"DEVELOPMENT, OPTIMIZATION AND CHARECTERIZATION OF MICROEMULSION BASED GEL USING ECTOIN FOR MANAGEMENT OF ATOPIC DERMATITIS"

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

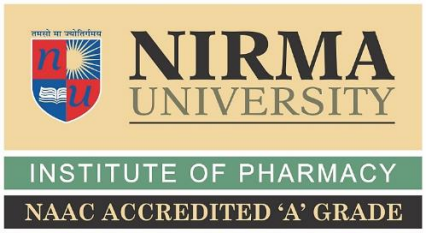
PHARMACEUTICS

BY

HIMANSHU H. MADHAVANI (19MPH106), B. PHARM.

Under the guidance of

Dr. SHITAL BUTANI
Associate Professor, Department of Pharmaceutics



Department of Pharmaceutics
Institute of Pharmacy
Nirma University
Ahmedabad-382481
Gujarat, India.
May 2021

CERTIFICATE

This is to certify that the dissertation work entitled "Development, optimization and characterization of micro emulsion based gel using ectoin for management of atopic dermatitis" submitted by Mr. Himanshu H. Madhavani with Regn. No. (19MPH106) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under my/our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

Guide

Dr. Shital Butani
M. Pharm., Ph.D.,
Associate Professor,
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University

Prof. Tejal Mehta
M. Pharm., Ph.D.,
Professor, & Head,
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University

Prof. Manjunath Ghate
M. Pharm., Ph.D.
Director
Institute of Pharmacy,
Nirma University

26th May, 2021

CERTIFICATE FROM INDUSTRY

COSMETICS CONCEPT RESEARCH

This is to certify that Mr Himanshu Harishbhai Madhvani student from Institute Of Pharmacy, Nirma University has undergone training from 04/08/2020 to 04/04/2021 in our organization.

He has done his training in the area of cosmeceuticals in R & D department on the topic "Development, optimization and characterization of micro emulsion based gel using ectoin for management of Atopic Dermatitis".

He has completed the same successfully with dedication.

During the training period his performance and attitude was found to be satisfactory.

We wish him All the Best for his bright future.

Best wishes
For,

Cosmetics Concept Research Pvt Ltd
Ahmedabad

D-603, Ganesh Meridian, Opp. Gujarat High Court, SG Highway, Ahmedabad, Gujarat - 380060, India
Phone: +91-79-49039200 | Toll Free No. 1800 233 0603
www.ccrinnovations.com | E-mail: info@brillarescience.com

Cosmetics Concept Research Pvt. Ltd.
CIN No. U74900GJ2016PTC095051
PAN No. AAGCC3718E
Service Tax No. AAGCC3718ESD001

CERTIFICATE OF ORIGINALITY OF WORK

This is to undertake that the dissertation work entitled "Development, optimization and characterization of micro emulsion based gel using ectoin for management of atopic dermatitis" Submitted by Himanshu H. Madhavani (19MPH106) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutics" is a bonafide research work carried out by me at the "Department of Pharmaceutics", Institute of Pharmacy, Nirma University under the guidance of "Dr. Shital Butani". I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, this work is original and not reported anywhere as per best of my knowledge.

Mr. Himanshu H. Madhavani (19MPH106)
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University

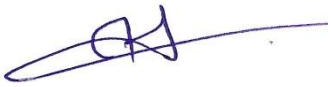
Guide

Dr. Shital Butani
M. Pharm., Ph.D.,
Associate Professor,
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University

26th, May, 2021

DECLARATION

I hereby declare that the dissertation entitled "Development, optimization and characterization of micro emulsion based gel using ectoin for management of atopic dermatitis" is based on the original work carried out by me under the guidance of Dr. Shital Butani, Assistant Professor, Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.



Mr. Himanshu H. Madhavani (19MPH106)
Department of Pharmaceutics
Institute of Pharmacy,
Nirma University,
Sarkhej - Gandhinagar Highway,
Ahmedabad-382481,
Gujarat, India

26th, May, 2021

ACKNOWLEDGEMENTS

This work is the result of prudent group efforts, intellect and experience of all those who have contributed their knowledge found beyond the covers of this dissertation. They have supported and encouraged me in all my efforts for successfully completing my project. A contribution value will forever be indebted and thus I express my gratitude to the people who have been instrumental in the successful completion of this project. It is my pleasure to thank everyone for those who have in any way have contributed to thesis and made it possible for me to complete it.

It is my great pleasure to express deep sense of gratitude and offer my most sincere and humble thanks to my dissertation guide ***Dr. Shital Butani****. Without her efforts, constant assistance and backing this project work would not be so fruitful in the way it is. Thank you mam!*

I am very fortunate to be a student of Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat and thankful to our ***HOD Dr. Tejal Mehta*** *and all the faculty members* ***Dr. Mayur Patel, Dr. Dhaivat Parikh, Dr. Mohit Shah, Dr. Jigar Shah*** *for their keen interest and valuable support. I also thankful to our director* ***Dr. Manjunath Ghate****.*

I am very grateful to ***my father Mr. Harishbhai Madhavani and my mother Mrs. Kamlaben Madhavani****for their love and faith in me that "I can do" added huge courage to my spirit.*

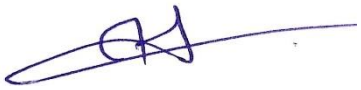
In the mean course of time, I have collaborated with very veteran personality, ***Vimal Patel*** *and* ***Vivek Mewada*** *for whom I have due respect and I hold a warmest thanks.*

I always will be thankful to ***GOD*** *for giving me the strength and wisdom to carry out the project work successfully.*

I would be failing in my duties if I do not thank to my beloved friends ***Rishabh, Jay, Rudranshu, Jitendra, Jay, Nilay, Palak, Minha, Anuja, Aaradhna, Drashti, Aarti, Urvashi, and Shweta****for their constant support in every endeavor of mine and provide me with necessary stimulus for keeping the driving force integrated for successful completion of this project. Thank you friends!*

I also wish to acknowledge our lab assistant, ***Shaileshbhai, Mukeshbhai and Drashtiben****for providing all materials that required in my work.*

Once again, I would like to thank all those mentioned here and those who I have unknowingly failed to mention in making this project a worthy endeavor.



Himanshu H. Madhavani

Table of content

List of figure

List of Table

List of Abbreviation

Sr. No.	Abbreviation	Full Name
1	AD	Atopic Dermatitis
2	IgE	Immunoglobulin
3	Th	Thelper cells
4	HLB	Hydrophilic Lipophilic Balance
5	QbD	Quality by design
6	NLC	Nanostructured lipid carrier
7	SLN	Solid Lipid Nanoparticle
8	UV	Ultraviolet
9	PEG	Polyethylene glycol
10	EDTA	Ethylene diamine tetra acetic acid
11	FTIR	Fourier transfer infra-red
12	DSC	Differential Scanning Calarometry
13	PDI	Polydispersibility index
14	SD	Standard deviation
15	IPM	Isopropyl Myristate
16	RPM	Rotation per minute
17	CDR	Cumulative drug release
18	CPR	Cumulative % drug release

ABSTRACT

Atopic dermatitis is a very relapsing, chronic, itchy and inflammatory topical disease that generally affecting infants and small children as well as adults. The main symptoms of dermatitis are red itchy skin generally inflammation which turns into eczema. The main triggers of dermatitis are allergens, infection, stress, heat and sweating. Generally topical corticosteroids are used for treatment of atopic dermatitis and also topical calcineurine inhibitor used. But these cause harmful side effects. Ectoin is a natural active that obtain from bacteria and it is a natural stress protect molecule that used in atopic dermatitis. The objective of preparation of microemulgel is to increase permeation. This is used as permeation enhancer. Gel gives ease of use of formulation on skin. There are advantages of preparing microemulgel because it increases penetration also give stability and reduces stickiness. Currently only cream of ectoin available in market. Ectoin also used in cosmetic industry. The main objective of this thesis is to formulate dosage form that having good penetration and better efficacy for treatment of atopic dermatitis. Spontaneous emulsification method used for preparation of micro emulsion. Jojoba oil used as oil phase, simulsol 1293 used as surfactant and polyethylene glycol 400 used as co surfactant. Carbopol or xanthan gum used as gelling agent. The formulation was evaluated for viscosity, pH, particle size, zeta potential, and In vitro release. In this formulation, harmful parabans are not used. Prepared 1% ectoin emulgel gives good result in drug release, appearance, texture and viscosity.

1. Introduction

1.1 Introduction to atopic dermatitis

1.1.1 Introduction:

Atopic dermatitis is a very chronic, inflammatory skin disease that affects most of the children and adults in industrialist country. It commonly affects the infants, child as well as adults. It is frustrating condition for patients because it affects physical and psychological life of patients. Over the past 30 years, it has been increasing 2-3 folds and currently it affecting 18 and 5% children and adults depending upon population. The main triggers for atopic dermatitis are heating, allergens, sweating, irritants, dry skin and infections. (1)

1.1.2 Epidemiology:

The prevalence of atopic dermatitis is estimated to be 15 to 20% in children and 2 to 3% in adults. According to American academy of dermatology, 90% of people get atopic dermatitis before age of 5 and it is rare that somebody will be diagnosed with AD if they didn't have it as a child. (1)

1.1.3 Risk factor:

The exact etiology of atopic dermatitis is still under investigation. Both genetic and environmental factors act as risk factors for developing atopic dermatitis. Environmental pollution, family history, excessive hygiene, impaired skin barrier dysfunction, nervous system imbalance, immune dysfunction and stress can cause atopic dermatitis. (1)

1.1.4 Pathophysiology:

The pathophysiology of atopic dermatitis is very complex process and multifactorial that involves elements like barrier dysfunction, cell mediated immune response, IgE mediated hypersensitivity and environmental factors. It is characterized by epidermal changes in immunological abnormalities due to changes in increasing level of circulating IgE antibodies and histamine release from basophiles. At cellular level of immunity both helper cells Th1 and Th2 cells goes to proliferation and resulting into hyper cutaneous T cells which causes over production of inflammatory cytokines that initiate and retain dermal inflammation. The resultant atopic dermatitis associated

impairment skin dysfunction increases its water permeability, which increases transdermal epidermal water loss resulting in dry skin and relapsing eczema. The water loss and dryness can cause increasing penetration of various allergens like microbes which leads to skin infection. (2)

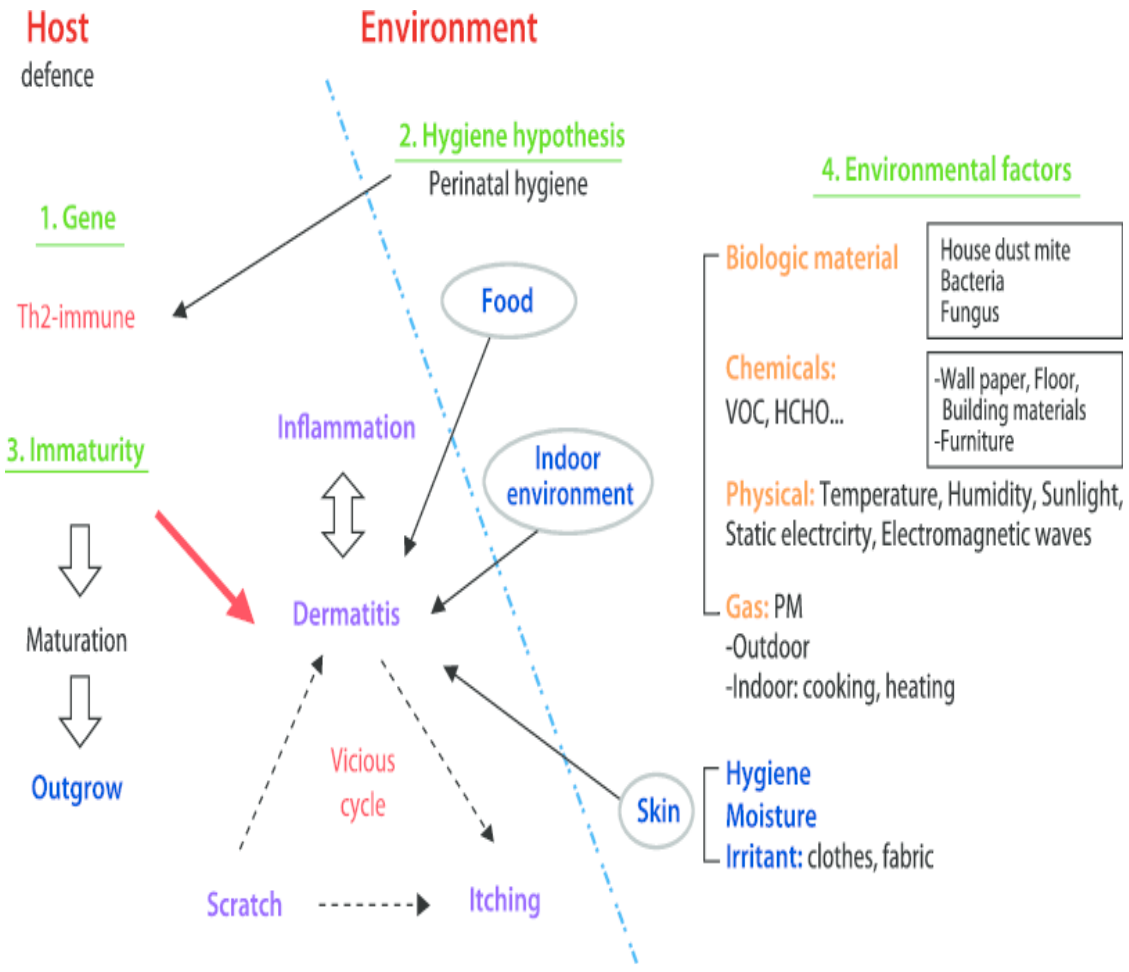


Figure 1: Pathophysiology of Atopic Dermatitis (1)

1.1.5 Symptoms and treatments available for atopic dermatitis:

Symptoms: Red, itchy, dry skin, rashes, flakiness, small red bumps which on scratch leak fluid. (3)

Treatments available: (3)

A. Topical corticosteroids
B. Emollients
C. Topical calcineurine inhibitor
D. Phototherapy
E. Antibacterial drugs
F. Antiallergic drugs

1.2 Introduction of micro emulsion

1.2.1 Introduction of emulsion:

Basically, emulsions are defined as mixture of two immiscible liquids, in that dispersed phase is uniformly dispersed in continuous phase like liquid-liquid, solid-liquid and liquid-gas etc. In convectional system this term only used for liquid-liquid

dispersion. As per United States pharmacopoeia, emulsion is defined as dispersed phased is uniformly dispersed in continuously in form of small droplets. Emulsions are thermodynamically unstable because of large difference in interfacial tension between two liquids. This problem can be solved by adding surfactant and co-surfactant which can decrease interfacial tension between two phases. The dispersed phase is available in low amount and continuous phase available in large amount. Pharmaceutical emulsion either low viscous in form of lotion or high viscous in form of cream. The particle size of dispersed phase is ranges from 0.1 to 100µm. There are three types of emulsions oil in water (O/W), water in oil (W/O) and multiple emulsions. (4)

1.2.2 Introduction of micro emulsion:

Micro emulsions defined as isotropic and thermodynamically stable system that consists of oil, water, surfactant and co-surfactant. The term micro emulsion was first given by Schulman in 1959. The droplet size in micro emulsion is between 10 nm to 300nm. Micro emulsion is characterized by constructing pseudo phase ternary diagram. Three components present in micro emulsion oil phase, various oils like isopropyl myristate, isopropyl palmitate, olive oil, oleic acid, sunflower oil etc. various surfactants used, generally nonionic surfactant used that having high HLB value. Propylene glycol and polyethylene glycol generally used as co-surfactant in micro emulsion. (5)

1.2.3 Advantages of using micro emulsion: (5)

This is thermodynamically stable system

Micro emulsions have capacity to solubilize both lipophilic as well as hydrophilic drug.

Increases bioavailability

Less amount of energy required

Rapid and efficient penetration of drug moiety

Ease of manufacturing process

Wide range of applicability

Micro emulsions are clear or translucent

1.2.4 Preparation methods of micro emulsion:

There are two methods to prepare micro emulsion:

1) Phase inversion method
2) Phase titration method

Phase inversion method:

In phase inversion method addition of excessive dispersed phase happened or response to temperature. In this method, phase inverse so there is sudden change in physical parameter like decreased in particle size can affect the drug release both in vivo and in vitro. This method can change the curvature of surfactant. For the non-ionic surfactant it is achieved by change in temperature like transition from lower temperature (O/W) to higher temperature (W/O). During cooling, system crosses point of zero spontaneous curvature and minimum surface tension, promoting creation of finely dispersed oil droplets. This method is known as phase inversion temperature method. Not only temperature but also other parameters like change in pH or change in salt concentration also considered. Also changes in transition of radius curvature can be obtained by changes in fraction of volume of water. By addition of water into oil, finely water droplets are formed in oil phase. By increasing water volume fraction, changes the spontaneous curvature of surfactant from initially stabilizing w/o micro emulsion to o/w at inversion locus. (5,6)

Phase titration method:

Micro emulsion can be prepared by spontaneous emulsification method that is called phase titration method. This can be prepared by phase ternary diagram. Construction of ternary phase diagram is useful approach to learn complex reaction when two or more components are involved. This diagram helps to find micro emulsion zones in which each corner represents 100% component. (5,6)

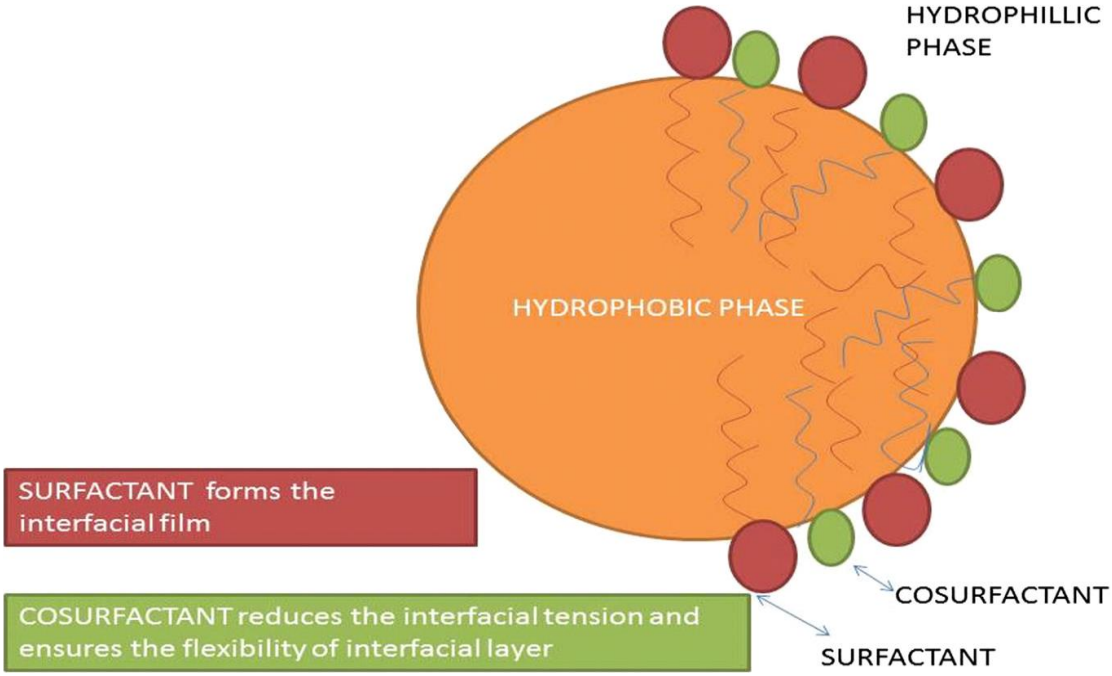


Figure 2: Structure of micro emulsion (31)

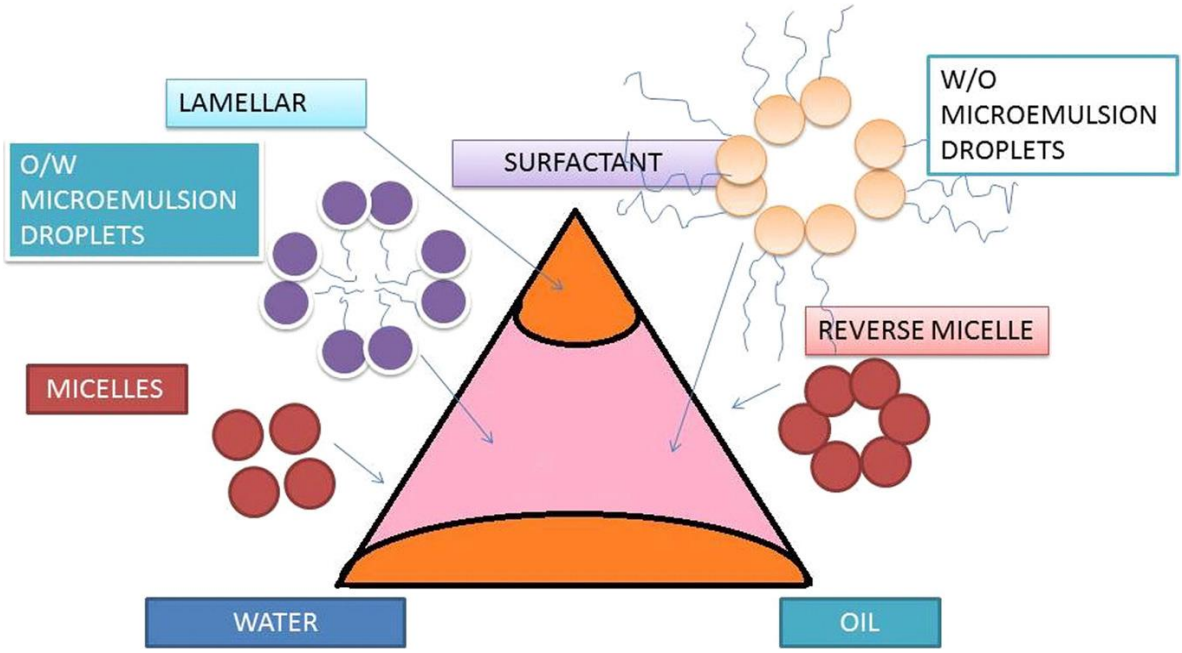


Figure 3: How to create micro emulsion (31)

The drug is to be dissolved in oil phase and water phase can be combined with surfactant and co-surfactant. This mixture added into oil phase slowly under stirring until clear system obtained. Amount of oil incorporate is determined with help of pseudo phase ternary diagram by titration method. Microemulgel can be prepared by adding gelling agent into micro emulsion. In W/O micro emulsion drug is to be dissolved in aqueous phase. (5)

1.2.5 Introduction of emulgel:

Emulgel is a combination of emulsion and gel. Nowadays emulgel is more beneficial than gel. Emulgel can be used for both hydrophobic as well as hydrophilic drug. The presence of gelling agent into emulsion that is called emulgel. Both o/w and w/o emulsions used as vehicle to deliver drugs. Emulgels have some good dermatological properties like thixotropicity, gracelessness, spread ability, removability, emollient, long shelf life and pleasant appearance. Emulgel also contain good patient acceptability because of it contain both emulsion as well as gel. Micro emulsions reduces the diffusion barrier of stratum corneum and show good drug release, good acceptability and clear appearance, low skin irritancy. Recently micro emulsion based gel shows great potential for topical drug delivery system. These are more stable than emulgel because reduced particle size. (7,8)

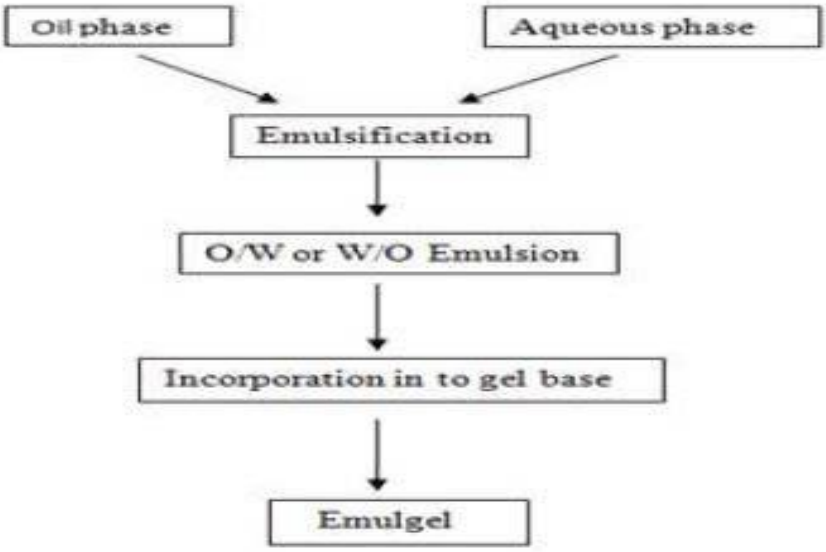


Figure 4: Preparation method of emulgel (7,8)

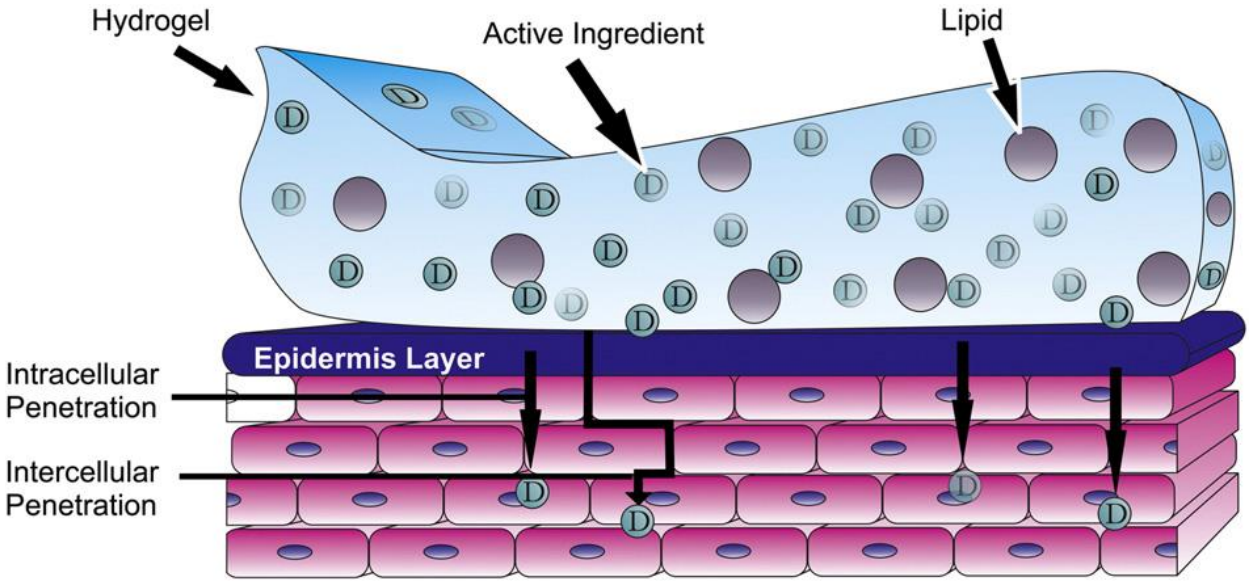


Figure 5: How emulgel works on skin layers (8)

2. Aim, Objective and Rationale

Aim:

To formulate and characterize micro emulgel of Ectoin

Rationale behind use of Ectoin:

- Other topical corticosteroids can give harmful effects on skin like stretch marks, bruising, discoloration or thin spidery blood vessels (tilangiectagiasis).
- Ectoin is natural active obtained from bacteria can treat atopic dermatitis naturally.
- It is used as dermatologically and as a cosmeceutical in other countries.
- It not shows any harmful side effects like steroids.

Rationale to formulate micro emulgel:

- ✓ Micro emulsion can be helpful to increase permeation and penetration of the drug through skin.
- ✓ Micro emulgel gives better stability.
- ✓ It gives better loading capacity.
- ✓ Low preparation cost and effective production feasibility.
- ✓ No need of intensive sonication.
- ✓ It can shows controlled release of drug.

Objective:

- To perform preformulation studies of Ectoin.
- To develop and characterize micro emulgel of Ectoin.
- To optimize and evaluate final formulation of Ectoin.

3. Literature Review

3.1 Literature review on drug Ectoin:

Galia lavi et al, 2006 "They developed sub-micron emulsion of hydrophilic molecule ectoin. Formulation was prepared with help of different emulsification method. O/w submicron emulsion with droplet size of 320 nm and narrow size distribution achieved by membrane emulsification method. Both methods membrane emulsification and high pressure homogenization give small droplets size but membrane method give more uniform distribution of droplets. Permeation of ectoin into skin is more depended on droplet size and also components present in formula. By addition of oleic acid or propylene glycol, increasing the permeation of ectoin into skin." (9)

Adam bownik et al, 2016 "Ectoin is water soluble compatible hydrophilic molecule that is produced by natural bacteria in response to stressful condition. This amino acid derivative has ability to accumulate inside the cell and can

protect cell from radiation and stress. It is useful because it has not any toxic side effects. Ectoin used in cosmetics, anti-inflammatory actions, biotechnology and in neurodegenerative diseases. Ectoin may inactivate some molecule therefore it is limited used in pharmaceutical industry." (10)

3.2 Literature review on disease:

Kangmo ahn et al, 2020 "Atopic dermatitis is chronic inflammatory skin disease characterized by skin barrier dysfunction, systemic immune degeneration. Atopic dermatitis can cause significantly impairment the quality of life. It is also connected with mental disorders and cardiovascular diseases. Many factors can trigger Atopic dermatitis like stress, sweat, microbial infection, immune system imbalance, environment and rash. Recently newer drugs are also available for treatment of atopic dermatitis." (1)

3.3 Literature review on micro emulsion based gel:

Nguyene thach Tung et al, 2019 "They developed micro emulsion based gel of betamethasone propionate. Also they applied QbD approach to determine effects of in depended variables on flux and skin deposition of betamethasone, micro emulsion droplet size and optimize the percentage ratio of Smix and oleic acid in the micro emulsion. After the evaluation it is indicated that carboxymethyl cellulose was most suitable agent for hydrogel. The produced gel not having any skin irritation in rabbit skin model and shows better anti-inflammatory activity rather than gel of NLC or SLN. The low level of oleic acid and high level of Smix show optimal skin deposition of betamethasone." (11)

Huabing Chen et al, 2006 "They used ethyl oleate as oil phase for micro emulsion due to powerful solubilizing and permeation enhancing effect for ibuprofen. Micro emulsion can increase permeation of ibuprofen 30 times more as compared conventional system. The addition of xanthan gum into micro emulsion can increase its viscosity. They used 1.5% xanthan gum to prepare hydrogel. It shows good stability. Optimum formula contains 3% ibuprofen, 6% ethyl oleate and 30% (2:1) Tween 80 as surfactant and propylene glycol as co surfactant." (12)

Rienhart neubert et al, 2004 "They did this study to check penetration of highly hydrophilic drug diphenhydramine as micro emulsion system with glycolipid as a penetration modifier. They used isopropyl palmitate as oil phase in micro emulsion and with glycolipid, without glycolipid as well as hydrogel used as standard formulation. Penetration is increased by the use of penetration modifier and also micro emulsion enhances penetration of drug." (13)

Mehmet evren okur et al, 2020 "They did research on micro emulsion based gel of fusidic acid for wound healing treatment. They used ethyl oleate as oil phase, tween 80 as surfactant and ethanol as co surfactant. The optimized formula of micro emulsion based gel shows good stability over three months. This formulation is more good comparison to marketed derma creams for wound healing. The healing percent of lesion area is 41.39% of this formulation. It shows better therapeutic activity at end of 10^{th} day in vivo experiment also shows good spreadibility." (14)

Airlla L.M. et al, 2016 "They prepared micro emulsion of pentoxifylline for skin disorders. The transparent formula was developed and optimized by pseudo ternary phase diagram. They used tween 80 and brij 52 mixtures as surfactant and concentration was 44%. 51% capryl caprice triglyceride used as oil phase and 5% water used in formula. That was w/o micro emulsion. Also inflammatory activity was increased with this micro emulsion of pentoxifylline. It also increases patient compliance and reduce side effects." (15)

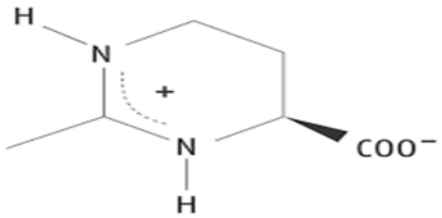
4. Drug & Excipients details

4.1 Drug related information:

4.1.1 General information (10,16)

Table 1: General information of drug

Name	Ectoin
Synonym	Ectoin the natural stress protection molecule
Chemical name	1,4,5,6 tetra hydro 2 methyl 4 pyrimidine carboxylic acid

Structure	
Molecular formula	$C_6H_{10}N_2O_2$
Molecular weight	142.16g/mol
Category	Cosmeceutical topical

4.1.2 Description of the drug:

Ectoin is a stress protective small molecule that is extremolytes. Ectoin obtained from extremophile microorganism like bacteria which are grown in very stressful conditions. Ectoin used in allergic and dermatological conditions. Melting point of ectoin is 230°C and decomposition point is 280°C. Ectoin is colorless, white slight hygroscopic and crystalline material. It is very stable in high pH and temperature conditions. It is soluble in water. (9,10)

4.1.3 How drug works?

Ectoin stabilizes the structural membrane in skin. It also reduces skin inflammation. It increases the hydration effect on skin. Ectoin form a complex with surrounding water molecules. These complexes are known as ecto hydro complex. Ectoin is Cosmo tropic molecule and give stabilization to water interaction on skin. This ectoin water complex reduces the dryness on skin thus this molecule is useful in many topical skin conditions like UV protection, atopic dermatitis. (9,10)

4.2 Excipients related information:

4.2.1 Jojoba oil:

Jojoba oil is extracted from the seeds of jojoba plant. This plant is mainly seen in California, United States and Mexico. Jojoba oil used in cosmetics and skin & hair preparations. It is used as emollient, moisturizer, antibacterial, and antioxidant. It is mainly used for atopic dermatitis. Color of jojoba oil is yellow.

4.2.2 Simulsol-1293:

Simulsol-1293 is used as surfactant for my formulation. It is basically nonionic surfactant which is widely used in formulation. It is also used as solubilizing agent. HLB value of this surfactant is 14. Common name of simulsol is PEG 40 hydrogenated castor oil.

4.2.3 PEG-400:

PEG-400 is used as co-surfactant in my formulation. PEGs are hydrophilic so they are used as penetration enhancer and frequently used in topical preparations like creams, lotions, patches. PEG 400 is low atomic weight polyethylene glycol. It is extremely hydrophilic. It is very viscous liquid. Density is 1.13g/cm^3.

4.2.4 Xanthan Gum (X Natural 75):

Xanthan gum is generally used as thickener and binder in food, cosmetic and pharma industries. It is used in creams, lotions and shampoos. It is available in various grades. It is high molecular polysaccharide gum. It is soluble in both hot and cold water. Generally appeared in powder form.

5. Materials and Methods

5.1 List of material

Table 2: List of material

Sr.no.	Name of material	Manufacturer
1	Simulsol-1293	Seppic
2	PEG-400	Rankem
3	Ectoin	Merck
4	Jojoba oil	Satvaras
5	Distilled water	
6	Disodium EDTA	Merck
7	Galgard Trident	Galaxy
8	Xanthan Gum	Jungbunzlauer

5.2 List of equipment

Table 3: List of equipment

Sr. No.	Equipment used	Model	Manufacturer
1	Weighing balance	CITIZEN	
2	Magnetic Stirrer	2MLH	REMI equipment
3	Particle size analyzer	SZ 100	HORIBA
4	UV Spectrophotometer	UV 1800	SHIMADZU
5	Digital pH meter	PHcal(2008/0952)	ANALAB
6	Differential scanning calorimeter	DSC-7020	HITACHI
7	FTIR	FTIR-6100	JASCO
8	Viscometer	DVLV	Brookfield
9	Texture Analyzer	QTS 50	Brookfield
10	Diffusion cell	Franz diffusion cell	ELECTROLAB

5.3 Preformulation parameters of ectoin:

Preformulation study is the first step in project after the literature review. The preliminary step of founding rationale of dosage form is called preformulation. In preformulation we can estimate both chemical and physical properties of both drug and combined with excipients. This study helps in formulation development. Also this study can impact on performance of safe, effective and efficacious dosage form.

5.3.1 Organoleptic Properties:

Primary characteristics of ectoin like,

- Color
- Odour
- Taste
- State

Can be studied by using some practically performed methods.

5.3.2 Solubility study:

The solubility of any solid substance can be defined as “Quantity of solute that completely dissolved in unit volume of liquid solvent to create saturated solution at given temperature and pressure.” The unit of solubility is moles of solute dissolves in 100 gm of solvent. Ectoin is a very highly soluble in water it is hydrophilic molecule. In this method ectoin is gradually dissolved in different solvent at predetermined amount on magnetic stirrer at room temperature.

5.3.3 Melting Point determination:

Melting point of ectoin was determined by capillary method. Capillary was sealed at one point. Small amount of drug powder filled at another point and make sure powder touches close end of capillary. Then thermometer put into beaker which is filled with paraffin oil and capillary also tied with thermometer. Then gradually increases the temperature and when powder turned into liquid that temperature called melting point.

5.3.4 UV Spectroscopy of drug:

The UV visible spectrophotometer shimadzu model UV-1800 used for determination of λ_{max} of the ectoin and getting standard curve. Identification of ectoin was performed and compared with literature. Also standard curve was taken in water and buffer pH 6 at skin condition.

5.3.5 FT-IR analysis of drug:

FT-IR Jasco 6100 Germany was used to identification of drug and to check any impurities present in the sample or not. To perform study first ectoin was diluted with dried potassium bromide (KBr) in a 1:100 ratio and triturate it. Sample scan at resolution of 400 to 4000cm^{-1}. The characteristic peak was recorded and compared.

5.3.6 DSC analysis of drug:

DSC Hitachi-7020 was used to determine thermal behavior of ectoin. 4 mg ectoin was taken for scanning. This was kept on aluminum pan which was initially hermetically sealed. The sample was heated up to 300°C with blank reference Note the crystalline nature, melting point and glass transition temperature.

5.4 Formulation and Evaluation of Micro emulsion:

5.4.1 Method of preparation:

Micro emulsion was prepared using magnetic stirrer. First took aqueous phase in which drug ectoin was dissolved, and then put on magnetic stirrer at high speed at room temperature. Then prepared mixture of oil, surfactant and co surfactant. Then added smix and oil into aqueous phase slowly drop wise and allowed to form micro emulsion for some minutes.

5.4.2 Screening of oil, surfactant and co surfactant:

As drug ectoin is soluble in water, so there is no need to check solubility of ectoin in various oils.

First isopropyl myristate took as oil because it gives emollient effect. But isopropyl myristate form micro emulsion with tween at higher concentration, so isopropyl myristate was rejected. Then coconut oil was selected, but it having some temperature and stability issue so it was also rejected. At end jojoba oil was finalized because it is useful in dermatitis condition, and it can form micro emulsion with surfactant at lower concentration. Simulsol1293 selected as surfactant and polyethylene glycol 400 was selected as co surfactant.

5.4.3 Physical appearance:

Prepared micro emulsion must be clear.

5.4.4 Physical Stability:

Physical stability of emulsion based system is very important for its performance. Phase separation might be possible and drug may be precipitating out. Phase separation affects the physical appearance of micro emulsion. Physical stability of micro emulsion was checked by put micro emulsion 24 hr at room temperature.

5.4.5 Droplet size and polydispersibility index:

Particle size and poly dispersibility index was determined using horiba SZ-100 particle size analyzer which is based on light scattering method. Polydispersibility

index shows particle distribution throughout system. Target particle size was less than 500 nm and polydispersibility index were less than 1.

5.5 Formulation and Evaluation of micro emulgel:

5.5.1 Basic methods of preparation:

Micro emulgel was prepared by mixing two phases' micro emulsion and gel. There are generally two way to prepare micro emulgel either gelling agent directly mixed with prepared micro emulsion on stirrer or first prepare gel with water then adding this gel into micro emulsion with proper stirring.

5.5.2 Drug content:

Drug content was estimated by performing assay of formulation. To perform an assay, weigh quantity of ectoin gel was dissolved in phosphate buffer solution pH 6. Then make required dilution and each sample was analyzed by taking absorbance in UV visible spectroscopy and does needful calculations.

5.5.3 pH determination:

pH of ectoin micro emulgel was determined by using digital pH meter. pH rode directly put into prepared gel and note pH.

5.5.4 Rheological measurement:

A rheological measurement of optimized gel formulation was determined by Brookfield viscometer. That is DLVL model Brookfield rheometer. Viscosity of gel gives idea about spread ability of gel on skin. 94 spindle no. was used at 60 RPM speed to check viscosity.

5.5.5 Physical stability study:

Physical stability study of optimized gel was carried out by checking various parameters like phase separation, particle size, viscosity, drug content, pH, appearance etc. Stability study was carried out by storing ectoin micro emulgel in proper container at atmospheric condition.

5.5.6 In vitro diffusion study:

This study also called in vitro drug release study and this study was performed by using Franz diffusion cell. Cellophane membrane was used as semipermeable membrane and placed between receptor and donor compartment. Phosphate pH 6 buffer solution was filled in receptor department; 1 gm ectoin gel was spread in donor compartment. 1 ml of aliquot was replaced with fresh buffer solution at regular time interval. The withdrawn sample was analyzed using UV visible spectroscopy by taking absorbance.

5.5.7 Texture Analyzer:

Texture analyzer, gel strength of gel was determined by Brookfield QTS 50 texture analyzer. In this equipment ectoin gel was sampled in small container then this container placed under rode that having round flat end then run software so this rode from some height gradually come down and go into gel container, so got graph about extrudability and gel strength.

6. Results

6.1 Preformulation Study

6.1.1 Organoleptic Properties:

Table 4: Organoleptic properties of drug

Parameters	Inference
State	Solid
Color	White crystalline powder
Odour	Odorless
Taste	Characteristics

6.1.2 Melting point determination:

Melting point: 230°C

6.1.3 UV-VIS spectroscopy:

The determination of λ_{max} is necessary for future analytics works. For determination of λ_{max} first took absorbance in water then prepared pH 6 phosphate buffer solution and took absorbance. pH 6 is because skin condition in atopic dermatitis.

From experiment λ_{max} of ectoin: 207nm

A. Standard curve in water:

Table 5: Absorbance data with water

Concentration (ppm)	Absorbance (1)	Absorbance (2)	Absorbance (3)	Avg of Abs.	SD
1.5	0.175	0.18	0.177	0.177	0.0025
2	0.212	0.231	0.24	0.227	0.0142
2.5	0.225	0.245	0.251	0.240	0.0136
3	0.257	0.261	0.269	0.262	0.0061
3.5	0.304	0.321	0.33	0.318	0.0132
4	0.325	0.336	0.341	0.334	0.0081

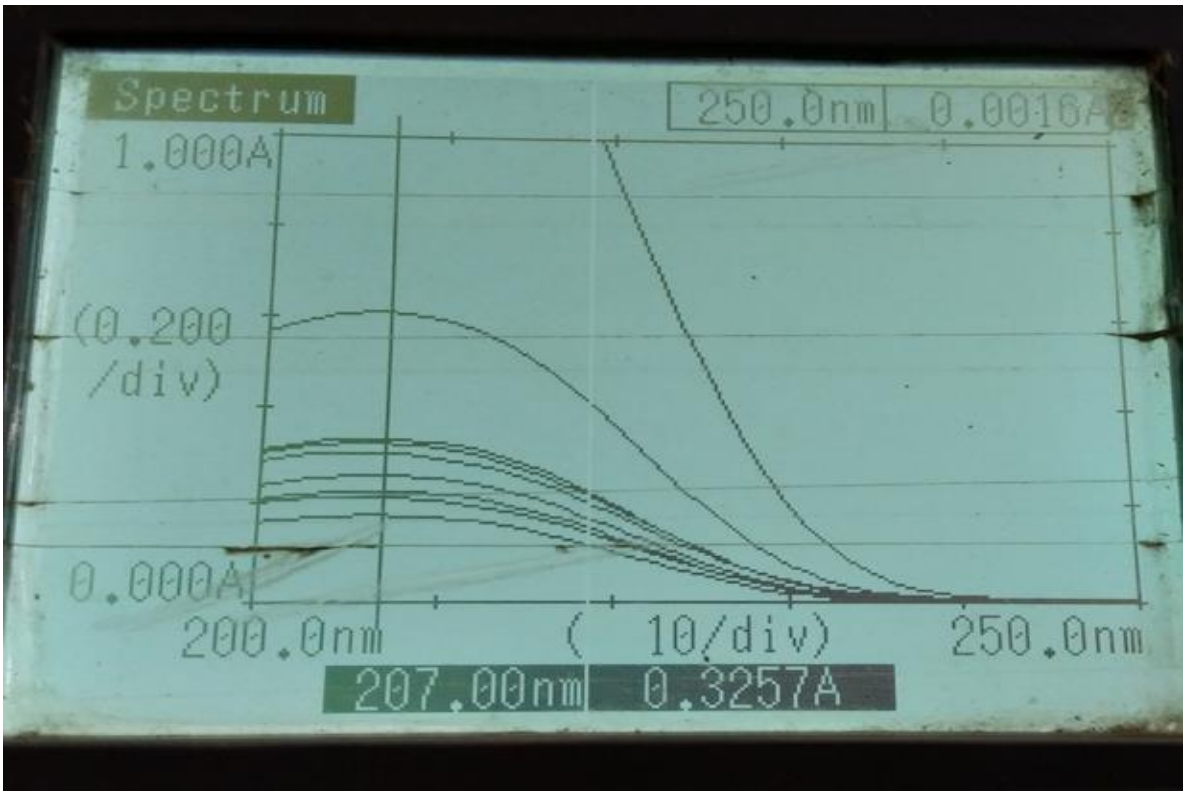


Figure 6: UV spectrum with water

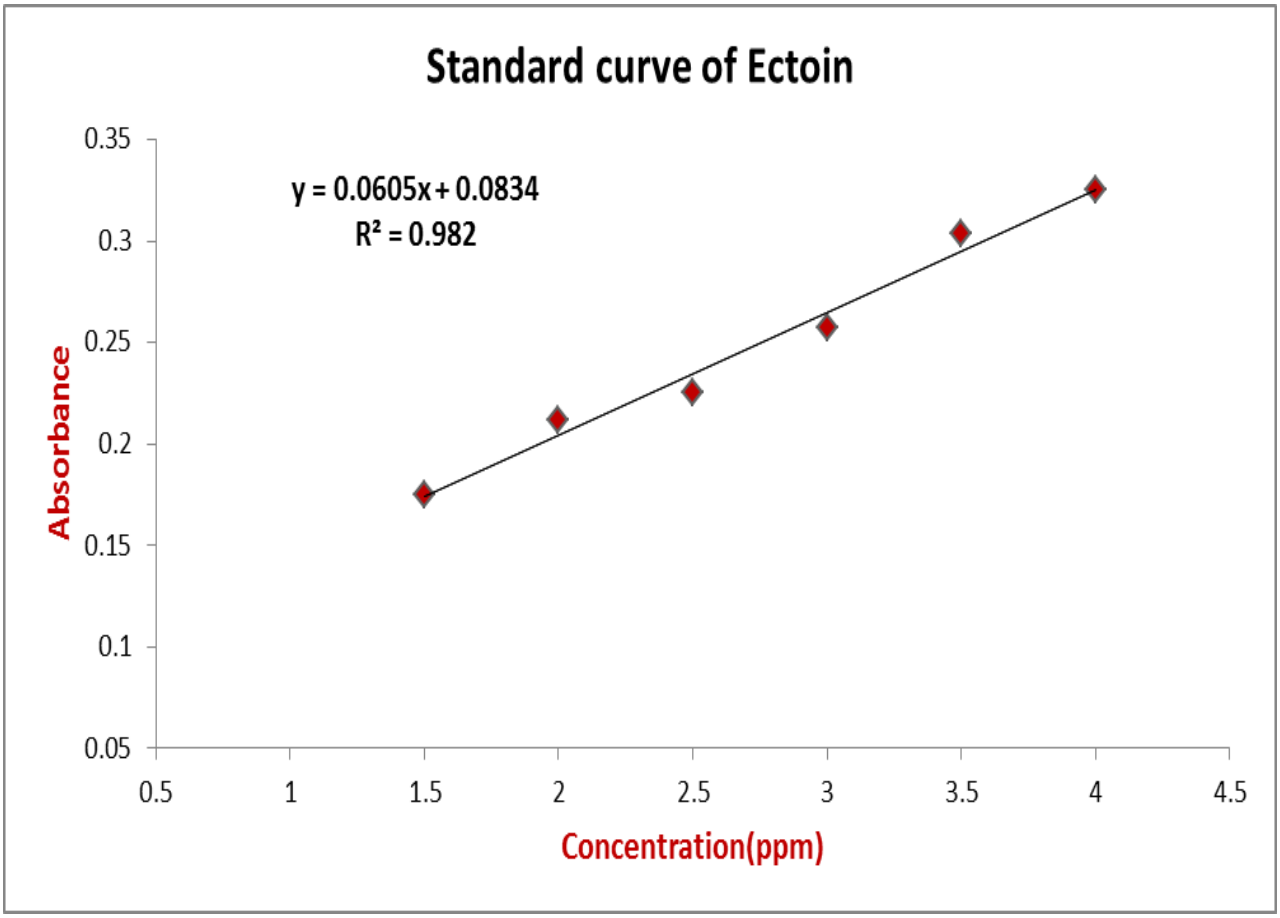


Figure 7: Standard curve of ectoin in water

B. Standard curve in phosphate buffer pH 6:

Table 6: Absorbance data with phosphate buffer pH 6

Concentra	Absorbance	Absorban	Absorb	Avg of Abs.	SD

tion (ppm)	(1)	ce (2)	ance (3)		
1	0.1	0.12	0.15	0.123	0.025166
2	0.193	0.2	0.22	0.204	0.0140.12
4	0.25	0.29	0.295	0.278	0.024664
6	0.317	0.325	0.335	0.325	0.009018
8	0.407	0.412	0.421	0.413	0.007095
10	0.501	0.52	0.531	0.517	0.015177
12	0.641	0.652	0.658	0.650	0.008622

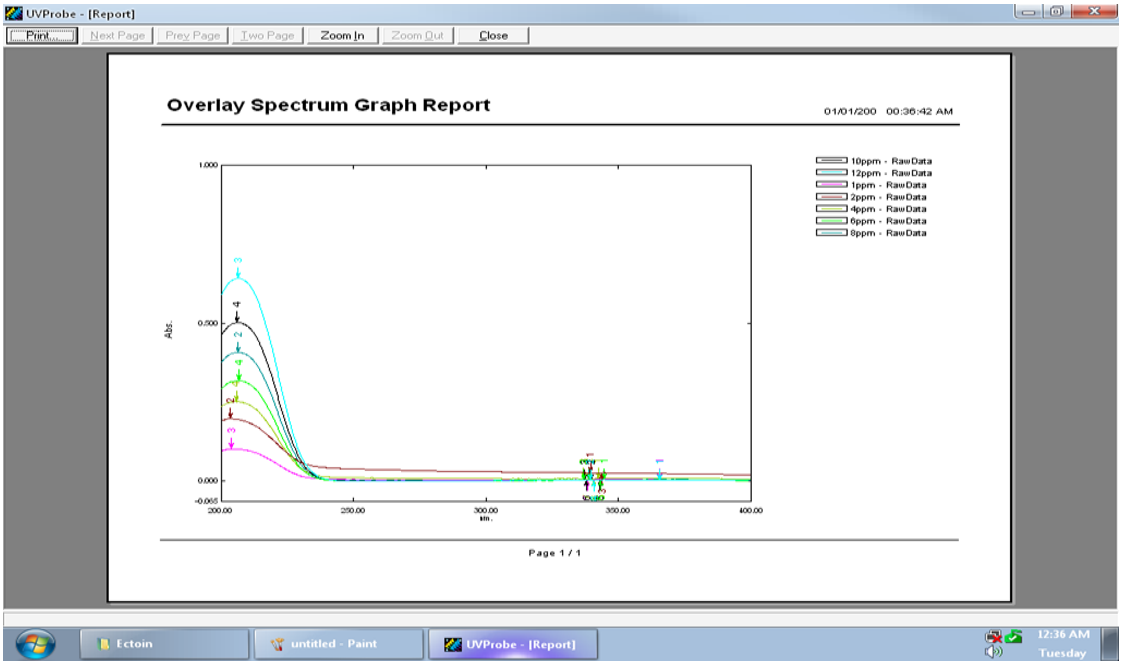


Figure 8: UV spectrum with phosphate buffer

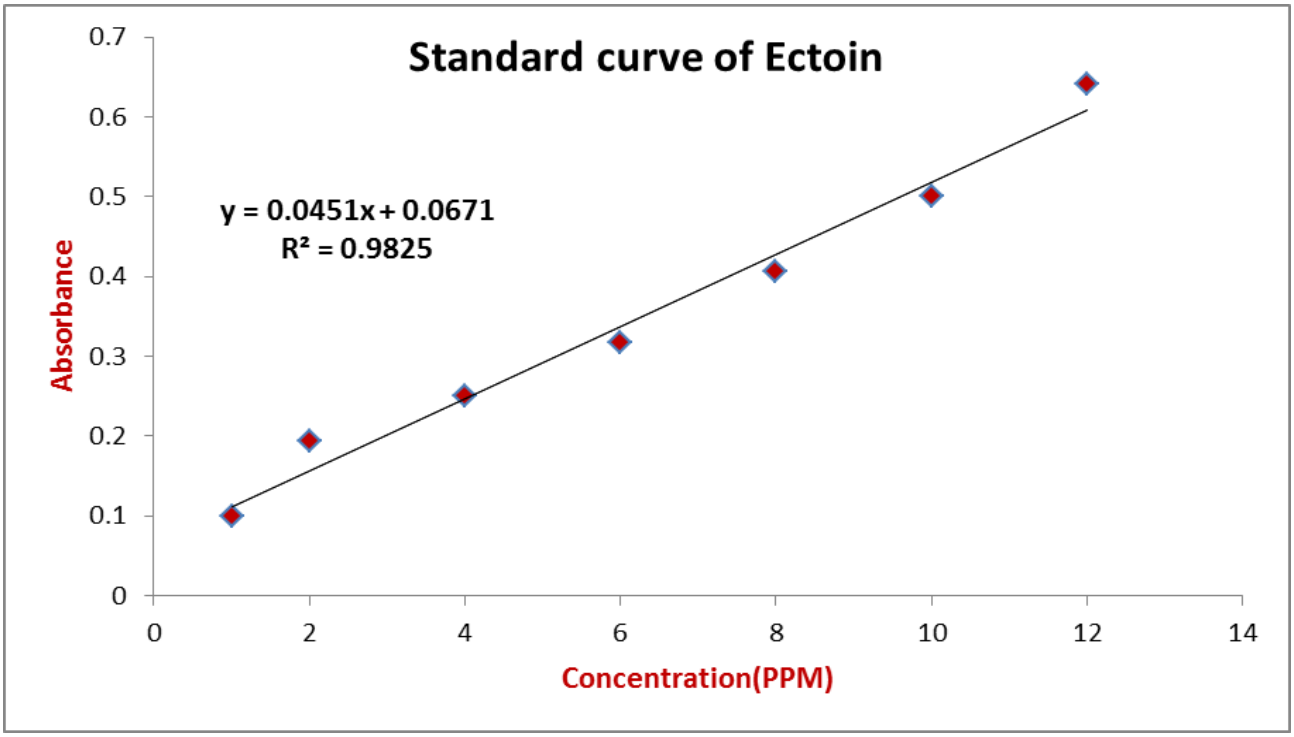


Figure 9: Standard curve of ectoin in phosphate buffer

6.1.4 FT-IR analysis of drug:

For FTIR study, drug was mixed with KBr and scan was run at resolution of 4000cm^{-1} to 400cm^{-1} in FTIR spectrophotometer. The spectra of pure drug can be obtained by this manner,

Experimental condition:

Sample: Ectoin

Light source: Standard

Detector: TGS

Resolution: 4000 to 400cm^{-1}

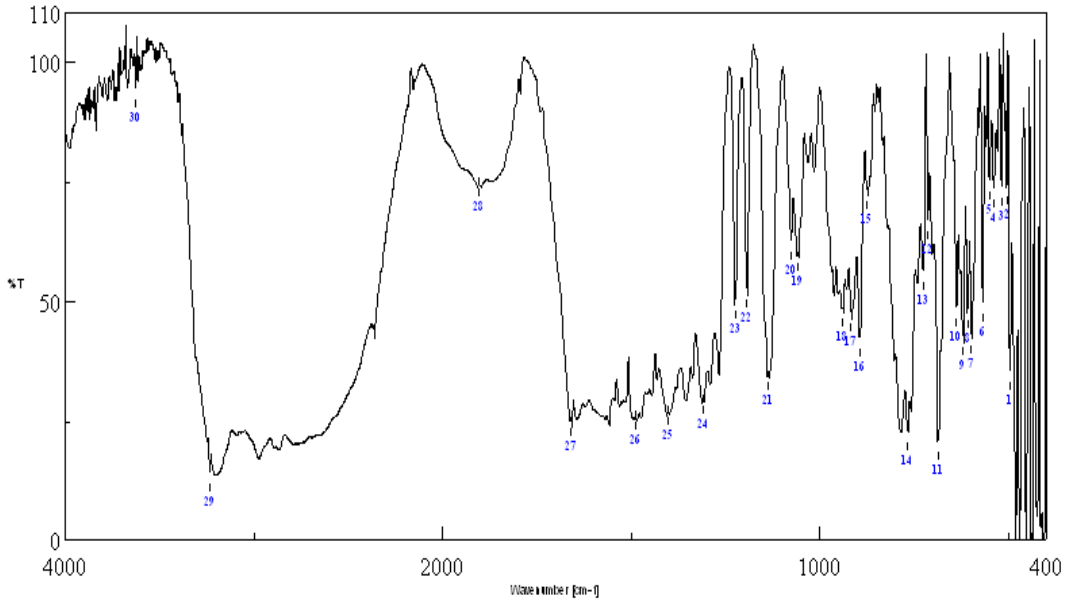


Figure 10: FTIR spectra of ectoin

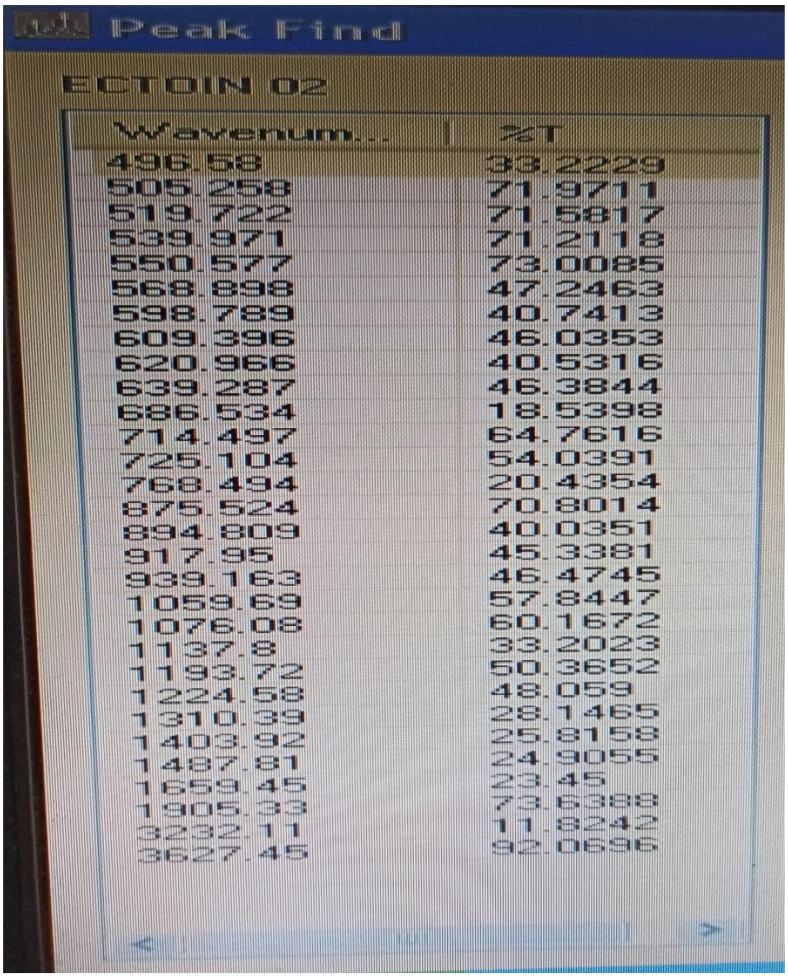


Figure 11 FTIR peaks data

6.1.5 DSC Analysis of drug:

The DSC thermo gram of ectoin shows thermal peak at 230°C with energy 0.295mW.

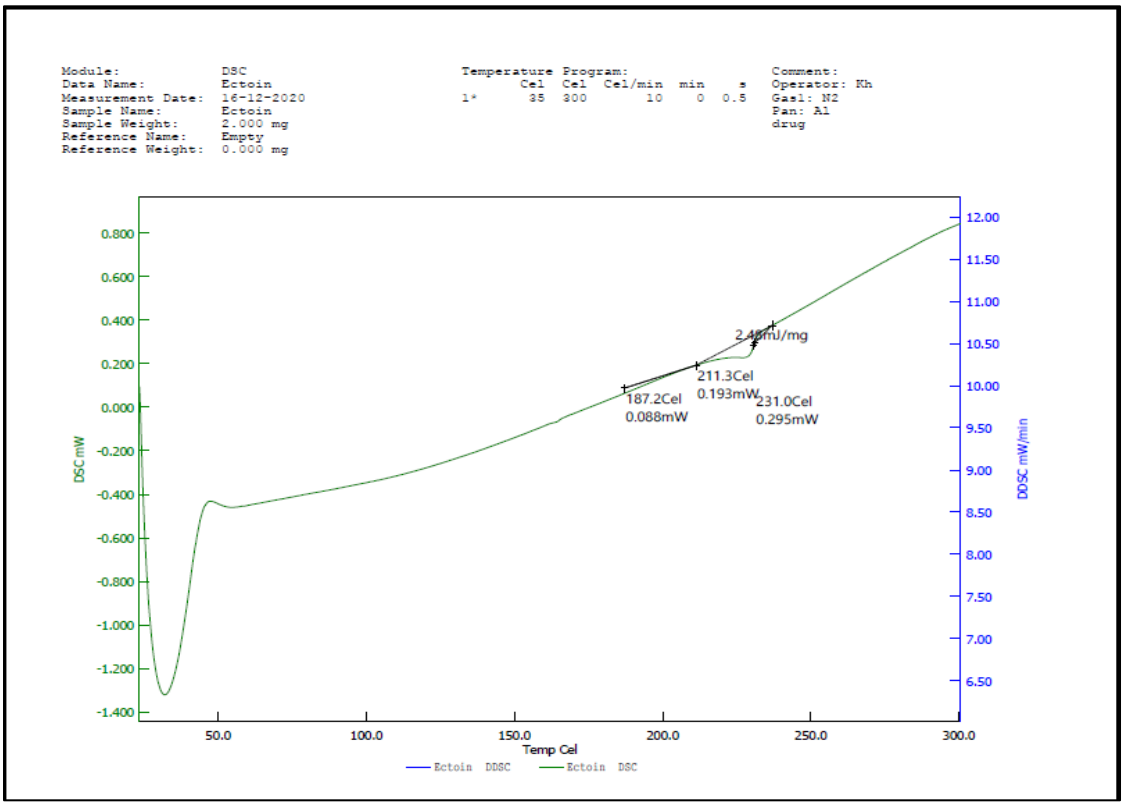


Figure 12: DSC thermo gram of ectoin

DSC thermo gram of xanthan gum (X Natural 75):

Xanthan gum shows thermal peak at 279.4°C with energy -2.072mW

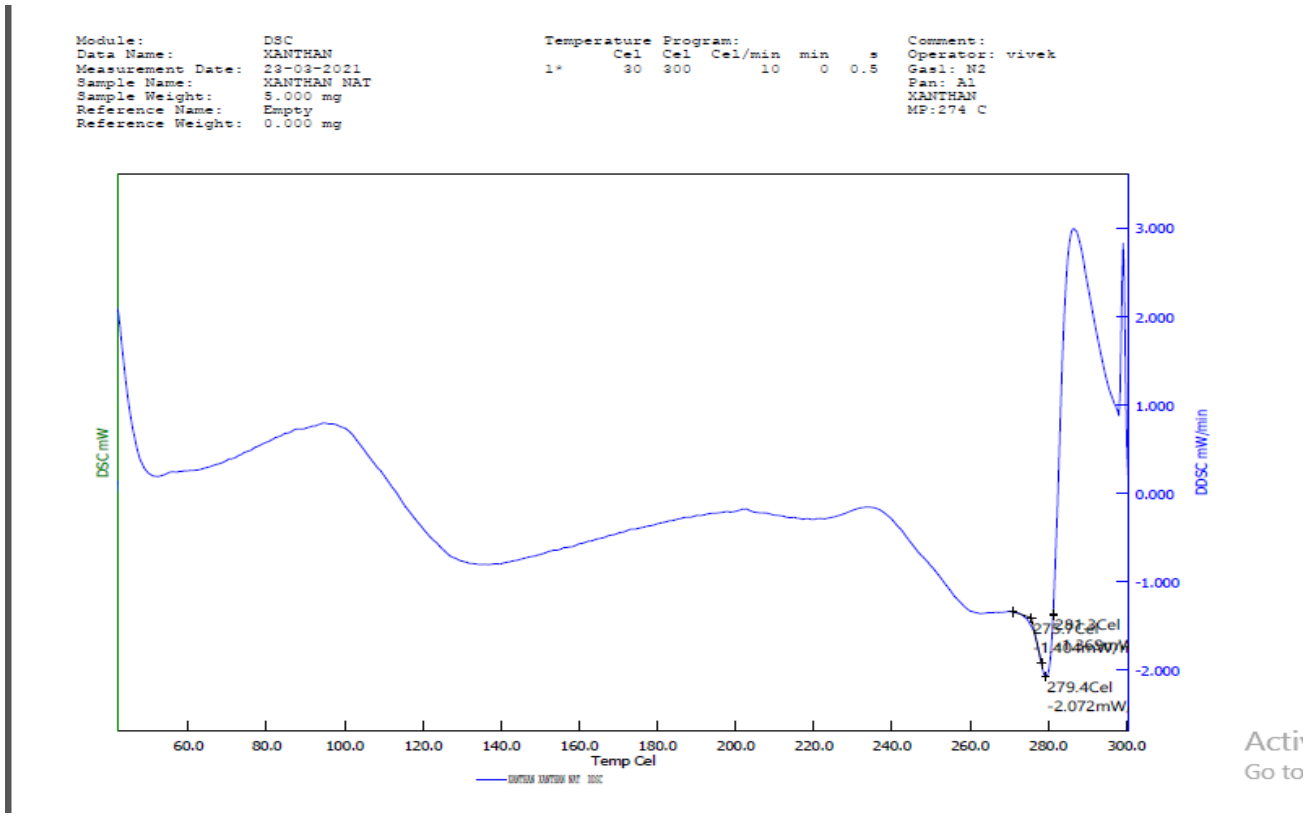


Figure 13: DSC thermo gram of xanthan gum

DSC compatibility of mixture of Ectoin and Xanthan gum:

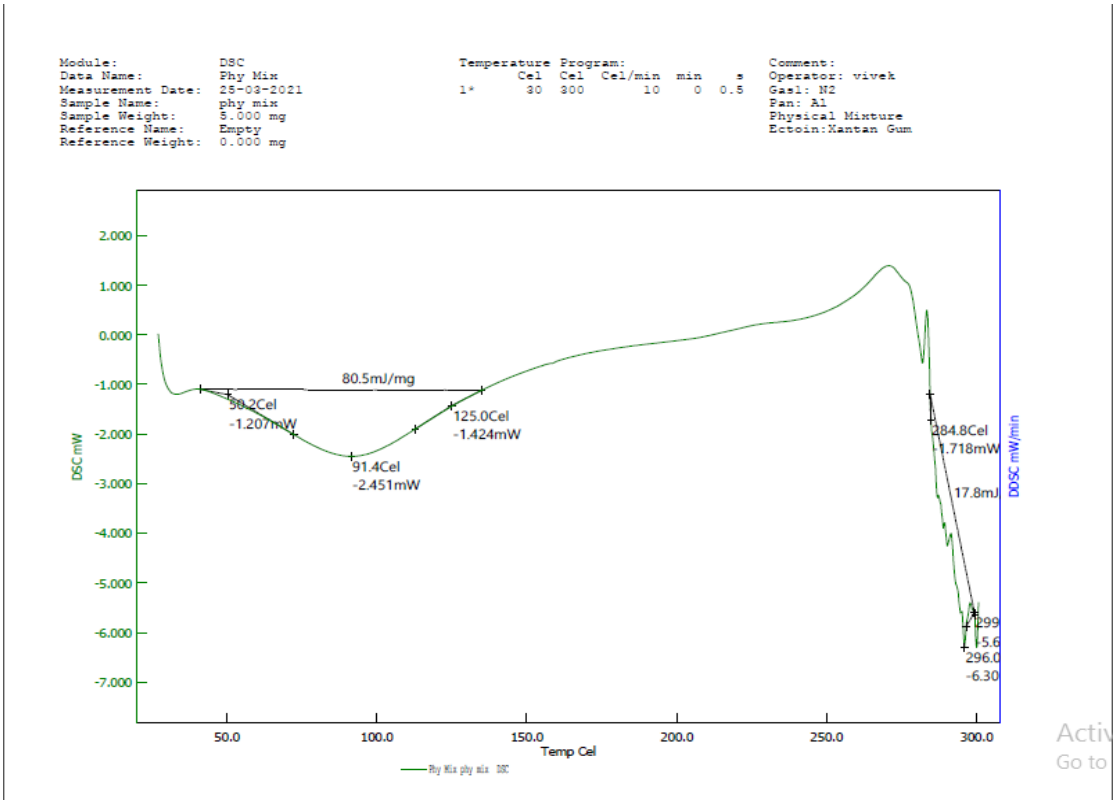


Figure 14: DSC thermo gram of mixture

Conclusion: From DSC study it was observed that drug Ectoin and polymer Xanthan gum both are compatible with each other.

6.2 Formulation and Evaluation of Micro emulsion:

6.2.1 Different oils, surfactants and co-surfactants used for trials of micro emulsion:

Table 7: List of different oils, Surfactants, Cosurfactants

Oils	Surfactants	Co-surfactants
Isopropyl myristate	Tween 80	Propylene glycol
Coconut oil	Tween 20	Transcutol
Jojoba oil	Labrasol	PEG 400
	Simulsol-1293	

6.2.2 Trials:

- ✓ Isopropyl Myristate took as oil; Tween 20 took as a surfactant and PEG 400 as a co-surfactant.
- ✓ Oil IPM 3% was fixed, this oil used as an emollient.

- ✓ Smix ratio varies 1:1, 2:1 and 3:1 with different concentrations like 30, 35, 40, 45 and 50%.
- ✓ Then took trials and it gave clear micro emulsion at 3:1(50%) with 3% IPM oil.
  - ❖ **Because Smix is 50% so I have decided to take other trials with different oil and surfactants.**

- ✓ Coconut took as oil, Simulsol-1293 and labrasol as a surfactant and transcutol, PEG 400 took as co- surfactant.
- ✓ Oil 3% was fixed used coconut oil because it is good for dermatitis condition.
- ✓ Smix ratio varies like1:1, 2:1 and 3:1 with concentrations like 25, 28, 30, 32 and 35%.
- ✓ Then took trials and it gave clear micro emulsion at 2:1(30%) with 3% coconut oil.

- ❖ **Because there was problem of temperature control with coconut oil, this oil was changed from liquid to solid and not gave good appearance to micro emulsion, so I have decided to take other trials with jojoba oil.**

6.2.3 Composition of Optimized batches:

Table 8: Composition of Optimization batches

Micro emulsion	Oil (ml)	Surfactant (ml)	Cosurfactant (ml)	Water (ml)
MEIPM01	3	25	25	47
MEIPM02	3	33.33	16.66	47
MEIPM03	3	37.5	12.5	47
MEIPM04	3	20	20	57
MEIPM05	3	26.66	13.33	57
MEIPM06	3	30	10	57
MEC01	3	20	10	67
MEC02	3	16.66	8.33	72
MEC03	3	18.66	9.33	69
MEC04	3	21.33	10.66	65
MEJ01	3	20	10	67
MEJ02	3	16.66	8.33	72
MEJ03	3	18.66	9.33	69
MEJ04	3	21.33	10.66	65

Here 3 ml oil was kept constant and Smix and water changed and jojoba oil gives clear micro emulsion with 2:3 ratio of Smix. Others batches are hazy and unstable.

6.2.4 Different oils in micro emulsion with Zavg & PDI value:

Table 9: Different oils with Zavg and PDI value

Oil	Ratio of Smix	Zavg	PDI
Isopropyl myristate	3:1(50%)	6982.5nm	>1
Coconut oil	2:1(30%)	279.2nm	0.485
Jojoba oil	2:1(30%)	93.9nm	0.444

6.2.5 Pseudo ternary phase diagram:

Pseudo ternary diagram was plotted by using chemix software. By taking different concentration of oil and Smix this graph was generated: (Jojoba oil, Simulsol-1293, PEG 400 and water)

Table 10: Phase ternary diagram data

Oil (ml)	Smix (ml)	Water (ml)
1	20	79
2	35	63
3	30	67
4	25	71
5	30	65
6	20	74

7	25	68
8	28	64
9	30	61
10	35	55

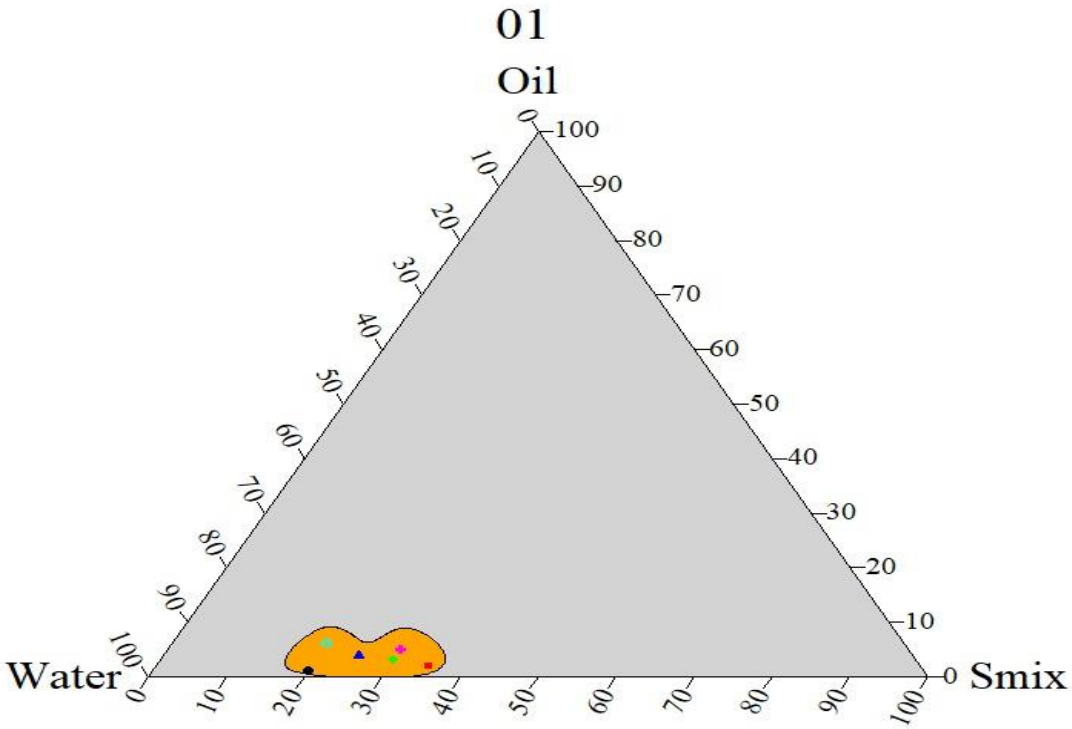


Figure 15: Phase ternary diagram

Figure 16: Image of microemulsions

- ✓ Jojoba oil took as oil, Simulsol-1293 as a surfactant and PEG 400 as a co-surfactant.
- ✓ Oil also varies from 1 to 8%, jojoba oil is widely used for atopic dermatitis.
- ✓ Smix ratio also varies like 1:1, 2:1 and 3:1 with different concentrations like 25, 28, 30, 32 and 35%.
- ✓ It gave clear micro emulsion at 2:1(30%) with 3% jojoba oil.

Final formula of my micro emulsion:

Table 11: Final formula of micro emulsion

Ingredients	Percentage

Jojoba oil	3
Simulsol-1293	20
PEG-400	10
Ectoin	3
Water	64

6.2.6 Particle size and Zeta potential of final formula:

- Zavg: 93.9 nm
- PDI: 0444
- Zeta potential (mean): -0.4 mV

Here in particle size and zeta potential study, jojoba oil gives appropriate results.

Globule size and Zeta potential study showing good result with good clear microemulsion of jojoba oil.

Here jojoba oil 3 ml and Smix ratio is 2:1 with 30% concentration.

2021.03.02 13:23:04

HORIBA Scientific

HORIBA SZ-100 for Windows [Z Type] Ver2.20

SZ-100

final jojoba ME 2,1 30 3_6163.nsz

Measurement Results

Date	:	Tuesday, March 02, 2021 12:05:03 PM
Measurement Type	:	Particle Size
Sample Name	:	final jojoba ME 2:1 30 3
Scattering Angle	:	90
Temperature of the Holder	:	25.0 °C
Dispersion Medium Viscosity	:	0.895 mPa·s
Transmission Intensity before Meas.	:	8544
Distribution Form	:	Standard
Distribution Form(Dispersity)	:	Monodisperse
Representation of Result	:	Scattering Light Intensity
Count Rate	:	127 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	95.8 nm	29.7 nm	87.4 nm
2	—	— nm	— nm	— nm
3	—	— nm	— nm	— nm
Total	1.00	95.8 nm	29.7 nm	87.4 nm

Histogram Operations

% Cumulative (1)	:	10.0 (%) - 61.1 (nm)
% Cumulative (2)	:	50.0 (%) - 90.4 (nm)
% Cumulative (3)	:	90.0 (%) - 139.3 (nm)

Cumulant Operations

Z-Average	:	93.9 nm
PI	:	0.444

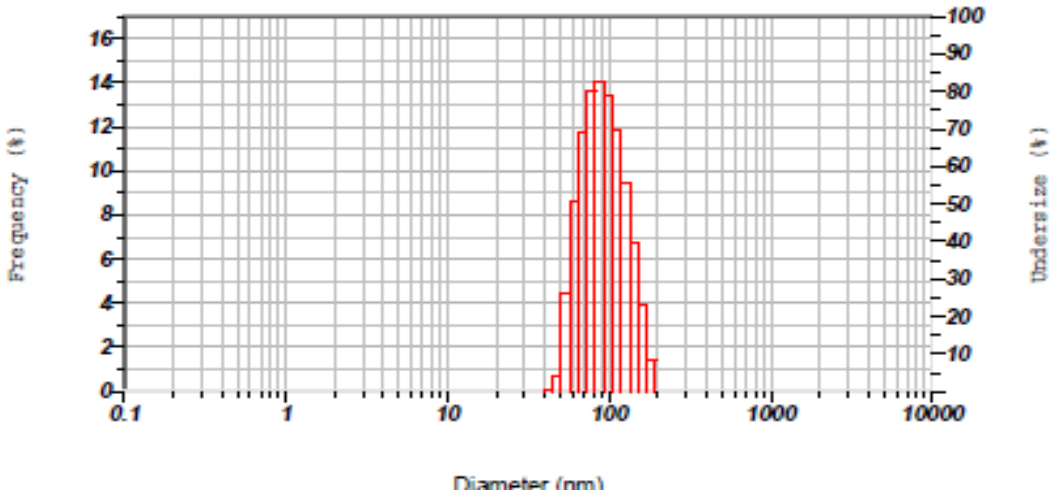


Figure 17: Particle size graph

2021.03.02 13:17:11

HORIBA SZ-100 for Windows [Z Type] Ver2.20

HORIBA Scientific

SZ-100

Measurement Results

Final jojoba oil 2,1 30 3 Zeta.nzt

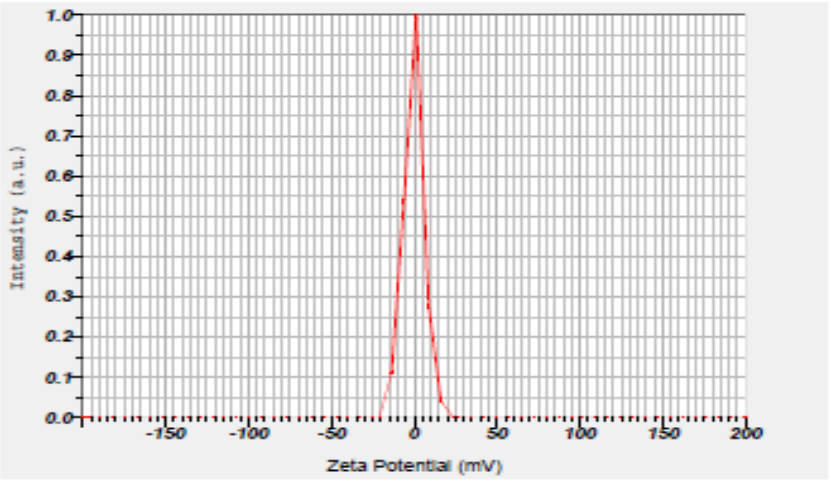
Measurement Results

Date	: Wednesday, May 15, 2019 10:06:11 AM
Measurement Type	: Zeta Potential
Sample Name	: CD-188-B-1:10-8/5/2019
Temperature of the Holder	: 25.0 °C
Dispersion Medium Viscosity	: 0.895 mPa·s
Conductivity	: 0.149 mS/cm
Electrode Voltage	: 3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-0.4 mV	-0.000003 cm2/Vs
2	— mV	— cm2/Vs
3	— mV	— cm2/Vs

Zeta Potential (Mean)	: -0.4 mV
Electrophoretic Mobility Mean	: -0.000003 cm²/Vs



Explore the future Automotive Test Systems | Process & Environmental | Medical | Semiconductor | Scientific HORIBA

1 / 1

Figure 18: Zeta potential graph

6.3 Formulation and Evaluation of micro emulgel:

6.3.1 Trials taken with different gelling agent:

Table 12: Trails with different gelling agent

Micro emulsion	Gelling agent	Result
Micro emulsion	Carbopol ultrez 20	Hazy and viscosity break
Micro emulsion	Hydroxyl ethyl cellulose	Gel was not formed
Micro emulsion	Xanthan gum (xnatural 75)	Clear transparent gel was formed

6.3.2 Final formulation of product:

Table 13: Final formula of product

Ingredients	Qty
Micro emulsion	97.9%
Gelling agent: Xnatural 75	1%
Preservative: Galgard trident	1%
Chelating agent: EDTA	0.1%

6.3.3 pH of emulgel:

Table 14: Data of pH

Concentration of xanthan gum in gel	pH
1%	5.7
1.5%	5.8
2%	6.0
2.5%	6.1

6.3.4 Viscosity of emulgel:

- ✓ 1% ectoin gel: At 60 RPM 2350cp using S94 spindle
- ✓ 1.5% ectoin gel: At 10 RPM 16440cp using S94 spindle
- ✓ 2% ectoin gel: At 6 RPM 29306cp using S94 spindle
- ✓ 2.5% ectoin gel: At 6 RPM 35330cp using S94 spindle

6.3.5 Physical appearance of micro emulgel:

Final prepared gel (with xanthan gum) is clear, transparent and good in appearance.

6.3.6 Drug content:

Table 15: Data of % drug content

% Gel	%DC
1	82.49
1.5	100.72
2	99.14
2.5	87.40

6.3.7 In vitro diffusion study of gel:

Diffusion study of different concentration of xanthan gum in emulgel:

Diffusion of 1% gel:

Table 16: Data of % drug release of 1% gel

Time	CPR 1	CPR 2	AVG
1	2.621713	5.847936	4.234825

3	3.257181	6.760403	5.008792
5	8.829748	9.31857	9.074159
10	6.222699	16.27776	11.25023
15	14.53267	18.72187	16.62727
20	10.78503	26.05419	18.41961
25	22.14362	23.93597	23.03979
30	18.07011	29.15006	23.61009
45	20.18833	39.41532	29.80182
60	30.45359	46.91058	38.68209
90	49.19175	55.70937	52.45056
120	52.28762	78.35811	65.32286
150	56.84996	64.18228	60.51612
180	75.0993	71.02578	73.06254
240	70.04814	76.7287	73.38842
300	92.85982	79.98751	86.42367
360	74.93636	78.03223	76.48429
420	80.47633	54.40585	67.44109
480	75.75106	86.83102	81.29104
540	95.14098	106.3839	100.7624
600	119.0932	96.60745	107.8503

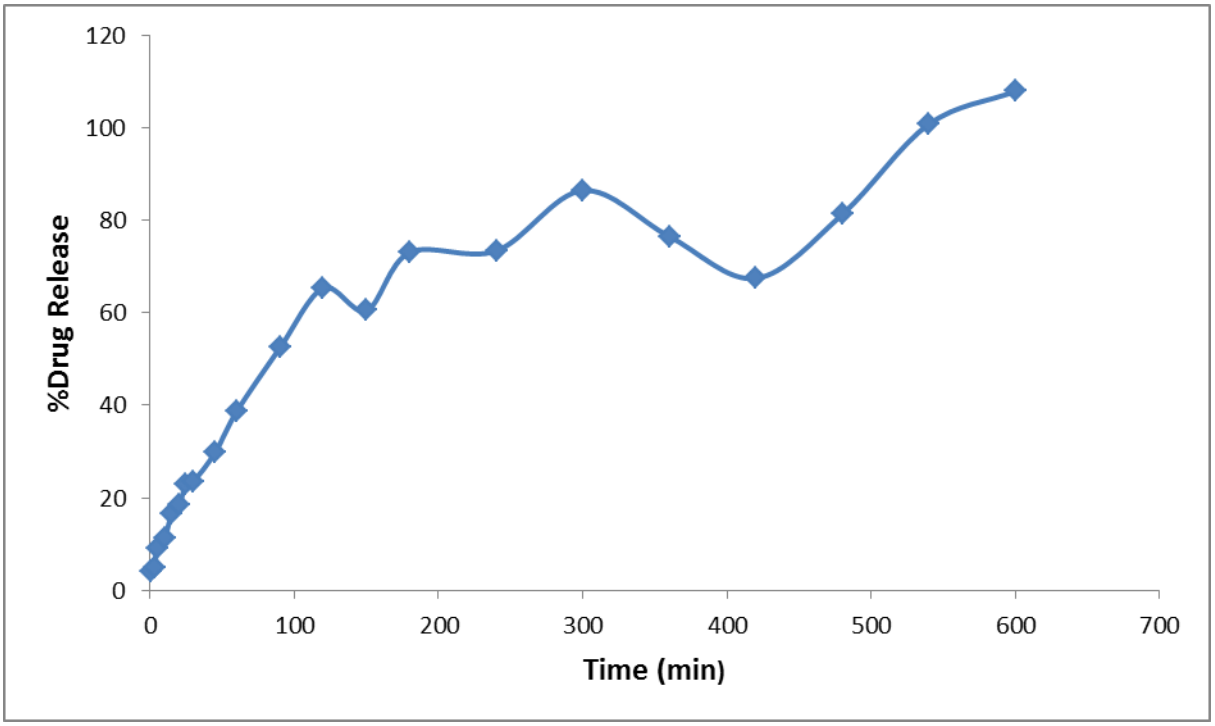


Figure 19: % Ectoin release from 1% emulgel

Diffusion of 1.5% gel:

Table 17: Data of % drug release of 1.5% gel

Time (min)	Absorbance	Concentration (µg/ml)	df	conc	amt in 50	CDR (mg)	CPR
1	0.72	14.4767184	5	72.38359	3619.18	3.61918	9.35914
3	0.093	0.574279379	50	28.71397	1435.698	1.435698	3.712693
5	0.117	1.106430155	50	55.32151	2766.075	2.766075	7.153027
10	0.192	2.76940133	50	138.4701	6923.503	6.923503	17.90407
15	0.211	3.190687361	50	159.5344	7976.718	7.976718	20.62767
20	0.312	5.430155211	50	271.5078	13575.39	13.57539	35.10574
25	0.288	4.898004435	50	244.9002	12245.01	12.24501	31.6654
30	0.35	6.272727273	50	313.6364	15681.82	15.68182	40.55293
45	0.346	6.184035477	50	309.2018	15460.09	15.46009	39.97954
60	0.398	7.337028825	50	366.8514	18342.57	18.34257	47.4336
90	0.43	8.046563193	50	402.3282	20116.41	20.11641	52.02071
120	0.466	8.844789357	50	442.2395	22111.97	22.11197	57.18121
150	0.544	10.57427938	50	528.714	26435.7	26.4357	68.36229
180	0.526	10.1751663	50	508.7583	25437.92	25.43792	65.78204
240	0.594	11.68292683	50	584.1463	29207.32	29.20732	75.52965
300	0.563	10.99556541	50	549.7783	27488.91	27.48891	71.08589
360	0.684	13.67849224	50	683.9246	34196.23	34.19623	88.4309
420	0.782	15.85144124	50	792.5721	39628.6	39.6286	102.4789
480	0.688	13.76718404	50	688.3592	34417.96	34.41796	89.00429
540	0.73	14.69844789	50	734.9224	36746.12	36.74612	95.02488
600	0.63	12.48115299	50	624.0576	31202.88	31.20288	80.69015
660	0.703	14.09977827	50	704.9889	35249.45	35.24945	91.1545
720	0.79	16.02882483	50	801.4412	40072.06	40.07206	103.6257

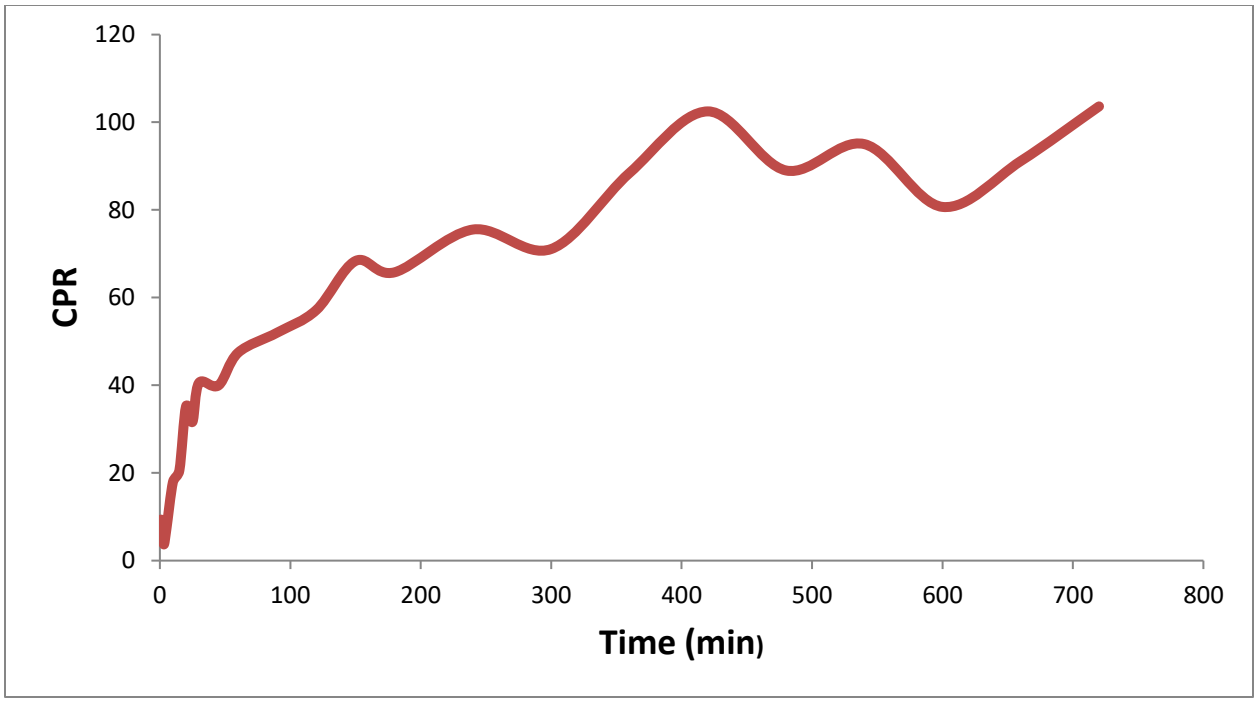


Figure 20: % Ectoin release from 1.5% emulgel

Diffusion of 2% gel:

Table 18: Data of % drug release of 2% gel

Time (min)	Absorbance	Concentration (µg/ml)	df	Conc	amt in 50	CDR (mg)	CPR
1	0.49	9.376940133	5	46.8847	2344.235	2.344235	7.413773
3	0.93	19.13303769	5	95.66519	4783.259	4.783259	15.12732
5	0.143	1.682926829	50	84.14634	4207.317	4.207317	13.30587
10	0.18	2.503325942	50	125.1663	6258.315	6.258315	19.79227
15	0.294	5.031042129	50	251.5521	12577.61	12.57761	39.77737
20	0.338	6.006651885	50	300.3326	15016.63	15.01663	47.49092
25	0.331	5.851441242	50	292.5721	14628.6	14.6286	46.26377
30	0.345	6.161862528	50	308.0931	15404.66	15.40466	48.71808
45	0.432	8.090909091	50	404.5455	20227.27	20.22727	63.96987
60	0.439	8.246119734	50	412.306	20615.3	20.6153	65.19703
90	0.495	9.487804878	50	474.3902	23719.51	23.71951	75.01427
120	0.51	9.820399113	50	491.02	24551	24.551	77.64389
150	0.57	11.15077605	50	557.5388	27876.94	27.87694	88.16237
180	0.549	10.68514412	50	534.2572	26712.86	26.71286	84.4809
240	0.546	10.61862528	50	530.9313	26546.56	26.54656	83.95498
300	0.623	12.32594235	50	616.2971	30814.86	30.81486	97.45369
360	0.748	15.09756098	50	754.878	37743.9	37.7439	119.3672

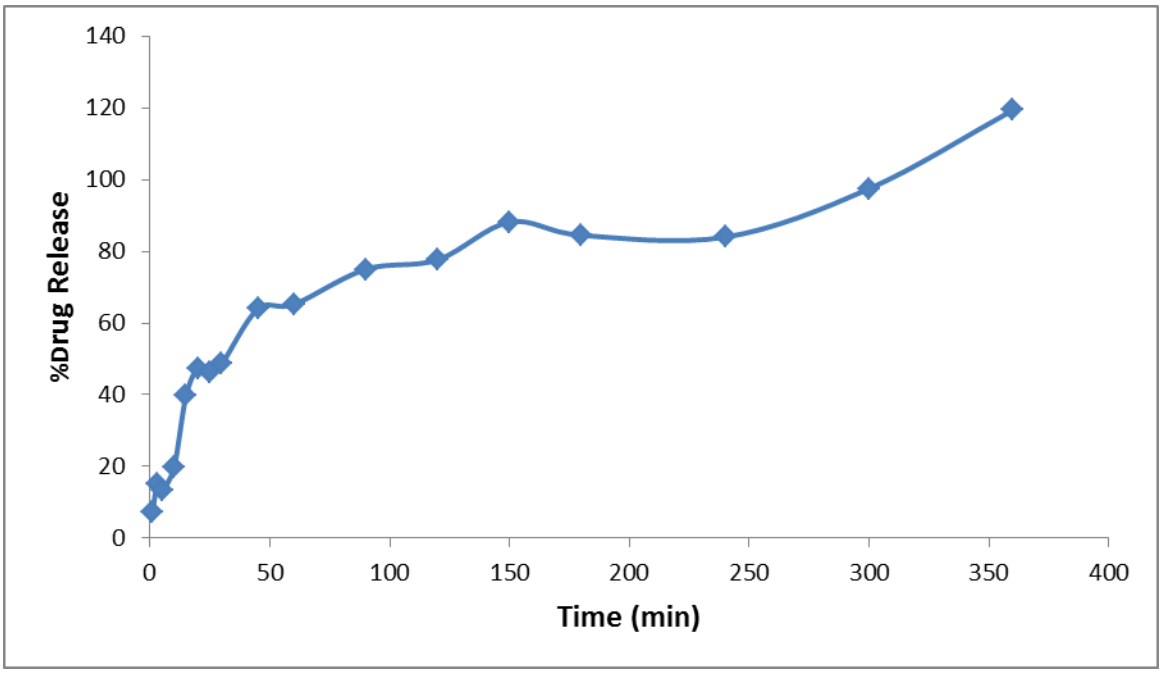


Figure 21: % Ectoin release from 2% emulgel

Diffusion of 2.5% gel:

Table 19: Data of % drug release of 2.5% gel

Time (min)	Absorbance	Concentration (µg/ml)	df	Conc	amt in 50	CDR (mg)	CPR
1	0.141	1.638580931	50	81.92905	4096.452	4.096452	10.30554
3	0.18	2.503325942	50	125.1663	6258.315	6.258315	15.74419

5	0.151	1.860310421	50	93.01552	4650.776	4.650776	11.70007
10	0.206	3.079822616	50	153.9911	7699.557	7.699557	19.36995
15	0.259	4.254988914	50	212.7494	10637.47	10.63747	26.76094
20	0.283	4.78713969	50	239.357	11967.85	11.96785	30.1078
25	0.296	5.075388027	50	253.7694	12688.47	12.68847	31.92068
30	0.317	5.541019956	50	277.051	13852.55	13.85255	34.84918
45	0.646	12.83592018	50	641.796	32089.8	32.0898	80.72906
60	0.64	12.70288248	50	635.1441	31757.21	31.75721	79.89234
90	0.694	13.90022173	50	695.0111	34750.55	34.75055	87.42278
120	0.726	14.6097561	50	730.4878	36524.39	36.52439	91.88526

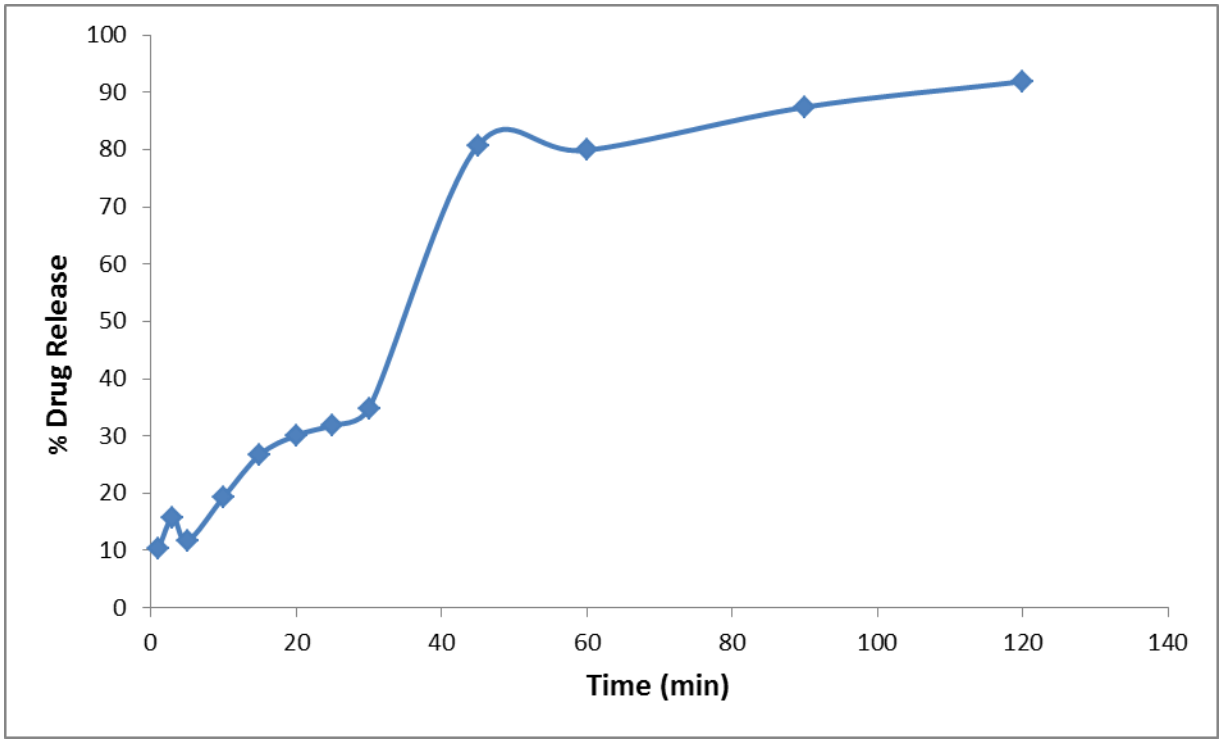


Figure 22: % Ectoin release from 2.5% emulgel

Overlay of drug release data:

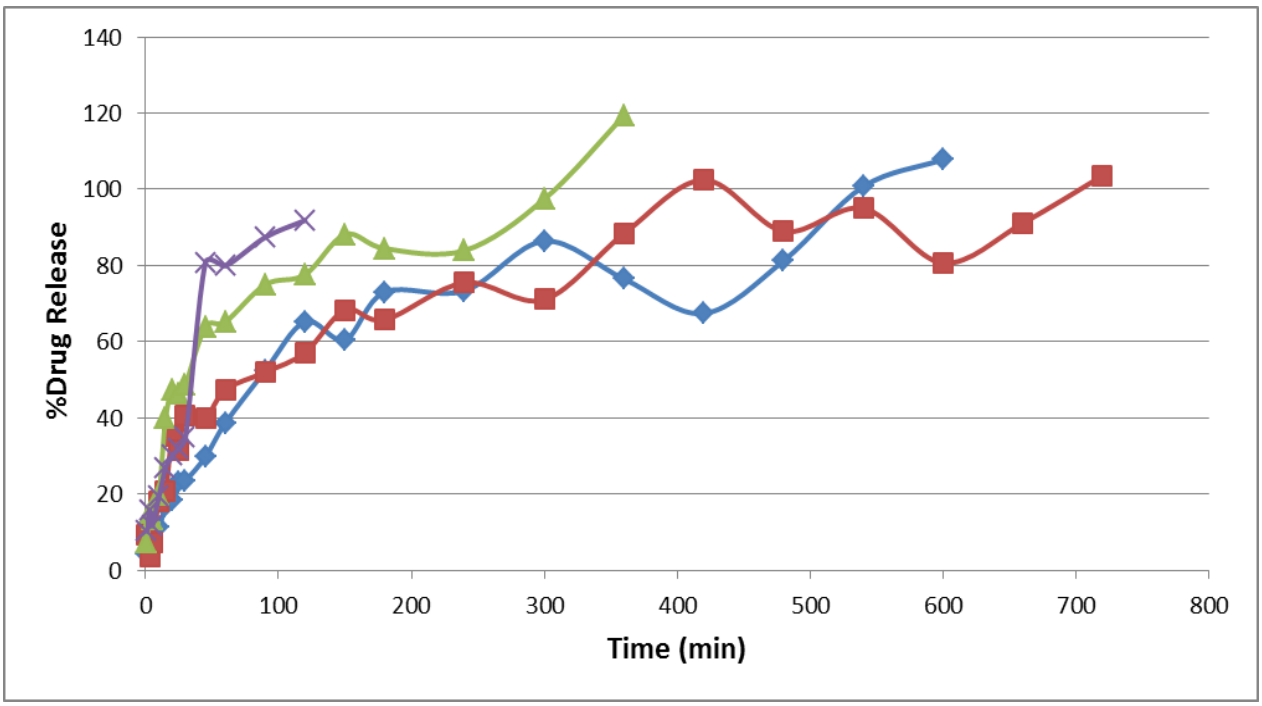


Figure 23: Overlay of % Ectoin release

From above studies, it is concluded that drug release is good in 1% gel which is final concentration of xanthan gum in final formula.

6.3.8 Texture analysis study of final emulgel:

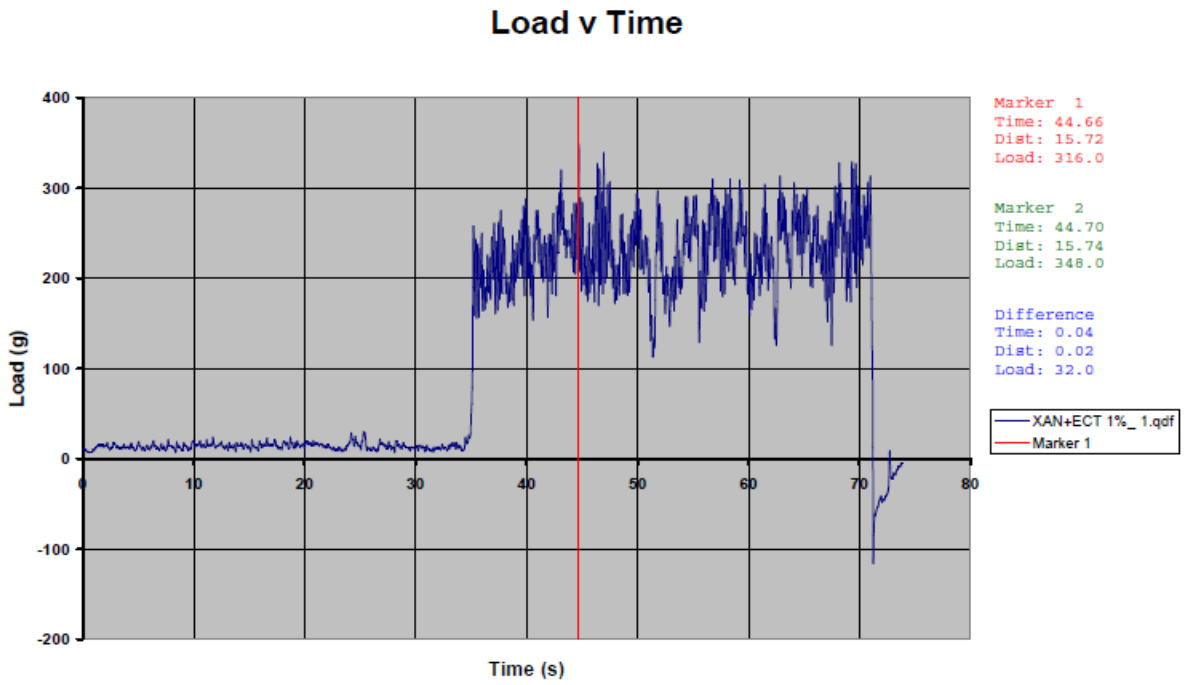


Figure 24: Analysis of texture and gel strength

- ✓ From this study, gel strength and texture can be obtained.
- ✓ I did this study on my final 1% emulgel formulation.
- ✓ 1% emulgel have good gel strength like 348g in 45 sec.

7. Conclusion

Ectoin in microemulgel for treatment of atopic dermatitis:

Nowadays, we should avoid harmful topical steroids for the treatment of atopic dermatitis and find way to treat dermatitis naturally. So, ectoin is a good natural option for treatment of atopic dermatitis. Ectoin is also used cosmeceutically and used in other countries for treatment of topical diseases. In India we should formulate different dosage form of ectoin. It not gives any side effects like topical steroids. Ectoin 3% used in various formulations to treat topical diseases. Micro emulgel of ectoin is not available in market, so this emulgel can increase penetration of ectoin through skin. In this formulation we used jojoba oil which is widely help in atopic dermatitis. Instead of tweens, simulsol is used as surfactant. Natural xanthan gum used for emulgel. Also in formulation harmful parabans are not used as preservative but galguard trident is used. Prepared 1% micro emulgel of ectoin gives good result.

8. References

1. Ahn K, Kim BE, Kim J, Leung DY. Recent advances in atopic dermatitis. Curr Opin Immunol. 2020;66:14–21.

2. Patel N, Feldman SR. Management of atopic dermatitis. Adherence in atopic Dermatitis. Introduction. Adv Exp Med Biol. 2017;1027:139–59.

3. Fishbein AB, Silverberg JI, Wilson EJ, Ong PY. Update on Atopic Dermatitis: Diagnosis, Severity Assessment, and Treatment Selection. J Allergy Clin Immunol Pract. 2020;8(1):91–101.

4. Khan BA, Akhtar N, Khan HMS, Waseem K, Mahmood T, Rasul A, et al. Basics of pharmaceutical emulsions: A review. African J Pharm Pharmacol. 2011;5(25):2715–25.

5. Jadhav A, Daundkar A, Morale D, Bhujbal N. Review Article Review on: Microemulsion a Novel Approach for Drug Delivery. Int J Pharm Sci Rev Res,. 2018;52(11):60–5.

6. Singh PK, Kashif Iqubal M, Shukla VK, Shuaib M. Microemulsions: Current Trends in Novel Drug Delivery Systems. J Pharm Chem Biol Sci J Pharm Chem Biol Sci J Pharm Chem Biol Sci. 2014;1(11):39–5139.

7. Mohammed Haneefa KP, Easo S, Hafsa P V., Prasad Mohanta G, Nayar C.

Emulgel: An advanced review. J Pharm Sci Res. 2013;5(12):254–8.

8. Akki R, Susmitha B, Kiranmai J. A novel approach for topical delivery using emulgel. 2019;8(4):35–42.

9. Lavi G, Ebrahimi M, Schmidts T, Runkel F, Czermak P. Development and production of oil-in-water vehicles submicron emulsion the dermal application of ectoin. AIChE Annu Meet Conf Proc. 2006;(January).

10. Bownik A, Stępniewska Z. Ectoine as a promising protective agent in humans and animals. Arh Hig Rada Toksikol. 2016;67(4):260–5.

11. Tung NT, Vu VD, Nguyen PL. DoE-based development, physicochemical characterization, and pharmacological evaluation of a topical hydrogel containing betamethasone dipropionate microemulsion. Colloids Surfaces B Biointerfaces. 2019;181(June):480–8.

12. Chen H, Chang X, Du D, Li J, Xu H, Yang X. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int J Pharm. 2006;315(1–2):52–8.

13. Neubert RHH, Schmalfuß U, Wolf R, Wohlrab WA. Microemulsions as colloidal vehicle systems for dermal drug delivery. Part V: Microemulsions without and with glycolipid as penetration enhancer. J Pharm Sci. 2005;94(4):821–7.

14. Okur ME, Ayla Ş, Yozgatlı V, Aksu NB, Yoltaş A, Orak D, et al. Evaluation of burn wound healing activity of novel fusidic acid loaded microemulsion based gel in male Wistar albino rats. Saudi Pharm J. 2020;28(3):338–48.

15. Cavalcanti ALM, Reis MYFA, Silva GCL, Ramalho ÍMM, Guimarães GP, Silva JA, et al. Microemulsion for topical application of pentoxifylline: In

vitro release and in vivo evaluation. Int J Pharm. 2016;506(1–2):351–60.

16. Pacific T, Information S, Museum BPB, Foundation NS, Anthro P, Botanists P, et al. Scientific Information. 1967;(August 1966).

17. Yuan L, Pan M, Lei M, Zhou X, Hu D, Liu Q, et al. A novel composite of micelles and hydrogel for improving skin delivery of hydrocortisone and application in atopic dermatitis therapy. Appl Mater Today. 2020;19:100593.

18. Kale SN, Deore SL. Emulsion micro emulsion and nano emulsion: A review. Syst Rev Pharm. 2016;8(1):39–47.

19. Yadav V, Jadhav P, Kanase K, Bodhe A, Dombe S. Preparation and evaluation of microemulsion containing antihypertensive drug. Int J Appl Pharm. 2018;10(5):138–46.

20. Gupta RR, Jain SK, Varshney M. AOT water-in-oil microemulsions as a penetration enhancer in transdermal drug delivery of 5-fluorouracil. Colloids Surfaces B Biointerfaces. 2005;41(1):25–32.

21. Khiljee S, Rehman NU, Khiljee T, Ahmad RS, Khan MY, Qureshi UA. Use of traditional herbal medicines in the treatment of eczema. J Pakistan Assoc Dermatologists. 2011;21(2):112–7.

22. Rozman B, Gasperlin M, Tinois-Tessoneaud E, Pirot F, Falson F. Simultaneous absorption of vitamins C and E from topical microemulsions using reconstructed human epidermis as a skin model. Eur J Pharm Biopharm. 2009;72(1):69–75.

23. Xing H, Su B, Wang Y, Yang Y, Ren Q, Xiao W, et al. Separation and determination of asiaticoside, asiaticoside-B and madecassoside in centella asiatica total triterpenoid saponins by HPLC. J Liq Chromatogr Relat

Technol. 2009;32(13):1891–900.

24. Nakagawa H. Treatment of Atopic Dermatitis with Immunomodulatory Drugs. 2002;126(1):477–82.

25. Shewaiter MA, Hammady TM, El-Gindy A, Hammadi SH, Gad S. Formulation and characterization of leflunomide/diclofenac sodium microemulsion base-gel for the transdermal treatment of inflammatory joint diseases. J Drug Deliv Sci Technol. 2021;61(October):102110.

26. Das S, Lee SH, Chow PS, Macbeath C. Microemulsion composed of combination of skin beneficial oils as vehicle: Development of resveratrol-loaded microemulsion based formulations for skin care applications. Colloids Surfaces B Biointerfaces. 2020;194(April):111161.

27. Praça FG, Viegas JSR, Peh HY, Garbin TN, Medina WSG, Bentley MVLB. Microemulsion co-delivering vitamin A and vitamin E as a new platform for topical treatment of acute skin inflammation. Mater Sci Eng C. 2020;110(January):110639.

28. Aggarwal N, Goindi S, Khurana R. Formulation, Characterization and evaluation of an optimized microemulsion formulation of griseofulvin for topical application. Colloids Surfaces B Biointerfaces. 2013;105:158–66.

29. Lv X, Cong Z, Liu Z, Ma X, Xu M, Tian Y, et al. Improvement of the solubility, photostability, antioxidant activity and UVB photoprotection of trans-resveratrol by essential oil based microemulsions for topical application. J Drug Deliv Sci Technol. 2018;48:346–54.

30. Patel RB, Patel MR, Bhatt KK, Patel BG. Formulation consideration and characterization of microemulsion drug delivery system for transnasal

administration of carbamazepine. Bull Fac Pharmacy, Cairo Univ. 2013;51(2):243–53.

31. Sharma AK, Garg T, Goyal AK, Rath G. Role of microemuslsions in advanced drug delivery. Artif Cells, Nanomedicine Biotechnol. 2016;44(4):1177–85.

32. Zhao L, Wang Y, Zhai Y, Wang Z, Liu J, Zhai G. Ropivacaine loaded microemulsion and microemulsion-based gel for transdermal delivery: Preparation, optimization, and evaluation. Vol. 477, International Journal of Pharmaceutics. Elsevier B.V.; 2014. 47–56 p.

33. Sabale V, Vora S. Formulation and evaluation of microemulsion-based hydrogel for topical delivery. Int J Pharm Investig. 2012;2(3):140.

34. Badihi A, Frušić-Zlotkin M, Soroka Y, Benhamron S, Tzur T, Nassar T, et al. Topical nano-encapsulated cyclosporine formulation for atopic dermatitis treatment: Topical cyclosporine NCs for AD. Nanomedicine Nanotechnology, Biol Med. 2020;24:102140.

35. Zhao J, Jiang K, Chen Y, Chen J, Zheng Y, Yu H, et al. Preparation and characterization of microemulsions based on antarctic krill oil. Mar Drugs. 2020;18(10).

36. Moon PD, Han NR, Lee JS, Kim HM, Jeong HJ. p-coumaric acid, an active ingredient of Panax ginseng, ameliolates atopic dermatitis-like skin lesions through inhibition of thymic stromal lymphopoietin in mice. J Ginseng Res. 2021;45(1):176–82.

37. Sugita K, Akdis CA. Recent developments and advances in atopic dermatitis and food allergy. Allergol Int. 2020;69(2):204–14.

38. Teichmann A, Heuschkel S, Jacobi U, Presse G, Neubert RHH, Sterry W, et al. Comparison of stratum corneum penetration and localization of a lipophilic model drug applied in an o/w microemulsion and an amphiphilic cream. Eur J Pharm Biopharm. 2007;67(3):699–706.

39. Sahoo S, Pani NR, Sahoo SK. Microemulsion based topical hydrogel of sertaconazole: Formulation, characterization and evaluation. Colloids Surfaces B Biointerfaces. 2014;120:193–9.

40. Master AM, Rodriguez ME, Kenney ME, Oleinick NL, Sen Gupta A. Delivery of the photosensitizer Pc 4 in PEG–PCL micelles for in vitro PDT studies. J Pharm Sci. 2010;99(5):2386–2398.

41. Čižinauskas V, Elie N, Brunelle A, Briedis V. Skin Penetration Enhancement by Natural Oils for Dihydroquercetin Delivery. Molecules. 2017;22(9):1–16.

42. Lei W, Yu C, Lin H, Zhou X. Development of tacrolimus-loaded transfersomes for deeper skin penetration enhancement and therapeutic effect improvement in vivo. Asian J Pharm Sci. 2013;8(6):336–45.

43. Lauterbach A, Müller-Goymann CC. Comparison of rheological properties, follicular penetration, drug release, and permeation behavior of a novel topical drug delivery system and a conventional cream. Eur J Pharm Biopharm. 2014;88(3):614–24.

44. Lalan MS, Laddha NC, Lalani J, Imran MJ, Begum R, Misra A. Dose Reduction of a Potent Topical Corticosteroid with Microemulsion Based Cream. J Nanopharmaceutics Drug Deliv. 2013;1(1):52–63.

45. Shevachman M, Shani A, Garti N. Formation and investigation of microemulsions based on jojoba oil and nonionic surfactants. JAOCS, J Am

Oil Chem Soc. 2004;81(12):1143–52.

46. Alves LP, da Silva Oliveira K, da Paixão Santos JA, da Silva Leite JM, Rocha BP, de Lucena Nogueira P, et al. A review on developments and prospects of anti-inflammatory in microemulsions. J Drug Deliv Sci Technol. 2020;60(March).

47. Peira E, Carlotti ME, Cavalli R, Trotta M. Azelaic acid sodium salt in the formulation of microemulsions for topical applications. J Drug Deliv Sci Technol. 2006;16(5):375–9.

48. Sahle FF, Wohlrab J, Neubert RHH. Controlled penetration of ceramides into and across the stratum corneum using various types of microemulsions and formulation associated toxicity studies. Eur J Pharm Biopharm. 2014;86(2):244–50.

49. Froelich A, Osmałek T, Snela A, Kunstman P, Jadach B, Olejniczak M, et al. Novel microemulsion-based gels for topical delivery of indomethacin: Formulation, physicochemical properties and in vitro drug release studies. J Colloid Interface Sci. 2017;507:323–36.

50. Puri A, Kaur A, Raza K, Goindi S, Katare OP. Development and evaluation of topical microemulsion of dibenzoylmethane for treatment of UV induced photoaging. J Drug Deliv Sci Technol. 2017;37:1–12.

51. Wan T, Xu T, Pan J, Qin M, Pan W, Zhang G, et al. Microemulsion based gel for topical dermal delivery of pseudolaric acid B: In vitro and in vivo evaluation. Int J Pharm. 2015;493(1–2):111–20.

52. Yan F, Li F, Liu J, Ye S, Zhang Y, Jia J, et al. The formulae and biologically active ingredients of Chinese herbal medicines for the treatment of atopic

dermatitis. Biomed Pharmacother. 2020;127(January):110142.

53. Volpe V, Giacomodonato MN, Sordelli DO, Insausti M, Buzzola FR, Grünhut M. Ciprofloxacin loaded o/w microemulsion against Staphylococcus aureus. Analytical and biological studies for topical and intranasal administration. J Drug Deliv Sci Technol. 2020;57(March):101705.

54. Das S, Lee SH, Chia VD, Chow PS, Macbeath C, Liu Y, et al. Development of microemulsion based topical ivermectin formulations: Pre-formulation and formulation studies. Colloids Surfaces B Biointerfaces. 2020;189(January):110823.

55. Shukla T, Upmanyu N, Agrawal M, Saraf S, Saraf S, Alexander A. Biomedical applications of microemulsion through dermal and transdermal route. Biomed Pharmacother. 2018;108(October):1477–94.

56. Wang Y. Preparation on Nano- and Microemulsions using Phase Inversion and Emulsion Titration Methods. 2014;1–133.

57. Paolino D, Ventura CA, Nisticò S, Puglisi G, Fresta M. Lecithin microemulsions for the topical administration of ketoprofen: Percutaneous adsorption through human skin and in vivo human skin tolerability. Int J Pharm. 2002;244(1–2):21–31.

58. Sh A, B AA, M RO. Nanoemulsion of Jojoba Oil, Preparation, Characterization and Insecticidal Activity against Sitophilus oryzae (Coleoptera: Curculionidae) on Wheat. Int J Agric Innov Res. 2015;4(1):2319–1473.

59. Szumała P, Jungnickel C, Kozłowska-Tylingo K, Jacyna B, Cal K. Transdermal transport of collagen and hyaluronic acid using water in oil

microemulsion. Int J Pharm. 2019;572:118738.

60. Kumar MR, Teelavath M, Yellanki SK. Development and evaluation of polyherbal emulgel formulation (A preventive hair care preparation). 2019;7(1):8–10.

61. Katoh N, Ohya Y, Ikeda M, Ebihara T, Katayama I, Saeki H, et al. Japanese guidelines for atopic dermatitis 2020. Allergol Int. 2020;69(3):356–69.

62. Badawi AA, Sakran WS, Ramadan MA, El-Mancy SMS. Improvement of the microbiological activity of topical ketoconazole using microemulsion systems. J Drug Deliv Sci Technol. 2012;22(6):473–8.

63. Callender SP, Mathews JA, Kobernyk K, Wettig SD. Microemulsion utility in pharmaceuticals: Implications for multi-drug delivery. Int J Pharm. 2017;526(1–2):425–42.

64. Tartaro G, Mateos H, Schirone D, Angelico R, Palazzo G. Microemulsion microstructure(s): A tutorial review. Nanomaterials. 2020;10(9):1–40.

65. Syed HK, Peh KK. Identification of phases of various oil, surfactant/co-surfactants and water system by ternary phase diagram. Acta Pol Pharm - Drug Res. 2014;71(2):301–9.

66. Moner V, Fernández E, Calpena AC, Garcia-Herrera A, Cócera M, López O. A lamellar body mimetic system for the treatment of oxazolone-induced atopic dermatitis in hairless mice. J Dermatol Sci. 2018;90(2):172–9.

67. Siddique MI, Katas H, Amin MCIM, Ng SF, Zulfakar MH, Jamil A. In-vivo dermal pharmacokinetics, efficacy, and safety of skin targeting nanoparticles for corticosteroid treatment of atopic dermatitis. Int J Pharm. 2016;507(1–2):72–82.

68. Zhu W, Guo C, Yu A, Gao Y, Cao F, Zhai GX. Microemulsion-based hydrogel formulation of penciclovir for topical delivery. Int J Pharm. 2009;378(1–2):152–8.

69. Lin YH, Tsai MJ, Fang YP, Fu YS, Huang Y Bin, Wu PC. Microemulsion formulation design and evaluation for hydrophobic compound: Catechin topical application. Colloids Surfaces B Biointerfaces. 2018;161:121–8.

70. Butani D, Yewale C, Misra A. Amphotericin B topical microemulsion: Formulation, characterization and evaluation. Colloids Surfaces B Biointerfaces. 2014;116:351–8.

71. Pandey SS, Maulvi FA, Patel PS, Shukla MR, Shah KM, Gupta AR, et al. Cyclosporine laden tailored microemulsion-gel depot for effective treatment of psoriasis: In vitro and in vivo studies. Colloids Surf B Biointerfaces. 2020;186:110681.

72. Patel HK, Barot BS, Parejiya PB, Shelat PK, Shukla A. Topical delivery of clobetasol propionate loaded microemulsion based gel for effective treatment of vitiligo: Ex vivo permeation and skin irritation studies. Colloids Surfaces B Biointerfaces. 2013;102:86–94.

2

ORIGINALITY REPORT

8%	7%	5%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Institute of Technology, Nirma University Student Paper	4%
2	Muhammad Irfan Siddique, Haliza Katas, Mohd Cairul Iqbal Mohd Amin, Shiow-Fern Ng, Mohd Hanif Zulfakar, Adawiyah Jamil. "In-vivo dermal pharmacokinetics, efficacy, and safety of skin targeting nanoparticles for corticosteroid treatment of atopic dermatitis", International Journal of Pharmaceutics, 2016 Publication	1%
3	Nguyen-Thach Tung, Van-Duy Vu, Phuong-Lan Nguyen. "DoE-based development, physicochemical characterization, and pharmacological evaluation of a topical hydrogel containing betamethasone dipropionate microemulsion", Colloids and Surfaces B: Biointerfaces, 2019 Publication	1%
4	africasportnews.com Internet Source	1%

5	pt.scribd.com Internet Source	<1%
6	repository-tnmgrmu.ac.in Internet Source	<1%
7	accp.co.in Internet Source	<1%
8	docplayer.net Internet Source	<1%
9	Khurram Rehman, Mohd Hanif Zulfakar. "Recent advances in gel technologies for topical and transdermal drug delivery", Drug Development and Industrial Pharmacy, 2013 Publication	<1%
10	Akshay R. Koli, Ketan M. Ranch, Hetal P. Patel, Rajesh K. Parikh, Dinesh O. Shah, Furqan A. Maulvi. "Oral bioavailability improvement of felodipine using tailored microemulsion: Surface science, ex vivo and in vivo studies", International Journal of Pharmaceutics, 2021 Publication	<1%

Exclude quotes	Off	Exclude matches	< 20 words
Exclude bibliography	On		