"PREPARATION AND EVALUATION OF RESVERATROL MICROEMULSION BASED GEL FOR ACNE TREATMENT"

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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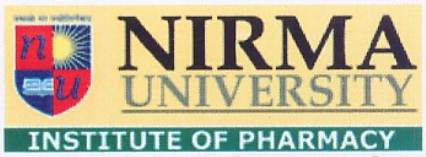

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MAY 2015

CERTIFICATE

This is to certify that the dissertation work entitled "preparation and evaluation of resveratrol microemulsion based gel for acne treatment " submitted by Ms. Anuradhabahen javaji vanjara with Regn. No. (13mph102) 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 "preparation and evaluation of resveratrol microemulsion based gel for acne treatment" is based on the original work carried out by me under the guidance of Dr. Tejal Mehat, professor & head of 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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Acknowledgements

By blessing of Almighty I came in an amazing world of art of making medication and research that enthralled me, I worship his love and care for all time through which he has showered every auspicious creation in my life. It is a matter of pride and ecstasy to convey my gratitude to one and all who have contributed in assorted ways and endowed me precious knowledge to be successful in my undertaking.

Foremost, I adorably express my noble regards to my parents Mrs. Hanshaben J Vanjara and Mr. Javaji D Vanjara, for their love, care, dynamic moral and economical support well timed shaping my dream career and creating my spirits alive at dwelling and anywhere I need. I am pleased to thank my nephew Aarav & Ananya for marvellous talks, pleasing moments and enormous altruistic facilitation.

My deepest & most loving thanks go to my grandfather for his care, warmth, constant motivation, source of wisdom and worthwhile support.

I reveal my genuine gratitude to my esteemed guide Dr. Tejal Mehta, Head and Professor, Department of Pharmaceutics & Pharmaceutical Technology, Nirma University, Ahmedabad for her intellectual guidance, timely vital suggestion, scrupulousness, unflinching encouragement and critical annotations for the entire span of my project work.

place on record, my sincere thanks to Prof. Manjunath Ghate (Director,

Insitute of Pharmacy, Nirma University) for his unconditional support and providing all necessary help and facility during my project.

I am fortunate to have the opportunity to work at Institute of Pharmacy, Nirma University and I am thankful to all the faculties like Dr. Mayur Patel, Dr. Dhaivat Parikh, Dr. Jigar Shah, Dr. Shital Butani, Dr. Renuka Mishra for their support. They always encourage me to do better work., Dr. Priti J Mehta Associate Professor, Head, Dept. of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University for their constant moral support and kind cooperation.

With an huge pleasure, I heartly thankful to my colleagues Ankit Gotecha, Bhavna singh, Bhranti Patel,Darshan Goswami, Dhara Desai,Jitendra Sharma,Khushali Modi,Khushbu Trivedi, Kripali Soni, Mimansha Jhaveri,Mo Kasim Shaikh,Monil Soni,Parth dave, Pankti Vasani, Parth Patel, Ronak Vashi, Pooja Patel, Shreyash Shah & Zahid Tanwar for their responsive support, friendly disposition, having nice cerebral moments together & for being everytime with me whenever I needed.I would like to give a special thanks to my all juniors to help me during my project work.

How can I forget to thank members at Department, amongst them my special thanks to Shaileshbhai, Mukeshbhai, Dipeshbhai, Shilpaben for their ever helping hands. I sincerely thanks to Mrs. P. Lalitha and Rajubhai for library facilities and constant encouragement during my work.I am also very thankful to Nityanandbhai and Hasmukhbhai for helping us.

Finally I would acknowledge all those who have knowingly and unknowingly helped me in flourishing completion of this endeavor.

Ms. Anuradha J Vanjara

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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

“Preparation and evaluation of resveratrol microemulsion based gel for acne treatment”

Abstract

The aim of this study was to develop and characterize resveratrol loaded microemulsion based hydrogel for topical delivery and to enhance the solubility and penetration of the resveratrol. Using UV spectroscopy, the solubility of resveratrol in oil and surfactant was determined. The ratio and effects of the oil, surfactant, and co-surfactant on forming a transparent and stable microemulsion were investigated using pseudo- ternary phase diagrams. By using pseudo-ternary diagram triacetin as oil phase, tween 80 as a surfactant and PEG 400 was selected. Simplex lattice mixture design was adopted to optimize the oil content(X1), surfactant and co-surfactant content(X2), water content (X3). Globule size and % drug release was evaluated for the design batches. The optimal formulation was composed of 8% triacetin (oil), 74% tween 80 and PEG 400(Smix) and 19% water (aq.phase) and 1% resvveartrol. The optimized globule size and % drug release was found to be 22.36 nm and 80.2%. Further optimized microemulsion was incorporated into the gel formulation using carbopol 940 as gelling agent without affecting aits characteristics to increase the viscosity of the microemulsion and thereby improve the topical applicability of the formulation. In-vitro drug release of hydrogel was compared with the marketed formulation and drug release shown was 85.6% and 61.8% respectively. Thus, microemulgel can be a better alternative for the treatment of acne as compared to conventional formulations.

1.1Introduction to skin:

1.1.1 What is Skin?

Skin is the largest organ of the human body. It is the outermost tissue of the body it has the 15% of the body weight and 16000cm2 surface area. It serves very important functions like it protects our body against external physical, chemical and biological attacker, it also prevent excess water loss from our body play role in heat regulation.as shown in figure skin has a very complex structure it consists of many components ,cells ,fibbers and other several layers that make skin multi-layered structure. Veins, capillaries make vast network inside the skin. Hair follicles are present over the skin to protect the skin from sun rays and regulate the body temperature and also protect form the particles of debris and foreign materials that could damage the skin or can enter in the body.

The skin composed of three layers:

1) Epidermis
2) Dermis
3) Subcutaneous tissue

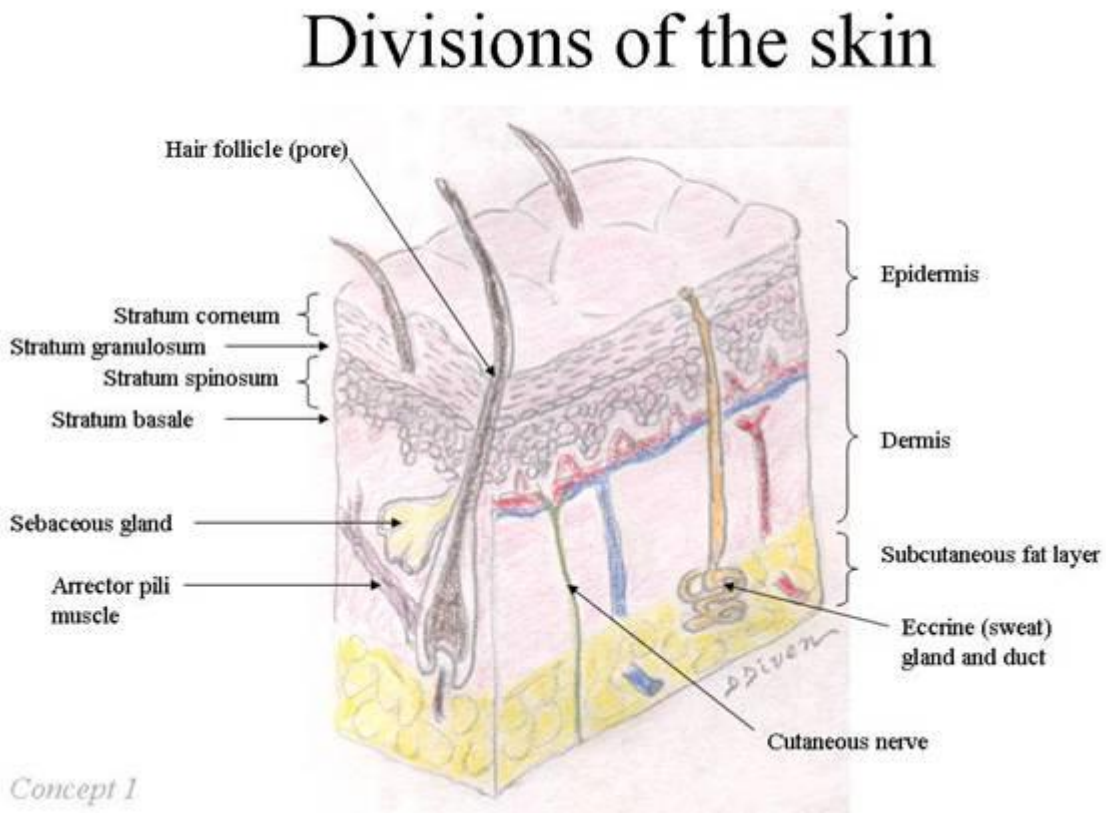


Fig 1.1 structure of skin

1) Epidermis:

The epidermis is composed of two types of cells keratinocytes and dendritic cells. Keratinocyte is the major cell of the epidermis it synthesizes the long threadlike protein called keratin, it has interconnected bridges that are known as the desmosomes that joins keratinocytes and it has adequate amount of stainable cytoplasm which differ from the clear dendritic cell layer. This keratinocytes which form different layers of epidermis by changes in morphology and position of the keratinocytes as they move peripherally. There are four layers of epidermis:

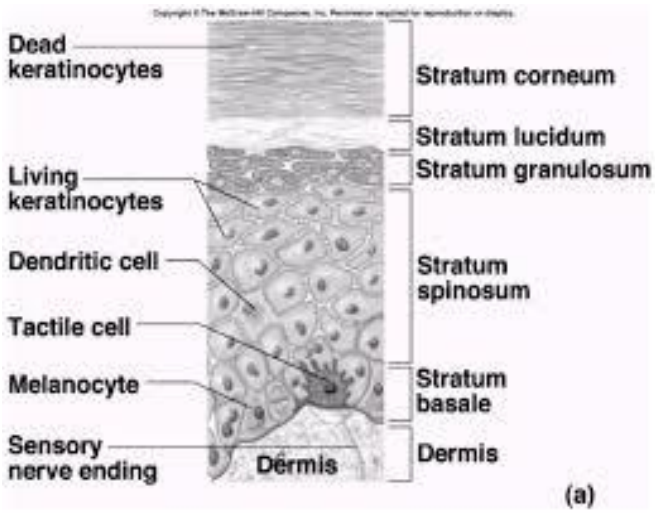


Fig 1.2 structure of epidermis

1) Stratum basal (basal or germinativum cell layer):

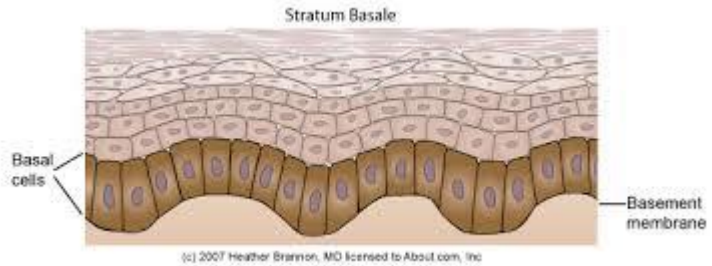


Fig 1.3 structure of stratum basal

The deepest layer of the epidermis which lies adjacent to the dermis comprises mainly dividing and non-dividing keratinocytes, which are attached to the basement membrane by hemi desmosomes. As keratinocytes divide and differentiate, they move from this deeper layer to the surface. Making up a small proportion of the basal cell population is the pigment (melanin) producing melanocytes. These cells are characterised by dendritic processes, which stretch between relatively large numbers of neighbouring keratinocytes. Melanin accumulates in melanosomes that are transferred to the adjacent keratinocytes where they remain as granules. Melanin pigment provides protection against ultraviolet (UV) radiation; chronic exposure to light increases the ratio of melanocytes to keratinocytes, so more are found in facial skin compared to the lower back and a greater number on the outer arm compared to the inner arm. The number of melanocytes is the same in equivalent body sites in white and black skin but the distribution and rate of production of melanin is different. Intrinsic ageing diminishes the melanocyte population. Merkel cells are also found in the basal layer with large numbers in touch sensitive sites such as the fingertips and lips. They are closely associated with cutaneous nerves and seem to be involved in light touch sensation.

2) Stratum spinosum (spinous or prickle cell layer):

Fig 1.4 structure of stratum spinosum

As basal cells reproduce and mature, they move towards the outer layer of skin, initially forming the stratum spinosum. Intercellular bridges, the desmosomes, which appear as `prickles' at a microscopic level, connect the cells. Langerhans cells are dendritic, immunologically active cells derived from the bone marrow and are found on all epidermal surfaces but are mainly located in the middle of this layer. They play a significant role in immune reactions of the skin, acting as Antigen-presenting cells.

3) Stratum granulosum (granular cell layer):

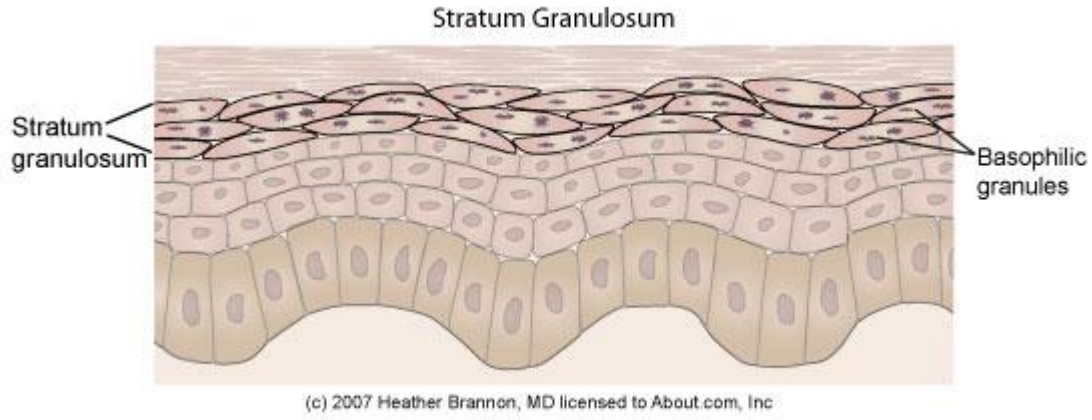


Fig 1.5 structure of stratum granulosum

Continuing their transition to the surface the cells continue to flatten, lose their nuclei and their cytoplasm appears granular at this level.

4) Stratum corneum (horny layer):

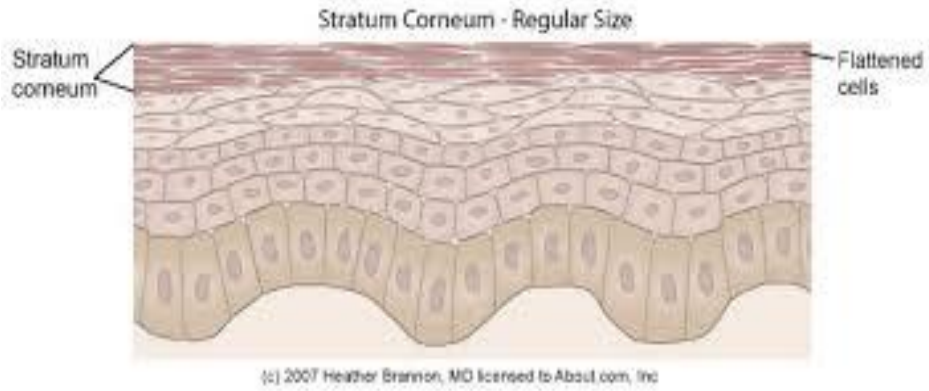


Fig 1.6 structure of stratum corneum

The final outcome of keratinocyte maturation is found in the stratum corneum,

This is made up of layers of hexagonal-shaped, non-viable cornified cells known as corneocytes. In most areas of the skin, there are 10±30 layers of stacked coenocytes with the palms and soles having the most. Each corneocyte is surrounded by a protein envelope and is filled with water-retaining keratin proteins. The cellular shape and orientation of the keratin proteins add strength

To the stratum corneum. Surrounding the cells in the extracellular space are stacked layers of lipid bilayers .The resulting structure provides the natural physical and water-retaining barrier of the skin. The corneocyte layer can absorb three times its weight in water but if its water content drops below 10% it no longer remains pliable and cracks. The movement of epidermal cells to this layer usually takes about 28 days and is known as the epidermal transit time.

2) Dermis:

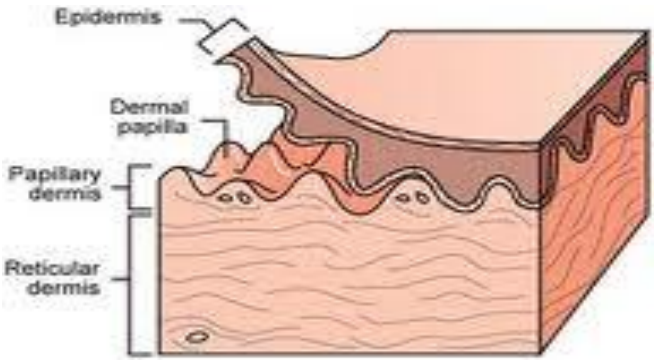


Fig 1.7 structure of dermis

Dermis found beneath the epidermis. It varies in the thickness on different parts ranging from 0.6 mm on eyelids to 3 mm on the back, palms and sole. There are two layers in the dermis:

1) Papillary layer: it lies just below the epidermis and connects with it. Thin collagen fibres are situated loosely in the papillary layer.

2) Reticular layer: it extended from the base of papillary layer to the subcutaneous tissue thicker collagen fibres are situated parallel to the skin surface in the deepest reticular layer.

The dermis is consists of fibroblasts which produce collagen, elastin, proteoglycans, mast cells and macrophages. Up to 70% of dermis was made up of collagen fibres which give strength and toughness to the dermis layer. Elastin provides elasticity and flexibility while proteoglycans maintain hydration and viscosity of the skin. The other components of the dermal layer are dermal vasculature, lymphatics, nerve cells and fibres, sweat glands, hair follicle roots and striated muscles.

3) Subcutaneous tissue:

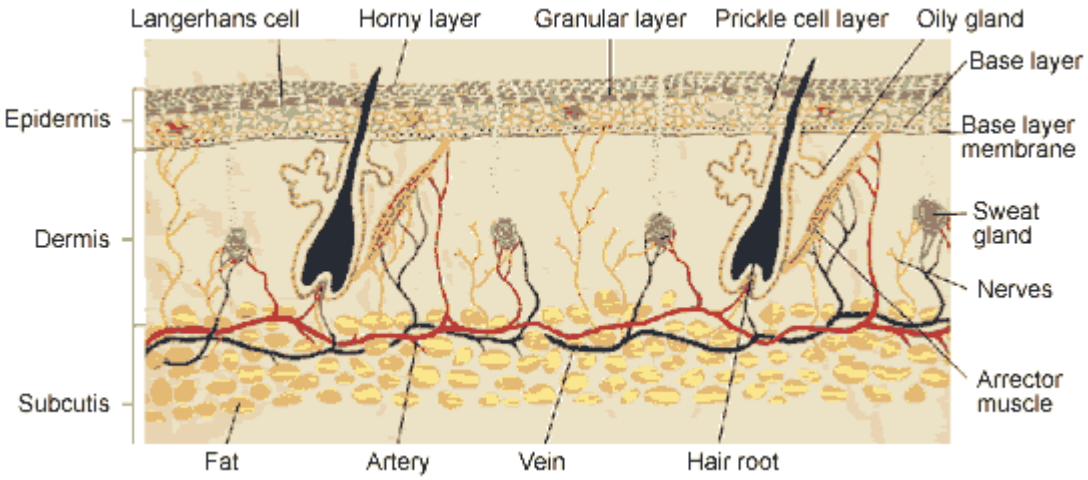


Fig 1.8 structure of subcutaneous tissue

The subcutaneous tissue or hypodermis is third layer of the skin it is a fatty layer loosely connected with dermis. The subcutaneous layer contains fat, connective tissue, larger blood vessels and nerves. It maintains temperature of the body and skin also acts as a protective layer. The thickness of this layer varies depending on anatomical sites, age, and from person to person. It constitutes about 10% of body weight.

Note: Skin-specific diseases are often seen in the epidermis and papillary dermis, whereas systemic disease is seen in the reticular dermis and subcutaneous tissue.

1.1.2 Appendages of skin:

The appendages of the skin are sweat glands, sebaceous glands, and mammary glands, hair, nails.

Hair follicles:

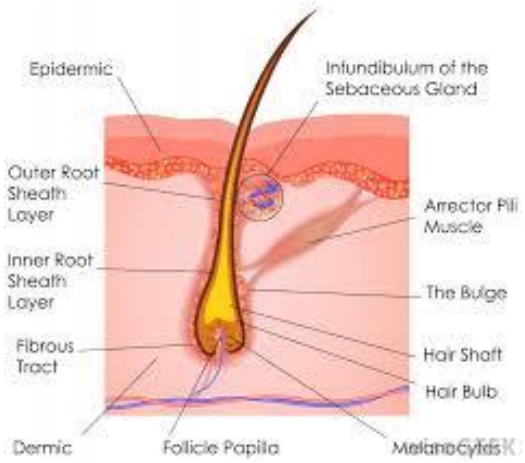


Fig 1.9 structure of hair follicles

It is an epidermal derivative responsible for hair growth. Hairs are widely distributed on all over body exception being on the palms, soles, lips, and around the anal and urethral orifices. The base of the hair follicle is known as a hair bulb, it is situated deeply in the dermis or hypodermis layer. Hair bulb is surrounded by root sheath which is composed of outer and inner layer. An erector pili muscle is connected with the hair shaft and contracts with cold, fear, emotion to pull the hair erect giving the skin 'goose bumps'. Germinative cell is associated with hair follicle which produces keratin and melanocytes which is responsible for the synthesis of pigment.

Sweat gland:

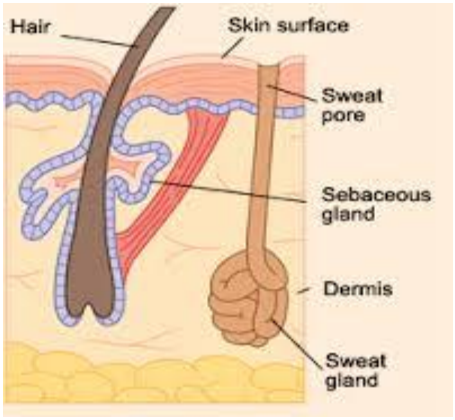


Fig 1.10 structure of sweat glands

Sweat glands are of two types:

1) Eccrine sweat gland: this is a major thermoregulatory device it is controlled by sympathetic nervous system and regulates body temperature. When internal temperature raises eccrine gland secret water to the skin surface it evaporates heat and give cooling effect. Sweat gland is coiled tubular gland situated deep in in the dermis layer adjacent to the hypodermis. Duct extends upward and opens at the surface of skin.

2) Apocrine sweat gland: apocrine glands are situated in the under arms and genital regions.it is associated with hair follicles, it secrets fatty sweat in tubular gland and bacteria break it down in odorous smell the gland are active after adolescences.

Sebaceous gland:

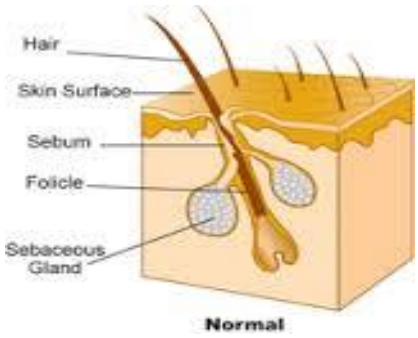


Fig 1.11 structure of sebaceous gland

Sebaceous gland is associated with hair follicles, and situated on all over body but widely at the face, back and scalp. Sebaceous gland is big and opens at the time of birth but shrink during the childhood an again open at puberty period. The opening will depend on the secretion of the hormones. Sebaceous gland secrets sebum consisting of fatty substance and cellular debris. It will secret the sebum in the sebaceous gland duct and then it will come at the skin surface. It provides the greasy layer on the skin by that it will restrict the evaporation water from the skin and it will maintain the flexibility of the skin.

1.1.3 Function of skin:

Table 1.1 functions of skin

Sr.no	Function	Example	Mechanism
1	Protection	Protects the body from: UV damage, dehydration, microorganism, bacterial invasion, mechanical trauma, physical injuries	❖ Stratum corneum layer form the physical barrier of the skin. ❖ Keratin protein also available in the skin. ❖ Pigments found in the skin one of the pigment is melanin that protects against UV radiation and sun damage.

2	Sensation	Feeling of pressure, touch, heat, pain and cold	❖ Somatic sensory receptor is responsible for sensation
3	Temperature regulation	Retention or release of heat depending on outside heat	❖ Sweat release or the thermoregulation and afterwards evaporates the sweat ❖ Regulates the blood flow in skin regions specially limbs
4	Immunity	Demolition of microorganism	❖ Different cells work for the immunity they are Langerhans cells, phagocytic cells, epidermal dendritic cells
5	Permits movement and growth	Growth of body tissue and modification of skin during movement	❖ Elastic and recoil properties of the epidermis, dermis and subcutaneous tissue.
6	Excretion	Excretion of urea, water, ammonia and uric acid	❖ Sweat regulates the waste of the body
7	Endocrine	Produce vitamin D	Vitamin d3 is produce under the skin by the reaction of organic chemical known as dehydrocholesterol with UV radiation light and form the vitamin D3.normally day UV light could form it but artificial sources can also be used.

1.2 Introduction to acne:

1.2.1 Introduction:

Acne vulgaris is commonest dermatological problem found in the people. 90% of the people face acne during their adolescence. Acne vulgaris is common non inflammatory skin

condition mostly found on the face and upper trunk. Acne main disorder of pilosebaceous unit (it consists of hair shaft, hair follicle and sebaceous gland which produce sebum) and also due to propionic bacterium acne it cause plugging of the hair follicle due to abnormal keratinization of upper part.

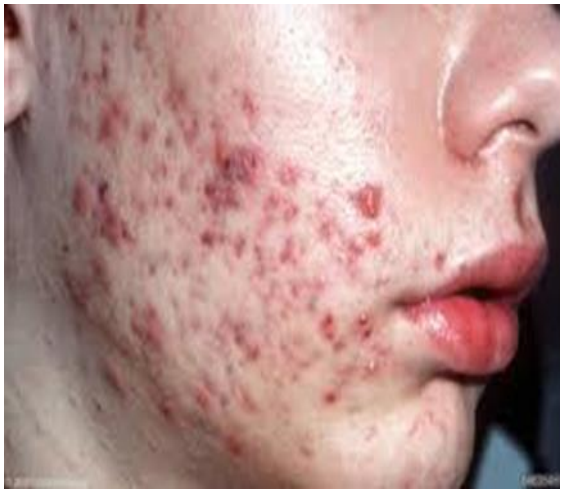


Fig 1.12 acne vulgaris on face

1.2.2 Pathogenesis of acne vulgaris:

Pathophysiology of acne is multifactorial process that mainly causes the clogging of pilosebaceous follicle. Therefore acne is mainly occurring at the sebaceous gland concentrated area. It normally start at the age of adolescence 8-13 and peaks at the age of 15-21 it may resolve in many persons at the age of 24-27 but may occur in some person post adolescence also.

Main 3 factors are related in acne pathophysiology:

1) Stimulation of sebaceous gland:

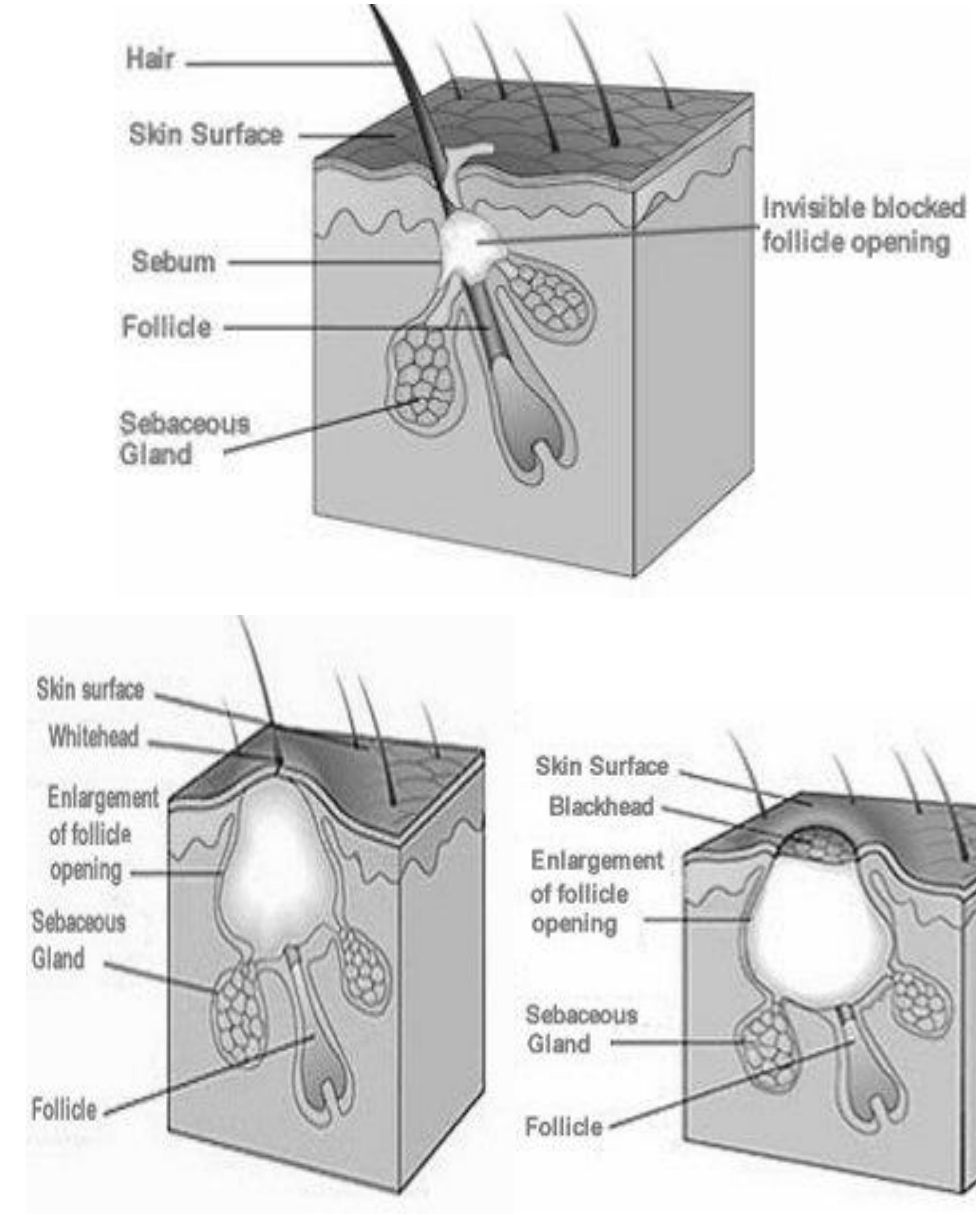
   Increased secretion of the androgens a metabolite of testosterone hormone will stimulate the secretion of the sebum in the sebaceous gland. There are many enzyme systems present in the sebaceous gland that convert cholesterol or weak androgens in to the strong androgens that can activate these glands. When activity of this increase it will increase the production of acne. Many receptors are also present that can increase

the sebum production by proliferation of sebocytes and secretion of specific substance .the receptors are PPAR system that act through retinoid receptor neuromediators (substance P).

2) Micro comedone formation:

Clogging of hair follicle occur due to keratinocytes. Keratinocyte doesn't separate from each other and form layering inside the follicular lining and block the follicles. That will cause clogging inside the ducts that will lead to the formation of micro comedones. As the production of sebum continues, sebum and bacteria come in contact with clogged dead cells that will not allow sebum to come at the surface so dilation of the follicle occurs that will lead to the formation comedone from micro comedone. Abnormalities in the androgen production lead to the defects in the keratinocytes proliferation. Interleukin 1α and cytokine released in response to the local irritation by the keratinocytes will believe to play role in the formation of the

Fig 1.13 structure of micro comedones, white head and black head



3) Inflammatory phase:

First two steps occur in all individuals when acne will form but inflammatory lesion will not occur in each and every individual. Propionic bacterium acne will play major role in the formation inflammatory acne. . P acne multiplies in the micro comedone and it also contains lipase that split triglyceride in to free fatty acids and glycerol. This free fatty acid and bacterial fragments move on the wall of comedones that will start inflammatory response. Metalloproteinase enzyme was secreted by the polynuclear neutrophils this enzyme disrupt the follicle wall so inflammation will spread in the deeper layers.t-lymphocytes, cytokines, leukotriene, macrophages are also other inflammatory mediators.

Papules: postules:

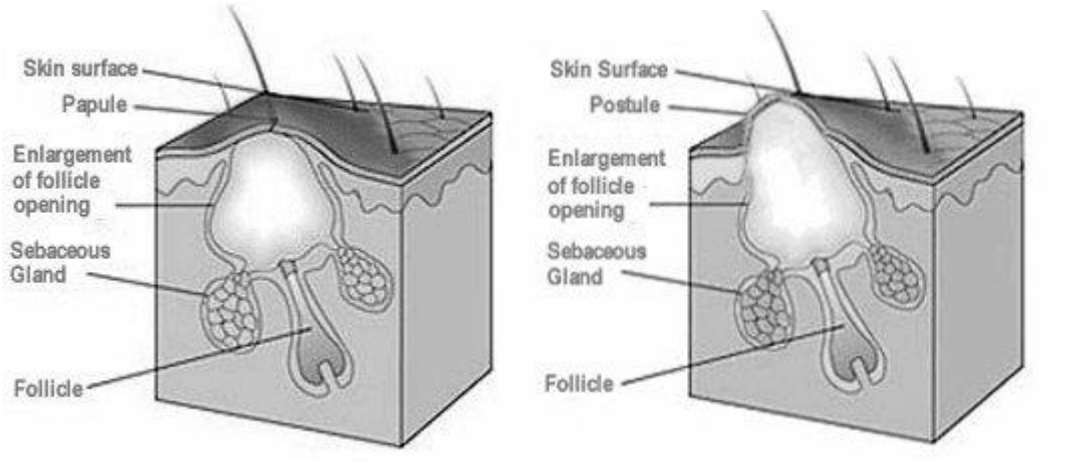


Fig 1.14 structure of papules and postules

Nodules:

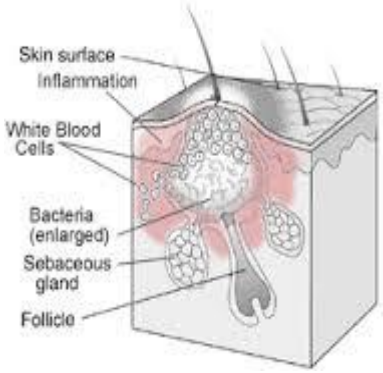


Fig 1.15 structure of nodules

Symptoms:

- Whiteheads (closed comedon)
- Blackheads (open comedon— the oil turns brown when it is exposed to air)
- Papules (Small red, tender bumps)
- Pimples (pustules-papules with pus at their tips)
- Nodules (Large, solid, painful lumps beneath the surface of the skin)
- Cystic acne (Painful, pus-filled lumps beneath the surface of the skin)

Diagnosis:

Diagnosis of acne vulgaris is made based on the appearance of the skin. Other condition such as pustular drug eruption, bacterial and fungal folliculitis also resembles acne distinguished by absence of comedones. Acne rosacea also differentiates from the acne by lack of comedones and nodules.

In this disorder, hair follicles become clogged which results in formation of comedones (black or white head), which leads to papules, pustules and nodules on inflammation. After inflammation pigmentation and scar are usually result of nodule or cystic acne.

1.2.3Treatment:

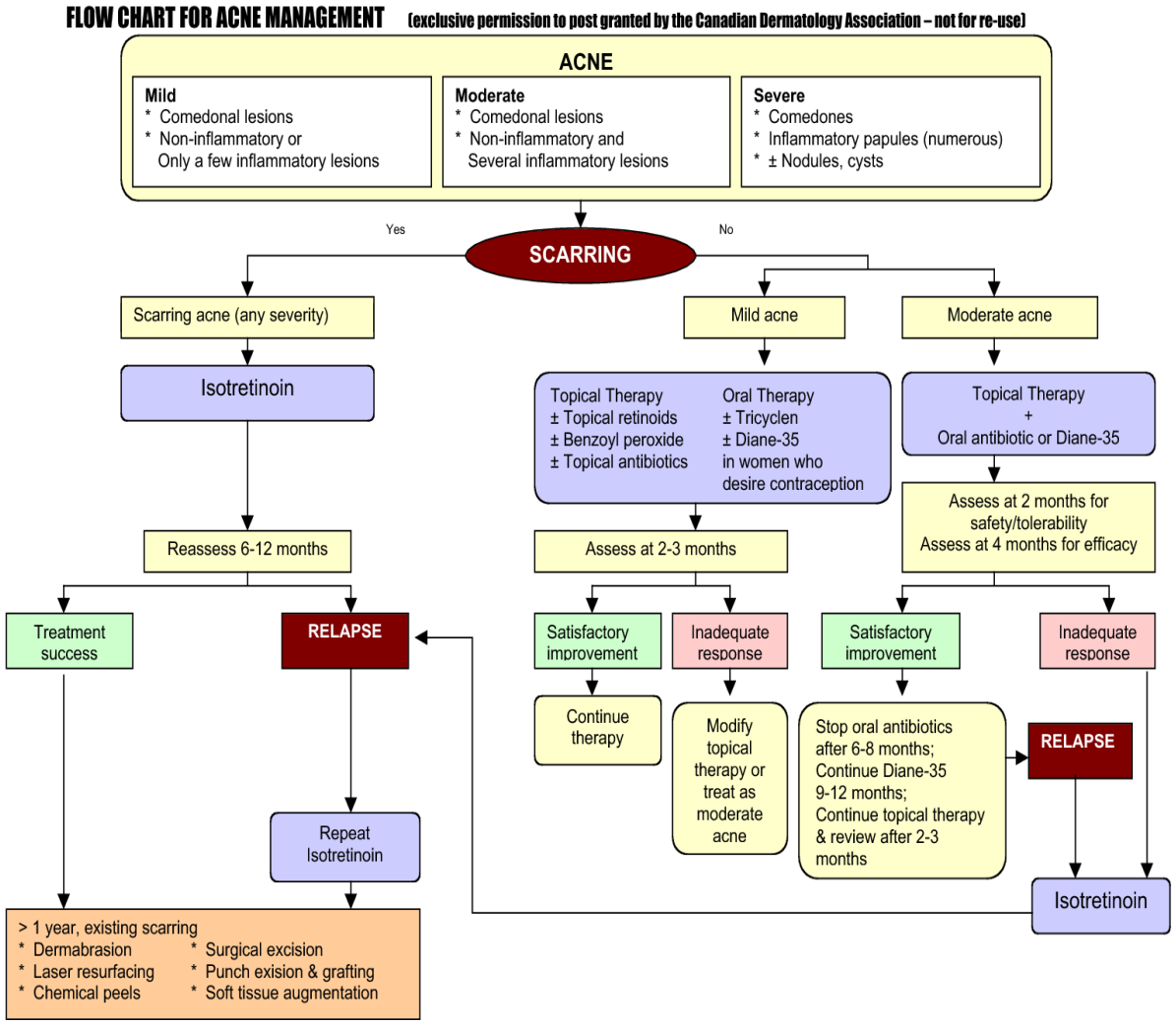


Fig1.16 flow chart of acne treatment

There are four therapies:

- Topical retinoid therapy
- antibiotic therapy
- Benzoyl peroxide
- Hormonal therapies.

Treatment for mild acne:

One of the most effective treatments is keratolytic agents when acne is not sever. These agents mainly target the plugging of the follicles.

The Main agents are:

Retinoids: most potent keratolytic agent is tretinoin. It prevents comedone formation by normalizing epithelial cell desquamation. But it has side effects like skin irritation, photosensitivity.

Adaplene: it has comedolytic and anti-inflammatory properties and it also has less irritancy effect.

Azelaic acid: it is naturally occurring dicarboxylic acid ,it has antibacterial, anti-inflammatory, and hyperkeratinisation effect it cause itching and burning sensations.

Severe acne treatment:

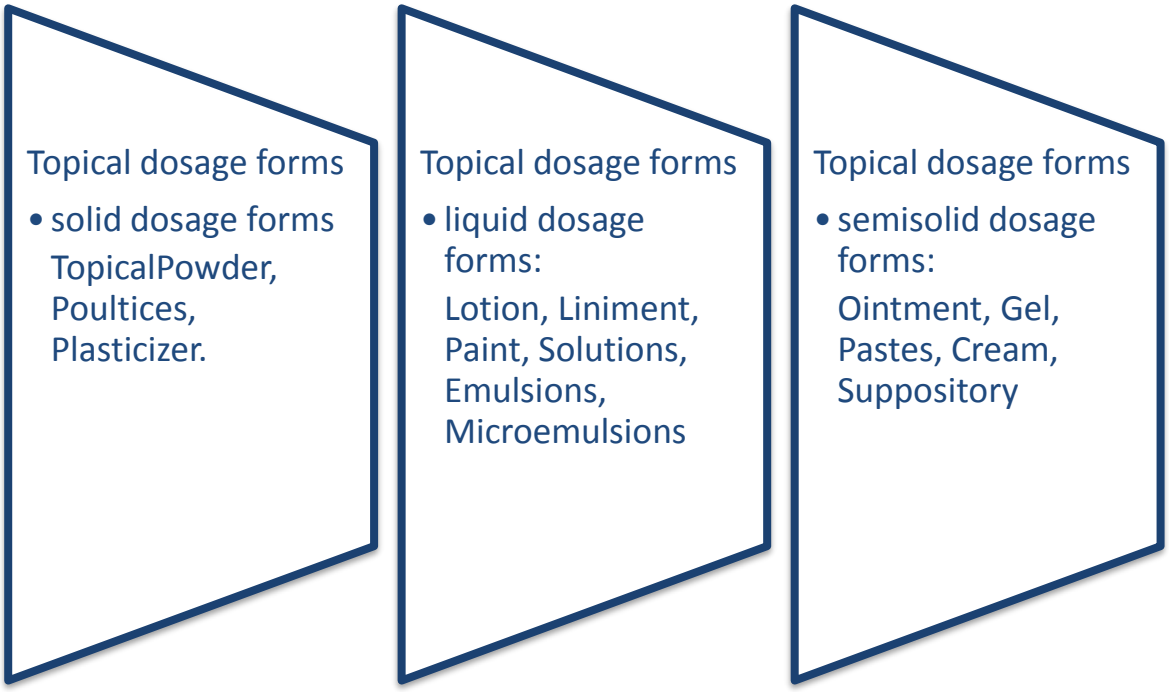
Anti-inflammatory and antibiotic are used in the severe acne treatment:

- Benzoyl peroxide: This is the most widely used over the counter drug for the acne treatment. It has a bactericidal and comedolytic effect. It causes dry skin and allergy as side effects.
- Topical antibiotics: clindamycin and erythromycin have antibacterial and anti-inflammatory actions. Combination of these two with benzyl peroxide will increase the effect of these agents and also reduce resistance to the P acnes.
- Oral antibiotics: these are used for the moderate to severe acne treatment in which there is risk of scarring. The drugs are tetracycline, erythromycin, and trimethoprim. Side effects observed are hepatic and renal impairment with long term use it can be resolved by biochemical monitoring.

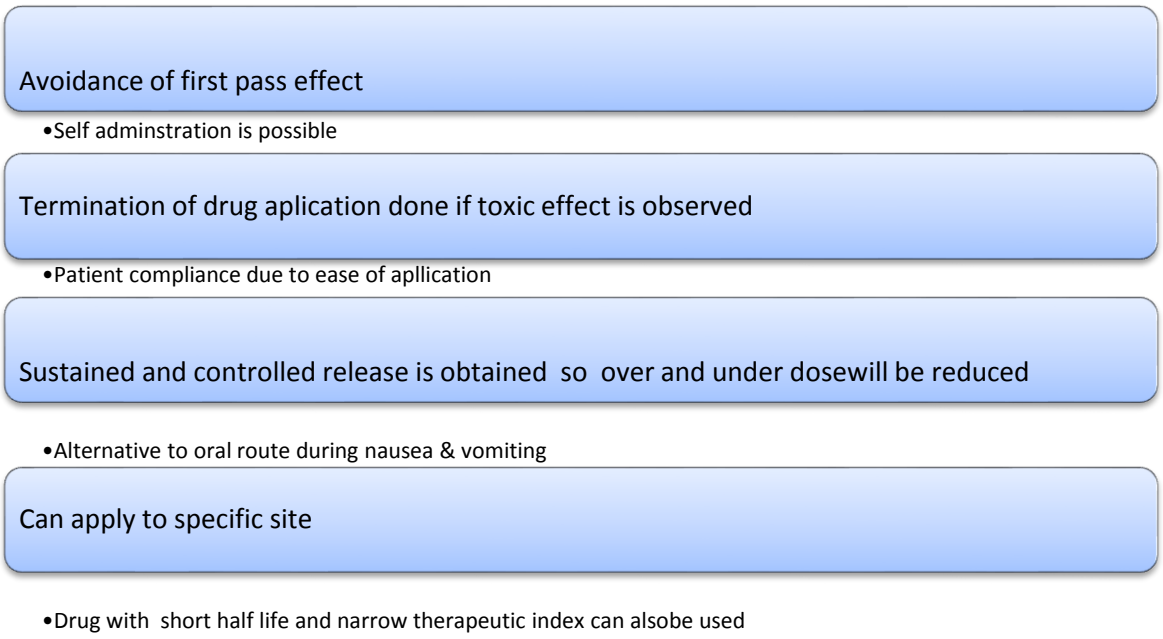
1.3 Topical dosage forms:

Topical dosage forms are a dosage form that applied on the skin surface to treat the skin disease or for the some other non-medical skin conditions. They can be is used in many skin conditions like itching, rosacea, sun tanning, infection, acne etc.

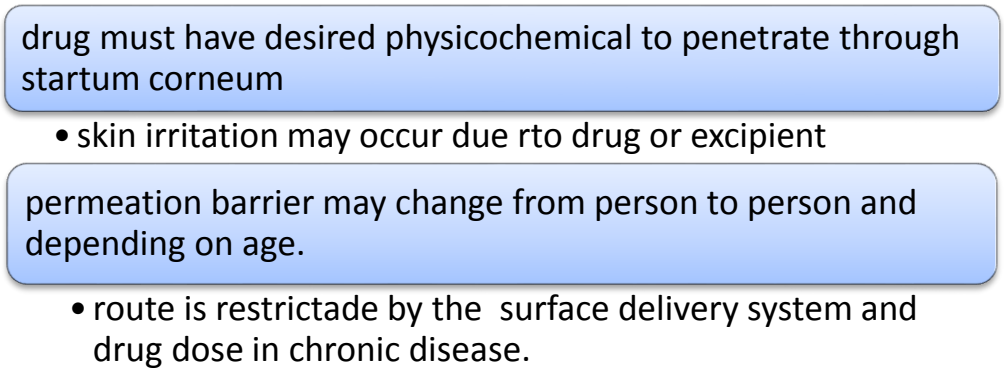
The topical dosage forms are classified as below:



1.3.1 Advantages:



1.3.2Disadvantages:



1.4 Microemulsion:

T. P. Hoar and J. H. Shulman was the first who has used microemulsion term they were professor of chemistry at the Cambridge University in 1943. They produced a clear monophasic solution by titrating milky emulsion with hexanol. They prepared oil in water type of microemulsion by dispersing oil in aqueous solution containing surfactant and used alcohol as a co-surfactant that system formed clear and stable microemulsion. Transparent emulsion, swollen micelle, micellar solution and solubilized oil are the alternate names used for this system.

The term microemulsion is defined as "**Microemulsions** are clear, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a co-surfactant"

Table 1.2 Comparison of micro, macro and nano emulsion:

Properties	Emulsion	microemulsion	nanoemulsion
appearance	milky	transparent	Milky or transparent
Optical isotropy	anisotropic	isotropic	isotropic
Interfacial tension	high	Very low	Very low
Phases	Biphasic	monophasic	monophasic
Droplet size	>500 nm	2-200 nm	0-100 nm
Stability	Thermodynamically unstable, , kinetically stable	Thermodynamically stable	Thermodynamically stable, kinetically unstable
Cost	Not cost effective	Cost effective	Not cost effective
Viscosity	Highly viscous	Less viscous	Less viscous

Energy input	High shear stress required	No energy input	High energy put is required

1.4.1 Advantages of microemulsion:

- Thermodynamically stable an allow self emulsification
  - Improves solubility of the lipophilic and hydrophilic drugs and drugs which is insoluble in both aqueous and lipophlic phase
- Pseudo zero order drug release can also be obtained depending on the drug dispersing phase
  - Absorption can be increased due to higher interfacial tension
- Both hydrophilic and lipophilic drug can be incorporated
  - Has low viscosity compared to macroemulsion and thermodynamicalt stable
- Less energy shource is required to prepare so it is easy to prepare
  - has higher efficacy soside effect will be less

1.4.2 Disadvantages of microemulsion:

- Require higher amount of s/cos ratio to stabilize the formulation
  - Nontoxic surfactant should be used
- Drug with higher melting point is not used because of less solubility
  - Microemulsion formulation is affected by environmental factor.

1.4.3 Types of microemulsion:

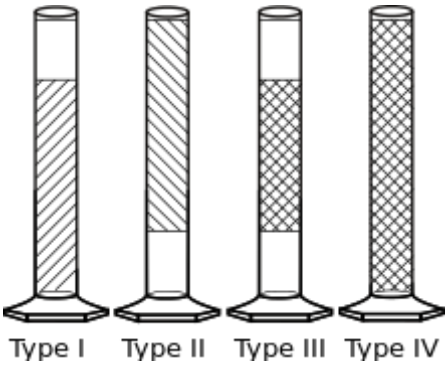


Fig 1.17 types of microemulsion

A well-known classification system is by which microemulsion classified is winsor who identified four types of microemulsion these are referred as Winsor phases:

Type I: It has two phases oil in water microemulsion form. The surfactant is soluble in water and this phase coexists with oil phase small concentration of surfactant is present as monomers.

Type II: with two phases water in oil it form microemulsion. surfactant is solublise in oil phase so surfactant containing oil phase coexists with water phase.

Type III: this is a middle phase microemulsion .it exists with three phases in which surfactant is present between the water and oil surfactant poor phases.

Type IV: it is in a single phase and form isotropic miceller solution that is formed by addition sufficient amount of amphiphile or co-surfactant.

1.4.4 Theories of microemulsion:

There are three approaches for the formation of microemulson:

1) Interfacial or mixed film theories.

2) Solubilisation theories.
3) Thermodynamic treatment

1) Interfacial film theories:

The complex film formed by surfactant and co-surfactant at oil and water interface that spontaneously form microemulsion droplets.so it will decrease the oil water tension to the very low from zero to negative. This film is equilibrium with the oil and water and it was considered to be liquid and duplex in nature with two dimensional spreading pressure n i, which determine the interfacial tension Y,

Yi =Yo/w – ni

Where,

Y o/w = oil in water interfacial tension without film
ni = spreading pressure

When large amount of surfactant and co-surfactant will adsorb at the surface of oil and water interface the spreading pressure become larger than Yo/w so interfacial tension will become negative .energy available will increase the interfacial area and reduce the droplet size. This negative interfacial is transient phenomenon it will become zero or small positive value at the equilibrium.

2) Solubilisation theory

Shinoda and friberg considered microemulsion as a thermodynamically stable miceller solution of water swollen (w/o) or oil swollen (o/w) spherical micelles. Rance and friberg described relationship of reverse micelles and w/o microemulsion system with the phase

diagram, where ternary systemcomposed of water, pentanol, and SDS gave transparent microemulsion by addition of p-xylene to inverse the miceller region.

3) Thermodynamic treatments:

The extent to which surfactant lowers the surface tension of the oil water system that will change the entropy of the system such that:

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

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

In the conventional emulsion this interfacial tension is much larger than the entropy so there is energy or shear stress is required for the formation of emulsion. The free energy of the emulsion is positive as energy is required for the breakage of the flocculation and coalescence, that will reduce the interfacial energy.to reduce this emulsifiers are used as energy barrier. In the microemulsion the droplets are dispersed in the continuous phase that will increase the entropy of the system and give negative free energy change which is not significant when droplets are larger. As the concentration of surfactant and co-surfactant increase at the interface it cause reduction in the chemical potentialand produce additional negative entropy change, it will give 'dilution effect'. So microemulsion is formed due to negative free energy change that is because of surfactant and co- surfactant accumulation at the interface entropy of the droplet dispersion in the continuous phase, so it will overcome the

small interfacial tension and large interfacial area. In this situation free energy of the system become zero or negative.

This describes the thermodynamic stability of the microemulsion it majorly depends on the ultra-low interfacial tension which is achieved by using surfactant and co-surfactant that will reduce the interfacial tension.

1.4.5 Microemulsion for topical application:

Microemulsion is a novel drug delivery system it has many advantages over conventional drug delivery system like cream, gel etc. it can incorporate both hydrophilic and lipophilic drugs there by increase the solubilisation of the insoluble drugs that can increase the therapeutic availability of that drug.

In microemulsion surfactant and co-surfactants are used that can increase the penetration of the drug by decreasing diffusion barrier of the skin. Water is used as an aqueous phase that can hydrate the skin and thereby increase the bioavailability of the drug. It will increase the therapeutic activity of the drug so the side effect will be decreased. In the microemulsion droplet size is very less (by dispersing o/w or w/o) so they are very good carrier for the transport of lipophilic compound in to the skin. They are considered as the ideal vehicle for the penetration of the active component inside the lipophilic environment of the pilosebaceous unit in the acne.

1.5 Microemulgel:

Hydrogels are cross-linked 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 cross-linked 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 gelatine, and polysaccharides such as alginate and agarose. Synthetic polymers

that form hydrogels are traditionally prepared using chemical polymerizationmethods. 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.

1.6 Drug profile:

- **Introduction to drug:**

  - Resveratrol (3,5,4′-trihydroxystilbene) is a non-flavonoid polyphenolic compound abundant in grapes, peanuts and other foods that are commonly consumed as part of human diet.
  - The compound was first isolated from the root of Polygonum cuspidatum, a plant used in traditional Chinese and Japanese medicine

  - **Structure:**

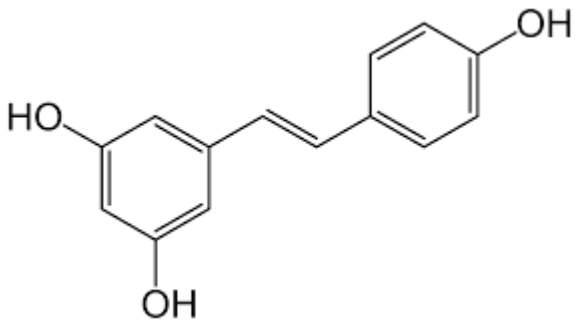


Fig1.18 structure of resveratrol

- **Physicochemical properties:**

Parameter	Resveratrol
Chemical formula	c14H12o3
IUPAC name	5-[(E)-2-(4-hydroxyphenyl)ethyl] benzene-1,3-diol
Molecular weight	228.24gm/mole
Description	Solid off white powder
Solubility	Soluble in methanol and ethanol
Melting point	253-255 ^{0}C
Partition coefficient log p	2.57
Dissociation constant Pka	8.99

- **Pharmacology:**

Resveratrol is known for its beneficial pharmacological properties like anti- aging, antioxidant, analgesic, anti-inflammatory, chemo-preventive, anti-platelet aggregation, anti-atherogenic, immunomodulation, cardioprotective and neuro-protective actions .

- The antioxidant effect of resveratrol is due to its free radical scavenging actions.
- The anti-radical activity of this polyphenol is responsible for most of its beneficial actions.
- The exact mechanism of action is still unclear but various research groups have pointed out the involvement of sirtuin SIRT1 receptors. Resveratrol activates SIRT1 receptors directly or may inactivate phosphodiesterase enzymes (PDEs) leading to a cascade which leads to activation of SIRT1. It has also been hypothesized to scavenge reactive oxygen species (ROS) and reduce facial redness which might have been caused by sun damage or atopic/contact dermatitis.

1.7 Polymer profile

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:**

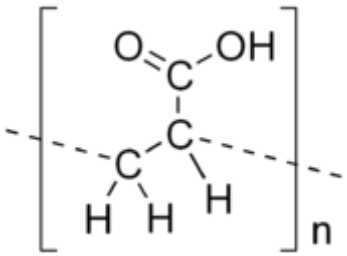


Fig 1.19 structure of carbopoal 940

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 odour.

- **Physical Properties:**

- pH: 2.5–4.0 for a 0.2% w/v aqueous dispersion; 2.5–3.0 for Acrypol 1% w/vaqueous dispersion.

- Melting Point: Decomposition occurs within 30 minutes at 2600C.

- Moisture content: Typical water content is up to 2% w/w. However, carbomers are hygroscopic and 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 glycerine 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 1.3 uses of carbopol 940

Use	% used
Emulsifying Agent	0.1-0.5
Gelling Agent	0.5-1.0
Suspending Agent	0.5-1.0
Tablet Binder	0.75-3.0
Controlled release agent	5.0-30

- **Stability:**

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 1040C 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, corrosion resistant 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 discoloured 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.

1.8 Excipient profile

1.8.1 Triacetin:

- **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:**

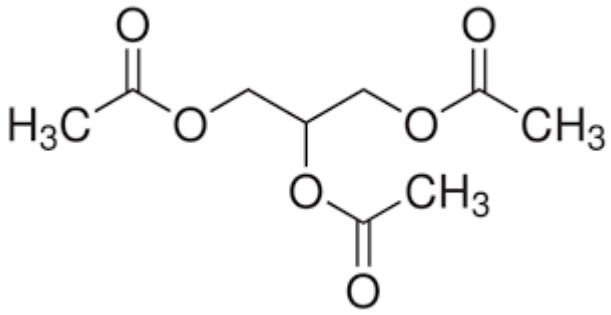


Fig 1.21 structure of triacetin

- **Molecular Weight:**

  218.21

- **Description:**

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

- **Physical Properties:**

Autoignition temperature : 4320C

Boiling point:2580C

Density: 1.16 g/cm3 at 250C

Flash point: 1530C (open cup)

- Freezing point: 3.20C (supercool to about –700C)

- Melting point : 780C
- Refractive index nD25 = 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 flavours.

- **Stability:**

Triacetin is stable.

- **Storage Conditions**

It should be stored in a well-closed, non-metallic 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 non-irritant 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.

1.8.2 Tween 80

- **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:**

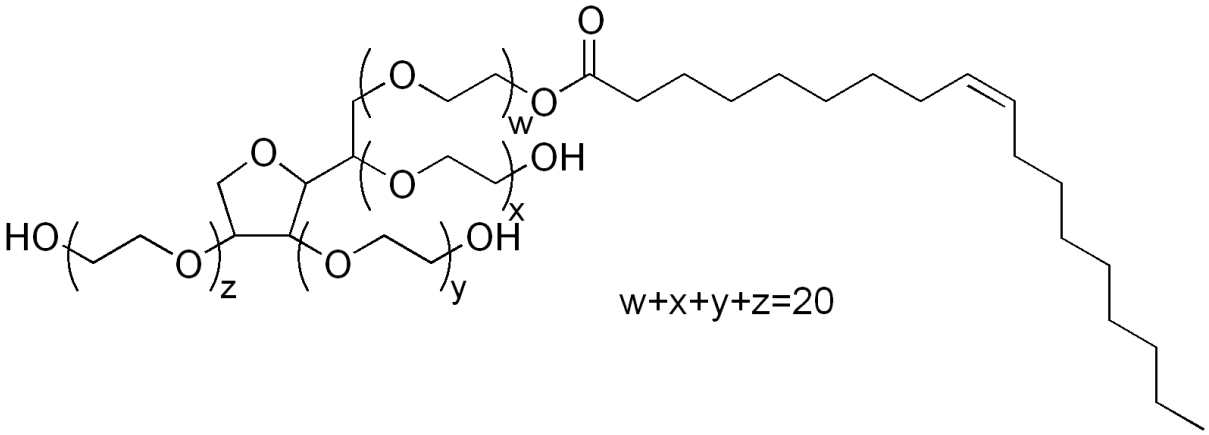


Fig 1.22 structure of tween 80

- **Molecular Weight:** 1310 g/ mol

- **Description:**

Tween 80 has a characteristic odor and a warm, somewhat bitter taste. Its colour and physical form at 250C is Yellow oily liquid.

- **Physical Properties:**

HLB Value : 15
Specific Gravity: 1.08 at 250C
Viscosity (dynamic): 425 mPa S at 250C;
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.

- **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-birth weight 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

1.8.3 Poly ethylene glycol (PEG 400):

- **Non-proprietary names:**

PhEur: Macrogols

JP: Macrogol 400

USP-NF: PEG

BP: Macrogols

- **Synonyms :**

Pluriol E, Carbowax Sentry, Carbowax, Lipoxol, Lutrol E, macrogola, PEG. Biochemical name: α-Hydro-ω-hydroxypoly(oxy-1,2-ethanediyl)

- **CASnumber :** (25322-68-3)

- **Experimental formula:** HOCH2(CH2OCH2)mCH2OH

- **molecular weight** : 380-420 g/mol

- **Structural formula:**

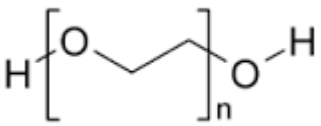


Fig 1.23 structure of polyethylene glycol

- **Uses :**

  Excipient in parenteral, topical, ophthalmic, oral, and rectal preparations, Ointment base.

- **Description :** Polyethylene glycol 400 are liquid at ambient temperatures. PEG 400 arises as clear, somewhat yellow-colored or colourless, viscous fluids.

- **Physicochemical properties:**

Form : liquid

Colour : colourless

Odor : characteristic

Boiling point : °c: > 250

Flash point: °c: > 245

Specific gravity @ 20°c: ~1.

pH (5% aq. sol.): 4.5 – 7.5

Solubility in water: soluble in water and insoluble in aliphatic hydrocarbon

Ignition temperature °C: ~ 380

- **STABILITY AND REACTIVITY**

Chemical stability: Normally stable

Reactivity: Does not react at ambient temperature

Compatibility with other chemicals: all polar solvents

Hazardous reaction products: No hazardous products known

- **ADVERSE EFFECTS:**

Respiratory: Not likely to occur

Skin: Prolonged or frequent contact may give transient redness and skin cracking

Eyes: Slightly irritating

Ingestion: Low acute toxicity

- **Storage:** avoid the containers in open condition

- **Regulatory information:**

Substance name: PEG 400 (Polyethylene Glycol)

Eu directive: Safety Data sheet According to EC-directive 2001/58/EC

Other information: Not classified as dangerous according to EEC Dangerous Substances Directive and Dangerous Preparation Directive

Aim and objective of present work:

Resveratrol is a beneficial compound found in the red wine red grape skins, purple grape juice, mulberries, and in smaller amounts in peanut, it is associated with life extension and some of the other health benefits. Resveratrol is polyphenolic phylotexin compound derivative of stillben class.it has multiple health benefits it is used for the atherosclerosis, heart disease, insulin resistance, lowering of bad cholesterol and increase good cholesterol, prevents cancer. It also has anti -inflammatory and anti -microbial effect which is useful in the cane treatment. It has antioxidant properties that can decrease the aging. According to pathophysiology of the acne the ideal treatment for such a condition would be single drug capable of inhibiting p acne bacterial growth and has anti- inflammatory activity. Resveratrol has both properties and also comparatively safe drug than conventional drug for acne treatment.

Despite its therapeutic efficacy in acne, clinical use of resveratrol is limited due to its physicochemical properties. Resveratrol is a BCS class-II drug. Resveratrol is less soluble or comparatively insoluble in the hydrophilic phase and it has high absorption power but bioavailability is very less. This characteristic is unfavourable for the production of resveratrol formulations with adequate bioavailability. An unexplored therapeutic approach for administration of resveratrol is the topical route. Utilization of topical applications provides an efficient strategy for the treatment of acne.

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.

Among the various colloidal nanosystems available, microemulsions are notable because they offer recognized advantages by increasing the solubility of hydrophobic drugs such as resveartrol. 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.

Thus, the aim of the present investigation was to design, develop & characterization of the microemulsion based hydrogel of reveartrol 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, by incorporating into gel.

3.1 Literature review for resveratrol :

Sr. no	Title / author	Description
1	Administration of resveratrol: What formulation solutions to bioavailability limitations? A. Amri , J.C. Chaumeil , S. Sfar , C.Charrueau .	It showed physicochemical and pharmacokinetic limitations to resveratrol bioavailability and describes formulations tested for resveratrol administration, controlled release and targeting, and identifies future opportunities for resveratrol delivery.
2	Simultaneous Determination of 14 Phenolic Compounds in Grape Canes by HPLC-DAD-UV Using Wavelength Ang Zhang , Li Wan ,Cuiyun Wu , Yulin Fan Wang.	It showed standard wavelength and method for uv spectroscopy detection of resveratrol
3	Resveratrol Photoisomerization : An Integrative Guided-Inquiry Experiment Elyse Bernard and Philip Britz-McKibbin.	Uv spectrometry standardisation method.

4	Preparation and physicochemical characterization of trans-resveratrol nanoparticles by temperature-controlled antisolvent precipitation Sanggu Kim , Wai Kiong , Yuancai Dong , Surajit Das , Reginald B.H. Tan .	It showed topical formulation of resveratrol for improvement of bioavailability and solubility of the drug .

3.2 Literature review for microemulsion:

5	**Preparation and Characterization of Dexamethasone Microemulsion Based on Pseudoternary Phase Diagram**	**The purpose of this study was the preparation and evaluation of novel microemulsion as a topical delivery system for dexamethasone by mixing appropriate amount of surfactant including Tween 80 and Labrasol, cosurfactant such as capryol 90 and oil phase including labrafac lipophile wl-transcutol P**
6	N. Aggarwal et al., 2013 Excipient used: 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.

3.3 Literature review for microemulsion gel:

7	**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″) ofthe 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 invitro permeation test in mice.**
8	**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. Theyshowed 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.**

4.1 materials & equipments

Table 4.1List of materials used

MATERIALS	COMPANY NAME
Resveratrol	Central drug house pvt.ltd. 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 study:

4.2.1 Identification of resveratrol

4.2.1.1Melting point:

Melting point of resveratrol was measured by thiel's tube method and observed melting point of drug was compared with standard.

Result:

Table no 4.3 melting point analysis of resveratrol

Sr. no	Drug	Reported Melting Point	Observed Melting Point
1	Resveratrol	254	253-255

Conclusion:

The observed melting point of resveratrol was found to be similar to the standard melting point so it confirmed the identity of procured drug sample.

4.2.1.2 UV spectrometric analysis:

7µg/ml drug solution was scanned in the range of 200 nm to 400 nm using double beam UV/Visible spectrophotometer.

The wavelength maxima was found and compared with reported wavelength of resveratrol

Result:

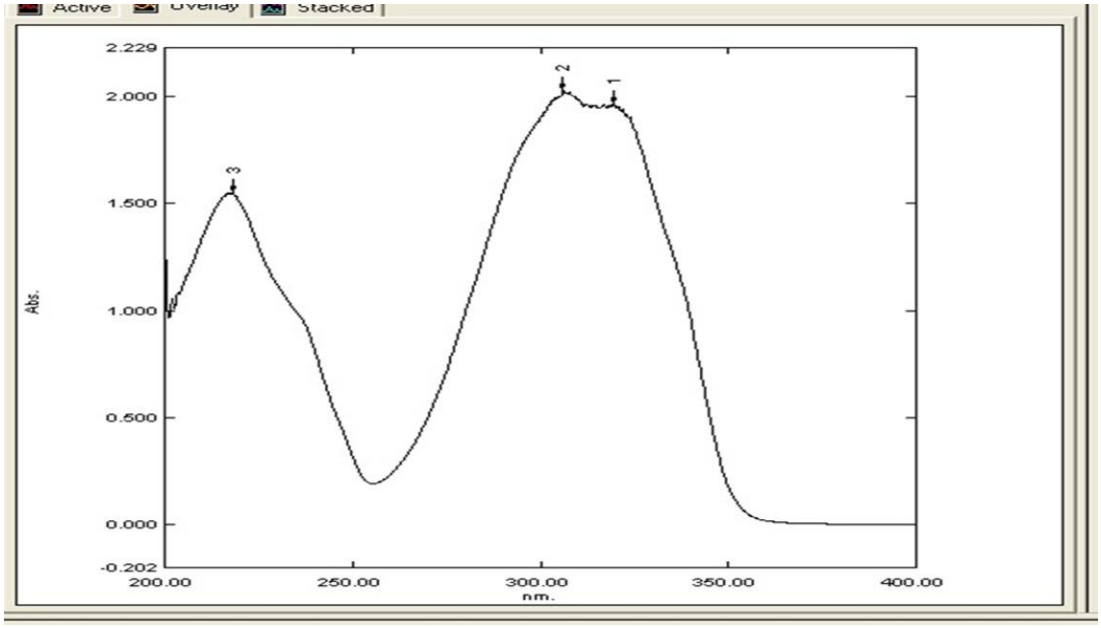


Fig4.1: standard curve of resveratrol

Table 4.4 wavelength maxima of (λ_{max}) resveratrol

Sr.no	**Drug**	**Reported wavelength maxima (λ_{max})**	**Observed wavelength maxima (λ_{max})**
1	Resveratrol	306nm	308nm

Conclusion:

The wavelength maxima of resveratrol were found to be 306nm which was almost identical to the reported wavelength maxima of drug, which again confirm the identity of acquired drug sample.

4.2.1.3 FT-IR SPECTRA OF RESVERATROL:

Identification of drug sample was done by infrared spectroscopy. The IR spectra were recorded in the solid state as a KBr dispersion medium using the FTIR spectrophotometer. The spectrum was run in the range of 4000 to 400 cm-1.

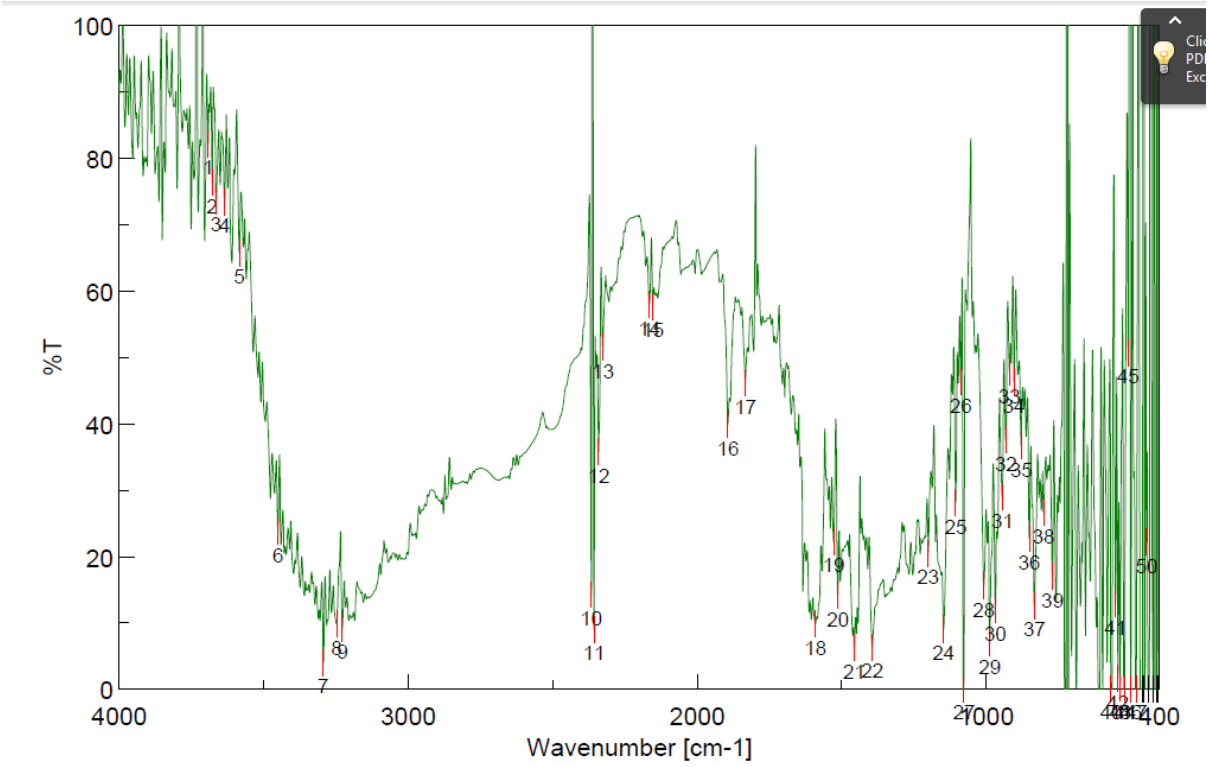


Fig 4.2 : Ir spectra of resveratrol

Development of standard spectrophotometric method for estimation of resveratrol:

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 resveratrol.

4.2.1.4 Calibration curve of resveratrol:

The standard stock solution of resveratrol was prepared by dissolving 10mg of drug in 100ml of methanol.

Suitable dilutions were made from the prepared stock solution to obtain concentration in the range of 1 to 7 µg/ml.

Absorbance maxima (λ_{max}) was measured by taking absorbance spectra of 7µg/ml solution on UV visible spectrophotometer and absorbance maxima was found at 306nm. The absorption of all the prepared solutions was then measured at the λmax, 306 nm, against blank. The readings were recorded in triplicate and the regressed Calibration curve was developed & equations generated to estimate the drug content.

Calibration plot of resveratrol in methanol:

Table 4.5 calibration of resveratrol in methanol

concentration	Absorbance 1	Absorbance 2	Absorbance 3	Average	SD
0	0	0	0	0	0
1	0.122	0.12	0.123	0.122	0.00126
2	0.273	0.275	0.272	0.273	0.00126
3	0.401	0.4	0.401	0.401	0.0005
4	0.573	0.515	0.518	0.516	0.028361
5	0.646	0.644	0.646	0.646	0.001
6	0.809	0.807	0.806	0.807	0.001258
7	0.959	0.958	0.955	0.959	0.001893

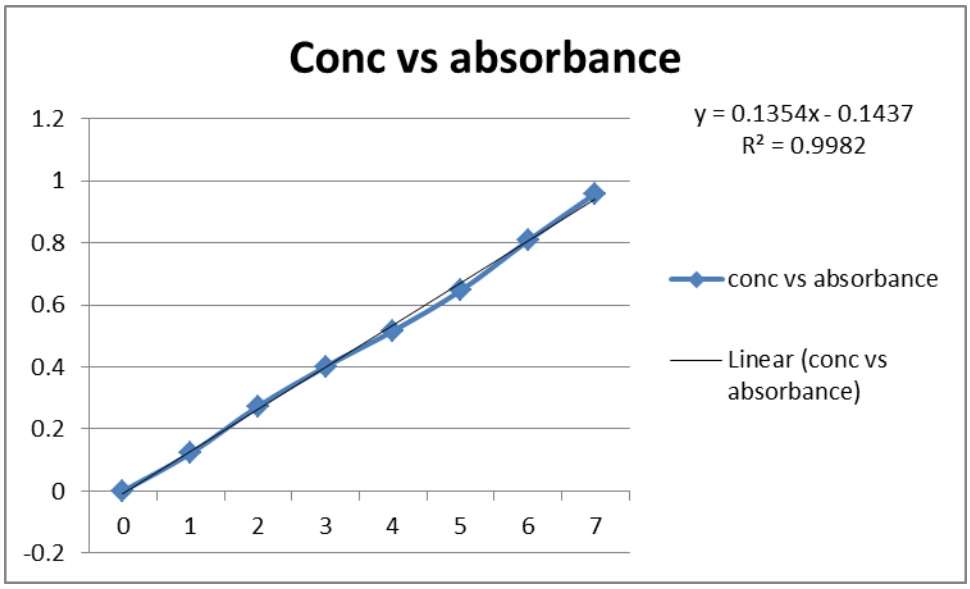


Fig 4.3 : calibration curve of resveratrol in methanol

Regression analysis

Table 4.6 Regression Analysis of the calibration plot of resveratrol in methanol

Sr. no	Regression parameter	Values
1	Correlation coefficient	0.9982
2	Slope	0.1354
3	Intercept	-0.1437

4.2.1.5 Calibration curve of resveratrol in phosphate buffer saline pH7.4:

Table 4.7 Preparation of phosphate buffer saline (pH7.4):

Sr.no	Ingredients	Amount
1	Potassium dihydrogen ortho phosphate0.2M	250ml
2	Sodium hydroxide0.2M	195.5ml
3	Distilled water	q.s.1000ml

Standard stock solution of resveratrol was prepared by dissolving 10mg of accurately weighed resveratrol in 100ml phosphate buffer saline (pH7.4).

Then, suitable dilutions were made from prepared stock solutions in the concentration range of 1to 10µg/ml .

The absorbance spectra was run at 10µg/ml concentration against blank sample and wavelength maxima was found at 304nm.then absorbance of all prepared sample was taken at 304nm against blank sample. Reading was recorded in triplicate and calibration curve is developed.

Calibration plot of resveratrol in phosphate buffer (pH 7.4):

Table 4.8calibration of resveratrol in phosphate buffer

concentration	**Absorbance 1**	**Absorbance 2**	**Absorbance 3**	**Average**	**SD**
0	0	0	0	0	0
1	0.137	0.135	0.133	0.135	0.001633
2	0.246	0.247	0.245	0.246	0.000816
3	0.379	0.376	0.378	0.377	0.001291
4	0.426	0.428	0.425	0.426	0.001258

5	0.567	0.569	0.565	0.567	0.001633
6	0.648	0.649	0.650	0.649	0.000816
7	0.759	0.761	0.756	0.758	0.002082
8	0.835	0.832	0.831	0.832	0.001732
9	0.927	0.929	0.927	0.927	0.001
10	0.958	0.960	0.961	0.959	0.001291

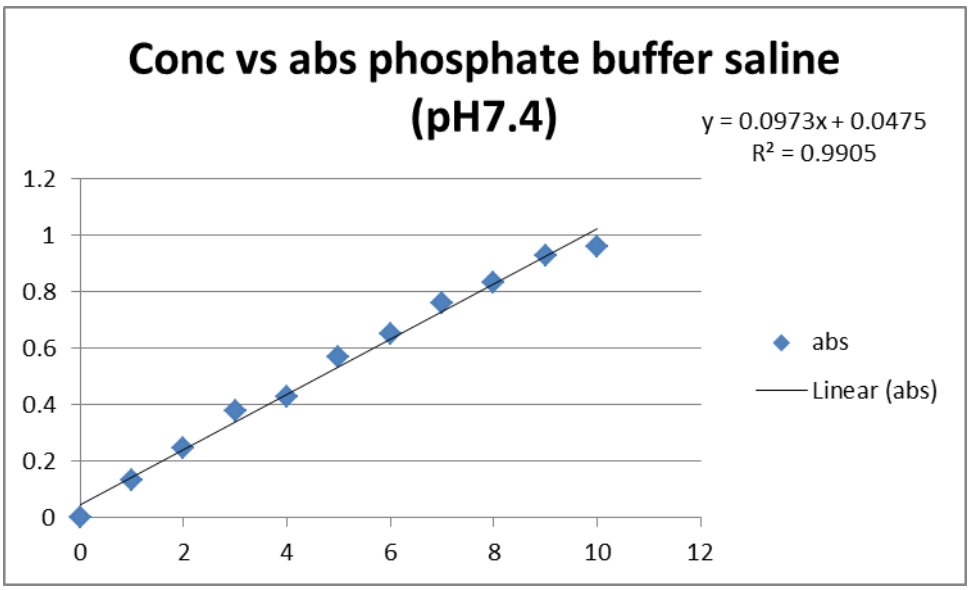


Fig4.4 : calibration curve of resveratrol in phosphate buffer

Table 4.9 Regression Analysis of the calibration plot of resveratrol in PBS (pH 7.4):

Sr.no	Regression parameter	Value
1	Correlation coefficient	0.990
2	Intercept	0.097
3	Slope	0.0475

Conclusion:

The correlation coefficient found near to 1 indicates good correlation.

4.3 Methods:

In order to get the concentration range of different component oil , surfactant/co-surfactant mixture, water used in the microemulsion to get microemulsion region. Pseudo ternary diagram was constructed using aqueous water titration method .Surfactant/co-surfactant (Smix) mixture were prepared in proportion of 1:1, 2:1 & 1:2.

4.3.1 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 were used.

4.3.1.1 Solubility study:

To determine the saturated solubility of resveratrol in different oils, surfactant and co-surfactant excess amount of resveratrol was added in dark brown tightly closed glass vials containing 5 ml of vehicle. The mixture was vortexed to facilate the solubilisation. Then glass vials were placed on shaker bath at 37°C for 48 h. To remove the undissolved resveratrol samples were centrifuged at 4000 rpm for 15 min. Then the supertant was removed and filtered through 0.45 µm filter paper. Then aliquot dilution were made with methanol to get the concentration of resveratrol by taking absorbance at 306 nm in UV spectrophotometer.

Results:

Solubility studies of resveratrol in oils:

Table 4.10 Solubility of resveratrol in oils

Sr.no	Oils	Solubility(mg/ml)
2	Ethyl oleate	0.08±0.1
3	Coconut oil	0.5±0.16

4	Oleic acid	0.85±0.15
5	Soyabean oil	0.76±0.23
6	Isopropyl myristate (IPM)	1.6±1.2
7	Triacetin	32.8±1.6

*(n=3, mean±SD)

Discussion:

Resveratrol shows higher solubility in triacetin oil compared to other oils

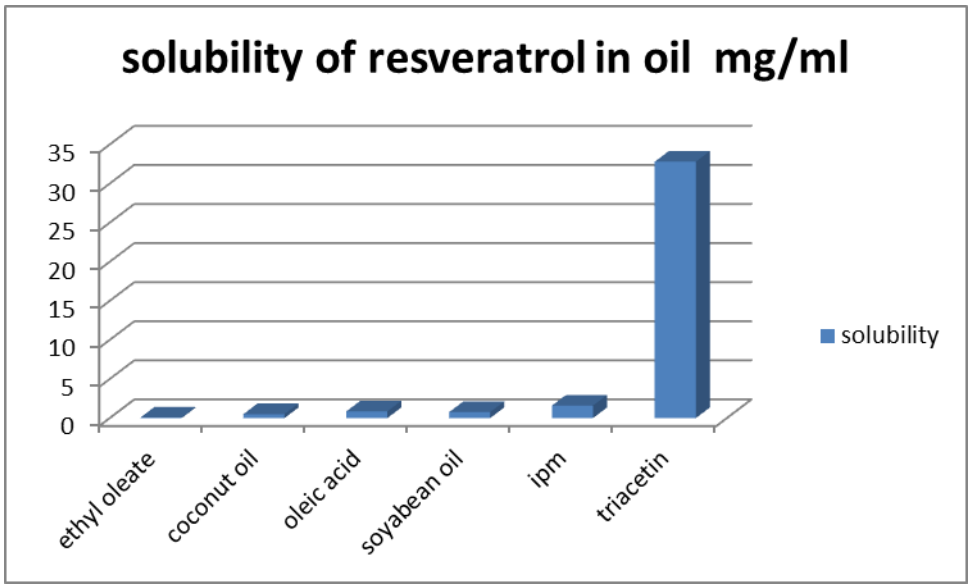


Fig 4.5: solubility of resveratrol in oils

Solubility of resveratrol in surfactants:

Table 4.11 solubility of resveratrol in surfactants

Sr.no	surfactants	Solubility(mg/ml)
1	PEG 400	705.3±1.2
2	Span 80	23.5±0.8
3	Span 20	37.8±2.5
4	Tween 80	830.7±5.6
5	Tween 20	42.6±3.5
6	Labrasol	609.6±8.4
7	Propylene glycol	316.2 ±6.8

*(n=3, mean±SD)

Discussion :

Resveratrol shows higher solubility in tween 80 compared to other surfactants

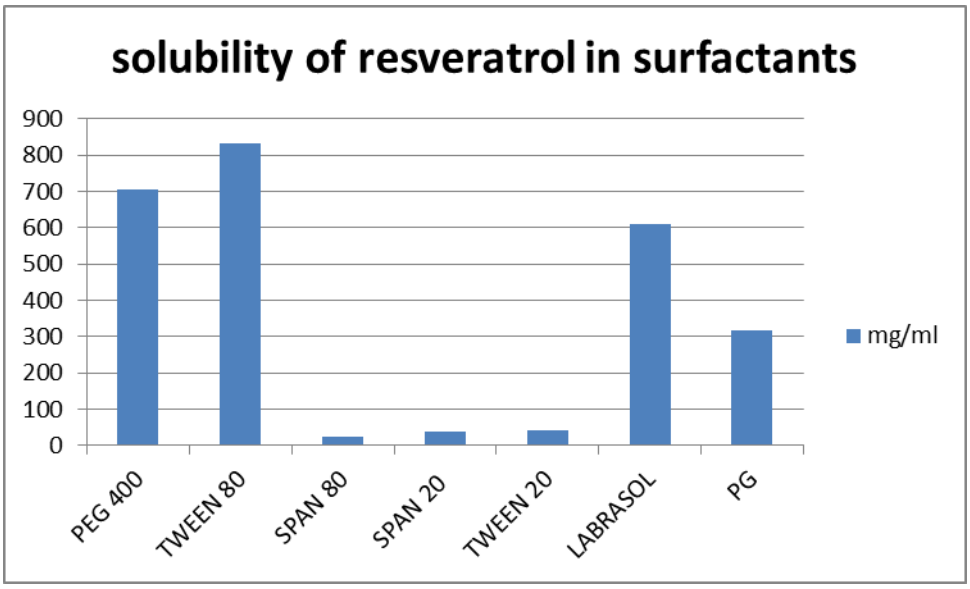


Fig 4.6: solubility of resveratrol in surfactants

Conclusion:

From above observation it was found that resveratrol has highest solubility in triacetin oil and tween 80 with 32.8±1.6mg/ml and 830.7±5.6 mg/ml respectively, so triacetin was selected as oil phase and tween 80 was selected as surfactant.

4.3.1.2 Visual miscibility of surfactant with co-surfactant:

Miscibility method was used to select the co-surfactant for microemulsion preparation. Visual miscibility was checked by mixing surfactant with co-surfactant. Weaker the interphase formed higher will be the miscibility between them. Co-surfactant which has higher miscibility with surfactant was found necessary to form large microemulsion domain.

Selection of co-surfactant:

Some studies have shown that mixture of two surfactant can enlarge the microemulsion region in phase diagram. Therefore mixture of surfactant and co-surfactant was taken in this research to enlarge the microemulsion region. From preliminary studies, triacetin and tween 80 were taken as oil phase and surfactant respectively.

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 (Smix) were then mixed with Triacetin at room temperature (25^0 C). The ratio of oil to Smix 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.

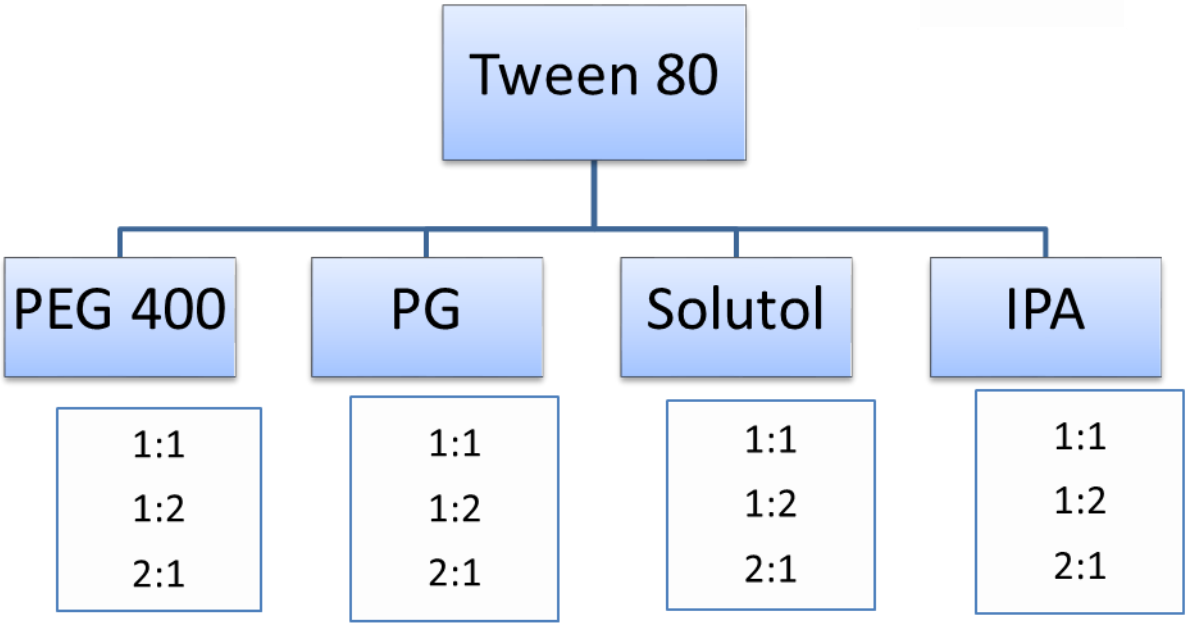


Fig 4.7 selection of co-surfactants

Results:

Table 4.12 phase separation studies

Ratio	PEG400	PG	Solutol	IPA
1:1	No phase separation	phase separation	phase separation	phase separation
1:2	No phase separation	phase separation	No phase separation	phase separation
2:1	No phase separation	No phase separation	No phase separation	phase separation

Conclusion:

From above observation it was concluded that PEG 400 containing microemulsion in any ratio has good stability up to one week with triacetin as oil phase and surfactant tween 80.

So from above all observations final optimized formula was:

Table 4.13 optimized components

Sr.no	Component
1	Triacetin (oil phase)
2	Tween 80 (surfactant)
3	PEG400 (co-surfactant)
4	Distilled water (aq.phase)

4.3.1.3 Construction of pseudo ternary phase diagram:

Pseudo ternary phase diagram was constructed using aqueous water titration method to get the microemulsion region. The ratio of surfactant to co-surfactant was used in different proportions 1:1, 2:1and 1:2 and then Smix mixture was prepared. The oil was then mixed with above prepared Smix in different concentration range of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 & 1:9. Then mixture of oil and Smix was vortexed for 2-3min at ambient temperature titrated with in5% increment at each adition of water. It was examined for the appearance. The end point is where the mixture becomes turbid or milky to clear. The end point was noted. The quantity required to make the mixture clear was noted and then percentage of each component was calculated. Same procedure was followed for the remaining ratios of Smix. Then pseudo ternary diagram was plotted using prossim software according to the observations and microemulsion region was identified.

Method 1: Following steps were used for optimization:

Step1: Surfactant /co-surfactant mixture (Smix) was prepared in 1:1, 2:1 and 1:2 ratios

Step 2: Then oil was mixed with Smix in different concentration range 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 & 1:9.

Step 3: Mixture of oil and Smix was titrated with water until it become milky to clear.

The formulation of mixture and appearance were shown in table:

Results:

Table 4.14 Smix1:1and oil: Smix concentration 9:1

SR. NO	OIL(ML)	SMIX(ML)	WATER(ML)	APPEARANCE
1	0.9	0.1	0.5	Milky
2	0.9	0.1	1	Milky
3	0.9	0.1	1.5	Milky
4	0.9	0.1	2	Milky
5	0.9	0.1	2.5	Milky
6	0.9	0.1	3	Milky
7	0.9	0.1	3.5	Milky
8	0.9	0.1	4	Milky
9	0.9	0.1	4.5	Milky
10	0.9	0.1	5	Milky
11	0.9	0.1	5.5	Milky
12	0.9	0.1	6	Milky
13	0.9	0.1	6.5	Milky
14	0.9	0.1	7	Milky

15	0.9	0.1	7.5	Milky
16	0.9	0.1	8	Milky
17	0.9	0.1	8.5	Milky
18	0.9	0.1	9	Milky
19	0.9	0.1	9.5	Milky
20	0.9	0.1	10	Milky
21	0.9	0.1	10.5	Milky
22	0.9	0.1	11	opaque
23	0.9	0.1	11.5	Opaque
24	0.9	0.1	12	Opaque
25	0.9	0.1	12.5	Opaque
26	0.9	0.1	13	Opaque
27	0.9	0.1	13.5	opaque
28	0.9	0.1	14	Clear
29	0.9	0.1	14.5	Clear

Table 4.15 Microemulsion containing Smix 1:1 and oil: Smix 8:2

SR.NO	OIL(ML)	SMIX(ML)	WATER(ML)	APPEARANCE
30	0.8	0.2	0.5	Milky
31	0.8	0.2	1	Milky
32	0.8	0.2	1.5	Milky
33	0.8	0.2	2.5	Milky
34	0.8	0.2	3	Milky
35	0.8	0.2	3.5	Milky
36	0.8	0.2	4	Milky
37	0.8	0.2	4.5	Milky
38	0.8	0.2	5	Milky

39	0.8	0.2	5.5	Milky
40	0.8	0.2	6	Milky
41	0.8	0.2	6.5	Milky
42	0.8	0.2	7	Milky
43	0.8	0.2	7.5	Milky
44	0.8	0.2	8	Milky
45	0.8	0.2	8.5	Milky
46	0.8	0.2	9	Milky
47	0.8	0.2	9.5	Milky
48	0.8	0.2	10	Milky
49	0.8	0.2	10.5	Milky
50	0.8	0.2	11	Opaque
51	0.8	0.2	11.5	Opaque
52	0.8	0.2	12	Opaque
53	0.8	0.2	12.5	Opaque
54	0.8	0.2	13	Clear

Table 4.16 Microemulsion containing Smix 1:1 and oil: Smix :7:3

SR. NO	OIL(ml)	SMIX(ml)	WATER(ml)	APPEARANCE
55	0.7	0.3	0.5	Milky
56	0.7	0.3	1	Milky
57	0.7	0.3	1.5	Milky
58	0.7	0.3	2	Milky
59	0.7	0.3	2.5	Milky
60	0.7	0.3	3	Milky
61	0.7	0.3	3.5	Milky

62	0.7	0.3	4	Milky
63	0.7	0.3	4.5	Milky
64	0.7	0.3	5	Milky
65	0.7	0.3	5.5	Milky
66	0.7	0.3	6	Milky
67	0.7	0.3	6.5	Milky
68	0.7	0.3	7	Opaque
69	0.7	0.3	7.5	Opaque
70	0.7	0.3	8	Opaque
71	0.7	0.3	8.5	Opaque
72	0.7	0.3	9	Opaque
73	0.7	0.3	9.5	Opaque
74	0.7	0.3	10	Clear
75	0.7	0.3	10.5	Clear

Table 4.17 Microemulsion containing Smix 1:1 and oil: Smix: 6:4

SR.NO	OIL(ml)	SMIX(ml)	WATER(ml)	APPEARANCE
76	0.6	0.4	0.5	Milky
77	0.6	0.4	1	Milky
78	0.6	0.4	1.5	Milky
79	0.6	0.4	2	Milky
80	0.6	0.4	2.5	Milky
81	0.6	0.4	3	Milky
82	0.6	0.4	3.5	Milky
83	0.6	0.4	4	Milky

84	0.6	0.4	4.5	Milky
85	0.6	0.4	5	Milky
86	0.6	0.4	5.5	Milky
87	0.6	0.4	6	Milky
88	0.6	0.4	6.5	Opaque
89	0.6	0.4	7	Opaque
90	0.6	0.4	7.5	Clear

Table 4.18 Microemulsion containing Smix 1:1 and oil: Smix: 5:5

SR.NO	OIL(ml)	SMIX(ml)	WATER(ml)	APPEARANCE
91	0.5	0.5	0.5	Milky
92	0.5	0.5	1	Milky
93	0.5	0.5	1.5	Milky
94	0.5	0.5	2	Milky
95	0.5	0.5	2.5	Milky
96	0.5	0.5	3	Milky
97	0.5	0.5	3.5	Milky
98	0.5	0.5	4	Milky
99	0.5	0.5	4.5	Milky
100	0.5	0.5	5	Milky
101	0.5	0.5	5.5	Milky
102	0.5	0.5	6	Milky
103	0.5	0.5	6.5	Opaque
104	0.5	0.5	7	Opaque
105	0.5	0.5	7.5	Opaque
106	0.5	0.5	8	Opaque
107	0.5	0.5	8.5	Clear

Table 4.19 Microemulsion containing Smix 1:1 and oil: Smix: 4:6

SR.NO	OIL(ml)	SMIX(ml)	WATER(ml)	APPEARANCE
108	0.4	0.6	0.5	Milky
109	0.4	0.6	1	Milky
110	0.4	0.6	1.5	Milky
111	0.4	0.6	2	Milky
112	0.4	0.6	2.5	Milky
113	0.4	0.6	3	Milky
114	0.4	0.6	3.5	Milky
115	0.4	0.6	4	Milky
116	0.4	0.6	4.5	Opaque
117	0.4	0.6	5	Opaque
118	0.4	0.6	5.5	Opaque
119	0.4	0.6	6	Clear

Table 4.20 Microemulsion containing Smix 1:1 and oil: Smix: 3:7

SR.NO	OIL(ml)	SMIX(ml)	WATER(ml)	APPEARANCE
120	0.3	0.7	0.5	Milky
121	0.3	0.7	1	Milky
122	0.3	0.7	1.5	Milky
123	0.3	0.7	2	Milky
124	0.3	0.7	2.5	Milky
125	0.3	0.7	3	Opaque

126	0.3	0.7	3.5	Clear
127	0.3	0.7	4	Clear

Table 4.21 Microemulsion containing Smix 1:1 and oil: Smix: 2:8

SR.NO	OIL(ml)	SMIX(ml)	WATER(ml)	APPEARANCE
128	0.2	0.8	0.5	Milky
129	0.2	0.8	1	Milky
130	0.2	0.8	1.5	Opaque
131	0.2	0.8	2	Opaque
132	0.2	0.8	2.5	Clear

Table 4.22 Microemulsion containing Smix 1:1 and oil: Smix: 9:1

SR.NO	OIL(ml)	SMIX(ml)	WATER(ml)	APPEARANCE
133	0.1	0.9	0.5	Milky
134	0.1	0.9	1	Opaque
135	0.1	0.9	1.5	Clear

Table 4.23 % of the component used in the microemulsion: OIL: SMIX (1:1)

SR.NO	OIL (%)	SMIX (%)	WATER (%)	TOTAL (%)
1	5.88	0.65	93.46	100
2	5.71	1.43	92.85	100
3	6.19	2.65	91.15	100
4	7.05	4.07	88.23	100
5	5.31	5.31	89.36	100
6	5.97	8.95	85.07	100
7	5.7	13.96	80.7	100
8	5.71	22.85	71.4	100
9	6.67	33.33	60	100

Table 4.24 % of the component used in the microemulsion: OIL:SMIX (2:1)

SR.NO	OIL(%)	SMIX(%)	WATER(%)	TOTAL(%)
1	6.20	0.69	93.10	100
2	6.15	1.53	92.30	100
3	6.25	2.67	91.07	100
4	5.76	3.84	90.38	100

5	5.88	5.88	88.23	100
6	6.25	9.37	84.38	100
7	6.52	15.21	78.26	100
8	6.25	25.00	68.75	100
9	4.54	40.9	54.54	100

Table 4.25 % of the component used in the microemulsion: OIL:SMIX (1:2)

SR.NO	OIL(%)	SMIX(%)	WATER(%)	TOTAL(%)
1	5.59	0.62	93.78	100
2	5.51	1.37	93.01	100
3	5.78	2.47	91.73	100
4	5.71	3.80	90.47	100
5	6.32	6.32	87.34	100
6	5.79	8.69	85.50	100
7	6.38	14.89	78.72	100
8	7.40	29.62	62.96	100
9	6.67	33.33	60.00	100

Fig 4.7 Pseudo ternary plot for Oil: Smix (1:1)

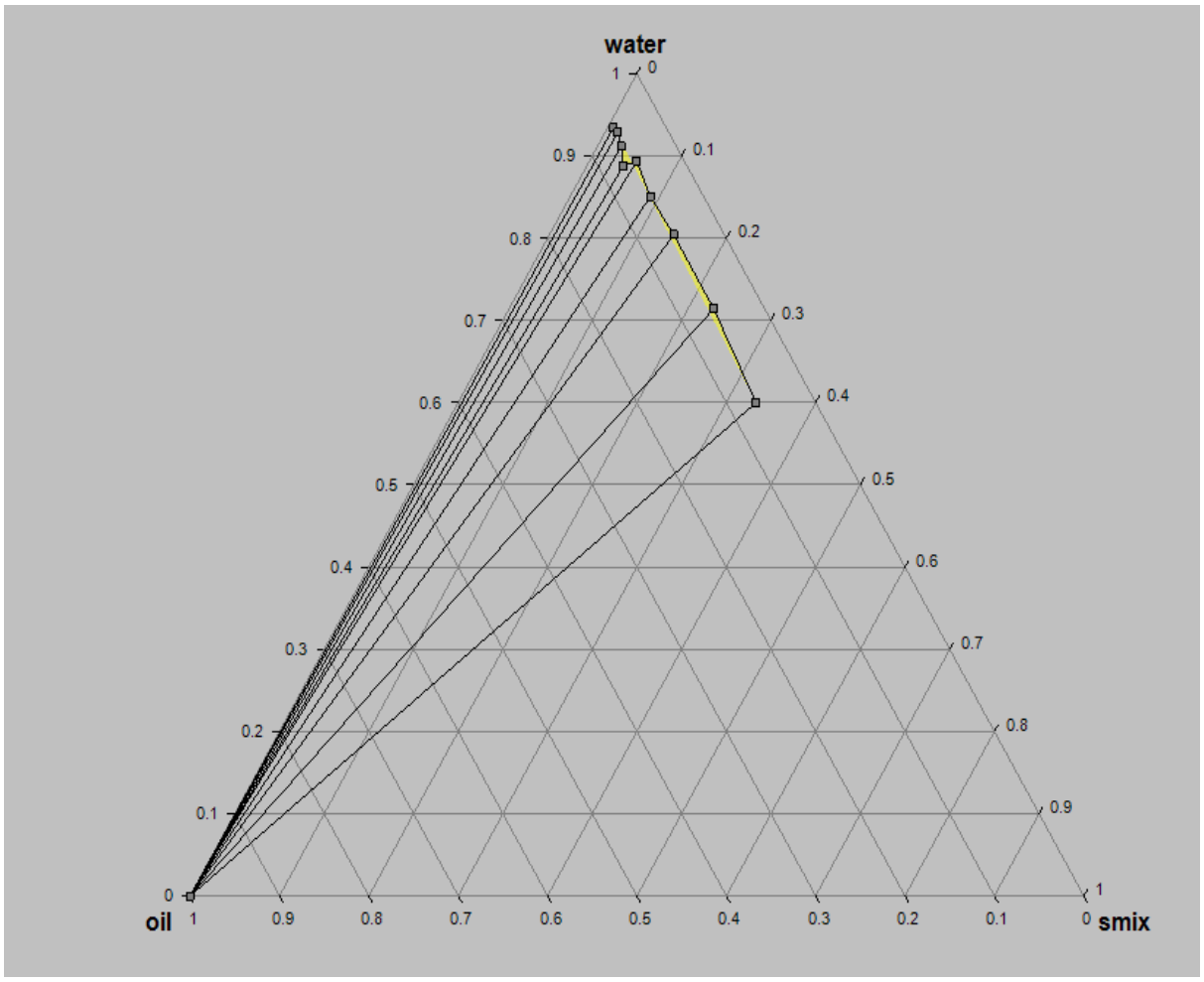


Fig 4.8 Pseudo ternary plot for Oil: smix 2:1

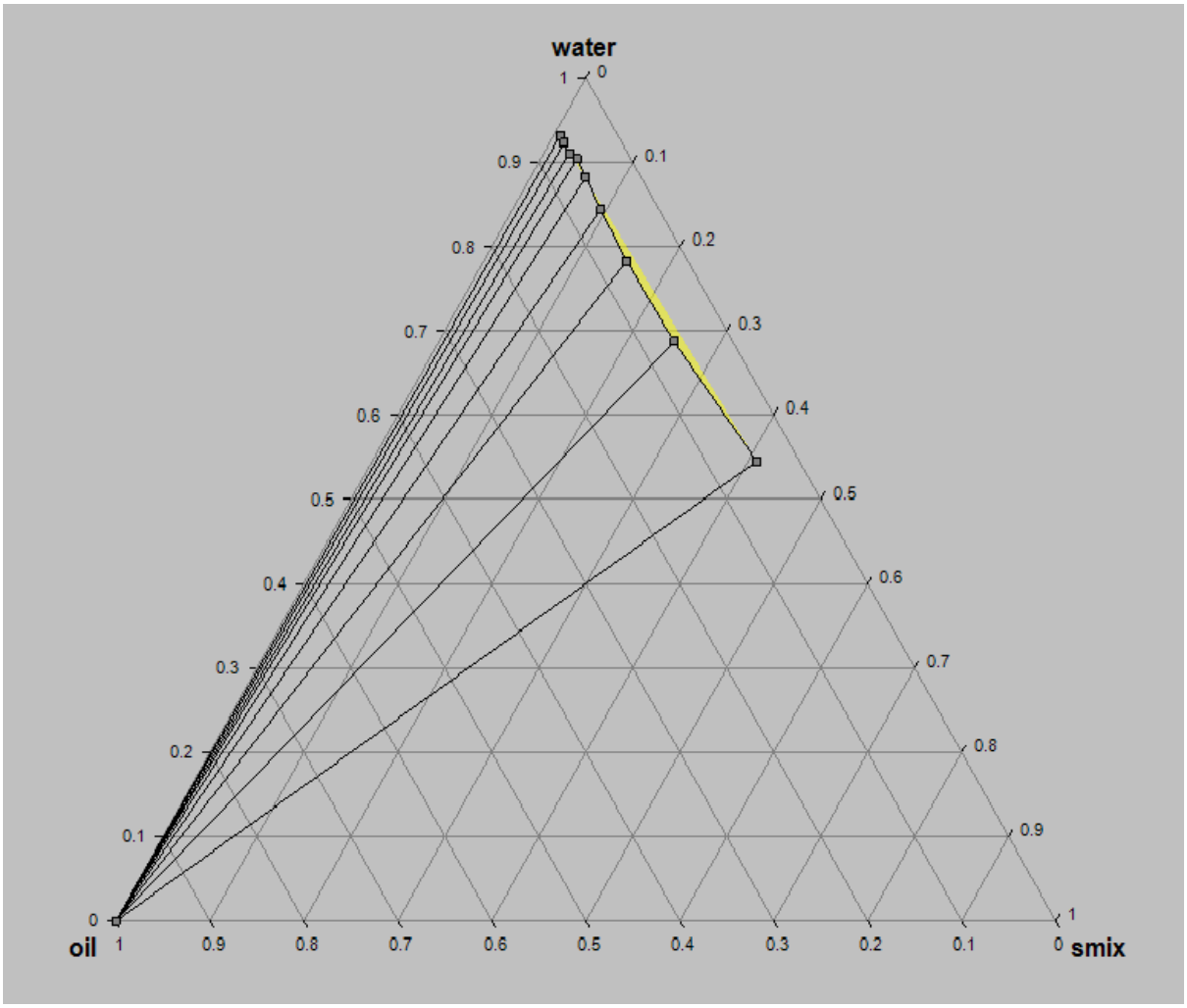
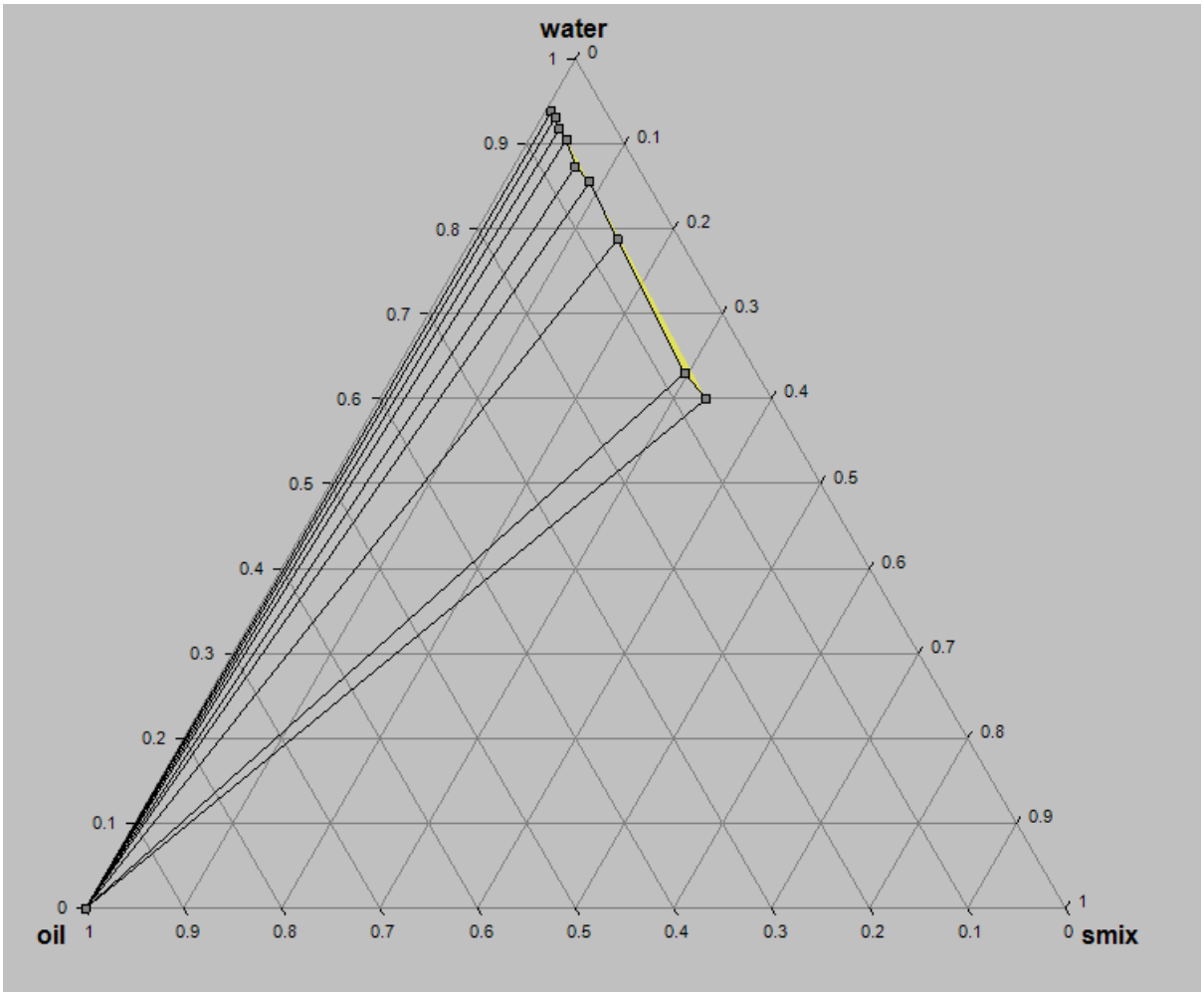


Fig 4.9 Pseudo ternary plot for Oil: smix 1:2



Discussion:

Pseudo ternary diagrams were plotted from observed results by applying first method desired microemulsion region was not obtained.

Method 2: following steps were used to optimize:

1) Step1: Surfactant /co-surfactant mixture (smix) was prepared in 1:1, 2:1 and 1:2 ratios

2) Step 2: Then oil was mixed with water(instead of smix done in method 1) in different concentration range 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 & 1:9.
3) Step 3: Mixture of oil and water was titrated with Smix until it become milky to clear

Table 4.26 % of the component used in the microemulsion: oil: water (smix1:1)

SR.NO	OIL (%)	SMIX (%)	WATER (%)	TOTAL (%)
1	56.25	37.5	6.25	100
2	40	50	10	100
3	29.16	58.33	12.5	100
4	23.07	61.53	15.38	100
5	17.85	64.28	17.85	100
6	13.33	66.67	20	100
7	9.67	67.74	22.58	100
8	5.88	70.58	23.53	100
9	2.94	70.58	26.47	100

Table 4.27 % of the component used in the microemulsion: oil: water (smix2:1)

SR.NO	OIL (%)	SMIX (%)	WATER (%)	TOTAL (%)
1	45	50	5	100
2	29.62	62.96	7.4	100
3	22.58	67.74	9.67	100
4	17.64	70.58	11.76	100
5	15.15	69.69	15.15	100
6	14.28	64.28	21.4	100
7	12	60	28	100
8	8.33	58.33	33.33	100
9	5.55	44.44	50	100

Table 4.27 % of the component used in the microemulsion: oil: water (smix1:2)

SR.NO	OIL (%)	SMIX (%)	WATER (%)	TOTAL (%)
1	5.55	44.44	50	100
2	9.09	54.54	36.36	100
3	12.05	58.33	29.16	100
4	15.38	61.53	23.07	100
5	15.15	69.69	15.15	100
6	17.64	70.58	11.76	100
7	22.58	67.74	9.67	100
8	26.9	61.53	11.53	100

9	29.62	62.96	7.4	100

Fig 4.11 Pseudo ternary plot for oil: smix ratio 1:1

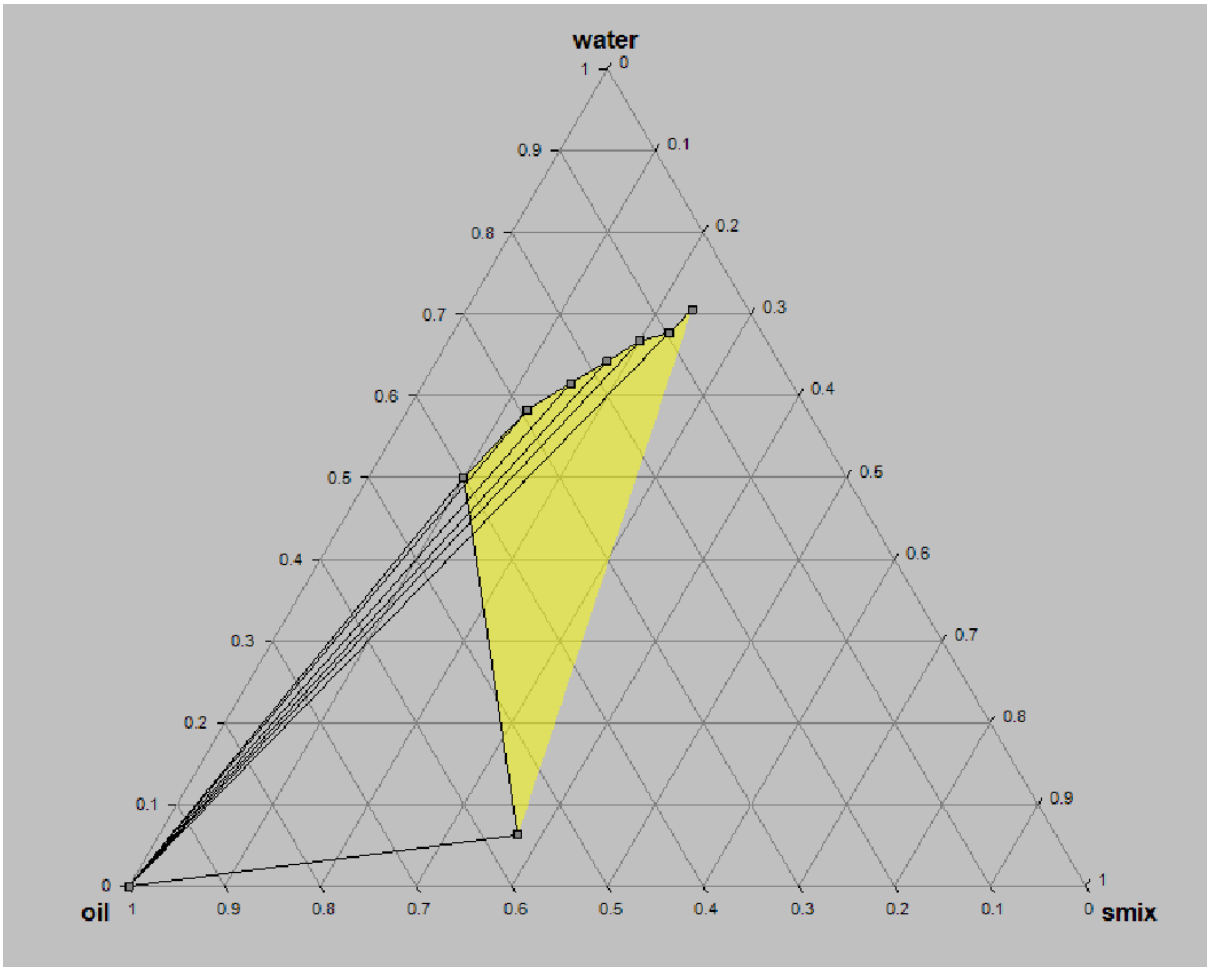


Fig 4.12 Pseudo ternary plot for oil: smix ratio 2:1

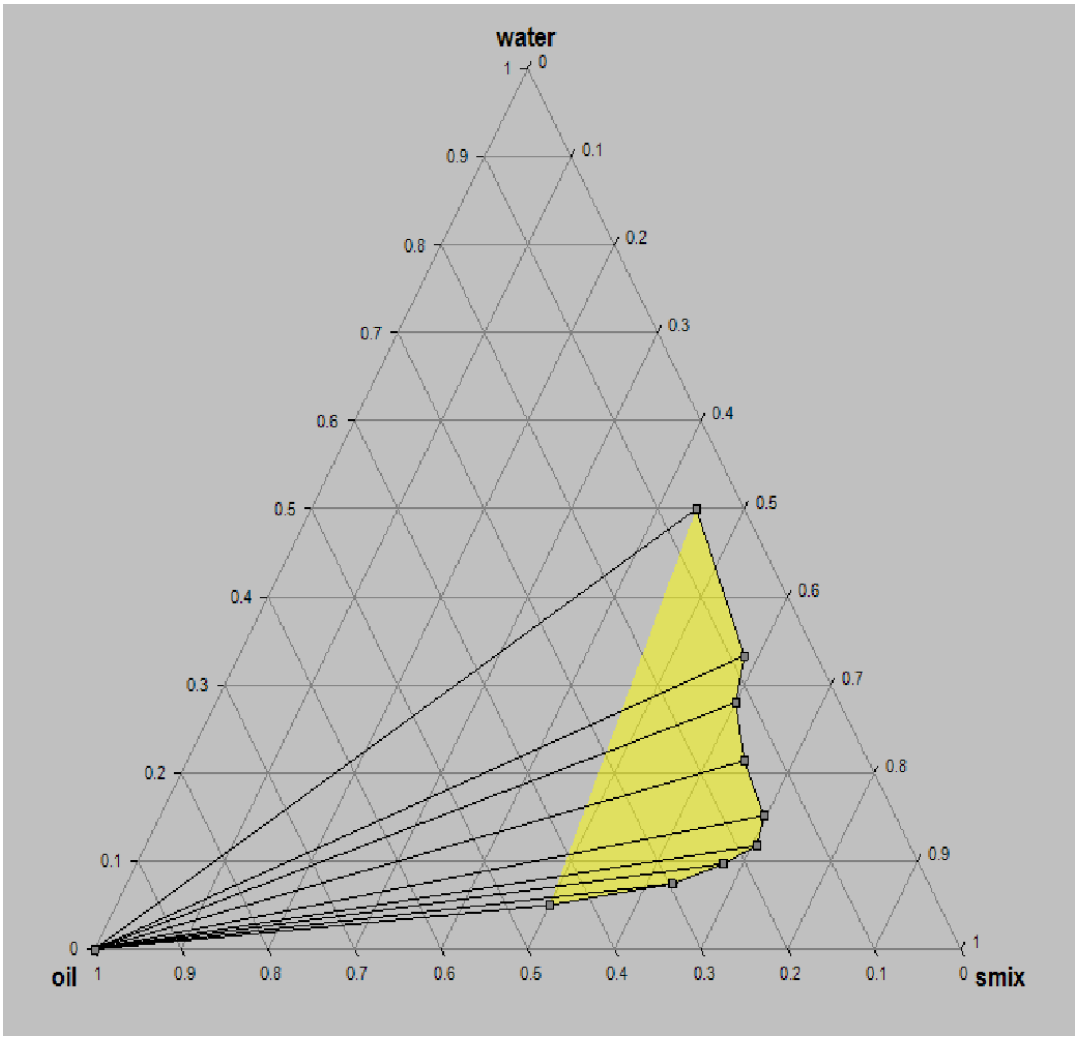
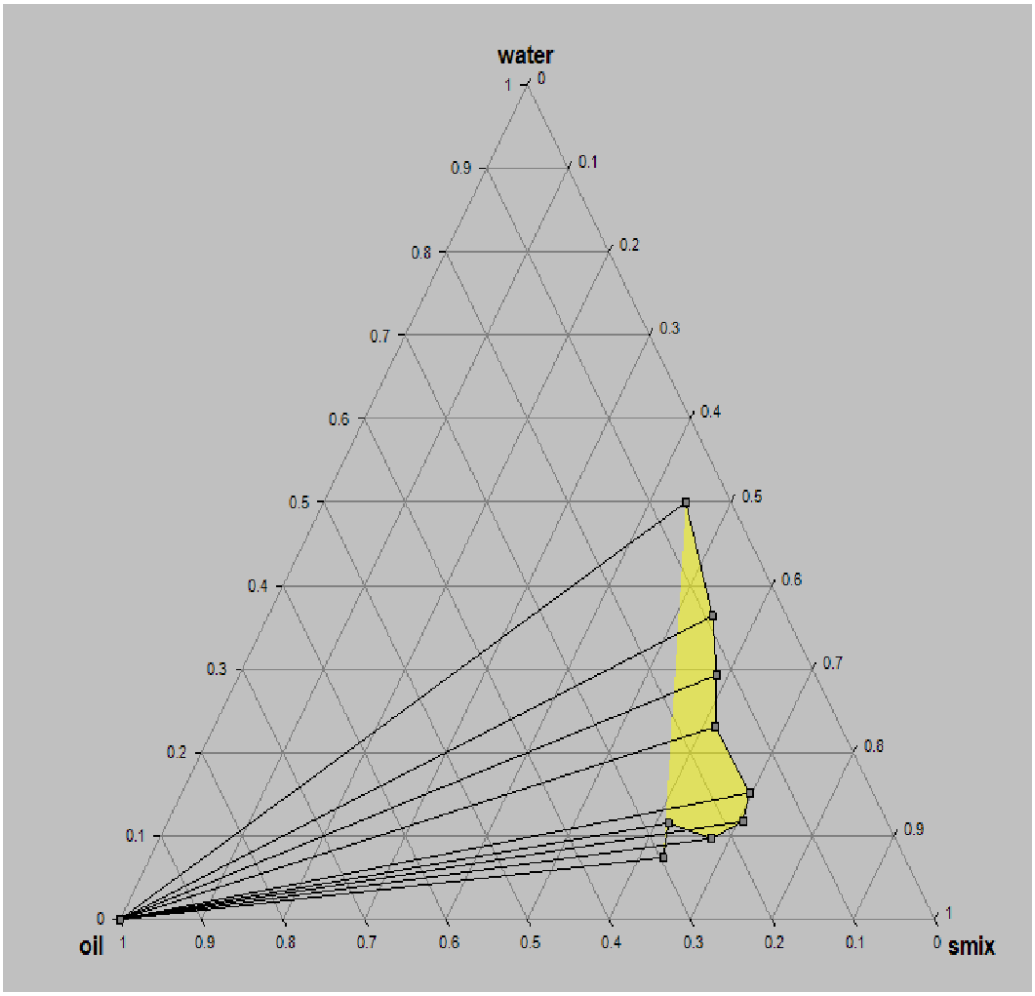


Fig 4.13 Pseudoternary plot for oil:water ratio smix 1:2



Discussion :

Pseudo ternary diagram was plotted based on the result observed for different ratios 1:1, 2:1, 1:2. From this ratios 2:1 shows higher microemulsion region compared to other ratios by using method 2.

Particle size analysis of optimized ratio:

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	17.61	Peak 1:	12.12	93.6	3.142
PdI:	0.175	Peak 2:	5186	5.1	483.1
Intercept:	0.947	Peak 3:	881.5	1.3	167.8
Result quality :	Refer to quality report				

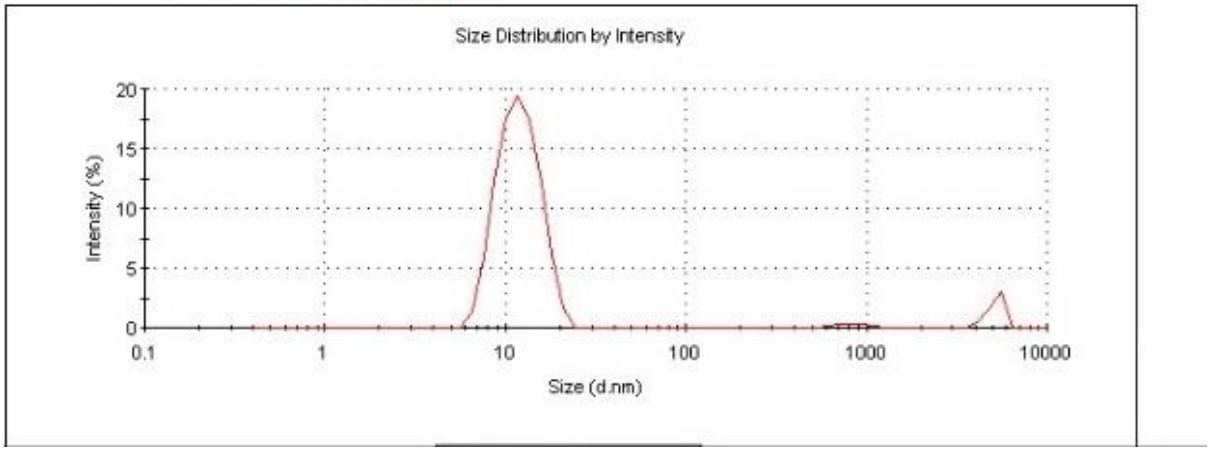


Fig 4.14 graph of particle size analysis

Conclusion:

From above all observations it was concluded that the microemulsion region found with 2:1 ratio of smix was larger and the particle size observed with this ratio is also in the range (17.61 nm) so it was considered as optimized ratio.

4.4 Mixture design:

To further control the particle size and reducing surfactant amount there was need to optimize microemulsionusing simplex lattice design.

Preparation of microemulsion using Simplex lattice design:

- Simplex lattice mixture experimental study was designed based on a three component system:
- The oil phase X1 (Triacetin), Smix X2 (a mixture of Tween 80/PEG 400 (2:1) and aqueous phase X3 (water).
- The total concentration of the three components summed to 100%.
- The constraints 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 5to 20 %. The Smix ranged from 45 to 60%. Since hydration of the stratum corneum significantly affects penetration of drug into the skin, water range was selected to be 10 to 25%.
- The particle size (nm) (Y1) and % drug release (Y2) of resveratrol were selected as the dependent variables (responses).
- The base design consists of 14 runs.

Table 4.29 Choosen constraints of simplex lattice design space :

Mixture Coding:	**Actual**	
Low	**Constraint**	**High**
5.000	A:oil	20.000
45.000	B:smix	60.000

10.000	C:water	25.000

Table 4.30 Formulation matrix and results of microemulsion formulations :

		Component 1	Component 2	Component 3	Response 1	Response 2
Std	Run	A:oil	B:smix	C:water	particle size	%cumulative drug release
		%	%	%	nm	%
9	1	7.5	47.5	20	22.31	82.5
1	2	20	45	10	148.7	58.9
12	3	5	60	10	18.5	79.1
13	4	5	45	25	46.23	67.14
8	5	7.5	55	12.5	14.56	84.23
6	6	5	52.5	17.5	31.8	71.6
4	7	12.5	52.5	10	65.37	61.9
2	8	5	60	10	80.12	78.5
10	9	10	50	15	14.22	71.4
7	10	15	47.5	12.5	74.2	52.31
3	11	5	45	25	38.5	64.2
14	12	12.5	52.5	10	59.17	60.8
11	13	20	45	10	167.2	56.3
5	14	12.5	45	17.5	117.31	58.17

Fig 4.15 Contour graph of particle size analysis :

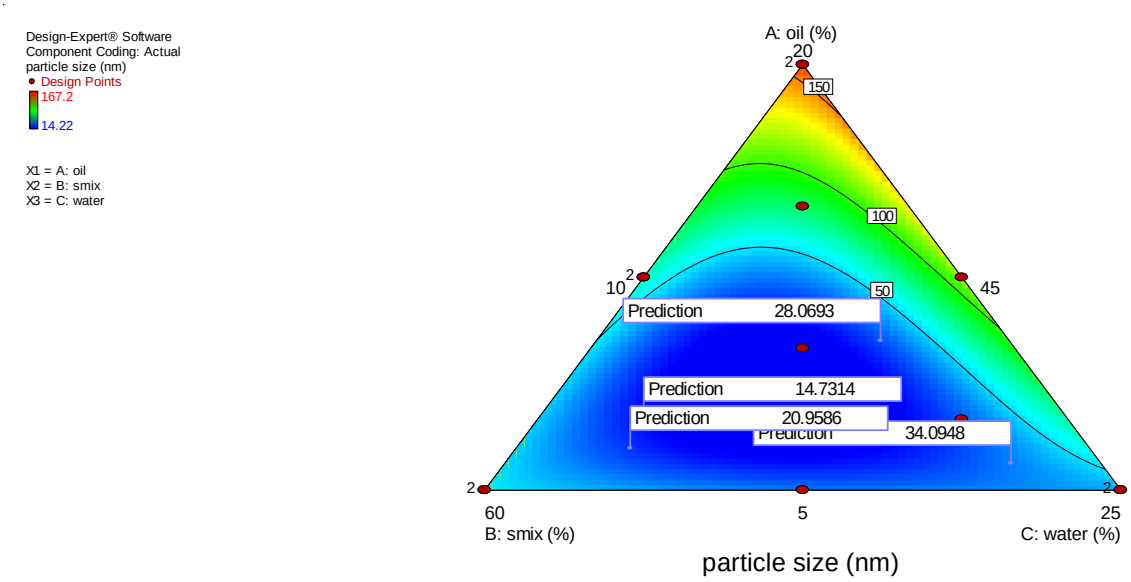


Fig 4.16 3-D surface graph:

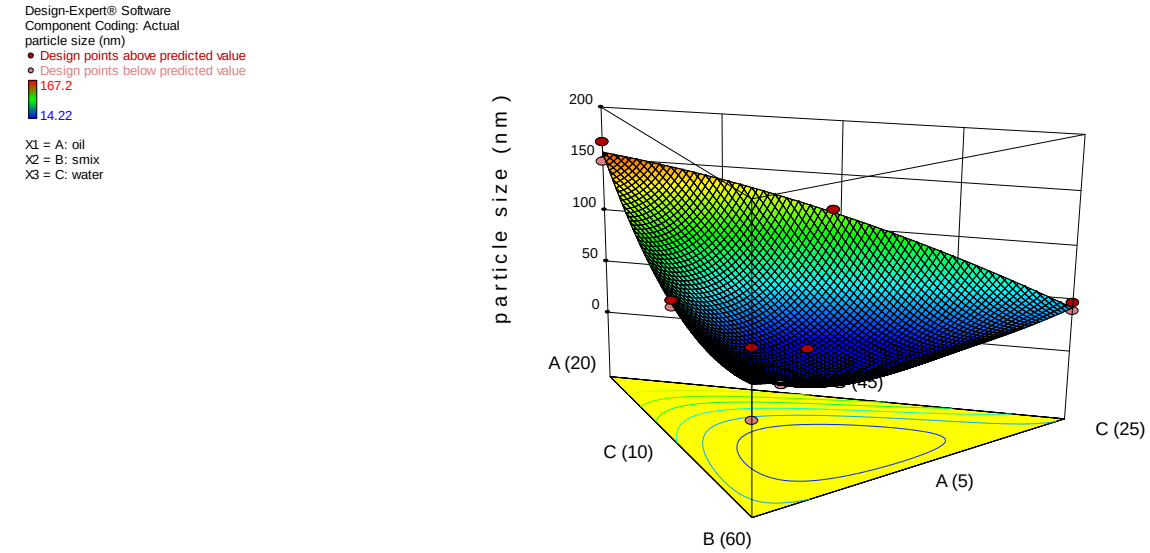
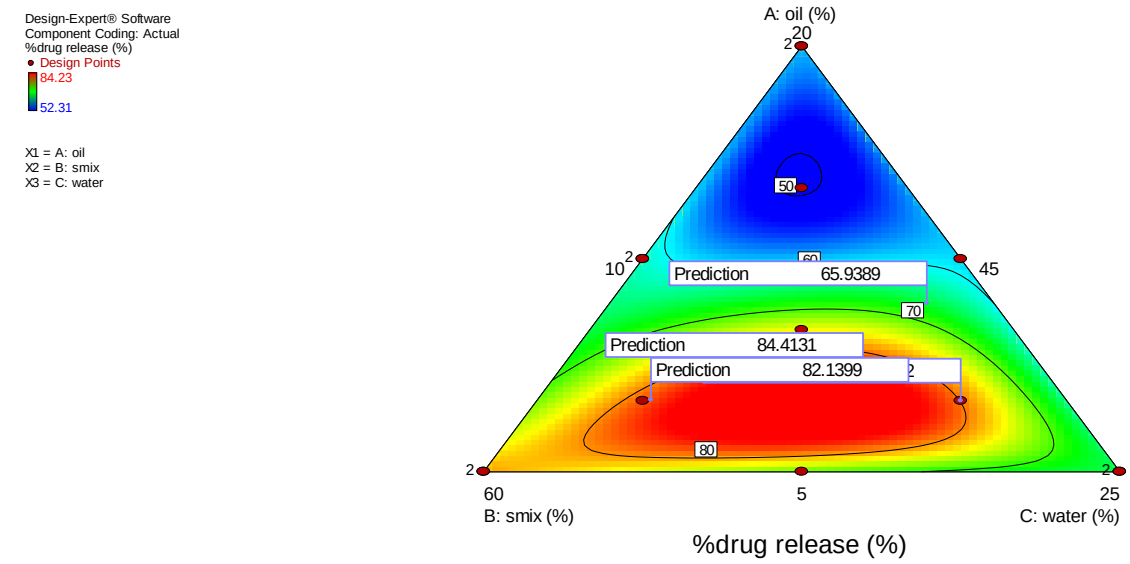


Fig 4.17 Contour graph of % drug release:



Result & Discussion:

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).

Polynomial equation:

Particle size(Y1)= +157.53* A+49.61* B+41.49* C-165.68* AB+60.83* AC-59.58* BC-1521.47* ABC

%drug release=+57.82* A+79.02* B+65.89* C-26.53* AB-11.25* AC+0.073* BC-1273.10* A^2BC+927.58* AB^2C+1312.22* ABC^2

Fig 4.18 3-D surface graph of %drug release:

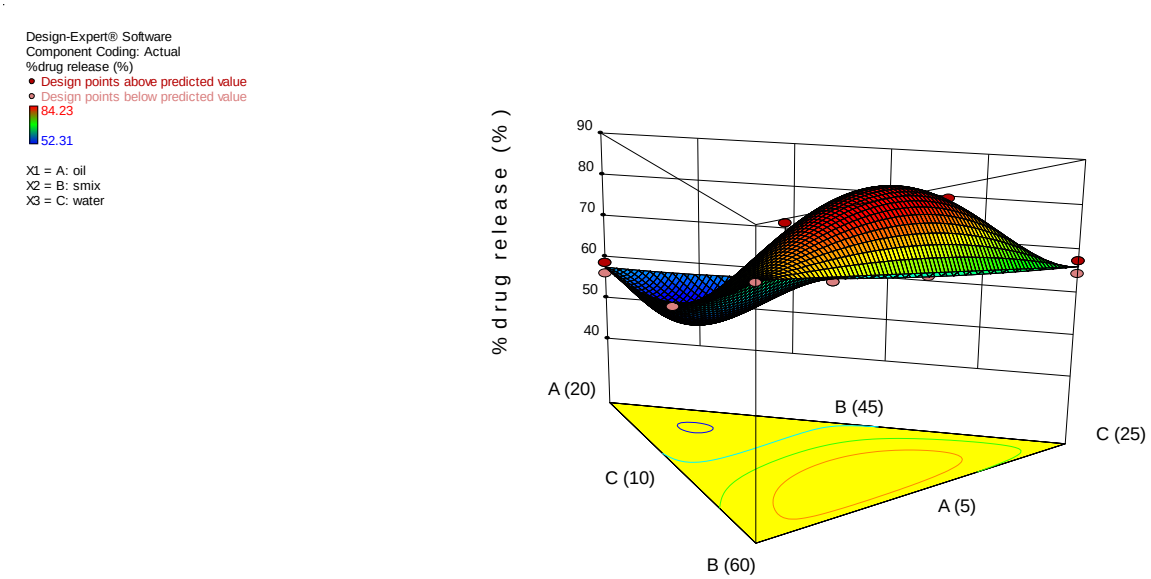
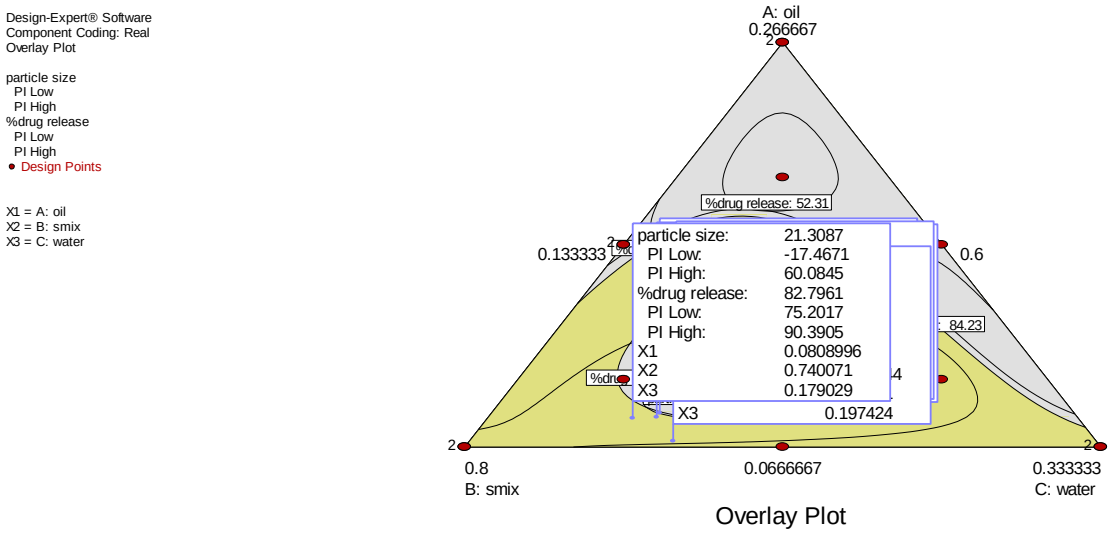


Fig 4.19 Predicted value in overlay plot:



Result and discussion:

The goal of the study was to get the stable microemulsion formulation with lower particle size and higher penetration in skin to increase the drug release of the microemulsion formulation. From all the above observation it was found that three independent component X1(oil %), X2(Smix %) & X3(water %) has the main effect on the dependent variables Y1 (particle size), Y2 (% drug release). The amount of Smix has main effect on particle size of the formulation as the amount of Smix increases there was decrease in the particle size. For the % drug release at the intermediate amount of Smix and water it was found that penetration of drug improved as surfactant can decrease the diffusion barrier and water hydrate the skin that will improve the transdermal penetration of the drug.

Table 4.31 Optimum formulation found to be as follows:

SR.NO	COMPONENT(ml)	VALUES	Component(%)
1	OIL	0.08	8
2	SMIX	0.74	74
3	WATER	0.19	19

Table 4.32 Observed results:

Responses	Predicted value	Observed value
Particle size(nm)	21.02	22.36
%drug release	82.79	80.2

Conclusion:

Check point batch was prepared and this batch was further characterised. From above all observation it was concluded that observed particle size and drug release was nearly similar to the predicted value. So it validates the applied design.

4.5 Characterization of the optimized microemulsion:

1) **pH:** pH of the mcromulsion was measured with digital pH meter. And the calibration of pH meter was done with standard buffer pH 4 and pH 7 solutions.

2) **Viscosity:** Brookfield viscometer was used to measure the viscosity. Spindle no 18 was dipped in the ME and rotated at 5, 10, 30, 50 rpm.

3) **Particle size, conductivity & zeta potential:** Malvern zetasizer was used to measure the particle size, conductivity & zeta potential of microemulsion.

   The Malvern Zetasizer series measures particle and molecule size from below a nano meter to several microns using dynamic light scattering, zeta potential and electrophoretic mobility using electrophoretic light scattering, and molecular weight using static light scattering method.

4) **%Transmittance:** It was measured to check the transparency of the formulation in the UV spectrophotometer against distilled water.

5) **%Drug content:** Accurately measured amount of sample was dissolved in the 100ml methanol and absorbance was taken in the UV spectrophotometer against methanol as a blank and % of drug was calculated from the absorbance.

6) **Dilution potential:** Microemulson was diluted 100 times with distilled water and checked for the transparency of the formulation.

7) **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 6 hours. At 0.5, 1, 2, 3, 4, 5, & 6 hrs. 2 ml aliquots from acceptor compartment were withdrawn and immediately 2 ml of phosphate buffer was replaced in the receptor compartment . The solution in the receptor compartment was continuously stirred throughout the process and temperature of the assembly was maintained at the 37 ± 0.05^0 C samples withdrawn were diluted and absorbance was measured in the UV spectrophotometer.

8) **Stability study:** Stability studies of microemulsion was tested by placing microemulsion in the tightly closed container at the different temperature for 15 days

and checked for the stability of the formulation. Stable systems were identified as those which has 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 15 days and samples were evaluated for physicochemical parameters like clarity & globule size.

Table 4.33 The physic-chemical characterization of the optimized microemulsion:

Sr.no	parameter	result
1	pH	5.4±0.05
2	viscosity	258.09± 4.28
3	Particle size	22.36
4	Percentage transmission	99.2±0.028
5	Drug content	91.5±0.5%
6	%Drug release	80.2%
7	conductivity	0.0698- (ms/cm)

8	Zeta potential	0.0379(mv)
9	Dilution potential	>100

Graph of particle size analysis:

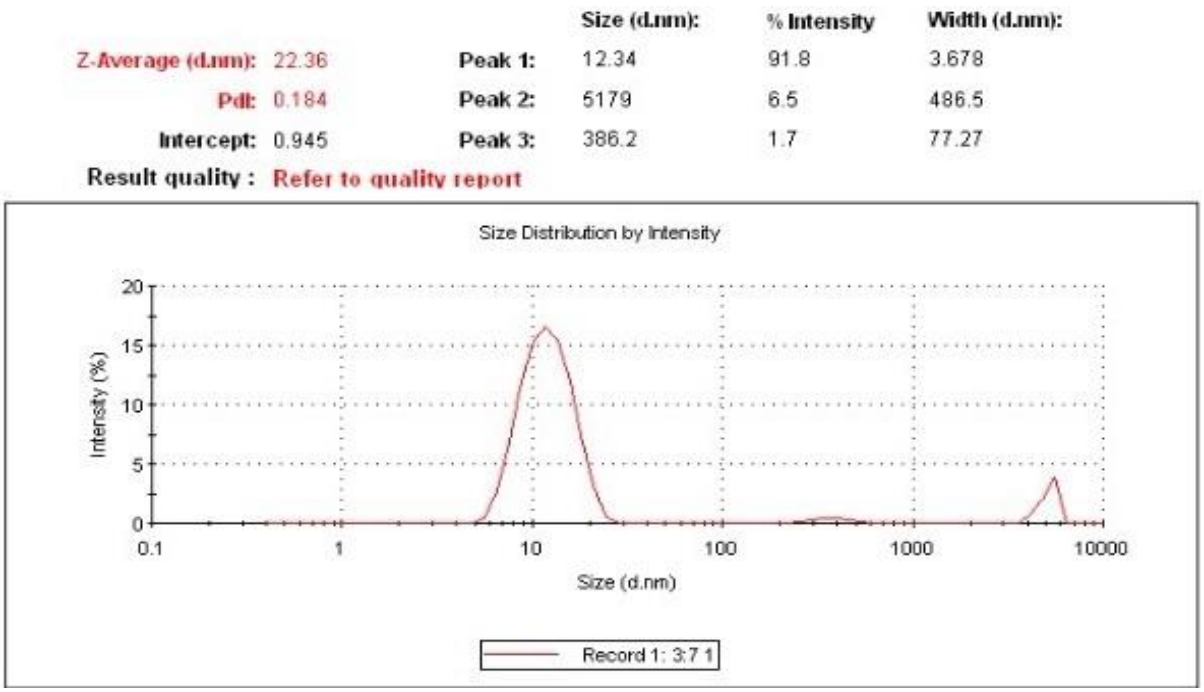


Fig 4.20 graph of particle size analysis

Graph of zeta potential analysis:

Zeta Potential (mV):	-0.0379	Peak 1:	-9.22	83.6	6.44
Zeta Deviation (mV):	21.6	Peak 2:	46.7	16.4	3.24
Conductivity (mS/cm):	0.0698	Peak 3:	0.00	0.0	0.00
Result quality :	See result quality report				

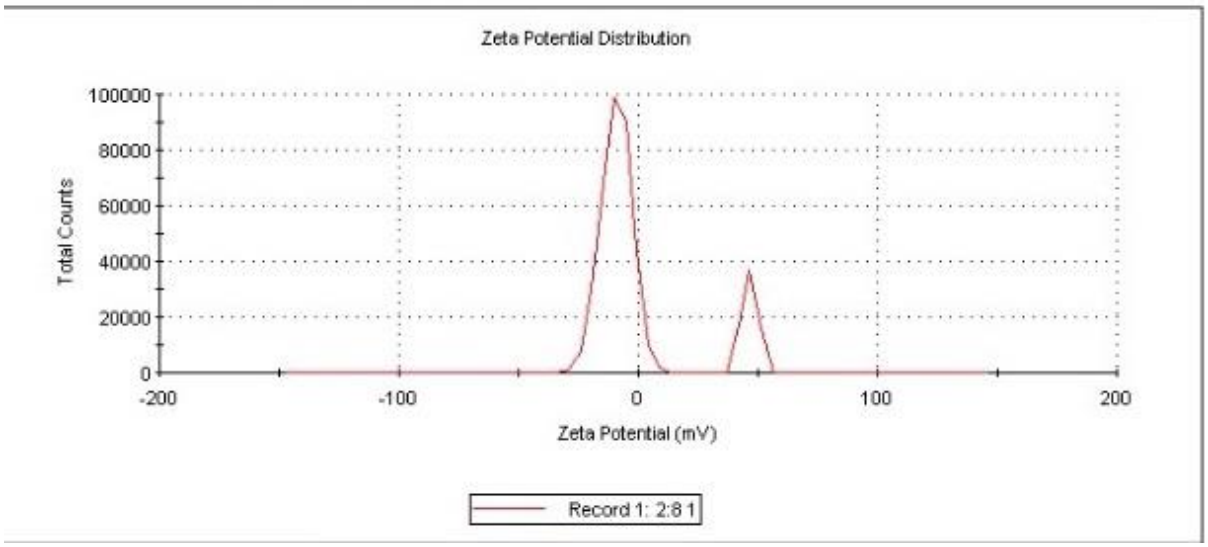


Fig 4.21 graph of zeta potential

Graph %CDR vs. time of optimized formula:

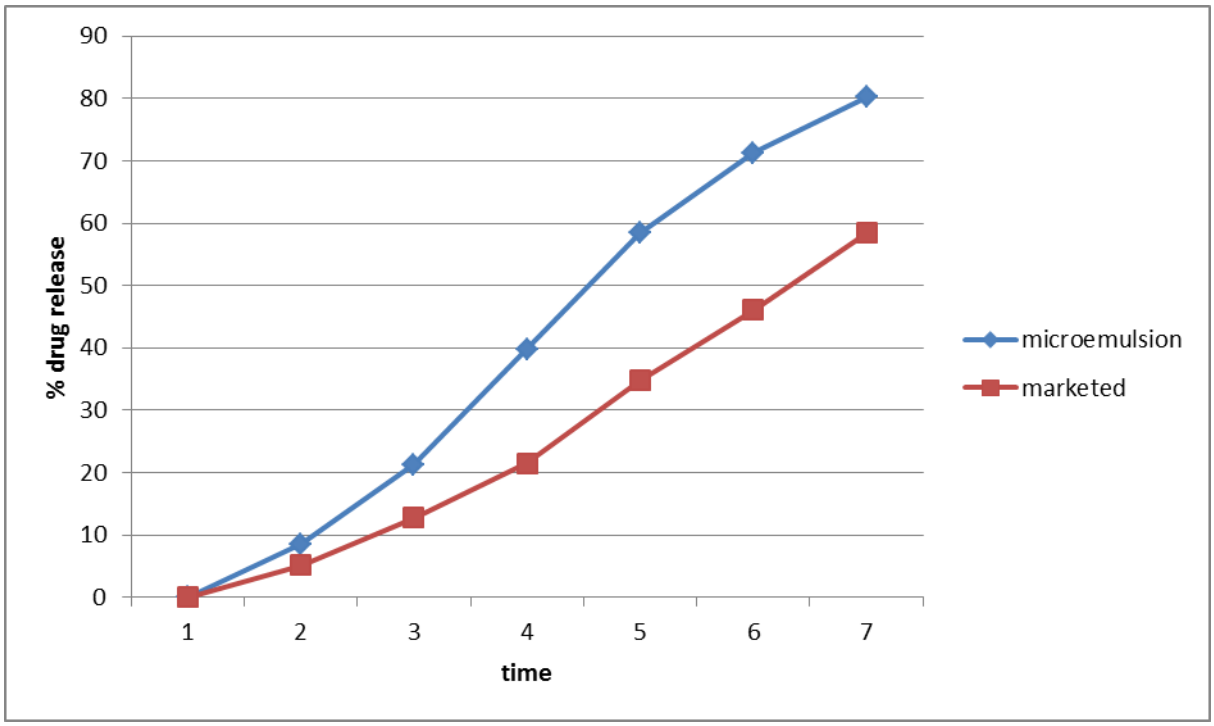


Fig 4.22 graph of % CDR vs. time

Conclusion: From above all observations it was found that the optimized microemulsion formulation was found physically stable and clear formulation and %CDR observed was also higher compared to the marketed formulation. Particle size of the microemulsion was also in very smaller size and it was coming in the desired size range. Zeta potential observed was also negatively charge which shows that the formulation was stable and there was no flocculation formed.

Physical stability:

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 15 days and samples were evaluated for physicochemical parameters like clarity, globule size and drug content after 15 days.

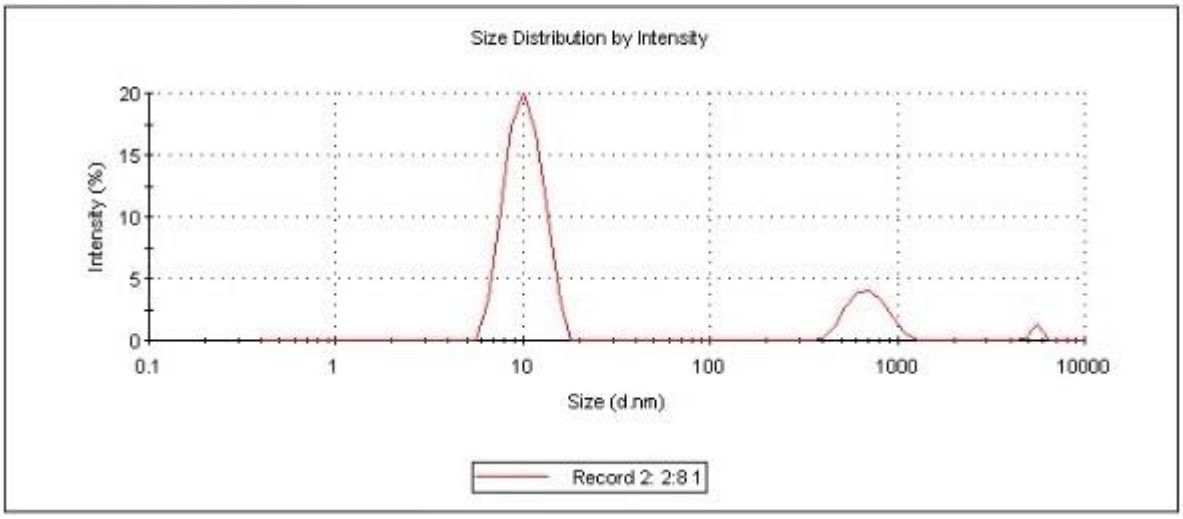
Results:

Table 4.34 evaluation of stability study of microemulsion

Sr.no	Evaluation parameter	Before	After
1	Clarity	Clear	Clear
2	Phase separation	Monophasic	Monophasic
3	Particle size(nm)	14.22	14.65

Size analysis before stability study:

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 14.22	Peak 1:	10.31	81.1	2.227
PdI: 0.433	Peak 2:	703.7	17.4	160.5
Intercept: 0.949	Peak 3:	5454	1.5	262.4
Result quality : Good				



Size analysis after stability study:

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 14.65	Peak 1:	10.76	76.4	2.699
PdI: 0.391	Peak 2:	432.2	17.3	146.0
Intercept: 0.946	Peak 3:	4654	6.2	808.3
Result quality : Good				

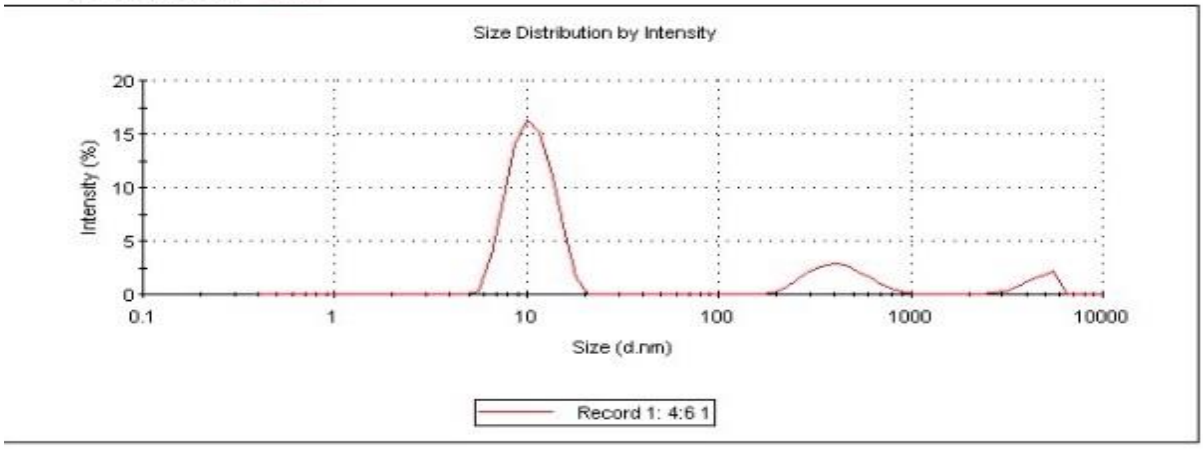


Fig 4.23 graph of particle size analysis

Conclusion:

Physical stability which performed at 12000 rpm for 30 minutes showed no change in the physical state. There was no significant change in particle size and phase behaviour of micro emulsion at 4°C and 45°C for 15 days. Hence, it was concluded that resveratrol loaded ME was stable.

4.6 Development and characterisation of microemulsion based gel:

Carbopoal 940 was used as the gelling agent due to its good gelling property .Carbopol 940 was dispersed in water and soaked in water for 24hrs and then stirred well and microemulsion was added in this solution under magnetic stirring. Triethanolamine was added in this solution to form the gel and maintain the pH of the solution. Gel was prepared indifferent concentration ranges from 0.5 to 2%.

Formulation of microemulsion based gel:

Table 4.35 formulation of microemulsion gel

Sr.no	Component (%)	0.5%	1%	1.5%	2%
1	Drug	1%	1%	1%	1%
2	Oil	6.5	6.5	6.5	6.5
3	Smix	53.5	53.5	53.5	53.5
4	Carbopoal 940	0.5	1	1.5	2
5	triethenolamine	1%	1%	1%	1%
6	Water	q.s.to 100 ml	q.s.to 100ml	q.s.to 100ml	q.s.to 100 ml

Physico chemical properties of gel:

Table 4.36 comparison of different conc. gel

Sr.no	parameter	0.5%	1%	1.5%	2%	Marketed gel
1	pH	7.1	7.05	7.2	6.9	7.0
2	Viscosity (cPs)	23854	25906	26580	29742	25296
3	Adhesive force	186.58	281.77	412.3	688.82	254.3
4	Peak load	10	10.60	11.72	15.15	10.41
5	%Drug release	70.5	85.6	75.4	72.8	61.8

In vitro drug release:

Table 4.37 in vitro release profile of gel

Time interval (hr.)	Marketed formulation	0.5%	1%	1.5%	2%
0	0	0	0	0	0
1	2.68	4.89	7.54	5.23	3.92
2	7.15	11.2	15.3	13.5	10.2
3	13.9	21.7	24.9	20.63	18.2
4	22.5	34.8	38.12	31.9	27.4
5	30.2	42.15	45.72	40.6	36.8
6	38.26	51.6	63.4	49.23	45.1
7	46.9	62.75	76.32	60.9	58.42
8	61.8	70.5	85.6	75.4	72.8

Comparison of drug release:

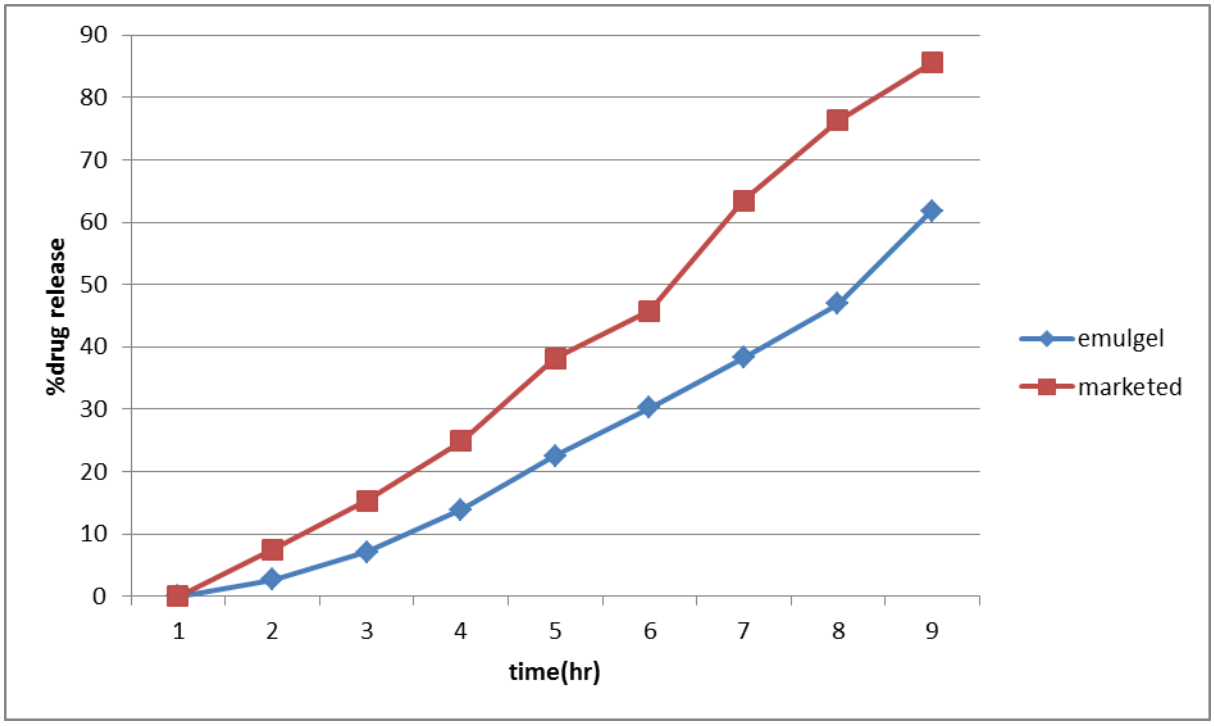


Fig 4.24 graph of in vitro drug release profile

1) **Texture analysis:** Gel strength was determined using a Brookfield Texture Analyser (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.

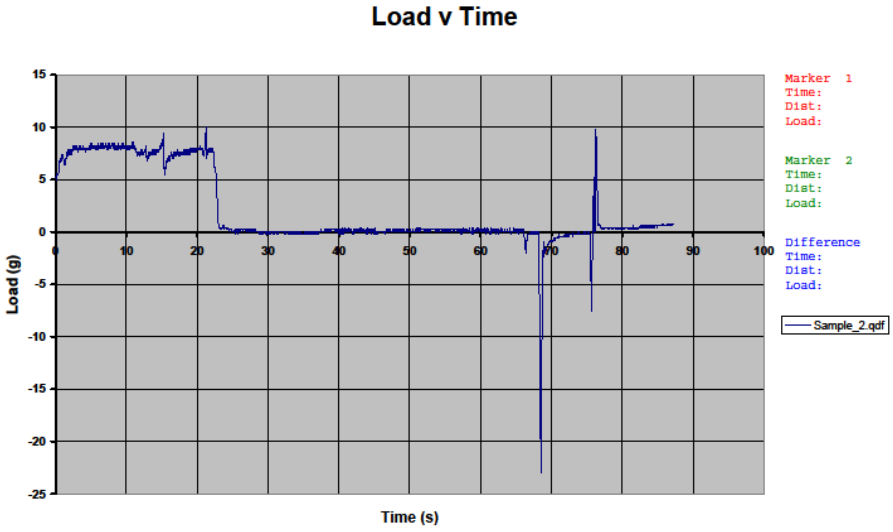


Fig 4.25 graph of texture analysis

Conclusion:

From above observation it was concluded that adhesive force for 1% gel was nearer to marketed formulation and drug release observed was also high so1% carbopol 940 gel was selected as gelling agent.

5. Summary

Acne vulgaris is the most common skin disease, with 80 % of reported occurrence, of adolescents and young adults. Due to low water solubility & side effects associated with oral route,resveratrol 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. Resveratrol 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 & PEG 400 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) + PEG 400 (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 2:1.

The “Mixture design” was used to optimization of microemulsion and to obtain the relationship between the particle size distribution, %CDR and components of the mixture in the formulation. A mixture 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 & %CDR nearer to 80%. Optimized batch was found with 8% oil, 74%

Smix, 19% water with droplet size & drug solubility of 22.36 nm & 80.2 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 1 % w/v Carbomer 940 containing MBG was found 25906 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 1 % w/v Carbomer 940 containing MBG, with 80.22 %, was higher than all remaining prepared MBGs and conventional gel. Hence, 1 % w/v Carbomer 940 containing MBG was selected for further studies.

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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.

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