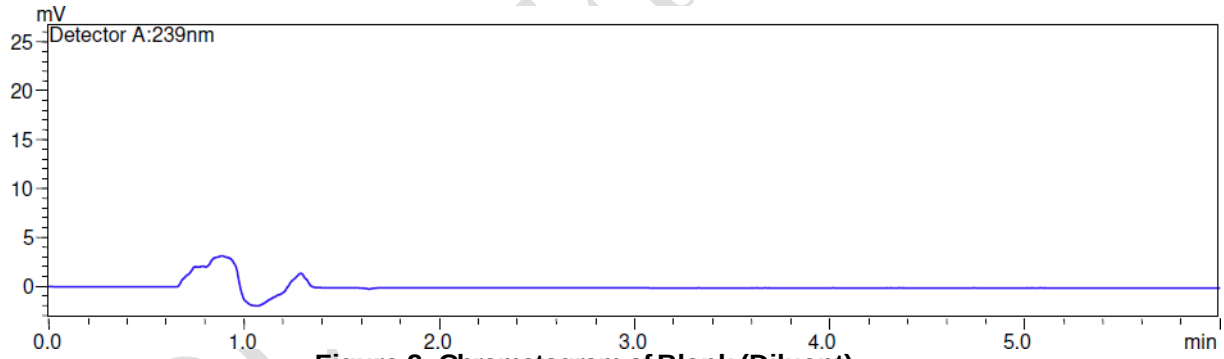

Figure 2: Chromatogram of Blank (Diluent)

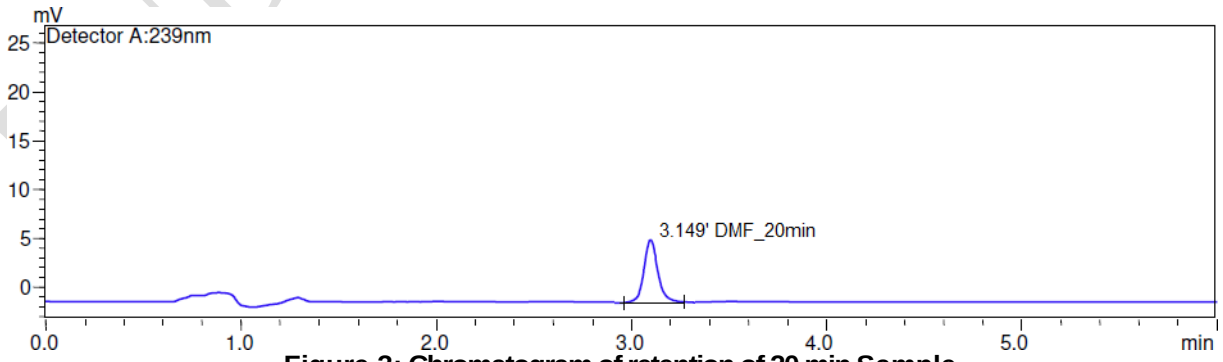

Figure 3: Chromatogram of retention of 20 min Sample

Table :6 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	-

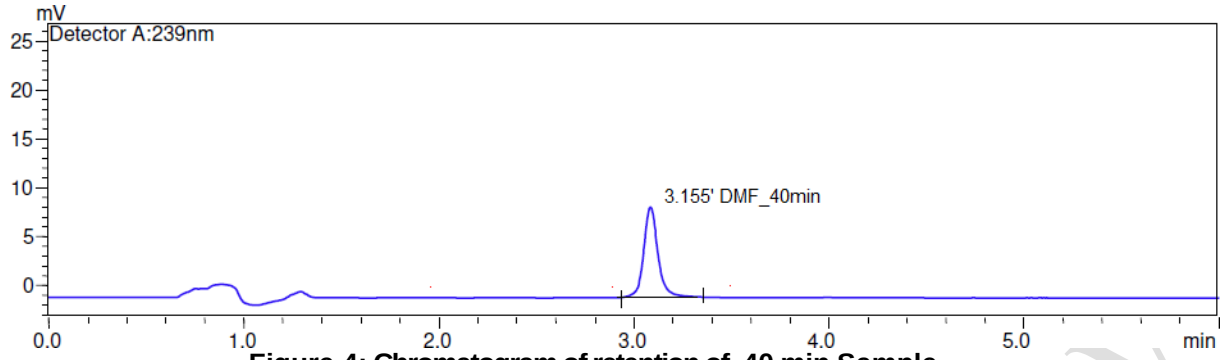

Figure 4: Chromatogram of retention of 40 min Sample

Table:7 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	-

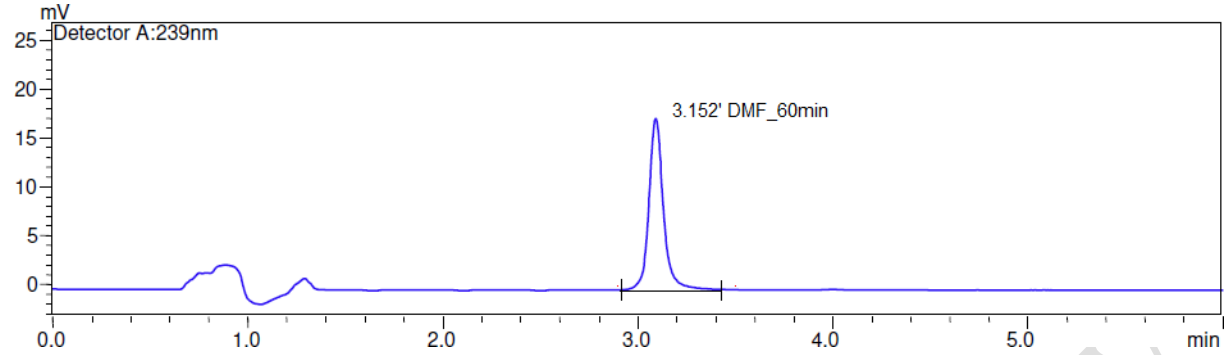

Figure 5: Chromatogram of retention of 60 min Sample

Table 8 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	-

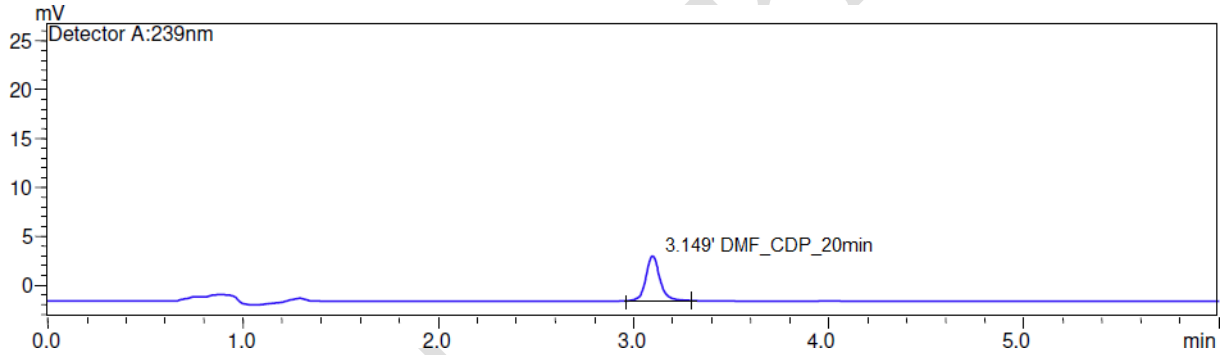

Figure 6: Chromatogram of Permeation 20 min Sample

Table 9 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	-

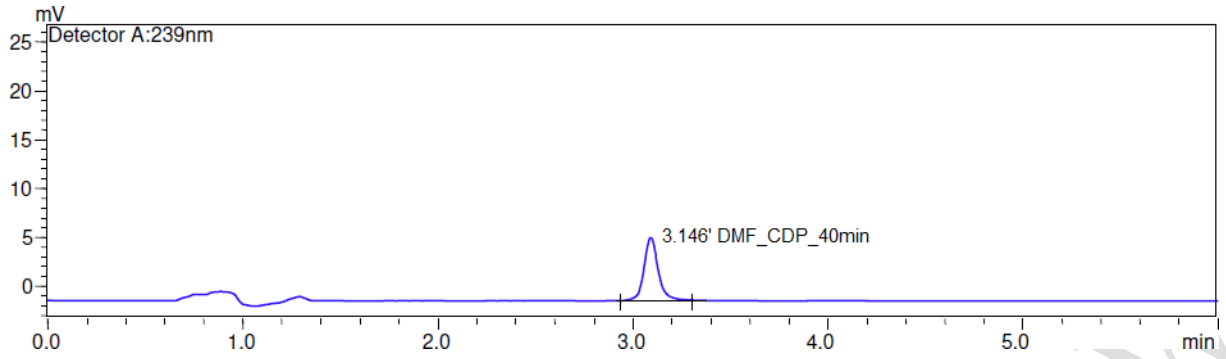

Figure 7: Chromatogram of Permeation 40 min Sample

Table 10 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	-

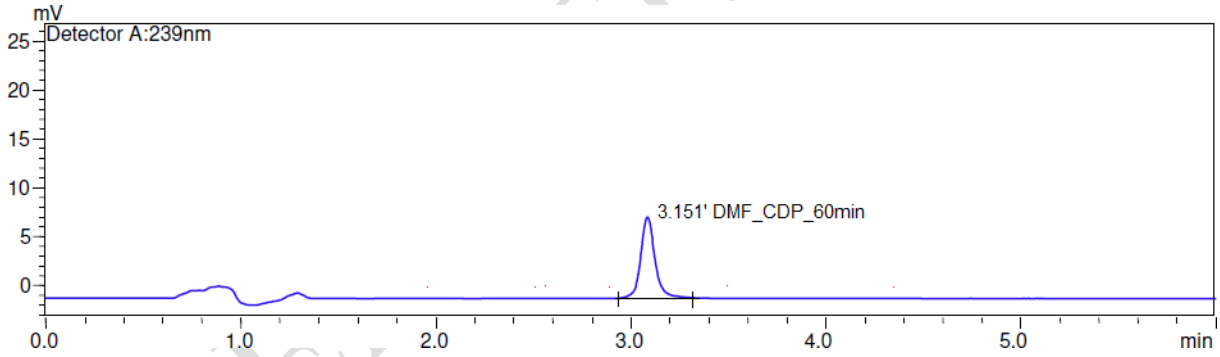

Figure 8: Chromatogram of Permeation 60 min Sample

Table 11 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	-

Table 12 System Suitability / Repeatability of DMF:

System Suitability / Repeatability:						
ComponentName	**Injection No.**	**Retention time**	**Area**	**Tailing Factor**	**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 13 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 14 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 15 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

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.

The DMF loaded in situ lyophilized nanogel formulation concluded that the drug retention observed 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

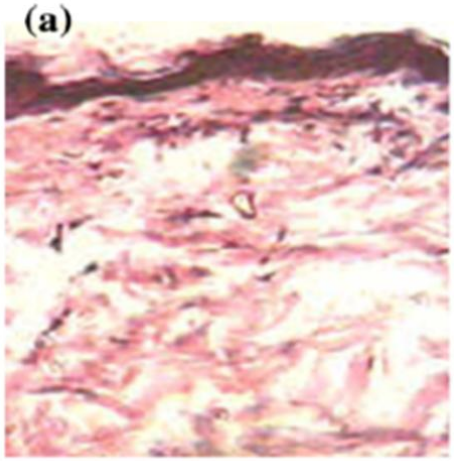

Figure 9:Negative Control Sample skin

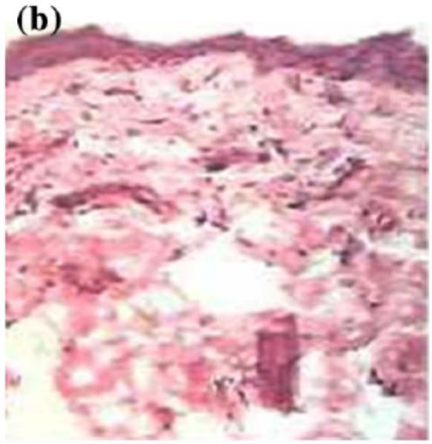

Figure 10: DMF *in situ* nanogel treated Skin

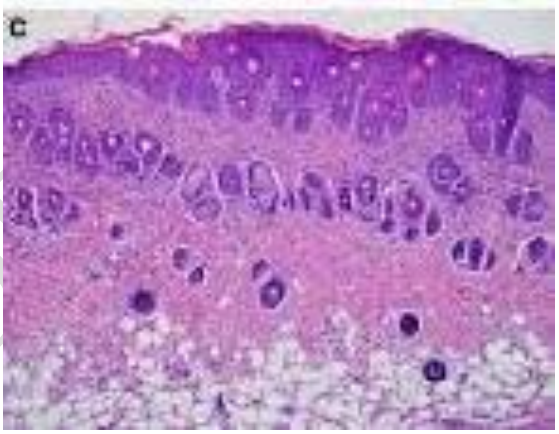

Figure 11 Imiquimod applied skin

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.

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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