"DESIGN, DEVELOPMENT & CHARACTERIZATON OF MICROEMULSION BASED GEL FOR THE TREATMENT OF ACNE"

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL TECHNOLOGY &

BIOPHARMACEUTICS

BY

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CERTIFICATE

This is to certify that the dissertation work entitled "Design, Development & Characterizaton of Microemulsion Based Gel for The Treatment of Acne" submitted by Mr. Shah Santur Tushar with Regn. No. (12MPH113) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Technology and Biopharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under 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.

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DECLARATION

I hereby declare that the dissertation entitled "Design, Development & Characterization of Microemulsion Based Gel for The Treatment of Acne", is based on the original work carried out by me under the guidance of Prof. Tejal A. Mehta, Professor & Head, 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.

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Mr. Santur T. Shah

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List of Abbreviations

Short Name	Abbreviation
IP	Indian Pharmacopoeia
BP	British Pharmacopoeia
USP	United States Pharmacopoeia
UV	Ultra Violet
NaCl	Sodium Chloride
KBr	Potassium Bromide
Conc.	Concentration
^{0}C	Degree Centigrade
%CDR	Percentage Cumulative Drug Release
µg	Microgram
SD	Standard Deviation
Avg.	Average
cPs	Centi poise
nm	Nanometers
g	Gram
cm	Centimeter
Hr.	Hour
Mins.	Minutes
FTIR	Fourier Transform Infrared Microscopy
λ_{max}	Absorbance maxima
ME	Microemulsion
MBG	Microemulsion Based Gel

"Design, Development and Characterization of Microemulsion Based Gel for the Treatment of Acne"

Abstract

The purpose of this study was to develop microemulsion based hydrogel formulation for topical delivery of Dapsone (MBG) with an objective to increase the drug solubility and its skin permeation. Triacetin was screened as the oil phase for microemulsion (ME), because of its good solubilizing capacity. The pseudo-ternary phase diagrams for microemulsion region was constructed using Triacetin (oil), Tween 80 (surfactant) and Labrasol (cosurfactant). D-Optimal mixture experimental design was adopted to optimize the amount of oil (X1), amount of Smix (mixture of surfactant and cosurfactant) (X2) and amount of water (X3) in the ME. The design batches were assessed for globule size (nm) (Y1) and solubility of Dapsone in microemulsion (ME) (mg/ml) (Y2). The microemulsion consisting of 5% oil, 64.17% Smix and 30.83% water was selected as the optimized batch of Dalsone loaded ME. The globule size and solubility of Dapsone for optimized DME were 13.20 nm and 173.10 mg/ml respectively. Transmission electron microscopy analysis of optimized batch confirmed the size and sphericity of ME globules. Further, Carbomer 934 was used to convert ME into gel form, without affecting its characteristics, for improving the viscosity of ME to improve its topical applicability. In-vitro permeation study of Dapsone from Dapsone loaded ME, MBG of Dapsone and control gel had shown 88.78%, 81.78% and 58.12% respectively after 8 h. The skin irritation study on rabbit model revealed that MBG of dapsone was safer as compared to Dapsone loaded ME because of its least/no erythema or edema and slight skin irritation in comparison to DME. Thus, it was concluded that developed MBG of Dapsone could be a promising formulation for effective treatment of acne compared to conventional formulations.

AIM AND OBJECTIVE OF PRESENT INVESTIGATION

1. Aim & objective of present work:

Dapsone (4, 4′-diaminodiphenylsulfone) is a drug of the sulfone class and having wide medical applications for more than 7 decades. A unique property of Dapsone is that it has dual therapeutic activity and demonstrates antimicrobial and anti-inflammatory properties. Since 1943, it has been the drug of choice in the treatment of leprosy although recently, due to its dual activity, it is used in acne especially for patients exhibiting sensitivities or intolerance to conventional anti-acne agents. It has bacteriostatic action by inhibition of bacterial dihydropterase synthase in the folic acid metabolic pathway and anti-inflammatory actions due to inhibition of neutrophil myeloperoxidase and eosinophil peroxidase activity, suppression of hypochlorous acid production, scavenging of reactive oxygen species, suppression of neutrophil activity, and inhibition of chemoattractant-induced signal transduction. [1]

Despite its obvious therapeutic efficacy in acne, clinical use of Dapsone is limited due to its physicochemical properties and side effects associated with oral route. Dapsone is a BCS class-II drug.[2] This characteristic is unfavorable for the production of Dapsone formulations with adequate bioavailability. Oral administration of Dapsone is associated with several adverse effects, including hemolytic anaemia, peripheral neuropathy, nausea, and headache. These side effects diminish its feasibility for treating skin diseases by the oral route. Many of the adverse effects of Dapsone are related to the production of metabolites. In the liver, Dapsone is acetylated by N-acetyltransferase which produces monoacetyl Dapsone, and upon enzymatic hydroxylation, Dapsone hydroxylamine is produced, which is primarily responsible for the development of adverse effects.[3]

Because of the therapeutic relevance of Dapsone, it is desirable to reduce its adverse effects using nanotechnology. An unexplored therapeutic approach for administration of Dapsone is the topical route. Utilization of topical applications provides an efficient strategy for the treatment of acne.[1]

Acne vulgaris is a multifactorial disease affecting the pilosebaceous follicle and characterised by comedomes, papules, pustules, nodules, and scars. Follicular

keratinisation, seborrhoea, and colonisation of the pilosebaceous unit with Propionibacterium acnes are fundamental to the development of lesions.[4] Furthermore, stratum corneum of the skin has remarkable barrier properties which block entry of most topically applied drugs, this poses a significant challenge to administering medications via the skin either for local cutaneous effects or as systemic therapy following their entry into superficial dermal capillaries.[5]

Among the various colloidal nanosystems available, microemulsions are notable because they offer recognized advantages by increasing the solubility of hydrophobic drugs such as Dapsone. Microemulsions can be administered by various routes, but topical application has gained prominence due to its advantages. Microemulsions are thermodynamically stable compared with traditional formulations such as creams and ointments, and also have a various nanostructure ideal for skin permeation. Further, microemulsion systems form spontaneously, which facilitates industrial production and scaling.[6]

Thus, the aim of the present investigation was to design, develop & characterize of the microemulsion based hydrogel of Dapsone for the treatment of acne.

The objective of the present investigation was……..

- ✓ Improve solubility of the drug
- ✓ Improve skin permeability of the drug
- ✓ To reduce systemic toxicity of drug
- ✓ To deliver the drug to specific site
- ✓ Further stabilize the microemulsion, increase viscosity & retention capacity & avoid waste of scarce by incorporating into gel.

INTRODUCTION

2. Introduction

2.1 Introduction to Skin

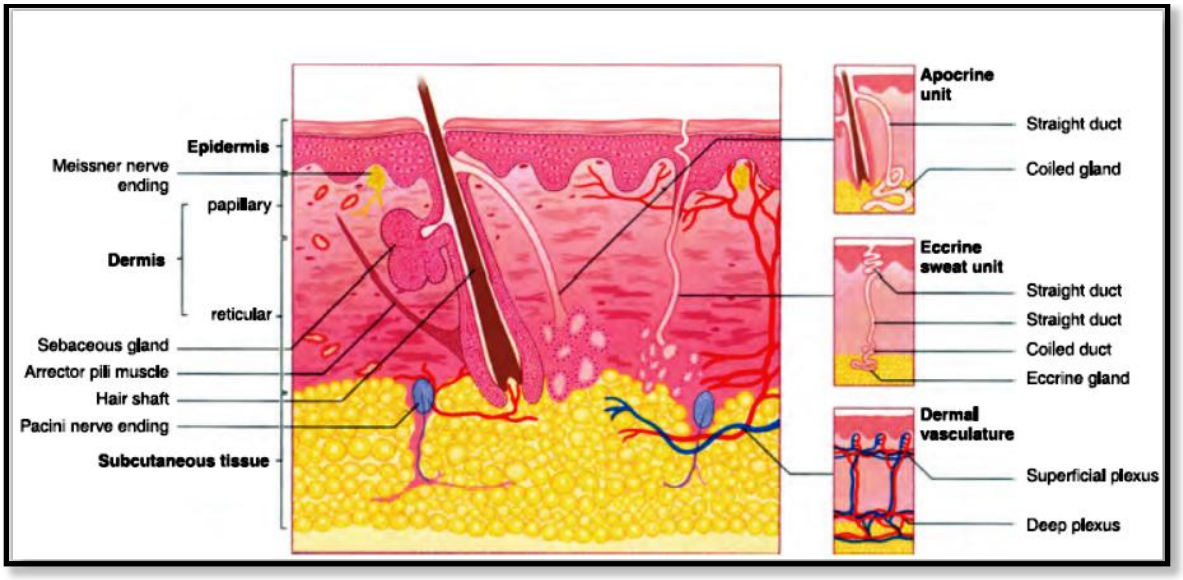


Figure 2.1Skin structure and function[7]

The integument (from the Latin integere, meaning to cover) is often said to be the largest organ in the body, comprising 16% of total body weight. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation etc. So an understanding of the anatomy and physiology of skin is fundamental. The skin is composed of three layers: the epidermis, the dermis, and subcutaneous tissue. The outer most level, the epidermis, consists of a specific constellation of cells known as keratinocytes, which function to synthesize keratin, a long, threadlike protein with a protective role. The middle layer, the dermis, is fundamentally made up of the fibrillar structural protein known as collagen. The dermis lies on the subcutaneous tissue, or panniculus, which contains small lobes of fat cells known as lipocytes. The thickness of these layers varies considerably, depending on the geographic location on the anatomy of the body. The eyelid, for example, has the thinnest layer of the epidermis, measuring less than 0.1 mm, whereas the palms and soles of the

feet have the thickest epidermal layer, measuring approximately 1.5 mm. The dermis is thickest on the back, where it is 30-40 times as thick as the overlying epidermis.

1) Epidermis:

The skin consists of two layers, the epidermis and the dermis. Epidermis is a terminally differentiated stratified squamous epithelium, the major cell type of which is the keratinocyte. Keratinocytes synthesize keratin, a protein-containing coiled polypeptide chains which combine to formsupercoils of several polypeptides linked by disulphide bonds between adjacent cysteine amino acids. Keratinocytes also produce cytokines in response to injury. The epidermis may be divided into four layers (Figure 2.1).

- *Stratum basale (basal cell layer):* This layer is generally only one cell thick, but in glabrous skin and hyperproliferative epidermis it can be two to three cells thick. The main cell type is the keratinocyte that may be dividing or non-dividing. Melanocytes are present in the basal layer and make up 5-10% of the cell population.
- *Stratum spinosum (spinous or prickle cell layer):* Basal cells move towards the surface and form a layer of polyhedral cells which are connected by desmosomes. These are the'prickles' seen under the microscope. Within this layer Langerhans cells can be identified.
- *Stratum granulosum (Granular cell layer):* Keratinocytes in the granular layer contain intracellular granules of keratohyalin. The cytoplasm also contains smaller lamellated granules (Odland bodies). The cells discharge their lipid components into the intercellular space which plays an important role in barrier function and intercellular cohesion within the stratum corneum.
- *Stratum corneum (horny layer):* This is the outermost layer of the epidermis. It is comprised of cells that have migrated from the stratum granulosum. The cells (now called corneocytes) have lost their nuclei and cytoplasmic organelles. The cells appear flattened and the keratin filaments align into disulphide cross-linked macrofibres. This layer may be several cells thick on the palms and the soles, but is less thick elsewhere. In palmoplantar skin there is an additional zone, the stratum lucidum. The cells found in this layer are still nucleated and are termed

transitional cells. The time from cell division to shedding from the horny layer is approximately 28 days, but this can be altered in various disease processes.

2) Dermis

The dermis is bounded externally by its junction with the epidermis and internally by subcutaneous fat. The dermis varies widely in its thickness being less than 1 mm thick on the eyelids but over 5 mm on the back. The dermis is a tough, resilient layer that protects the body against mechanical injury and contains specialized structures. The papillary dermis is the thin upper layer of the dermis. This lies below and interdigitates with the epidermal rete ridges. Deeper to this is the reticular dermis. Being connective tissue, the dermis contains cells, ground substance and fibres. The ground substance consists of polysaccharides and proteins which interact to produce hygroscopic proteoglycan macromolecules. The cells are fibroblasts that synthesize collagen and elastin fibres. Collagen represents 75% of the dry weight and up to 30%of the volume of the dermis. Seventy five percent is type I collagen and 15% type III collagen. The properties of collagen change both qualitatively and quantitatively with ageing. Elastin fibres are also present within the dermis and these provide a degree of elasticity to the skin.

- **Nails**

Nails serve to protect the fingertips, improve tactile sensation and are useful for scratching the skin. The nail itself is more correctly termed the nail plate and is made of keratin. It arises from under the nail fold and grows along the nail bed. It is bordered by the paronychium and ends at the hyponychium. The hyponychium contains the highest density of dermal lymphatics of anywhere in the body. The germinal matrix of the proximal nail bed produces 90% of the nail plate, the remainder being produced by the sterile matrix of the distal part which provides adherence for the nail. Fingernails grow at an average rate of 0.1 mm per day, two to three times faster than the rate of toenail growth.[8]

- **Hair Follicles:**

Hair follicles vary considerably in size and shape, depending on their location, but they all have the same basic structure. The number and distribution of hair follicles over the body and the future phenotype of each hair is established during fetal development; no

extra follicles are added after birth. The basophilic cells in the basal cell layer of the epidermis overlying mesenchymal cell sites are induced to grow at a downward angle into the dermis. The follicle continues to develop until finally widening at the base and forming a bulb around the group of mesenchymal cells from which the dermal papilla is formed.

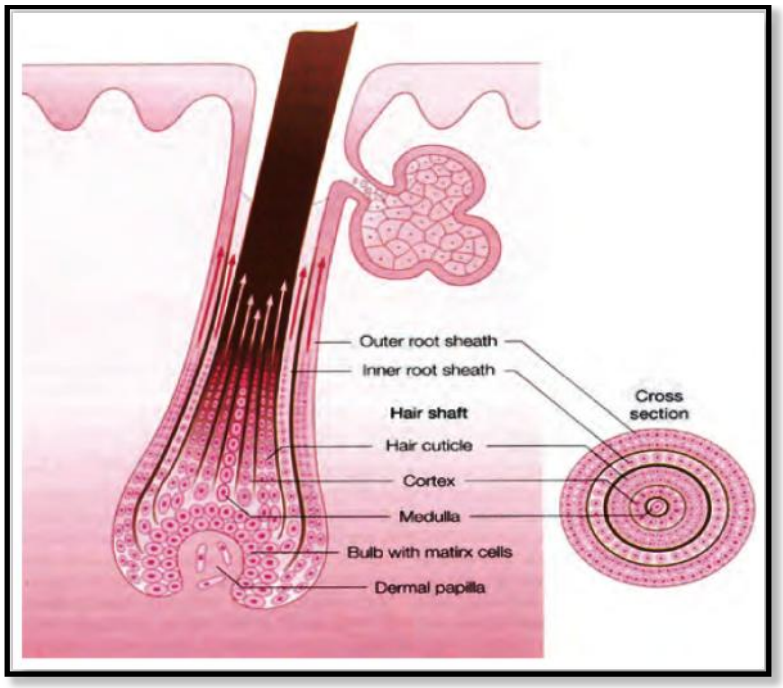


Figure 2.2Hair Follicle Structure[9]

Differentiation at the lower portion of the hair follicle forms the hair cone and later the hair, the cuticle, and the two inner root sheaths and in the upper segments of the follicle producing the hair canal in the upper dermis through the epidermis. The sebaceous gland forms from a bud in the fetal hair follicle. Along the same side of the follicle but below the sebaceous gland, another bud develops into an attachment for the arrector pili muscle which are a smooth muscle bundle that attaches to the external root sheath of the follicle. The region of the follicle above the sebaceous gland is known as the infundibular segment, and the region between the sebaceous duct and AP attachment is known as the isthmus. The region below the isthmus is known as the inferior portion which undergoes

cycles of involution and regeneration throughout life, whereas the infundibular and isthmus portions remain permanently. Rapidly proliferating cells in the hair bulb, called matrix cells, are responsible for the production of the hair shaft as well as the inner and outer root sheaths. Hair color is determined by the distribution of melanosomes in the hair shaft.

Hair growth occurs in a cyclical manner, but each follicle functions as an independent unit. There are three phases of hair growth. In anagen, hair actively grows. Anagen lasts approximately 1000 days in men and 2-5 years longer in women. In catagen, the hair follicle degenerates, a process taking 2-3 weeks. In telogen, the hair is shed and the follicle enters a resting phase that lasts 3-4 months. Ten percent of hairs are usually in this phase leading to 50-100 hairs falling out on a daily basis.

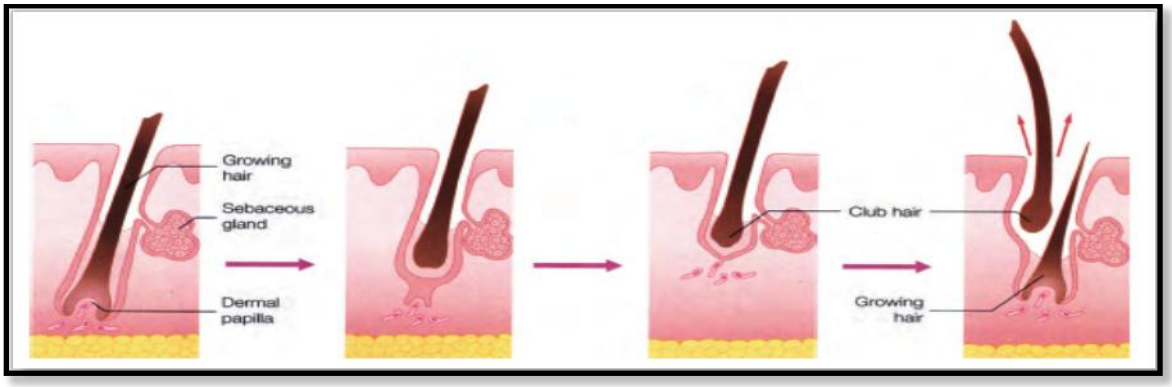


Figure 2.3Phases of Hair Growth[9]

- **Sebaceous Gland:**

  The human sebaceous gland is a multiacinar, holocrine-secreting tissue present in all areas of the skin, found in greatest number on the face and scalp but are present on nearly all other locations of the body with the exception of the tarsal plate of the eyelids, the buccal mucosa and vermilion borders of the lip, the prepuce and mucosa lateral to the penile frenulum, the labia minora, and the female areola. The number of sebaceous glands remains approximately constant throughout life, whereas their size tends to increase with age. Cells of the sebaceous glands contain abundant lipid droplets known as sebum in their cytoplasm and are arranged into lobules off the

upper segment of the hair follicle. Basaloid germinative cells surrounding the lobule give rise to the lipid-filled cells, which are then expelled into the infundibular segment of the hair follicle via the sebaceous duct.[9]

❖ **Functions of the Skin:**

1) Protection:
   - ✓ Physical Barrier: The stratum corneum of the epidermis is relatively impermeable provides the protection from the environment due to the keratinocytes are arranged in a scaffold-like lattice, bound together by the fibrous protein keratohyalin and a histidine-rich protein involucrin. Secondly, the intercellular spaces are filled with a lipid-rich matrix arranged in a laminar fashion providing a robust and waterproofing barrier.
   - ✓ Immune functions: The skin functions as a first line of defence against invading microorganisms. The mechanisms by which it is able to do this include the production of anti-microbial peptides, resident epidermal Langerhans cells, and transient epidermal T-cells. In addition, the dryness of the outer layer of the epidermis and the continual shedding of keratinocytes assists in preventing any sustained growth of organisms on the skin.
   - ✓ Ultraviolet Radiation: Ultraviolet radiation is composed of electromagnetic energy with wavelengths from 400 nm to 200 nm. The skin functions as a protective layer for UV radiation in two ways. The stratum corneum reflects radiation, so reducing the exposure dose. Sun exposure increases the activity of melanocytes, the number of melanosomes produced and the rate of transfer of melanin to the epidermal keratinocytes. This helps to decrease absorption of UV radiation by DNA and cellular constituents.

2) Sensation:
   - ✓ Cutaneous innervation is highly complex and is involved in perception of external stimuli, thermoregulation and sociosexual communication. Sensory afferent modalities include touch, vibration, temperature, pressure, pain and itch. Various receptors detect and transmit stimuli to the central nervous system.
   - ✓ For Example, In hairless (glabrous) skin, Meissner's corpuscles detect changes in light touch and vibration, Merkel cell receptors detect light touch and sustained

pressure; In the deep dermis and subcutaneous fat, Pacinian corpuscles detect pressure and vibration changes and Ruffini receptors detect skin stretch and contribute to joint position sense.

- ✓ Pain receptors are free nerve endings and are polymodal in their function, that is they can detect a number of stimuli.
- ✓ Itch is a complex sensation that is poorly understood. It can be defined as a sensation that produces the urge to scratch and is mediated by c-fibres.
- ✓ Thermoreceptors exist for cold and warmth as free nerveendings, irregularly distributed in the skin.

3) Skin circulation:

- ✓ Anangiosomeis a composite block of tissue with overlying skin that is supplied by an underlying source artery and associated draining veins. The body is composed of numerous angiosomes that fit together like a jigsaw. The angiosomes are linked by communicating vessels (choke arteries and oscillating veins) that allow blood flow between them under certain circumstances. The blood supply of the dermis far outstrips that which is required for its nutritional needs.[8]

2.2 Introduction to Acne:

2.2.1 Introduction:

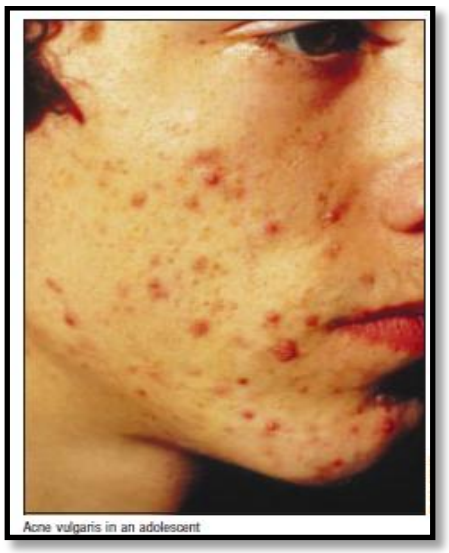


Figure 2.4Acne Vulgaris in adolescent

- Acne vulgaris, one of the most common skin disorders, is the result of a chronic inflammation of a sebaceous follicle and is characterized by tender inflammatory papules and nodules mainly scattered on the face, chest, and upper back.
- It may be caused by cutaneous micro-organisms such as *Propioni- bacterium acnes* (*P. acnes*) and usually appears in adolescence and early adulthood. *P. acnes* is a gram-positive and propionic acid-producing bacterium that colonizes anaerobically within the hair follicles of the skin.[10]

2.2.2 Pathogenesis:

- Acne is described as a uniform disorder in many dermatology textbooks, as it onsets in the adolescence period. It can also start in any post-adolescence period.
- There are four processes in the pathogenesis of acne:
  1. Increased sebum production;
  2. Perifollicular hyperkeratinization and follicular obstruction;
  3. Colonization with Propionibacterium acnes;
  4. Release of enzymes which induce humoral and cell mediated inflammations.[11]

- In lesion initiation, abnormal proliferation and differentiation leads to the occurrence of microcomedone in the initial lesion. This is followed by...

  (1) the accumulation of sebum in the follicle lumen, causing a plug, either open or closed, of a clinical comedone;

  (2) inflammatory components leaking from a follicle to the dermis. An acne lesion thus forms. A patient's sensitivity can also affect the initiation of acne lesion.
- Sebum production depends on local androgen levels and androgen sensitivity. Two subtypes of 5-alpha-reductase convert testosterone to the more active dihydrotestosterone (DHT): type 1 isozyme is expressed in the scalp, chest and sebaceous glands, whereas type 2 isozyme is expressed in genitourinary tissue, dermal papillae and hair follicles. DHT stimulates sebum production by sebocytes.
- There are several molecular cues that cause the progression of acne virulence. One is the presence of Christie, Atkins, Munch-Peterson (CAMP) factor of *P. acnes*, a secretory protein with its co-hemolytic activity of the host acid sphingomyelinase (ASMase). These two, CAMP and ASMase can be utilized for the development of drugs to inhibit the progressionof acne or even eradicate bacterial overgrowth.The synergistic lysis of erythrocytes via the CAMP reaction has been found in *P. acnes.* The CAMP reaction was originally described as a synergistic lysis of sheep erythrocytes by *Staphylococcus aureus* sphingomyelinase C and CAMP factor (extracellular protein) produced by some streptococcal species. The constituents of the plasma membrane, sphingomyelin and phospholipids, are first hydrolyzed by the enzyme, followed by cell lysis. One cause acne virulence is sialidase, a bacterial cell-wall anchoring factor produced by *P. acnes*. It can catalyze the hydrolysis of sialic acid from the surface of mammalian cellsand lead to cell death. Looking at the micro-environment of acne lesions, e.g.,free fatty acids hydrolyzed by the gene product, *P. acnes*lipase plays a role in the colonization of bacteria in sebaceous follicles. . Other inflammatory reactions localized in the acne lesion include chemoattractant molecules that recruit polymorphonuclear leukocytes and lymphocytes, the production of the inflammatory cytokines, and the complement activation.
- The pathogenesis of acne can also be triggered through the toll-like receptor 2 (TLR2), which regulates many immune response genes. *P.acnes*activates the pilosebaceous unit and induces the production of IL-12 and IL-8 of monocytes via the

TLR2 pathway. TLR2 and TLR4 expression is increased in the acne lesions of the epidermis. This inflammation, in turn, can lead to hyperproliferation of the ductal epidermis. IL-8 can recruit neutrophils to the pilosebaceuous unit, in which degradative enzymes lead to the rapture of the follicular epithelium. This leads to the clinical symptoms of the acne.[12]

2.2.3 Diagnosis and Treatment of Acne:

2.2.3.1 Diagnosis:

- Acne most commonly affects the face, especially the T-zone, and trunk. Non-inflammatory lesions include open, "black" comedones (papules with central darkened impaction) and closed, "white" comedones (flat, pale papules). Inflammatory lesions include papules, pustules and nodules (Figure 2.5).

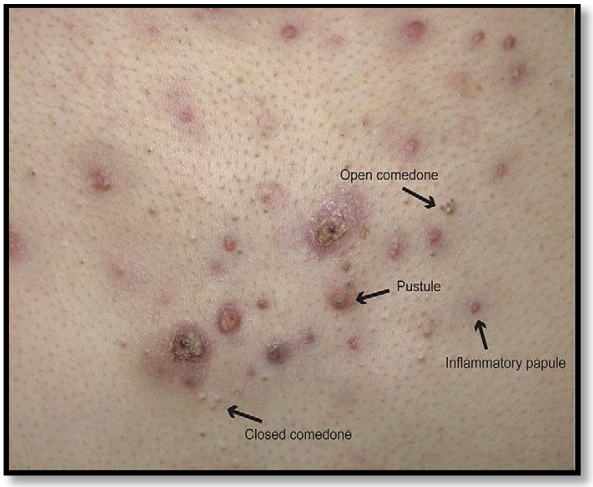


Figure 2.5Comedones, papules and pustules in mixed comedonal and inflammatory acne

- Secondary bacterial infection (e.g. Staphylococcal aureus) may rarely increase crusting and inflammation. There are five main types of scars. Ice-pick scars are narrow, tapering deeply into the dermis. Rolling scars are wide and shallow. Boxcar scars are well demarcated, punched out depressions (Figure 2.6).
- Hypertrophic and keloid scars are raised, the latter extending beyond the area of original inflammation. Perifollicular elastolysis presents as truncal follicular atrophy. Despite inter-observer and intra-observer variability, grading the severity of acne can be useful for monitoring response to therapy. The Leeds-Cunliffe technique is a facial

photo-numeric scale that assigns a number from a manual to the patient's presentation. Serial photography is also helpful. Psychological assessment is pertinent for those with severe scarring, acne excoriee and dysmorphophobia.

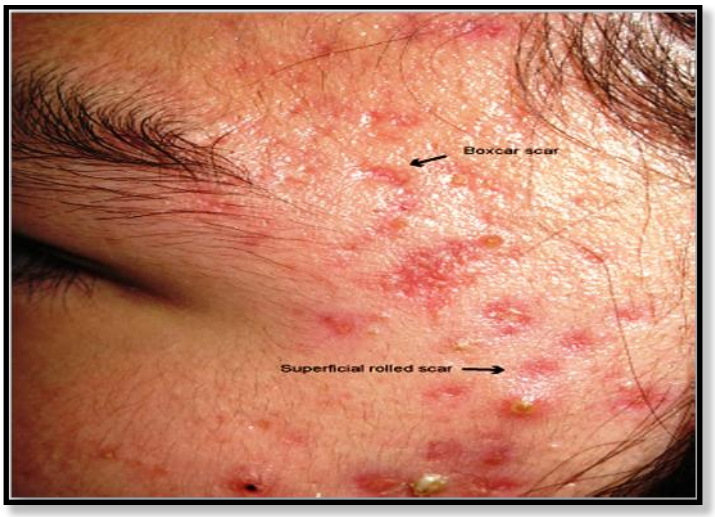


Figure 2.6Common Acne Scars

- Signs of possible hyperandrogenism include, (1) late onset, severe acne, (2) marked seborrhoea, (3) acanthosis nigricans (HAIR-AN syndrome), (4) dysmenorrhoea and infertility (polycystic ovary syndrome), (5) dyslipidaemia and diabetes (HAIR-AN, PCOS) and (6) Cushingoid habitus (Cushing's syndrome).[11]
- In 1990, the American Academy of Dermatology developed a classification scheme for primary acne vulgaris. This grading scale delineates three levels of acne:

  - ✓ Mild: characterized by the presence of few to several papules and pustules, but no nodules.
  - ✓ Moderate: Patients with moderate acne have several to many papules and pustules, along with a few to several nodules.
  - ✓ Severe: patients have numerous or extensive papules and pustules, as well as many nodules.[13]

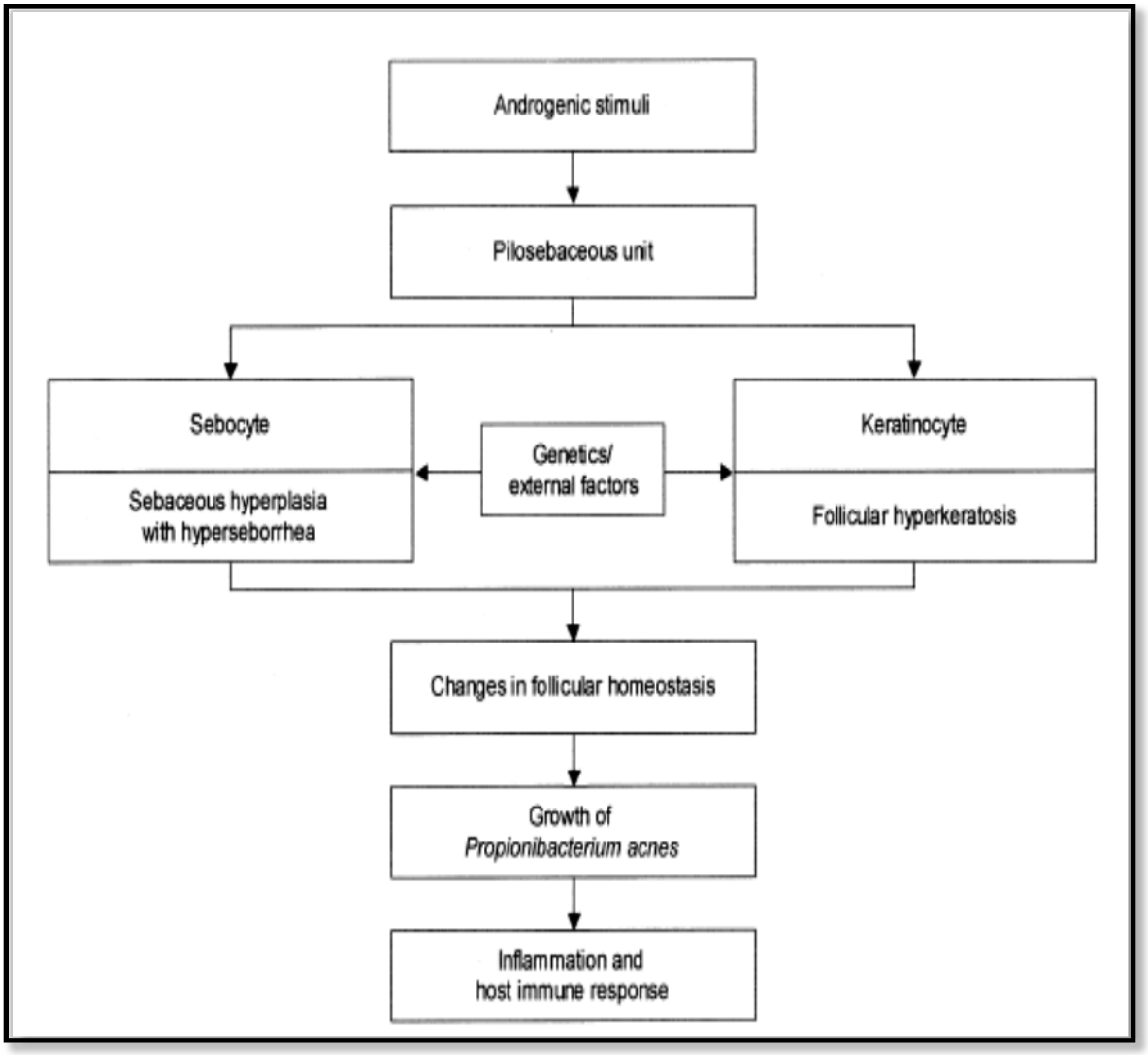


Figure 2.7Acne Treatment Algorithm[13]

2.2.3.2 Treatment:

Successful management of acne needs careful selection of anti-acne agents according to clinical presentation and individual patient needs.

a) Topical Therapy:

Topical therapy is useful in mild and moderate acne, as monotherapy, in combination and also as maintenance therapy.

i. Benzyl Peroxide:

- ✓ Benzoyl peroxide is a broad spectrum bactericidal agent which is effective due to its oxidizing activity. The drug has an anti-inflammatory, keratolytic, and comedolytic activities, and is indicated in mild-tomoderate acne vulgaris. The

main limitation of benzoyl peroxide is concentration dependent cutaneous irritation or dryness and bleaching of clothes, hair, and bed linen. Mostly subsides with continued use.

ii. Topical Retinoids:

- ✓ Tretinoin, adapalene, tazarotene, isotretinoin, metretinide, retinaldehyde, and β-retinoyl glucuronide are currently available topical retinoids.
- ✓ Topical retinoids target the microcomedo–precursor lesion of acne. There is now consensus that topical retinoid should be used as the first-line therapy, alone or in combination, for mild-to-moderate inflammatory acne and is also a preferred agent for maintenance therapy.
- ✓ Its effectiveness is well documented, as it targets the abnormal follicular epithelial hyperproliferation, reduces follicular plugging and reduces microcomedones and both noninflammatory and inflammatory acne lesions.
- ✓ The main adverse effects with topical retinoid is primary irritant dermatitis, which can present as erythema, scaling, burning sensation and can vary depending on skin type, sensitivity, and formulations.

iii. Topical Antibiotics:

- ✓ Topical antibiotics such as erythromycin and clindamycin are the most popular in the management of acne and available in a variety of vehicles and packaging.
- ✓ They inhibit the growth of *P. acne* and reduce inflammation. Clindamycin and erythromycin were both effective against inflammatory acne in topical form in combination of 1–4% with or without the addition of zinc.
- ✓ Side effects though minor includes erythema, peeling, itching, dryness, and burning, pseudomembranous colitis which is rare, but has been reported with clindamycin.
- ✓ A most important side effect of topical antibiotics is the development of bacterial resistance and cross resistance; therefore, it should not be used as monotherapy.

iv. Newer Topical agents:

- Combination therapy:
  - ✓ Benzoyl peroxide has the advantage to prevent and eliminate the development of *P. acne* resistance. Therefore it is being more preferred as combination therapy. Its efficacy and tolerability are enhanced when combined with topical erythromycin or clindamycin, confirmed on various trials.
  - ✓ Benzoyl peroxide can be combined with tretinoin and found to be superior to monotherapy. Both the molecules should not be applied simultaneously as benzoyl peroxide may oxidize tretinoin.
  - ✓ A combination of topical retinoid and topical antimicrobial is more effective in reducing both inflammatory and noninflammatory acne lesions than either agent used alone.
- Salicylic acid: It has been used for many years in acne as a comedolytic agent, but is less potent than topical retinoid.
- *Azelaic acid:* It is available as 10–20% topical cream which has been shown to be effective in inflammatory and comedonal acne.
- *Dapsone gel 5%:* It is a sulfone with anti-inflammatory and antimicrobial properties. The trials have confirmed that topical dapsone gel 5% is effective and safe as monotherapy and in combination with other topical agents in mild-to-moderate acne vulgaris.

b) Systemic Therapy:

i. Systemic Antibiotics:

- ✓ Oral antibiotics are indicated in mainly moderate-to-severe inflammatory acne. Tetracyclines and derivatives still remain the first choice. Tetracycline (500 mg–1 g/day), doxycycline (50–200 mg/day), minocycline (50–200 mg/day), lymecycline (150–300 mg/day), erythromycin (500 mg–1 g/day), co-trimoxazole, trimethoprim, and recently azithromycin (500 mg thrice weekly) are being used successfully in acne.
- ✓ Gastrointestinal upset and vaginal candidiasis are most common side effects. Doxycycline can be associated with photosensitivity. Minocycline may produce pigment deposition in the skin, mucous membrane, and teeth. Autoimmune

hepatitis, systemic lupus erythematosus-like syndrome, and serum sickness-like reactions occur rarely with minocycline. Long-term therapy with oral antibiotic not only threat to resistant of *P. acne*, but also to coagulase negative staphylococci on the skin, *Staphylococcus aureus* in the nares, and streptococci in the oral cavity.

ii. Hormonal Therapy:

- ✓ It may be needed in female patients with severe seborrhoea, clinically apparent androgenetic alopecia, seborrhoea/acne/hirsuitism/alopecia (SAHA) syndrome, late-onset acne (acne tarda), and with proven ovarian or adrenal hyperandrogenism.

❖ Oral Contraceptives:

- ✓ Estrogen is commonly combined with progestin to avoid the risk of endometrial cancer. Anti-acne effect of oral contraceptive governed by decreasing level of circulatory androgens through inhibition of luteinizing hormones (LH) and follicle stimulating hormone (FSH).

❖ Spironolactone:

- ✓ They functions primarily as a steroidal androgen receptor blocker. It may cause hyperkalemia (when higher doses are prescribed or when there is cardiac or renal compromise), menstrual irregularities.

❖ Cyproterone Acetate:

- ✓ It is the first androgen receptor blocking agent to be well studied and found to effective in acne in females. It is also combined (2 mg) with ethinyl estradiol (35 or 50 μg) as an oral contraceptive formulation to treat acne.

❖ Flutamide:

- ✓ It is useful in acne when given in females with hirsuitism.

❖ Oral isotretinoin:

- ✓ Oral retinoid is indicated in severe, moderate-to-severe acne or lesser degree of acne producing physical or psychological scarring, unresponsive to adequate conventional therapy. It is the only drug that affects all four pathogenic factors implicated in the etiology of acne.

- ✓ Side effects include those of musculoskeletal, mucocutaneous, and ophthalmic systems, as well as headache, and central nervous system effects. Oral isotretinoin is a potent teratogen.

c) Physical Treatment:

i. Leison removal:

- ❖ Comedones:
- ✓ Both open and closed comedones can be removed mechanically with comedone extractor and a fine needle or a pointed blade. Preprocedure topical retinoid application makes the procedure easier. Gentle cautery and laser puncture of macrocomedones are also useful procedure.
- ✓ The limitations of comedo extraction include incomplete extraction, refilling, and the risk of tissue damage.
- ❖ Active deep inflammatory lesions:
- ✓ Aspiration of deep inflamed lesion may be needed in few cases which are followed by IL steroid injection in cysts and sinus tract.

ii. Photo therapy:

- ❖ Visible Lighr:
- ✓ They are indicated for mild-to-moderate inflammatory acne. *In vitro* and *in vivo* exposure of acne bacteria to 405–420 nm of ultraviolet free *blue light* results in the photo-destruction through the effect on the porphyrin produced naturally by *P. acne*.
- ✓ Use of limited spectrum wavelength, such as blue light (peak at 415 nm), and mixed blue and red light (peak at 415 and 660 nm) have been found to be effective in reducing acne lesions after 4–12 weeks.
- ❖ Photodynamic therapy:
- ✓ This includes pulsed dye laser (585 nm), also effective in acne.[10]

2.3 Topical dosage form:

Topical dosage forms are those which are applied to the skin. These preparation are applied to the skin either for their physical effects, that is for their ability to act as skin protectants, lubricants, emollients, drying agents, etc. or for their specific effect of

medicinal agents present. Preparations sold over the country frequently contain mixtures of medicinal substance used in the treatment of such condition as minor skin infection, itching, bruise, acne, psoriasis and eczema. Skin application, which require a prescription generally contain a single medicinal agent intended to counter a specific diagnosed condition. Topical dosage forms have been used since very ancient times. The application of medicinal substance to skin or to various body orifices is a concept as old as humanity. Various ointments, creams, gels, lotions, pastes, powders and plasters have been used for many years. The primary topical drug delivery system (TDDS) is that they could provide controlled constant administration of a medicament by simple application to the skin surface. The topical delivery has been attempted and made successful using a number of lipid based systems viz., vesicular systems, lipid, microsphere, lipid nanoparticles, lipid emulsion and polymeric gels.

Figure 2.8Various approaches to Topical Dosage forms[14]

2.3.1 Advantages of topical systems:

They are of least therapeutic interest but of practical relevance is good patient compliance. The systems are easy to apply and remove. It avoids risks and inconveniences associated with intravenous therapy.

- ✓ They eliminate the variables, which influences gastrointestinal absorption such as food intake, stomach emptying, intestinal motility and transit time.
- ✓ Produces sustained and controlled level of drug in plasma thus reduces the chance of over or under-dosing.
- ✓ Reduces frequency of drug dosing.
- ✓ Topical systems are easily retractable thereby termination of drug inaproxenut, if toxic effects are observed.
- ✓ Offers an alternative route when oral therapy is not possible as in case of nausea and vomiting.
- ✓ Helps in achievement of more constant blood levels with lower dosage of drug by continuous drug in aproxenut and by by-passing hepatic first-pass metabolism and consequent degradation.
- ✓ In certain circumstances, enzymatic transformation within epidermis may be used to improve permeability of certain hydrophilic drugs when applied to the skin in the form of prodrug.

2.3.2 Limitations of topical systems:

- ✓ Drugs with reasonable partition coefficient and possessing solubility both in oil and water are most ideal, as drug must diffuse through lipophilic stratum corneum and hydrophilic viable epidermis to reach the systemic circulation. Only drugs, which are effectively absorbed by the percutaneous routes as such or by using penetration promoters, can be considered.
- ✓ The route is not suitable for drugs that irritate or sensitize the skin.
- ✓ The route is restricted by the surface area of delivery system and the dose that needs to be administered in the chronic state of disease.

- ✓ Topical drug delivery systems are relatively expensive compared to conventional dosage forms. They may contain a large amount of drug, of which only a small percentage may be used during the application period. Apart from these limitations other problems include pharmacokinetics and pharmacodynamic restrictions. Thus clinical need has to be examined carefully before developing a TDDS.

2.4 Introduction to Emulsion:

2.4.1 Introduction:

Emulsion consists of two immiscible liquids (e.g. oil and water) that are brought together into one pseudo phase using surfactants. They are prepared using shearing force or shaking.[15] The O/W emulsion consists of oil droplets dispersed in water phase. Similarly, W/O emulsion consists of water droplets dispersed in the oil phase. The word "Emulsion" can be found in "Macroemulsion" as well as in "Microemulsion".[16] The differences between macroemulsion and microemulsion (Figure 8) are listed in Table 1.1.[17]

Table 2.1Difference between Microemulsion and Macroemulsion

Sr. No.	PROPERTY	MICROEMULSION	MACROEMULSION
1	Appearance	Transparent	Cloudy
2	Optical Isotropy	Isotropic	Anisotropic
3	Interfacial tension	Ultra low	High
4	Microstructure	Dynamic (interface is continuously and spontaneously	Staic
5	Droplet size	20-200 nm	> 500 nm
6	Stability	Thermodynamically stable, long shelf-life	Thermodynamically unstable (kinetically stable), will eventually phase separate
7	Phases	Monophasic	Biphasic

8	Preparation	Facile preparation, relatively lower cost for commercial production	Require a large input of energy, higher cost
9	Viscosity	Low viscosity	Higher viscosity

Figure 2.9Difference between Microemulsion and Macroemulsion

2.4.2 Types of emulsion:

Emulsions can be commonly classified as water-in-oil (W/O) emulsion or oil-in-water (O/W) emulsion. Generally, hydrophilic surfactant forms O/W emulsion easily and hydrophobic surfactant is likely to form W/O emulsion.

Double emulsions are "emulsions of emulsions". The droplets of double emulsions contain a number of inner droplets and are much greater in size as compared with the droplets of single emulsions. Two main types of double emulsions are: O/W/O emulsions and W/O/W emulsions.

Bicontinuous microemulsions consist of a net structure which is twined by oil, water, and surfactants.[16]

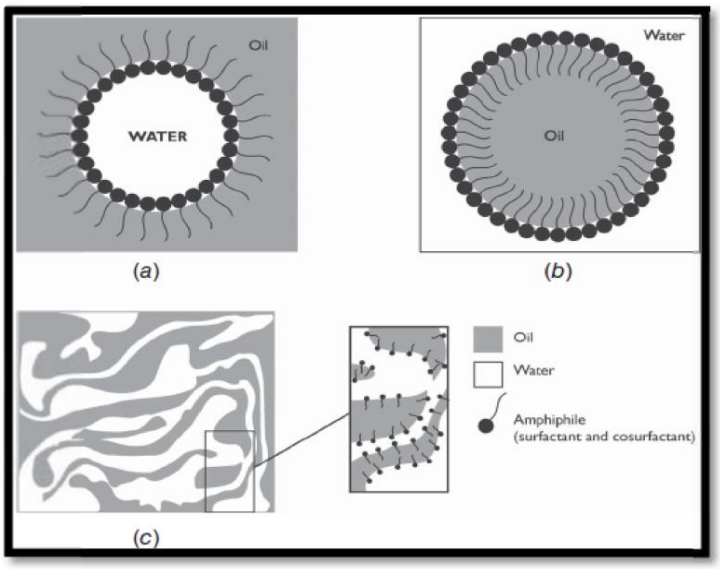


Figure 2.10Diagrammatic representation of different types of ME systems: (a) w/o ME, (b) o/w ME, (c) water-and-oil bicontinuous ME[18]

2.4.3 Microemulsion

2.4.3.1 *Microemulsion as drug delivery vehicle*

Microemulsions have unique physical properties. They are composed of water, oil and a mixture of surfactants making a homogeneous, optically isotropic and thermodynamically stable solution. Microemulsions can be sterilized by filtration and their production is relatively simple and inexpensive. Because of these properties, they have attracted a great interest as drug delivery vehicles. Microemulsions can be applied as liquid membrane carriers to transport lipophilic substances through an aqueous medium or to carry hydrophilic substances across lipoidal medium. They are proposed for oral, topical, dermal, transdermal, parentenal and pulmonary administration of drugs. Although microemulsions have been known for a long period, their potential as vehicles for topical ocular drug delivery has been investigated only within the last decade. Preparing a pharmaceutical acceptable dosage form demands a clear understanding of the microemulsion structure, phase behavior, factors leading to its thermodynamic stability, factors influencing drug release from the formulation, requirements of ideal microemulsion excipients, and the potential uses and limitations of the microemulsion system.[19]

2.4.3.2 Microemulsions vs. nanoemulsions:

The main difference between microemulsions and nanoemulsions is that microemulsions are self-assembling nano-scale emulsions whereas nanoemulsions are nano-scale emulsions formed by energy input, generally from mechanical devices or from the chemical potential of the components. Microemulsions are isotropic solutions of oil and water and are prepared using a high surfactant concentration of around 40 % under gentle stirring or shaking. Microemulsions form spontaneously without mechanical shear. An extremely high concentration of surfactants ensures self-assembling with particle size at the nano-scale level. Nano-emulsion generating processes are divided into two groups. The first includes the 'high-energy' processes which use high mechanical shear to reach very small droplet sizes, whereas the second 'low-energy' process benefits from the intrinsic physico-chemical properties of surfactants for generating nanoemulsions.

Unlike microemulsions, nanoemulsions are thermodynamically unstable systems as the interfacial tension between oil and water phase is high. It has high kinetic stability against creaming or sedimentation and a large interfacial area.[20]

2.4.3.3 Theories of microemulsion formulation

Three different approaches have been proposed to explain microemulsion formation and the stability aspects. However, no single theory explains all aspects of microemulsion formation but each has its own significance in understanding of microemulsion formation. The important features of the microemulsion are thermodynamic stability, optical transparency, large overall interfacial area (about 100 m^2/mL), variety of structures like ordered droplets on lamellar mixtures with wide range of phase equilibria with excess oil/water phases, low interfacial tension and increased solubilization of oil/water dispersed phase. Microemulsion requires more surfactant than emulsion to stabilize a large overall interfacial area.

- **Thermodynamics of microemulsion**

The interfacial tension between the oil and water can be lowered by the addition and adsorption of surfactant. When the surfactant concentration is increased further, it lowers the interfacial tension till CMC (Critical Micelle Concentration), after which micelles are formed. This negative interfacial tension leads to a simultaneous and spontaneous increase in the area of the interface. The large interfacial area formed may divide

itself into a large number of closed shells around small droplets of either oil in water or water in oil and further decrease the free energy of the system. In many cases, the interfacial tension is not yet ultra low when the CMC is reached. It has been studied and observed by Schulman and workers that the addition of a co surfactant (medium sized alcohol or amine) to the system reduces the interfacial tension virtually to zero and further addition of a surfactant (where γ is zero) leads to negative interfacial tension.

i. Mixed Film theories

The relatively large entropy of mixing of droplets and continuous medium explains the spontaneous formation of microemulsion. Schulman (Hoar and Schulman, 1942; Schulman & Strokenius, 1959) emphasized the importance of the interfacial film. They considered that the spontaneous formation of microemulsion droplets was due to the formation of a complex film at the oil-water interface by the surfactant and cosurfactant. This caused a reduction in oil-water interfacial tension to very low values (from close to zero to negative) which is represented by following equation.

$$\gamma_i = \gamma_{o/w}^{-\pi i}$$

Where, $\gamma_{0/w}$= Oil-water interfacial tension without the film present

$^{\pi i}$= Spreading pressure

γ_i=interfacial tension

❖ **Mechanism of curvature of a duplex film**

The interfacial film should be curved to form small droplets and to explain both the stability of the system and bending of the interface. A flat duplex film would be under stress because of the different tension and spreading of pressure on either side of it. The reduction of this tension gradient by equalizing the two surface pressures and tensions is the driving force for the film curvature. Both sides of the interface expand spontaneously with penetration of oil and co surfactant until the pressures become equal. The side with higher tension would be concave and would envelop the liquid on that side, making it an internal phase. It is generally easier to expand the oil side of an interface than

the waterside i.e. by penetration of the oil or co surfactant into the hydrocarbon chain area hence W/O microemulsion can be formed easily than O/W microemulsion.[21]

ii. Solubilization theories

The group of Shinoda considered microemulsion to be thermodynamically stable mono phasic solution of water-swollen (W/O) or oil swollen (O/W) spherical micelles. Rance and Friberg illustrated the relation between reverse micelles and W/O microemulsion with the help of phase diagrams. The inverse micelle region of ternary system i.e. water, pentanol and sodium dodecyl sulphate (SDS) is composed of water solubilized reverse micelles of SDS in pentanol. Addition of O-xylene up to 50% gives rise to transparent W/O region containing a maximum of 28% water with 5 % pentanol and 6% surfactant (i.e. microemulsions). The quaternary phase diagram constructed on adding p-xylene shows relationship of these areas to the isotropic inverse micellar phase. These four component systems could be prepared by adding hydrocarbon directly to the inverse micellar phase by titration. Thus the system mainly consists of swollen inverse micelle rather than small emulsion.

iii. Thermodynamic theories

The formation of a ME system can be explained using a simplified thermodynamic approach and with reference to the equation

$$\Delta G = \gamma\, \Delta A - T\Delta S$$

Where, ΔG = Free energy of ME formation

γ = Interfacial tension at oil-water interface

ΔA = Change in interfacial area (associated with reducing droplet size)

S = System entropy

T = Absolute temperature

The process of ME formation is associated with a reduction in droplet size, which results in an increase in the value of ΔA due to an overall increase in surface area that is associated with droplet size reduction. This is compensated by a very low interfacial

tension that is normally achieved by using relatively high amphiphile concentrations. Furthermore, the process of ME formation is accompanied by a favorable entropy contribution (increased value of ΔS) that is due to the mixing of the two immiscible phases, surfactant molecules partitioning in favour of the interface rather than the bulk and monomer-micelle surfactant exchange. The net outcome is a negative value for ΔG which translates into a spontaneous ME formation. Whether an o/w or w/o ME forms is dependent to a great extent on the volume fraction of oil and water as well as the nature of the interfacial film as reflected by the geometry of the amphiphile molecules forming the film. It follows that the presence of o/w ME droplets is more likely to happen in systems where the oil volume fraction is low, whereas w/o ME droplets form when the water volume fraction is low and oil is present in abundance. Interestingly, in systems containing comparable amounts of water and oil, a bicontinuous ME may exist (Figure 9 *c*). In such systems both oil and water exist as microdomains that are separated by an amphiphile - stabilized interface with a zero net curvature.[22]

iv. Theory of self-assembly of surfactant molecules

It is based solely on geometric considerations. Accordingly, if the volume of the surfactant is *v*, its head group surface area *a,* and its length *l*, it follows that when the critical packing parameter (CPP = *v/al)* has values between 0 and 1, o/w MEs are likely to be formed. On other hand, when CPP is greater than 1, w/o MEs are favoured. When using surfactants with critical packing parameters close to unity (CPP $\approx$ 1) and at approximately equal volumes of water and oil, the mean curvature of the interfacial film approaches zero and droplets may merge into a bi-continuous structure. The ratio of hydrophilic and hydrophobic groups of the surfactant molecules, that is, their hydrophile-lipophile balance (HLB), is also important in determining interfacial film curvature and consequently the structure of the ME. The HLB system has been used for the selection of surfactants to formulate MEs and accordingly the HLB of the candidate surfactant blend should match the required HLB of the oily component for a particular system. In brief, a match in the lipophilic part of the surfactant used with the oily component should be favourable.[23]

2.4.3.4 Phase Diagrams

- **Ternary systems**

The phase behaviour of surfactant-oil-water (S/O/W) is exactly presented by using ternary diagram. Here, two independent composition variables are sufficient, since third one is complement to 100% (Fig 2.10). The phase diagram allows one to determine ratio of oil: water, surfactant-cosurfactant at the boundary of microemulsion region. To plot the composition of four component systems, a regular tetrahedron composed by fixing and varying the other three or by using a constant ratio of two components (surfactant and cosurfactant or co solvent). Fig. 2.10 shows the pseudo ternary diagram at constant surfactant to cosurfactant ratio. It also shows that single phase or multiphase regions of microemulsion domain are near the centre of diagram in areas containing large amounts of surfactant that is toxic. The phase behavior of surfactants, which form microemulsions in absence of cosurfactant, can be completely represented by ternary diagram.

- **Winsor's regions**

Winsor (1954) reported the relationship between the phase behaviour of amphiphiles-oil-water and nature of the different components of ternary system. Different regions of a phase diagram are shown in Fig. 2.9

Figure 2.11Different regions of phase diagram

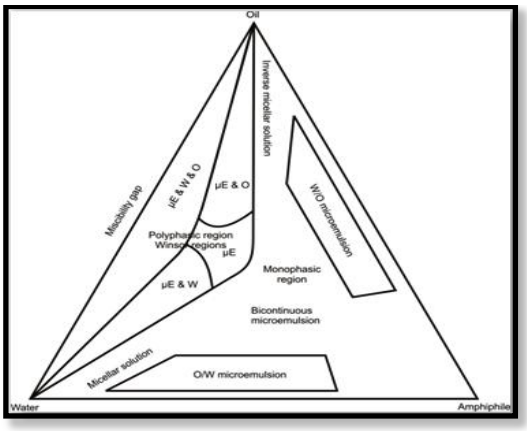
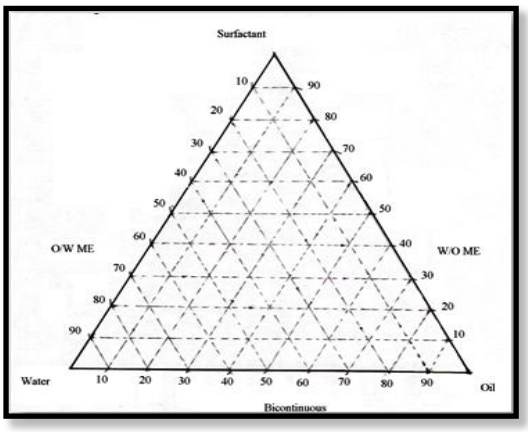


Figure 2.12Ternary system



***Winsor I*:** The microemulsion composition corresponding to Winsor I is characterized by two phase, the lower oil/water(O/W) microemulsion phase in equilibrium with excess oil.

Winsor II: The microemulsion composition corresponding to Winsor II is characterized by very low interfacial tension and maximal solubilization of oil and water for a given quantity of surfactant. Since, in this phase, microemulsion coexists with both excess phases and no one can distinguish the dispersed phase from the continuous phase.

***Winsor III*:** This phase comprises of three phases, middle microemulsion phase (O/W plus W/O, called bicontinuous) in equilibrium with upper excess oil and lower water.

***Winsor IV*:** Microemulsions can be distinguished from the micelles by its inner core swollen with oil. The microemulsion structure depends on the chemical composition, temperature and concentration of the constituents.

Different surfactants stabilize different microstructures, because of their aggregation pattern in a particular medium leading to a system with a minimum free energy and thermodynamically stable. Even though the spherical micelles are considered to have minimal water-hydrocarbon contact area for a given volume, the inter micellar free

energy and the impossibility of the existence of voids in the hydrophobic region leads to other amphiphillic assemblies like cylinders and planes. They are organized in the form of liquid crystalline phases or liquid isotropic phases. A wide variety of surfactant molecules obeys the geometric rules embodied in the packing parameter. In concentrated aqueous solutions, amphiphiles exist as: Lamellar phase with two configuration (planar and continuous lamellar phase), Hexagonal phase (surfactant molecules aggregate into circular cylinder micelles that pack onto the hexagonal lattice). Cubic phases, Nematic phases. In dilute solution they exist as a worm or thread like micelles, anomalous isotropic (sponge) structure and vesicles.

- **Quaternary Phase Diagrams**

Microemulsion is a type of quaternary system. To study their phase behavior, pseudo ternary phase diagram consisting of the oil-water amphiphiles is commonly drawn in which amphiphile surfactant/ cosurfactant ratio. Optimization was done by using pseudo ternary diagram which was not an accurate technique. Hence, it is better to use quaternary phase diagram for such system.[24]

2.4.3.5 Applications of microemulsions

During the last two decades, microemulsions have been extensively researched because of their tremendous potential in many applications. The role of microemulsions in drug delivery is major concern for pharmaceutical development.

- **Oral delivery**

Microemulsions have the potential to enhance the solubilization of the poorly soluble drugs and overcome the dissolution related bioavailability problems. This is particularly important for the BCS class II or class IV drugs. The successful formulation of such drugs is highly dependent on the performance of the formulated product. Microemulsions act as super solvent of these drugs and can be optimized to ensure consistent bioavailability. In addition, they can be used for the delivery of hydrophilic drugs including macromolecules such as proteins and peptides. This is due to the existence of polar, nonpolar and interfacial domains which allow encapsulation of drugs with varying solubility.

Moreover, these systems have been reported to protect the incorporated drugs against oxidation, enzymatic degradation and enhance the membrane permeability. Presently, Sandimmune Neoral® (Cyclosporine A), Fortovase® (Saquinavir), Norvir® (Ritonavir), etc. are the commercially available oral SMEDDS formulations.

- **Parenteral delivery**

The formulation of lipophilic and hydrophobic drugs into parenteral dosage forms has proven to be difficult. O/W microemulsions are beneficial in the parenteral delivery of sparingly soluble drugs where the administration of suspension is not desirable. They provide a means of obtaining relatively high concentration of these drugs which usually requires frequent administration. Other advantages are that they exhibit a higher physical stability in plasma than liposomes or other vesicles and the internal oil phase is more resistant against drug leaching. Several sparingly soluble drugs have been formulated into o/w microemulsion for parenteral drug delivery. Microemulsions can also be used as intravenous delivery systems for the fat soluble vitamins and lipids in parenteral nutrition.

- **Topical delivery**

Microemulsions have been reported to enhance the transdermal permeation of drugs significantly compared to conventional formulations such as solutions, gels or creams. They are able to incorporate both hydrophilic (5-fluorouracil, apomorphine hydrochloride, diphenhydramine hydrochloride, tetracaine hydrochloride, methotrexate etc.) and lipophilic drugs (estradiol, finasteride, ketoprofen, meloxicam, felodipine, triptolide etc.) and enhance their permeation. The advantages of microemulsion for the transdermal delivery of a drug are: A large amount of drug can be incorporated in the formulation due to the high solubilizing capacity that might increase thermodynamic activity towards the skin, the permeation rate of the drug from microemulsion may be increased, since the affinity of a drug to the internal phase in microemulsion can be easily modified to favour partitioning into stratum corneum, using different internal phase, changing its portion in microemulsion, the surfactant and co surfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as penetration enhancers, the percutaneous absorption of drug will also increase due to hydration effect of the stratum corneum if the water content in microemulsion is high.

Opthalmic delivery

Microemulsions offer a promising alternative in ocular drug delivery). In point of view of production and sterilization, microemulsions are simple and inexpensive. Moreover, they are comprised of aqueous and oily components and therefore can accommodate both hydrophilic as well as lipophilic drugs. Water-in-oil microemulsions may be of value as vehicles for ocular drug delivery of irritant hydrophilic compounds as they appear to have a protective effect. Microemulsions could become especially favourable for water-continuous ophthalmological carrier systems because of their aqueous consistence, their transparency and thermodynamical stability. Further advantages result from a possible improvement of solubility and stability of drugs with a potential increase in bioavailability, especially for poorly soluble drugs. In addition, no impairment of visibility can be expected in comparison with eye oils. Because of these circumstances the compliance to the patient could be improved. The most used surfactants in the preparation of ophthalmic microemulsions are the poloxamers, polysorbates, tyloxapol, polyethylene glycol and their derivatives.

Periodontal delivery

The periodontium, which anchors the teeth to the jaws, consists of the gingiva, periodontal ligament, cementum and alveolar bone. It is normally in a balanced state with the periodontal microbiota in the dental plaque. Human periodontal diseases (i.e. gingivitis and periodontitis) result from heterogenous etiologies, including changes to the complex biofilm in the subgingivial microenvironment, social and behavioral modulations, and genetic or epigenetic traits of the host's immune and inflammatory responses. Periodontitis is a chronic inflammatory disease that is characterized by destructive inflammatory processes affecting the supporting structures of the teeth, causing resorption of alveolar bone and formation of periodontal pockets. It is a major cause of tooth loss. The microemulsion formulation comprising local anaesthetic in oil form, surfactant, water and optionally a taste masking agent could be used as a local anaesthetic for pain relief within the oral cavity in conjunction with periodontal scaling and root planning. The formulation can overcome the problem with the existing topical products (jelly, ointment or spray) such as lack of efficacy due to inadequate depth of

penetration, too short duration and difficulties in administration due to spread, taste etc. Microemulsion alone or in conjunction with in situ gelling system is promising tool for drug delivery in periodontitis.

- **Nasal delivery:**

Microemulsions are now being studied as a delivery system to enhance penetration/uptake across nasal mucosa. Addition of a mucoadhesive polymer helps in prolonging the residence time on the mucosa. Nasal route for administration of diazepam microemulsion might be a useful approach for the rapid onset of action during the emergency treatment of status epilepticus due to better penetration and improved bioavailability.

2.4.4 Topical microemulsions

Microemulsion systems are now being investigated zealously for topical delivery. They have been reported to enhance the transdermal permeation of drugs significantly compared to conventional formulations such as solutions, gels or creams. Several plausible mechanisms have been proposed to explain the advantages of microemulsion for the transdermal delivery of a drug. A large amount of drug can be incorporated in the formulation due to the high solubilizing capacity that might increase thermodynamic activity towards the skin. The permeation rate of the drug from microemulsion may be increased, since the affinity of a drug to the internal phase in microemulsion can be easily modified to favor partitioning into stratum corneum, using different internal phase, changing its portion in microemulsion. The surfactant and co surfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as penetration enhancers. The percutaneous absorption of drug will also increase due to hydration effect of the stratum corneum if the water content in microemulsion is high enough. Due to the small droplet size and large amount of inner phase in microemulsions, the density of droplets and their surface area are assumed to be high. Therefore, droplets settle down to close contact with the skin providing high concentration gradient and improved drug permeation. Moreover, low surface tension ensures good contact to the skin. Also, the dispersed phase can act as a reservoir making it possible to maintain an almost constant concentration gradient over the skin for a long time.

2.4.4.1 Formulation considerations of topical microemulsion

- The challenges in formulating topical microemulsions are:
  - ✓ Determining systems that are non-toxic, non-irritating, non-comedogenic and non-sensitizing.
  - ✓ Formulating cosmetically elegant microemulsions. The microemulsion formulation must have low allergic potential, good physiological compatibility and high biocompatibility. The components involved in the general formulation of microemulsions include.
  - ✓ An oil phase
  - ✓ An aqueous phase containing hydrophilic active ingredients (preservatives and buffers may be included)
  - ✓ A primary surfactant [anionic, non-ionic or amphoteric]
  - ✓ Secondary surfactant or co-surfactants.

Generally non-ionic surfactants are chosen because of their good cutaneous tolerance, lower irritation potential and toxicity. Microemulsions can be formulated using single chain surfactants or double chain surfactants. Single chain surfactants do not lower the oil water interfacial tension sufficiently and hence co-surfactants are required. Double chained surfactants like sulfosuccinates can form microemulsions in the absence of co-surfactants but are too toxic for general pharmaceutical applications. The co-surfactants even though being indispensable in the formulation of microemulsions, have exhibited toxicity e.g. medium chain length alcohols. Hence judicious choice of surfactants and co-surfactants is of great importance. The use of polyoxyethylenealcohol ethers has been reported as co-surfactants. Microemulsions prepared from phospholipids such as lecithins are preferred over synthetic surfactants from the toxicity point of view. The biocompatibility requirements of the amphiphiles are fulfilled by lecithins and non-ionic surfactants.[19]

2.5 Topical gels

The U.S.P. defines gels as a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid. Most topical gels are prepared with organic polymers, such as carbomers, which impart

an aesthetically pleasing, clear sparkling appearance to the product, and are easily washed of the skin with water. Gels have a variety of applications in the administration of medications orally, topically, intranasally, vaginally and rectally. A gel may be defined as a semi-solid formulation having an external solvent phase, apolar (organogels) or polar (hydrogel), immobilized within the spaces available of a three dimensional networked structure. Gels are two-component semisolids system rich in liquid. Their one characteristic feature is the presence of a continuous structure providing solid-like properties. In a typical polar gel, a natural or synthetic polymer builds a three dimensional matrix throughout a hydrophilic liquid. Typical polymers used include the natural gums tragacanth, carrageenin, pectin, agar and alginic acid; semisynthetic materials such as methylcellulose, hydroxyl ethylcellulose, hydroxyl propyl methylcellulose, and carboxy methylcellulose; and a synthetic polymer carbopol. We may also use certain clays such as bentonite, veegum, and laponite. Provided that the drug does not bind to the polymer or clay, such gels release medicaments well; the pores allow relatively free diffusion of molecules, which are not too large.

2.5.1 Microemulsion based topical gels

Microemulsion based gels are the microemulsions gelled by gelling agents. They provide high patient acceptability by providing previously mention advantages of both microemulsions and gels. Topical microemulsion improves skin permeation and can incorporate both water soluble and insoluble drugs while gel improves its application to the topical site by providing high viscosity.

2.5.1.1 Microemulsion based hydrogels

Hydrogels are crosslinked polymer networks that absorb substantial amounts of aqueous solutions. Hydrogels can be divided into two categories based on the chemical or physical nature of the crosslink junctions. Chemically crosslinked networks have permanent junctions, while physical networks have transient junctions that arise from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds, or hydrophobic interactions. Hydrogels can also be separated into two groups based on their natural or synthetic origins. Hydrogel-forming natural polymers include proteins such as collagen and gelatin, and polysaccharides such as alginate and agarose. Synthetic polymers that form hydrogels are traditionally prepared using chemical polymerization

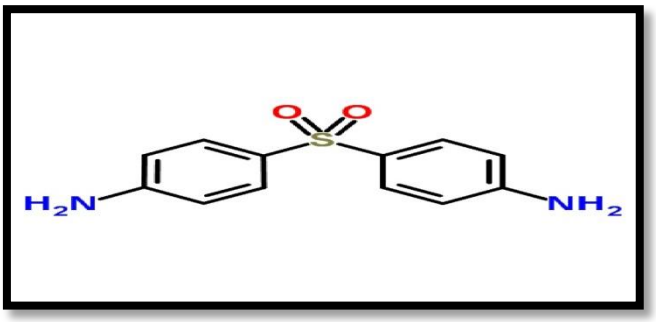
methods. Approaches using genetic engineering and biosynthetic methods to create unique hydrogel materials have been recently reported. In biosynthetic methods, predetermined amino acid sequences of artificial proteins are encoded into recombinant DNA, and the target proteins are expressed using host cells such as *E. coli* bacteria. Fidelity of biosynthesis machinery ensures protein products of precisely defined molecular weight, composition, and sequence. The modularity of recombinant DNA technology allows biological determinants such as cell-binding domains and enzyme recognition sites to be incorporated readily. These advantages offered by biosynthetic methodology are not easily realized in chemically synthesized materials.

2.5.1.2 Organogels

In the current review, attempts will be made to have an insight on the mechanism of formation and applications of the organogels as a delivery system. The organogels may be regarded as bi-continuous systems consisting of gelators and apolar solvent, which may or may not contain water-molecules entrapped within the self-assembled structures of the gelator. The gelators, when used in concentration < 15 % (approx.), may undergo physical or chemical interactions so as to form self-assembled fibrous structures which get entangled with each other resulting in the formation of a three-dimensional networked structure. The three-dimensional networked structure, hence formed, prevents the flow of external apolar phase. Some common examples of gelators include sterol, sorbitan monostearate, lecithin and cholesteryl anthraquinone derivates. The thermo-reversible property of the organogels has generated much interest for the potential use of the organogels as drug delivery system. [22]

2.6 Drug profile[25, 30]

- **Name**: Dapsone
- **Chemical Name: 4**-[(4-aminobenzene)sulfonyl]aniline
- **Molecular Formula:** C_{12} H_{12} N_2 O_2 S
- **Molecular Weight:** 248.301 g
- **Structural Formula:**



- **Physical and Chemical Properties:**
  - ✓ **Melting Point:175.5^{0}C**
  - ✓ **Water Solubility:** 380 mg/L (at 37 °C)
  - ✓ **Log P: 0.97**
  - ✓ **Log S:-2.28**
  - ✓ **pKa:2.41**
- **Mechanism of Action:**

Dapsone acts against bacteria and protozoa in the same way as sulphonamides, that is by inhibiting the synthesis of dihydrofolic acid through competition with para-amino benzoate for the active site of dihydropteroate synthetase. The anti-inflammatory action of the drug is unrelated to its antibacterial action and is still not fully understood.

- **Adverse effects and Precautions:**

Nausea, vomiting, loss of appetite, dizziness, or blurred vision may occur. unusually fast heartbeat, unusually fast breathing, bluish lips/skin, chest pain, mental/mood changes, muscle weakness, difficulty urinating.This drug may rarely cause very serious low blood counts (bone marrow suppression) or liver disease. Dapsone can commonly cause a rash that is usually not serious. Oral dapsone treatment has produced dose-related hemolysis and hemolytic anemia. Individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency are more prone to hemolysis with the use of certain drugs. There was no evidence of clinically relevant hemolysis or anemia in patients treated with Dapsone

gel including patients who were G6PD deficient. Peripheral neuropathy (motor loss and muscle weakness) has been reported with oral dapsone treatment. No events of peripheral neuropathy were observed in clinical trials with topical Dapsone gel treatment.

- **Interactions:**

Increased toxicity with Trimethoprim & Sulfomethoxazole, with Rifabutin & Rifampicin Dapsone level in the blood decreases..

- **Pharmacokinetics:**

Dapsone is completely absorbed after oral administration and is widely distributed in the body, though penetration in CSF is poor. It is 70% plasma protein bound, but more importantly concentrated in skin (especially lepromatous skin), muscle, liver and kidney. Dapsone is acetylated as well as glucuronide and sulfate conjugated in liver. Metabolites are excreted in bile and reabsorbed from intestine, so that ultimate excretion occurs mostly in urine. The plasma $t_{1/2}$ of dapsone is variable(5-10 hrs), though often > 24 hrs. The drug is cumulative due to retention in tissues and enterohepatic circulation. Elimination takes 1-2 weeks or longer.

- **Topical Pharmacokinetics:**

When applied topically twice daily, Dapsone gel (5%) gives AUC of 415 ± 224 ng•h/mL which is 100 times lower than oral Dapsone 100 mg.

- **Uses and Administration:**
  - ✓ In the treatment of leprosy.
  - ✓ In combination with pyrimethamine, dapsone can be used for chloroquine-resistant malaria.

2.7 Polymer profile[26]

- **Carbopol 940**

- **Nonproprietary Names:**
  - ✓ BP: Carbomers
  - ✓ PhEur: Carbomers
  - ✓ USP-NF: Carbomer
- **Synonyms:** Acrypol; Acritamer; acrylic acid polymer; carbomera; Carbopol; carboxy polymethylene; polyacrylic acid; carboxyvinyl polymer; Pemulen; Tego Carbomer
- **Chemical Name and CAS Registry Number:**
  Carbomer 940 [9007-17-4]
- **Functional Category:**

Bioadhesive material; controlled-release agent; emulsifying agent; emulsion stabilizer; rheology modifier; stabilizing agent; suspending agent; tablet binder.

- **Structural Formula:**

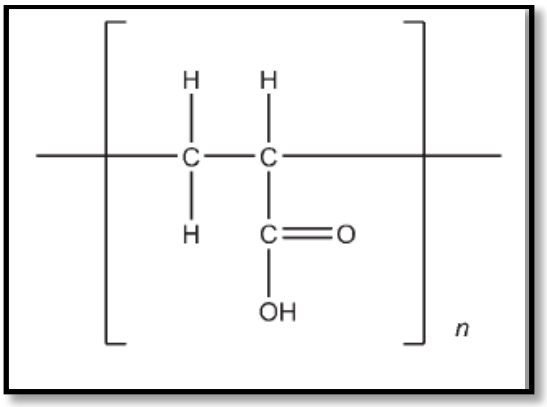


Fig. Acrylic Acid monomer unit in carbomer polymer

Carbomer polymers are formed from repeating units of acrylic acid. The monomer unit is shown above. The polymer chains are crosslinked with allyl sucrose or allyl pentaerythritol.

- **Molecular weight:** Approximately 104 400 g/mol
- **Description:** Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a characteristic slight odor.

- **Physical Properties:**
  - ✓ pH: 2.5–4.0 for a 0.2% w/v aqueous dispersion; 2.5–3.0 for Acrypol 1% w/v aqueous dispersion.
  - ✓ Melting Point :Decomposition occurs within 30 minutes at 260^0C.
  - ✓ Moisture content : Typical water content is up to 2% w/w. However, carbomers are hygroscopic and a typical equilibrium moisture content at 258C and 50% relative humidity is 8–10% w/w. The moisture content of a carbomer does not affect its thickening efficiency, but an increase in the moisture content makes the carbomer more difficult to handle because it is less readily dispersed.
  - ✓ Dissociation constant pKa = 6.0±0.5
  - ✓ Solubility: Swellable in water and glycerin and, after neutralization, in ethanol (95%).
- **Application:**

  Carbomers are used in liquid or semisolid pharmaceutical formulations as rheology modifiers. Formulations include creams, gels, lotions and ointments for use in ophthalmic, rectal, topical and vaginal preparations. Various uses are described in following table.

Table 2.2Use of Carbomers

Sr. No.	Use	Concentration (%)
1	Emulsifying Agent	0.1-0.5
2	Gelling Agent	0.5-1.0
3	Suspending Agent	0.5-1.0
4	Tablet Binder	0.75-3.0
5	Controlled-release agent	5.0-30.0

- **Stability:**

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104^0C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. In contrast,

microorganisms grow well in unpreserved aqueous dispersions, and therefore an antimicrobial preservative such as 0.1% w/v chlorocresol etc. Exposure to light causes oxidation that is reflected in a decrease in dispersion viscosity.

- **Storage conditions:**

Carbomer powder should be stored in an airtight, corrosionresistant container and protected from moisture. The use of glass, plastic, or resin-lined containers is recommended for the storage of formulations containing carbomer.

- **Incompatibility:**

Carbomers are discolored by resorcinol and are incompatible with phenol, cationic polymers, strong acids, and high levels of electrolytes. Certain antimicrobial adjuvants should also be avoided or used at low levels. Trace levels of iron and other transition metals can catalytically degrade carbomer dispersions. Certain amino-functional actives form complexes with carbomer; often this can be prevented by adjusting the pH of the dispersion and/or the solubility parameter by using appropriate alcohols and polyols. Carbomers also form pH-dependent complexes with certain polymeric excipients. Adjustment of pH and/or solubility parameter can also work in this situation.

- **Safety:**

Carbomers are used extensively in nonparenteral products, particularly topical liquid and semisolid preparations. Grades polymerized in ethyl acetate may also be used in oral formulations. There is no evidence of systemic absorption of carbomer polymers following oral administration. Acute oral toxicity studies in animals indicate that carbomer 934P has a low oral toxicity, with doses up to 8 g/kg being administered to dogs without fatalities occurring. Carbomers are generally regarded as essentially nontoxic and nonirritant materials, there is no evidence in humans of hypersensitivity reactions to carbomers used topically.

- **Regulatory status:**

Included in the FDA Inactive Ingredients Database (oral suspensions, tablets; ophthalmic, rectal, topical, transdermal preparations; vaginal suppositories). Included in nonparenteral medicines licensed in Europe. Included in the Canadian List of Acceptable Nonmedicinal Ingredients.

2.8 Excipient profile

2.8.1 Triacetin:[27]

- **Nonproprietary Names**

✓ BP: Triacetin

✓ PhEur: Triacetin

✓ USP: Triacetin

- **Synonyms**

Captex 500; E1518; glycerol triacetate; glyceryl triacetate; triacetinum; triacetyl glycerine.

- **Chemical Name and CAS Registry Number**

1,2,3-Propanetriol triacetate [102-76-1]

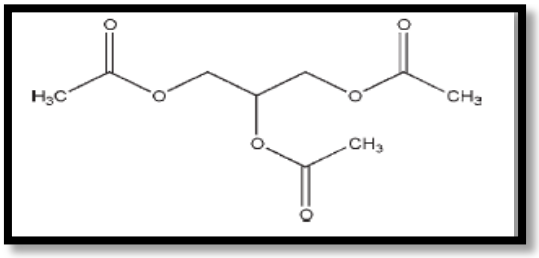
- **Functional Category:**

Humectant; plasticizer; solvent.

- **Empirical Formula:**

  C9H14O6

- **Structural Formula:**



- **Molecular Weight:**

  218.21

- **Description:**

  Triacetin is a colorless, viscous liquid with a slightly fatty odor.

- **Physical Properties:**

  ✓ Autoignition temperature : 432^0C

  ✓ Boiling point: 258^0C

  ✓ Density: 1.16 g/cm3 at 25^0C

- ✓ Flash point: 153^0C (open cup)
- ✓ Freezing point: 3.2^0C (supercools to about -70^0C)
- ✓ Melting point : 78^0C
- ✓ Refractive index $n_D^{25} = 1.4296$
- ✓ Viscosity (dynamic): 17.4 mPa s (17.4 cP) at 258C;
- ✓ Solubility: Miscible with chloroform, ethanol & 1 in 14 with water.

- **Application:**
  - ✓ Triacetin is mainly used as a hydrophilic plasticizer in both aqueous and solvent-based polymeric coating of capsules, tablets, beads, and granules; typical concentrations used are 10–35% w/w.
  - ✓ Triacetin is used in cosmetics, perfumery, and foods as a solvent and as a fixative in the formulation of perfumes and flavors.
- **Stability:**

  Triacetin is stable.
- **Storage Conditions**

  It should be stored in a well-closed, nonmetallic container, in a cool, dry place.
- **Incompatibilities**

  Triacetin is incompatible with metals and may react with oxidizing agents. Triacetin may destroy rayon fabric.
- **Safety**

  Triacetin is used in oral pharmaceutical formulations and is generally regarded as a relatively nontoxic and nonirritant material at the levels employed as an excipient.
- **Regulatory Status:**

  GRAS listed. Accepted in Europe as a food additive in certain applications. Included in the FDA Inactive Ingredients Database (oral capsules and tablets and gels). Included in nonparenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

2.8.2 Tween 80[28]

- **Nonproprietary Names:**

✓ BP: Polysorbate 80

✓ PhEur: Polysorbate 80

✓ USP-NF: Polysorbate 80

- **Synonyms:**

  Atlas E; Armotan PMO 20; Capmul POE-O; Cremophor PS 80; Crillet 4; Crillet 50; Drewmulse POE-SMO; Drewpone 80K; Durfax 80; Durfax 80K; E433; Emrite 6120; Eumulgin SMO; Glycosperse O-20; Hodag PSMO-20; Liposorb O-20; Liposorb O-20K; Montanox 80; polyoxyethylene 20 oleate; polysorbatum 80; Protasorb O-20; Ritabate 80; (Z)-sorbitan mono-9-octadecenoate poly(oxy1,2- ethanediyl) derivatives; Tego SMO 80; Tego SMO 80V; Polysorbate 80.

- **Chemical Name and CAS Registry Number**

  Polyoxyethylene 20 sorbitan monooleate [9005-65-6]].

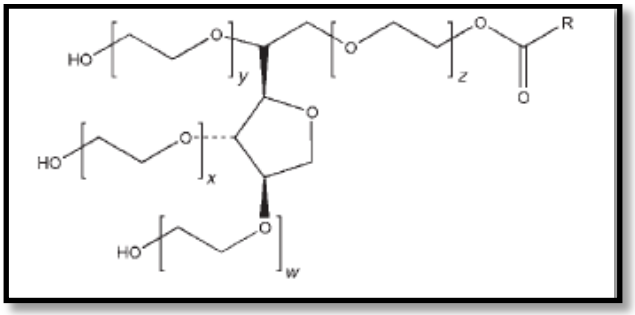
- **Functional Category:**

  Dispersing agent; emulsifying agent; nonionic surfactant; solubilizing agent; suspending agent; wetting agent.

- **Empirical Formula:**

  C64H124O26

- **Structural Formula:**



$w \times x \times y \times z = 20$

R = fatty acid

- **Molecular Weight:**

  1310

- **Description:**

  Tween 80 has a characteristic odor and a warm, somewhat bitter taste. Its color and physical form at 25^0C is Yellow oily liquid.

- **Physical Properties:**
  - ✓ HLB Value : 15
  - ✓ Specific Gravity: 1.08 at 25^0C
  - ✓ Viscosity (dynamic): 425 mPa S at 25^0C;
  - ✓ Solubility: Soluble in water, ethanol & insoluble in mineral oil, vegetable oil with water.

- **Application:**

  Polyoxyethylene sorbitan fatty acid esters (polysorbates) are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5, or 4 moles of ethylene oxide for each mole of sorbitol and its anhydrides. The resulting product is therefore a mixture of molecules of varying sizes rather than a single uniform compound.

Table 2.3Uses of Tween 80

Sr. No.	USE	Concentration (%)
1	Emulsifying agent • Used alone in oil-in-water emulsions • Used in combination with hydrophilic emulsifiers in oil-in-water emulsions • Used to increase the water-holding properties of ointments	1-15 1-10 1-10
2	Solubilizing agent • For poorly soluble active constituents in lipophilic bases	1-15
3	Wetting agent • For insoluble active constituents in lipophilic bases	0.1-3

- **Stability:**

  Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.

- **Storage Conditions**

  Tween 80 should be stored in a well-closed container, protected from light, in a cool, dry place.

- **Incompatibilities**

  Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tarlike materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates.

- **Safety**

  Polysorbates are widely used in cosmetics, food products, and oral, parenteral and topical pharmaceutical formulations, and arehave, however, been occasional reports of hypersensitivity to polysorbates following their topical and intramuscular use.Polysorbates have also been associated with serious adverse effects, including some deaths, in low-birthweight infants intravenously administered a vitamin E preparation containing a mixture of polysorbates 20 and 80. When heated to decomposition, the polysorbates emit acrid smoke and irritating fumes.

- **Regulatory Status:**

  Polysorbates 80 is GRAS listed. Polysorbate 80 is accepted as food additives in Europe. Polysorbate 80 is included in the FDA Inactive Ingredients Database (IM, IV, oral, rectal, topical, and vaginal preparations). Polysorbate 80 is included in parenteral and nonparenteral medicines licensed in the UK. Polysorbate 80 is included in the Canadian List of Acceptable Non-medicinal Ingredients.

2.8.3 Labrasol[29]

- **Nonproprietary Names:**

✓ BP: Caprylocaproyl Macrogolglycerides

✓ PhEur: Caprylocaproyl Macrogolglycerides

✓ USP-NF: Caprylocaproyl Polyoxylglycerides

- **Synonyms:**

  Labrasol; macrogolglyceridorum caprylocaprates; PEG 400 caprylic/ capric glycerides.

- **Chemical Name and CAS Registry Number:**

  Caprylocaproyl polyoxylglycerides [73398-61-5] [223129-75-7].

- **Functional Category:**

  Dissolution enhancer; emulsifying agent; nonionic surfactant; penetration agent; solubilizing agent; sustained-release agent.

- **Chemical nature:**

  Glyceryl and polyethylene glycol esters; Caprylocaproyl Macrogolglycerides (Polyoxylglycerides).

- **Molecular Weight:**

  Mean relative molecular mass between 200 and 400.

- **Description:**

  Caprylocaproyl polyoxylglycerides are pale-yellow oily liquids.

- **Properties:**

✓ **Form -** Oily liquid

✓ **Colour -** Roughly white

✓ **Odour -** Light

✓ **Boiling point/Boiling range -** > 150°C

✓ **Flash point -** > 150°C

✓ **Self igniting -** Product is not selfigniting

✓ **Relative density -** 1,060 - 1,070 (20°C)

✓ **Solubility in / Miscibility with water -** Soluble

✓ **Organic solvents -** Soluble in many organic solvents.

✓ **Functional category:** Surfactant and Co-surfactant

✓ **HLB value:** 14

- **Applications:**

  Labrasol® is used in oral and topical formulations. It is a solubilizer/bioavailability enhancer for oral formulations, and can be used in Self Emulsifying Lipidic Formulations (SELF) as a surfactant. ointments, microemulsions, emulsions and gels. It is also used in transdermal patches.

- **Stability and reactivity:**

  No decomposition if used according to specifications. Avoid mixture of the products with strong acids, oxidants and alkalis.

- **Storage and packaging:**

  Store in its original package hermetically closed. Recommended packing or flasks materials are polyethylene.

- **Incompatibility:**

  Incompatible with strong acids, oxidizing agents. Incomplete combustion releases monoxide carbon and dioxide carbon.

- **Safety:**

  No particular hazards known. Not irritating to the skin. Not irritating to the eyes. Ingestion may cause gastrointestinal disturbances.

- **Regulatory status:**

  EP, USP recorded in the US inventory: TSCA (Toxic Substance Control Act). of the substance are recorded into european inventory EINECS (European Inventory of Existing Chemical Substances) directive 67/548/EEC.

3

LITERATURE REVIEW

3. Review of Literature :

3.1 Microemulsion:

Sr. No.	Reference	Polymer / Excipient Used	Work Done
1	S. Peltola et al., 2003	Oleic acid, Isopropyl Myristate, Epicuron 20, Tween 80, Tween 20, Span 80	They developed O/W microemulsion for the transdermal delivery of Estradiole, due to its higher first pass metabolism and lower bioavailability by oral route. The permeation data showed that microemulsion formulations increased estradiol flux 200–700-fold over the control. The superior transdermal flux of estradiol was due to 1500-fold improvement in solubilization of estradiol by microemulsions. The results suggested microemulsions as potential vehicles for improved topical delivery of estradiol.
2	N. Aggarwal et al., 2013	Oleic acid, Tween 80, Ethanol	They prepared microemulsion containing griseofulvin for topical application for the treatment of dermatophytosis. They showed that microemulsion, containing 5% oil & 40 % of 1:1 surfactant to co-surfactant, has 7, 5 and almost 3-fold higher drug permeation as compared to aqueous suspension, oily solution and conventional cream respectively.

3	M. R. Patel et al., 2011	Isopropyl Myristate, Caprylocaproyl macrogol-8-glyceride, Polyglyceryl oleate	They prepared microemulsion containing Isotretinoin, which is a topical retinoid having photoinstability used for the treatment of Acne. Prepared Microemulsion was evaluated for photostability of Isotretinoin, which showed improved photostability in comparision to methanol solution. Further, degradation kinetic parameters of isotretinoin-loaded microemulsion formulation were demonstrated increase isotretinoin half-life about five-times in comparison with a methanol solution under a direct sun light.
4	Y.-S. Rhee et al., 2011	Oleic acid, Labrasol, Cremophore RH 40	They prepared transdermal O/W microemulsion containing Ketoprofen with various concentration ranges of oil, surfactant & co-surfactant & showed the effect of these additives on skin permeation of ketoprofen was evaluated with excised rat skins. Terpenes were added to the microemulsion at the level of 5% and their effect on the skin permeation of ketoprofen from the microemulsion was evaluated. Of the four terpenes used, only microemulsion with limonene resulted in a powerful enhancing activity with 3-fold increase over the control.

5	A.C. Sintov et al., 2004	Isopropyl Myristate, glyceryl oleate & polyoxyl 40 fatty acid derivatives (surfactants), tetraglycol (co-surfactant)	They prepared Lidocaine microemulsion of quaternary system. Percutaneous penetration studies using rat skin in vitro showed that the transdermal flux of lidocaine was significantly improved by microemulsion. And by analyzing skin layers (epidermis and dermis) for lidocaine content, significantly higher concentrations were found after rats were treated in vivo with liquid microemulsions compared to those measured after application of marketed cream. So, it has been suggested that these microemulsions loaded with lidocaine would provide adequate analgesia in relatively shorter periods of time.
6	Y. Yuan et al., 2006	Isopropyl Myristate, Tween 85, Ethanol	They developed meloxicam microemulsion for better skin permeability. They investigated for the effect of the content of isopropyl myristate (IPM) and the effect of the mass ratio of the surfactant/cosurfactant (Km) on skin permeation with excised rat skins. The optimized formulation showed highest skin permeation rate.
7	R.M. Al Abood et al., 2013	Oleic acid, Tween 20, PEG 400	They developed microemulsion of ondansetron for transdermal delivery for treatment of nausea & vomiting

			induced by chemotherapy & in order to predict the efficacy, pharmacokinetic studies were performed and pharmacokinetic profile was compared with conventional ondansetron gel & oral marketed syrup.The absorption of ondansetron from ondansetron microemulsion gel resulted in 6.03 fold increase in bioavailability as compared to oral conventional syrup and 9.66 times with reference to the conventional ondansetron gel.
8	W. Zhu et al., 2008	Oleic Acid, Cremophore EL, Ethanol	They prepared microemulsion loaded with pencyclovir for dermal delivery which was oprimized by simplex lattice mixture design including the concentrations of surfactant, cosurfactant and water (independent variables) and the solubility and the cumulative amount of penciclovir permeated through excised mouse skins per unit area (response variables). The conclusion was that the permeating ability of penciclovir was significantly increased from the microemulsion formulation compared with commercial cream.

3.2 Microemulsion Based Gel:

Sr. No.	Reference	Polymer / Excipient Used	Work Done
1	H. Chen et al., 2006	Ethyl Oleate, Rween 80, Propylene Glycol, Xanthan Gum	They developed microemulsion based hydrogel for topical delivery of ibuprofen. Various microemulsion formulations were prepared and the abilities of various microemulsions to deliver ibuprofen through the skin were evaluated in vitro using Franz diffusion cells fitted with porcine skins. The in vitro permeation datashowed that microemulsions increased the permeation rate of ibuprofen 5.72–30.0 times over the saturated solution. Xanthan gum as a gel matrix was used to construct the microemulsion-based hydrogel for improving the viscosity of microemulsion for topical administration. The studied microemulsion-based hydrogel showed a good stability. These results indicate that the studied microemulsion-based hydrogel may be a promising vehicle for topical delivery of ibuprofen.
2	V. Sabale et al., 2013	Oleic Acid, Tween 80, IPA, HPMC K100 M	They developed microemulsion based hydrogel for topical delivery of bifonazole with an objective to increase the solubility and skin permeability optimized through 3^2 fractional factorial design. The HPMC K100 M as a gel matrix was used to construct the microemulsion based hydrogel for improving the viscosity of microemulsion for topical administration. They found that drug release from microemulsion based hydrogel was observed to follow zero order kinetics.

3	L. Chen et al., 2013	Oleic acid, Tween 80, Labraol, aquous ethanol, Poloxamer 407	They developed microemulsion based hydrogel of triptolide for transdermal drug delivery to reduce its toxicity. WhentheconcentrationofPoloxamer407increased, the rheological properties such as the yield stresses (sy), storage and loss moduli (G′, G″) of the formulations increased, and the network structures became more compact. They also showed that MBH preparations gave good sustained-release profile when compared to microemulsions, which was proved by an in vitro permeation test in mice.
4	H. Chen et al., 2007	IPM, Tween 80, PG, Carbopol 940	They designed microemulsion based hydrogel for topical delivery of triptolide (HTM) at extremely low concentration. They described that HTM had good stability. The permeation rates of triptolide from various HTM were 2.2–3.6 times over that from the control hydrogel. And finally they concluded that The powerful permeation enhancing ability of HTM with a suitable viscosity makes it promising alternative carrier for transdermal administration of drug molecule at an extremely low concentration.
5	S.A. Fouad et al., 2013	Capryol, Labrasol, Transcutol, Poloxamer	They developed microemulsion based hydrogel using Poloxamer of to enhance transport of diclofenac epolamine (DE) into the skin forming in-skin drug depot for sustained transdermal delivery of drug. D-optimal mixture experimental design was applied to optimize ME that contains maximum amount of oil, minimum globule size and optimum drug solubility. They

			showed that optimized ME showed the highest cumulative amount of DE permeated after 8 h and the in vivo anti-inflammatory efficacy in rat paw edema was sustained to 12 h after removal of ME applied to the skin confirming the formation of in-skin drug depot.
6	H.K. Patel et al., 2013	IPM, Cremophore EL, IPA, Carbopol 934P	They developed microemulsion based hydrogel for dermal delivery of clobetasol propionate. They applied D- optimal design for optimization Ex-vivo permeation studies showed that cumulative amount of clobetasol propionate permeated (Qn) from Microemulsion, Microemulsion based hydrogel and market formulation at 8 h after application were 53.6 ± 2.18, 28.43 ± 0.67 and 37.73 ± 0.77 g cm−2 respectively. They found that there was significant interaction of microemulsion components with skin resulting in permeation enhancement and retention of CP into skin layers.

3.3 Dapsone:

Sr. No.	Reference	Polymer / Excipient Used	Work Done
1	K. Panduranga Rao et al., 1994	Oleic acid, stearic acid, Chitosan	They developed liposomes containing oletc and steartc acids and sequestered in chitosan gel. Dapsone and bromothymol blue were entrapped in the liposomes. The carboxyl group of sequestered liposomes were subsequently coupled to the ammo group of chitosan by carbodiimide. In vitro release studies of the drug from liposomes and liposomes sequestered m chitosan gel in phosphate buffer and mice plasma were carried out. liposomes coupled to chitosan gel displayed much slower release of Dapsone than uncoupled liposomes.
2	N. Kaila et al., 2003	Sodium CMC, Veegum HV	They prepared extamporaneos suspension from tablets & cheked its stability. The 91-day analytical stability testing study was conducted at 4, 30, 50, 60, and 70°C. The energy of activation for the suspension was determined to be –23288.35 J/K/M. The zero-order rate of degradation for dapsone (k0,25) in suspension at 25°C was found to be 0.040845 day-1. The shelf life for the suspension was calculated to be 31.67 days at 25°C and 230.76 days under refrigeration at 4°C.
3	L. M. Monteiro et al., 2012	Isopropyl Myristate, Tween 80, Span 80, Propylene glycol	They prepared dapsone nanoemulsion for oral delivery & studied for permeability & in silico bioavailability studies.The release profiles of the dapsone nanoemulsions using different combinations of surfactants and cosolvent

			showed a higher dissolution rate in simulated gastric and enteric fluid than did the dispersed dapsone powder. The drug release kinetics were consistent with a Higuchi model.
4	V. Borges et al., 2013	IPM, NMP, ISO, Tween 80, Span 20	They developed nanoemulsion loaded with dapsone for the topical delivery for the treatment of leprosy. They showed that Topical administration of Dapsone can be an alternative route for treatment of leprosy and can also provide new therapeutic applications for an established drug. They also showed that Physicochemical characterization demonstrated that nanosystems were formed, which had a uniform droplet distribution and a pH compatible with the skin surface. Use of n-methyl-pyrrolidone provided a greater nanoemulsion region and higher solubilization of dapsone, and increased the in vitro release rate when compared with a nanoemulsion prepared using isopropyl myristate. However, use of isopropyl myristate promoted an increase in in vitro epidermal permeation that followed the Higuchi model.

4

EXPERIMENTAL WORK

4. Experimental work

4.1 Materials and Equipments

Table 4.1List of materials used

MATERIALS	COMPANY NAME
Dapsone	Gifted by Zydus Cadila Pharmaceuticals, India
Triacetin AR	Central drug house Pvt. Ltd, India
Labrasol (Polyethylene glycol-8-glycol caprylate)	Gifted by Gattefosse, France
Labrafil M 2125 (PEG -6 corn oil)	
Capryol 90 (Propylene glycol monocaprylate)	
Cremophor EL (Polyethoxylated castor oil)	Gifted by BASF, Germany
Carbomer 940	Gifted by Corel Pharmachem Ltd, India
Isopropyl Myristate	S.D.Fine-chem Ltd, India
Soyabean oil	S.D.Fine-chem Ltd, India
Ethyl oleate	S.D.Fine-chem Ltd, India
Oleic acid	Central drug house Pvt. Ltd, India
Tween 20	S.D.Fine-chem Ltd, India
Tween 80	S.D.Fine-chem Ltd, India
Span 80	S.D.Fine-chem Ltd, India
Span 20	S.D.Fine-chem Ltd, India
Propylene glycol	Central drug house Pvt. Ltd, India
PEG 400	Central drug house Pvt. Ltd, India
Triethanolamine	Central drug house Pvt. Ltd, India

Methanol AR	S.D.Fine-chem Ltd, India
Sodium hydroxide	Central drug house Pvt. Ltd, India
Potassium dihydrogen ortho phosphate	Central drug house Pvt. Ltd, India

Table 4.2List of equipments used

INSTRUMENTS	**MODEL COMPANY NAME**
Digital Balance	Citiweigh- Tejas Exports, India
Mechanical Stirrer	Remi Motors Ltd. India
Vortex Shaker Hot	Remi Motors Ltd. India
Hot Air Oven	EIE Instruments Pvt. Ltd., India
Ultraviolet Spectrophotometer	Shimdzu UV 1800 Corporation, Japan
Refrigerated Micro Centrifuge	Rajendra Electrical Industries Ltd, India
Humidity Control Oven	Nova Instruments Pvt. Ltd, India
Ultrasonicator	Trans-O-Sonic D-Compact, India
pH Meter	Analab Scientific Instruments, India.
Malvern-Zetasizer	Nano ZS90, Malvern Instruments Ltd, UK
Brookfield Viscometer	Brookfield Engineering Laboratories, USA
Texture Analyser	QTS 250, Brookfield Engineering Laboratories, USA

Fourier Transformed Infra Red Spectrophotometer	FTIR 6100 Type A, Jasco, Japan
Multi-diffusion Cell Apparatus	Orchid Scientific & Innovative India Pvt Ltd, India
Transmission Electron Microscope	Tecnai 20, Phillips, Holland
Dynamic Light Scattering	Nanotrec® 525, Microtrec, UK

4.2 Pre-formulation studies

4.2.1 Identification of dapsone

4.2.1.1 Melting point analysis

The melting point of the Dapsone was determined by thiel's tube method and it was compared with reported melting point of standard.[31]

Result

Table 4.3Melting point analysis of dapsone

Sr. No.	Drug	Reported Melting Point (^{0}C)	Observed Melting Point (^{0}C)
1	Dapsone	175.5	172-175

Conclusion

The melting point of the Dapsone was found similar to the reported value of melting point, which confirmed identity of procured drug sample.

4.2.2 UV spectrophotometric analysis

UV Spectra of 8 µg/ml of drug solution was taken from 200-400 nm in Methanol AR using UV-Visible spectrophotometer. The wavelength maxima was found and it was compared with reported wavelength maxima of Dapsone.[32]

Result

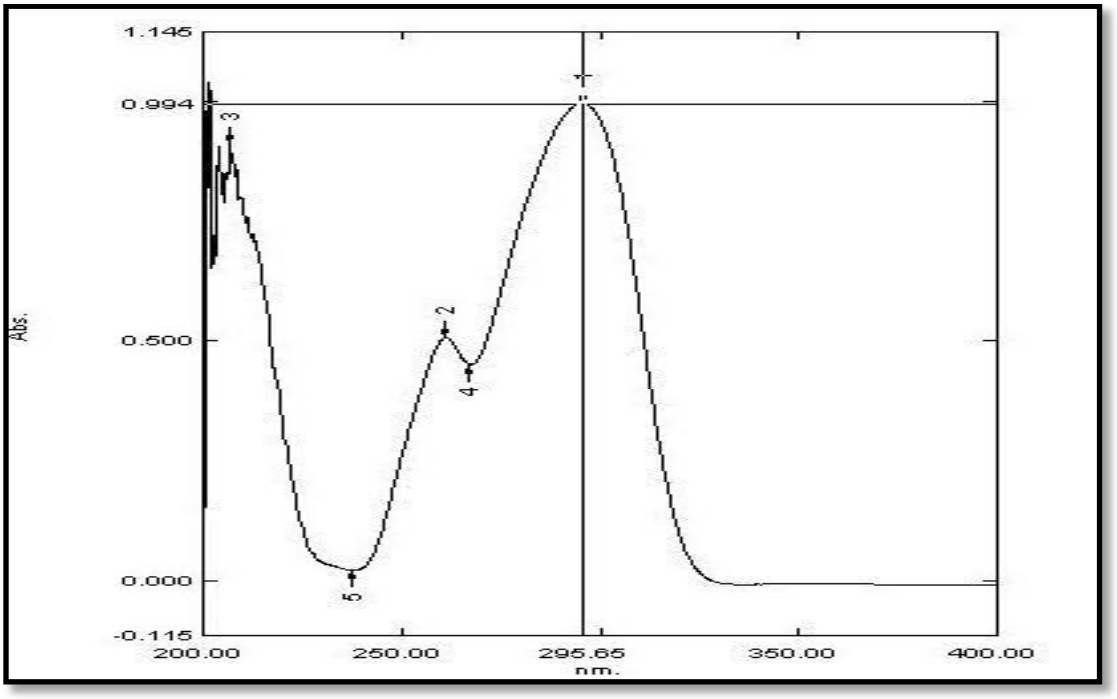


Figure 4.1UV Spectrum of Dapsone Showing absorption maxima at 295 nm

Table 4.4wavelength maxima (λmax) of dapsone

Sr. No.	Drug	Reported wavelength maxima (λ_{max})	Observed wavelength maxima (λ_{max})
1	Dapsone	295	295.65

Conclusion

The UV absorbance of Dapsone was found 295.65 nm, which was similar to reported value of wavelength maxima (λ_{max}), which again confirmed identity of the procured drug sample.

4.2.3 FTIR spectra of dapsone

Identification of procured drug was done by Infrared spectroscopy. KBr disc containing the drug was prepared and the spectra was recorded in a range between 4000 & 400 cm^{-1}.

Result

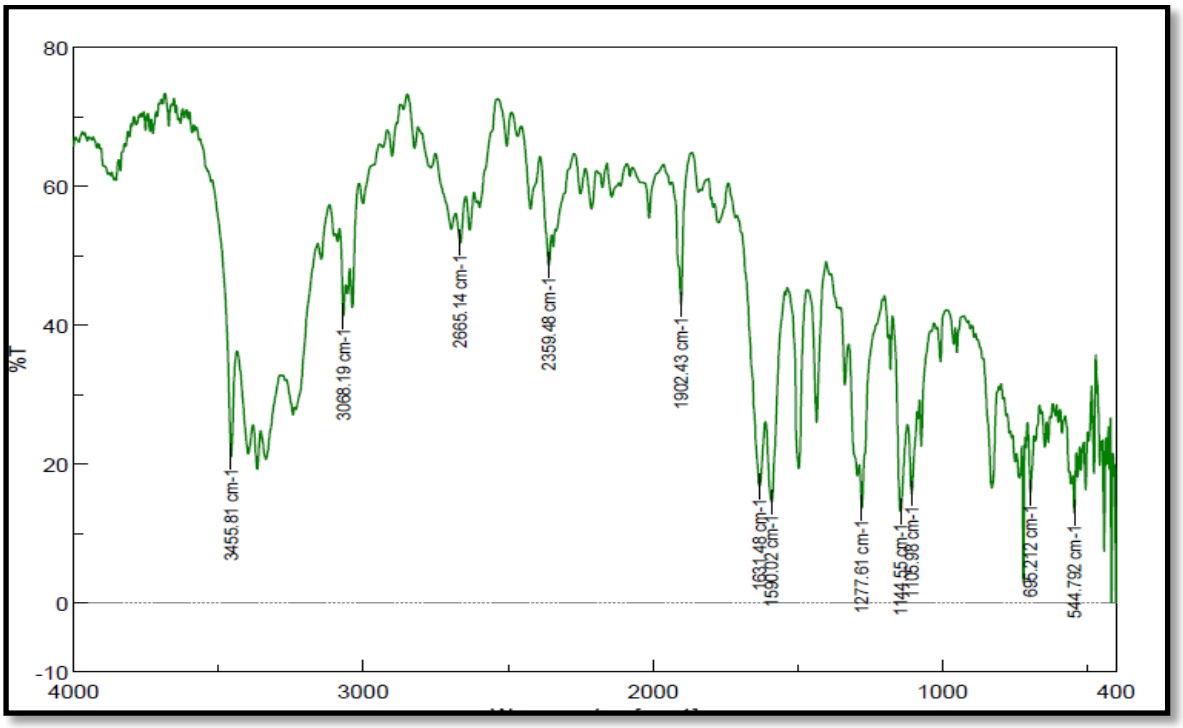


Figure 4.2FTIR Spectrum of Dapsone

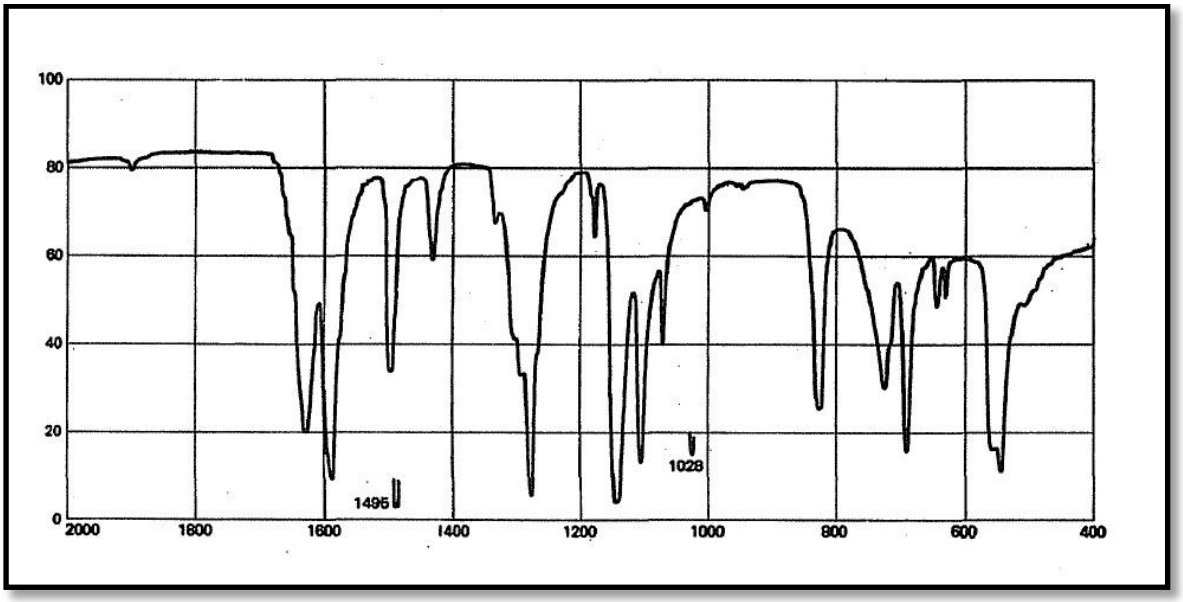


Figure 4.3Standard FTIR Spectrum[33]

Table 4.5Interpretation of the FTIR spectra of Dapsone Sample

Sr. No.	Standard Frequency (cm^{-1})	Observed frequency (cm^{-1})	Inference
1	1633	1631	N-H bending
2	1592	1590	C-C stretching
3	1150	1144	O=S=O stretching
4	1107	1106	C-N streching
5	685	696	C-H bending

Conclusion

The sample spectrum of Dapsone was compared with standard and both spectra were found similar in peak values representing wave numbers. Thus, it was concluded that procured Dapsone sample was a pure drug.

4.2.4 Drug-polymer compatibility study using FTIR spectroscopy

Compatibility study of procured drug & polymer was done by Infrared spectroscopy. KBr disc containing the drug and polymer mixture was prepared and the spectra was recorded in a range between 4000 & 400 cm^{-1}.

Result

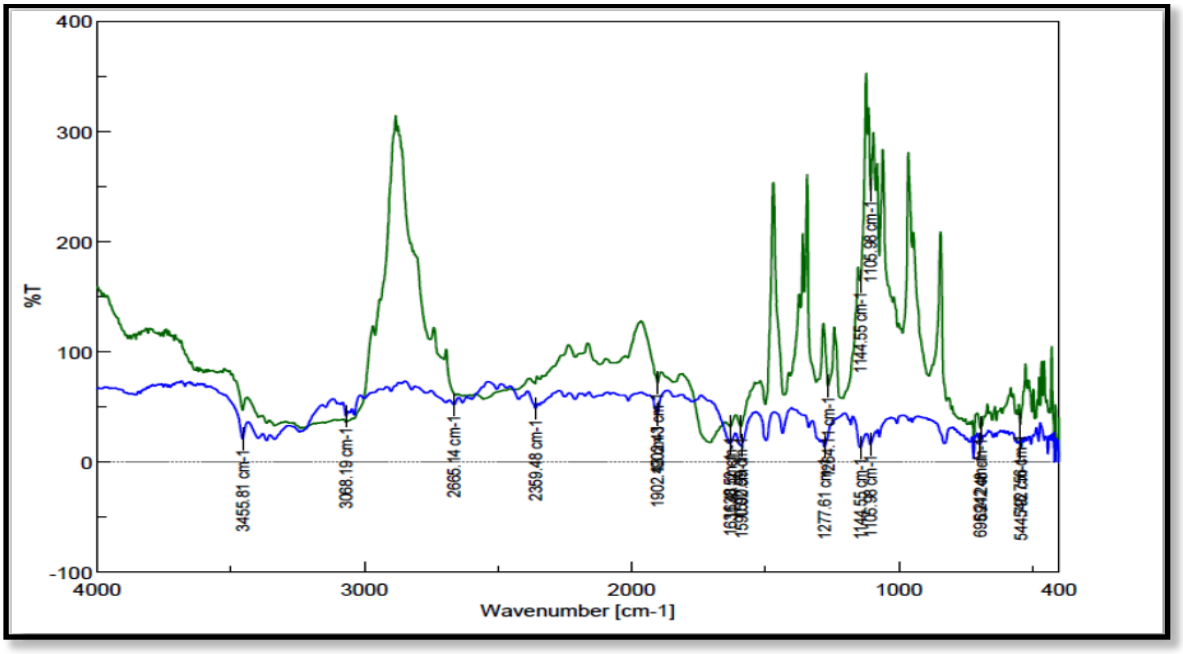


Figure 4.4Overlay of FTIR Spectra of drug & drug + carbomer 940

Conclusion

The intensity of characteristic peaks of Dapsone and carbopol 940 physical mixture found similar as FTIR spectra of Dapsone, which confirmed drug-polymer compatibility.

4.2.5 Development of spectrophotometric method for estimation of Dapsone

To have quantitative data on various studies such as purity, evaluation of the drug, compatibility studies, in-vitro diffusion studies etc, it is essential to develop analytical methods which are precise, specific and accurate. Therefore, the following analytical methods were developed and validated for Dapsone.

4.2.5.1 Calibration curve of Dapsone in methanol

- **Preparation of stock solution:**

A standard stock solution of Dapsone was prepared in methanol by dissolving 100 mg Dapsone in 100 ml of methanol.

- **Preparation of dilutions:**

Suitable dilutions were made from the standard solution to get concentrations in the range of 1-8µg/ml.

The absorption maxima (λ_{max}) was determined by scanning 8 µg/ml solution against the reagent blank on UV-visible spectrophotometer and the absorbance maxima was found out at 295 nm. The absorption of all the prepared solutions was then measured at the λ_{max}, 295 nm, against blank. The readings were recorded in triplicate and the experiment was repeated on 3 consecutive days using freshly prepared stock solutions each time. Mean values (n=3) along with the standard deviation were recorded and the regressed calibration curve was developed.

Table 4.6Calibration plot of Dapsone in Methanol

Conc.(µg/ml)	**Absorbance**			**Average**
	1	**2**	**3**	
0	0.00	0.00	0.00	0.00
1	0.14	0.13	0.14	0.13
2	0.25	0.25	0.25	0.25
3	0.37	0.38	0.38	0.38
4	0.52	0.51	0.51	0.51
5	0.63	0.64	0.64	0.64
6	0.77	0.77	0.77	0.77
7	0.90	0.90	0.90	0.90
8	0.99	1.00	1.00	0.99

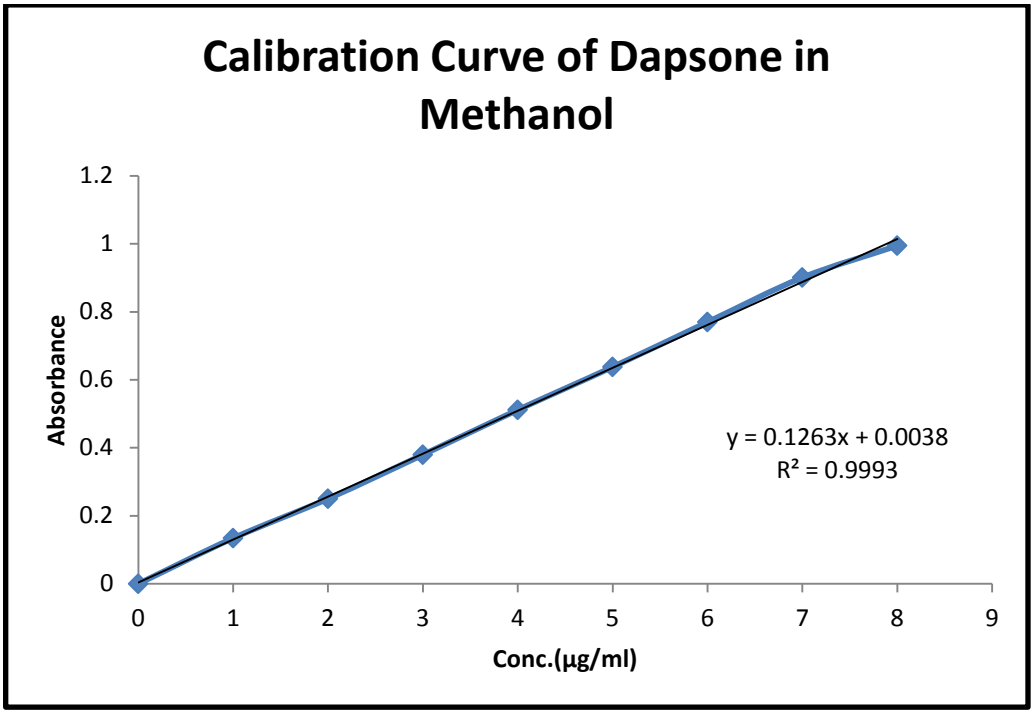


Figure 4.5Calibration Curve of Dapsone in methanol

Regression analysis

Table 4.7Regression Analysis of the calibration plot of Dapsone in methanol

Sr. No.	Regression Parameter	Values
1	Correlation Coefficient	0.9993
2	Slope	0.1263
3	Intercept	0.0038

4.2.5.2 Calibration curve for analysis of Dapsone in phosphate buffer saline (pH 7.4)

- **Preparation of stock solution:**

50 mg Dapsone was weighed into 50 ml volumetric flask and was dissolved in Methanol AR and volume was made up to 50 ml.

- **Preparation of dilutions:**

First 1ml of the stock solutions were pipetted out into a 100 ml volumetric flask and the volume was made up with Phosphate buffer saline pH7.4 (composition shown in table 4.8)

Then, Suitable aliquots of the first dilution solutions were pipetted out into a 10 ml volumetric flask and the volume was made up with Phosphate buffer solution (pH7.4).
The absorption maxima (λ_{max}) was determined by scanning 9 µg/ml solution against the reagent blank on UV-visible spectrophotometer and the absorbance maxima was found at 291 nm. The absorption of all the prepared solutions was then measured at the absorption maxima, 291 nm, against blank. The readings were recorded in triplicate and the experiments was repeated on 3 consecutive days using freshly prepared stock solutions each time. Mean values (n=3) along with the standard deviation was recorded and the calibration curve is developed.

Table 4.8Preparation of Phosphate buffer saline (pH 7.4)

Sr. No.	Ingredients	Quantity
1	Potassium dihydrogen ortho phosphate, 0.2 M	250 ml
2	Sodium hydroxide, 0.2 M	195.5 ml
3	Distilled water	q.s. to 1000 ml

Table 4.9Calibration plot of Dapsone in PBS (pH 7.4)

Sr. No.	Absorbance			Average
	1	2	3	
0	0.00	0.00	0.00	0.00
1	0.11	0.11	0.11	0.11
2	0.23	0.22	0.22	0.22
3	0.32	0.31	0.31	0.31
4	0.44	0.44	0.41	0.43
5	0.53	0.53	0.53	0.53
6	0.64	0.64	0.63	0.64
7	0.74	0.74	0.73	0.74
8	0.87	0.87	0.86	0.87
9	0.96	0.96	0.96	0.96

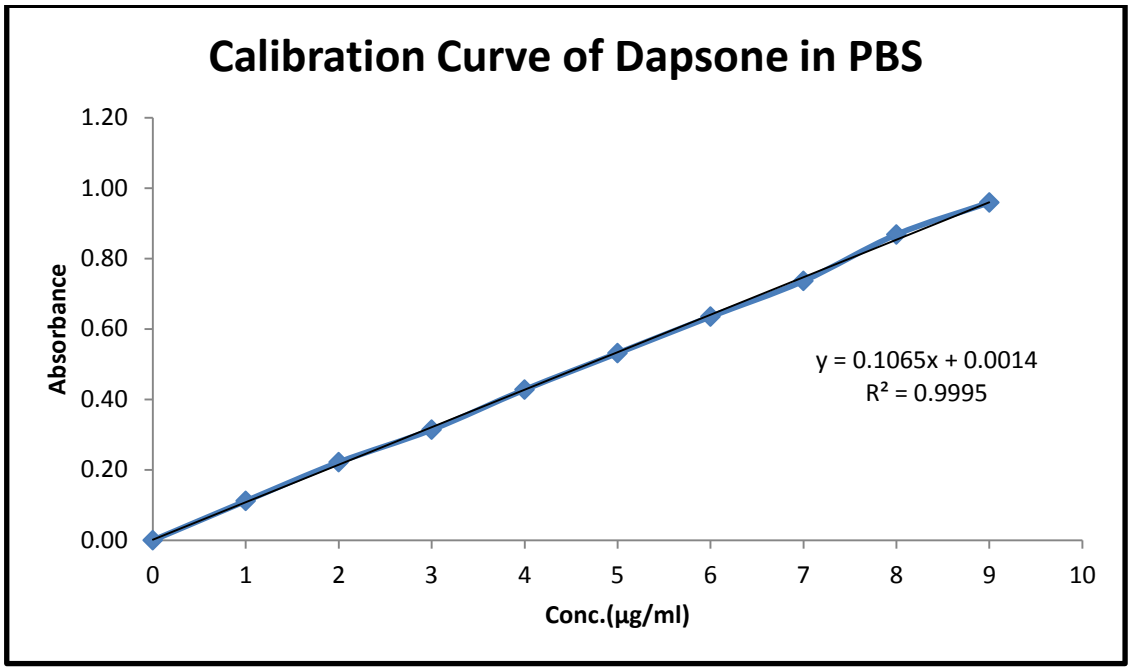


Figure 4.6Calibration Curve of Dapsone in PBS (pH 7.4)

Regression analysis

Table 4.10Regression Analysis of the calibration plot of Dapsone in PBS (pH 7.4)

Sr. No.	Regression Parameter	Values
1	Correlation Coefficient	0.9995
2	Slope	0.1065
3	Intercept	0.0014

4.3 Methods

4.3.1 Construction of pseudo-ternary phase diagrams

The aqueous titration method was adopted for construction of pseudo-ternary phase diagrams to determine the microemulsion (ME) region and to obtain the concentration range of components for the existing range of microemulsions with different possible compositions of oil, surfactant/co-surfactant, and water. The ratio of surfactant to co-surfactant (Smix) was changed at 1:1, 1:2 and 2:1 and such mixtures were prepared. The oil in the concentration range of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 & 1:9 was mixed with above prepared Smix .

Applying the aqueous titration method, distilled water was titrated drop by drop with 5% increment to the oil and Smix mixture & vortexed for 2-3 minutes at ambient temperature. After each addition, the mixture was examined for the appearance. The end point of the titration was the point where the solution becomes cloudy or turbid. The quantity of the aqueous phase required to make the mixture turbid was noted. The percentages of the different incorporated components were then calculated and the same procedure was followed for the other Smix ratios to plot the pseudo-ternary phase diagram.[34]

Pseudo- ternary phase diagrams were constructed with Sigma Plot Software Version 11 (Systate Software, Inc). The clear ME zones were identified and marked.

4.3.2 Preparation of dapsone loaded microemulsion[35]

Appropriate amount of oil, surfactant and co surfactant was mixed together in accordance to ME domain, and equilibrated with gentle vortex shaking to get the initial concentrate. Then appropriate Dapsone was dissolved in the initial concentrate under ultra-sonication. Then water was added in the resulting mixture with 5% increment at room temperature.

Drug name	Dose
Dapsone	5% or 50 mg/ml

4.3.3 Evaluation of optimized microemulsion

4.3.3.1 pH[36]

The pH was measured for each formulation using a pH meter, which was calibrated before use with buffered solutions of pH 4 and pH 7.

4.3.3.2 Viscosity[36]

The rheological property of the microemulsion formulations was evaluated using small sample adaptor and spindle no. 18 of the Brookfield Viscometer (LVDV-I Prime model). The viscosity was measured at 0.5 rpm speed. Evaluations were conducted in triplicate.

4.3.3.3 Particle size distribution[37]

The particle size distribution of the oil droplets in the microemulsion was analyzed using a Dynamic Light Scattering (DLS) technique using Microtrec Nanotrec® 525 without dilution at 25^{o}C.

DLS technique, also known as Photon Correlation Spectroscopy, is one of the most widely used methods to measure the size of nano range. This technique assumes that all the particles are in Brownian motion in the solution and that all the particles are very small and spherical. Scattering of light (normally a laser) takes place when particles come in the path of light. The particle size can be determined based on the physical properties of the scattered light: the angular distribution, frequency shift, the polarization and the intensity of the light.

4.3.3.4 Percentage transmittance[38]

The percent transmittance of the system was measured using colorimeter. % transmission was set to zero using filter and % transmission was set to 100% using transparent cuvette filled with water. Then, different microemulsion samples were kept in the transparent cuvette and % transmission was noted.

4.3.3.5 Conductivity and zeta potential[38]

Conductivity and zeta potential of the microemulsion formulations was determined at 25^{o}C using Malvern Zetasizer.

4.3.3.6 Drug content[37]

Drug content in formulation was determined by dissolving 100µl quantity of formulation in 10 ml of methanol. The solution was then filtered through 0.45µm membrane filter and analyzed for Dapsone content by UV-visible spectrophotometer at 295 nm.

4.3.3.7 Determination of drug solubility in the prepared microemulsion

The solubility of Dapsone in microemulsion was determined as follow:

Excess amount of Dapsone was added in 3 g of each of the previously prepared ME in 10-ml-capacity stoppered vials. The resultant mixture was mixed initially by vortex mixer then, all the vials were shaken in the shaker bath for 24 h at 25°C. Afterwards, centrifugation was done at 4000 rpm for 10 min and the concentration of Dapsone in the supernatant was determined by UV spectrophotometer after appropriate dilution with methanol at λ_{max} 295nm. The plain microemulsion without Dapsone with the same composition was taken as blank after appropriate dilution with methanol.[39]

4.3.3.8 Dilution potential

The prepared formulation was diluted 10 times with continuous media and the effect of dilution on occurrence of phase separation was noted via visual determination.[37]

4.3.3.9 Transmission electron microscopy (TEM) of the optimized dapsone loaded microemulsion

The morphology of the optimized ME systems was observed using transmission electron microscopy. A drop of each ME was placed on a copper grid and the excess amount was removed with a filter paper. One drop of 1 % aqueous solution of phosphotungistic acid (PTA) was added onto the grid and left for 1-2 minutes to allow staining. The excess was removed with a filter paper. The grid was finally examined under the transmission electron microscope (Tecnai 20, Phillips, Holland).[37]

4.3.3.10 In-Vitro permeation study

The study was carried out using a Franz Diffusion cell. Here, a cellophane membrane previously soaked in phosphate buffer 7.4 was used. The donor compartment was filled with 1 g of microemulsion. The receptor compartment was filled with 20 ml of Phosphate Buffer pH 7.4 and the permeation study was carried out for 8 hours. At 0.5, 1, 2, 3, 4, 5, 6, 7 & 8 hours, 3 ml aliquots from acceptor compartment were withdrawn and

appropriately diluted if required. After each withdrawal, the volume of receptor compartment was compensated by 3 ml of fresh phosphate buffer saline pH 7.4. The temperature of assembly was kept constant at $37^0 \pm 0.05^0$C and the volume of receiver compartment was constantly stirred using magnetic stirrer. The concentration of drug in the withdrawn samples were checked with the help of UV-Visible spectrophotometer. [40]

4.3.3.11 Stability assessment

Stability of microemulsion, both as a function of time and storage temperature was routinely evaluated by visual inspection of the samples initially on a daily and later on a weekly basis. Stable systems were identified as those free of any physical change, such as phase separation, flocculation or precipitation. Particle size of the microemulsion upon storage was also determined to assess microemulsion stability in terms of drastic changes in the mean droplet diameter due to droplet coalescence or aggregation. Stability was monitored at ambient temperature.

Physical stability of optimized microemulsion was also checked by centrifugation at 12000 rpm for 30 mins. Occurrence of phase separation of the microemulsion on centrifugation suggests that the system was not stable.

The optimized microemulsion formulation was stored at 4°C and 45°C for 1 months and samples were evaluated for physicochemical parameters like clarity, globule size and drug content after 1 month.[41]

4.3.4 Formulation of microemulsion based hydrogel

Carbopol 934 was selected as a gelling agent due to its widespread use in pharmaceutical formulations and fast dispersion in water. Carbopol 934 was allowed to hydrate in sufficient quantity of water for 24 h at room temperature. Further, microemulsion containing Dapsone was gradually added to the Carbopol 934 dispersion under magnetic stirring. The dispersion was neutralized with triethanolamine to obtain microemulsion based hydrogel of Dapsone (MBG) with 0.5, 0.75, 1, 1.5 & 2% concentration. The final MBG formulations contained 5% (w/v) Dapsone.[42]

4.3.5 Characterization and optimization of microemulsion based hydrogel of dapsone

4.3.5.1 pH measurements

The pH was measured for each formulation using a pH meter, which was calibrated before use with buffered solutions of pH 4 and 7.[38]

4.3.5.2 Drug content

Drug content in formulation was determined by dissolving 10 mg quantity of formulation in 10 ml of methanol. The solution was then filtered through 0.45µm membrane filter and analyzed for Dapsone content by UV-visible spectrophotometer at 295 nm.[40]

4.3.5.3 Rheological studies

The viscosity of the optimized formulation was determined at different angular velocities at 30±1 ^{0}C using of the Brookfield Viscometer. A typical run involved changing the angular velocity from 0.5 to 50 rpm at a controlled ramp speed with 10, 20 30 & 50 rpm. After 6 s at 0.5 rpm, the velocity was successively increased to 50 rpm, with a similar period at each speed. The angular velocity was then decreased (50-0.5 rpm) for a similar period of 6 s. The average of two readings was used to calculate the viscosity. Evaluations were conducted in triplicate.[43]

4.3.5.4 Texture analysis

Gel strength was determined using a Brookfield Texture Analyzer (USA) in compression mode. Different formulations were transferred into cylindrical holder (figure 4.7) and care was taken to avoid the introduction of air into the samples. A cylindrical analytical probe (38 mm diameter) was forced down into each sample at a defined rate (20 mm/min) and to a defined depth (10 mm). At least three replicate analyses of each sample were performed for each formulation. From the resulting load–time plots, the gel strength (the maximum force required to attain a given deformation i.e. peak load) and adhesive force (the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe) were derived.[44]

Table 4.11Various parameters for texture analyzer

Test type:	Compression
Trigger point:	5g
Target value:	10mm
Test speed:	20mm/min
Probe:	38 mm

Figure 4.7Brookfield Texture Analyzer

4.3.5.5 In-Vitro permeation study

The study was carried out using a Franz Diffusion cell. Here, a cellophane membrane previously soaked in phosphate buffer Saline (pH 7.4) was used. The donor compartment was filled with 1 g of microemulsion based hydrogel. The acceptor compartment was filled with 20 ml of Phosphate Buffer pH 7.4 and the permeation was carried out for 8

hours. At 0.5, 1, 2, 3, 4, 5, 6, 7 & 8 hours, 2ml aliquots from acceptor compartment were withdrawn and appropriately diluted if required. After each withdrawal, the volume of receptor compartment was compensated by 2ml of fresh phosphate buffer saline pH 7.4. The temperature of assembly was kept constant at $37^0 \pm 0.05^0$C and the volume of receiver compartment was constantly stirred using magnetic stirrer at 200 rpm. The concentration of drug in the withdrawn samples was checked with the help of UV-Visible spectrophotometer.[45]

4.3.5.6 Release kinetic of the optimized batch[46]

In order to analyze the release mechanism, several release models were tested such as:

Zero Order : $Q_t = Q_0 + K_0 t$

Q_t = Amount of drug released at time t

K_o = Apparent diffusion rate constant or zero order release constant

Q_o = Initial concentration of drug in the solution resulting from burst effect

First Order : $\ln Q_t = \ln Q_o + K_1 t$

Q_t = Amount of drug released at time t

Q_o = Initial concentration of drug in the dosage form

K_l = First order release constant

Higuchi : $Q_t = KH \sqrt{t}$

Q_t = Amount of drug released at time t

KH = Higuchi release rate constant

Hixon – Crowell : $Q_o^{1/3} - Q_t^{1/3} = K_s t$

Q_o = Initial concentration of drug in the dosage form.

Q_t = Amount of drug remaining in the dosage form at time t

K_s = Constant incorporating the surface or volume relationship

Korsmeyer – Peppas : $Q_t / Q_\infty = Kk\ t_n$

Kk = Constant incorporating structural and geometric characteristic of the drug dosage form

n = Release exponent indicative of the drug release mechanism

4.3.5.7 Skin irritancy test

Skin irritation potential of Microemulsion based hydrogels were assessed by carrying out *Draize primary skin irritation test* on albino rabbits. New Zealand white rabbits (weighing 2.5–3 kg) were acclimatized for 7 days before the study. The fur from the dorsal surface of rabbits was removed with hair remover without damaging the skin, 24 h prior to the experiment. Just prior to the test, the rabbits will be randomly divided into two groups, one intact-skin group and one skin-injury group, obtained by scarifying intact skin until capillary hemorrhage. For each group, animals were divided into three groups: Group 1: 0.9% (w/v) NaCl solution (Control); Group 2: Dapsone loaded microemulsion; Group 3: Microemulsion based hydrogel of dapsone. The formulations were applied to approximately 2 square in area of the skin. The animals were then returned to their cages and were examined at 24, 48 and 72 h after the application of formulation. The sites were inspected for dermal reactions such as erythema and edema. The mean erythemal and edemal scores were recorded on the basis of degree of severity: no erythema/edema = 0, slight erythema/edema = 1, moderate erythema/edema = 2, severe erythema/edema = 3.

$$Average\ irritation\ scores = \frac{erythema\ recation\ scores + dropsy\ reaction\ scores}{amount\ of\ animals}$$

The intensity criterion of skin irritation followed the scheme that scores of <0.5 meant no irritation, 0.5–3 for slight irritation, >6 showed severe irritation.[41]

4.3.5.8 Stability assessment

Physical stability of optimized MBG was checked by centrifugation at 12000 rpm for 30 minutes.

The optimized MBG formulation was stored at 4°C and 45°C for 1 months and samples were evaluated for physicochemical parameters like clarity, globule size and drug content after 1 month.[38]

4.4 Preliminary studies to formulate microemulsion

A selection of components for microemulsion (ME) suitable for pharmaceutical use involves a consideration of their toxicity and, if the systems are to be used topically, their irritation and sensitivity properties. The ionic surfactants are generally too toxic to be used for preparation of MEs; therefore, non ionic surfactants, such as the poloxamers, polysorbates, polyethylene glycol are preferred.

4.4.1 Solubility studies and selection of surfactant and oil component

The saturated solubility of Dapsone in different oils, surfactants, and co-surfactants was determined visually and then they were quantified. Excess amount of Dapsone was added in 5 g of oils, surfactants and co-solvents in 10-ml-capacity stoppered glass vials and shaken on a shaker for 48 hours at ambient temperature. Suspension was centrifuged at 4000 rpm for 10 min.[37] The concentration of Dapsone was determined by taking 0.1 ml supernatant with micropipette and diluting with methanol by UV spectrophotometer at λ_{max} 295 nm.

Result

Table 4.12Solubility of Dapsone in oils

Sr. No.	Oils	Solubility(mg/ml)*
1	IPM	0.11±0.07
2	Capryol	24.40±1.31
3	OA	0.12±0.14
4	SO	1.60±0.35
5	**Triacetin**	**44.67**±1.68
6	EO	0.16±0.14

*(n=3, mean±SD)

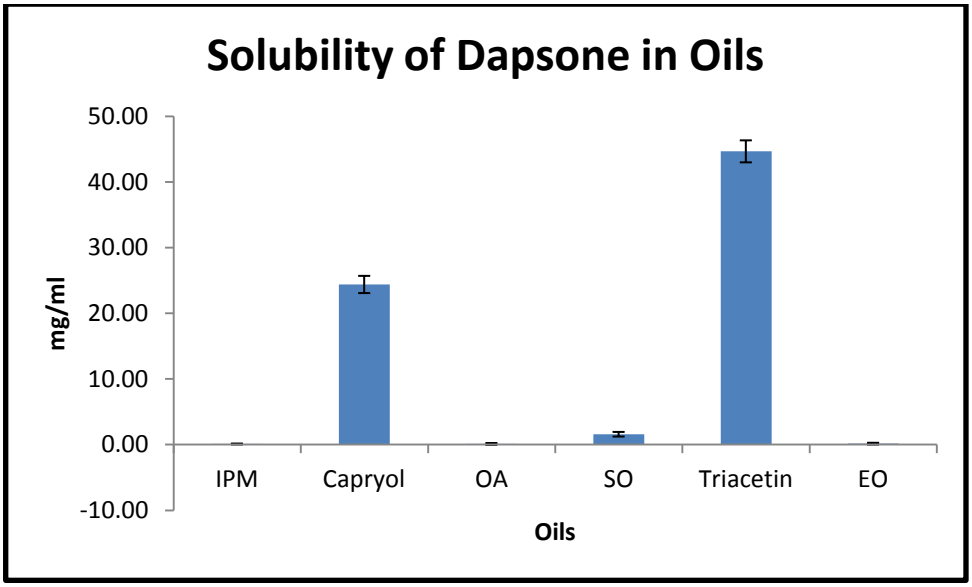


Figure 4.8Solubility of Dapsone in different oils

Table 4.13Solubility of Dapsone in surfactants

Sr. No.	Surfactants	Solubility (mg/ml)*
1	Labrasol	694.54±5.39
2	Labrafil	2.06±1.50
3	Cremophor EL	206.81±14.33
4	Tween 20	48.63±6.19
5	**Tween 80**	**761.84±16.87**
6	Span 80	1.60±0.71
7	Span 20	3.50±1.26
8	PG	26.78±2.21
9	PEG 400	50.06±4.13

*(n=3, mean±SD)

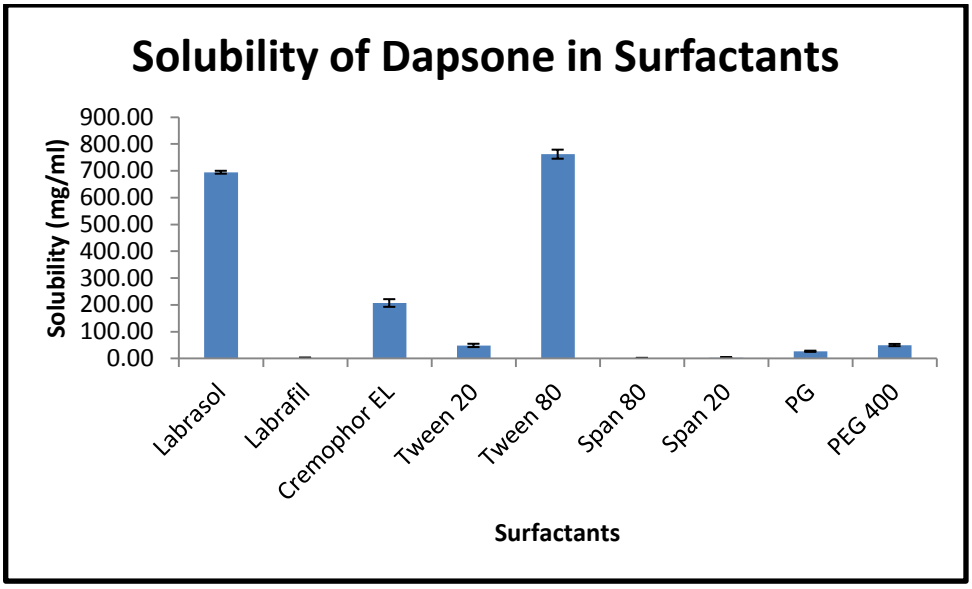


Figure 4.9Solubility of Dapsone in various surfactants

Conclusion

It was found that oil triacetin & surfactant tween 80 had highest solubility of Dapsone with 44.67±1.68mg/ml & 761.84±16.87 mg/ml respectively. Therefore, Triacetin & Tween 80 were selected as oil phase & surfactant respectively.

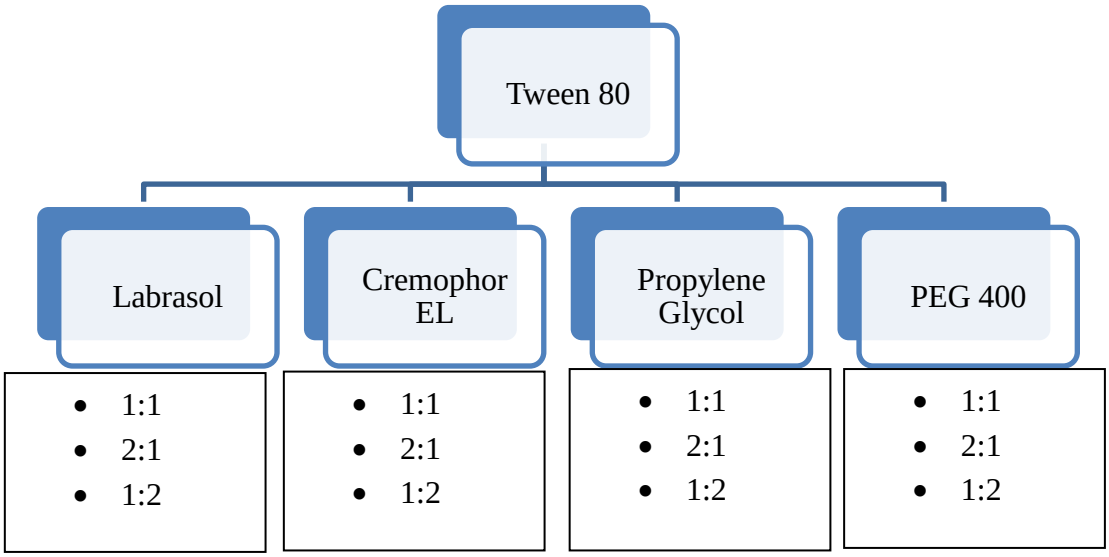
4.4.2 Visual inspection of miscibility of selected surfactant with other co-surfactants

Co-surfactants were screened by miscibility tests with tested surfactant. Visual inspection was carried out and the formation of interphase between two liquids was noted. Weaker the interphase formed, higher the miscibility between them. Cosurfactant with higher miscibility with surfactant was required so as to form large ME domain.

- **Selection of co surfactant**

Some studies have shown that mixtures of two surfactants can enlarge microemulsion region significantly in pseudo-ternary phase diagram.[47] Therefore, mixture of surfactants was used in this research to explore enlargement of ME region. From preliminary studies, Triacetin was selected as oil component and Tween 80 was selected as a surfactant.

Various combinations of Triacetin and Tween 80 with different co-surfactants were screened for the formation of microemulsion. Tween 80 was blended with each co-surfactant in fixed weight ratios (1:2, 1:1, 2:1). Aliquots of each Tween 80 and co-surfactant mixture (S_{mix}) were then mixed with Triacetin at room temperature ($25^{0}C$). The ratio of oil to S_{mix} was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 7:3, 2:8 and 1:9. Then, water was added to above mixture in 5% increment and checked for formation of microemulsion or liquid crystal or gel. The resulting MEs were tightly sealed and stored at ambient temperature. Their physical stability was measured by observing periodically the occurrence of phase separation.[34]



For each Surfactant to Cosurfactant ratio , different Smix-Oil proportions (9:1, 8:2 …1:9) were screened for the formation of microemulsion.

Results

Table 4.14Physical stability of the microemulsion containing different co-surfactant

Ratio	ME containing			
	Labrasol	**Cremophor EL**	**Propylene Glycol**	**PEG 400**
1:2	No phase separation	Phase separation	Phase separation	Phase separation
1:1	No phase separation	No phase separation	Phase separation	Phase separation
2:1	No phase separation	No phase separation	No phase separation	No phase separation

Conclusion

From the above, it can be concluded that microemulsion containing Labrasol had good physical stability for the selected oil (Triacetin) & surfactant (Tween 80). Hence, Labrasol was chosen as co-surfactant.

So, the final optimized composition of the microemulsion was…….

Table 4.15Final composition of blank microemulsion

Sr. No.	Composition	
1	Oil Phase	Triacetin
2	Surfactant	Tween 80
3	Co-surfactant	Labrasol
4	Aqueous Phase	Distilled water

4.4.3 Construction of pseudoternary phase diagrams

The pseudoternary phase diagram for the surfactant to cosurfactant ratio of 1:2 was prepared & shown below. Same was prepared for surfactant to cosurfactant ratio of 1:1 & 2:1. Shaded area shows formation of clear microemulsions.

Table 4.16Composition of microemulsion containing ratio 9:1 of oil:smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	450.00	50.00	5.00	50.00	550.00	81.82	9.09	9.09	100.00
2	450.00	50.00	10.00	100.00	600.00	75.00	8.33	16.67	100.00
3	450.00	50.00	15.00	150.00	650.00	69.23	7.69	23.08	100.00
4	450.00	50.00	20.00	200.00	700.00	64.29	7.14	28.57	100.00
5	450.00	50.00	25.00	250.00	750.00	60.00	6.67	33.33	100.00
6	450.00	50.00	30.00	300.00	800.00	56.25	6.25	37.50	100.00
7	450.00	50.00	35.00	350.00	850.00	52.94	5.88	41.18	100.00
8	450.00	50.00	40.00	400.00	900.00	50.00	5.56	44.44	100.00
9	450.00	50.00	45.00	450.00	950.00	47.37	5.26	47.37	100.00
10	450.00	50.00	50.00	500.00	1000.00	45.00	5.00	50.00	100.00
11	450.00	50.00	55.00	550.00	1050.00	42.86	4.76	52.38	100.00
12	450.00	50.00	60.00	600.00	1100.00	40.91	4.55	54.55	100.00
13	450.00	50.00	65.00	650.00	1150.00	39.13	4.35	56.52	100.00
14	450.00	50.00	70.00	700.00	1200.00	37.50	4.17	58.33	100.00
15	450.00	50.00	75.00	750.00	1250.00	36.00	4.00	60.00	100.00
16	450.00	50.00	80.00	800.00	1300.00	34.62	3.85	61.54	100.00
17	450.00	50.00	85.00	850.00	1350.00	33.33	3.70	62.96	100.00
18	450.00	50.00	90.00	900.00	1400.00	32.14	3.57	64.29	100.00
19	450.00	50.00	95.00	950.00	1450.00	31.03	3.45	65.52	100.00
20	450.00	50.00	100.00	1000.00	1500.00	30.00	3.33	66.67	100.00

Table 4.17Composition of microemulsion containing ratio 8:2 of oil:smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	400.00	100.00	5.00	50.00	550.00	72.73	18.18	9.09	100.00
2	400.00	100.00	10.00	100.00	600.00	66.67	16.67	16.67	100.00
3	400.00	100.00	15.00	150.00	650.00	61.54	15.38	23.08	100.00
4	400.00	100.00	20.00	200.00	700.00	57.14	14.29	28.57	100.00
5	400.00	100.00	25.00	250.00	750.00	53.33	13.33	33.33	100.00
6	400.00	100.00	30.00	300.00	800.00	50.00	12.50	37.50	100.00
7	400.00	100.00	35.00	350.00	850.00	47.06	11.76	41.18	100.00
8	400.00	100.00	40.00	400.00	900.00	44.44	11.11	44.44	100.00
9	400.00	100.00	45.00	450.00	950.00	42.11	10.53	47.37	100.00
10	400.00	100.00	50.00	500.00	1000.00	40.00	10.00	50.00	100.00
11	400.00	100.00	55.00	550.00	1050.00	38.10	9.52	52.38	100.00
12	400.00	100.00	60.00	600.00	1100.00	36.36	9.09	54.55	100.00
13	400.00	100.00	65.00	650.00	1150.00	34.78	8.70	56.52	100.00
14	400.00	100.00	70.00	700.00	1200.00	33.33	8.33	58.33	100.00
15	400.00	100.00	75.00	750.00	1250.00	32.00	8.00	60.00	100.00
16	400.00	100.00	80.00	800.00	1300.00	30.77	7.69	61.54	100.00
17	400.00	100.00	85.00	850.00	1350.00	29.63	7.41	62.96	100.00
18	400.00	100.00	90.00	900.00	1400.00	28.57	7.14	64.29	100.00
19	400.00	100.00	95.00	950.00	1450.00	27.59	6.90	65.52	100.00
20	400.00	100.00	100.00	1000.00	1500.00	26.67	6.67	66.67	100.00

Table 4.18Composition of microemulsion containing ratio 7:3 of oil: smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	350.00	150.00	5.00	50.00	550.00	63.64	27.27	9.09	100.00
2	350.00	150.00	10.00	100.00	600.00	58.33	25.00	16.67	100.00
3	350.00	150.00	15.00	150.00	650.00	53.85	23.08	23.08	100.00
4	350.00	150.00	20.00	200.00	700.00	50.00	21.43	28.57	100.00
5	350.00	150.00	25.00	250.00	750.00	46.67	20.00	33.33	100.00
6	350.00	150.00	30.00	300.00	800.00	43.75	18.75	37.50	100.00
7	350.00	150.00	35.00	350.00	850.00	41.18	17.65	41.18	100.00
8	350.00	150.00	40.00	400.00	900.00	38.89	16.67	44.44	100.00
9	350.00	150.00	45.00	450.00	950.00	36.84	15.79	47.37	100.00
10	350.00	150.00	50.00	500.00	1000.00	35.00	15.00	50.00	100.00
11	350.00	150.00	55.00	550.00	1050.00	33.33	14.29	52.38	100.00
12	350.00	150.00	60.00	600.00	1100.00	31.82	13.64	54.55	100.00
13	350.00	150.00	65.00	650.00	1150.00	30.43	13.04	56.52	100.00
14	350.00	150.00	70.00	700.00	1200.00	29.17	12.50	58.33	100.00
15	350.00	150.00	75.00	750.00	1250.00	28.00	12.00	60.00	100.00
16	350.00	150.00	80.00	800.00	1300.00	26.92	11.54	61.54	100.00
17	350.00	150.00	85.00	850.00	1350.00	25.93	11.11	62.96	100.00
18	350.00	150.00	90.00	900.00	1400.00	25.00	10.71	64.29	100.00
19	350.00	150.00	95.00	950.00	1450.00	24.14	10.34	65.52	100.00
20	350.00	150.00	100.00	1000.00	1500.00	23.33	10.00	66.67	100.00

Table 4.19Composition of microemulsion containing ratio 6:4 of oil:smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	300.00	200.00	5.00	50.00	550.00	54.55	36.36	9.09	100.00
2	300.00	200.00	10.00	100.00	600.00	50.00	33.33	16.67	100.00
3	300.00	200.00	15.00	150.00	650.00	46.15	30.77	23.08	100.00
4	300.00	200.00	20.00	200.00	700.00	42.86	28.57	28.57	100.00
5	300.00	200.00	25.00	250.00	750.00	40.00	26.67	33.33	100.00
6	300.00	200.00	30.00	300.00	800.00	37.50	25.00	37.50	100.00
7	300.00	200.00	35.00	350.00	850.00	35.29	23.53	41.18	100.00
8	300.00	200.00	40.00	400.00	900.00	33.33	22.22	44.44	100.00
9	300.00	200.00	45.00	450.00	950.00	31.58	21.05	47.37	100.00
10	300.00	200.00	50.00	500.00	1000.00	30.00	20.00	50.00	100.00
11	300.00	200.00	55.00	550.00	1050.00	28.57	19.05	52.38	100.00
12	300.00	200.00	60.00	600.00	1100.00	27.27	18.18	54.55	100.00
13	300.00	200.00	65.00	650.00	1150.00	26.09	17.39	56.52	100.00
14	300.00	200.00	70.00	700.00	1200.00	25.00	16.67	58.33	100.00
15	300.00	200.00	75.00	750.00	1250.00	24.00	16.00	60.00	100.00
16	300.00	200.00	80.00	800.00	1300.00	23.08	15.38	61.54	100.00
17	300.00	200.00	85.00	850.00	1350.00	22.22	14.81	62.96	100.00
18	300.00	200.00	90.00	900.00	1400.00	21.43	14.29	64.29	100.00
19	300.00	200.00	95.00	950.00	1450.00	20.69	13.79	65.52	100.00
20	300.00	200.00	100.00	1000.00	1500.00	20.00	13.33	66.67	100.00

Table 4.20Composition of microemulsion containing ratio 5:5 of oil:smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	250.00	250.00	5.00	50.00	550.00	45.45	45.45	9.09	100.00
2	250.00	250.00	10.00	100.00	600.00	41.67	41.67	16.67	100.00
3	250.00	250.00	15.00	150.00	650.00	38.46	38.46	23.08	100.00
4	250.00	250.00	20.00	200.00	700.00	35.71	35.71	28.57	100.00
5	250.00	250.00	25.00	250.00	750.00	33.33	33.33	33.33	100.00
6	250.00	250.00	30.00	300.00	800.00	31.25	31.25	37.50	100.00
7	250.00	250.00	35.00	350.00	850.00	29.41	29.41	41.18	100.00
8	250.00	250.00	40.00	400.00	900.00	27.78	27.78	44.44	100.00
9	250.00	250.00	45.00	450.00	950.00	26.32	26.32	47.37	100.00
10	250.00	250.00	50.00	500.00	1000.00	25.00	25.00	50.00	100.00
11	250.00	250.00	55.00	550.00	1050.00	23.81	23.81	52.38	100.00
12	250.00	250.00	60.00	600.00	1100.00	22.73	22.73	54.55	100.00
13	250.00	250.00	65.00	650.00	1150.00	21.74	21.74	56.52	100.00
14	250.00	250.00	70.00	700.00	1200.00	20.83	20.83	58.33	100.00
15	250.00	250.00	75.00	750.00	1250.00	20.00	20.00	60.00	100.00
16	250.00	250.00	80.00	800.00	1300.00	19.23	19.23	61.54	100.00
17	250.00	250.00	85.00	850.00	1350.00	18.52	18.52	62.96	100.00
18	250.00	250.00	90.00	900.00	1400.00	17.86	17.86	64.29	100.00
19	250.00	250.00	95.00	950.00	1450.00	17.24	17.24	65.52	100.00
20	250.00	250.00	100.00	1000.00	1500.00	16.67	16.67	66.67	100.00

Table 4.21Composition of microemulsion containing ratio 4:6 of oil:smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	200.00	300.00	5.00	50.00	550.00	36.36	54.55	9.09	100.00
2	200.00	300.00	10.00	100.00	600.00	33.33	50.00	16.67	100.00
3	200.00	300.00	15.00	150.00	650.00	30.77	46.15	23.08	100.00
4	200.00	300.00	20.00	200.00	700.00	28.57	42.86	28.57	100.00
5	200.00	300.00	25.00	250.00	750.00	26.67	40.00	33.33	100.00
6	200.00	300.00	30.00	300.00	800.00	25.00	37.50	37.50	100.00
7	200.00	300.00	35.00	350.00	850.00	23.53	35.29	41.18	100.00
8	200.00	300.00	40.00	400.00	900.00	22.22	33.33	44.44	100.00
9	200.00	300.00	45.00	450.00	950.00	21.05	31.58	47.37	100.00
10	200.00	300.00	50.00	500.00	1000.00	20.00	30.00	50.00	100.00
11	200.00	300.00	55.00	550.00	1050.00	19.05	28.57	52.38	100.00
12	200.00	300.00	60.00	600.00	1100.00	18.18	27.27	54.55	100.00
13	200.00	300.00	65.00	650.00	1150.00	17.39	26.09	56.52	100.00
14	200.00	300.00	70.00	700.00	1200.00	16.67	25.00	58.33	100.00
15	200.00	300.00	75.00	750.00	1250.00	16.00	24.00	60.00	100.00
16	200.00	300.00	80.00	800.00	1300.00	15.38	23.08	61.54	100.00
17	200.00	300.00	85.00	850.00	1350.00	14.81	22.22	62.96	100.00
18	200.00	300.00	90.00	900.00	1400.00	14.29	21.43	64.29	100.00
19	200.00	300.00	95.00	950.00	1450.00	13.79	20.69	65.52	100.00
20	200.00	300.00	100.00	1000.00	1500.00	13.33	20.00	66.67	100.00

Table 4.22Composition of microemulsion containing ratio 3:7 of oil:smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	150.00	350.00	5.00	50.00	550.00	27.27	63.64	9.09	100.00
2	150.00	350.00	10.00	100.00	600.00	25.00	58.33	16.67	100.00
3	150.00	350.00	15.00	150.00	650.00	23.08	53.85	23.08	100.00
4	150.00	350.00	20.00	200.00	700.00	21.43	50.00	28.57	100.00
5	150.00	350.00	25.00	250.00	750.00	20.00	46.67	33.33	100.00
6	150.00	350.00	30.00	300.00	800.00	18.75	43.75	37.50	100.00
7	150.00	350.00	35.00	350.00	850.00	17.65	41.18	41.18	100.00
8	150.00	350.00	40.00	400.00	900.00	16.67	38.89	44.44	100.00
9	150.00	350.00	45.00	450.00	950.00	15.79	36.84	47.37	100.00
10	150.00	350.00	50.00	500.00	1000.00	15.00	35.00	50.00	100.00
11	150.00	350.00	55.00	550.00	1050.00	14.29	33.33	52.38	100.00
12	150.00	350.00	60.00	600.00	1100.00	13.64	31.82	54.55	100.00
13	150.00	350.00	65.00	650.00	1150.00	13.04	30.43	56.52	100.00
14	150.00	350.00	70.00	700.00	1200.00	12.50	29.17	58.33	100.00
15	150.00	350.00	75.00	750.00	1250.00	12.00	28.00	60.00	100.00
16	150.00	350.00	80.00	800.00	1300.00	11.54	26.92	61.54	100.00
17	150.00	350.00	85.00	850.00	1350.00	11.11	25.93	62.96	100.00
18	150.00	350.00	90.00	900.00	1400.00	10.71	25.00	64.29	100.00
19	150.00	350.00	95.00	950.00	1450.00	10.34	24.14	65.52	100.00
20	150.00	350.00	100.00	1000.00	1500.00	10.00	23.33	66.67	100.00

Table 4.23Composition of microemulsion containing ratio 8:2 of oil:smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	100.00	400.00	5.00	50.00	550.00	18.18	72.73	9.09	100.00
2	100.00	400.00	10.00	100.00	600.00	16.67	66.67	16.67	100.00
3	100.00	400.00	15.00	150.00	650.00	15.38	61.54	23.08	100.00
4	100.00	400.00	20.00	200.00	700.00	14.29	57.14	28.57	100.00
5	100.00	400.00	25.00	250.00	750.00	13.33	53.33	33.33	100.00
6	100.00	400.00	30.00	300.00	800.00	12.50	50.00	37.50	100.00
7	100.00	400.00	35.00	350.00	850.00	11.76	47.06	41.18	100.00
8	100.00	400.00	40.00	400.00	900.00	11.11	44.44	44.44	100.00
9	100.00	400.00	45.00	450.00	950.00	10.53	42.11	47.37	100.00
10	100.00	400.00	50.00	500.00	1000.00	10.00	40.00	50.00	100.00
11	100.00	400.00	55.00	550.00	1050.00	9.52	38.10	52.38	100.00
12	100.00	400.00	60.00	600.00	1100.00	9.09	36.36	54.55	100.00
13	100.00	400.00	65.00	650.00	1150.00	8.70	34.78	56.52	100.00
14	100.00	400.00	70.00	700.00	1200.00	8.33	33.33	58.33	100.00
15	100.00	400.00	75.00	750.00	1250.00	8.00	32.00	60.00	100.00
16	100.00	400.00	80.00	800.00	1300.00	7.69	30.77	61.54	100.00
17	100.00	400.00	85.00	850.00	1350.00	7.41	29.63	62.96	100.00
18	100.00	400.00	90.00	900.00	1400.00	7.14	28.57	64.29	100.00
19	100.00	400.00	95.00	950.00	1450.00	6.90	27.59	65.52	100.00
20	100.00	400.00	100.00	1000.00	1500.00	6.67	26.67	66.67	100.00

Table 4.24Composition of microemulsion containing ratio 1:9 of oil:smix

Sr. No.	Oil (µl)	Smix (µl)	Water (%)	Water (µl)	Total (ml)	Oil (%)	Smix (%)	Water (%)	Total (%)
1	50.00	450.00	5.00	50.00	550.00	9.09	81.82	9.09	100.00
2	50.00	450.00	10.00	100.00	600.00	8.33	75.00	16.67	100.00
3	50.00	450.00	15.00	150.00	650.00	7.69	69.23	23.08	100.00
4	50.00	450.00	20.00	200.00	700.00	7.14	64.29	28.57	100.00
5	50.00	450.00	25.00	250.00	750.00	6.67	60.00	33.33	100.00
6	50.00	450.00	30.00	300.00	800.00	6.25	56.25	37.50	100.00
7	50.00	450.00	35.00	350.00	850.00	5.88	52.94	41.18	100.00
8	50.00	450.00	40.00	400.00	900.00	5.56	50.00	44.44	100.00
9	50.00	450.00	45.00	450.00	950.00	5.26	47.37	47.37	100.00
10	50.00	450.00	50.00	500.00	1000.00	5.00	45.00	50.00	100.00
11	50.00	450.00	55.00	550.00	1050.00	4.76	42.86	52.38	100.00
12	50.00	450.00	60.00	600.00	1100.00	4.55	40.91	54.55	100.00
13	50.00	450.00	65.00	650.00	1150.00	4.35	39.13	56.52	100.00
14	50.00	450.00	70.00	700.00	1200.00	4.17	37.50	58.33	100.00
15	50.00	450.00	75.00	750.00	1250.00	4.00	36.00	60.00	100.00
16	50.00	450.00	80.00	800.00	1300.00	3.85	34.62	61.54	100.00
17	50.00	450.00	85.00	850.00	1350.00	3.70	33.33	62.96	100.00
18	50.00	450.00	90.00	900.00	1400.00	3.57	32.14	64.29	100.00
19	50.00	450.00	95.00	950.00	1450.00	3.45	31.03	65.52	100.00
20	50.00	450.00	100.00	1000.00	1500.00	3.33	30.00	66.67	100.00

Table 4.25Composition of microemulsions for Smix ratio 2:1

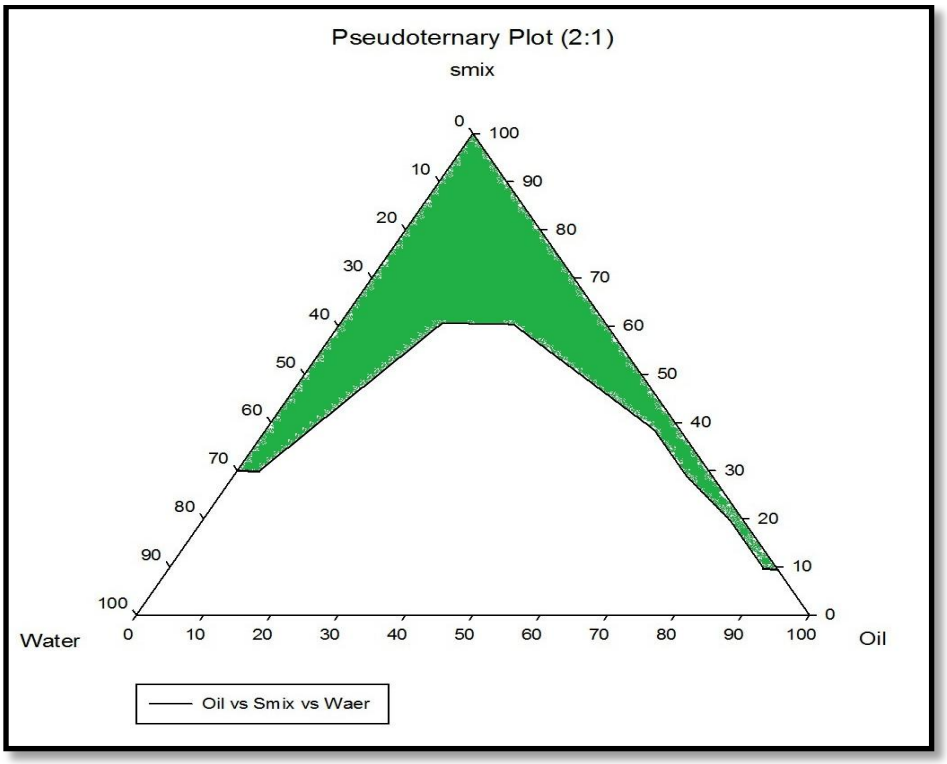
Sr. No.	Oil (%)	Smix. (%)	Water (%)
1	88.23	9.80	1.96
2	78.43	19.61	1.96
3	67.31	28.85	3.85
4	57.69	38.46	3.84
5	46.29	46.29	7.41
6	35.71	53.57	10.71
7	25.86	60.34	13.79
8	15.15	60.61	24.24
9	3.33	30.00	66.67

Table 4.26Composition of microemulsions for Smix ratio 1:1

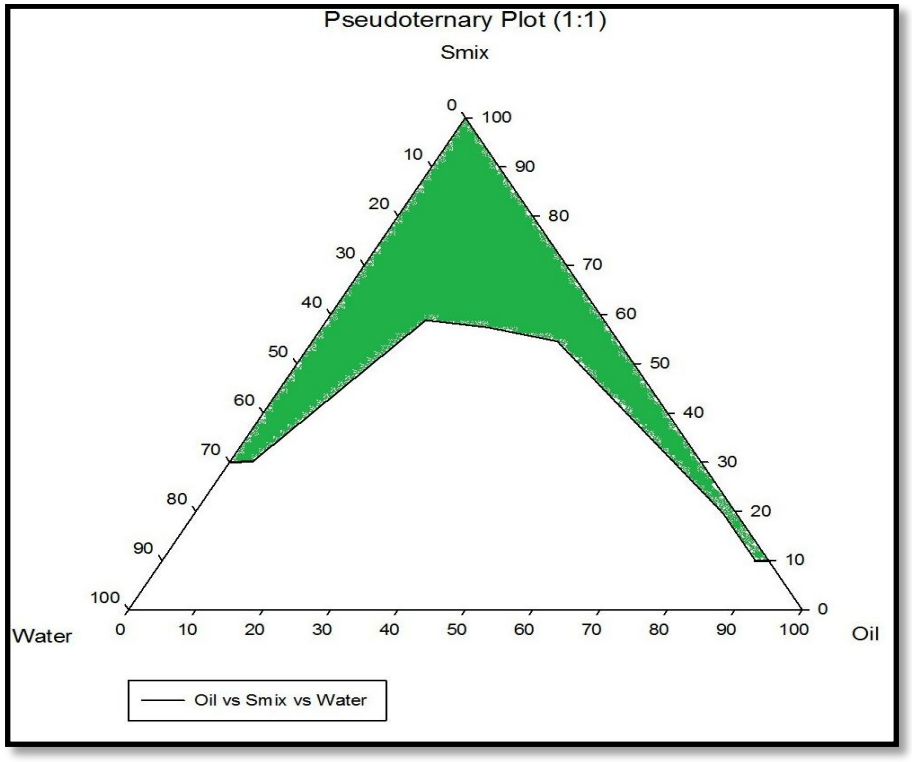
Sr. No.	Oil (%)	Smix. (%)	Water (%)
1	88.24	9.80	1.96
2	78.43	19.61	1.96
3	67.31	28.84	3.84
4	56.60	37.74	5.66
5	46.29	46.29	7.41
6	36.36	54.55	9.09
7	24.59	57.37	18.032
8	14.71	58.82	26.47
9	3.33	30.00	66.67

Table 4.27Composition of microemulsions for Smix ratio 1:2

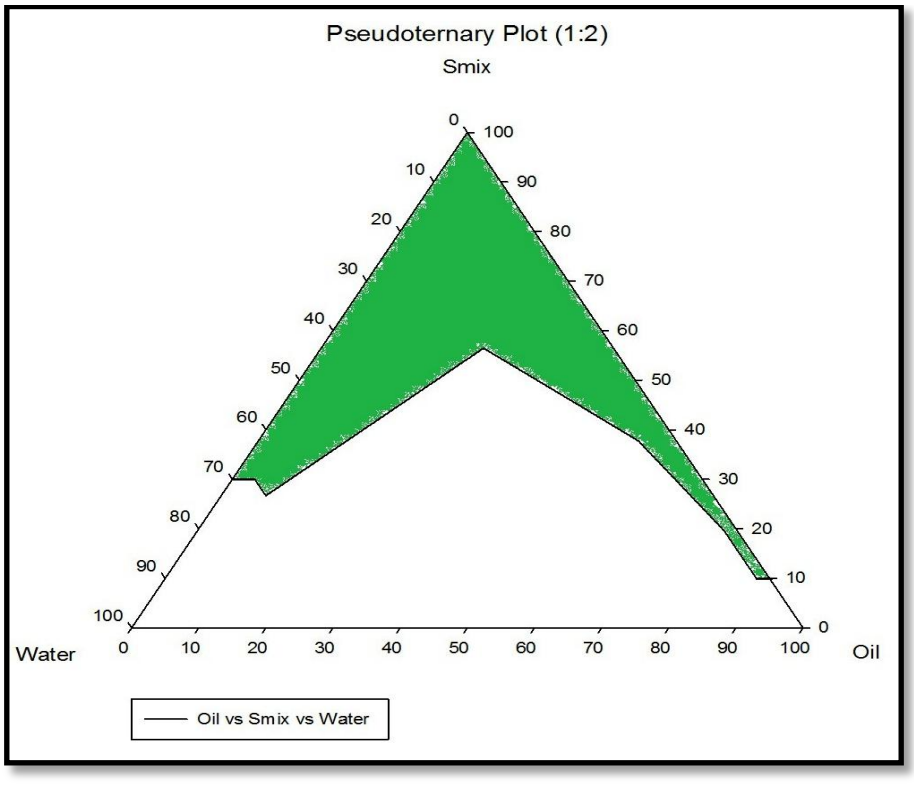
Sr. No.	Oil (%)	Smix. (%)	Water (%)
1	88.23	9.80	1.96
2	78.43	19.61	1.96
3	67.31	28.85	3.85
4	56.60	37.74	5.66
5	44.64	44.64	10.71
6	33.89	50.85	15.25
7	24.19	56.45	19.35
8	10.00	40.00	50.00
9	3.3333	30.00	66.67



(a)



(b)



(c)

Figure 4.10Pseudo-ternary Phase diagrams of Smix (a) 2:1, (b) 1:1, (c) 1:2.

C**onclusion**

The optimum microemulsion region was found when microemulsion formed using surfactant to co-surfactant ratio of 1:2. Therefore, the microemulsion with surfactant to co-surfactant ratio of 1:2 was selected for the further studies.

4.5 Formulation optimization of microemulsion by D-optimal design

4.5.1 Introduction

4.5.1.1 Optimal mixture design

The Design of experiment (DOE) technique provides an efficient means of optimizing the processes as well as determining the optimal formulation of a specific mixture. The levels of experimental design could not be chosen arbitrarily, where the composition is a factor of interest, because the sum of all the fractions of components equals to unity. Classical experimental designs such as full factorial designs do not consider specific experimental constraints, and thus they lack prediction power. The main distinction between Mixture DOE and other types of DOE is that with the former, the input variables or components are non-negative proportionate amounts of the mixture. If the relationship is expressed as fractions of the mixture, the sum must be equal to one. Furthermore, in mixture DOE, the measured response is assumed to depend only on the relative proportions of the ingredients or components in the mixture and not on the volume of the mixture. In most mixture designs, there are some bound restrictions on the component proportions (Xj) which limit the feasible space of variables between the lower (Lj) and upper (Uj) constraints. The general form of the constrained mixture problem is as below

$$\Sigma_f X_f = 1 \text{ and } L_f \leq X_f \leq U_f \quad \textbf{(i)}$$

In mixture DOE, optimizing the response variable (Y) is desirable based on the experimental values of the independent factors, X_f.

$$Y = f(X_1, X_2, X_3, X_4, \ldots X_n) \quad \textbf{(ii)}$$

- A mixture DOE typically involves the following 6 steps:
  1. Selection of a suitable technique of mixture DOE: There are several available mixture design techniques. Based on the ranges of the independent variables or bound restrictions, an appropriate technique should be selected. Simplex designs can be used whenever the components form a simplex region; in other words,

when the ranges of independent variables are equal. On the other hand, an optimal design is the right selection when the bound restrictions are non-simplex or the same size.

2. Identification of the name, unit and the bound restrictions of mixture components.
3. Identification of the name and unit of the responses.
4. To propose an appropriate model to find the relationship between the responses and the mixture components.
5. To run all the determined experiments designed by the model one by one according to the run numbers.
6. To enter the achieved responses from the experimental results.

- There are three types of optimal design in the Design Expert software namely D-optimal, A-optimal, and IV-optimal.
  - **D-optimality** is desirable for the factorial and screening designs to identify the most crucial variables; the algorithm picks points that minimize the volume of the confidence ellipsoid of the coefficients.
  - **A-optimal design** minimizes the average variance of the polynomial coefficients.
  - **'IV' optimal or 'I' optimal design** makes use of an integrated variance criterion that minimizes the average variance of the responses throughout a specific region of interest. Since the IV-optimal designs provide lower average prediction variance across the region of experimentation, the IV-optimality was determined as the desirable design type.

For instance, the possible experimental runs are displayed by an equilateral triangle in a three-component mixture design, where the real value of the responses could be then represented as distance orthogonal to factor space. Moreover, the range covering the components is limited in the design space, which could be represented by irregular polyhedron delimited by extreme vertices. In such cases, D-optimal design would be appropriate, as maximum prediction power could be obtained in selected set of experimental runs, minimizing the variance associated with the estimates of the coefficients in the model.[48]

4.5.1.1.1 D- optimal mixture design

In the design of experiments for estimating statistical models, optimal designs allow parameters to be estimated without bias and with minimum-variance. A non-optimal

design requires a greater number of experimental runs to estimate the parameters with the same precision as an optimal design. In practical terms, optimal experiments can reduce the costs of experimentation.

Advantages of optimal designs

1. Optimal designs reduce the costs of experimentation by allowing statistical models to be estimated with fewer experimental runs.
2. Optimal designs can accommodate multiple types of factors, such as process, mixture, and discrete factors.
3. Designs can be optimized when the design-space is constrained, for example, when the mathematical process-space contains factor-settings that are practically infeasible (e.g. due to safety concerns).

- D-optimal mixture experimental study was designed based on a three component system: the oil phase X1 (Triacetin), Smix X2 (a mixture of Tween 80/Labrasol®, 1:2 w/w) and aqueous phase X3 (water). The total concentration of the three components summed to 100%. The constrains were chosen on the basis of pseudo-ternary phase diagrams and preliminary experiments, which is as follows: The amount of oil was chosen in the range 5-15 %. The Smix ranged from 50% to 80%. Since hydration of the stratum corneum significantly affects penetration of drug into the skin, water range was selected to be 10–40%.

Table 4.28Chosen constrains of D-optimal design space

5 ≤	**X1**	**≥ 15**
50 ≤	X2	≥ 80
10 ≤	X3	≥ 40

- The globule size (nm) (Y1) and solubility of Dapsone in the microemulsion (Y2) were selected as the dependent variables (responses). The globule size (Y1) was determined using photon correlation spectroscopy (Microtrec Nanotrec® 525) at 25°C. The solubility of Dapsone in microemulsion (Y2) was determined by extracting Dapsone in methanol, which was analyzed by UV spectrometry.

- The Design-Expert® software (Version 7; Stat-Ease Inc., MN, USA) was employed to treat the responses. The software selected a set of candidate points as a base design consisting of factorial points (high and low level from the constraints on each factor), centers of edges, constraint plane centroids, axial check point, and an overall center point. The base design consisted of 16 run (Table 4.29).[40]

Table 4.29Formulations of mixture design and their characterization results

Formulation	X1:Triacetin (%)	X2:Smix (%)	X3:Water (%)	Y1:Droplet Size (nm)	Y2:Drug Solubility (mg/ml)
1	10.08	74.13	15.79	65.50	286.78
2	14.30	75.70	10.00	91.8	297.27
3	5.07	80.00	14.93	51.2	226.44
4	10.96	56.26	32.78	66.9	163.90
5	14.30	75.70	10.00	97	299.246
6	5.10	54.90	40.00	61.5	124.545
7	15.00	59.29	25.71	110.01	302.301
8	11.58	50.00	38.42	79.88	271.596
9	5.00	62.34	32.66	18.10	162.79
10	11.58	50.00	38.42	84.40	270.15
11	5.00	73.83	21.17	10.13	239.509
12	15.00	68.56	16.44	101.30	310.98
13	15.00	59.29	25.71	100.00	300.51
14	9.32	66.66	24.02	63.80	291.933
15	5.07	80.00	14.93	53.12	283.59
16	5.10	54.90	40.00	62.20	121.62

As shown in the table, lowest globule size (Y1) was obtained with run 11 (X1: 5%, X2: 73.83%, X3: 21.13%), where proportion of water was lowest and the proportion of oil and Smix were intermediate. It implied that microemulsion globules tend to constrict and get stabilized with higher amount of Smix when proportion of oil decreases significantly. The

highest globule size was 110.01 nm. The wide range of globule size in the design space implied the effect of selected independent variables on the globule size. On the contrary, solubility (Y2) was highest when proportion of oil was at high level (run 1; X1: 15%, X268.56: %, X3: 16.44%). This was due to the higher oil requirements of the system to solubilize Dapsone. Graphical presentation of the data helped in interpretation of results Shown in Fig. 4.11 & 4.12.

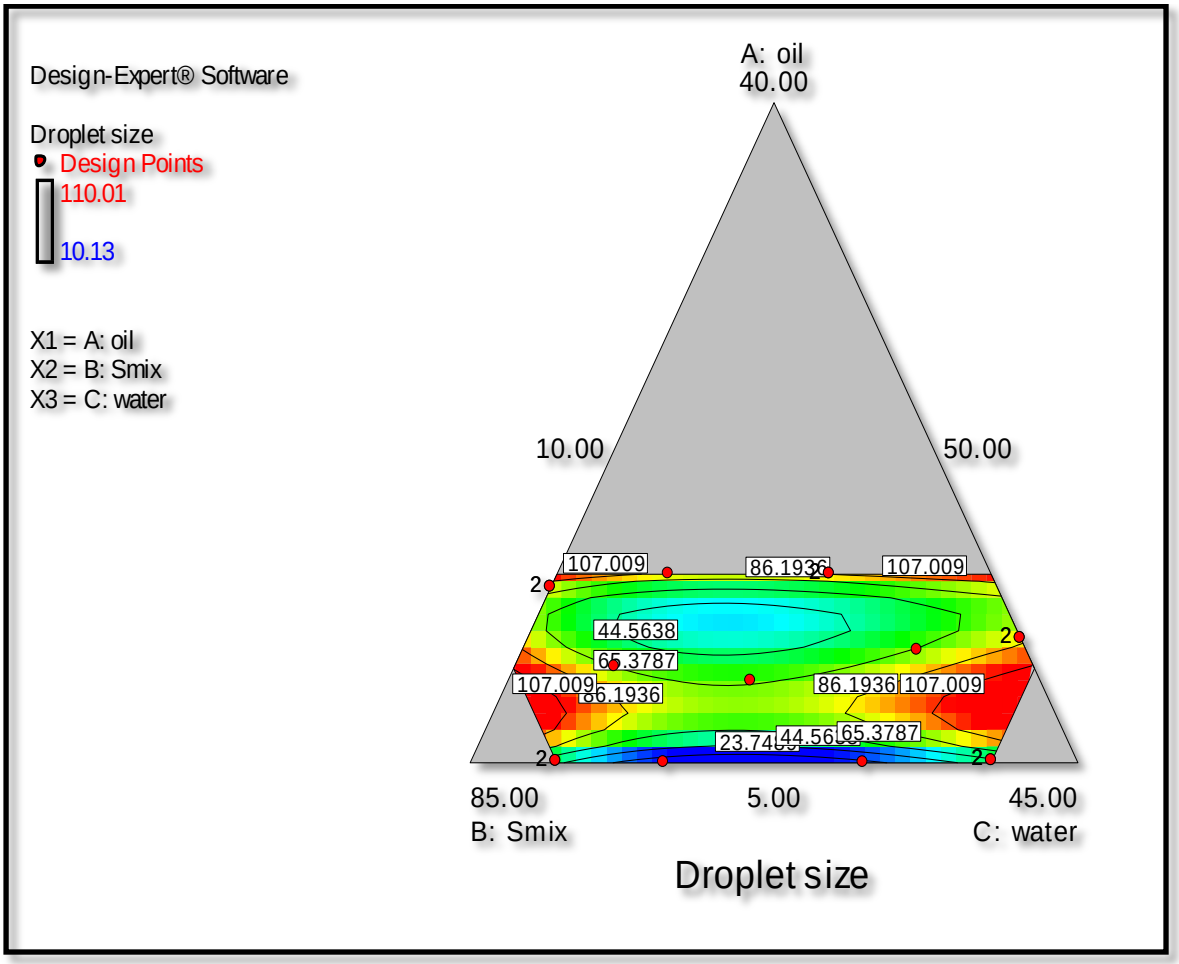


Figure 4.11Contour plot of effect of variables on Droplet size

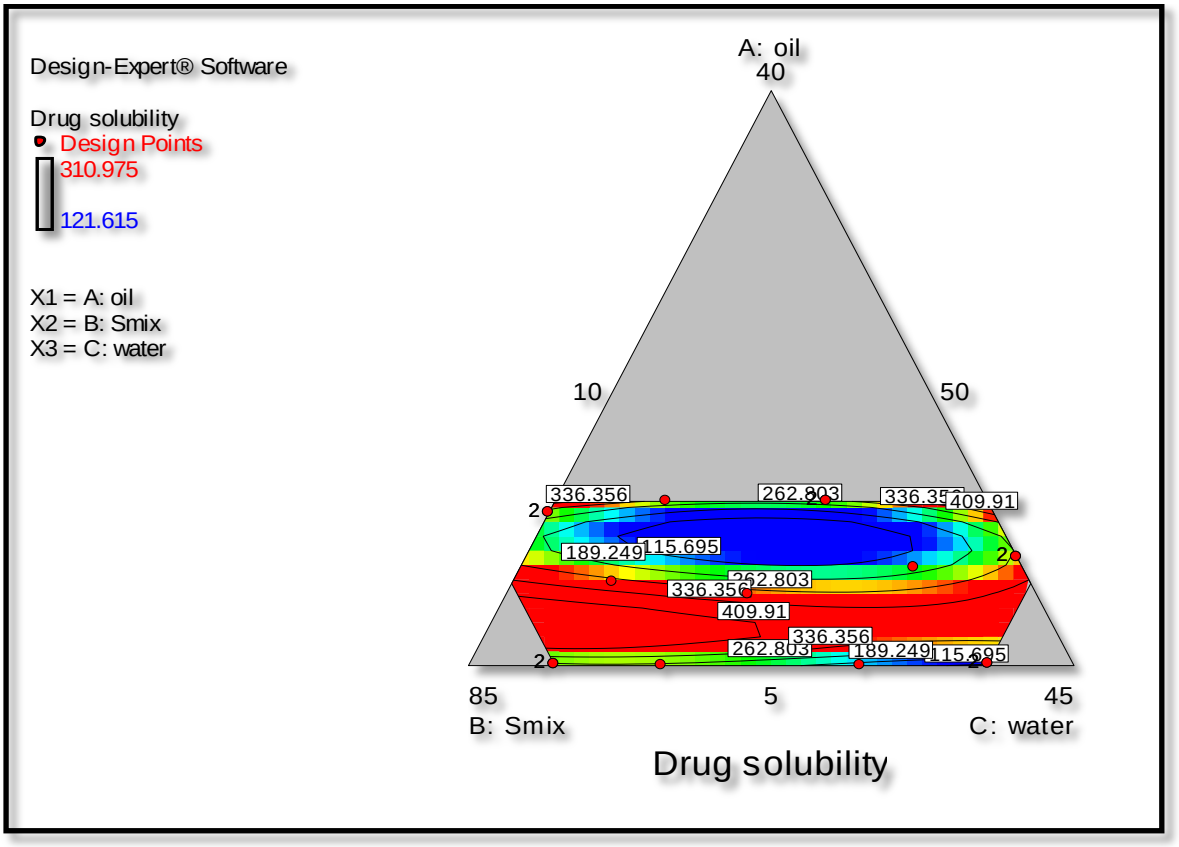


Figure 4.12Contour plot of effect of variable on drug solubility

Suitable mathematical models of the mixture design such as linear, quadratic, cubic and special cubic models were analyzed by the software. Significance of the model was determined by comparisons of statistical parameters like standard deviation (SD), R^2, adjusted R^2, predicted R^2, and predicted residual error sum of squares (PRESS). The best model was decided on the basis of higher values of adjusted R^2 and predicted R^2. Moreover, the difference between adjusted R^2 and predicted R^2 should not be more than 0.2 in order to ensure the validity of the model. PRESS demonstrates the excellence of model fitting which should be small for the best model. Table represents the model summary statistics of the measured responses. [37]

Table 4.30Model summary statistics of the measured responses

Response	Model	SD	R^2	Adjusted R^2	Predicted R^2	PRESS
Y1	Linear	14.37	0.7783	0.7441	0.6542	4187.02
	Quadratic	13.68	0.6193	0.8455	0.7683	4608.59
	Special cubic	5.77	0.9752	0.9587	0.9244	914.94
	Cubic	3.56	0.9937	0.9843	0.9759	291.84
Y2	Linear	39.85	0.6905	0.6429	0.5335	31112.87
	Quadratic	34.54	0.8211	0.7317	0.5915	27242.49
	Special cubic	35.93	0.8258	0.7097	0.5717	28562.55
	Cubic	17.47	0.9725	0.9314	0.7807	14626.61

- For both Y1 & Y2, cubic model showed a superior fit and was selected as best model. The adjusted R^2 and predicted R^2 values of the selected models were comparatively higher than other models for both the responses. Also, PRESS values of quadratic model of both the responses were smallest. Analysis of Variance (ANOVA) was carried out by the software to generate the polynomial equation of the responses. A factor was considered as significantly influencing the response because the magnitudes of the effects significantly deviate from zero and the p-value is less than 0.05.
- For response Y1, all the factors were significant, while for response Y2, the factors X2X3 (X2-X3) were not significant, as their p-values were greater than 0.05. Eqs. represent the refined regression equation of the responses which were used to calculate the predicted values for other formulations in the design space.[39]
- The relationship between responses (dependent variables) and factors (independent variables) was established using polynomial equation generated through statistical analysis by the software to determine the composition that yields microemulsion formulation with ideal attributes.

- Positive sign in front of the factors indicates synergistic effects while negative sign indicates antagonistic effect of the factors. Model equations were calculated after converting the actual constraints into the coded levels ($0 \leq X1, X2, X3 \leq 1$). The conversion was done to overcome the complexity of the non-simplex models, where one of the components (X1) relatively varied less than the other component. (X2).

Y1=+25066.92X1+108.42X2+87.13X3-43461.94X1X2-43522.90X1X3-374.37 X2X3 + 41087.66X1X2X3-20059.47X1X2(X1-X2)-20395.96X1X3(X1-X3)-131.30X2X3(X2-X3)

Y2=+1.136E+005X1+189.74X2+84.44X3-1.976E+005X1X2-1.960E+005X1X3 +266.82X2X3+1.784E+005X1X2X3-93265.47X1X2(X1-X2)-92036.26X1X3(X1-X3)+366.83X2X3(X2-X3)

4.5.2 Selection of optimized batch

- The aim of the optimization of pharmaceutical formulations is to determine the levels of the variable from which a robust product with high quality characteristics may be produced. Some of the measured responses have to be minimized. The globule size of microemulsion should be as small as possible to penetrate into the deeper layers of skin. The contour plot of the globule size (Y1) proved that oil proportion needs to be lesser in amount to have the minimum globule size of microemulsion. It has been reported that retention of drug in the skin depends on the distribution of drug between the vehicle of microemulsion and layers of stratum corneum. Thus, the composition of microemulsion showing highest solubility of drug would probably result into its reduced partitioning into the skin layers, and majority of the drug will be retained in the vehicle after application of the formulation. So, the composition of microemulsion with following globule size & drug solubility was selected.[37]

Table 4.31Selected responses for optimization

Sr. No.	Responses	Values
1	Droplet size (Y1)	10-50 nm
2	Drug Solubility (Y2)	150-200 mg/ml

The optimum composition was selected on the basis of overlaid contour plot, which is shown in figure. 4.13.

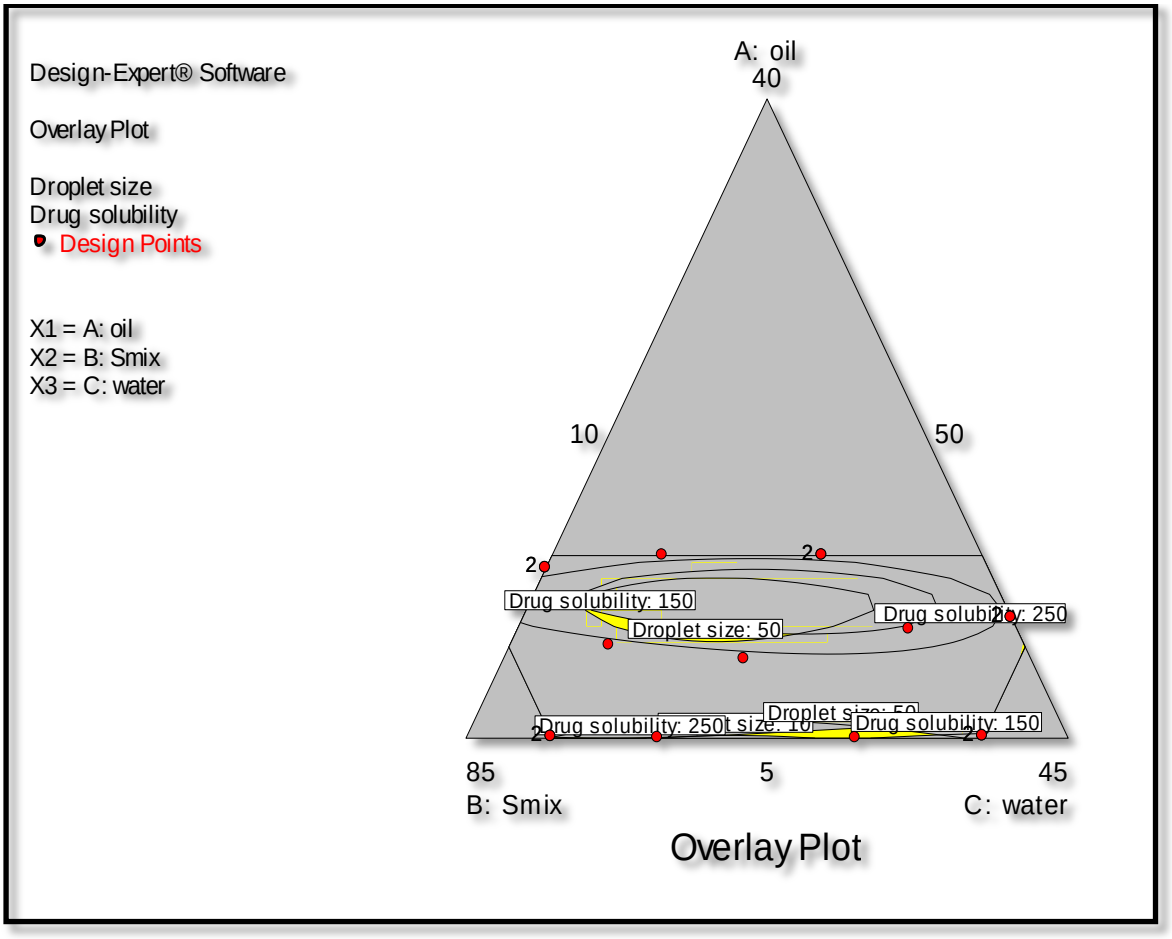


Figure 4.13Overlay plot of the effect of variables on droplet size (Y1) and solubility (Y2)

Conclusion

The optimum formulation was found to be as follows:

Table 4.32Formulation of the optimized ME

Sr. No.	Composition	Values (%)	Droplet Size (nm)	Drug Solubility (mg/ml)
1	Oil (X1)	5	11.56	174.58
2	Smix(X2)	64.17		
3	Water (X3)	30.83		

4.5.3 Validation of applied design

In order to assess the reliability of the developed mathematical model, microemulsion formulation with optimized components (ME) was formed corresponding to optimized factor levels. The % relative error (PPE) between predicted values and experimental values of each response was calculated using the following equation:[39]

Percentage Relative Error =

$$\left(\left| \text{Predicted value} - \text{Experimental Value} \right| / \text{Predicted value} \right) \times 100$$

Result

The results of the experimental values were found to be as follows:

Table 4.33Experimental and Predicted Values for the Optimized ME Formulation

Response	Experimental values	Predicted Value	PPE
Y1 (nm)	13.20	11.56	14.19
Y2 (mg/ml)	173.10	174.58	0.85

Conclusion

From the above results, it was concluded that there was no significant difference between the experimental values and the predicted values, which validated the selected model.

4.5.4 Characterization of optimized microemulsion

4.5.4.1 Physico-chemical characterisation

The physic-chemical characterization of the developed ME is shown in the following table.

Table 4.34Physico-chemical characterization of optimized ME

Sr. No.	Parameter	Results*
1	pH	5.9±0.05
2	Viscosity	1324±11.9 cPs
3	Droplet size	13.20
4	Percentage Transmission	99.2±0.16%
5	Polydispersity Index	0.267
6	Zeta Potential	-1.98 mV
7	Conductivity	0.185 mS cm^{-1}
8	Drug Content	97.70 ±0.5%
9	Drug Solubility	173.10 (mg/ml)
10	Dilution Potential	> 100

*(n=3, mean±SD)

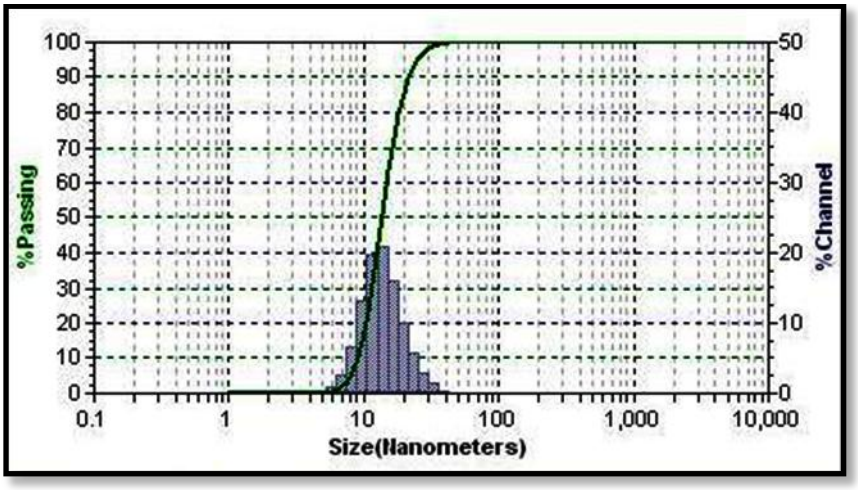


Figure 4.14Particle size distribution of oil droplets in Dapsone loaded ME

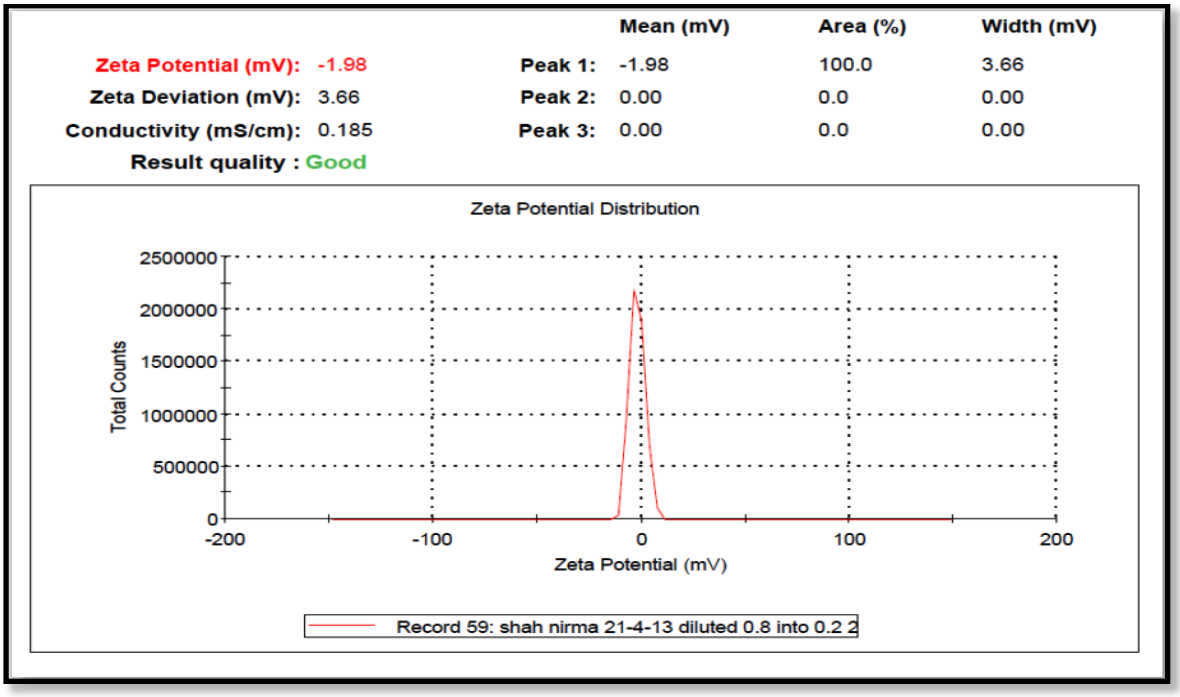


Figure 4.15Report of the conductivity & zeta potential of optimized Dapsone loaded ME

Conclusion

The developed ME had very low viscosity, which was not feasible for the topical application. It showed Transmission of >99 %, which showed the transparency of the microemulsion. The droplet size of the ME found to be in the nanometer range. The PDI shows that ME was monophasic. The negative zeta potential indicates that globules of

microemulsion had no charge, that is, the system was stable. As there was no charge on globules, no flocculation of globules occurred and hence, microemulsion was found to be stable. Conductivity data showed that the formulation was o/w type of microemulsion.

4.5.4.2 Transmission electron microscopy (TEM) of the optimized dapsone loaded microemulsion

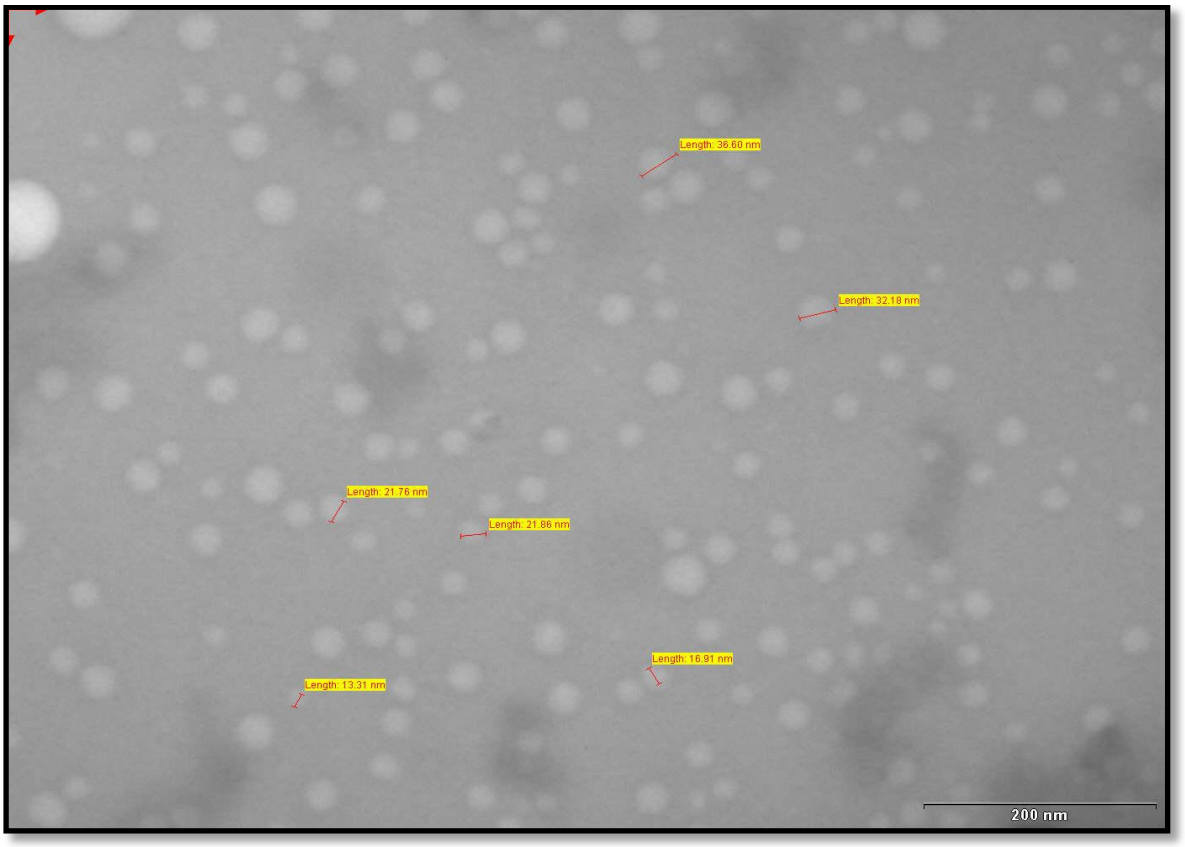


Figure 4.16TEM image of the optimized microemulsion

Conclusion

TEM image of the optimized Dapsone loaded ME showed that prepared Dapsone loaded ME had droplet size distribution of 10-100 nm, with the mean diameter of 13.20 nm.

4.5.4.3 Stability Study

Results

- **Physical stability**

Table 4.35Evaluation parameters showing physical stability of ME

Sr. No.	Evaluation parameter	Before	After
1	Clarity	Clear	Clear
2	Phase separation	Monophasic	Monophasic
3	Droplet size (nm)	13.20	13.33

- **Stability Study**

Table 4.36Evaluation parameters showing stability study of ME

Sr. No.	Evaluation parameter	Before	After
1	Phase separation	Monophasic	Monophasic
2	Droplet size (nm)	13.20	13.64
3	Drug content (%)	97.70±0.5%	96.98±0.63%

Conclusion

Physical stability which performed at 12000 rpm for 30 minutes showed no change in the physical state. There was no significant change in properties like Droplet size & Drug content at 4°C and 45°C for 1 months. Hence, it was concluded that Dapsone loaded ME was stable.

4.6 Development and Characterization of Microemulsion Based Gel

4.6.1 Formulation of microemulsion based hydrogel

Table 4.37Composition of MBGs & Conventional gel

Sr. No.	Components	Conventional gel	0.5 (%)	0.75 (%)	1 (%)	1.5 (%)	2 (%)
1	Drug (%w/v)	5	5	5	5	5	5
2	Oil (% v/v)	-	5	5	5	5	5
3	Smix (% v/v)	-	64.17	64.17	64.17	64.17	64.17
4	Carbopol 940 (%w/v)	1	0.5	0.75	1	1.5	2
5	Triethanolamine (%w/v)	1%	1 %	1 %	1 %	1 %	1 %
6	Water	q.s. 100 ml	q.s. 100 ml	q.s. 100 ml	q.s. 100 ml	q.s. 100 ml	q.s. 100 ml

4.6.2 Characterization and optimization of microemulsion based hydrogel of dapsone

4.6.2.1 Physico-chemical characterization of microemulsion based hydrogel of dapsone

Table 4.38Evaluation parameters of the microemulsion based hydrogel of Dapsone

MBGs / Parameters*	0.5 %	0.75 %	1%	1.5 %	2%	Marketed**
pH	7.1	7.1	7	7.2	7.1	6.9
Viscosity (cPs)	20322 ±115.47	24218 ±251.66	25918 ±173.21	26738 ±115.47	28634 ±152.75	23899 ±455.69
Droplet size (nm)	13.02	13.54	14.00	13.50	13.89	NA
Drug Content (%)	97.55 ±0.56	97.52 ±0.91	97.41 ±0.87	97 ±0.53	98.02 ±0.23	NA
Adhesive force (g)	67	228	400	704	621	216
Peak load (g)	73	392	687	1069	1257	389

*(n=3, mean±SD), **(marketed: Benzoac AC 5%, Gladerma)

Table 4.39Viscosity of the different MBGs

RPM	Viscosity (cPs)*				
	0.5 %	**0.75 %**	**1 %**	**1.5 %**	**2 %**
0.5	20322 ±115.47	24218 ±251.66	25918 ±173.21	26738 ±115.47	28634 ±152.75
10	16532 ±208.17	19218 ±260.8	20121 ±152.75	22425 ±251.66	23125 ±208.17
20	12636 ±200	14990 ±321.46	15230 ±219.39	16660 ±200	16900 ±161.66
30	9320 ±321.46	11580 ±251.66	12435 ±307.53	13490 ±300	13735 ±222.88
50	4090 ±152.75	7075 ±1850.23	8025 ±208.17	9022 ±152.75	9082 ±208.17

(*N=3, mean±SD)

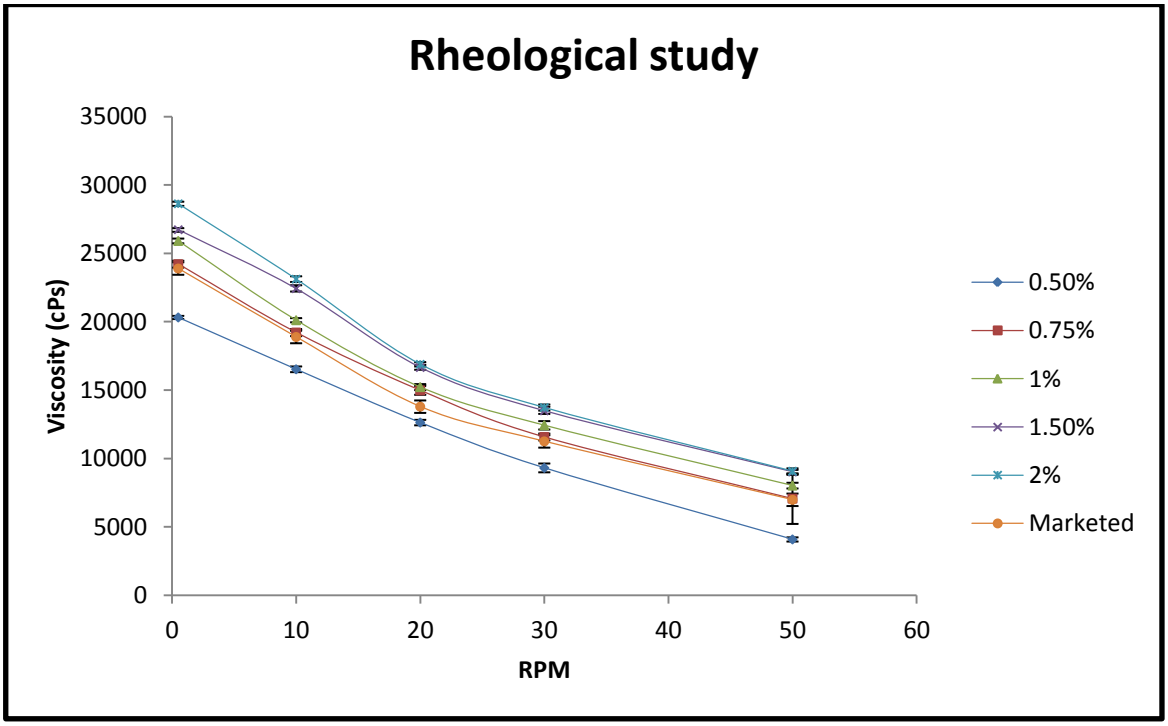


Figure 4.17Overlay of Rheological study of different MBGs

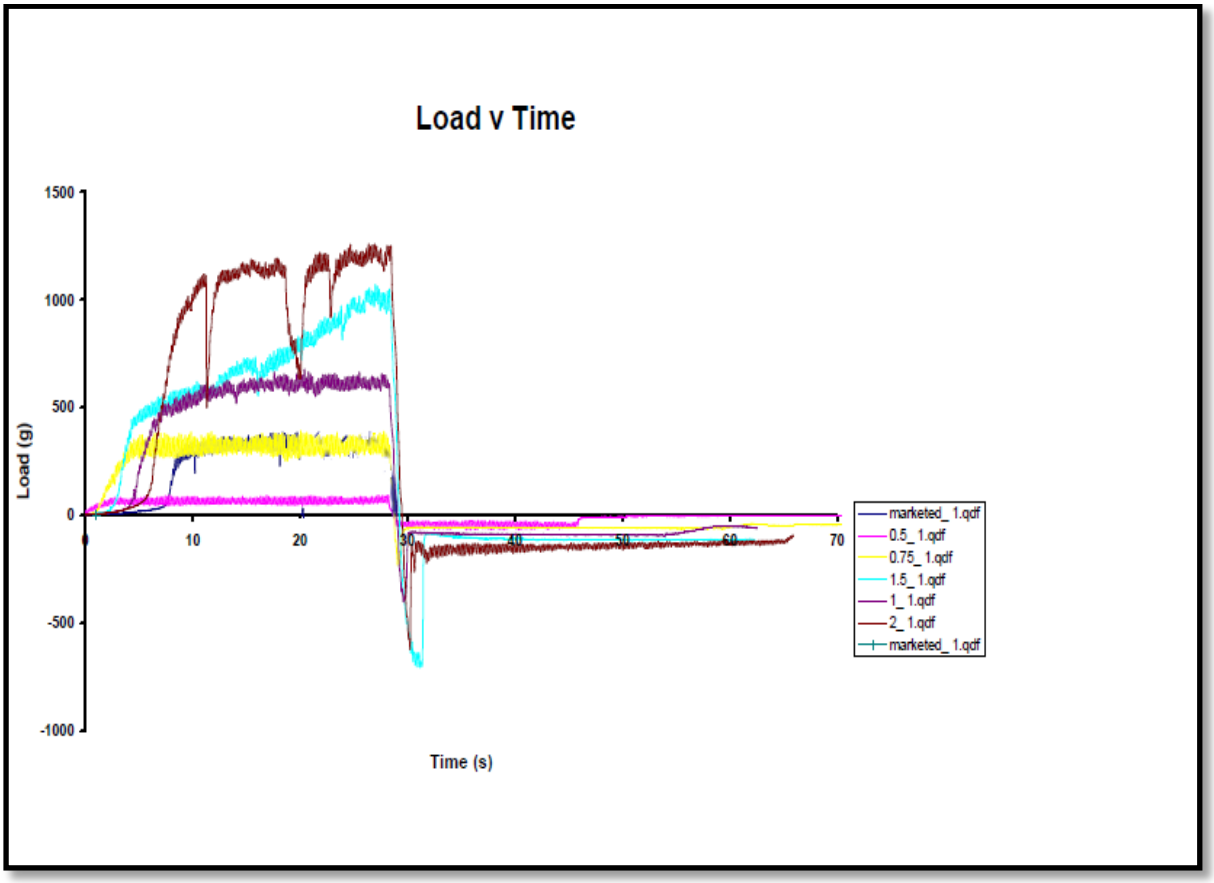


Figure 4.18Overlay of Load vs. Time plot showing gel strength & adhesive force

Conclusion

The pH of all batches was found near to 7.0 indicated that MBG could result in more less stimulation to skin than Dapsone loaded ME. The peak load (gel strength) & adhesive force of the 0.75 % w/v Carbomer 940 was containing MBG found to be nearly similar to the marketed formulation. Furthermore, the viscosity, peak load (gel strength) & adhesive force was found very small for 0.5 % w/v carbomer 940 containing MBG. Hence, further studies were continued without 0.5 %w/v carbomer 940 containing MBG.

4.6.2.2 In-Vitro permeation study

Table 4.40Comparison of % CDR of Conventional gel, ME & Different MBGs

Sr. No.	Time (hr)	% CDR*					
		Conventional gel	**Dapsone loaded ME**	**0.75 % w/w MBG**	**1.0 % w/w MBG**	**1.5 % w/w MBG**	**2.0 % w/w MBG**
1	0	0	0	0.00	0.00	0.00	0.00
2	0.5	1.80 ±1.22	6.21 ±1.01	4.86 ±0.62	3.85 ±1.51	2.66 ±0.7	2.66 ±0.57
3	1	5.81 ±0.59	11.88 ±1.46	7.55 ±1.30	6.21 ±1.42	5.79 ±1.10	5.29 ±0.58
4	2	10.55 ±1.24	48.51 ±1.25	23.40 ±3.44	36.31 ±1.36	33.87 ±1.07	33.32 ±1.06
5	3	15.91 ±2.05	56.47 ±2.92	42.01 ±4.04	41.34 ±1.95	38.78 ±0.65	38.44 ±1.06
6	4	26.59 ±3.17	62.29 ±3.05	48.39 ±2.50	47.66 ±2.17	43.81 ±1.63	43.60 ±1.36
7	5	33.75 ±2.63	70.33 ±1.39	55.86 ±1.07	55.07 ±2.11	52.24 ±2.12	49.96 ±1.54
8	6	40.37 ±3.54	80.77 ±1.12	61.01 ±2.0	60.20 ±2.41	58.40 ±1.86	57.21 ±1.64
9	7	51.69 ±2.06	84.83 ±1.21	67.83 ±2.09	66.93 ±1.56	63.93 ±2.11	62.64 ±1.98
10	8	58.12 ±2.01	88.78 ±1.65	81.78 ±1.08	79.14 ±0.93	78.79 ±2.25	77.22 ±1.19

*(n=3, mean±SD)

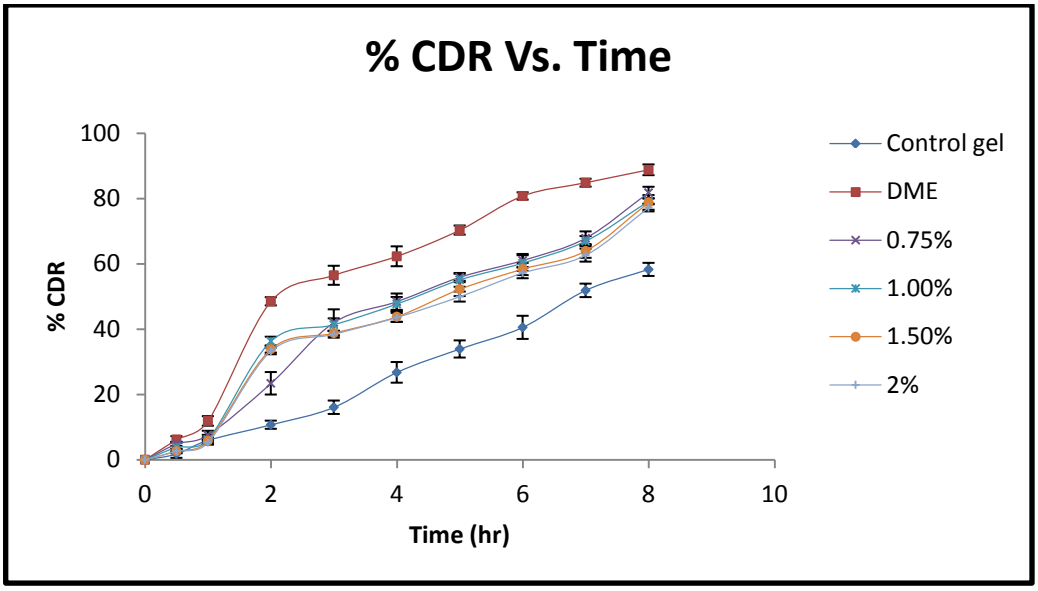


Figure 4.19Comparison of % CDR of Conventional gel, Dapsone loaded ME& Different MBGs

Result & Discussion

From the above results, it can be concluded Dapsone loaded ME has % CDR of 88.78 % in 8 hours. The 0.75 % w/v Carbopol 940 has the % CDR of 81.78 % in 8 hours, which was more than other batches of MBG. The result showed that addition of Carbomer 940 into microemulsion decreased markedly the permeability of Dapsone. It might attribute to the increased viscosity and transformation of microemulsion to lamellar structure or a highly ordered microstructure.

Conclusion

So a conclusion could be drawn that addition of Carbomer 940 in microemulsion would delay drug release. Further, The similarity factor was found, f2 = 39(<50). The paired t-test was performed and t-test was found greater than t critical [t stat (4.69) > t critical (2.26)]. Both, f2 value and t-test showed that there was significant difference between drug release from conventional gel and MBG. Hence, 0.75 % w/v Carbomer 940 containing MBG was selected for further studies.

4.6.2.3 Release kinetic data for optimized MBG

The correlation coefficient value (R^2) of each formulation for zero order, first order, higuchi, korsmeyer peppas and Hixon-crosswel model are shown in Table 4.41.

Table 4.41Release kinetic data for optimized MBG

Model name	Multiple R	R Square	X variable	Slope	SSR	Fischer Ratio
Zero order	0.9866	0.9734	10.1013	2.4007	198.0320	24.7540
First order	0.9792	0.9588	-0.0831	2.0343	175.5252	21.9407
Higuchi	0.9725	0.9458	30.5278	-12.6670	403.8680	50.4835
Korsmeyer - Peppas	0.9866	0.9734	1.0580	-1.0039	331.4806	47.3544
Hixson - Crowell	0.9894	0.9789	0.2349	-0.0464	103.0864	12.8858

Result

The release kinetics data indicates that the release of drug from MBG follows Hixson - Crowell drug release because the correlation coefficient values are higher in case of Hixson – Crowell equation.

4.6.2.4 Stability Study

Results

- **Physical Stability**

Table 4.42Evaluation parameters showing physical stability of MBG

Sr. No.	Evaluation parameter	Before	After
1	Clarity	Clear	Clear
2	Droplet size (nm)	13.54	13.59

- **Stability Study**

Table 4.43Evaluation parameters showing stability study of MBG

Sr. No.	Evaluation parameter	Before	After
1	Droplet size (nm)	13.54	13.77
2	Drug content (%)	97.52+0.91 %	96.98+0.69%

Conclusion

Physical stability which performed at 12000 rpm for 30 minutes showed no change in the physical state. There was no significant change in properties like Droplet size & Drug content at 4°C and 45°C for 1 month. Hence, it was concluded that MBG was stable.

4.6.2.5 Skin irritation studies

Results

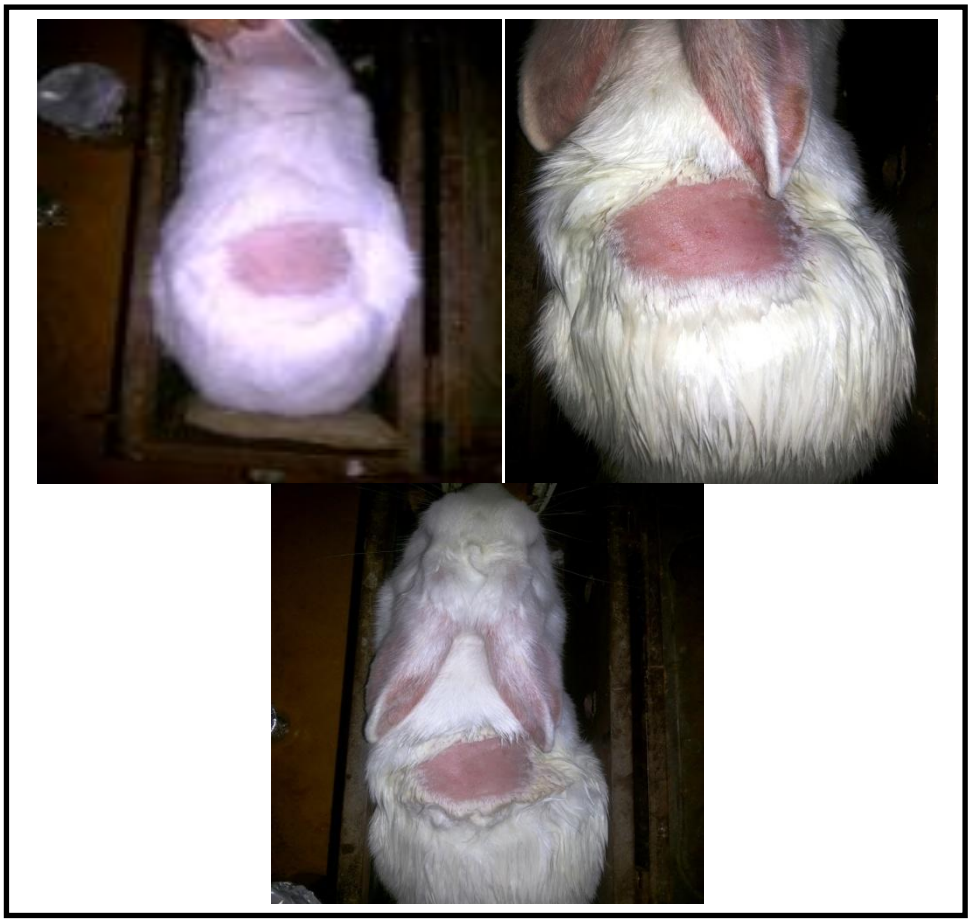


Figure 4.20Skin irritation study on intact skin group

Table 4.44Average irritation scores on intact skin group

Sr. No.	Intact Skin group			
	Formulation	Avg. Scores		
		0 h	24 h	72 h
1	control	0	0	0
2	Dapsone loaded ME	0.33	0	0
3	MBG	0	0	0

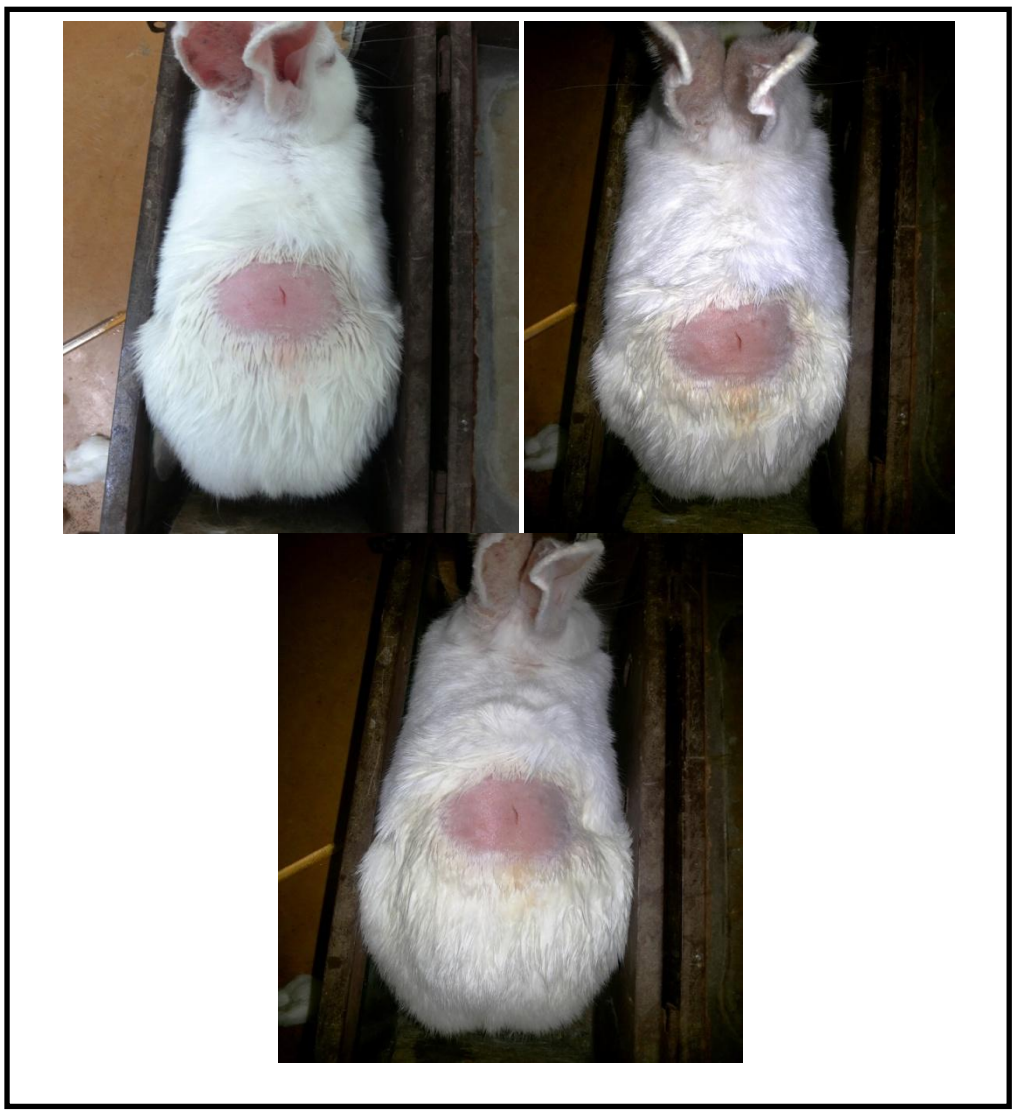


Figure 4.21Skin irritation study on skin injury group

Table 4.45Average irritation scores on skin injury group

Sr. No.	Skin Injury group			
	Formulation	Avg. Scores		
		0 h	24 h	72 h
1	control	0.33	0	0
2	Dapsone loaded ME	0.66	0.33	0
3	MBG	0.33	0	0

Discussion

For intact skins after application, the three tested formulations had no irritation. However, to injured skins, after application of microemulsion, slight skin irritation occurred. The possible reason was that the decrease of the metabolic capability for damaged skins induced accumulation of surfactant mixture and oil phase, which led to occurrence of irritation reaction. There was change of microemulsion property after adding Carbomer 940. The network structure formed and the increase of the viscosity decreased the contact chances between skins and microemulsion. Thus the irritation of MBG was much weaker.

Conclusion

Therefore, it could be concluded that MBG had no irritation to skins under the conditions of the study.

SUMMARY

5. Summary

Acne vulgaris is the most common skin disease, with 80 % of reported occurrence, of adolescents and young adults. Dapsone is used to treat acne especially for the patients who are unresponsive to conventional anti-acne agents. Due to low water solubility & side effects associated with oral route, Dapsone was a right candidate to formulate it with a topical microemulsion system. Further, to limit the disadvantages of the microemulsion systems like low viscosity, low physical stability & poor patient compliance, microemulsion was incorporated into hydrogel.

Therefore, the objective was to design, develop and characterize the microemulsion based gel for topical delivery, to limit the side effects of drug, improve the solubility & permeability of drug, for the treatment of acne.

Oil and surfactant were selected on the basis of solubility of drug. Dapsone showed higher solubility in Triacetin and Tween 80. So, they were selected as oil and surfactant phase respectively for formulation of microemulsion.

The influence of different co surfactants (Labrasol, Cremopher EL, Propylene glycol & PEG 400) on the formation of microemulsion was studied. From this study, Combination of Tween 80 & Labrasol showed enhanced region of ME domain in the phase diagram. Therefore, it was selected as co-surfactant.

The pseudo-ternary phase diagrams for different surfactant to co surfactant ratio were successfully developed for the following system: Triacetin (Oil) + Tween 80 (Surfactant) + Labrasol (Co surfactant) + Water. Initially, surfactant and co-surfactant were mixed together in the ratio of 1:2, 1:1 & 2:1; and finally mixed with oil in the ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 & 1:9. The system showed the largest ME region in pseudo ternary phase diagram when surfactant to co-surfactant ratio was 1:2.

The “Mixture design” or D- optimal design was used to optimization of microemulsion and to obtain the relationship between the particle size distribution, drug solubility and components of the mixture in the formulation. A D-optimal equation was obtained and contour plot of response was plotted over D-optimal space. An optimum formulation was selected on the basis of droplet size in range of 10-50 nm & drug solubility in the range of 150 to 200 mg/ml. Optimized batch was found with 5% oil,

64.17 Smix, 30.83% water with droplet size & drug solubility of 13.20 nm & 173.10 mg/ml respectively.

For optimized microemulsion, microemulsion based hydrogel was developed using carbomer 940 and evaluated for drug content, pH, viscosity, gel strength, adhesive force, in-vitro diffusion studies.

The pH of all the formulated MBGs was near to skin pH. Viscosity, gel strength & adhesive force results of the 0.75 % w/v Carbomer 940 containing MBG was found 24218 cPs (at 0.5 rpm), 392 g & 228 g respectively which were nearly to the marketed formulation.

In-vitro diffusion study showed that % CDR of 0.75 % w/v Carbomer 940 containing MBG, with 81.78%, was higher than all remaining prepared MBGs and conventional gel. The similarity factor, f2 = 39(<50) and t-test {t stat (4.69) > t critical (2.26)} showed that there is significant difference between drug release from conventional gel and MBG. Hence, 0.75 % w/v Carbomer 940 containing MBG was selected for further studies.

Skin irritation study revealed that MBG containing Dapsone had no skin irritation with slight irritation for Dapsone loaded ME.

The present study was done with an aim to develop MBG for the treatment of acne in view to overcome the problems associated with conventional topical gels. Microemulsion was found a suitable vehicle due to its excellent permeability in the MBG. The study concludes the potentiality of developed formulation for the treatment of acne.

REFERENCES

6. References

1. Borges, V. R.; Simon, A.; Sena, A. R.; Cabral, L. M.; de Sousa, V. P., Nanoemulsion containing dapsone for topical administration: a study of in vitro release and epidermal permeation. *International journal of nanomedicine* **2013,** *8*, 535-44.
2. Dahan, A.; Miller, J. M.; Amidon, G. L., Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs. *The AAPS journal* **2009,** *11* (4), 740-6.
3. Monteiro, L. M.; Lione, V. F.; do Carmo, F. A.; do Amaral, L. H.; da Silva, J. H.; Nasciutti, L. E.; Rodrigues, C. R.; Castro, H. C.; de Sousa, V. P.; Cabral, L. M., Development and characterization of a new oral dapsone nanoemulsion system: permeability and in silico bioavailability studies. *International journal of nanomedicine* **2012,** *7*, 5175-82.
4. Zouboulis, C. C., Acne and sebaceous gland function. *Clinics in dermatology* **2004,** *22* (5), 360-6.
5. Prausnitz, M. R.; Elias, P. M.; Franz, T. J.; Schmuth, M.; Tsai, J.-C.; Menon, G. K.; Holleran, W. M.; Feingold, K. R., Skin barrier and transdermal drug delivery. *Dermatology, 3rd edn. Philadelphia, PA: Elsevier Saunders* **2012**, 2065-74.
6. Naik, A.; Kalia, Y. N.; Guy, R. H., Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science & technology today* **2000,** *3* (9), 318-326.
7. Odom, R. B.; James, W. D.; Berger, T. G., *Andrews' Diseases of the Skin: Clinical Dermatology*. W.B. Saunders Company: 2000; p 1.
8. Venus, M.; Waterman, J.; McNab, I., Basic physiology of the skin. *Surgery (Oxford)* **2011,** *29* (10), 471-474.
9. Kolarsick, P. A. J.; Kolarsick, M. A.; Goodwin, C., Anatomy and Physiology of the Skin. *Journal of the Dermatology Nurses' Association* **2011,** *3* (4), 203-213 10.1097/JDN.0b013e3182274a98.
10. Rathi, S. K., Acne vulgaris treatment : the current scenario. *Indian journal of dermatology* **2011,** *56* (1), 7-13.

11. Nguyen, R.; Su, J., Treatment of acne vulgaris. *Paediatrics and Child Health* **2011,** *21* (3), 119-125.
12. Hsieh, M.-F., Review: Delivery of Pharmaceutical Agents to Treat Acne Vulgaris: Current Status and Perspectives. *Journal of Medical and Biological Engineering* **2012,** *32* (4), 215.
13. Feldman, S.; Careccia, R. E.; Barham, K. L.; Hancox, J., Diagnosis and treatment of acne. *American family physician* **2004,** *69* (9), 2123-30.
14. Singla, V.; Saini, S.; Joshi, B.; Rana, A., Emulgel: A New Platform For Topical Drug Delivery. *International Journal of Pharma & Bio Sciences* **2012,** *3* (1).
15. Bibette, J., *Emulsion science: basic principles : an overview*. Springer: 2002.
16. Nielloud, F., *Pharmaceutical Emulsions and Suspensions: Second Edition, Revised and Expanded*. Taylor & Francis: 2000.
17. Talegaonkar, S.; Azeem, A.; Ahmad, F. J.; Khar, R. K.; Pathan, S. A.; Khan, Z. I., Microemulsions: a novel approach to enhanced drug delivery. *Recent patents on drug delivery & formulation* **2008,** *2* (3), 238-57.
18. Bequette, B. W., From Pilot Plant to Manufacturing: Effect of Scale-up on Operation of Jacketed Reactors. In *Pharmaceutical Manufacturing Handbook*, John Wiley & Sons, Inc.: 2007; pp 137-158.
19. Fanun, M., Microemulsions as delivery systems. *Current Opinion in Colloid & Interface Science* **2012,** *17* (5), 306-313.
20. Anton, N.; Vandamme, T. F., Nano-emulsions and micro-emulsions: clarifications of the critical differences. *Pharmaceutical research* **2011,** *28* (5), 978-85.
21. Luckham, P. F.; Vincent, B.; Tadros, T. F., The controlled flocculation of particulate dispersions using small particles of opposite charge. III. Investigation of floc structure using rheological techniques. *Colloids and Surfaces* **1983,** *6* (2), 101-118.
22. Bagwe, R. P.; Kanicky, J. R.; Palla, B. J.; Patanjali, P. K.; Shah, D. O., Improved drug delivery using microemulsions: rationale, recent progress, and new horizons. *Critical reviews in therapeutic drug carrier systems* **2001,** *18* (1), 77-140.
23. Lawrence, M. J.; Rees, G. D., Microemulsion-based media as novel drug delivery systems. *Advanced Drug Delivery Reviews* **2012,** *64*, 175-193.
24. Madhav, S.; Gupta, D., A Review On Microemulsin Based System. *Int. J. Pharm.*

Sci. Res. 2011; 2: 8: 1888 **1899**.

25. Tripathi, K. D., *Essentials Of Medical Pharmacology*. Jaypee Brothers Medical Publishers: 2008.
26. Rowe, R. C.; Sheskey, P. J.; Quinn, M. E.; Association, A. P., *Handbook of Pharmaceutical Excipients, 6th Edition*. Pharmaceutical Press: 2009; p 110-114.
27. Rowe, R. C.; Sheskey, P. J.; Quinn, M. E.; Association, A. P., *Handbook of Pharmaceutical Excipients, 6th Edition*. Pharmaceutical Press: 2009; p 748-749.
28. Rowe, R. C.; Sheskey, P. J.; Quinn, M. E.; Association, A. P., *Handbook of Pharmaceutical Excipients, 6th Edition*. Pharmaceutical Press: 2009; p 549-553.
29. Rowe, R. C.; Sheskey, P. J.; Quinn, M. E.; Association, A. P., *Handbook of Pharmaceutical Excipients, 6th Edition*. Pharmaceutical Press: 2009; p 557-561.
30. www.drugbank.ca/drugs/DB00250
31. Salama, N. N.; El Ries, M. A.; Toubar, S.; Abd El Hamid, M.; Walash, M. I., Thermoanalytical Investigation of Some Sulfone-Containing Drugs. *Journal of Analytical Methods in Chemistry* **2012,** *2012*, 9.
32. De, A.; Dey, S.; Pradhan, P. K.; Chaudhari, F.; Patel, M., Esimation of Dapsone in Bulk & Dosageform by UV Spectroscopic Method. *Indo American Journal of Pharmaceutical Research* **2014,** *4* (1), 312-319.
33. Indian Pharmacopoeia **2010.** Government of India, Ministry of Health and Family Welfare; Vol. 1, p 304.
34. Peltola, S.; Saarinen-Savolainen, P.; Kiesvaara, J.; Suhonen, T. M.; Urtti, A., Microemulsions for topical delivery of estradiol. *International Journal of Pharmaceutics* **2003,** *254* (2), 99-107.
35. Al Abood, R. M.; Talegaonkar, S.; Tariq, M.; Ahmad, F. J., Microemulsion as a tool for the transdermal delivery of ondansetron for the treatment of chemotherapy induced nausea and vomiting. *Colloids and Surfaces B: Biointerfaces* **2013,** *101* (0), 143-151.
36. Zhu, W.; Yu, A.; Wang, W.; Dong, R.; Wu, J.; Zhai, G., Formulation design of microemulsion for dermal delivery of penciclovir. *International Journal of Pharmaceutics* **2008,** *360* (1–2), 184-190.
37. Fouad, S. A.; Basalious, E. B.; El-Nabarawi, M. A.; Tayel, S. A., Microemulsion and poloxamer microemulsion-based gel for sustained transdermal delivery of

diclofenac epolamine using in-skin drug depot: in vitro/in vivo evaluation. *Int J Pharm* **2013,** *453* (2), 569-78.

38. Sabale, V.; Vora, S., Formulation and evaluation of microemulsion-based hydrogel for topical delivery. *International journal of pharmaceutical investigation* **2012,** *2* (3), 140-9.
39. Barot, B. S.; Parejiya, P. B.; Patel, H. K.; Gohel, M. C.; Shelat, P. K., Microemulsion-based gel of terbinafine for the treatment of onychomycosis: optimization of formulation using D-optimal design. *AAPS PharmSciTech* **2012,** *13* (1), 184-92.
40. Patel, H. K.; Barot, B. S.; Parejiya, P. B.; Shelat, P. K.; Shukla, A., Topical delivery of clobetasol propionate loaded microemulsion based gel for effective treatment of vitiligo: ex vivo permeation and skin irritation studies. *Colloids and surfaces. B, Biointerfaces* **2013,** *102*, 86-94.
41. Zhu, W.; Guo, C.; Yu, A.; Gao, Y.; Cao, F.; Zhai, G., Microemulsion-based hydrogel formulation of penciclovir for topical delivery. *Int J Pharm* **2009,** *378* (1-2), 152-8.
42. Chen, H.; Mou, D.; Du, D.; Chang, X.; Zhu, D.; Liu, J.; Xu, H.; Yang, X., Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration. *Int J Pharm* **2007,** *341* (1-2), 78-84.
43. Gan, L.; Gan, Y.; Zhu, C.; Zhang, X.; Zhu, J., Novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of lipophilic cyclosporine A: in vitro and in vivo results. *Int J Pharm* **2009,** *365* (1-2), 143-9.
44. Hurler, J.; Engesland, A.; Poorahmary Kermany, B.; Škalko-Basnet, N., Improved texture analysis for hydrogel characterization: Gel cohesiveness, adhesiveness, and hardness. *Journal of Applied Polymer Science* **2012,** *125* (1), 180-188.
45. Chen, L.; Zhao, X.; Cai, J.; Guan, Y.; Wang, S.; Liu, H.; Zhu, W.; Li, J., Triptolide-loaded microemulsion-based hydrogels: physical properties and percutaneous permeability. *Acta Pharmaceutica Sinica B* **2013,** *3* (3), 185-192.
46. Dash, S.; Murthy, P. N.; Nath, L.; Chowdhury, P., Kinetic modeling on drug release from controlled drug delivery systems. *Acta poloniae pharmaceutica* **2010,** *67* (3), 217-23.

47. Li, P.; Ghosh, A.; Wagner, R. F.; Krill, S.; Joshi, Y. M.; Serajuddin, A. T., Effect of combined use of nonionic surfactant on formation of oil-in-water microemulsions. *Int J Pharm* **2005,** *288* (1), 27-34.

48. Jeirani, Z.; Mohamed Jan, B.; Si Ali, B.; Mohd. Noor, I.; Chun Hwa, S.; Saphanuchart, W., The optimal mixture design of experiments: Alternative method in optimizing the aqueous phase composition of a microemulsion. *Chemometrics and Intelligent Laboratory Systems* **2012,** *112* (0), 1-7.

Annexure I

Item No: 4
Project Number: IP/PCEU/MPH/14-1/004

CERTIFICATE

This is certify that the project title "Formulation Optimization of Microemulsion Based Hydrogel for the Treatment of Acne" has been approved by the IAEC on 10/01/2014 in the 14th IAEC meeting.

Name of Chairman/Member Secretary IAEC:
Dr.Manjunath Ghate
Chairperson

Name of CPCSEA nominee:
Dr. Ramtej Verma
CPCSEA nominee

Signature with date 10.1.2014

Chairman/Member Secretary of IAEC:

CPCSEA nominee:

Annexure II

Presented poster titled "Formulation Development & Characterization of Microemulsion Based Hydrogel of Naproxen" in 2nd Nirma Institute of Pharmacy International Conference held on 24th January, 2014.

Formulation Development & Characterization of Microemulsion Based Hydrogel of Naproxen

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Microemulsions at present are of concern to the pharmaceutical field due to their substantial potential as a mean of delivering drugs by incorporating a broad range, both hydrophilic & hydrophobic, of drug molecules. Naproxen, which is chemically (s)-6-methoxy-α-methyl-2-naphthalenenacetic acid, is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic effects which is utilized in the management of rheumatoid arthritis, osteoarthritis and dysmenorrheal. Lower solubility, Gastritis and peptic ulceration are the main limitation upon oral delivery of Naproxen likewise other NSAIDs. In addition, there is inconvenience in application & waste of scarce due to the low viscosity of microemulsion and affected by the environmental condition. In the view of above problems, Formulation of topical microemulsion based hydrogel (MBH) of naproxen is developed. Based on the solubility of the Naproxen, Isopropyl Myristate as oil phase, mixture of Tween 80 & Brij 35 as surfactant, propylene glycol as co-surfactant & double distilled water as aqueous phase was selected. Prepared MBH was characterized for droplet size, viscosity, gel strength, short-term stability, in-vitro drug release etc. The optimized batch showed satisfactory results.

PT-70

FORMULATION DEVELOPMENT & CHARACTERIZATION OF MICROEMULSION BASED HYDROGEL OF NAPROXEN

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Abstract

Microemulsions at present are of concern to the pharmaceutical field due to their substantial potential as a mean of delivering drugs by incorporating a broad range, both hydrophilic & hydrophobic, of drug molecules. Naproxen, which is chemically (s)-6-methoxy-α-methyl-2-naphthalenenacetic acid, is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic effects which is utilized in the management of rheumatoid arthritis, osteoarthritis and dysmenorrheal. Lower solubility, Gastritis and peptic ulceration are the main limitation upon oral delivery of Naproxen likewise other NSAIDs. In addition, there is inconvenience in application & waste of scarce due to the low viscosity of microemulsion and affected by the environmental condition. In the view of above problems, formulation of topical microemulsion based hydrogel (MBH) of naproxen is developed. Based on the solubility of the Naproxen, Isopropyl Myristate as oil phase, mixture of Tween 80 & Brij 35 as surfactant, propylene glycol as co-surfactant & double distilled water as aqueous phase was selected. Prepared MBH was characterized for droplet size, viscosity, gel strength, short-term stability, in-vitro drug release etc. The optimized batch showed satisfactory results.

Objective

The objective of the present study is to formulate the microemulsion based Hydrogel for the topical delivery of naproxen in order to minimize the oral side effect, to improve the solubility of the drug & to improve the stability of the microemulsion by incorporating in to gel.

Introduction

Naproxen, chemically (s)-6-methoxy-α-methyl-2-naphthalenenacetic acid, is non-steroidal anti-inflammatory drug (NSAID) used in the treatment of the rheumatoid arthritis, osteoarthritis and dysmenorrheal. Naproxen is poorly soluble & has adverse effects like gastritis & peptic ulceration when taken by oral route. Topical delivery of Naproxen can prevent these side effects & may help to maintain consistent plasma levels for long-term therapy from a single dose. Microemulsion is defined as a dispersion containing oil, surfactant, Co-surfactant & aqueous phase, which is a single optically isotropic & thermodynamically stable liquid solution. microemulsions can be used to deliver drugs to patients via several routes, but the topical application of microemulsions has gained increasing interest. Therefore, in this study, microemulsion systems were prepared for topical application.

Materials & Method

Screening of Oils & Surfactants:

Oils & surfactants	Solubility (mg/ml)
IPM	12.820±0.44
Oleic acid	4.1233±1.75
Ethyl Oleate	0.9983±3.40
Tween 80	13.697±0.536
Span 80	3.1465±2.25
Brij 35	12.550±1.00
PEG 400	10.7458±2.75
PG	11.3300±1.75

Table 1. Solubility of Naproxen in different oils, surfactant & cosurfactants

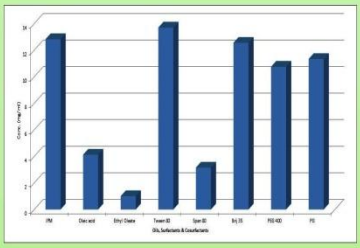


Fig. 1. Comparison of Solubility of Naproxen in different oils, surfactant & cosurfactants

Construction of Pseudoternary Phase Diagram:

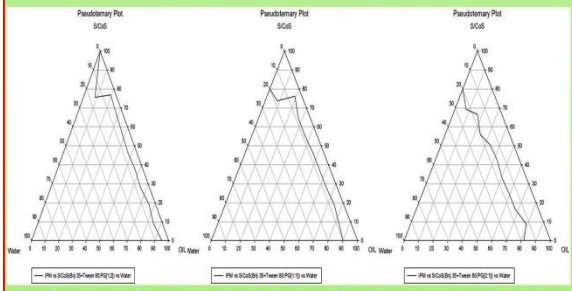


Fig 2. The Pseudoternary Phase diagrams of the Oil-Surfactant-Cosurfactant-Water System at the 1:2, 1:1, & 2:1 weight ratio of Brij 35+ Tween 80 to Propylene glycol at ambient temperature

Formulation	Drug (1%)	S/C	% Oil	% S/CoS	% Water
ME-1	1	1:1	37.7358	56.6038	5.6604
ME-2	1	1:1	28.5714	66.6667	4.7619
ME-3	1	2:1	33.6134	50.4202	15.9664
ME-4	1	2:1	24.0000	56.0000	20.0000
ME-5	1	1:2	27.5229	64.2202	8.2569
ME-6	1	1:2	36.6972	55.0459	8.2569

Table 2. Compositions of the selected Microemulsions

Formulation	Carbopol 940 (g)	Water (g)	Triethanolamine (g)	Microemulsion (g)
MBH-1	0.75	50	1	50 g of ME-1
MBH-2	0.75	50	1	50 g of ME-2
MBH-3	0.75	50	1	50 g of ME-3
MBH-4	0.75	50	1	50 g of ME-4
MBH-5	0.75	50	1	50 g of ME-5
MBH-6	0.75	50	1	50 g of ME-6

Table 3. Compositions of the selected Microemulsion Based Hydrogels

Result & Discussion

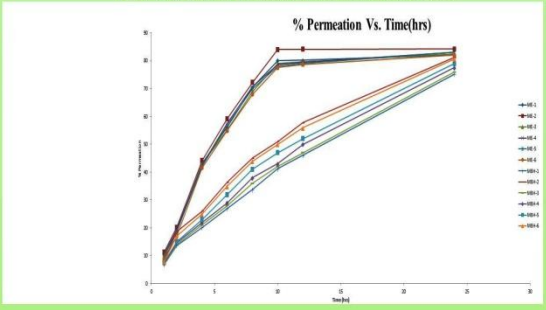
Table 4. Characterization of Selected Naproxen Microemulsion (NME) batches

Formulation	Droplet size	PI	pH	Viscosity
ME-1	10.3±0.5 nm	0.348±0.021	6.72	36.17 ± 1.60
ME-2	34.5±1.3 nm	0.348±0.023	6.89	40.60 ± 1.15
ME-3	11.6±0.3 nm	0.347±0.014	6.60	48.13 ± 1.98
ME-4	53.6±0.5 nm	0.346±0.016	6.85	51.63 ± 1.66
ME-5	21.9±0.6 nm	0.347±0.018	6.70	25.73 ± 1.88
ME-6	79.8±0.7 nm	0.347±0.025	6.76	28.90 ± 1.91

Table 5. Characterization of Selected Naproxen Microemulsion Based Hydrogel (NMBH) batches

Formulation/ Characterization	MBH-1	MBH-2	MBH-3	MBH-4	MBH-5	MBH-6
Viscosity (cPs)	7096±25.29	7817±14.38	8256±12.59	9276±13.49	5289±23.69	6548±13.19
pH (±0.5)	6.99	7.01	7.10	7.24	7.59	7.44
Stability(30°C for 6 months)	Stable	Stable	Stable	Stable	Stable	Stable

Fig. 3. Comparison of % Permeation of different NME & NMBH Batches



Conclusion

The results of the present study revealed that the Microemulsion based hydrogel of Naproxen for the topical delivery shows the good solubility of the drug which leads to improved permeability. By incorporating in the gel, microemulsion shows improved stability.

References:

1. Qingping Tiana, Fude Renb, Zhen Xua, Ying Xiea, Shuqiu Zhang. Preparation of high solubilizable microemulsion of naproxen and its solubilization mechanism. International Journal of Pharmaceutics.426 (2012) 202–210.
2. Neslihan Ustundag Okura, Sebnem Apaydınb, N. Ulku Karabay Yavasogluc, Altug Yavasoglud, H. Yesim Karasulu. Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations. International Journal of Pharmaceutics 416 (2011) 136–144.
3. Weiwei Zhua, Chenyu Guoa, Aihua Yua, Yan Gaoa, Fengliang Caoa, GuangXi Zhaia, Microemulsion-based hydrogel formulation of penciclovir for topical delivery. International Journal of Pharmaceutics 378 (2009) 152–158.

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