"TO FORMULATE AND CHARACTERIZE NANOEMULGEL CONTAINING ANTI-ACNE AGENT (DAPSONE)"

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in Partial Fulfillment for the Award of the Degree of

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IN
PHARMACEUTICS

BY

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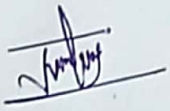

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List of Abbreviation

Sr. No	Abbreviation	Name
1.	NE	Nanoemulsion
2.	NEG	Nanoemulgel
3.	NSAID	Non steroidal anti-inflammatory agent
4.	ppm	Parts per million
5.	nm	Nanometer
6.	®	Registered trademark
7.	0C	Degree celcious
8.	PBS	Phosphate buffer saline
9.	PDI	Poludispervity index
10.	CoS	Cosurfactant
11.	UV	Ultraviolet

"TO FORMULATE AND CHARACTERIZE NANOEMULGEL CONTAINING ANTI-ACNE AGENT (DAPSONE)"

ABSTRACT

Acne is a very common inflammatory disorder mostly occurred in teenagers. Main reasons behind inflammation are overproduction of sebum, hormonal changes, bacteria infection, etc. Topical products are the first choice of healthcare practitioners to treat the acne because of some advantages like easy to apply, local action, fewer side effects, etc. Dapsone is the sulfone based antibiotic and has an antibacterial activity with minimum side effects. Currently, two different strengths of Dapsone 5% and 7.5% gel are available in the market. Dapsone has a log P value near to 1; it is less lipophilic so facing difficulties during penetration. Preparation of Nanoemulgel is a convincing concept to improve the penetrability of Dapsone. Nanoemulgel is a combination of Nano emulsion and gel. In which drug is incorporated in the Nano emulsion by preparing o/w emulsion, while gel phase mostly provides proper consistency for the easy application on the face. There are some advantages for preparing Nanoemulgel like increase the drug penetration because of oil droplets acts as lipidic carriers and can incorporate a large amount of drug compared to other nano systems. The main objective of this thesis is to formulate dosage form that has good penetration and better efficacy in the treatment of acne. Spontaneous emulsification method was used to prepare Nano emulsion. While triacetin used as oil phase, labrasol as surfactant and two different co-surfactant transcutol & Tween 80 was used in formulation development while Carbopol 934 was used as a gelling agent. The formulation was evaluated in viscosity, drug content, in-vitro release and ex-vivo release profile

1. Introduction

1.1 Introduction to Acne

1.1.1 Introduction:

Acne is also known as Acne vulgaris (AV). It is one of the most common skin diseases during adulthood with a prevalence ratio of almost 90% starting at the age of 12 years. Patients with such a young age like 8 years may have Acne and this condition would persist till adulthood about 45 years of age. Acne is generally present with psychological comorbidities like low self-esteem, anxiety and depression; specifically, when it happened during adolescent. (1)

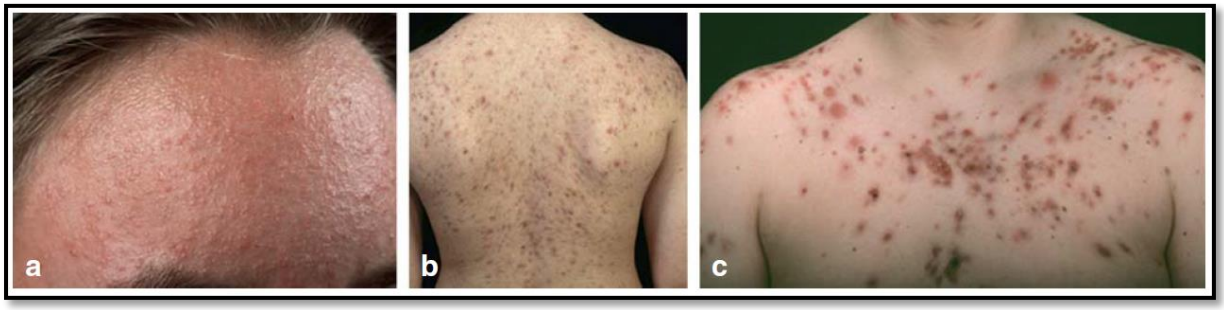


*Figure 1*Appearance of Acne (2)

1.1.2 Epidemiology: (3)

- Mostly acne appears into 64% population in 20s and 43% population during 30s.
- Heritability is also major reason behind acne with around 80% prevalence in first degree relatives. Furthermore, it not only occurs earlier but more severely in these patients.
- Around 20% of young people will experience moderate to severe acne.
- In USA, people spend more than 3 billion dollars per year behind the treatment of acne.

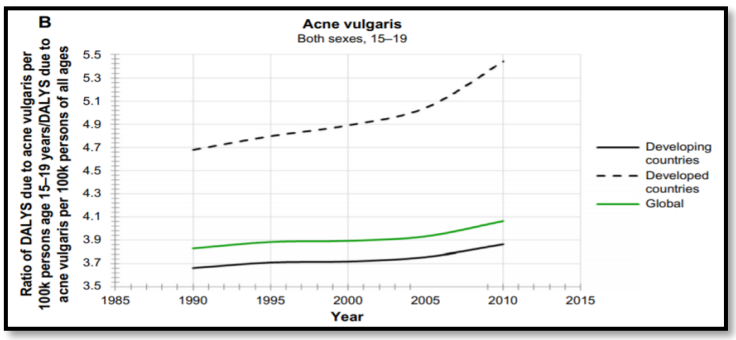


Figure 2 Representation of Acne epidemiology in Developed and developing contries (4)

1.1.3 Pathophysiology:

The pathophysiology of Acne is multidimensional and was rooted in Inflammatory processes, Propionibacterium acnes, skin keratinization and excess sebum production. More specifically in adolescent patients, acne development should result of endocrine changes during puberty period, increased sebum production and accession of androgenic hormones. Most common reason behind AV is overproduction of oils from the sebaceous gland, those are mostly found on face and upper back. Secondly, any alteration in follicular development process such as hyperkeratinisation will contribute in Acne development. Then gram positive bacteria like P. Acnes can inhibit sebaceous follicles and the skin; it releases some enzymes like lipases, proteases should be responsible for the acne related inflammation and play a critical part in creation of likewise inflammatory mediators. These all inflammatory mediators combinedly cause plugging on epidermis and thus produce inflammatory lesions like papules, pustules, nodules, macule, patch, vesicle, bulla, cyst and wheals. (5,6)

Different Skin Lesions OR Symptoms are, (7)

1. **Macule:** It is a flat, not raised and coloured lesions with diameter of less than 2 cm.
2. **Patch:** A macule with larger diameter; generally, more than 2 cm.
3. **Papule:** It is a small solid palpable lesion with less than 1 cm of diameter.
4. **Nodule:** Papule with more than 1 cm in diameter and thus easily palpable.
5. **Vesicle:** It is a small fluid filled lesion and translucent with diameter of less than 1 cm.
6. **Pustules:** Vesicles with leucocytes.
7. **Bulla:** A fluid filled and raised lesion with more than 1 cm in diameter.
8. **Cyst:** It is a soft, raised, encapsulated lesion which contain liquid or semisolid content.
9. **Wheal:** A raised erythematous papule because of oedema.
10. **Comedone:** They are the skin-coloured, small bumps (papules) frequently found on the forehead and chin of those with acne. A single lesion is a comedo.
    a. Open comedone are blackheads; black because of surface pigment (melanin), rather than dirt.
    b. Closed comedone are whiteheads; the follicle is completely blocked.

c. Microcomedone are so small that they are not visible to the naked eye.
d. Macrocomedone are facial closed comedones that are larger than 2–3 mm in diameter.
e. A giant comedo is a type of cyst in which there is a clear blackhead-like opening in the skin.

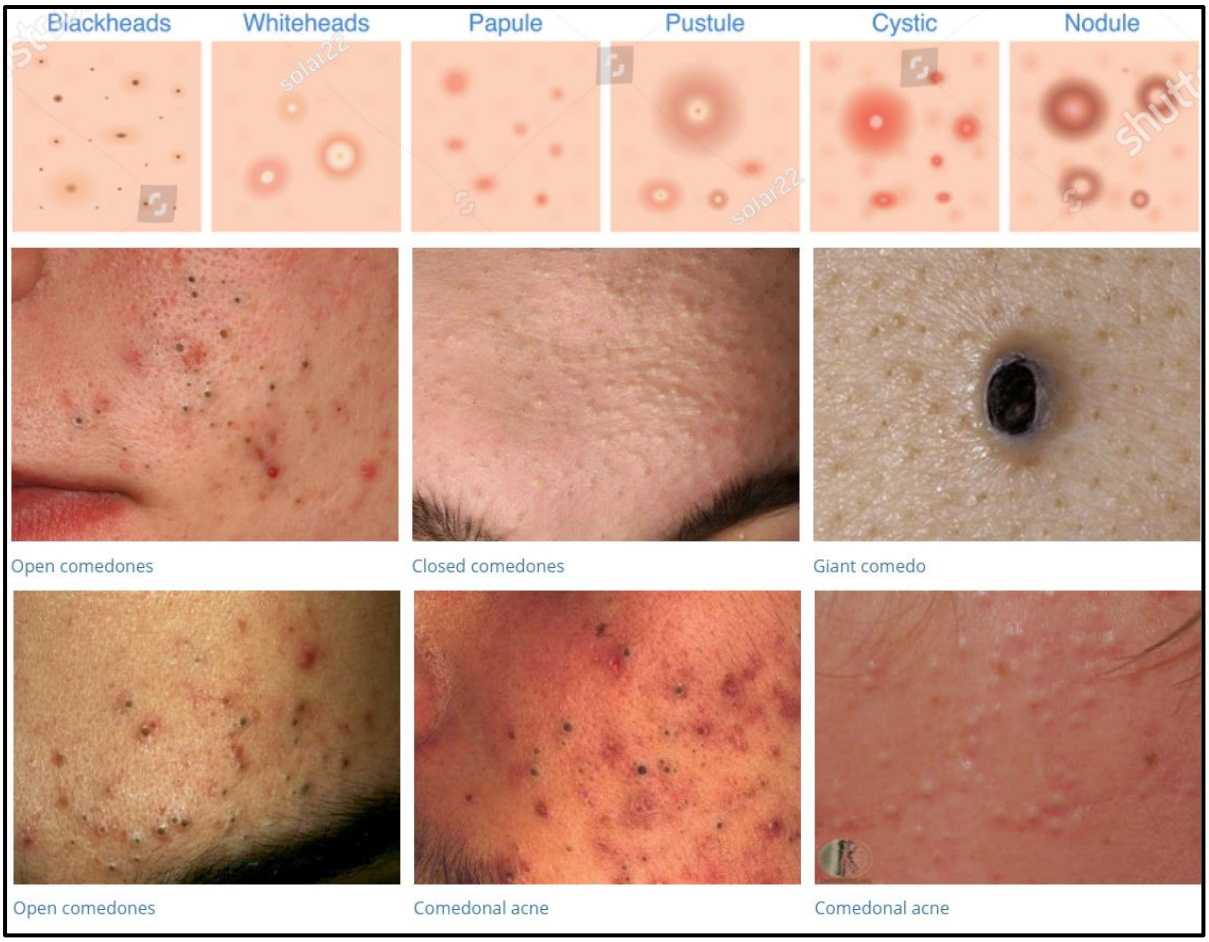


Figure 3 Types of different Acne lesion(8,9)

Other than these, endocrine abnormalities during pregnancy or polycystic ovarian syndrome during reproductive age also causes acne. Mostly, polycystic ovarian syndrome is characterizing by hyperandrogenism that increase the sebum production and thus producing the acne. Those patients have acne lesions on not only on their face but also on neck, chest and upper back. (10)

1.1.4 Goal of Therapy:

Regardless of the age, goal for the treatments are;

1. To resolve and target the pathological condition of Acne
2. Decrease in the inflammatory lesions

European dermatological forum (EDF) mentioned in their guideline that 10% reduction in number of lesions should be considered as successful therapy, but ultimately patients' perspective on clinical success of therapy varies. (11) At last expected that patient's quality of life is improve.

1.1.5 Classification of Acne Severity:

There is not any standard method available for determination of the acne severity. Generally, acne is manifest based on present numbers of comedone either close or open, or both. It is seen that microcomedone serve as a precursor to comedone, specifically when follicle would start inhibited by excess of sebum produced, keratinized cells and bacteria combinedly. On accumulation of these mixture in follicle, a comedone will form and it is known as closed comedo or Whitehead. If further build up on follicular duct happens, it will expose to the air and oxidize to become black; it is known as open comedo or blackhead.

Inflammatory lesions would not only painful but it could lead to a permanent scarring. Thus, it is important to identify the appropriate treatment base on types and density of inflammatory lesions.

Normally acne's severity should be judge on mild, moderate and severe stages. Guidelines suggest the use of these stages based on either types of inflammatory lesions or its quantity or both. Two different studies give note to use this grading system but none of them will provide general consensus. (11)

Generally, an acne is noninflammatory and comedone based; still patient can perceive large number of comedone and inflammatory lesions during moderate to severe cases. Thus, it is important for doctors to classify the acne and choose the best possible method for treatment. Most severe type of acne is Conglobate acne as per European Dermatology Forum (EDF); that

is characterized by group of inflammatory nodules and papules, comedones, etc. Also, conglobate acne primarily found on upper limbs, trunk and in lesser extend on face. (12)

Mostly acne at the adolescents and adult age is classified in four groups:

1. Comedonal acne
2. Mild-moderate papulopustular acne
3. Severe papulopustular acne/moderate nodular acne
4. Severe nodular acne/conglobate acne

Table 1 Sevearity based classification system of Acne

Guideline			
General Classification	**Global Alliance**	**European Dermatology Forum**	**American Association of Pediatrics**
Mild	Comedonal or Mixed and papular / Pustular	Comedonal or Mild to moderate papulopustular	Comedonal or Inflamatory / mixed lesions
Moderate	Mixed and papular / pustular or Nodular	Mild to moderate papulopustular	Comedonal or Inflamatory / mixed lesions
Severe	Nodular or Conglobate	Severe papulopustular/ moderate nodular or Severe nodular / conglobate	Inflammatory / mixed and/or nodular lesions

1.1.6 Marketed Formulations for Acne Treatment: (2)

Table 2 Currently available molecules in market in suitable dosage form

Drugs	Available Formulation	Dose	Common Adverse Effect

Topical Retinoids			
Adapalene	Cream, Gel, Lotion	Once daily	Dryness, Erythema, Photosensitivity, Pruritis, Stinging
Tazarotene	Cream, Foam, Gel	Once daily	
Tretionin	Cream, Gel, Micronized gel	Once daily	
Topical Antibiotics			
Clindamycin	Foam, Gel, Lotion, Pledget, Solution	Twice daily	Burning, Dryness, Erythema, Oiliness
Erythromycin	Gel, Pad, Solution	Twice daily	
Topical Combination			
Adapalene / BPO	Gel	Once daily	Burning, Dryness, Erythema, Oiliness, Pruitus
Clindamycin / Tretinoin	Gel	Once daily	
Clindamycin / BPO	Gel	Once daily	
Erythromycin / BPO	Gel	Twice daily	
Oral Antibiotics			

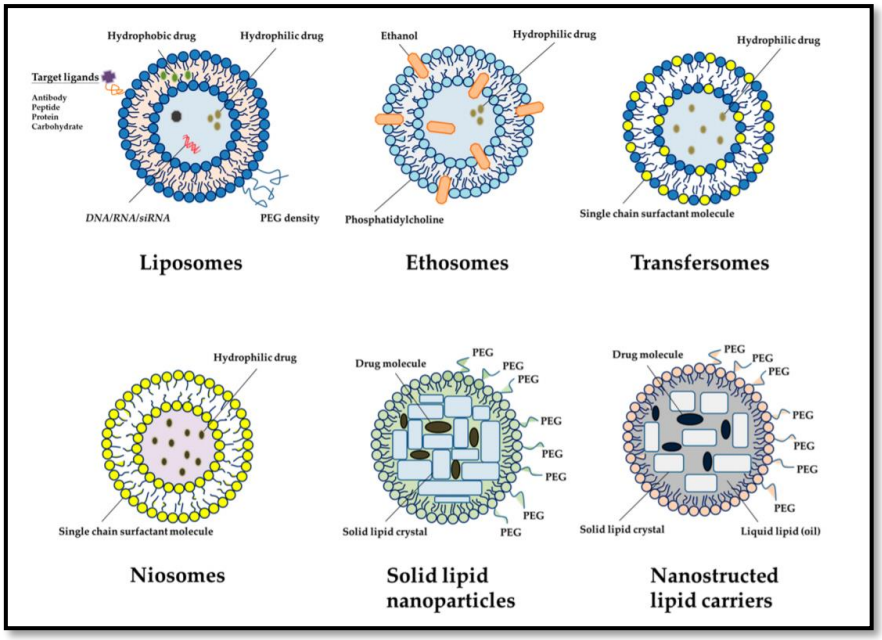
Doxycycline	Capsule, tablet	Once or twice daily	GI upset, esophagitis, Photosensitivity, Tooth staining
Erythromycin	Capsule, tablet	twice daily	GI upset, hepatotoxicity
Minocycline	Tablet	1mg/kg/day for 12 weeks	Autoimmune disorder, Dizziness, fatigue, headache
Tetracycline	Capsule	200-250mg twice daily	GI upset, tooth staining
Trimethoprime / Sulphamethoxazole	Tablet	800/160mg twice daily	Anemia, Hypersensitivity, rash
Hormonal Therapy			
Combined contraceptives	variable	Estrogen lowest dose possible; Progestin variable once daily	Breakthrough bleeding, breast tenderness, weight gain
Spironolactone	Tablet	20-100mg daily	Breast tenderness, hyperkalemia, hypotension, menstrual irregularities
Other			
Azelaic acid	Cream	Twice daily	Hypopigmentation, Pruritus, Stinging
Dapsone	Gel	Twice daily or once daily	Erythema

Oral isotretinoin	Capsule	0.5-1 mg/kg/day in two divided doses	Blood dyscrasia, hair, skin and mucous membrane dryness, hepatotoxicity

1.2 Introduction to Nanoemulgel

1.2.1 Nanotechnology - Introduction:

Nanomedicine is a branch of pharmaceutical sciences that uses the science of nanotechnology for prevention and cure of different diseases made by using special lipids and equipments, such as nanoparticles like Liposome, Niosomes, Nanoemulsion, Metallic nanoparticles, etc and nanorobots; for various applications like diagnosis, drug delivery, sensory, etc. Nanoparticles are defined as a particle have size between 1 to 100 nm. Large size particles used in convectional dosage possesses some major problems including poor bioavailability, poor solubility, less efficacy, probable adverse reaction, etc. (13)



*F*igure 4 Different types of Nano based systems (14)

Nanoparticles possesses a special structural, chemical, mechanical, magnetic, biological and electrical properties. One of the reasons behind success of nanoparticles is its capability of delivering the drug by encapsulating it in lipidic carrier to a specific target tissues with pre specified rate (Controlled or Sustained release). (15) Major limitations of convectional system such as drugs having very low solubility produce some delivery issues on oral administration, less diffusion capacity through outer membrane, large quantity of IV intake required, unwanted

side effects, etc. are overcome by applying nanotechnological approaches in different dosage forms. (13)

Initially nanocomposite is used as carriers for anticancer drugs and vaccines and then it gives significantly enhanced bioavailability and better toxicodynamic of drugs. This would be improving the in-vivo delivery of many drugs that previously possesses delivery problems. Furthermore, modification in nanocomposite could also improve the delivery of drug through blood brain barrier and thus target the brain tumours. For example, nanocomposite of Doxorubicin with polysorbate 80 and modified with polybutylcyanoacrylate shows better penetration through blood brain barrier compare to the convectional dosage form. In addition to this, nanocomposite can also play an important role in DNA delivery of vectors because they can efficiently penetrate into tissues and due to their nanosized and lipidic nature they can easily uptake by cells. Nanocomposite also used as potential carrier for different drugs like anti-hypertensive, hormonal therapy, etc. (15)

Nanocomposite not only change the drug's physicochemical properties but also changed the pharmacokinetic of pharmaceutically active agents. For example, secretion of insulin from pancreases would increases by using spray drying based aggregates of trans-retinoic acid nanoparticles, that is coated with $CaCO_3$. These aggregates will re-disperse in water and stimulate the insulin secretion from islets.

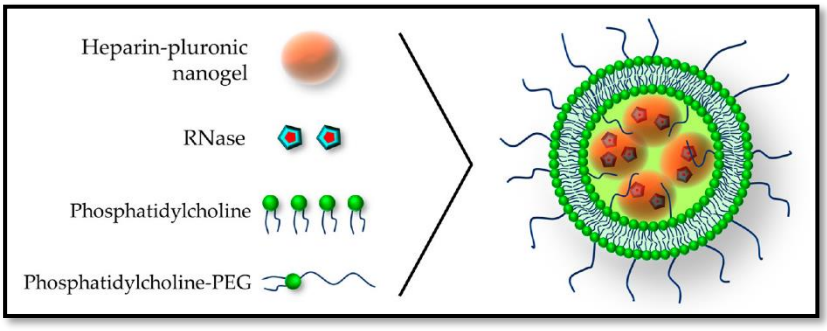


Figure 5 Demonstration of Nanocomposite that contains RNAs (16)

Selection of ideal nano drug delivery system is based on biophysical and biochemical properties of the active pharmaceutical ingredient selected for the treatment. Apart from all these benefits, nanocomposite also shows some toxicity effect that cannot be ignored. To overcome the toxicity issue now-a-days nanocomposite made up with the combination of

natural products. Recently green chemistry route of nanocomposite synthesis widely used by scientist because of its ability to minimize the hazardous constituents during biosynthetic process. (13)

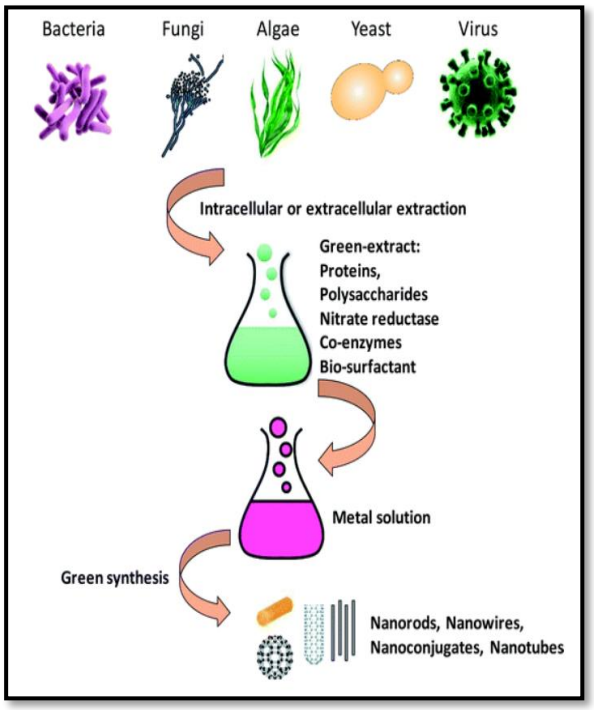


Figure 6 Process of systhesis nanocomposite by green chemistry route (17)

1.2.2 Introduction of Emulsion:

Basically, emulsions are defined as the mixture of two immiscible phases in that dispersed phase is uniformly dispersed in continuous phase like liquid-liquid, solid-liquid, liquid-gas, etc. In convectional system this term only used for the liquid-liquid dispersion. United states Pharmacopeia defines emulsions as, “emulsions are two-phase systems in which one liquid is dispersed throughout another liquid in the form of small droplets.” Emulsions are thermodynamically unstable system because of large difference in interfacial tension between two liquids. This can be overcome by adding surfactant and cosurfactant. Main function of using surfactant and cosurfactant is to reduce the interfacial tension between two liquids and make a stable emulsion. Normally two phases are known as Continuous phase and Dispersed phase. In that dispersed phase mostly present in less amount compare to the continuous phase.

Mostly emulsions are of two types: first is Oil in water (o/w) that are more prevalent and second is Water in oil (w/o). In addition to these, double emulsions are also available like water in oil in water (w/o/w). (18)

1.2.3 Introduction to Microemulsion OR Nanoemulsion:

Microemulsions are isotropic and thermodynamically stable system made up of oil, surfactant and water. The term "Microemulsion" was first coined by Schulman in 1959. (19) Although many scientists found its nomenclature confusing. (20) The main hallmark behind the microemulsion is thermodynamically stable but does not have any compulsion about particle size. However mostly droplet size is below 100 nm. (18)

In past, not much known classification of emulsion for delineate the size of dispersed phase, mostly below 1 mm, with unique nomenclature such as miniemulsion, ultrafine emulsion and submicron emulsion. Currently, the term Nanoemulsion have been used in three ways: first in replacement of term Microemulsion but smaller in size, second is more precise incarnation of the system such as miniemulsion having size less than 1 mm and third usage is in terms of nanotechnology, has a particle size around 100 nm. Here the prefix micro in microemulsion term giving wrong connotation of micron size particle range but actually the particles are around 100 nm in size. Term microemulsion generally known as thermodynamically stable system; so, when Nanoemulsion is use in substitute of microemulsion, it too will be considered as thermodynamically stable. (21)

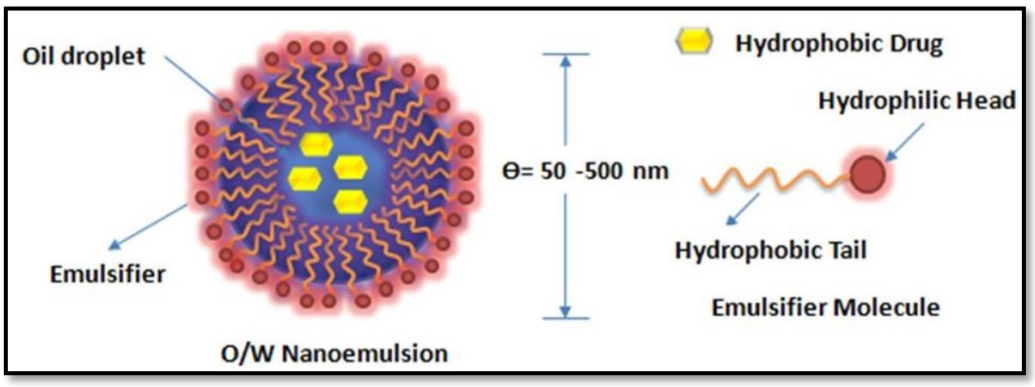


Figure 7 Graphical presentation of dispersed phase of o/w Nanoemulsion (22)

The US government's institute called National Nanotechnology Initiative defined the criteria for the nanotechnology as, "The understanding and control of matter at dimensions of roughly 1-100 nm." With respect to the nanotechnology, Nanoemulsion must have particle size below 100 nm. Diameter would either be particle's radius or its diameter. However small change in the size of particles does not cause significant change in emulsion property.

Nanoemulsion should be transparent or translucent, in contrast to the opaque or milky white appearance of convectional emulsions. One of the reasons behind transparent or translucent appearance of Nanoemulsion is blockage of transition of optical wavelength of visible light from such a small size particle precisely of 100 nm in range. Quantity of dispersed phase also affect the optical property of emulsion.

Some problems associated with emulsion like creaming and sedimentation are not seen in the Nanoemulsion. The most basic reason behind not occurring of sedimentation is the energy generated by Brownian motion of the droplets is fair enough to counter the gravitational force. Secondly, creaming is due to particle size and difference in the densities of continuous and dispersed phase. Though it might be possible that optical property in that there is not any specific cut-off at 100 nm; responsible for creaming did not seen in Nanoemulsion.

The Nanoemulsion should defined as, "Heterogeneous system composed of one immiscible liquid dispersed as droplets within another liquid, where the average droplet diameter is below 100 nm."

1.2.4 Method of Preparation – Nanoemulsion:

Nanoemulsion should be prepared by mainly two methods: High energy method and Low energy method. These two methods are differing by energy used in preparation of emulsion. In high energy method, different mechanical devices like high shear homogenizer (HSH), high pressure homogenizer (HPH), microfluidizer and probe sonicator are used to generate vast disruptive forces. Due to the ease of production high energy methods are usually used in preparation of Nanoemulsion. Now-a-days, use of low energy methods should increase in formulating temperature sensitive drugs.

A. High Energy Method:

High energy methods used various mechanical devices to generate large amount of disruptive force to produce Nanoemulsion. They consume large amount of energy so they are costly but showing good industrial scalability. The main principles in using high energy methods for preparation of Nanoemulsion are milling, high frequency sound wave (>20 kHz), high pressure displacement pump (500 – 50000 psi), etc. Amongst them high pressure valve homogenizer is widely used in food and pharmaceutical industries. In this instrument particle size would be decreases by applying various disruptive forces like turbulence, cavitation and shear. Increase in homogenization pressure will produce reduced particle size. Similarly, microfluidizer works on the same principle; only difference between two instruments is flow path of particles. One more instrument widely used is Ultrasonicator, this uses high energy sound waves to reduce the particle size of Nanoemulsion by increasing the sonication time and wave intensity. Generally high energy methods should be used with any type of oils but specifically used for the oils having high viscosity and high molecular weight because they are difficult emulsified. One of the benefits of this system is that it required less amount of surfactant. While using this method for formulating heat sensitive drugs could be difficult because of generation of heat during manufacturing process. (23)

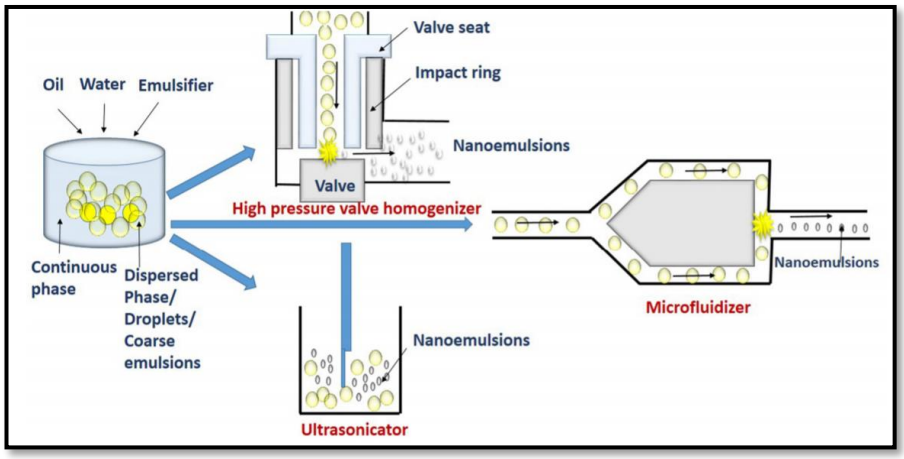


Figure 8 Graphical representation of high energy methods like high pressure homogenization, microfluidizer and ultrasonicator (23)

B. Low energy method:

Nanoemulsion is prepared by using either internal chemical energy of the system or chemical potential of components called low energy method. Chemical energy released during

emulsification is responsible for the emulsification in low energy methods. Release of the energy is due to the spontaneous change in curvature of surfactant molecules from positive to negative (w/o) or from negative to positive (o/w). Normally low energy method includes stirring of emulsion at slower rate for long period of time; that will result in low energy consumption. Principally low energy method should be classified as Isothermal and thermal methods. For emulsification of temperature sensitive bioactive compounds generally Isothermal method is used like spontaneous emulsification, phase inversion composition, D phase emulsification and microemulsion dilution. On the other side thermal method mostly applicable in solid lipid containing nanoparticles where heating is requiring for maintaining liquid phase. (24)

i. Spontaneous Emulsification:

This is the most simplest method of emulsification. In this method rapid mixing is required for emulsification method. This process starts as soon as organic phase came in contact with water phase. This fast migration will produce an immense turbulence at the surface of both the phases and that leads to increasing in oil-water interfacial area. Here fine droplets of oils are generated spontaneously. Irrespective of surfactant, solvent shall play an important part in this process. Spontaneous emulsification in absence of surfactant called Ouzo effect. In this type of emulsification method order of mixing does not show any significant effect on emulsion formation. (25)

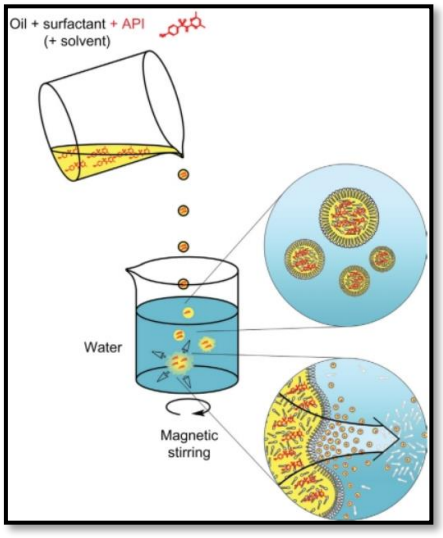


Figure 9 Nanoemulsion generation by spontaneous emulsification (25)

ii. Phase Inversion Composition (PIC):

It is an type of spontaneous emulsification method. This method used to prepare Nanoemulsion at room temperature. In this method water phase is slowly adding in drop wise manner into the oil phase on magnetic stirrer at room temperature. Thus, initially w/o emulsion will form which will covert into o/w emulsion on increase in volume of water. Various factors like interfacial tension, surfactant structure, bulk viscosity and its concentration, etc affect the emulsification method. In this method, phase inversion occurring on increase in the volume of water phase so it is also knowing as Catastrophic inversion.

iii. Phase Transition Temperature (PIT):

Temperature is the key factor in preparation of Nanoemulsion by PIT method. In this method surfactant, oil and water were continuously stirred and heated in specific manner until phase inversion ice bath and finally o/w Nanoemulsion will form. Phase transition temperature is normally 20-65 ^{0}C higher than its storage temperature when Nanoemulsion was o/w type and phase transition temperature was 10-40 ^{0}C higher than its storage temperature in case of w/o Nanoemulsion. Nanoemulsion is sensitive to the temperature near PIT so to produce stability cosurfactant is added in the system. Generally, non-ionic surfactant will show temperature dependent changes in molecular geography and thus it will suitable for this emulsification method. Sometimes inorganic salts also have been used for adjustment of PIT. This method will produce high emulsification capcity and low polydispersity index compare to PIC method. (26)

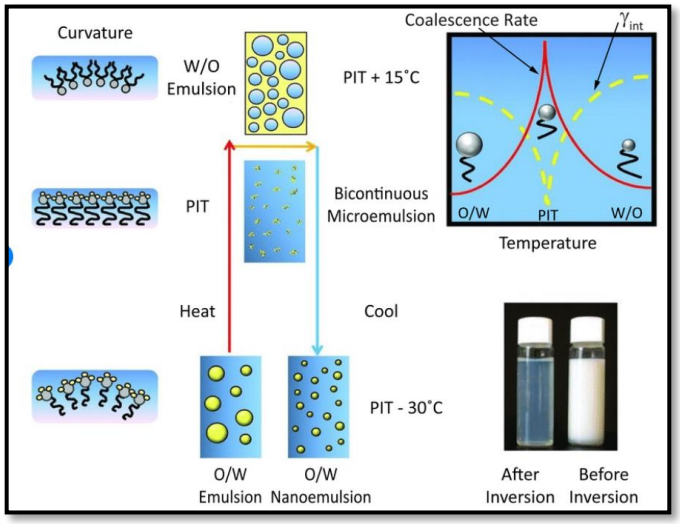


Figure 10 Formation of Nanoemulsion by phase transition temperature (26)

iv. Microemulsion Dilution:

It is the simplest type of emulsification method and also called self-emulsification method because emulsification occurs upon dilution with water at specific temperature. Mostly o/w type of emulsion is directly prepared by adding large amount of water in oil and surfactant mixer. Large amount of water would reduce the surfactant concentration and thus produce thermodynamically stable emulsion. Scaled up is easy in this type of emulsification method.

v. D – Phase Emulsification (DPE):

Sagitani, Hattori, Nabeta and Nagai in 1983 are pioneer in preparation of emulsion by this method. This method needs one special ingredient called alkyl polyols with oil, surfactant and water to form o/w Nanoemulsion. In contrast to other method this method requires no strict adherence to HLB, low surfactant concentration or its mixing and less energy to form a Nanoemulsion. (27)

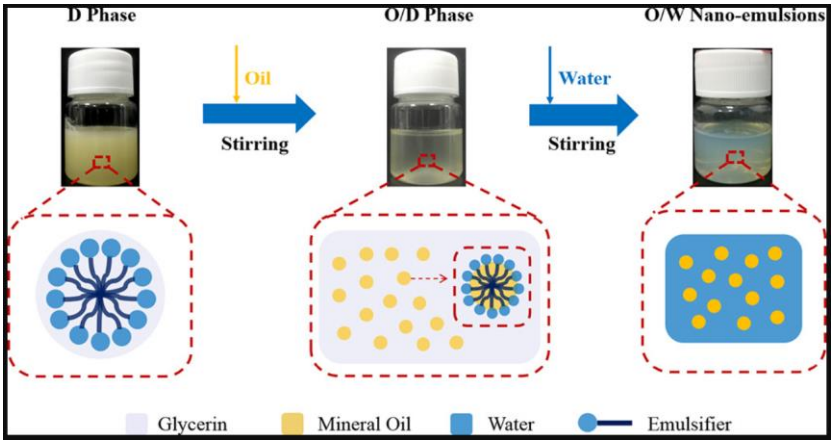


Figure 11 **Method of preparation of Nanoemulsion by D phase emulsification (27)**

1.2.5 Nanoemulgel:

Nanoemulgel is basically a combination of Nanoemulsion and gel in a single dosage form. Emulgel is commonly used in the topical preparation. Nanoemulgel is as a dosage form more patient compliant than traditional emulsion system. Moreover, this system widely uses for delivering the drugs which have low lipophilicity and high dose. Due to the presence of drug into oil globules, penetration of BCS class III and due to the lipidic nature of the drug carrier (oil phase) solubility and penetration of BCS class IV drugs increases. Simple reason behind

increasing penetration of drug is that oil phase due to its lipidic nature it fluidizes the lipid layer of skin for the short period of time and drug will transport into the skins. (28)

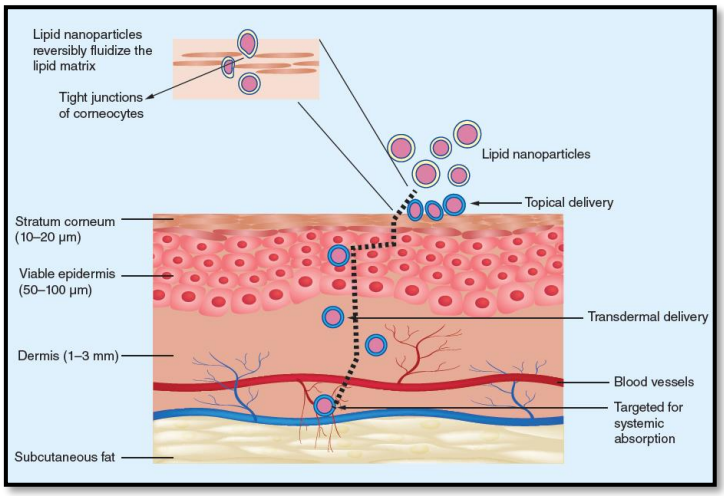


Figure 12 **Figure illustrate the penetration of active pharmaceutical ingredients from lipid nanoparticles** ***(28)***

Emulgels have certain eye touching properties like thixotropic, easily spreadable, greaseless, easily removable, emollient, nonstaining, water soluble, transparent, pleasant appearance, etc. Nanoemulgel is prepared by adding preformulated Nanoemulsion into the mixer of gel and water. (29)

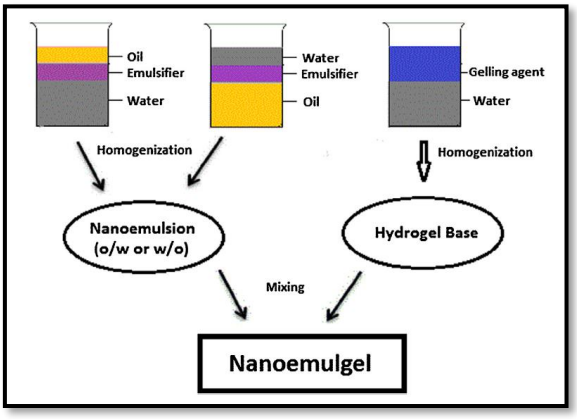


Figure 13 Method of preparation of Nanoemulgel (29)

1.2.6 Marketed Formulations – Emulgel:

Table 3 List of marketed formulation of nanoemulgel

Sr. No.	Name	Use
1.	Voltaren Emulgel	Topical analgesic
2.	Diclomax Emulgel	Tendons, ligaments
3.	Gaja Emulgel	Vaginal dryness, dehydration, redness

2. Aim, Objective and Rationale

Aim: To formulate and characterize of Nanoemulgel containing anti-acne agent (Dapsone).

Rationale behind use of Dapsone:

- Dapsone is a sulfone based antibiotic and proven efficient in treatment of Acne.
- It is proven that once daily use of Dapsone based gel efficient to treat acne lesions and produce less side effect compare to other anti-acne agents.
- Dapsone could be used to treat medium to severe cases of Acne lesions.
- Dapsone has log P value 0.97 that is slightly lipophilic and also suitable for topical formulation.

Rationale to formulate Nanoemulgel:

1. Nanoemulsions are one of the useful nanocarrier for achieving the highest drug penetration through skin.
2. The difference between convention gel and nano gel is that the later will show more bioavailability, reduce the size of dose and have lesser side effect and in future prospect for industrial purpose.
3. Surfactant present in Nanoemulsion has the capability to loosen the lipid bilayers by breaking hydrogen bonds while penetrating into the lipid bilayers. (30)

Objective:

I. To perform Preformulation study of Dapsone.
II. To prepare formulation and characterization of Nanoemulsion.
III. To perform optimization study and evaluation of Dapsone Nanoemulgel.
IV. To perform pharmacokinetic studies of optimize formulation of Nanoemulgel.

3. Literature Review

3.1 Literature Review on Dapsone – A drug used for Treatment of Acne

Christine Chim et al., describe the basics about Acne vulgaris. Basically, acne is a inflammatory disorder of skin mostly caused by Propionibacterium acne, hormonal changes and some inflammatory mediators. Scientist also describe the classification of acne as per European Dermatological Forum (EDF) and American Academy of Dermatology (AAD) into three categories: Mild, Moderate and Severe based on the number of comedone. Addition to this researcher also describe the marketed formulations and related side effects. (2)

Anna Hwee Sing Heng et al., briefly discussed the epidemiological study Acne obtained from Web od Science search and studied the risk factors associated with acne severity. Also study the risk factors like family history, BMI, age and skin type for acne presentation. Moreover, association between these factors with dietary ingredients, smoking and acne severity also studied. Scientist also give demographic study of acne prevalence in the world. (3)

Abhishek Budhiraja ey al., prepared a Rosmarinic acid loaded niosomal gel having particle diameter 814.42 nm and polydispersity index 0.329. Scientist design test for in-vitro release and in-vitro skin permeation study in diseased skin. In-vitro release study was performed on the franz diffusion shell using dialysis membrane having 12,000 dalton molecular weight. Methanolic phosphate buffer of pH 5.6 filled in a receptor compartment and maintained at around 37^0C and 1000 RPM. While in-vitro permeability study was performed using male albino rate skin of less than 2mm diameter. While receptor compartment filled with phosphate buffer pH 5.5. (31)

Clinical trial on Dapsone 7.5% gel shows once a day use of this gel give satisfactory result in patients older than 12 years. Dapsone is a sulfone-based anti-inflammatory agent which give effective results in treating Acne vugaris. Clinical result suggests that minimum 2 weeks use of once daily dapsone 7.5% gel shows reduced lesions on skin and it can use up to 4 to 8 weeks. This gel also shows reduced side effect on skin compare to other formulations. (32)

Mohammed Elmowafy et al., prepared the dapsone based nanostructured lipid carrier (NLC). The main objective behind preparing dapsone based NLC is to prepare dosage form that increase the permeability of dapsone. Emulsification / Sonication method is used for preparation of Dapsone NLC. 2% solution of Lecithin used as a lipophilic emulsifying agent. Particle size of NLC prepared by this method was found between 106 to 151 nm. The entrapment efficiency was found between 76% to 91%. Maximum steady state permeation of Dapsone was 5.3±0.86 μg/cm^2.h. (33)

3.2 Literature Review on Nanoemulsion in Treatment of Acne

Jonathan P. Fast et al., describe briefly the difference between Nanoemulsion, Microemulsion and related Nanoparticles. Microemulsion are the emulsion with particle size below 100 nm and thermodynamically stable. On the other side Nanoemulsion should not be use in substitute of microemulsion because both have almost particle size in the same range but it is thermodynamically unstable. Furthermore, Nanoemulsion are more towards the transparent or translucent as compare to the milky appearance of convectional emulsions. Reason behind that is, particles with nanometer in size become translucent or transparent because at particle size is lesser than optical wavelength of visible light. (18)

Mudra Saurabh Kapoor et al., discussed about the penetration of drug from the skin, barriers of skin and gave a brief about nanoparticle's penetration mechanism. Skin is the primary and largest barrier for skin targeted drug delivery system. Skin's upper most layer is Stratum corneum is tightly densed layer made up of corneocytes anchored lamellar lipid. Most preferred route for penetration through skin is extracellular route of lipid lamella. In this article scientists also describe the various methods for biophysical evaluation of skin and various transport mechanism through skin. (28)

Abhijit A. Date et al., describe in details about different class of oils, surfactant and cosurfactant. Normally oils having abnormally long hydrocarbon chains or very high molecular weight are difficult to emulsify, on the other hand oils having shorter chains or less molecular weight for example medium chain triglycerides (MCT) and medium chains mono- and di-glycerides are comparatively easy to emulsify. Opposite to this ability to solubilize the pharmaceutically active moieties should be higher in long chain triglycerides. (34)

Jamuna Bai Aswathanarayan et al., briefly discussed about the emulsification methods used in industry for the preparation of Nanoemulsion and its composition. Main focus of article is toward the method of preparation by high energy methods. Mostly various instruments like high pressure homogenizer, high shear homogenizer, probe sonicator and microfluidizer are

widely used for Nanoemulsion manufacturing at large scale. Scientist discussed in details about principles and working of these instruments. For example probe sonicator used high energy sound wave for decreasing the size in Nanoemulsion, high shear homogenizer creates high turbulence which produce shear force and thus particle size reduces, high pressure homogenizer and microfluidizer worked on the same principle that is particles are pressurize to pass through the slit and reduced the particle size. (23)

M. Safaya et al., mostly discussed about the law energy method for preparation of Nanoemulsion and effect of HLB on selection of surfactant. Low energy methods like spontaneous emulsification, phase transition temperature, microemulsion dilution, phase inversion composition and D – phase emulsification in brief. Mostly spontaneous emulsification method widely used while some systems like self-emulsification dosage form uses microemulsion dilution method for the Nanoemulsion preparation. Mostly these methods are used in formulating temperature sensitive pharmaceutical active agents. Scientist also discussed about various application of Nanoemulsion. (35)

S.A. Chime et al., discussed in brief about different Emusion based systems like Self emulsifying drug delivery system (SEDDS), Nano self-emulsified drug delivery system (NSEDDS), Microemulsion, Nanoemulsion, its manufacturing methods, characterization tests and application of these systems in treatment of various diseases. Basically, Nanoemulsion have inherent ability to dissolve large amount of insoluble drugs and naturally protect the pharmaceutically active agent from hydrolytic and enzymatic degradation made it preferred drug delivery system. (24)

Vivek Borhade et al., made a clotrimazole containing oral Nanoemulsion for the treatment of Malaria. The main objective of study was to improve the solubility and thus dissolution of lipophilic clotrimazole. Nanoemulsion prepared by using Capryol 90, Solutol HS 15 and Gelucire as oil, surfactant and cosurfactant respectively with 25 mg/ml of clotrimazole. The mean particle size of Nanoemulsion is less than 25 nm at specific pH. This Nanoemulsion released 100% of drug within 15 min of administering. (36)

Qian Xu et al., formulate a W/O Nanoemulsion of Baicalin for the treatment of Hepatitis B virus. Base of this research is that formation of lymphatics will eradicate the viruses. Researchers explain Ternary Phase Diagram in detail. A ratio of Phosphotidylcholine and propylene glycol used in different proportion (S_{mix}). Batches of ternary phase diagram was prepared by using dropwise titration method under magnetic agitation until mixture become clear. (37)

3.3 Literature Review on Nanoemulgel

Gururaj C. Aithal et al., developed a quercetin based Nanoemulgel for the treatment of periodontitis with the help of cinnamon oil, tween 80, Carbitol® and poloxamer 407. Overall, this formulation shows good stability, sol-gel transition and syringebility. Scientist also using computational study for evaluation of sol to gel phase transformation. In addition to this, ternary phase diagram for S_{mix} ratios 1:1, 2:1, 4:1, 1:4, 1:0 and 3:1 was applied to identify the perfect ratio of surfactant, co-surfactant and oil phase with respect to water. (38)

Yogeshwar G. Bachhav et al., prepared Microemulgel of fluconazole and evaluate it in-vitro and in-vivo. Main objective of preparing this system is proving that microemulsion based gel improve the drug penetration and thus control the inflammation quickly. Scientist used Cremophor EL and Capryol 90 as a surfactant in preparation of microemulsion having globule size 24 nm and polydispersity index 0.98. While microemulgel is formed by using the gelling agents like Hydroxypropyl methylcellulose (Methocel K4M) and CarbopolR ETD 2020. Bio adhesion study of this gel is performed by preparing special assembly using agar plate USP disintegration test apparatus. Addition to this, small clinical trial study was performed between Microemulgel and marketed product Candid-VR gel to check the efficacy. (39)

Patr´ıcia C. Pires et al., prepared a Nanoemulgel of phenytoin and fosphenytoin for intranasal administration of drug for treatment of convulsant. The main objective of preparing this dosage form is to achieve immediate and sustained drug release from the dosage form. The formulation has around 10% of non-aqueous phase which is responsible for sustain release of drug. Prepare of Nanoemulsion by using Miglyol 812 as oil phase, while Tween 80 and Transcutol P as surfactant and cosurfactant respectively. (40)

Chatchai Sungpud et al., prepared Mangostin loaded Nanoemulgel using virgin coconut oil that is used as anti-oxidant or antimicrobial. Mostly bioactive polyphenols from mangosteen peel was extracted by using organic solvent. Specifically, emulsion was stabilizing by using combination of surfactant and cosurfactant like Span 20 and Tween 20 respectively by preparing batches with different HLB values between 12 to 15. (41)

Manish Srivastava et al., prepared nanoemulgel of ketoprofen with eugenol to get synergistic action in treatment of periodontitis. Research was simply used oil phase as eugenol in preparation of Nanoemulsion which also have a antibacterial activity. Results of this research shows combination of eugenol with ketoprofen for the treatment of periodontitis give synergistic action and also show significant antibacterial, analgesic and anaesthetic properties. (42)

Manish Kumar Jeengar et al., formulate curcumin and emu oil based Nanoemulgel for the treatment of inflammation. Curcumin has a very good anti-inflammatory activity but it has poor solubility and poor permeability problem. Thus, preparation of Nanoemulgel provide a good alternative to formulate curcumin-based product because preparation of Nanoemulsion solve the solubility problem, while lipophilic oil droplets also improve the penetration through skin by acting as a lipidic carrier. (43)

Sonal Setya et al., formulated Selegilline hydrochloride based Nanoemulgel for the treatment of Parkinson's disease. Selegiline HCl containing w/o emulsion was prepared by combination of low energy and high energy methods. Selection of surfactant is based on its solubility in aqueous phase and HLB balance. Simple Selegiline gel was prepared and it was compared with Nanoemulgel at various level in in-vivo and in-vitro penetration study, thermodynamic stabilility study, etc. (44)

Omar Samia et al., formulated Carbamazepine Nanoemulgel for the treatment of Epilepsy. Normally, Carbamazepine is delivered through tablet but due to its highly hydrophobic nature, it is showing wide range of variation in dissolution time and interpatient variations. Thus, formulating an intranasal o/w mucoadhesive Nanoemulgel believe to be improve the drug's intersubjective variability. 0.1% Carbamazepine Nanoemulsion was prepared by law energy method, stirring on magnetic stirrer until clear solution will obtained. The findings of this research were satisfactory to target brain by olfactory mucosa route. (45)

Yujuan Mao et al., prepared Epriomectin containing o/w Nanoemulgel for the treatment of Endo and ectoparasites. Nanoemulgel is suitable for the transdermal delivery of API having highly lipophilicity and high dose. Scientists choose the method to prepare Nanoemulsion is continuous stirring on magnetic stirrer followed by Homogenizing it at 8000 RPM. Overall findings of study suggest that formulating Nanoemulgel is good approach to deliver the drug transdermally because of its mucoadhesive nature. (46)

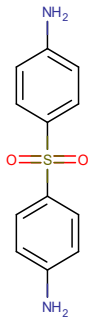
Swati Pund et al., prepared a Leflunomide Nanoemulgel for the treatment of psoriatic lesions and melanoma affected skin regions. Basically, this article is a mechanistic validation for the proof of concept of effective topical delivery of Leflunomide. Leflunomide is a newly approved disease modifying anti-rheumatic drug. It was hypothesized that it is useful in treatment of psoriasis and symbiotic relationship between keratinocytes and melanocytes. Nanoemulsion of leflunomide was prepared by low energy method. Prepared Nanoemulgel gave satisfactory result in various physicochemical tests, while toxicity study suggested that high therapeutic response which leads to reduced therapeutic dose and reduced systemic side effects. (47)

4. Drug & Excipients Details

4.1 Drug Related Information

4.1.1 Basic Information: (48)

Table 4 Information of Dapsone

Synonym:	• 1,1'-sulfonylbis(4-aminobenzene) • 1,1'-Sulfonylbis[4-aminobenzene] • 4-(4-amino-benzenesulfonyl)-phenylamine • 4-(4-aminophenylsulfonyl)aniline • 4-(4-aminophenylsulfonyl)benzenamine
Description:	Dapsone is a sulfone based antibiotic having wide range of anti-bacterial activity but mostly it is used to treat infection caused by mycobacterium leprae. Mechanism of action of Dapsone is similar to the sulfonamide class of antibiotic that involve in the folic acid synthesis inhibition. It should also used to treat malaria.
Type:	Small molecule, approved, Investigational
Structure:	
Molecular weight:	248.301 gm/mol
Chemical formula:	$C_{12}H_{12}N_2O_2S$
IUPAC Name:	4-(4-aminobenzenesulfonyl)aniline
Category:	Anti-acne Anti-bacterial Anti-infective

	Anti-mycobacterial
Chemical class:	Benzene and its substituted derivatives
Sub class:	Benzenesulfonyl compounds

4.1.2 Pharmacodynamic:

Table 5 Pharmacodynamic information of Dapsone (48)

Introduction of API:	Dapsone is a sulfone with antibacterial, anti-inflammatory and antibiotic properties. It is the principal drug that recommended by the WHO for the treatment of leprosy. It is used for treating malaria to as anti-inflammatory agent and Pneumocystic carinii pneumonia. It is rapidly and almost completely absorbed through GIT. However, it tends to be retained in skin and muscle and especially in the liver and kidney.
Absorption:	70 to 80% following oral administration.
Protein binding:	70 to 90%
Metabolism:	Hepatic, mostly CYP2E1-mediated.
Route of elimination:	Renal
Half life:	28 hours (range 10-50 hours)
Toxicity:	Overdosage might be expected to produce nasal congestion, syncope, or hallucinations. Measures to support blood pressure should be taken if necessary.
Affected organism:	Mycobacteria Mycobacterium leprae
Food Interaction:	Take without regard to meals.

4.1.3 Drug Interaction: (49)

Table 6 Drug interaction of Dapsone gel 7.5%

Drug	Interaction
Folinic acid	Combined used with dapsone will cause decrease in therapeutic activity.
Warfarin	Therapeutic activity decreases on combined use.
Albendazole	Dapsone could affect the metabolism of albendazole.
Aldosteron	Dapsone would decreses the metabolism of albendazole.
Alpha tocopherol	Combined use will decreases the metabolism of alpha tocopherol.
Ambroxol	The metabolism of Ambroxol decrease.
Amphotericin B	The metabolism of Amphotericin B decrease.
Verapamil	The metabolism of Verapamil decrease.
Trimethoprim	The serum concentration of Dapsone would increased on combined with Trimethoprim.

4.1.4 Physicochemical Properties:

Table 7 Physicochemical properties of Dapsone (48)

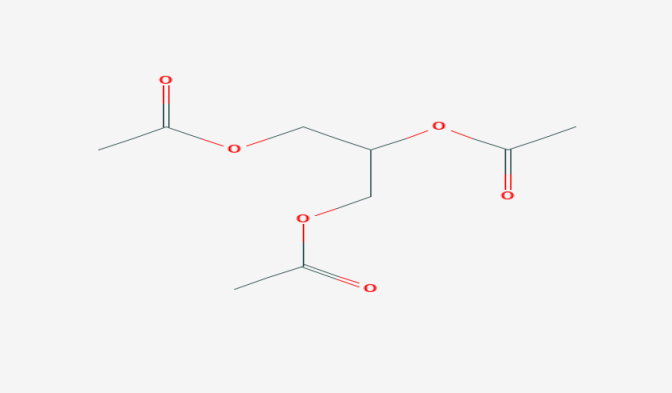
State:	Solid
Melting point:	175.5ºC
Water solubility:	380 mg/L
Log P:	0.97
pKa:	2.41
Rule of Five:	Yes
Veber's Rule:	NO
Ghose filter:	Yes

4.2 Excipients Related Information

4.2.1 Triacetin: (50)

Triacetin is a triglyceride obtained by acetylation of the three hydroxy groups of glycerol. It has fungistatic properties (based on release of acetic acid) and has been used in the topical treatment of minor dermatophyte infections. It has a role as a plant metabolite, a solvent, a fuel additive, an adjuvant, a food additive carrier, a food emulsifier, a food humectant and an antifungal drug. It derives from an acetic acid.

Table 8 Information regarding Triacetin

Synonyms	Glyceryl triacetate Glycerol triacetate Glycerin triacetate
Molecular Formula	$C_3H_5(OCOCH_3)_3$
Structure	
Molecular Weight	218.2 g/mol
Colour	Colorless somewhat oily liquid
Odour	Slightly fruity or fatty odor
Taste	MILD, SWEET TASTE, BITTER ABOVE 0.05%
Boiling Point	258-259 °C
Melting Point	-78°C

Solubility	Slightly soluble in carbon disulfide; miscible with alcohol, ether, chloroform
Density	1.1583 g/cu cm at 20 °C
Vapor Pressure	0.00 mmHg
Log P	0.25
Stability	Chemical stability: Stable under recommended storage conditions

4.2.2 Labrasol: (51)

Labrasol is a PEG derivative of medium chain fatty acid triglyceride of capric and caprylic acid.

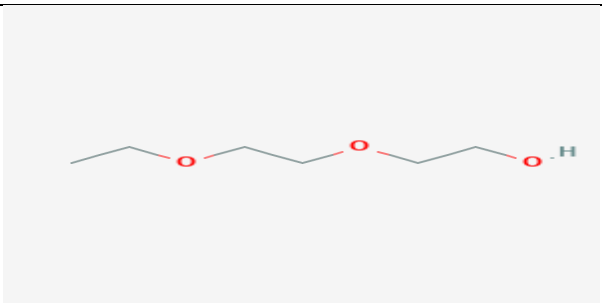
Table 9 Information regarding labrasol

Description	A nonionic oil-in-water surfactant used as solubilizer in topical formulations
Synonyms	Caprylocaproyl Polyoxyl-8 glyceride
Application	Solubilizer, Bioavailability enhancer, Surfactant in capsule filling, SMEDDS, Suspension, Solution, Topical penetration enhancer, Processing aid.
Colour	Transparent liquid
Odour	Characteritic
Density	80 – 110 mPa.s at 20^0C
HLB	12

4.2.3 Transcutol: (52)

Diethylene glycol monoethyl ether is a primary alcohol that is ethanol substituted by a 2-ethoxyethoxy group at position 2. It has a role as a protic solvent. It is a diether, a primary alcohol and a hydroxypolyether. It derives from a diethylene glycol.

Table 10 Information regarding Transcutol

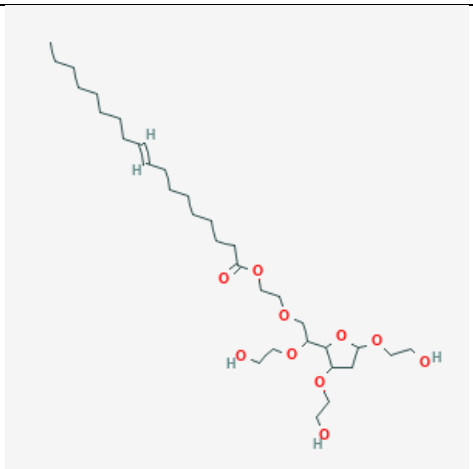
Synonyms	Diethylene glycol monoethyl ether 2-(2-Ethoxyethoxy)ethanol CARBITOL
Molecular Formula	$CH_3CH_2OCH_2CH_2OCH_2CH_2OH$
Structure	
Molecular Weight	134.17 g/mol
Colour	Colorless hygroscopic liquid
Odour	Mildly sweet odour
Taste	Bitter
Boiling Point	396 °F at 760 mm Hg
Melting Point	-108 °F
Solubility	Miscible with ethanol, acetone, benzene; very soluble in ethyl ether; water miscible
Density	0.99 at 68 °F
Log P	-0.54
Decomposition	When heated to decomposition it emits acrid smoke and irritating fumes

4.2.4 Tween 80: (53)

Polysorbate 80 is obtained from polyethoxylated sorbitan and oleic acid. The hydrophilic groups present in the compound are polyethers which also known as

polyoxyethylene groups. In the nomenclature of polysorbates, the numeric designation following polysorbate refers to the oleic acid, a lipophilic group.

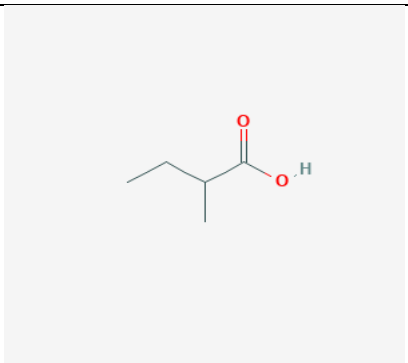
Table 11 Information regarding Tween 80

Synonyms	Polysorbate 80 (glycol), Polyoxyethylene 20 sorbitan monooleate, Inhibited ethylene glycol, 2-hydroxyethyl 2-deoxy-3,5-bis-O-(2-hydroxyethyl)-6-O-{2-[(9E)-octadec-9-enoyloxy]ethyl}hexofuranoside, PEG-3 Sorbitan oleate
Molecular Formula	$C_{32}H_{60}O_{10}$
Structure	
Molecular Weight	604.8 g/mol
Colour	Amber colored liquid
Odour	Characteristic
Boiling Point	$>100^0C$
Solubility	Very soluble in water, Soluble in ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene
Density	1.06–1.09 g/mL
HLB	15

4.2.5 Carbopol 934: (54)

2-methylbutyric acid is a methylbutyric acid comprising a butyric acid core carrying a 2-methyl substituent. Produced from amino acid leucine during nutrient starvation in bacteria. It has a role as a bacterial metabolite and a human metabolite. It is a conjugate acid of a 2-methylbutyrate. Ethylmethylacetic acid is a carboxylic acid found in low amounts in normal humans. It has been described in the urine of individuals with 2-ethylhydracrylic aciduria associated with short/branched-chain acyl-CoA dehydrogenase deficiency.

Table 12 Information regarding Carbopol 934

Synonyms	2-Methylbutanoic acid 2-METHYLBUTYRIC ACID DL-2-Methylbutyric acid Butanoic acid
Molecular Formula	$C_5H_{10}O_2$
Structure	
Molecular Weight	102.13 g/mol
Colour	White powder
Boiling Point	176.5 °C
Solubility	In water 45 mg/mL at 20 °C
Log P	1.18

4.3 Plan of Work

1. Literature Review:

2. Preformulation Study:
    a) Organoleptic Properties
    b) Solubility Study
    c) Melting Point
    d) Partition Coefficient
    e) UV Spectrum Analysis
    f) FT-IR
    g) DSC
    h) Antibacterial Assay / Activity Study

3. Selection of Oil Phase, Surfactant and Cosurfactant:

4. Formulation and Evaluation Nanoemulsion:
    a) Physical Appearance
    b) Physical Stability
    c) Droplet Size
    d) Zeta Potential
    e) Polydispersity Index

5. Formulation and Evaluation Nanoemulgel:
    a) Drug Content
    b) pH Determination
    c) Rheological Measurement
    d) Shelf life Determination
    e) In-vitro Diffusion Study
    f) Ex-vivo Diffusion Study

5. Materials & Methods

5.1 List of Materials

Table 13 List of materials

Sr. No.	Name of Material	Manufacturer
1.	Dapsone	Atul Chemicals ltd.
2.	Labrasol	Gattefosse
3.	Transcutol	Gattefosse
4.	Tween 80	Sigma-Aldrich
5.	Labrafil	Gattefosse

5.2 List of Equipment

Table 14 List of equipments used

Sr. No.	Equipment Used	Model	Manufacturer
1.	Weighing balance	CITIZEN	-
2.	Magnetic Stirrer	REMI Equipmetn's	-
3.	Particle Size Analyser	Horiba	SZ 100
4.	Double beam Spectrophotometer	Shimadzu	UV 1800
5.	pH Meter	Chemical lab	-
6.	Differential scanning calorimeter	Hitachi	DSC - 7020
7.	Brookfield Viscometer	Brookfield	-
8.	FT-IR	Jasco	FTIR/6100
9.	Vortex	Labpro	-

5.3 Evaluation Preformulation Parameters

Preformulation study is the study for determination of physicochemical properties of the drug powder and related parameters. This information will subsequently be helped in different approaches in formulation development. Moreover, this study has impact on the performance and development of safe, stable and efficacious dosage form.

5.3.1 Organoleptic Properties:

Around 500 mg of Dapsone powder was equally distributed on petri plate and organoleptic properties like appearance, colour and odour were evaluated manually.

5.3.2 Solubility Study:

Solubility study of Dapsone was performed by using Saturation solubility method. In this method Dapsone was gradually dissolving predetermined amount of drug in glass vial and particular solvent at room temperature and on magnetic stirrer. Solubility of Dapsone in different solvents was determined by ability of solvent to dissolve maximum quantity of drug.

5.3.3 Melting Point

Melting point of Dapsone was determined by using thin walled Capillary. Capillary was sealed at one end. Small amount of drug powder was carefully filled in to the capillary and make sure that power touches the close end of capillary. Then joint capillary and thermometer by thread and put it into the beaker containing paraffin oil. Its temperature was gradually increasing and the temperature at which powder turns into liquid noted as melting point.

5.3.4 UV Spectrum Analysis

UV-VIS Spectrophotometer of Shimadzu UV-1800 Japan was used for identification of compound and getting standard curve. Identification of Dapsone was perform by finding maximum wavelength of compound and compare it with literature. While standard curve of Dapsone was took in two different system one was in methanol and second was in phosphate buffer pH 5.5 which is equivalent to diseased skin.

5.3.5 FT-IR:

FTIR (Jasco FT-IR 6100 Germany) was used for identification of drug and check out any present impurities in the sample. To perform a study firstly Dapsone was diluted with dried potassium bromide (KBr) in a 1:100 ratio and triturate it properly. Each KBr disc scanned at a resolution of 2 cm and at 4 mm/s over a wave number region of 4000–400 cm-1 and recorded by using IR solution software. The characteristic peaks of Dpson were recorded and compared.

5.3.6 DSC:

Differential scanning calorimetric (DSC) (Hitachi DSC-7020) analysis was used for analyse the thermal behaviour of drug. 4 mg of Dapsone was taken for scanning the sample. This was kept on aluminium pan which was initially hermetically sealed. Sample kept was heated at a temperature rate of 10°C/ min under nitrogen with empty aluminium pan as a reference. Range of temperature is from 30-300°C.

5.4 Formulation and Evaluation of Nanoemulsion

Method of Preparation:

Nanoemulsion was prepared by using low energy method. Magnetic stirrer was used for the preparation of Nanoemulsion. Oil phase or discontinuous phase was prepared by dissolving dapsone in oil phase, surfactant and co-surfactant. While continuous phase or aqueous was prepared by dissolving co-surfactant in required quantity of distilled water. Then, add oil phase dropwise into water phase and stir on magnetic stirrer at room temperature for specific time and RPM (rounds per minutes).

5.4.1 Screening of Oil, Surfactant and Cosurfactant

Selection of oil phase, surfactant and co-surfactant is based on its solubility for dapsone. To find out the solubility of oil phase, surfactant and co-surfactant 1gm of each component taken one by one and gradually adding predefined amount of dapsone simultaneously stirring on magnetic stirrer. Maximum amount of drug loading

5.4.2 Physical Appearance

Prepared Nanoemulsion must be clear.

5.4.3 Physical Stability

The physical stability of emulsion-based system is very important for its performance. Phase separation might possibly end in precipitating out of API. Moreover, phase separation affecting physical appearance as well. Physical stability was checked by putting a Nanoemulsion at room temperature for 24 hours.

5.4.4 Droplet Size & Polydispersity Index

The average particle size and polydispersity index of Nanoemulsion was checked in particle size analyser (Horiba SZ-100) which is based on light scattering method. Polydispersity index shows particle size distribution throughout the system. Target particle size was 200 nm and polydispersity index were near to 1.

5.5 Formulation and Evaluation of Nanoemulgel

Basic Methods of Preparation:

Nanoemulgel was prepared by mixing previously prepared Nanoemulsion and gel phase. Mostly two different approaches: First one is simultaneously dispersed gelling agent into a water and this gel was directly added into a nanoemulsion while second method is to add gelling agent directly into the nanoemulsion and continuously stir it on magnetic stirrer till gelling agent was dissolved and form a gel.

5.5.1 Drug Content

Drug content was estimated by performing an assay of formulation. To perform an assay weighed quantity of dapsone gel was dissolved in methanol. Then make a required dilution and measure the absorbance of each sample using UV-VIS spectroscopy.

5.5.2 pH Determination

pH of Nanoemulgel was determined by using digital pH meter. To measure the pH of Nanoemulgel dispersed the 0.5 gm of Nanoemulgel in 50ml water.

5.5.3 Rheological Measurement

Rheological characterization of optimized gel formulation was performed by using Brookfield Rheometer. Viscosity is important parameter which gives idea about spread ability of Nanoemulgel. To determine the viscosity spindle C 50-1 was used at 25^0C and 80 RPM.

5.5.4 Physical Stability Study

Physical stability of Nanoemulgel was checked at various performance parameters like vesicular shape and size, phase separation, appearance, pH, drug content, etc. The stability assessment of optimized formulation was performed by storing Nanoemulgel in glass vial and in an atmospheric condition.

5.5.5 Antibacterial Activity Study

Antibacterial activity of dapsone gel was evaluated by cup plate method. Agar gel was taken as bacterial growth media while bacteria such as S. aureus and E. coli were used. Firstly, sterilized molten nutrient agar was poured into previously sterilized petri plate and allowed to solidify. Then plates were swabbed with 100 µl culture of microbes. Immediately after this made a cylinder cup and incubated at 37°± 0.5°C for 48 h. At last zone of inhibition of bacterial growth were measured.

5.5.6 In-vitro Diffusion Study

This study also called in-vitro drug release study and was performed by using Frans diffusion cell. Cellophane membrane was used as semipermeable membrane and placed between receptor and donor compartment. While methanolic phosphate buffer of pH 5.6 was filled in the receptor compartment. 1 gm of gel was equally spread on donor compartment. 1 ml of aliquot was replaced with fresh buffer solution at specific time interval. The withdrawn sample was analyzed using UV spectroscopy.

6. Results

6.1 Preformulation Study

6.1.1 Organoleptic Properties:

Table 15 Organoletic properties of Dapsone

Parameters	Inference
State	Solid
Colour	White Crystalline Powder
Taste	Characteristic
Odour	Odourless

6.1.2 Melting Point Determination:

Melting Point: 179^0C

6.1.3 UV-VIS Spectrum Study:

The Determination of λmax by UV spectroscopy is necessary for future analytical works. The absorption maxima λmax of drug was determined using UV spectroscopy. A stock solution of known concentration is prepared and analysed using double beam UV visible spectrophotometer with slit width of 1 cm using pH 6.8 simulated salivary fluid in range of 200-400 nm.

A. Standard Curve in Phosphate buffer pH 5.5:

Table 16 Absorption table for Phosphate buffer pH 5.5

Conc.	Absorption
1	0.1339
2	0.1976
3	0.3265
4	0.4111
5	0.5535
6	0.6742
7	0.7918
8	0.9294

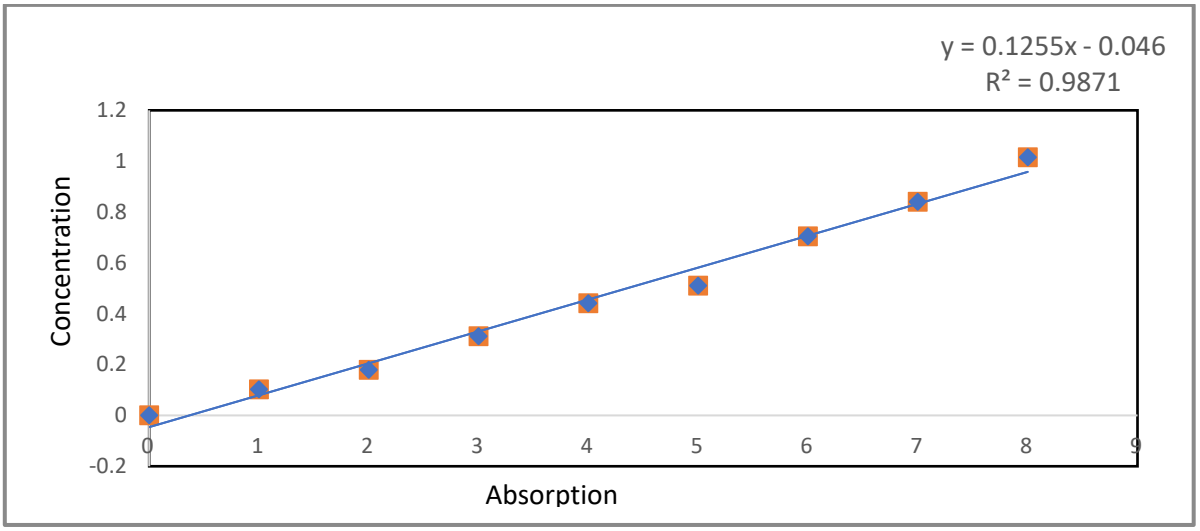


Figure 14 Standard curve of dapsone in Phosphate buffer pH 5.5

B. Standard Curve in Methanol:

Table 17 Absorption table for Methanol

Concentration	Absorption
2	0.2528
3	0.4401
4	0.5477
5	0.6254
6	0.8746
7	0.9752
8	1.4394
10	1.0922

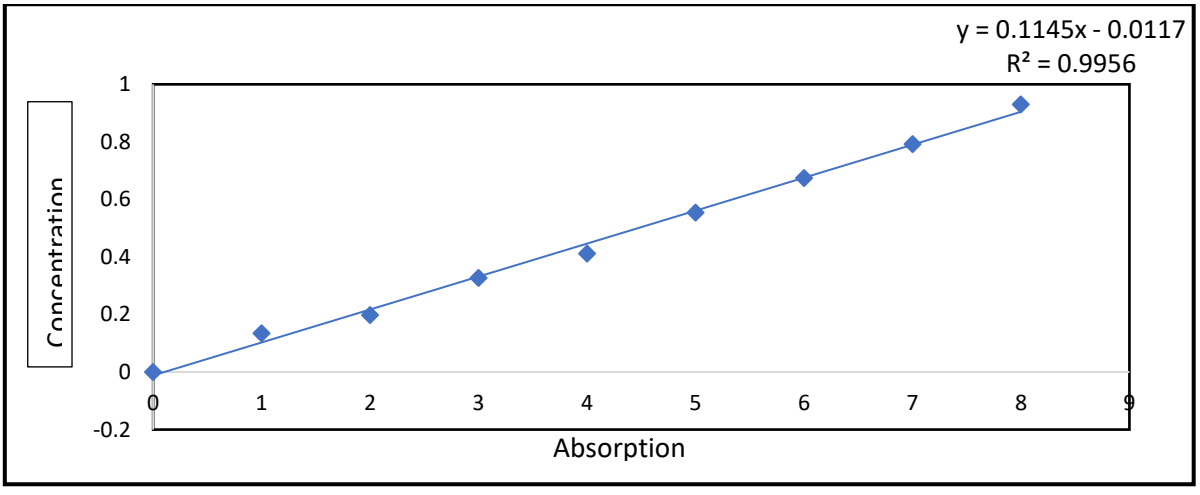


Figure 15 Standard curve of dapsone in Methanol

6.1.3 FT-IR:

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

Experimental Condition:

Table 18 **Concentration (ppm)** IR Study

Sample	Dapsone
Light Source	Standard
Detector	TGS
Resolution	4 cm^{-1}

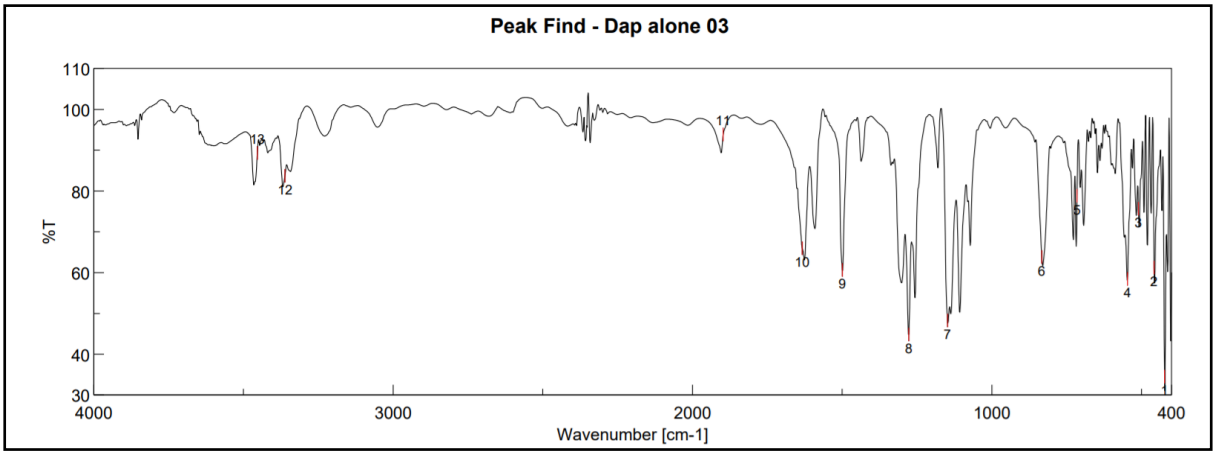


Figure 16 IR graph of Dapsone

Table 19 Various types of rotation of Dapsone

Functional Group	Type of Rotation	Range	Test Wave-number
N-H	Stretching	-	3453.88
N-H	Stretching	3250-3400	3361.32
C=C	Banding	1580-1680	1633.41
O-H	Stretching	3200-3500	3453.88

C-C	Stretching	1400-1500	1499.38
$-SO_2$	Asymmetric Stretching	1250-1335	1277.61
$-SO_2$	Symmetric Stretching	-	1148.4
=C-H	Aromatic Banding	860-680	715.61
$-SO_2$	Scissoring	-	546.72

6.1.4 DSC:

- The DSC thermogram of Dapsone showed thermal peack at 178.8^{0}C and with the energy --25.70 mW.

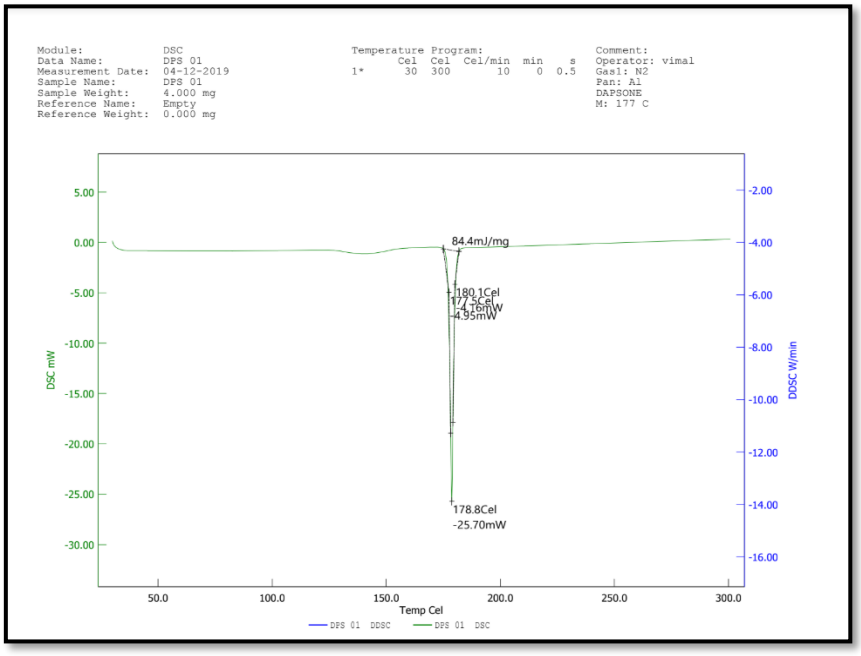


Figure 17 DSC thermogram of Dapsone

6.2 Formulation and Evaluation of Nanoemulsion

6.2.1 Screening of Oil, Surfactant and Co-surfactant:

Table 20 List of oil, surfactant and co-surfactant for solubility study

Oil Phase

Components	Concentration (mg)
Triacetin	110
Neem Oil	7
α-Tocopherol	20
Clove Oil	95
Eucalyptus Oil	60
Tea Tree Oil	50
Castor oil	15
Isopropyl palmitate	5
Seasam oil	5
Corn oil	10
Olive oil	10

Surfactant:

Components	Concentration (mg)
Glyceryl mono-oleate	20
Miglyol	7
Campryol 90	80
Prulol	25
Labrafil	15
Labrasol	130

Co-surfactant:

Components	Concentration (mg)
Transcutol	110
Tween 80	10

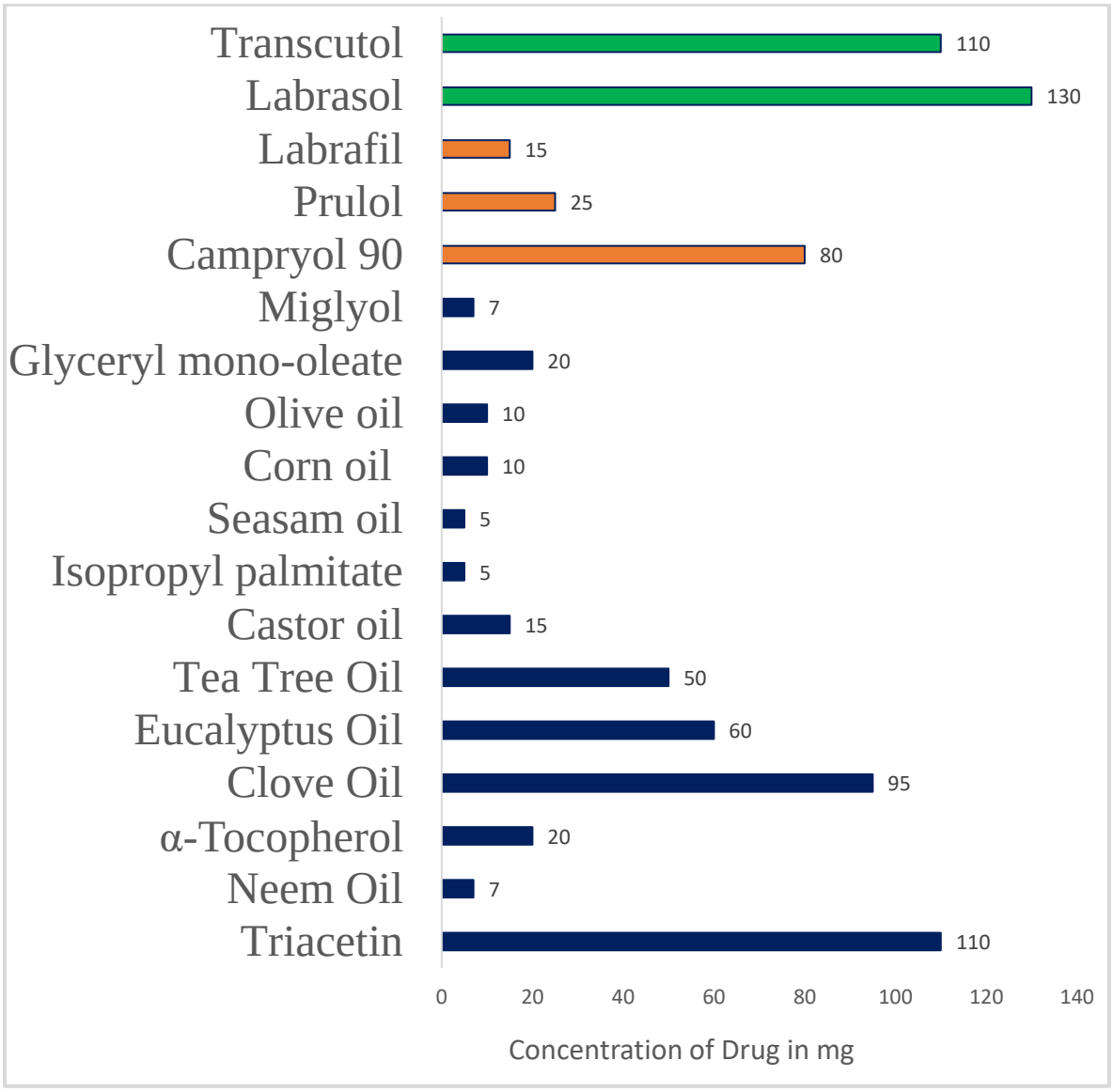


Figure 18 Graphical representation of solubility study

6.2.2 Batch Details

6.2.2.1 Selection of Surfactant and Co-surfactant:

Table 21 Batch Details for Selection of Surfactant and Co-surfactant

Sr No	Oil Phase	Surfactant	Cosurfactant	Water	Batch Quantity	Stirring Time	Inference
1	Olive+Clove+ Triacetin (TA) 2.5+1.3+1.2gm	Capryol 90 2.400gm	Tween 80 0.600gm	2.0gm	10.0gm	15-20 min	Emulsion was break.
2	Clove+Olive Oil 1.000+0.750gm	Capryol 90 0.750gm	~	7.5gm	10.0gm	15-20min	Emulsion was break.
3	Clove oil+TA 0.500+1.0gm	Capryol 90 0.750gm	Transcutol 0.750gm	7.0gm	10.0gm	15-20 min	Clear Emulsion + Phase Separation
4	Clove oil 0.750gm	Cremophor EL 0.350gm	Transcutol 0.350gm	3.5gm	5.0gm	15-20 min	Clear Emulsion + Phase Separation
5	Capryol 90 0.375gm	Cremophor EL 0.200gm	Labrasol 0.175gm	1.75gm	2.5gm	15-20 min	Milky Emulsion
6	Clove oil 90 0.375gm	Cremophor EL 0.200gm	Labrasol 0.175gm	1.75gm	2.5gm	15-20 min	Milky + Immediately Break
7	Clove oil 0.375gm	Capryol 90 0.200gm	Labrasol 0.175gm	1.75gm	2.5gm	15-20 min	Milky Emulsion

8	Clove oil 0.375gm	Tween 80 0.300gm	Span 80 0.075gm	1.75gm	2.5gm	15-20 min	Hazy Emulsion
9	Clove oil + TA 0.200+0.200gm	Tween 80 0.300gm	Span 80 0.075gm	1.75gm	2.5gm	15-20 min	Milky + Immediately Break
10	Clove oil + TA 0.200+0.200gm	Poloxamer 407 0.200gm	Transcutol 0.125gm	1.775gm	2.5gm	15-20 min	Milky + Stable Emulsion
11	Clove oil + TA 0.200+0.200gm	Cremophor 0.200gm	Transcutol 0.125gm	1.775gm	2.5gm	15-20 min	Milky + Phase Separation
12	Clove oil + TA 0.200+0.200gm	Capryol 90 0.200gm	Labrasol 0.125gm	1.775gm	2.5gm	15-20 min	Clear emulsion but phase separated
13	Clove oil + TA 0.125gm+0.25g	Capryol 90 0.180gm	Transcuol 0.180gm	1.75gm	2.5gm	30min	Milky and Break
14	Clove oil + TA 0.250+0.250gm	Poloxamer 407 0.125gm	Transcuol 0.125gm	1.75gm	2.5gm	30min	Milky

Inference:

Selected Oil Phase and Surfactant & Co-surfactant:

1. Triacetin + Capryol 90
2. Triacetin + Transcutol + Labrasol

Note: Selection of Oil phase and Surfactant & Co-surfactant is based on its capability of forming Clear and Stable Emulsion.

6.2.2.2 Surfactant and Co-surfactant Ratio Study:

Table 22 Batch details for Triacetin and Capryol 90 ratio study

Sr. No.	Oil	Surfactant	Water	Batch Size	Stirring Time	Inference
1	Triacetin 0.187gm	Capryol 90 0.187gm	2.12 gm	2.5 gm	30 min	Break after some time
2	Triacetin 0.260gm	Capryol 90 0.110gm	2.12 gm	2.5 gm	30 min	Break after some time
3	Triacetin 0.110gm	Capryol 90 0.260gm	2.12 gm	2.5 gm	30 min	Break after some time

Inference:

This combination of Oil Phase and Surfactant is selected for further study because it gives clear and stable emulsion.

Table 23 Batch details for triacetin and Transcutol ratio study

Sr. No.	Oil	Surfactant	Water	Batch Size	Stirring Time	Inference
1	Triacetin 0.375gm	Transcutol 0.375gm	1.75gm	2.5gm	30 min	Hazy but clear on slightly heating
2	Triacetin 0.225gm	Transcutol 0.525gm	1.75gm	2.5gm	30min	Slightly Hazy
3	Triacetin 0.300gm	Transcutol 1.200gm	3.50gm	5.0gm	30min	Clear Emulsion + Stable
4	Triacetin 0.150gm	Transcutol 1.35gm	3.50gm	5.0gm	30min	Clear Emulsion + Stable

Inference:

This combination of Surfactant and Co-surfactant is rejected for further study because emulsion form by them are break after some period of time mostly within 1-2 hours

6.2.2.3 Maximum Drug Loading Study:

Table 24 Batch details for drug loading studies

Sr. No	API	Oil	Surfactant	CoS - A	CoS - B	Water	Batch Size	Stirring Time	Inference
1	70mg	Triacetin 0.280gm	Labrasol 1.120gm	Transcutol 0.200gm	~	2.4gm	4.0gm	30min	Clear Emulsion + Break after some time + On slightly heating emulsion become hazy
2A	100mg	Triacetin 0.300gm	Labrasol 0.900gm	Transcutol 0.200gm	Tween 80 0.200gm	2.4gm	4.0gm	>24 hr	Clear emulsion on overnight stirring
2B	100mg	Triacetin 0.300gm	Labrasol 0.900gm	Transcutol 0.200gm	Tween 80 0.200gm	2.4gm	4.0gm	>48 hr	Clear emulsion on overnight stirring
3	100mg	Triacetin 0450gm	Labrasol 0.950gm	Transcutol 0.200gm	~	2.4gm	4.0gm	2 hr	~
4	150mg	Triacetin 0.150gm	Transcutol 1.35gm	~	~	3.35gm	5.0gm	30min	Milky + Break
5	150mg	Triacetin 0.250gm	Transcutol 1.00gm	Labrasol 0.250gm		3.15gm	5.0gm	30min	Milky + Stable for few hours
6	150mg	Triacetin 0.250gm	Transcutol 1.00gm	Labrasol 0.250gm	~	3.15gm	5.0gm	30min	Milky + Break Immediately
7	200mg	Triacetin 0.280gm	Transcutol 1.120gm	Labrasol 0.200gm	~	2.4gm	4.0gm	30min	Hazy Emulsion but after few hours it will be clear and Oil phase was slightly separated.

8A	~	Triacetin 0.280gm	Transcutol 1.120gm	Labrasol 0.35+0.35 gm	~	2.4gm	4.0gm	30min	Clear emulsion and comparatively stable emulsion
8B	100 mg	Triacetin 0.280gm	Transcutol 1.120gm	Labrasol 0.35+0.35 gm	~	2.4gm	4.0gm	30min	Clear emulsion and Stable on storing in Refrigerator; Phase separated on addition of drug
9A	150 mg	Triacetin 0.450gm	Labrasol 0.850gm	Transcutol 0.300gm	Tween 80 0.200gm	2.4gm	4.0gm	~24 hr (1000 RPM)	Milky + Separated
9B	150 mg	Triacetin 0.450gm	Labrasol 0.850gm	Transcutol 0.300gm	Tween 80 0.200gm	2.4gm	4.0gm	~48 hr(1000 RPM)	Milky + Separated
10 A	125 mg	Triacetin 0.400gm	Labrasol 0.700gm	Transcutol 0.300gm	Tween 80 0.200gm	2.4gm	4.0gm	~48 hr (800RPM)	Clear + Yellowish + Stable Emulsion
10 B	125 mg	Triacetin 0.400gm	Labrasol 0.700gm	Transcutol 0.300gm	Tween 80 0.200gm	2.4gm	4.0gm	~48 hr (800RPM)	Clear + Yellowish + Stable Emulsion
11 A	125 mg	Triacetin 0.500gm	Labrasol 1.000gm	Transcutol 0.250gm	Tween 80 0.200gm	3.0gm	5.0gm	~24 hr (800 RPM)	Clear + Yellowish + Stable Emulsion
11 B	125 mg	Triacetin 0.500gm	Labrasol 1.000gm	Transcutol 0.250gm	Tween 80 0.200gm	3.0gm	5.0gm	~24 hr (800 RPM)	Clear + Yellowish + Stable Emulsion

12	125mg	Triacetin 0.500gm	Labrasol 1.000gm	Transcutol 0.250gm	Transcutol 0.200gm	3.0gm	5.0gm	~24 hr (800 RPM)	Clear + Yellowish + Stable Emulsion

Inference:

1. **Maximum Drug Loading:** 125mg
2. **S_{mix} Ratio:** 9:4, 7:5, 2:1
3. **Probable Particle Size:** Around 300nm
4. **Probable Formulation:**

Table 25 Propable formulation for Nanoemulgel system

Ingredients	Concentration
Dapsone	125mg
Triacetin	10%
Labrasol	15%
Transcutol	10%
Tween 80	5%
Water	60%

6.2.3 Particle size and PDI measurement of Batches:

Table 26 List of Particle size analysis and PDI

Sr No	API	Oil	Surfactant	CoS-A	CoS-B	Aqueous Phase	Particle Size	PDI
2A	100 mg	Triacetin 0.300gm	Labrasol 0.900gm	Transcutol 0.200gm	Tween 80 0.200gm	Water 2.4gm	D90: 431 nm	0.326
2B	100 mg	Triacetin 0.300gm	Labrasol 0.900gm	Transcutol 0.200gm	Tween 80 0.200gm	Water 2.4gm	D90: 357 nm	0.420
10 A	125 mg	Triacetin 0.400gm	Labrasol 0.700gm	Transcutol 0.300gm	Tween 80 0.200gm	Water 2.4gm	D90: 709 nm	0.635

10 B	125 mg	Triacetin 0.400gm	Labrasol 0.700gm	Transcutol 0.300gm	Tween 80 0.200gm	Water 2.4gm	D90: 867 nm	0.687
11 A	125 mg	Triacetin 0.500gm	Labrasol 1.000gm	Transcutol 0.250gm	Tween 80 0.200gm	Water 3.0gm	D90: 8257 nm	0.602
11 B	125 mg	Triacetin 0.500gm	Labrasol 1.000gm	Transcutol 0.250gm	Tween 80 0.200gm	Water 3.0gm	D90: 926 nm	0.669

6.2.4 pH Analsysis:

Table 27 Table of pH

Sr. No.	Batch No.	pH
1.	2A	5.43
2.	2B	5.57
3.	10A	6.02
4.	10B	5.87
5.	11A	6.23
6.	11B	6.19

6.2.5 Physical Appearance of Nanoemulsion:

Visual Appearance: Clear and Slightly yellowish solution

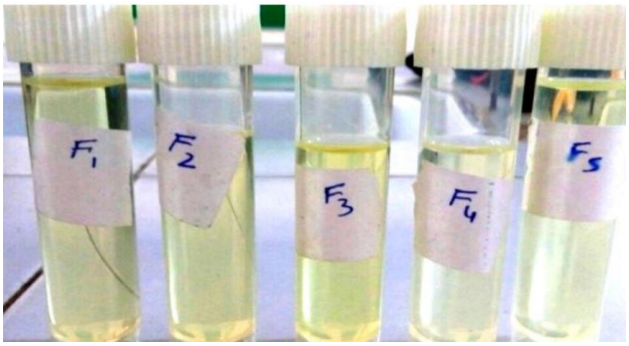


Figure 19 Physical appearance of nanoemulsion

6.3 Formulation and Evaluation of Nanoemulgel

6.3.1 Batch Details:

Table 28 Batch details for preparation of Nanoemulgel

Sr. No	API	Oil	Surfactant	CoS - A	CoS - B	Water	Gelling Agent	Inference
2A	100 mg	Triacetin 0.300gm	Labrasol 0.900gm	Transcutol 0.200gm	Tween 80 0.200gm	2.4gm	Carbopol 934 (1%)	Hazy but Good consistency
2B	100 mg	Triacetin 0.300gm	Labrasol 0.900gm	Transcutol 0.200gm	Tween 80 0.200gm	2.4gm	Carbopol 934 (1%)	Hazy but Good consistency
10 A	125 mg	Triacetin 0.400gm	Labrasol 0.700gm	Transcutol 0.300gm	Tween 80 0.200gm	2.4gm	Carbopol 934 (1%)	Hazy but Good consistency
10 B	125 mg	Triacetin 0.400gm	Labrasol 0.700gm	Transcutol 0.300gm	Tween 80 0.200gm	2.4gm	Carbopol 934 (1%)	Hazy but Good consistency
11 A	125 mg	Triacetin 0.500gm	Labrasol 1.000gm	Transcutol 0.250gm	Tween 80 0.200gm	3.0gm	Carbopol 934 (1%)	Hazy but Good consistency
11 B	125 mg	Triacetin 0.500gm	Labrasol 1.000gm	Transcutol 0.250gm	Tween 80 0.200gm	3.0gm	Carbopol 934 (1%)	Hazy but Good consistency

Inference:

Study shows 1% gel of Carbopol give hazy but good consistency gel.

6.3.2 pH Analysis:

Table 29 ph Analysis of Nanoemulgel

Sr. No.	Batch No.	pH
1.	2A	6.9
2.	2B	7.02
3.	10A	7.25
4.	10B	7.08
5.	11A	6.86
6.	11B	7.03

6.3.4 Particle size Analysis:

Table 30 Particle size analysis table for nanoemulgel

Sr No	API	Oil	Surfactant	CoS-A	CoS-B	Aqueous Phase	Particle Size
2A	100 mg	Triacetin 0.300gm	Labrasol 0.900gm	Transcutol 0.200gm	Tween 80 0.200gm	Water 2.4gm	D90: 750 nm
2B	100 mg	Triacetin 0.300gm	Labrasol 0.900gm	Transcutol 0.200gm	Tween 80 0.200gm	Water 2.4gm	D90: 654 nm
10 A	125 mg	Triacetin 0.400gm	Labrasol 0.700gm	Transcutol 0.300gm	Tween 80 0.200gm	Water 2.4gm	D90: 956 nm
10 B	125 mg	Triacetin 0.400gm	Labrasol 0.700gm	Transcutol 0.300gm	Tween 80 0.200gm	Water 2.4gm	D90: 1020 nm

6.3.5 Viscosity Measurement:

Table 31 Viscosity meaasurment data

Sr. No.	Batch No.	Viscosity
1.	2A	38 cps
2.	2B	42 cps

3.	10A	34 cps
4.	10B	36 cps

6.3.6 Physical Appearance:

Prepared Nanoemulgel is milky in appearance.

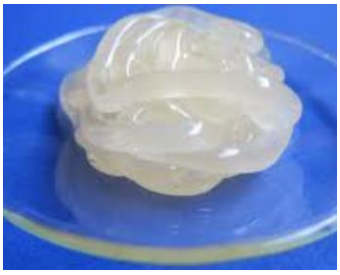


Figure 20 Physical appearance of Nanoemulgel

7. Conclusion

Dapsone in Nanoemulgel for treatment of Acne:

Dapsone widely used to treat acne topically. So Dapsone is selected for the formulating Nanoemulgel. Mostly in acne treatment topical products are used because of its ability to give local effect. Also topical products have some benefits like avoid first pass metabolism, no GI side effects, local action, etc.

The preliminary study shows Dapsone powder is in crystalline form and it has a solubility in very less oil phase and this are clove oil and triacetin. Solubility study also perform for the selection of surfactant and co-surfactant. Based on solubility study primary batches of dapsone was taken with different oil and surfactant combination with aim of preparing a stable and clear batch.

Then two combination of oil and surfactant were selected, one of them is Triacetin & Transcutol and second is Triacetin & capryol 90. From them, Triacetin and Transcutol was selected because they gave stable and clear emulsion.

Currently Dapsone gel available in two different ratio 5% and 7.5%. To get maximum drug loading, numbers of experiments were performed and the results shows 3.2% is the maximum drug that bear the Nanoemulsion system. On trying to add further drug emulsion would break. Also from results of performed experiments three different ratios of S_{mix} (9:4, 7:5, 2:1) was selected for performing ternary phase study.

Nanoemulgel was simply prepare by adding 1% Carbopol 934 gel into previously prepared Nanoemulsion which gives good results.

Pending Work:

1. Performing a phase diagram
2. Perform In-vitro release stud

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