DEVELOPMENT OF LIPID BASED CARRIER FORMULATION FOR POORLY SOLUBLE DRUG

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1.1 LIPID BASED DRUG DELIVERY SYSTEM

The term "lipid-based drug delivery system" (LBDDS) refers to formulations that comprise a dissolved or suspended medication in lipidic excipients for delivering a pharmaceutical substance in the body as needed to accomplish the intended therapeutic effects in a safe manner (Zaro JL.et al. 2015). LBDDS is a drug delivery method that differs from other drug administration systems. The goal of lipid-based pharmaceuticals is to improve the solubility and bioavailability of medications that are poorly water-soluble (Rawat M. et al., 2008). Lipid-based carriers have been demonstrated to be desirable choices for the formulation of medicines, as well as vaccines, diagnostics, and nutraceuticals, due to their efficiency and safety (Pouton CW. 2006).

1.1.1 Lipid-based medication delivery systems possess the following advantages.

The following are the advantages of lipid based drug delivery (Reithmeier H. et al., 2001, Mehanna M. et al., 2012)

1. Targeted and controlled drug release.

2. Stability in pharmaceuticals.

3. Significantly increased drug content (compared to other carriers).

4. Possibilities of transporting both hydrophilic and lipophilic medications.

5. Biocompatible and biodegradable.

6. The flexibility of excipients

7. Flexibility in formulation.

8. Low risk status.

9. Vesicular system formation that is passive, non-invasive, and ready for rapid commercialization

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1.1.2   Classifications of lipid based drug delivery systems

Lipid-based carriers can be divided into various categories depending on their physicochemical properties and the method that is used for their fabrication (Tapeinos C. et al., 2017)

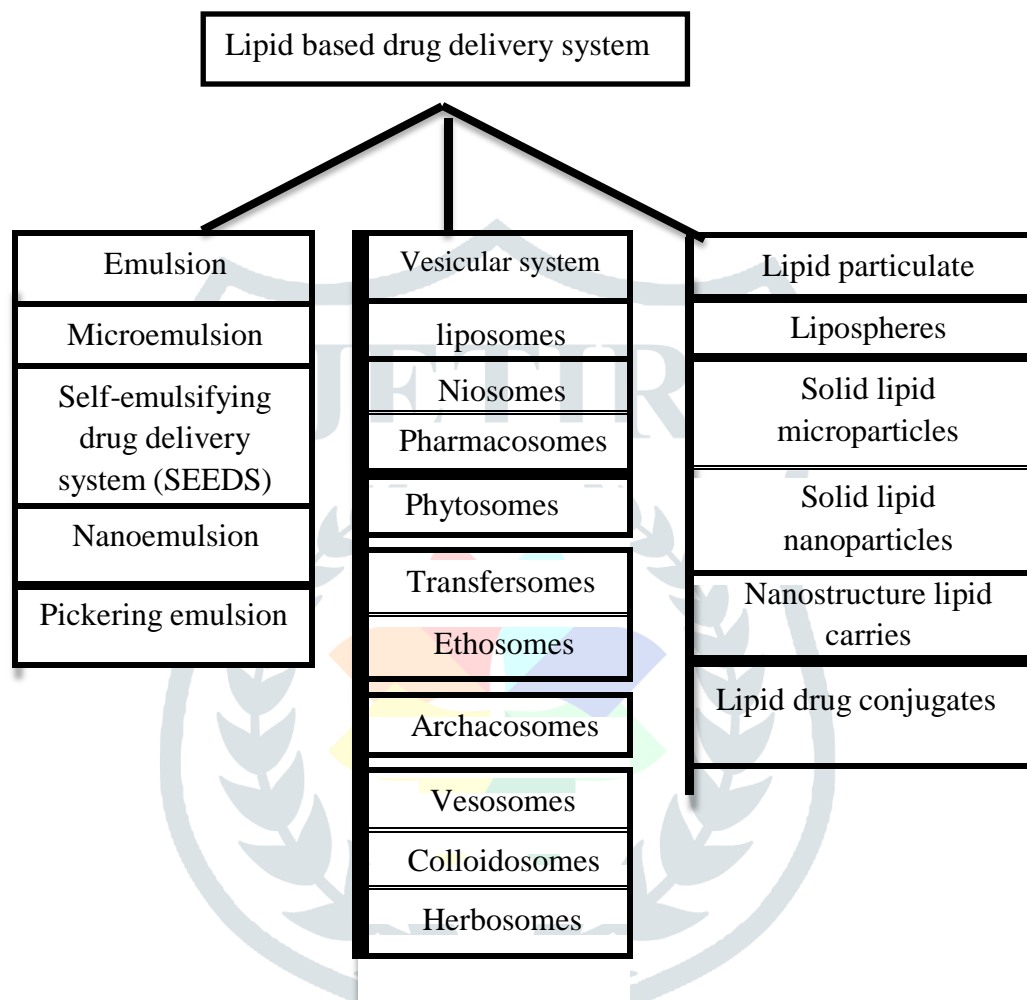LBDD systems are classified mainly into Emulsion system, vesicular systems, and lipidparticulate systems see figure -1

Figure No.1   Types of lipid based carrier formulations

1.1.3   The lipid formulation classification system:

Table No.1 Characteristic features, advantages, and disadvantages of the four essential types of "lipid" formulations

Formulation Type	Materials (Bunjes H. et al.,2010)	Characteristics (Chime A. et al., 2013	Advantages (Long C. et al., 2016	Disadvantages (Patidar A. et al., 2010)
Type I	Oils without surfactants(e.g.tri-,di-,and monoglycerides)	Non dispersing requires digestion	Generally recognized as safe (GRAS)status; simple; and excellent capsule compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic
Type II	Oils and water insoluble surfactants (Bradley M. et al., 2001	SEDDS formed without water-soluble components	Unlikely to lose solvent capacity on dispersion	Turbid o/w dispersion (particlesize0.25–2μm)
Type III	Oils, surfactants, and cosolvents (both water-insoluble and water-soluble excipients)	SEDDS/SMEDDS formed with water-soluble components	Clear or almost clear dispersion, drug absorption without digestion	Possible loss of solvent capacity on dispersion, less easily digested
Type IV	Water-soluble surfactants and cosolvents	Formulation disperses typicallyto form micellar solution	Formulation has good solvent capacity form any drugs	Likely loss of solvent capacity on dispersion may not be digestible (Beloqui A. et al., 2016)

1.2   TOPICAL MICROEMULSION BASED GEL

Topical drug delivery system is the application of a drug-containing formulation to the skin to treat cutaneous conditions directly. When alternative routes of medication administration, such as oral, sublingual, rectal, or parental, fail, or in cases of local skin illness, such as a fungal infection, the topical drug delivery method is used (Atiyeh B. et al., 2009). Human

skin is a specially designed organ that allows for terrestrial existence by controlling heat and water loss from the body and preventing the admission of unpleasant chemicals or germs. It is also the largest organ in the human body, accounting for around 10% of an average person's total mass, and covering an average area of 2 square metres (Sah S. et al., 2017). Many commonly used topical treatments, such as ointments, creams, and lotions, have various drawbacks. Because of all of these aspects, they also demonstrate the problem of stability within the principal category of semisolid preparations; the usage of transparent gels has expanded in both cosmetics and pharmaceutical preparations. A gel is a colloid that is normally 99% liquid by weight and is immobilised by surface tension between it and a macromolecular network of fibres constructed from a little quantity of a gelatinous component present. When opposed to ointments and cream pastes, gel formulations often enable faster medication release. Despite the numerous benefits of gels, one significant restriction is their inability to deliver hydrophobic medicines (Chang RK. et al., 2013). The medication is not immediately integrated into the gel base system. To circumvent this constraint, an emulsion-based technique is being employed to successfully integrate and distribute a hydrophobic medicinal component via gels. First, the emulsion or microemulsion is created and analysed, and then it is incorporated in an appropriate gelling agent (Tadwee I. et al., 2011). When gels and emulsions are mixed, the dosage forms are known as microemulsion-based gels. When gels and emulsions are mixed, the dosage forms are known as microemulsion based gels. In reality, the presence of a gelling agent in the aqueous phase transforms a traditional emulsion into a gel based on microemulsions. Lipophilic pharmaceuticals are encapsulated in the direct oil-in-water system, whereas hydrophilic medications are encapsulated in the reverse water-in-oil system. Microemulsions have a refined appearance and can be easily removed as needed. They can also enter the skin quite well (Kumar KK. Et al., 2011)

1.3   Drug permeation through the skin:

Pathway of transdermal permeation:

1.   Transdermal permeation, through the stratum corneum.

2.   Intercellular permeation, through the stratum corneum.

3.   Transappendageal permeation, via the hair follicle, sebaceous and sweat glands.

1.3.1   Mechanism of drug absorption:

Permeation of a drug involves the following steps:

1.   Sorption by stratum corneum.

2.   Penetration of drug through viable epidermis.

3.   Uptake of the drug by the capillary network in the dermal papillary layer.

1.3.2   Factors affecting drug absorption through the skin are as follow

Physiological Factors includes (Ansel H. et al., 1999)

a.   Skin thickness

b.   Lipid content

c.　　　　Density of hair follicles

d.　　　　Density of sweat glands

e.　　　　Skin pH

f.　　　　Disease condition

g.　　　　Blood flow

h.　　　　Hydration of skin

i.　　　　Inflammation of skin

Physiochemical Factors are as follow (Jain A. et al., 2010)

a.　　　　Partition coefficient

b.　　　　Molecular weight (<400 daltons)

c.　　　　Degree of ionization (only unionized drugs gets absorbed well)

d.　　　　Effect of vehicles

1.4　Optimal Characteristics of Microemulsion Based Gel

Microemulsion-based gel has the following characteristics (Anayatollah S. et al., 2012):Microemulsion-based gel should be;

1.　　　　Non-toxic,

2.　　　　Economical and efficient,

3.　　　　Inactive, compatible with other additives,

4.　　　　free from microbial contamination

5.　　　　maintained all rheological properties of the gel,

6.　　　　stable at storage condition,

7.　　　　washed with water and free from staining nature,

8.　　　　convenient in handling and its application

1.4.1　Advantages of Microemulsion Based Gel

1.　　　　**Better stability:** The microemulsion based gel has better stability when compared with other transdermal preparations (Ashara KC. Et al., 2016. It does not show phase inversion or breaking as found creams, creaming effect as in normal topical emulsion, rancidity (due to oily base) as in ointment and hygroscopic properties as in powders (Dhandore S. G. et al., 2018) .

2.　　　　**Greater loading capacity:** When compared with other novel approaches like liposomes and niosomes, gels have comparatively greater loading capacity of the drug due to vast network.

3.　　　　**Production feasibility:** Production of microemulsion-based gel is carried out in short and simple

steps, this increases the feasibility of the preparation also in the preparation of microemulsion-based gels, no specialized instruments needed for the production(Danielson I. et al., 1981).

4.   **Low production cost:** The production cost of microemulsion-based gels is low because the materials used are cheap and easily accessible.

5.   **Incorporation of hydrophobic drugs:** The major problem of incorporating most of the hydrophobic drugs (mainly Biopharmaceutical class ii drugs) directly into the gel base is solubility (Kogan A. et al., 2006). Microemulsion-based gel helps to avoid this constraint. Microemulsion-based gel incorporates these lipophilic drugs into the oil phase and then oily globules are dispersed in an aqueous phase bringing about o/w emulsion. Instances of such drugs are ketoconazole, fluconazole, and so on (NeubertHR. 2011,).

6.   **No intensive sonication:** Intensive sonication is needed in the preparation of vesicular molecules which may result in leakage and drug degradation. But this problem is not encountered during the preparation of microemulsion-based gel as sonication is not required (Stamatas G.N. et al., 2002).

7.   **Avoids first pass effect:** Concentration of drugs are reduced as the drug substance move through the portal circulation following gastrointestinal absorption. The deactivation of the drug by digestive and liver enzymes can be avoided by the use of microemulsion-based gels (Grampurohit M. et al., 2009).

8.   **Controlled release:** The effect of drugs having shorter half-lives can be prolonged by the use of microemulsion based gel (Asahi M. et al., 1993).

9.   Gastrointestinal drug absorption difficulties caused by gastrointestinal pH and enzymatic activity also drug interaction with foods and drinks can be avoided (Aggarwal N. et al., 2013).

10.   Oxidation and hydrolysis of drugs does not occur since microemulsion based gel provides protection as it is not exposed to attack by air and water (Patel M.R. et al., 2016).

11.   The efficacy of a drug can improve by the use of microemulsion based gels as a delivery system; this reduces side effects by allowing the total dose to be decreased (Tadros T.F. et al., 1992).

12.   The use of microemulsion based gels as a delivery system ultimately leads to increase in the rate of absorption and bioavailability of drug because microemulsion based gel increases the rate at which drug substances penetrate the skin barrier (Le Scriven., 1976).

13.   Microemulsion based gel can easily be removed from the skin since it is less greasy in nature.

14.   It is non-invasive and patient compliance is increased (Onah Chinwe M. et al., 2019).

1.4.2   Disadvantages of Microemulsion Based Gel

The disadvantages of microemulsion-based gel are as follows (Teo S. et al., 2017).

1.   Poor absorption because of poor permeability of some drugs through the skin.

2.   Microemulsion-based gel can only be used for drugs which need very small plasma concentration for action.

3.   Allergenic reactions may occur.

4.   The larger particle size drugs not easy to absorb through the skin.

1.4.3   Formulation Consideration for Microemulsion-Based Gel (MEG)

Factors to be considered during the process of formulating microemulsion-based gels are stated below.

1   Drug substance: For a successful development of MEG judicious choice of drug substance plays a significant role. There should be thorough examination of both the physicochemical properties (molecular weight, pH, etc.) and the biological properties (tolerance, irritation) (Onah Chinwe et al., 2019).

2   Vehicle (microemulsion): The vehicle selected should be able to deliver and release drug at the required site, sustain a therapeutic drug level for a sufficient period to provide a pharmacological effect and maintain even distribution of the drug substance on the skin. Depending majorly on the properties of the vehicle, the rate and extent of absorption vary.

3   Penetration enhancers: Penetration enhancers alter the structure of the skin thereby promoting skin permeability. These are considered as fundamental part of most topical formulations. Water, oils, surfactants and co-surfactants can be used as penetration enhancer in most cases.

4   Gelling agents: It is used to improve the consistency of any dosage form and can also be utilized as thickening agent (Dhruti P. et al., 2019).

5   Preservatives: To resist microbial attack, a preservative is used. Examples include methyl paraben, propyl paraben.

6   Chelating agents: Chelating agents are incorporated in the formulation to avoid any further reaction because bases and medications in gels are sensitive to heavy metal. Examples of chelating agents include ethylenediamine tetra acetic acid (EDTA) and methylatedcyclodextrin (Patel C. et al., 2013).

1.5   Method of preparation of microemulsion

1.5.1   Method of Phase Titration:

Microemulsions are created using the spontaneous emulsification method (phase titration) and can be represented using phase diagrams. The use of phase diagrams to explore the complex series of interactions that might occur when different components are blended is a useful method. Depending on the chemical content and concentration of each component, microemulsions are generated along with various association structures (including emulsions,micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion).

1.5.2   Method of Phase Inversion:

Microemulsion phase inversion occurs when an excess of the dispersed phase is added or in reaction to temperature. During phase inversion, dramatic physical changes occur, including particle size alterations, which can affect medication release both in vivo and in vitro (Nemichand S. K. et al., 2016).

1.6   Method of preparation of microemulsion based gel

1.   Preparation of microemulsion of O/W or W/O.

2.   Preparation of gel base

3.   Incorporation of the microemulsion phase into the gel phase with continuous and through stirring.
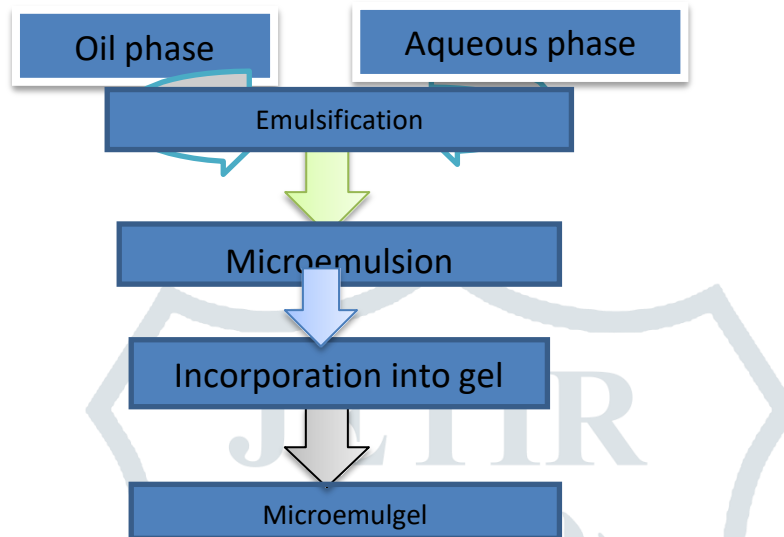
Figure 2:   Formation of Microemulgel

1.7.   Characterization of Microemulsion Gel (MEG)

Microemulsion-based Gel (MEG) can be characterized by determining the following factors like, pH, drug content, viscosity, spreadability, extrudability study, skin irritation studies, in vitro release, in vivo study, stability and consistency (Barot B. et al., 2012).

1.7.1   Measurement of pH

Digital pH meter is used to determine pH of various gel preparations. About one gram of gel is dissolved in 100 ml distilled water and allowed to stay for two hours. The measurement of pH of each preparation is normally done in triplicate and average value calculated.

1.7.2   Drug Content

100 ml of suitable solvent is mixed with about 1gm of the prepared gel. Suitable dilutions are made to prepare aliquots of different concentrations. After filtering the stock solution, absorbance is measured. The equation, obtained by linear regression analysis of calibration curve is used to calculate drug content.

1.7.3   Viscosity Study

Brookfield Viscometer is used to measure the viscosity of the prepared gel. The gels are rotated at 0.3, 0.6 and 1.5 rotations per minute. The corresponding dial reading is noted at each speed. Multiplication of the dial reading with factor given in the Brookfield Viscometer catalogues is carried out in order to obtain the viscosity of the gel.

1.7.4   Spreadability

Good spreadability is one of the criteria; a gel must possess in order to be effective. The extent of area to which a gel readily spreads when applied to skin or affected part is known as spreadability. The spreading value of a formulation greatly affects its therapeutic efficacy. It is expressed in terms of time in seconds taken by two slides to slip off from gel placed in between the slides under the direction of certain load. Spreadability will be better if the time taken to separate the two slides is small.

It is determined by using the following formula:

S = M.L/T

Where M=Weight tied to upper slide,L= Length of glass slides,

T=Time taken to separate the slides.

1.7.5   Extrudability Study

In extrudability study the formulations are allowed to set in a collapsible container after which they are filled in the collapsible tubes. The extrudability of the gel is determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds (Jadhay C. et al., 2014).

1.7.6   *In-vitro* release Studies

The in vitro diffusion studies of the gel formulations can be carried out using cellophane membrane. Franz diffusion cell is used for studying the dissolution release of gels through a cellophane membrane. Gel sample (0.5 gm) is placed in cellophane membrane and the diffusion studies are carried out at $37 \pm 1°$ using 250 ml of phosphate buffer (pH 7.4) as the dissolution medium. 5ml of each sample is withdrawn intermittently at 1, 2, 3, 4, 5, 6, 7 and 8 hrs and each sample is replaced with the same volume of fresh dissolution medium. The samples are then analysed by UV-spectrophotometer at 283nm for the drug content using phosphate buffer as blank (Kumar A. et al., 2014).
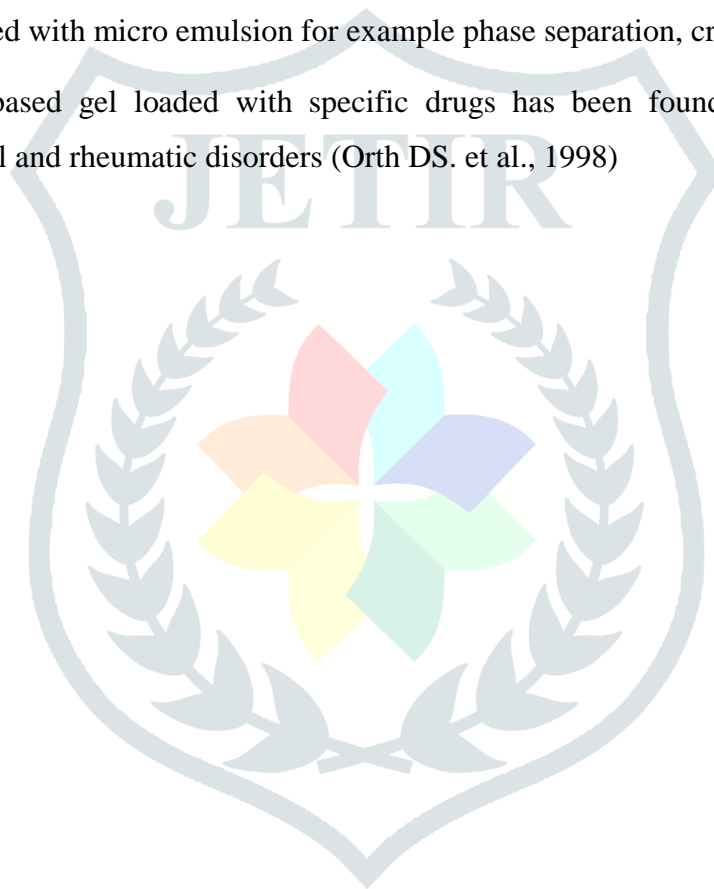
1.7.7   Stability

The stability studies for all gel formulations are carried out by freeze-thaw cycling. This involves subjecting the formulation to a temperature of 4°C for 1 month, then at 25°C for 1 month, then at 40°C for 1 or more month (s). Gel is exposed to ambient room temperature at the end and liquid exudates' separating is recorded.

1.8 Application of Microemulsion Based Gel

Microemulsion based gel has been utilized in the following areas:

1.   When compared to conventional formulations such as solutions, gels or creams, microemulsion based gel enhance the transdermal permeation of drugs significantly (Rajput R. et al., 2016). They are able to incorporate both hydrophilic (5-fluorouracil, apomorphine hydrochloride, diphenhydramine, hydrochloride, tetracaine hydrochloride, methotrexate etc.) and lipophilic drugs (estradiol, finasteride, ketoprofen, meloxicam, felodipine, triptolide, etc.).and enhance their permeation

2. High solubilizing capacity of the formulation makes it possible for the incorporation of large amount of drug.

3. The affinity of a drug to the internal phase in micro emulsion may be easily altered to favor partitioning into the stratum corneum, thereby increasing the rate of permeation of the drug from the micro emulsion as a result of increase in thermodynamic activity towards the skin.

4. The diffusional barrier of the stratum corneum may reduce by using different internal phases that act as penetration enhancers (Jachowicz J. et al., 1993).

5. If the water content in the gel preparation is sufficiently high, the percutaneous absorption of drug will increase because of the hydration effect of micro emulsion basedgel on the stratum corneum.

6. It is a dual controlled release system because of the incorporation of micro emulsioninto gel.

7. Problems associated with micro emulsion for example phase separation, creaming is avoided.

8. Micro emulsion based gel loaded with specific drugs has been found effective in some topical disorders for instance fungal and rheumatic disorders (Orth DS. et al., 1998)

CHAPTER 2
LITERATUREREVIEW

2.1 Formulation Specific Review

Microemulsion systems containing salicylic acid were formulated for their research. Different concentrations of salicylic acid were incorporated in a microemulsion base composed of isopropyl myristate, water, and tween 80: propylene glycol in the ratio of 15:1. Three micro emulsion systems were prepared: S2%, S5%, and S10% which contain 2%, 5% and 10% of SA respectively. Evaluation by examination under cross-polarizing microscope, measuring of percent transmittance, pH measurement, determination of the specific gravity, assessment of rheological properties, and accelerated stability study were carried out. They noted that all systems were not affected by accelerated stability test they also confirm that after stability study of S10% system for 6 months under ambient conditions. No remarkable changes were recorded except a decrease in the viscosity value after 1 month. They concluded by suggesting that based on results recorded ME could be a suitable vehicle for topical application of different concentrations of salicylic acid (*Alia A Badawi et al.*, 2009).

Developed micro-emulsion gel based topical delivery of salicylic acid and neem oil for the management of psoriasis, Labrasol was selected as surfactant, plurol oleique as co surfactant and neem oil as oil component based on solubility study. The dispersion solubility of SA was studied in various oils, surfactants and co-surfactants and by constructing pseudo phase ternary diagram, micro emulsion area was identified. The optimized formulations of micro emulsion were subjected to thermodynamic stability tests stable formulation was characterized for droplet size, pH determination, centrifugation, % drug content in micro emulsion, zeta Potential and vesicle size measurement and then micro emulsion gel were prepared and characterized for spreadability, measurement of viscosity, drug content, In-vitro diffusion, in-vitro release data. The optimized formulation contained SA 0.05 (%w/w), labrasol (24%), plurol oleique (8 %) and neem oil (8%). ME6 Particle size 16.9±0.3 nm Polydispersity index 0.122±0.01, zeta potential -36.99±2.03mv, drug content 99.98±1.87% and transmittance 99.14±0.52 %. The in vitro drug release from SA micro emulsion gel was found to be considerably higher in comparison to that of the pure drug. The in-vitro diffusion of microemulsion gel was significantly good. Based on their study, they concluded that solubility and permeability of SA can be increased by formulating into microemulsion gel (*Shweta Bharade et al., 2019*).

Antifungal microemulsion gels of benzoic acid and salicylic acid for the treatment of fungal infections were developed and evaluated in for their research. Carbopol 934 and HPMC

k15m were used as gelling agents, oil as a preservative and emulsifying agent a penetration enhancer in their work to create microemulgels of benzoic and salicylic acid. The formulated microemulgels were evaluated for drug excipient interactions syneresis, spreadability and drug content. In vitro study of the formulated six batches were prepared and one of the microemulgel formulation (F4) was chosen as the best candidate which demonstrated maximum drug release of 95.37 percent , resulting in an effective modified route of administration. It also shows improved formulation's stability elegance and effectiveness (.*Chandira. et al*., 2022).

Microemulgel Containing Flaxseed Extract was developed for the research and Its In Vitro and In Vivo Characterization. Ethanolic fraction was selected for further study by TPC (18.75 mg gallic acid equivalent/g) and TFC (1.34 mg quercetin equivalent/g). HPLC-UV analysis showed the existence of benzoic, quercetin, caffeic, vanillic, p-coumaric, gallic, cinnamic, syringic, and sinapic acids. Biological activities showed 87.00%, 72.00%, and

21.75 values for DPPH assay, tyrosinase inhibition, and SPF assays, respectively. An (O/W) microemulsion containing the flaxseed extract, with 99.20 nm particle size, -19.3 Zeta potential and 0.434 polydispersity index was developed and incorporated in Carbopol-940 gel matrix to formulate an active microemulgel with 59.15% release in in vitro studies. The successfully formulated stable active microemulgel produced statistically significant effects ($p < 0.05$), in comparison to a placebo, on skin erythema, melanin, sebum, moisture, and elasticity, in a non-invasive in vivo study performed on 13 healthy human female volunteers (*Tasneem, R. et al., 2022*).

Microemulsion based gel containing fluconazole (FLZ) for the vaginal deliveries were developed for their research. Chemicals used are Fluconazole (2%, w/w) Capryol 90 (14%, w/w) Cremophor EL (43.5%, w/w) Benzyl alcohol (2%, w/w) Chlorocresol (0.1%, w/w) and Water to make (100g), sodium alginate, hydroxyl propyl methyl cellulose (Methocel K4M) and Carbopol ETD 2020 were evaluated for their ability to gel FLZ microemulsion. The formulation was characterized for drug content, pH and spreadability, Rheological studies, intro studies and Primary vaginal irritation studies. The solubility of FLZ in oils and surfactants was evaluated to identify components of the microemulsion. The ternary diagram was plotted to identify the area of microemulsion existence. Various gelling agents were evaluated for their potential to gel the FLZ microemulsion without affecting its structure. The bio adhesive potential and anti-fungal activity of the FLZ microemulsion based gel (FLZMBG) was determined in comparison to the marketed clotrimazole gel (CandidVgel) by

invitro methods. The vaginal irritation potential of the FLZ-MBG was evaluated in rabbits. The clinical efficacyof the FLZ-MBG and Candid Vgel was evaluated in females suffering from vaginal candidiasis. The FLZ microemulsion exhibited globule size of 24nm and polydispersity index of 0.98. Carbopol ETD could successfully gel the FLZ microemulsion without disturbing the structure. The FLZ-MBG showed significantly higher (P <0.05) in vitro bioadhesion and anti-fungal activity as compared to that of CandidV gel. The FLZ- MBG did not show any signs of vaginal irritation in the rabbits. The small-scale clinical studies indicated that the FLZ-MBG shows faster on set of action than CandidV gel although no difference was observed in the clinical efficacy (*Yogeshwar G. Bachhav et al., 2009*).

Insulin-loaded micro emulsion topical gel with aloe vera gel for the treatment of dermatologic manifestation of diabetes were prepared and carried out I vivo study. The insulin-loaded microemulsion was prepared by 3square factorial design method, using oleic acid as oil phase, tween 80 as the surfactant and poly ethylene glycol -400 as a co-surfactant. Nine batches of insulin loaded microemulsion formulations were prepared among which only 3 formulations S4, S5, and S6 were found to be stable after centrifugation for 1h at 5000rpm due their desirable concentration of oil phase, surfactant and co-surfactant used. The best three formulations were selected for ex vivo insulin permeation study. The best permeation batch S5 was incorporated in aloe Vera gel and evaluated the zeta potential and particle size, assessment of drying time, skin irritation study etc. test were performed and percentage insulin content in insulin-loaded micro emulsion topical gel with and without aloe vera gel was found to be 99.86±0.22 and 100±0.32 which indicated that insulin formulations prepared were homogeneous . In their in-vivo experiment on four different groups of rat they concluded that on the basis of all evaluation parameters and in vivo animal studied results, the process or technology of insulin-loaded micro emulsion topical gel with aloe Vera gel can be used as dermatologic manifestation of diabetes significantly as well as to penetrate the higher molecular weight insulin through topical skin (*Chakraborty. T. et al., 2020)*

Microemulsion-based topical sulconazole gel was prepared for their research. Microemulsion formulation of sulconazole nitrate was prepared by water titration method using oil, surfactant, cosurfactant and water at different ratios. This was then subjected to clarity and particle size analysis, a centrifugation test, a dilution test, and freeze thawing. Tween 20 and PEG 400 we used as the surfactant and cosurfactant and olive oil as the oil and Carbopol 934P and propylene glycol were used as gelling agent. A Series of 6 formulations were prepared in all these formulations, the amount of sulconazole nitrate was kept constant (50 mg) and the amounts of surfactant, co surfactant, oil, and cosolvent were varied. The zeta potential of formulation F1 was -41.3 and stable. The pH of the microemulsion formulation was within the range of pH of skin. F1 showed a higher percentage amount of drug as compared with the other formulations. The viscosity showed that F1 was optimum. The freezing and thawing results showed there was no phase separation and the formulation was stable. In vitro drug release showed that the drug release from the microemulsion of F1 was higher when compared to the other formulations. It revealed F1 had the highest drug content of 95.88±0.3% and % cumulative drug release was 88.75% release in 8 h. The in vivo skin irritation study on rats confirmed that formulation was nontoxic and non-irritant. They concluded that formulated gel possessed good physicochemical

properties, high drug content, and sustained drug release. It was also confirmed that the formulated gel is safer for topical delivery by the in vivo studies. Based on these results, it can be concluded that a microemulsion based gel of sulconazole nitrate is promising for topical delivery against fungal infections (*PAYYAL Sumedha P. et al., 2020).*

Thyme oil- loaded microemulsion based gel was formulated for the treatment of fungal infections due to candida and trichophyton species using tween 80 as surfactant, isopropyl alcohol as co-surfactant and carbopol 934 as the gelling agent by phase titration method. This formulation was constructed by D-optimal design and the optimized final formulation contains 0.82% of oil, 9.22% of Smix, and 89.95% of water. At the course of evaluating their work they observed that the optimized microemulsions was pale yellow to amber transparent microemulsion with a globule size of 14.23 ± 0.3n, zeta potential of -0.69 mv and PDI value of 0.00143 indicating a stable microemulsion. The microemulsion based gel performed had a pH of 6.03, appreciated viscosity and rheological properties. The drug release of the formulation was 100.0 ± 0.22%. The % of drug permeated in skin layers was found to be

15.53 ± 0.22%. While % drug retention on the ski surface was found to be 26.32 ± 0.26% and within the skin layers was found to be 58.47 ± 0.22%. Still stable after 6 months of storage and demonstrated better efficacy as the marketed formulation of the antifungal agent, they concluded that a thyme oil loaded microemulsion based gel showed as a promising treatment alternative for the treatment of superficial fungal infections (*Jeet V. Gandhi et al., 2020*).

Acyclovir- loaded microemulsion gel, the objective of their work was to design and develop o/w microemulsion for transdermal delivery of poorly water soluble acyclovir by aqueous titration method using oleic acid: castor oil in the ratio 3:1, tween 80, and ethanol were selected as oily phase, surfactant and co-surfactant respectively while carbopol 934 was used as the gelling agent. The pseudo ternary phase was constructed by aqueous titration method. The co-surfactant affect the shape and extant of microemulsion regions. Ethanol (cosurfactant) is expected to disorder the interfacial film gave extended microemulsion zones by destabilizing the liquid crystalline phase. Largest Microemulsion single phase region was found at Smix (2:1) than the system at other Smix. They evaluated the microemulsion for droplet Shape and size, refractive index, pH, Viscosity, drug loading capacity. The mean droplet size of microemulsion was found below 50 nm. The maximum solubility of ACV in microemulsion system was found to be 47.4 mg/ml. The ex-vivo skin permeation studies were done using skin of Wistar albino rat by Franz diffusion cell, and microemulsion formulation MEC1 exhibited highest flux, was found to be 238.1±4.87 µg/cm2/hr, while flux of MEGel, aqueous solution and conventional emulsion of ACV were found to be 230.40±6.23 µg/cm2/hr, 2.47±0.76 µg/cm2/hr and 8.65 ±1.21 µg/cm2/hr respectively. The pharmacokinetic parameters of ME Gel after topical application to the Wistar albino rat skin were significantly different from those of ACV in aqueous solution (PD) and conventional emulsion (CE). After their findings they concluded that microemulsion of ACV prepared with Oleic acid: castor oil (3:1) as oily phase, tween 80 as surfactant, and ethanol as cosurfactant can be used as transdermal drug carrier for this and other poorly water soluble drug *(Brajesh Kumar et al., 2010).*

Lidocaine-loaded microemulsion –based topical gels using titration methods at different surfactant /cosurfactant

weight ratio and carbopol 940 as used as the gelling. Their formulation was evaluated for refractive index, electrical conductivity, droplet size, zeta potential, pH, viscosity, and stability while Microemulsion based gel were characterized for spreadability, pH, viscosity, and in vitro drug release measurements, and based on their results , selected best microemulsion based gels were subjected to ex-vivo rat skin permeation anesthetic effect and irritation studies. Their data indicated the formation of Nano-sized droplets of microemulsions ranging from 20-50nm with a polydispersity index of less than

0.5. ME droplet size, was less than 60nm, PDI less than 0.4 and it zeta potential was found to be -0.711 to ± 0.832 mV indicating that the interface had a low surface charge. In their work various formulation of loaded lidocaine-loaded MBGs and characterization they concluded that the MBGs could be considered as a more promising approach for the transdermal delivery of lidocaine due to their appropriate viscosity and rheological behaviour, spreadability, pH, high penetration ability with no irritation. MBGs showed significantly higher drug release and permeation compared to the marketed topical gel. They suggested further research be carried out to elucidate the possible mechanism of lidocaine delivery to the skin and to confirm the therapeutic efficacy (*Mahshid D. et al., 2021*)

Microemulsion-based hydrogel of bifonazole with the aim of increasing the solubility and skin permeability of the drug were formulated in their research, the pseudo ternary phase diagrams for microemulsion regions were constructed using oleic acid as oil, tween 80 as the surfactant and isopropyl alcohol (IPA) as the co-surfactant while hydroxyl propyl methyl cellulose (HPMC) K100 M was used the gelling agent. They prepared various microemulsion formulations and optimized by 3^2 factorial design on the basis of percentage transmittance, globule size, zeta potential, drug release and skin permeability. Ex vivo was also evaluated using franz diffusion cells fitted with rat skins. The optimized microemulsion-based hydrogel was evaluated for viscosity, spreadability, skin irritancy, skin permeability, stability, and antifungal activity by comparing to it with marketed bifonazole cream. In their result they noted that the formulation MBHG showed a good stability over the period of 3 months. Average globule size of selected F5) formulation was found to be 18.98 nm, zeta potential was found to be -5.56mv, and permeability within 8 h was observed 84%. They concluded that microemulsion based hydrogel F5 has a potential for sustained action of drug release and it may act as promising vehicle for topical of bifonazole (*Vidya S., Sejal V. et al., 2012).*

Nadifloxacin-loaded microemulsion gel for the ant acne agent, nadifloxacin were designed and developed to overcome the problems associated with the cutaneous delivery due to poor water solubility. The solubility of nadifloxacin in oils, surfactants and cosurfactants was evaluated to screen the components of the microemulsion. Formulations in which the oil content was 10% and surfactant–cosurfactant k_m (1:1) were 30, 35, 40 and 60%, respectively, were investigated for drug loading. The systems were assessed for drug-loading efficiency and characterised for optical birefringence, pH and refractive index, robustness to dilution, globule size, drug content and thermodynamic stability. Optimised microemulsion systems were formulated into gel form and evaluated for viscosity, spreadability, drug content, ex vivo skin permeation and antibacterial activity. The maximum solubility of nadifloxacin in the microemulsion system was found to be 0.25%. The nadifloxacin microemulsions had a small and uniform globule size (67.3-121.23 nm). The stability results

revealed that all formulations showed a stable globule size and the polydispersity index under stress conditions. Incorporation of nadifloxacin in microemulsion gel increased the ex vivo skin permeation and antibacterial activity when compared to marketed cream (*Ujwala S. et al., 2012*). Microemulsion-based gel of Betamethasone dipropionate (BD), the aim of their work was to test the hypothesis that the addition of corticosteroidd such as BD and a keratolytic agent such as salicylic acid in nano carrier based microemulsions formulation would result in enhancement and sustaining of corticosteroid delivery rate leading to better anti-psoriatic activity. Microemulsions were prepared by aqueous phase titration method, using oleic acid: sefsol (1.5:1), Tween 20, isopropyl alcohol, and distilled water as the oil phase, surfactant, cosurfactant and aqueous phase, respectively. Selected formulations were subjected to physical stability studies and consequently in vitro skin permeation studies. Surface studies of optimized formulation were done by transmission electron microscopy. In vivo anti- inflammatory activity was done by carageenan-induced raw paw edema method. The droplet size of microemulsions ranged from 60 to 190 nm. The optimized formulation exhibited viscosity 28.55 $\pm$ 2.03 cP, refractive index 1.409, pH 6.4, and conductivity 10-4 scm-1. The optimized microemulsion was converted into hydrogel using carbopol 934, and salicylic acid was incorporated into it. Drug deposition in skin was found to be 29.73μg/mg. Assessment of skin permeation was done by histopathology studies which indicated changes in the structure of epidermal membrane of skin. In vivo anti-inflammatory activity indicated 72.11% and 43.96% inhibition of inflammation in case of developed microemulsion gel and marketed gel, respectively. They concluded that the developed microemulsion gel containing BD and salicylic acid provided sustained and good anti-inflammatory activity for the treatment of psoriasis (*Sanjula B. et al., 2011*).

Curcumin-loaded microemulsions gel suitable for topical cosmetic applications were formulated in their research, using grape seed oil as the oily phase, which is often employed in pharmaceuticals, especially in cosmetics. The optimized microemulsion was formulated using Tween 80 and Plurol Diisostearique CG as a surfactant mix and ethanol as a co-solvent. Three different water soluble polymers were selected in order to increase the viscosity of the microemulsion: Carbopol 980 NF, chitosan, and sodium hyaluronate salt. Curcumin was chosen as a model drug. Microemulsion and gel microemulsions; approximately 1.7 (w/w)%. The gel microemulsions exhibited a pseudoplastic non-Newtonian rheological behaviour. At a similar mass concentration of thickening agent, Carbopol 980NF showed a lower viscosity value compared to the other two polymers. In the case of the gel microemulsions with hyaluronic acid and those with chitosan, close values of viscosity were obtained. The evaluation of cell viability for keratinocytes as a normal cells model showed that the gel microemulsions had a lower cytotoxicity than the simple microemulsion, and for the gel microemulsions, the percentage of viable keratinocytes increased in the order hyaluronic acid, Carbopol 980 NF, then chitosan. Taking into account the fact that the highest viscosity was obtained for gel microemulsions with chitosan, and their high biocompatibility with the skin, the CT4LVM sample would be the most suitable as a system for the delivery and controlled release of active ingredients in the skin. Due to the physico-chemical characteristics of gel microemulsions, high encapsulation capacities and the improvement of the penetration degree of the active principles, they concluded that these systems are suitable to be used as colloidal vectors for formulations in topical application of Curcumin in dermatocosmetic products (*Cristina S.*
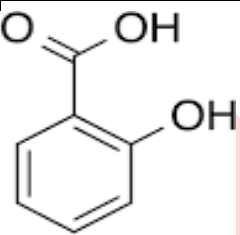
et al., 2021).

Prepared topical delivery of clobetasol propionate loaded-microemulsion based gel for effective treatment of vitiligo which showed that higher drug permeation into the skin microemulsion (60.33 ± 4.67%) compared with the marketed product (37.77 ± 0.77%) with better retention in the skin and minimal irritation potential thus proved to be a promising formulation for the effective treatment of vitiligo. The values of viscosity (Pas) and yield stress (Pa) were 25.47±2.27; 32.86±4.52 (MBC), 26.69±3.64; 34.42±3.91 (MFCP) and 14.56±1.08; 18.77±2.38 (CPG) respectively. Based on ME of CP has improved the solubility of drug and incorporation of the same into Carbopol 934P has a prominent effect in the treatment of vitiligo. The rheogram of MBC was developed in order to optimize its viscosity so as to have suitable extrudability and spreadability for topical application. In vitro drug release and ex vivo cutaneous deposition studies concluded that MBC has better penetration as well as retention capacity in the dermal layers than MFCP and CPG. The dermal targeting potential of MBC was additionally confirmed in vivo by visualization of skin localization of CP in various skin layers and dermal uptake by CLSM technique. Patients treated with MBC showed faster repigmentation than those treated with MFCP. Thus, it could be concluded that MBC could be a promising formulation to form the re-pigmentation of the skin and to cure vitiligo. The efficacy of MBC could be further established conducting multi-cantered clinical trial with large number of patient (*Patel et al., 2014*).

Desonide-loaded microemulsion based gel for effective management of atopic dermatitis microemulsions were developed and optimized using D-optimal mixture statistical experimental design for their research. They studied the morphology of ME using transmission electron microscopy, their microemulsion formulation contains 5%w/w of oil, 35.634%w/w Smix and 59.357% water while carbopol 940 was used as the gelling agent. The physicochemical characteristics exhibited by developed microemulsion were found to be optimal with the average droplet size of 163.2 +- 0.09 nm with PDI of 0.231 and zeta potential of -32.5 +- 0.6 mV indicating uniform particle size distributions and good physical colloidal stability. TEM revealed spherical droplets having nanoparticle size distribution with negligible coalescence. The ME formulation containing 5% w/w oil,35.643% w/w Smix, and 59.357% w/w water was selected. The physicochemical characteristics exhibited by developed ME were found to be optimal. Ex-vivo studies showed 3 fold increase in drug retention from MG in rat skin as compared to commercial formulation. MG resulted in significant reduction (p<0.05) in dermatitis score as compared to marketed gel with reduction in neutrophilic infiltration. Transepidermal water loss by application of drug loaded carbopol gel, marketed gel and MG on mice skin was found to be 3.01 ±0.08 g/m2h, 2.12± 0.06 g/m2h and 2.52 ± 0.12 g/m2h, respectively. The significant reduction in transepidermal water loss (p<0.05) indicated the potential of MG to minimize steroid associated epidermal barrier impairment due to entrapment of drug in ME droplets. Conclusion: The overall results elucidated that desonide loaded MG could be a successful carrier system for the treatment of AD (Kusha S. et al., 2021).

Metronidazole-loaded microemulsion gel, a poorly water soluble drug were developed for their research. The pseudoternary phase diagrams were developed for various microemulsion formulations composed of Capmul 908 P, Acconon MC8-2, and propylene glycol. The emulgel was optimized using a three-factor, two-level factorial design, the independent variables selected were Capmul 908 P, and surfactant mixture (Acconon MC8-

2 and gelling agent), and the dependent variables (responses) were a cumulative amount of drug permeated across the dialysis membrane in 24 h (Y_1) and spreadability (Y_2) based on solubility. The statistical validity of the polynomials was established, and optimized formulation factors were selected for physical appearance and pH determination, rheological studies, spreadability, in-vitro diffusion studies, ex vivo diffusion studies, skin irritation test, and stability studies. Based on their result it was concluded that the developed emulgels were efficacious for the delivery of lipophilic and poorly soluble drugs such as Metronidazole. The results demonstrated that the formulations were stable and showed improved permeation of the drug from the emulgel compared to conventional gel (*Rao M. et al., 2013*).

2.2   Drug profile – Salicylic acid

Synonyms	2-Hydroxybenzoic Acid, ortho-Hydroxybenzoic Acid,
IUPAC Name	2-Hydroxybenzoic Acid
Chemical Formula	$C_7H_6O_3$
Chemical Structure	
Molecular Weight	138.12
Solubility	soluble in ethanol, propanol, ether, benzene, acetone, turpentine oil, slightly soluble in water, soluble in boilingwater
Log P	2.26
Melting Point	158.6℃
Functional category	Salicylic Acid has direct activity as an anti-inflammatory agent and acts as a topical antibacterial agent because of its capacity to promote exfoliation.
Descriptions	Salicylic acid is an odourless white to light tan solid, Sinks and mixes slowly with water, has acrid taste. It is a compound obtained from the bark of the white willow and wintergreen leaves. It has bacteriostatic, fungicidal, and keratolytic actions.
Incompatibilities	It is incompatible with iodine, iron salts, and oxidizing substances

Applications	Salicylic acid is used to treat a number of skin conditions including acne, hyperkeratosis disorders (e.g., psoriasis, ichthyoses, etc.), warts, and corns. It is a beta hydroxyl acid that is used as a chemical peeling agent.

Pharmacodynamics Salicylic acid treats acne by causing cells to slough off more readily, preventing pores clogging up. This effect on the skin cells also makes salicylic acid an active ingredient in several shampoos meant to treat dandruff. Use of straight salicylic acid solution may cause hyperpigmentation on unpretreated skin for those with darker skin types as well as with the lack of use of a broad spectrum sunblock. Subsalicylates in combination with bismuth form the popular stomach relief aid known commonly as pepto-bismol. When combined the two key ingredients help control diarrhoea, nausea, heartburn and even gas. It is also very mildly anti-biotic. .

Mechanism of action Salicylic acid promptly irreversibly inhibits COX-1 and COX-2 to reduce transformation of arachidonic acid to precursors of prostaglandins and thromboxane. Salicylate's usefulness in rheumatic diseases is because of its analgesic and ant-inflammatory activity. Salicylic acid is an essential element in several skin-care products for the treatment of acne, psoriasis, calluses, corns, keratosis pilaris, and warts. Salicylic acid allows cells of the epidermis to more readily slough off. Salicylic acid is used in many shampoo formulation for the treatment of dandruff due its effect on skin cells. Salicylic acid is as well used as an active ingredient in gels that remove verrucas (plantar warts). Salicylic acid competitively inhibits oxidation of uridine-5-diphosphoglucose (UDPG) with nicotinamide adenosine dinucleotide (NAD) and noncompetitively with UDPG. It likewise competitively inhibits the transferring of the glucuronyl groups of uridine-5-diphosphoglucuronic acid (UDPGA) to phenolic acceptor. Inhibition of mucopoly saccharide synthesis is likely important for the slowing of wound healing wit salicylates.

Pharmacokinetics:

Distribution:

The volume of distribution is about 170 ml/kg of body weight.

Metabolism Salicylic acid is extensively metabolized. It undergoes metabolism by conjugating with glucose to SA glucoside and an ester. At low dosage, approximately 80% of salicylic acid is metabolized in the liver. Conjugation with glycine, form salicyluric acid and when conjugated with glucuronic acid, acyl and phenolic glucuronide are formed. Small amounts of salicylic acid are also hydroxylated to gentisic acid. With large dose, the kinetics switch from first order to zero order

Route of Elimination: About 10% is excreted unchanged in the urine.

Adverse effects Salicylic acid can cause skin dryness, irritation, itching, redness, warmth of skin, contact dermatitis and other similar skin reactions, nausea, vomiting, headaches, and diarrhoea.

2.3   Excipients profile

2.3.1   Soya Lecithin

Synonyms	Mixed soybean phosphatides, Phospholipon, soybean lecithin, soybean phospholipids, vegetables lecithin.
IUPAC Name	[3-hexadecanoylxyl-2-[9E,12E]-octadeca-9,12-dienoyl]oxypropyl)-[2-(trimethylazaniumyl)ethyl] phosphonate
MolecularFormula	$C_{42}H_{80}NO_8P$
ChemicalStructure	
MolecularWeight	759.1
Melting Point	231.1℃
Solubility	Soy Lecithin is soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, mineral oil, and fatty acids, but in cold vegetable and animal oils, polar solvents, and water it is nearly insoluble. Insoluble in water, soluble in chloroform, ethanol and ether. Lecithin hydrates to produce emulsions when mixed with water.
Functionalcategory	Lecithin in pharmaceutical technology is used emollient; emulsifying agent; solubilizing agent, stabilizing agent, and encapsulation and vesicle forming agent
Description	Solid, White or faintly yellow pearly granules or crystals Soy lecithin varies widely depending on the free fatty acid concentration, ranging from sticky semisolid to powders. Depending on the degree of purity, it can also vary in appearance from brown to pale yellow.
Incompatibilities	Excessive heat and flames, Avoid strong oxidizers, incompatible with esterases owing to hydrolysis.
Applications	Hypolipidemic ingredients, nutrition supplements, humectant and emulsifiers.

2.3.2   Tween 80

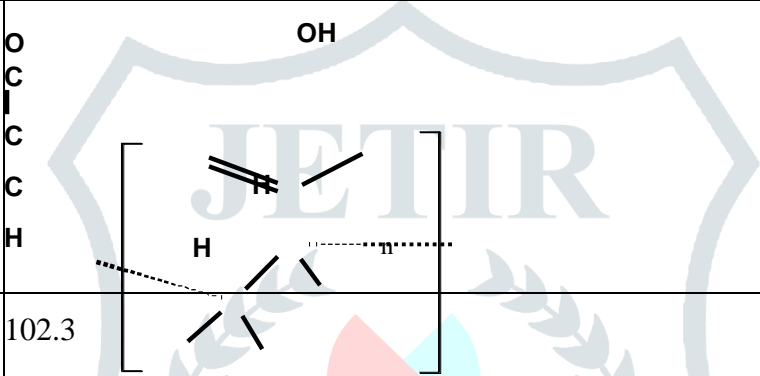|	
Synonyms	Glycol (PS polysorbate 80), polyethylene oxide sorbitan. monooleate, polyoxyethylene (20) sorbitan monooleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan oleate
IUPAC Name	Polyoxyethylene (20) sorbate monooleate
Chemical Formula	$C_{64}H_{124}O_{26}$
Chemical Structure	
Molecular Weight	1310g/mol
Melting Point	-25℃
Solubility	It is miscible with ethanol (95%), glycerin, and water. It is soluble in cottonseed oil, corn oil, ethyl acetate, methanol, toluene
Functional category	Hydrophilic non-ionic surfactant, emulsifier, solubilizer, wetting agent, dispersing/suspending agent
Descriptions:	Tween 80 is Viscous liquid of golden yellow/amber colour has a faint characteristic odour and a warm somewhat bitter taste. It is a mixture of partial esters of fatty acids. Mainly oleic acid (PS 80) or lauric acid (PS 20), respectively with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides
Incompatibilities	Discoloration and/or precipitation occur with phenols, tannins, tars, and tarlike materials. Reduce antimicrobial activity of paraben preservatives. Avoid strong oxidizers.
Applications	used in cosmetic, food and pharmaceutical industries as an emulsifier stabilizer, solubilizer or dispersant

2.3.3   Propylene Glycol

Synonyms	1, 2-dihydroxypropane, 1, 2-propanediol, methyl glycol, trimethyl glycol
IUPAC Name	Propane-1,2-diol
Chemical Formula	$C_3H_8O_2$
Chemical Structure	
Molecular Weight	76.10
Melting Point	-59°C
Solubility	It is miscible with acetone, chloroform, ethanol (95%), glycerin, and water. It is soluble at 1 in 6 parts of ether but not miscible with light mineral oil or fixed oils.
Functional category	It is a water-miscible co-solvent that act as antimicrobial preservative, humectant, disinfectant, plasticizer, solvent, and a stabilizer for vitamins
Descriptions	An odourless, colourless, hygroscopic viscous liquid, tasteless/a sweet, slightly acrid taste resembling that of glycerine
Incompatibilities	propylene glycol is incompatible with oxidizing reagents such as potassium permanganate
Applications	Propylene glycol has been used in the pharmaceutical formulations as a solvent, extractant, and preservative in a variety of parenteral and non-parenteral formulations. It is also used as a plasticizer in a aqueous film-coating formulation

2.3.4   Ethanol

Synonyms	Ethanolum, absolute ethyl alcohol, ethyl hydroxide, grain alcohol, methyl carbinol.
Chemical Formula	C_2H_6O
Chemical Structure	
Molecular Weight	46.07
Melting Point	-112°C
Solubility	It is miscible with chloroform, ether, glycerine, and water.
Description	Alcohol is a clear, colourless, mobile, and volatile liquid with a slight and characteristic odour.
Functional category	Ethanol and different concentration of ethanol aqueous solutions are widely employed in pharmaceutical and cosmetic products. Ethanol is primarily used as a solvent, disinfectant and antimicrobial preservative. In topical formulation, ethanol solutions are employed in the development of transdermal drug delivery systems as penetration enhancers
Application	Ethanol in pharmaceutical technology is used as an antimicrobial preservative, disinfectant, skin penetrant, solvent.

2.3.5   Carbopol 940

Carbopol is used in liquid or semisolid pharmaceutical formulations as a rheology or viscosity modifiers. It is used in the formulations of creams, gels, lotions and ointments for applications inophthalmic, rectal, topical and vaginal delivery.

Synonyms	Acrylic acid polymer, carbomer, carboxy polymethylene, polyacrylic acid.
Chemical Formula	$(C_3H_4O_2)_n$
Chemical Structure	
Molecular Weight	102.3
Melting Point	260°C
Solubility	It is swellable in water, glycerin and ethanol (95%), after neutralization. Carbopol do not dissolve but swells to a certain extent, because they are three-dimensionally crosslinked microgels.
Descriptions	It is white-colored, fluffy, acidic, hygroscopic powders with a characteristic odor
Application	Used as a Gelling agent, viscosity modifier, Bioadhesive, emulsifying agent, rheology modifier, stabilizing agent and suspending agent

CHAPTER 3
AIM OBJECTIVEAND PLAN OF WORK

Aim

The aim of this work is to develop salicylic acid-loaded microemulsion-based gel for topicalapplication.

Objective

To overcome the intrinsic defects related to conventional dosage form of salicylic acid, bydeveloping a topical salicylic acid Microemulgel which has the following benefits.

1.   Enhance solubility of salicylic acid
2.   Improve bioavailability and maintain controlled release
3.   Minimize the side effect.
4.   Better patient compliance.

Plan of work

1.   Preformulation studies

i.   Organoleptic properties

ii.   Melting point

iii.   UV-spectroscopy

iv.   FTIR spectroscopy

v.   DSC.

vi.   Determination of melting point

vii.   Solubility studies

viii.   Determination of partition coefficient

3.   Development of formulation

a.   Preparation of drug loaded microemulsion formulation

(By titration method)

b.   Preparation drug loaded microemulsion based gel

(By dispersion method)

4.   Characterization of formulation

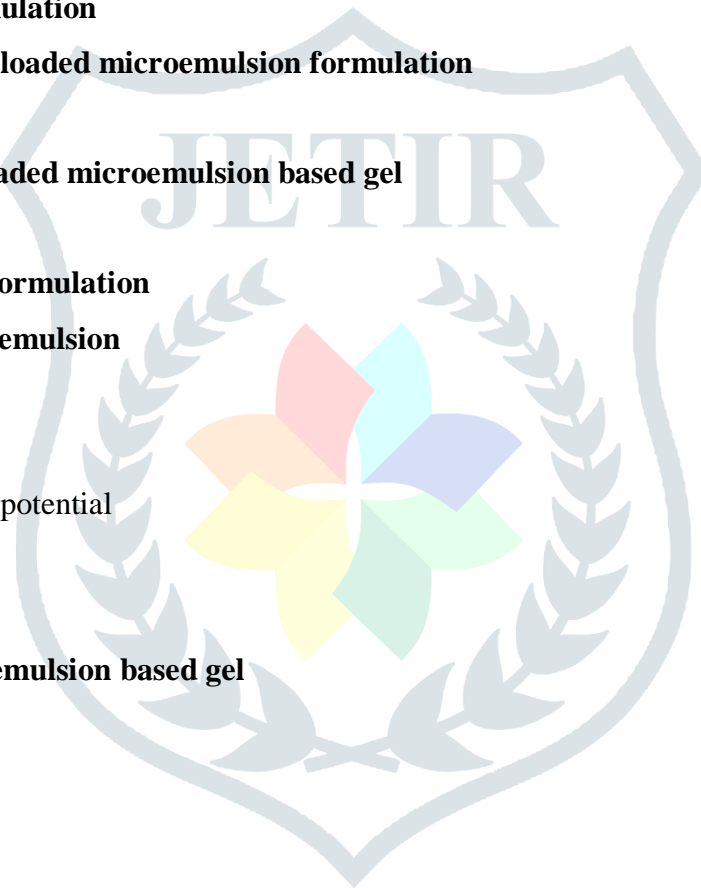**A.   Evaluation of microemulsion**

i.   Centrifugation

ii.   Morphology

iii.   Particle size and zeta potential

iv.   Drug content

v.   In-vitro release

B.   Evaluation of microemulsion based gel

i.   pH

ii.   Spreadability

iii.   Extrudability

iv.   Viscosity

v.   Drug content

vi.   Drug release study

5.   Storage and stability studies

CHAPTER 4
MATERIALSAND METHOD

4.1 MATERIALS

4.1.1 Chemicals

Table No 2: List of Chemicals

S. No	CHEMICALS	SOURCE
1	Salicylic acid	Merck SPL, Mumbia
2	Lecithin	Reg-labogens, Uttarakhand
3	Propylene glycol	Merck SPL Mumbai
4	Tween 80	SDFCE, Mumbai
5	Ethanol	Qualitech lab chemicals, Bangalore
6	Carbopol 940	Ases chemical, Rajasthan
7	Triethanolamine	Maya chemtech privat LTD ,Delhi
8	Sodium hydroxide	Merck SPL Mumbai
9	Potassium di hydrogen phosphate	Reg-labogens, Uttarakhand

4.1.2 Equipment

Table No 3: List of Instruments

S/No	INSTRUMENTS	SOURCE
1	Centrifuge	Remi lab world, Mumbia
2	UV spectrophotometer	Agilent Tech India
4	Zeta Sizer and particle Sizer	Beckman Coulter Delsa Nano New Delhi
5	FTIR	PerkinElmer Pvt Ltd, India
6	DSC	PerkinElmer DSC 2000, India
7	Dialysis membrane	Himedia, India

8	Franz diffusion cell apparatus	Orchid Scientific
9	Microscope	Popular India
10	Melting point apparatus	Popular India
11	Hot plate	Popular India
12	Brookfield viscometer	Ametek Brookfield India Mumbai
13	pH meter	

4.2   METHODOLOGY

4.2   Preformulation Studies

4.2.1   Organoleptic Properties

The physical appearance of salicylic acid such as nature, colour, and odour, were analysed by visual observations.

Nature: A small sample was placed on a butter paper to inspect the nature of the drug.

Colour: A small quantity of the medication was placed on butter paper and viewed in a lit area.

Odour: A little amount of the medication perceived to observe the odour.

4.2.3   Melting point:

To know the melting point of salicylic acid, five capillary tubes which has one end sealed were filled with a little amount of salicylic acid and placed one after the other inside the melting point apparatus along with a thermometer before the instrument was turned on to measure the melting point. Salicylic acid started to melt at a temperature of between 130 and 158 degrees Celsius, at the temperature of 159 the substance inside the tube was completely liquid form.

4.2.4   Preparation of standard buffer solution

A)   Preparation of 0.2 M Potassium dihydrogen phosphate:

27.218 g of potassium dihydrogen phosphate was accurately weighed and dissolved indistilled water and made up to 1000 ml with distilled water.

B)   Preparation of 0.2M Sodium hydroxide solution:

8.0 g of Sodium hydroxide was accurately weighed and dissolved in distilled water and madeup to 1000 ml with distilled water.

Phosphate buffer solution of pH 7.4 1 litre preparation

250 ml of 0.2 M potassium dihydrogen phosphate solution and 195.5 ml of 0.2 M sodium hydroxide solution were combined and diluted with distilled water to make 1000ml. The pH was then adjusted to 7.4.

4.2.5   Preparation of standard curve of salicylic acid by using pH 7.4 phosphate buffer

A)   Preparation of primary stock solution

To create a stock solution containing 1000μg/ml of salicylic acid, 100 mg of salicylic acid that had been precisely measured was dissolved in small amount of ethanol in a volumetric flask and made up to 100ml with phosphate buffer.

B)   Preparation of working solution:

To create a 100 μg/ml standard working solution, 10 ml of the stock solution were pipetted into a 100 ml volumetric flask and made up to mark with of phosphate buffer. Aliquots of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, and 9 ml, from the aforementioned working solution were pipetted out in a series of 10 ml volumetric flasks and made up to the mark with buffer solution to be equivalent to 10-90 μg/ml. The solution's absorbance at 200-800 nm wavelength was then determined using a UV double beam spectrophotometer against blank sample. The wavelength of maximum absorption was then determined to be the one at which the highest absorbance was recorded, which was found to be λmax = 276nm. The result is shown in the table 5 and Figure 3

4.2.6   Solubility studies

The formulation of the micro emulsion depends on the drug's solubility in the desired substance such as the oils, surfactants, cosurfactants and consolvents. Therefore, in order for the medication to be in the proper solubility range and for the phase diagram to begin, it is proper to first choose the oil, surfactant, and co-surfactant in which the drug displays maximum solubility. This is crucial for the creation of a micro emulsion drug delivery system. Salicylic acid's solubility was tested at room temperature using a mixture of 5ml of water, ethanol, tween 80, propylene glycol, methanol, span 80, and PBS along with 50mg of the drug. The drug was dissolved in each of the liquid substance. The mixture was left undisturbed for 24 hours after being stirred with a stirring stick for 30 minutes at a time. Each solution was centrifuged at 10,000 rpm for 15 minutes after 24 hours. The solubility for any deposit at the bottom of the centrifuge tube was observed in each system and under microscope for any crystal visibility; their solubility performance was graded on the scale of 10-50.

4.2.7   Determination of partition coefficient:

The partition coefficient of salicylic acid was calculated using the shake flask method and then titration procedures. In a 50 ml conical flask, 10mg of salicylic acid was weighed and dissolved in 10 ml of water and 10 ml of n-octanol, the flask was firmly shaken for 15 to 20 minutes. The solution was transferred into a separating funnel and left undisturbed for 24 hours to achieve equilibrium, following which it separated into two phases: aqueous phase and organic phase. After 24hours the knob of the separating funnel was loosen to separate the solution, the aqueous phase at the bottom of the separating funnel was poured into the conical flask, and the organic phase was done in the same manner. 5ml of each phase was titrated against 0.1M NaOH solution using 2 drops of phenolphthalein as an indicator; the titration was continued until the end point was reached by a colour shift to pink; the process was repeated three times.

The partition coefficient was determined and calculated with the formula: $K = \underline{C_{org}}$

C_{aq}

Where,

K =, partition coefficient or distribution constant

C_{org} = centration in organic phase

C_{aq} = concentration in aqueous phase

4.2.8   Drug-excipients interaction studies:

4.2.8.1   FT-IR spectra of pure drug salicylic acid and drug with all excipients were obtained using PerkinElmer Spectrum in order to detect probable interactions between the drug and excipients. The plain drug and excipients were combined with KBr in the ratio (0.1:3:3). Using a hydraulic press, the samples were compacted to create a pellet. The produced pellets were shaped into a disc and put to the centre of the sample holding device, where they were scanned from 4,000 to 450 cm-1 with an FT-IR spectrophotometer.

4.2.8.2   DSC- Differential Scanning Calorimetry was used to measure the temperature and heat flow of the sample, it provides direct information about how much energy the sample has absorbed and or released. The DCS of pure drug, drug + carbopol 940 + lecithin, and DSC of final gel formulation were scanned using PerkinElmer DSC 2000 at 0 to 350ºC. Each sample weighing 5mg was prepared and placed in the sample disk and a prepared empty reference pan was placed in the reference disk. The empty reference pan was used to remove the effects of internals of the machines. The samples and the reference were heated at the same rate from a single heating source. Temperature difference between the pans was recorded and converted to a power difference which gives different in heat flow. The result ofthe scanning is displayed on computer connected to the DSC machine.

4.3   Formulation of salicylic acid- loaded microemulsion

The salicylic acid-loaded microemulsion was prepared by titration method using lecithin as oil phase, tween 80 as surfactant, propylene glycol as co surfactant and ethanol for cosolvent and penetration enhancer. The quantity of the drug was kept at 3%w/w and Lecithin dose was kept 0.1% throughout the formulation. Ethanol was adopted and used as dissolving medium for Salicylic acid which is the model drug. Different batches of microemulsions were prepared by water titration method describe below, the composition for the formulation is given in the table no

The formulation was created once the microemulsion area was confirmed.

These processes were followed in order to generate a thermodynamically stable clear light yellow mixture of salicylic acid's most soluble microemulsion, and the best me procedure was selected for further research.

Procedure 1:

1.   In this procedure salicylic acid was dissolved in ethanol, the solution was covered and keptaside.

2.   A combined mixture of Tween 80 and propylene glycol was created.

3.   A weighed amount of lecithin was dispersed in water and was added to the step 2 liquid and stirred instantly and continuously till a clear solution was obtained.

4.   Then the solution of salicylic acid was added slowly to the above liquid with thorough mixing till a clear light yellow mixture was achieved. Thereafter the volume was made up as desired.

Procedure 2:

1.   In this procedure, lecithin was weighed and dispersed in defined water proportion.

2.   Tween 80, propylene glycol and ethanol were added to the mixture and stirred properly till clear solution was formed

3.   Then a weighed amount of salicylic acid was added in above solution and mixedthoroughly.

4.   The volume was made up as required.

Procedure 3

1.   Here salicylic acid was dissolved in ethanol and kept aside.

2.   A dispersion of lecithin in water was prepared.

3.   A clear solution of tween 80 and propylene glycol was mixed and added slowly to thedispersion medium with proper mixing till a clear solution was obtained.

4.   The solution of salicylic acid was added slowly to the above solution with thoroughmixing.

5.   The volume of the solution was made up as required.

The formulation which displayed maximum stability after physical centrifuge and stress analysis was selected as the best stable formulation after several compositions trial taken for further studies.

Table No 4: Composition of salicylic acid microemulsion formulations

Formulation code	Salicylic acid (%)	lecithin (%)	Tween 80 (%)	Propylene glycol (%)	Ethanol (%)	Water q.s(%)
M1	3	0.1	2	-	5	100
M2	3	0.1	4	-	10	100
M3	3	0.1	6	5	-	100
M4	3	0.1	8	10	10	100
M5	3	0.1	10	10	5	100
M6	3	0.1	12	10	12	100
M7	3	0.1	14	10	14	100
M8	3	0.1	15	10	15	100
M9	3	0.1	16	10	16	100

Table No.5 Compositions of optimize salicylic acid microemulsion Formulation (M9)

Chemicals	Quantity (mg) or ml	Quantity (%w/w)
Salicylic acid	300 mg	3
Lecithin oil	10 mg	0.1
Tween 80	1600 mg	16
Propylene glycol	1ml	10
Ethanol	1.6ml	16
Water q.s	q.s	q.s (100)

The selected optimized salicylic acid microemulsion (M9) procedure 3 containing the mentioned quantities of material in table no 5 was found to be most stable in comparison to other formulations this was used to prepare salicylic acid-loaded microemulsion based gel of different Carbopol 940 concentrations.

4.4   Preparation of salicylic acid loaded microemulsion gel

Gelling agent (carbopol 940) was pre-soaked separately in aqueous phase (water) for 24 hours. The pH was adjusted with drop wise addition of triethanolamine. The prepared optimized microemulsion (M9) was then added slowly to the gel phase to form microemulsion based gel and mixed continuously until the smooth, elegant microemulgel was obtained. As stated in the table no 8 below, 3% salicylic acid micro emulsion gel was created using different concentrations of carbopol 940 as a vehicle for integration formulation for topical medication delivery. Carbopol 940 was dissolved in hot water that was almost at the boiling point in the needed quantity, allowed to hydrate for hours, stirred, and neutralised by adding triethanolamine drops at a time to get a desirable pH of 7. Before adding the 80% volume of the salicylic acid solution, 1% ethanol and 1% tween 80 were added to the carbopol 940 and mixed thoroughly. The gel formulation was carefully combined to produce a homogeneous white semisolid mixture. It was kept in a desiccator for 24 hours to dry before being removed for further research. Different batches of microemulsion gel were created with different concentrations of gelling agent. The formulas for the gels were made for 10g, and 25g

Table No 6: Composition of Micro emulsion gel formulation

Formulation code	Salicylic acid (%)	Lecithin (%)	Carbopol 940 (%)	Tween 80 (%)	Propylene glycol (%)	Ethanol (%)	Water (q.s %)	Tea (q.s)
G1	3	0.1	1	17	10	17	100	q.s
G2	3	0.1	1.5	17	10	17	100	q.s
G3	3	0.1	2	17	10	17	100	q.s
G4	3	0.1	2.5	17	10	17	100	q.s
G5	3	0.1	3	17	10	17	100	q.s
G6	3	0.1	4	17	10	17	100	q.s

4.5   EVALUATION OF SALICYLIC ACID LOADED MICROEMULSION

4.5.1   Physical stability studies

4.5.1.1   Visual inspection: visual inspection was made after each addition of water to the surfactant or mixture of surfactant and co-surfactant and co-solvent the formulated ME system was viewed visually for homogeneity, clarity and fluidity.

4.5.1.2   Centrifugation: Physical stability studies were carried out to address the issue of metastable formulations. Centrifugation (Remi microcentrifuge) at 5000 rpm for 30 minutes was performed on the selected microemulsions. The heating and cooling cycles were performed on formulations that did not exhibit any phase separations.

4.5.1.3   Stress test: This test was carried out to determine the optimal microemulsion formulation under severe conditions. Each week, five cycles were performed between room temperature, refrigerator temperature (4°C), and (40-45°C), with storage at each temperature of at least 24 hours. A freeze-thaw cycle test was performed on the formulations that were determined to be stable. For 24 hours, the formulations were maintained in a deep freezer at - 20°C. The microemulsions were removed after 24 hours and stored at room temperature. Within 2-3 minutes, the physically stable microemulsions reverted to their former form. Three such cycles were carried out. Coalescence, flocculation, cracking, and phase separation were all examined in the samples.

4.5.2   Particle size and distribution analysis:

The particle size determination and particle size distribution of optimized micro emulsion was analysed using a Delsa[TM] Nano C analyser (Beckham Coulter) Dynamic light scattering (DLS) was equipped to measure the intensity of light scattered in the dispersion. 3ml of the microemulsion sample was taken for analysis and measured at 25^oC and 400nm

4.5.3　　Zeta potential:

The zeta potential of micro-emulsion was also measured using Delsa ^{TM} Nano C analyser by adding the sample into the high concentration cell equipped with a transparent electrode. 3ml of the microemulsion sample was taken for analysis the sample was placed in a clear disposable zeta cell and measure at 25°C and the result was recorded.

4.5.4　　Morphological analysis:

Under a microscope, the salicylic acid, prepared microemulsion and microemulsion gel formulations were examined for vesicle formation and discreteness of dispersed colloidal structure. A slide was created by placing each sample of the material on a glass slide, then covers it with a cover slip and viewed it under an optical microscope at 40X magnification. Photographs were taken with a digital camera for each prepared slide.

4.5.5　　Determination of drug content:

For determination of drug content, 0.5 ml of the M9 formulation was measured and diluted to 10ml using PBS in a 100 ml volumetric flask the solution was further diluted to 100 ml with PBS before filtration. The sample was analysed by UV spectrophotometer at 276nm using PBS as a blank. Drug content was calculated by linear regression analysis of the calibration curve.

4.5.6　　Drug release study

The diffusion cell with cellophane paper Himedia, India of 1inch was used for the in vitro drug/permeation release investigations. The release media was made with a PBS 7.4. Prior to the experiment, a 1 inch section of the dialysis membrane was cut and placed in boiling water for 30 minutes before being gently removed. The experiment for drug release tests were carried out in a modified Franz diffusion cell. A cellophane membrane was attached to the Franz diffusion cell. The formulation was introduced into the dialysis membrane through the donor compartment. 25 mL of pH 7.4 phosphate buffer was added to the reservoir compartment. The experiment lasted 12 hours and was carried out at 37 1°C and 100 rpm. 5 ml samples were withdrawn from the reservoir compartment at 0.25, 0.5, 1, 2, 4, 6, 8, and 10 h intervals via the side tube and the same amount of PBS was also replaced the absorbance was determined spectrophotometrically at 276 nm. The percentage (%) cumulative drug release was calculated by linear regression analysis of the calibration curve.

4.6　　EVALUATION OF SALICYLIC ACID LOADED MICROEMULSION BASEDGEL

4.6.1　　Physical evaluation

4.6.2　　Texture: The prepared gel formulations were observed and note the appearance.

4.6.3　　Consistency: Small amounts of each gel formulation were taken and slowly rub between thumb and forefingers in order to gauge the consistency of the gel formulation.

4.6.4　　Determination of pH: The pH of the microemulsion gel formulations were measured by immersing the electrode directly into the formulation using calibrated digital pH meter (VSI-IB, VSI electronics, India) at 25±1°C)

4.6.5   Determination of spreadability: The Spreadability of the gel was determined using the following technique: 0.5 gm gel was placed within a circle of 1cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 50 gm was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gels was noted and the mean diameter was taken by repeating the experiment three times.

It is calculated by using the following formula:

Spreadability = $\underline{M \times L}$

T

Where,

M= weight of tied to upper slide, L= length of the glass slide,

T= time taken to separate the slide.

4.6.6   Extrudability: This is a common empirical test for determining the force required to extrude the gel material from a tube. The method used to evaluate microemulsion-based gel formulation for extrudability is based on the amount of gel and gel extruded from an aluminium collapsible tube based on the weight in gram necessary to extrude at least 0.5 cm ribbon of microemulsion gel in 10 seconds. The greater the quantity extruded, the greater the extrudability (Vaibhavi M., et al. 2014).

The extrudability is calculated using the following formula;

Extrudability =Applied weight to extrude microemulsion gel from tube (in gm)

Area (in cm2)

4.6.7   Viscosity: The viscosities of stable formulations were determined by using Brookfield viscometer and results were noted. Brookfield viscometer consists of a cup, which is stationary and a spindle which is rotating. Rotating spindles are used and immersed in test material. For liquids with low viscosity large size spindles (large diameter and surface area) are used while for higher viscosity liquids small spindles (small diameter and surface area) are used. The spindle was rotated in the microemulsion/gel till a constant dial reading on the display of the viscometer was attained

4.6.8   Determination of drug content

For determination of drug content, 0.5 g of each gel formulation was measured and diluted to 10ml using PBS into a 100ml volumetric flask the solution was further diluted to 100ml with PBS before filtration. The sample was analysed by UV spectrophotometer at 276nm using PBS as a blank. Drug content was calculated by linear regression analysis of the calibration curve.

4.6.9   Drug release study

The diffusion cell with cellophane paper Himedia, India of 1inch was used for the in vitro drug/permeation release investigations. The release media was made with a PBS 7.4. Prior to the experiment, a 1 inch section of the dialysis membrane was cut and placed in boiling water for 30 minutes before being gently removed. The experiment for drug release tests were carried out in a modified Franz diffusion cell. A cellophane membrane was attached to the Franz diffusion cell. The formulation 0.5g was introduced into the dialysis membrane through the donor compartment. 25 mL of pH 7.4 phosphate buffer was added to the reservoir compartment. The experiment lasted 12 hours and was carried out at 37 1°C and 100 rpm. 5 ml samples were withdrawn from the reservoir compartment at 0.25, 0.5, 1, 2, 4, 6, 8, and 10 h intervals via the side tube and the same amount of PBS was also replaced the absorbance was determined spectrophotometrically at 276 nm. The percentage (%) cumulative drug release was calculated by linear regression analysis of the calibration curve.

In other to determine the release pattern of the formulation, the cumulative % result data of the highest release formulation was fitted in the different kinetics model like the zero other kinetic, the first order kinetic, the Higuchi matrix model and the Korsmeyer peppas model.

Zero-order model

The following equation is used for the calculation:

$Q_0 - Q_t = K_0 t$ (1)

Rearranging equation (1) results in:

$Q_t = Q_0 + K_0 t$ (2)

Where Qt is the amount of drug dissolved in time t, Q0 is the initial concentration of drug in the solution (most of the time, Q0 = 0), and K0 is the zero-order release constant expressed in concentration/time units. To analyse release kinetics, data from in vitro drug release studies were displayed as cumulative amount of drug released vs time

First order model

This model has also been used to show drug absorption and/or excretion, but this mechanismis difficult to understand theoretically.

The equation for the first order rate constant for drug release islog C = log C0 - Kt / 2.303.

Where, C0 denotes the starting drug concentration, k the first order rate constant, and t the time. The acquired data is shown as a straight line with a slope of -K/2.303 vs time (Narashmhan B. et al., 1999).

Higuchi model

The total proportion of drug released was plotted against the square root of time for Higuchikinetic model.

The equation used to calculate is written as:

$Q = K1/2$

Where K denotes the system's design parameters and t denotes the time in hours. As a result, the rate of drug

release is proportional to the square root of the reciprocal of time (Hugchi T.Jet al., 1963).

Korsmeyer-Peppas model

Korsmeyer et al. (1983) devised a simple equation for characterising drug release from a polymeric system. 60% of the drug release data were first fitted into the Korsmeyer-Peppas model to determine the mechanism of drug release.

$$Mt / M\infty = Ktn$$

The fraction of drug released at time t represents Mt / M∞, k represents release rate constant, and n represent release exponent. For cylindrical shaped matrices, the n value is utilised to characterise distinct releases. In the case of cylindrical tablets, $0.45 \leq n$ represents a Fickian diffusion mechanism, $45 \leq n$ corresponds to a Fickian diffusion mechanism, $0.45 < n$ 0.89 to super case II transport. Only $M_t / M\infty < 0.6$ should be utilised to calculate the exponent of n the part of the release curve. Data from in vitro drug release studies were shown as log cumulative percentage drug release versus log time taken to analysed release kinetics (Korsemeyer R.W *et. al*., 1983).

4.6.10   Storage – stability studies:

The stability of microemulsion formulations was studied through various parameters such as clarity, phase separation observation, for months at room temperature. In order to estimate metastable systems, the selected ME were centrifuged at 11,300 rpm for 60 minutes. Beside the physicochemical properties, the chemical stability of the investigated drug in the vehicle plays a major role. Therefore the drug content of optimized microemulsion and its gel formulation was analysed at defined time intervals during the observation period of three months for changes in appearance phase separation. During the observation period the formulations were stored at room temperature in centrifugal tubes to simulate patient usage conditions.

CHAPTER 5
RESULTS AND DISCUSSIONS

5.1   PREFORMULATION STUDIES

Pre-formulation studies were carried out in order to assess the physical and chemical properties of the drug in required to formulate a safe, effective, and stable dosage form. The purity of the medication powder was ensured by the Preformulation study. The physical characteristics of the salicylic acid were studied using the following criteria:

1   Organoleptic characteristic

2   Melting point

3   Determination of absorption maxima4 Partition coefficients

5 FTIR studies

5.1.1   Organoleptic properties of salicylic acid

Discussion: The preliminary investigation showed that salicylic acid is colourless or white acicular crystals, sweetish acrid/bitter taste and odourless solid. It is freely soluble in ethanol, methanol and 0.1 N HCl soluble, in chloroform, and slightly soluble in water. These observations confirms recorded confirms the specification of the drug in the pharmacopoeia

5.1.2   Melting point:

Discussion: The melting point of the drug was determined using capillary tube which was inserted in a digital melting pint apparatus along with thermometer. It was observed that the drug powder started melting at 130°C and when at 159°C the content in the capillary tube was completely liquid. The melting point was found in the range of 158°C - 159°C which is in compliance with the standard value of 158.6°C as per Indian Pharmacopoeia.

5.1.3   UV-Visible Spectrophotometer:

Discussion: The different concentrations (10, 20, 30, 40, 50, 60, 70, 80, and 90 µg/ml) of salicylic acid were

prepared with ethanol and diluted with phosphate buffer pH solution in table no.7 was analysed through UV at 200 to 400nm wavelength using corresponding media as a blank. This was done to obtain absorbance, slope and to determine the point of maximum absorbance wavelength. The maximum absorption was found to be at λmax 276nm and good linearity R^2 value of 0.9996 in figure 3 for Salicylic acid, which suggests that it obeys the Beer Lamberts law. So 276 nm absorption maximum of salicylic acid was used for further UV analysis of the formulation in this research work

Table 7: Absorbance of various concentration of SA drug in phosphate buffer

Volume of working Solution (ml)	Volume of PBS to make 10ml	Concentration of SA (µg/ml)	Absorbance (mean ± SD) (n=3)
1	9	10	0.110±0.001
2	8	20	0.298±0.003
3	7	30	0.319±0.002
4	6	40	0.417±0.001
5	5	50	0.509±0.001
6	4	60	0.599±0.002
7	3	70	0.698±0.002
8	2	80	0.797±0.001
9	1	90	0.897±0.001

Absorbance
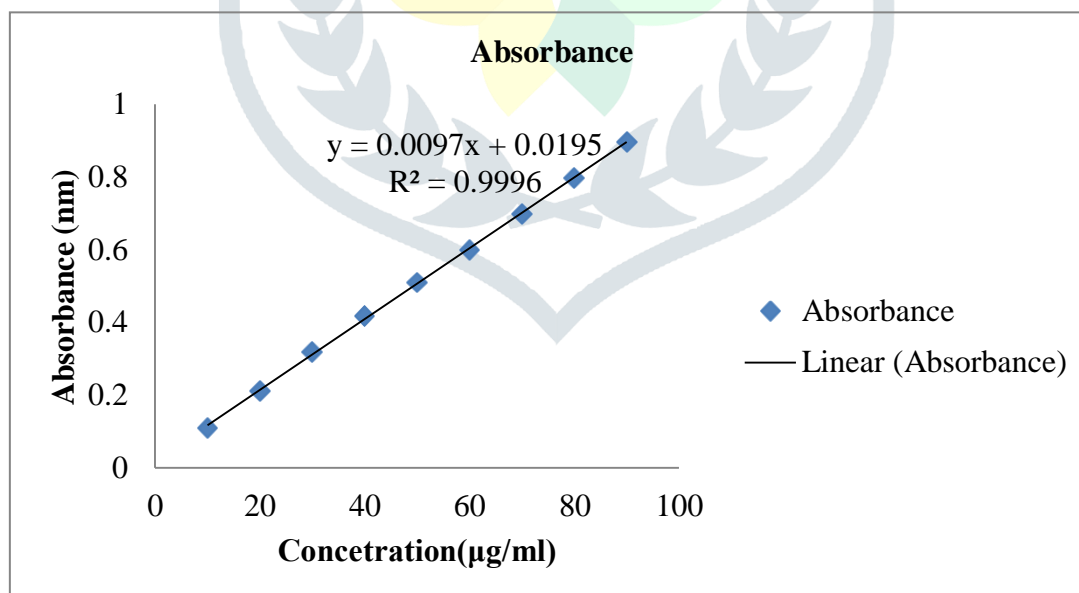
$y = 0.0097x + 0.0195$
$R^2 = 0.9996$

Figure No 3: Standard Calibration Curve for Salicylic Acid

5.1.4   Solubility studies : In formulation of microemulsion system which will be used as drug delivery system selection of excipients such as oil, surfactant and co-surfactant depends on the solubility of the drug in them. Solubility was studied by shake-flask method

Discussion: The solubility of salicylic in various media was analyzed in order to screen components of ME.

From these results in the table no.8 and figure no 4, it showed that SA has maximum solubility in ethanol, tween 80, span 80, PBS, and propylene glycol while it was less soluble in water and methanol. Therefore tween 80 was selected as surfactant, propylene glycol as cosurfactant, water solution of lecithin as the oil phase with ethanol s cosolvent were subsequently used for the formulation of microemulsion containing SA in this study.

Table No 8: Solubility range of salicylic acid in different system

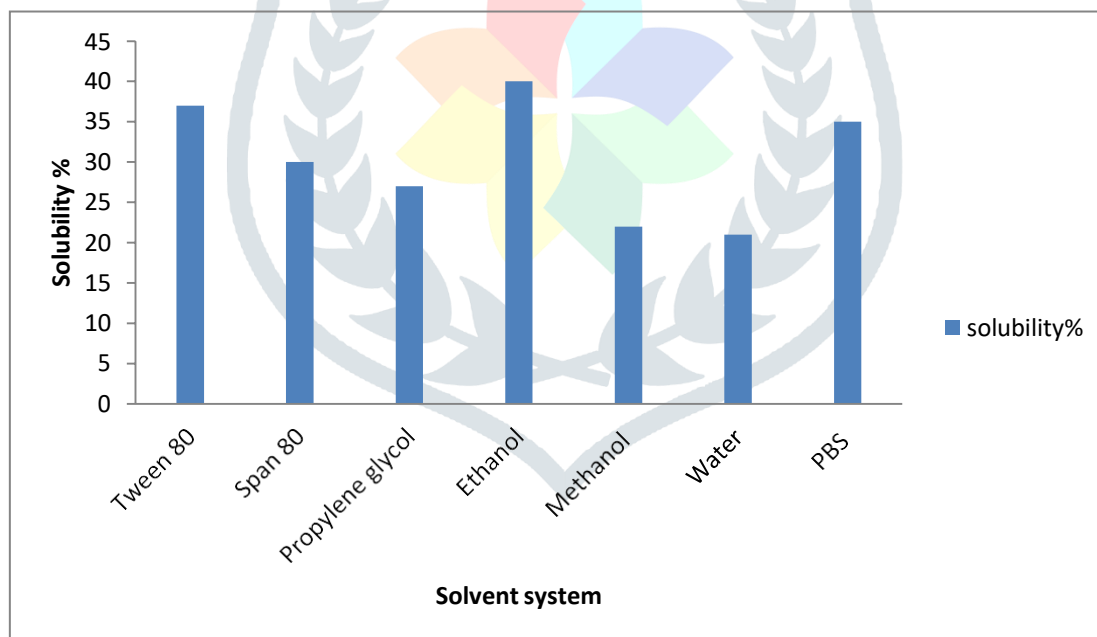| S /No	Surfactant/co-surfactant	Solubility (%)
1	Tween 80	35
2	Span 80	30
3	Propylene glycol	27
4	Ethanol	40
5	Methanol	22
6	Water	21
7	PBS	37

Figure No 4: % solubility in different system

5.1.5 Determination of partition coefficient:

The partition coefficient of salicylic acid was determined by shake flask method the lipophilic-hydrophilic of the drug was known by dividing concentration data obtained from the compound in organic phase to concentration in aqueous phase.

Discussion: The partition coefficient of salicylic acid in n-octanol: water was found to be 2.26. This indicated that the drug is lipophilic in nature because its affinity in organic solvent is higher than it is in aqueous phase. A log P value greater than 1 indicates that the compound is lipophilic whereas log P value less than 1 is said to be hydrophilic.

5.1.6 FT-IR Study: FTIR was used to analyse the drug interaction with it excipients. The FTIR analysis of pure drug and drug with excipients revealed that the medicine is compatible with the other substances in the formulation. The FT-IR measurements of the physical mixture show that the drug's functions, including peak figure intensities, have stayed unaltered. Figure 5 and figure 6
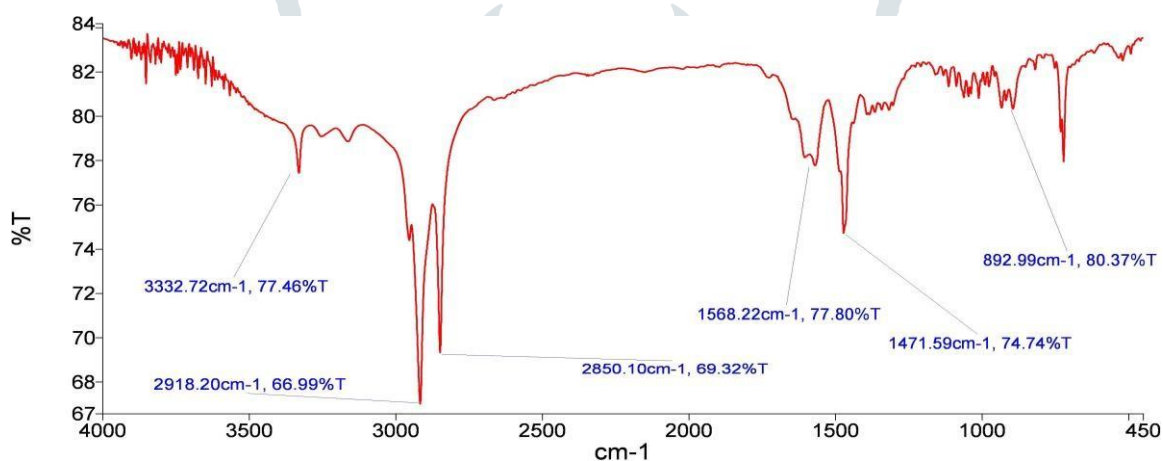

Figure No 5: **FT-IR of SA Table No 9. FTIR of Salicylic acid**

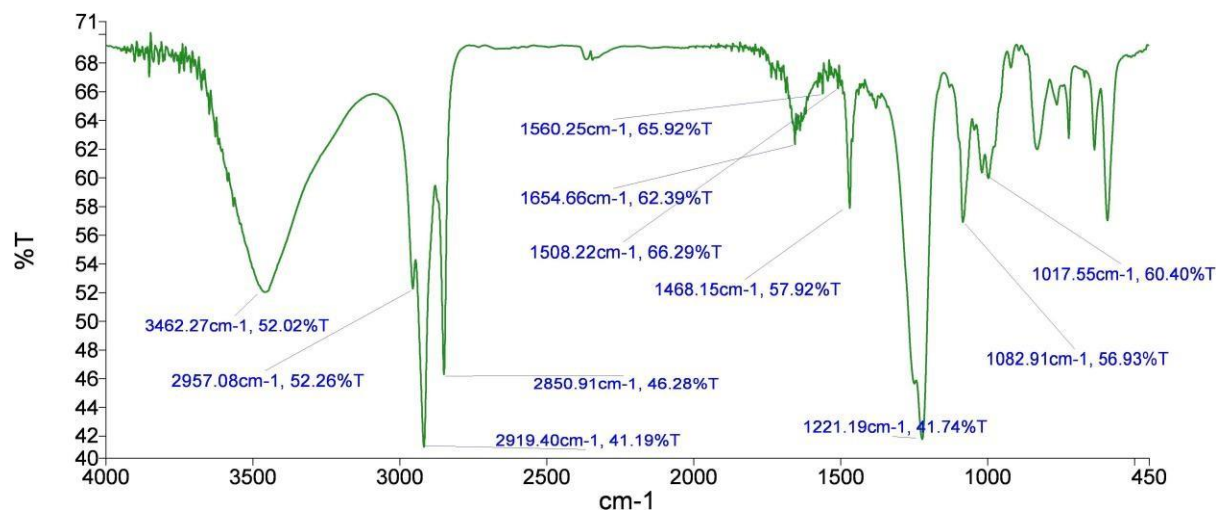| Functional group	Wavenumber (cm^{-1}) of peaks
O-H stretching	3332
C-H stretching	2850- 2918
C=C(phenolic) stretching	1568
C-C stretching	1471

Figure 6: FTIR of SA and the physical mixturesTable No 10. FTIR of SA and the physical mixtures

S/no	Functional group	Wavenumber(cm^{-1}) peaks
1	O-H	3462
2	CH stretching	2819-2957
3	Amino N-H bending	1654
4	C=C(phenolic) stretching	1560
	CH$_3$ bending alkanes	1468
5	C-N stretching	1221
6	C-O stretching	1082

5.1.7   Differential scanning Calorimeter

Discussion: Differential scanning calorimetry (DSC) analysis was used to determine the drug's compatibility with excipients. This study was conducted to detect any change in the chemical composition of the medicine following its combination with excipients in the ration (1:1) Figure 7 showed the temperature peak measured at 159°C and heat flow of 120.3 J/g for salicylic acid, figure 8 showed temperature peak 1 and 2 measured at 153.53^{o}C and 235.72^{o}C with heat flow of 41.06J/g and 89.29J/g respectively for salicylic acid and excipients, and figure 25 showed temperature peak measured at 200.25^{o}C and a heat flow of 15.18 J/g for microemulsion gel.
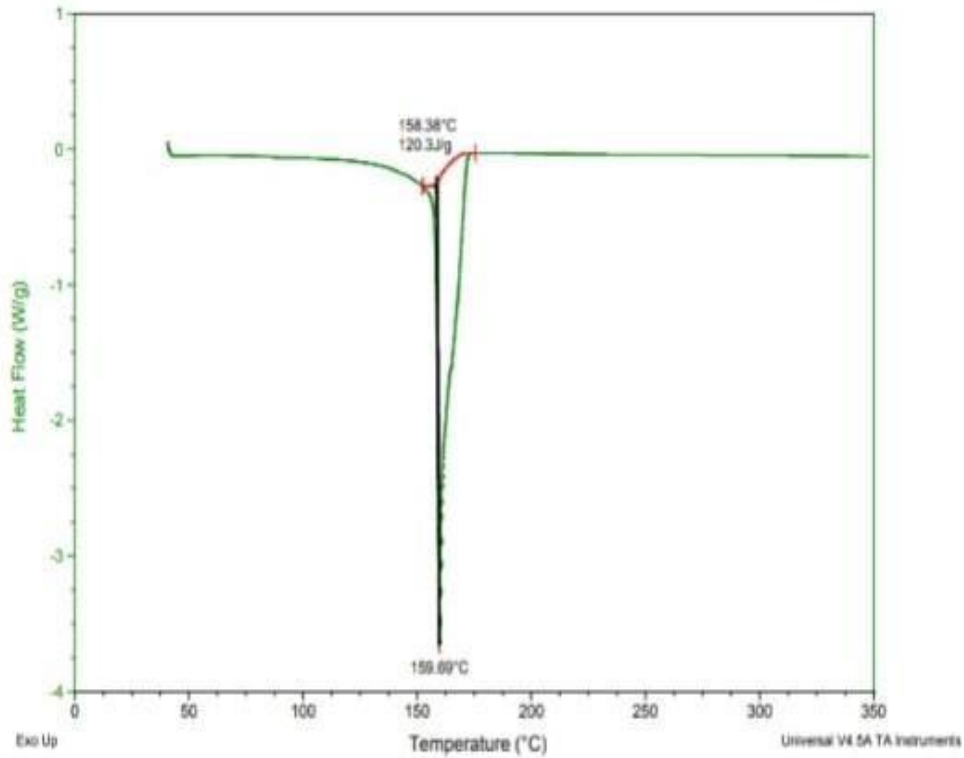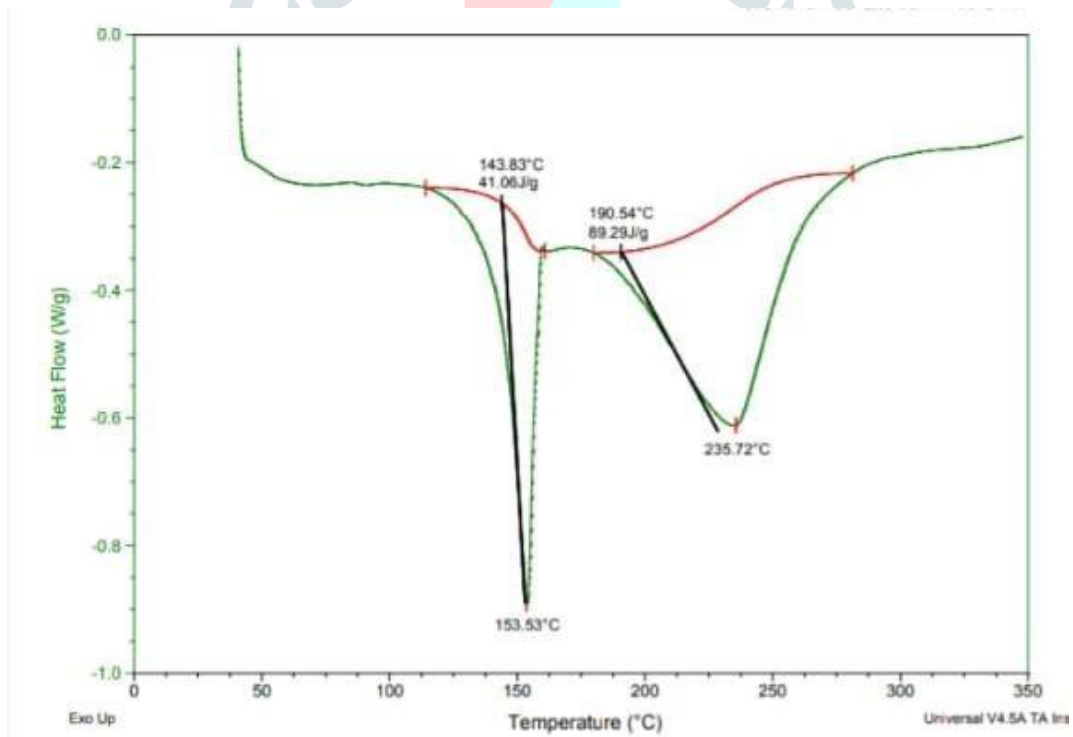
Figure 7: DSC of pure SA

Figure 8: DSC of SA and Excipients

5.2   Formulation salicylic acid-loaded microemulsion

5.2.1   Confirmation of microemulsion:

Discussion: Initially, 9 formulation of microemulsion was created using ethanol as a cosurfactant because short-chain alcohols stimulate microemulsion formation. The pseudoternary phase diagram of a microemulsion composed of tween 80 as surfactant (S), and oil dissolved in aqueous phase is shown in Figure no 9. Figure 9's shaded zone represents transparent microemulsions, while the rest of the region represents conventional or turbid emulsions. Microemulsion (ME) pseudo-ternary phase diagrams were created using the water titration method at room temperature, at a certain mixture of different ratios were altered as aqueous phase was added to each surfactant and cosurfactants combination drop by drop, with gentle agitation. Typically, after the clear mixture became turbid at a specific point (beginning of phase inversion region), the turbid mixture became clear (beginning of O/W ME area) and then turbid again (end of O/W ME area) with continual water addition. The experiment was repeated for each combination (1, 2 and 3 time). Based on these findings, optimum concentrations of oil, surfactant, and cosurfactant were chosen and used in the manufacture of microemulsions (MEs) containing salicylic acid. During the titration, the samples were continuously agitated for a sufficient period of time to homogenise them, and the end product was visually monitored against a dark backdrop by illuminating the samples with white light. After 24 hours of equilibration at room temperature, the mixes were visually examined and classified as ME, crude emulsions, or ME gels. The spontaneity of ME formation was tested by adding a known amount of water all at once to a known amount of oil, surfactant, and cosurfactant with controlled stirring. The ease with which clear ME might be formed was chosen as the spontaneity criterion.
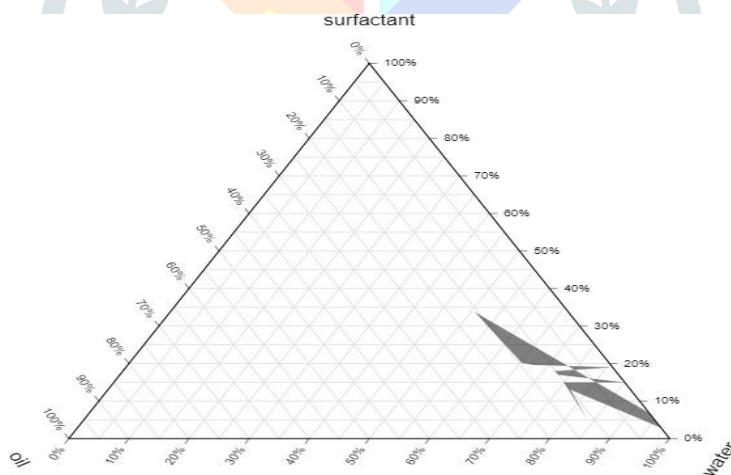

Figure No 9: Pseudo ternary phase diagram of microemulsion

5.3   EVALUATION OF SALICYLIC ACID LOADED MICROEMULSION

5.3.1   Physical stability studies

5.3.1.1   Visual, Centrifugation and Stress test

Microemulsions are confirmed to be thermodynamically stable systems that form at a specific concentration of oil, surfactant, and water and when it exhibits no phase separation, creaming, or cracking.

Discussion: Different stress stability tests, including as heating and cooling cycles, centrifugation, and freeze-thaw cycles, were performed on selected formulations from the phase diagram. Some formulations were turbid during physical stability tests, and some phase separation occurred. One cause of this instability in microemulsions could be ostwald ripening, which occurs when molecules move as a monomer and coalescence of small droplets occurs, leading in the production of large droplets through diffusion processes driven by surface free energy gain. Another reason could be that when temperature is quenched during a stress stability investigation, the microemulsion becomes unstable due to oil phase separation, and droplet distribution of smaller size is encouraged by the change in curvature free energy. The results of the physical investigation of the selected 8 formulations M1, M2, M3, M4, M5, M6, M7, M8 and M9 are listed the table no 11 and figure 10 showed that only M9 formulation of procedure 3 demonstrated no phase separation, creaming, cracking, coalescence, or phase inversion during centrifuge and stress stability testing, hence it was found to be more stable as compared to other formulations chosen for future research.

Table 11: Physical stability studies of microemulsion

Formulation code	Heating cooling cycles	Freeze thawcycles	Centrifugation studies	Appearance
M1	X	X	x	Crude
M2	X	▯	x	Turbid
M3	▯	▯	X	Turbid
M4	▯	X	▯	Clear
M5	▯	▯	X	Clear amber
M6	▯	X	▯	Clear amber
M7	▯	X	▯	Clear amber
M8	▯	▯	▯	Clear amber
M9	▯	▯	▯	Clear amber

x = fail,   ▯ = pass

M1M2   M3 M4   M5   M6   M7
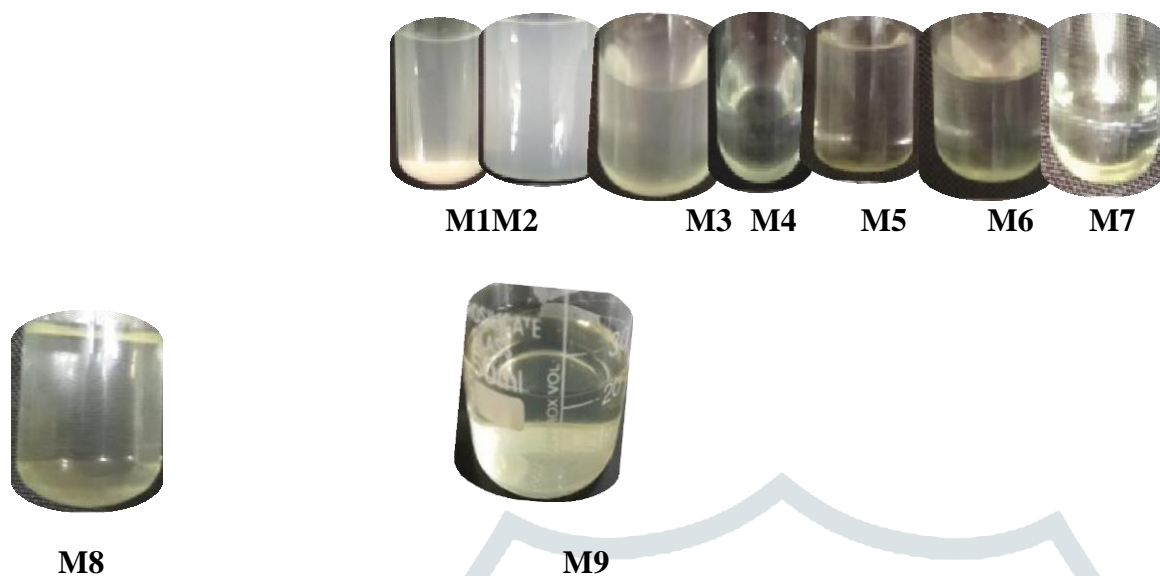

M8   M9

Figure No 10. Image of microemulsion gel from M1 to M9 Physicochemical parameters of salicylic acid loaded microemulsion

5.3.2   Particle size and distribution analysis: The average size and polydispersity index of the microemulsion droplets were determined by Delsa^{TM} Nano C analyser (Beckham Coulter).

Discussion: The droplets size of microemulsions was found to be 91 nm. The polydispersity index showed that the microemulsions had narrow size distribution. The average particle size and polydispersity index of the formulation M9 were found to be 91nm and 0.232 nm respectively see figure 11

5.3.3   Zeta potential: Zeta potential of the optimized formulation M9 was determined using particle size analyzer.

Discussion: Zeta potential of optimized formulation was found to be -0.43mV. This does not matter as in the cased of low concentration of non-ionic surfactant and cosurfactant used in this formulation. Low zeta potential is expected but did not in any way affect the stability of the optimized formulation.
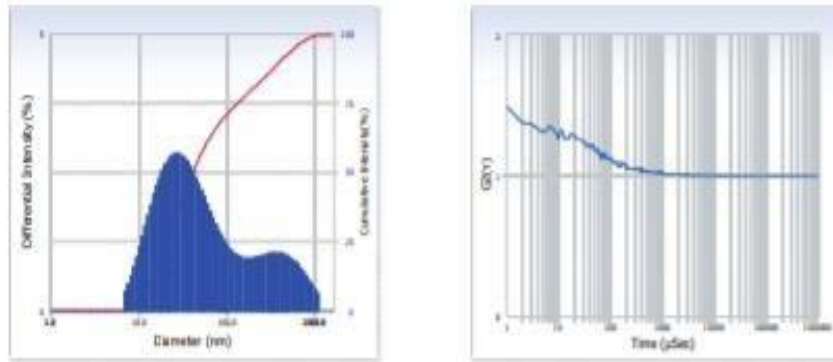
5.3.4   Refractive index, pH, conductivity, and viscosity of microemulsion: Refractive index is the net value of the components of microemulsion and indicates isotropic nature of formulation. Refractive index, Polydispersity index, Conductivity, Viscosity and diffusion

constant of microe mulsion was determined along with same particle and zeta sizer (BECKAM COULTLER Delsa Nano, New Delhi, India) at 25 ± 0.5 °C.

Discussion: Viscosity of the microemulsion (M9) formulation was very low as expected for o/w emulsion. The low viscosity may be due to presence of low amount of Tween 80 nonionic surfactant and non-ionic cosurfactant and also the low concentration of lipid. The mean value of the refractive index for the formulation M9 was found to be 1.330.The results of all these parameters are given in the table no 12.

Table No 12: Evaluation parameters of the optimized microemulsion.

S.No	Parameters	M9 Formulation
1	Particle size (nm)	91.4
2	Polydispersity index	0.232
3	Zeta potential (mV)	-0.42
4	Conductivity (pS/cm)	0.6931
5	Refractive index	1.3300
6	Dielectric constant	78.3
7	pH	3.7
8	Viscosity (cp)	0.8800

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	43.2	34.5
2	443.3	231.4
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	133.0	202.0
Residual :	1.904e-002	(O.K)

Cumulants Results

Diameter (d)	: 91.4	(nm)
Polydispersity Index (P.I.)	: 0.232	
Diffusion Const. (D)	: 5.431e-008	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: N W	
Refractive Index	: 1.3300	
Viscosity	: 0.8800	(cP)
Scattering Intensity	: 5913	(cps)
Attenuator 1	: 100.0	(%)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
1.0	0.0	0.0	6.4	0.0	0.0	41.5	2.5	50.3	267.5	1.0	84.4
1.1	0.0	0.0	6.9	0.4	0.4	44.7	2.4	52.7	288.2	1.0	85.4
1.2	0.0	0.0	7.5	0.5	0.8	48.2	2.3	55.0	310.5	1.0	86.4
1.3	0.0	0.0	8.1	0.6	1.5	51.9	2.2	57.2	334.5	1.0	87.5
1.3	0.0	0.0	8.7	0.8	2.2	55.9	2.0	59.2	360.4	1.0	88.5
1.5	0.0	0.0	9.4	1.0	3.2	60.3	1.9	61.1	388.3	1.0	89.6
1.6	0.0	0.0	10.1	1.2	4.4	64.9	1.8	62.9	418.4	1.0	90.6
1.7	0.0	0.0	10.9	1.3	5.7	70.0	1.7	64.6	450.7	1.0	91.6
1.8	0.0	0.0	11.7	1.5	7.2	75.4	1.6	66.2	485.6	1.0	92.6
2.0	0.0	0.0	12.6	1.7	9.0	81.2	1.5	67.6	523.2	1.0	93.6
2.1	0.0	0.0	13.6	1.9	10.9	87.5	1.4	69.0	563.7	0.9	94.5
2.3	0.0	0.0	14.6	2.1	13.0	94.2	1.3	70.3	607.3	0.9	95.4
2.4	0.0	0.0	15.8	2.3	15.2	101.5	1.2	71.4	654.2	0.8	96.2
2.6	0.0	0.0	17.0	2.4	17.7	109.4	1.1	72.6	704.9	0.8	97.0
2.8	0.0	0.0	18.3	2.5	20.2	117.9	1.1	73.6	759.4	0.7	97.7
3.1	0.0	0.0	19.7	2.6	22.8	127.0	1.0	74.7	818.2	0.6	98.3
3.3	0.0	0.0	21.2	2.7	25.6	136.8	1.0	75.6	881.5	0.5	98.8
3.5	0.0	0.0	22.9	2.8	28.4	147.4	1.0	76.6	949.7	0.5	99.3
3.8	0.0	0.0	24.6	2.8	31.2	158.8	1.0	77.6	1023.1	0.4	99.7
4.1	0.0	0.0	26.5	2.8	34.0	171.1	0.9	78.5	1102.3	0.3	100.0
4.4	0.0	0.0	28.6	2.8	36.9	184.3	0.9	79.5	1187.6	0.0	100.0
4.8	0.0	0.0	30.8	2.8	39.7	198.6	1.0	80.4	1279.5	0.0	100.0
5.2	0.0	0.0	33.2	2.8	42.5	213.9	1.0	81.4	1378.4	0.0	100.0
5.6	0.0	0.0	35.8	2.7	45.1	230.5	1.0	82.4	1485.1	0.0	100.0

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
6.0	0.0	0.0	38.5	2.6	47.8	248.3	1.0	83.3	1600.0	0.0	100.0
D (10%) :	13.10	(nm)	D (50%) :	41.20	(nm)	D (90%) :	401.00	(nm)			

Figure 11: Particle size analyses and distribution of M9

5.3.5 Morphological analysis: Microscopic Evaluation of all formulations was viewed under the optical microscope a 40× objective.

Discussion: The microscopic pictures Figures 12 below shows the needle-shaped crystal image of pure salicylic acid and figure showed spherical shape of microemulsion and that salicylic acid was completely dissolved in in the optimized microemulsion as no particles of salicylic acid was visible, under the microscope for the microemulsion formulation and in the microemulsion gel as well figure no 14 and 15.

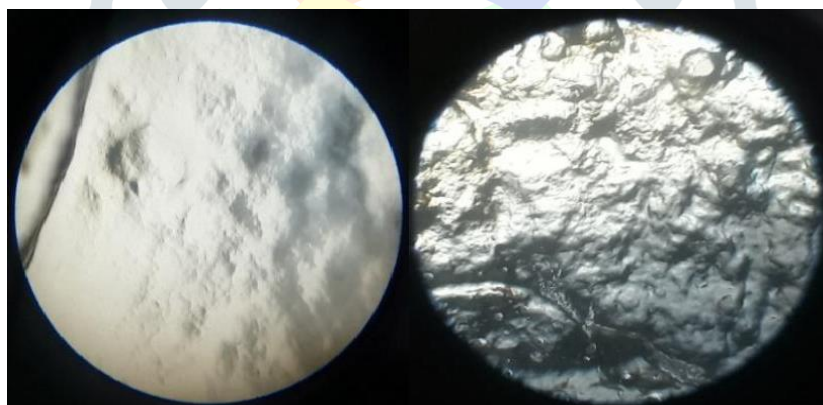**Figure 12: Image of pure SA**   **Figure 13 Image of SA ME (M9)**

Figure 14: G1   **Figure 15   G5**

5.3.6   Drug content determination of salicylic acid Microemulsion

Discussion: The drug content is the most significant factor in microemulsion formulation, the drug content of optimized formulation of microemulsion M9 was determined by UV spectrophotometry after appropriate dilution the results was calculated using slope of

calibration curve and results are satisfactory. The drug content was high and was discovered to be 99.82% indicating that the formulation has a good capacity to retain the medication see table no.13

Table No 13. % drug content of salicylic acid-loaded microemulsion M9

S/no.	Formulation code	% Drug content
1	M9	99.82±0.02

Mean ± SD; n=3

5.3.7 *In-vitro* drug release study of microemulsion

Discussion: The *in-vitro* release of the optimized microemulsion M9 was determined in time interval of 0, to 9hr the absorbance was measure and slope of calibration curve was used for the calculation of cumulative percent drug release, drug content of the microemulsion was found to be completely release within 9hr releasing 99.22%. Results of the in vitro release in each time intervals are mentioned in table no 14.

Table No 14. Microemulsion *in-vitro* release

S/no	Time (hr)	Cumulative % drug release of M9 formulation
1	0	0.00
2	0.25	10.76±0.002
3	0.5	18.74±0.002
4	1	27.30±0.002
5	2	37.42±0.021
6	4	55.44±0.022
7	6	73.48±0.024
8	8	90.82±0.023
9	9	99.22±0.021

Mean ± SD; n=3

5.4 Evaluation of Salicylic Acid loaded microemulsion gel

5.4.1 Physical evaluation of microemulsion gel

Texture: The prepared microemulsion gel shows elegant appearance.

Consistency: The prepared formulations consistency was found to be smooth.

Discusion: The gel compositions based on microemulsions have a viscous look. G1 to G3 were discovered to be white/milky, whereas G4, G5 and G6 were found to be transparent/off white. No phase separations were observed. All formulas were stable and consistent. The consistency of the formulation G1, G2 was found to be lower than that of the other formulations. The consistency of the formulations G4-G46 was found to be greater than that of the formulations G1-G3. Because of the increased concentration of carbopol 940 in the G4-G6 batch compared to the G1-G3 batch, the consistency of G5 was found to be higher. Figure 16-21 and table no 15 displays the outcomes.

Figure16.   **G1**   G2   G3

G4   G5   G6

Table No 15. Physical evaluation of salicylic acid-loaded microemulsion gel

Formulation code	Clarity	Odour	Phase separation	Washability	Consistency
G1	Milky	No	No	Washable	Less
G2	Milky	No	No	Washable	Less
G3	Milky	No	No	Washable	Yes
G4	Clear	No	No	Washable	Yes
G5	Clear	No	No	Washable	Yes
G6	Clear	No	No	Washable	Yes

5.4.2   Spreadability

Spreadability of the gel is a crucial factor. It would be easier to apply the formula to an affected area if the base spreads readily and exhibits the most slide and drag. A circle with a diameter that is connected to the spreadability of the gel was created when a weighed amount of gel was placed between two glass plates of known weight. The higher the diameter betters the spreadability.

Discussion: The prepared microemulgel formulation of G1 to G6 was found to spreads easily, with a spreadability of between 4.50 and 10.66 gm.cm/s. Comparing this formulation to other formulations, G5 10.66 gm.cm/s is superior to others. The information in table no 16 shows the diameter spreading of gel formulation. And figure 17 shows the chart %

Table no 16. Spreadability of microemulsion gel

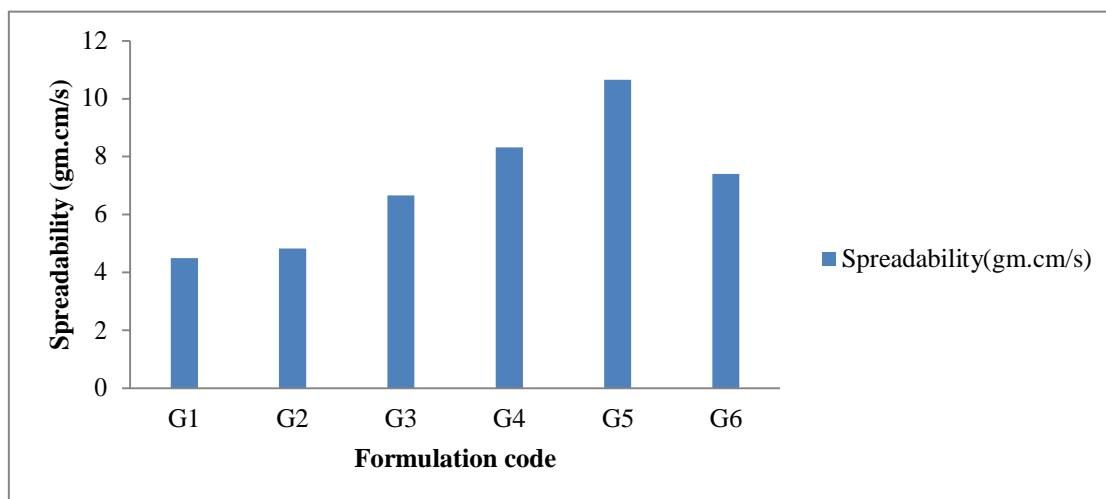| Formulation code	Spreadability(gm.cm/s)
G1	4.50±0.03
G2	4.83±0.05
G3	6.67±0.01
G4	8.33±0.04
G5	10.66±0.02
G6	7.41±0.03

Mean ± SD; n=3

Figure No 17. Spreadability of microemulsion gel

5.4.3 Extrudability Analysis:

Discussion: The microemulsion-based gel was filled in a collapsible tube and tested for extrudability. G1, G2, and G3 formulations had very high extrudability due to a low amount of carbopol 940, which was not desired for the optimum. G4 and G5 formulations, has a good extrudability while G6 was hard to extrude due to higher concentration of carbopol 940. In comparison to the other formulations, G5 possessed the requisite extrudability**.** The table no 17 shows the performance of gels formulation with respect to extrudability

Table no 17. Extrudability of microemulsion gel

Formulation code	Extrudability
G1	++
G2	++
G3	++
G4	+++
G5	+++
G6	++

+ Satisfactory, ++ Good, +++ Outstanding

5.4.4 Rheological Investigation

Discussion: For 5-10 minutes, the microemulsion-based gel was rotated with spindle no. 4 at 12rpm. Table 18 and figure 18 depict the viscosity range of the formulations. G1, G2, and G3 were less viscous, whereas G4 was quite viscous whereas G6 was more solid. As a result of the results, it was determined that the G5 formulation had the necessary viscosity when compared to other microemulsion-based gel formulations.

Table No 18. Viscosity of microemulsion gel

S.No	Formulation code	Viscosity (cps)
1	G1	4200±11
2	G2	4401±13
3	G3	4501±12
4	G4	5450±13
5	G5	5756±11
5	G6	7645±14

Mean ± SD; n=3

Figure No 18 .Viscosity of microemulsion gel

5.4.5   pH determination

Discussion: The gel formulation pH was adjusted to pH of range 7 using triethanolamine Table 21 and Figure 13 illustrate the pH of a microemulsion-based gel of G1 to G6. It was found to be between 7.1.to 7.3, which is the normal pH range of the skin. For all formulations, there was no significant change in pH values as a function of time.

Table No 19. PH range of SA microemulsion gel

S.No	Formulation code	pH
1	G1	7.12±0.01
2	G2	7.21±0.03
3	G3	7.23±0.05
4	G4	7.32±0.03
5	G5	7.15±0.04
6	G6	7.24±0.05

5.4.6   Drug content

The drug content is the most significant factor in microemulsion formulation, the drug content of microemulsion gel was determined by UV spectrophotometry after appropriate dilution the results was calculated using slope of calibration curve and results are satisfactory.

Discussion: The drug content was discovered to be G1 91.23%, G2 90.54%, G389.11%, G4 96.29%, G5 99.42% and G6 86.36% indicating that the formulation has a good capacity to retain the medication. G5 was found to have higher drug content in comparison to other gel formulation Table No.20

Table No 20. Drug content of Microemulsion gel

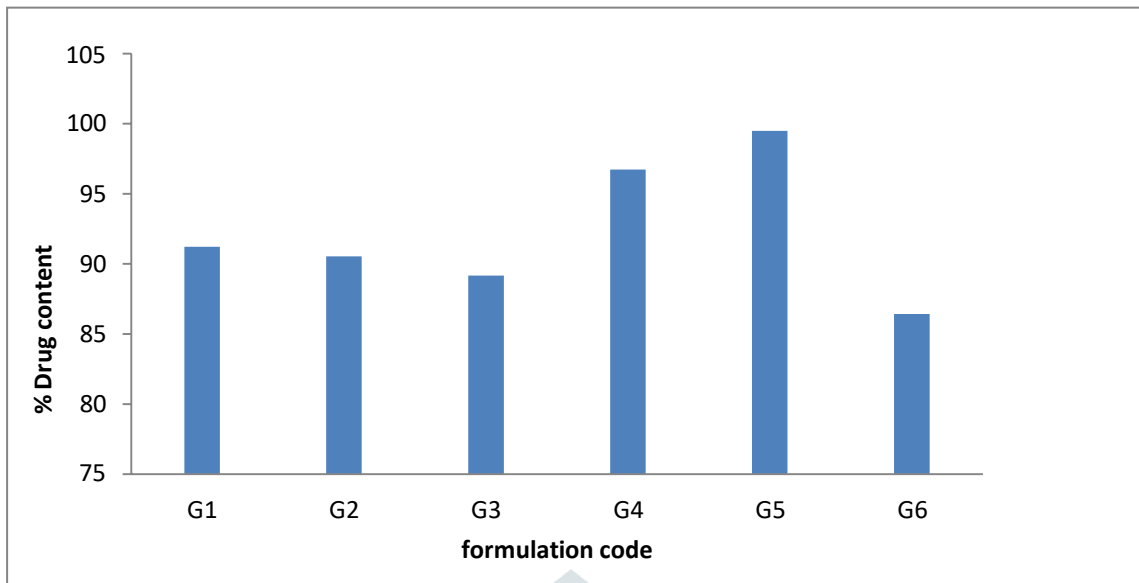| S.No	Formulation code	% Drug content
1	G1	91.23±0.002
2	G2	90.54±0.002
3	G3	89.11±0.001
4	G4	96.29±0.003
5	G5	99.42±0.002
6	G6	86.36±0.002

Mean ± SD; n=3

Figure 19: Drug content of salicylic acid microemulsion gel

5.4.7 *In-vitro* drug release study:

Discussion The concentration of the polymer affected the release of Salicylic acid from the microemulsion-based gel. The drug release from the microemulsion-based gel formulations was written in the following order: G5>G4>G1>G2>G3>G6>, with the levels of drug release after 10 hr being 96.82%, 92.73%, 91.28%, 89.36%, 86.95%, and 82.14%, respectively. The amount of drug diffused from the formulation across the diffusion membrane was proportional to the polymer concentration. Formulations having less carbopol 940, such as G1, G2, and G3, had higher release than formulations G6, which had more carbopol 940. Batch G5 formulation showed the best diffusion profile or drug release from the gel. It has been demonstrated that as polymer concentration increases, drug diffusion diminishes. Finally, the In-Vitro release investigation clearly indicated that the release of salicylic acid is dependent on the polymer concentration. Table 21 shows the cumulative % medication release of all formulations. Figure 20 depicts a graph plotting cumulative% medication release versus time.

Table No 21. Microemulsion based gel in vitro drug release

Time	cumulative % drug release of gel formulation					
	G1	G2	G3	G4	G5	G6
0.25	6.96	6.48	5.52	7.13	8.84	4.43
0.5	11.87	11.63	10.69	13.89	16.29	8.07
1	22.49	20.32	18.16	21.52	25.13	14.25
2	29.46	28.74	26.34	29.22	32.83	22.04
4	44.86	43.17	42.45	45.1	48.47	38.84
6	61.94	60.73	58.09	63.14	63.14	53.6
8	76.77	76.13	74.93	78.29	80.94	67.95
10	91.28	89.36	86.95	92.73	96.82	82.14

Figure No 20: Release study of microemulgel

5.4.8   Kinetic of Drug Release

There are many kinetic models available to illustrate the full release of the medication from dose forms. The models; zero order, first order, the korsmeyer-peppas model, and the higuchi matrix diffusion model were used to fit the *in-vitro* release of the best formulation.

Discussion: The plots below displays the results obtained for the cumulative % drug release of G5 formulation vs. time for zero order kinetic model, first order kinetic model displayed log cumulative % of drug remaining vs. time, Higuchi model displayed the cumulative % drug release vs. square root of time, while Korsmeyer-peppas displayed the log fraction drug release vs. log time figure no 21 to 24.

The significance of the derived regression coefficients served as the foundation for data interpretation. The data listed in table 22 represents the estimated regression coefficients for the zero order, first order, Higuchi matrix and Korsmeyer models. Since the plot had the maximum linearity, it was determined that the Korsmeyer peppas model best predicted the in vitro drug release of G5 formulation. The Korsmeyer peppas model's R^2 was found to be 0.9906 which is the highest value.
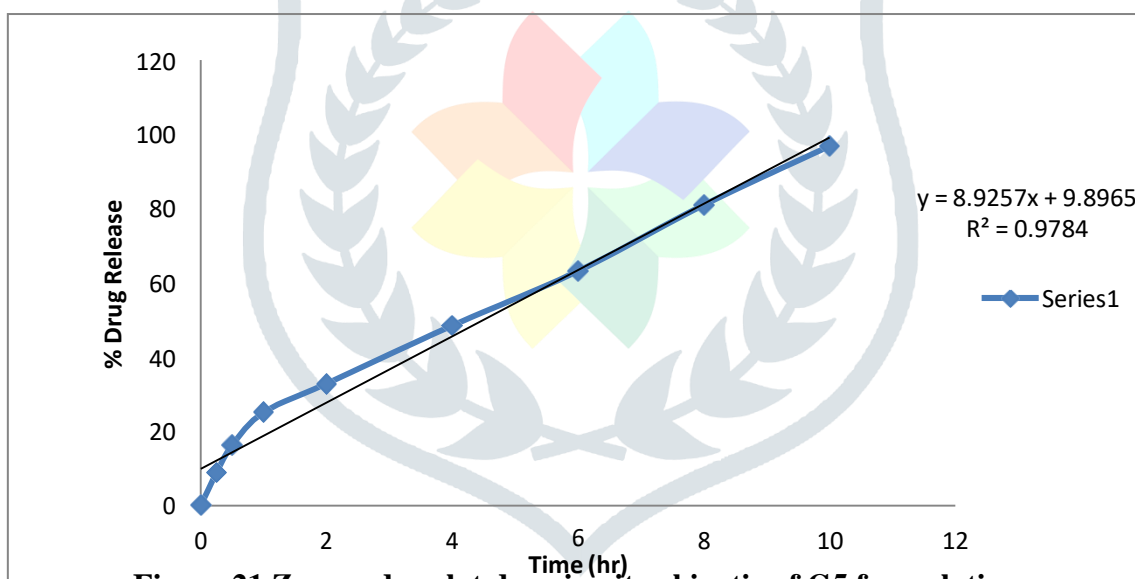

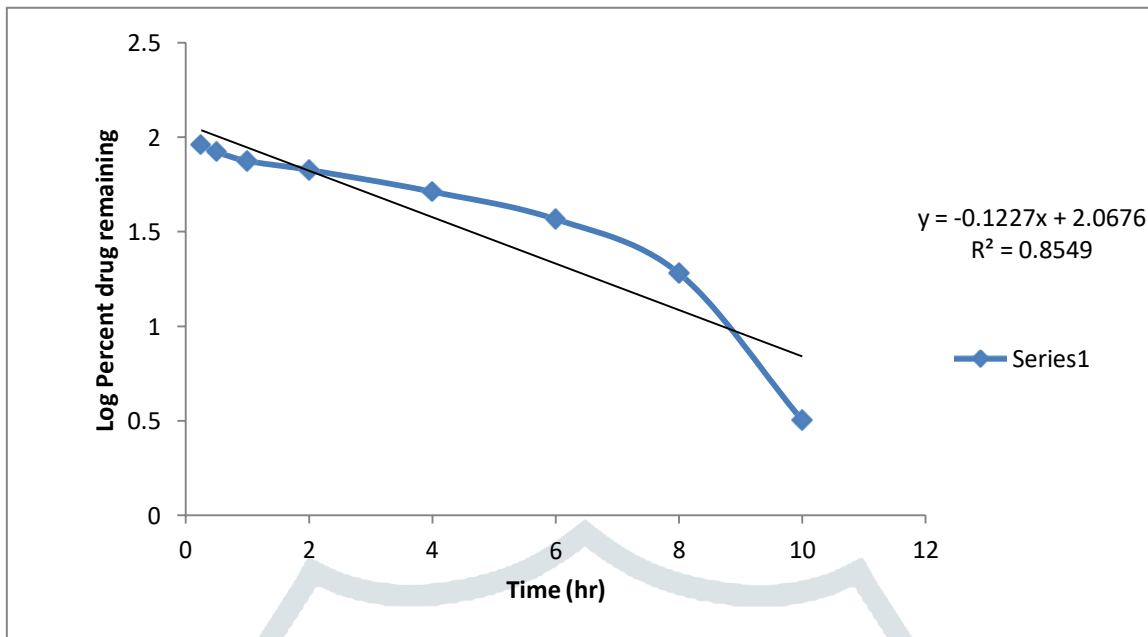**Figure 21 Zero order plot drug in vitro kinetic of G5 formulation**

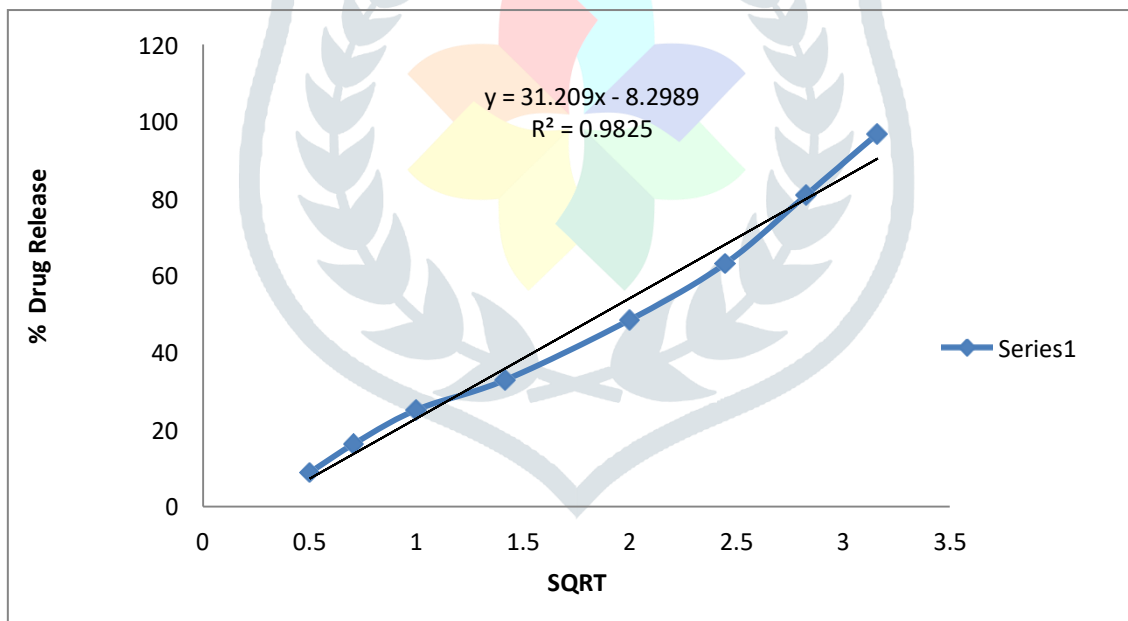**Figure No 22. First order plot drug in-vitro kinetic of G5 formulation**

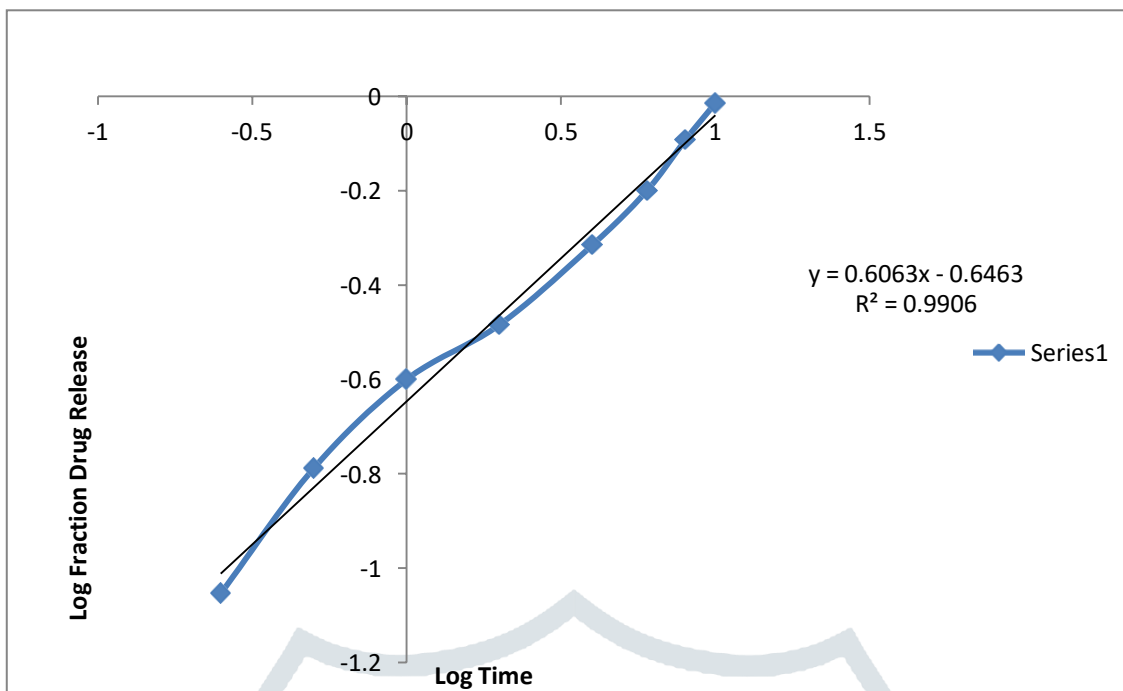**Figure 23 Higuchi matrix order plot drug in vitro kinetic of G5 formulation**

$y = 0.6063x - 0.6463$
$R^2 = 0.9906$

Figure No 24. Korsmeyer Peppas plot drug _in-vitro_ kinetic of G5 formulation

Table No 22. Kinetic equation parameter of formulation G5

Formulation	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0
SA-MBG5	0.9784	8.9257	0.8549	-0.1227	0.9825	31.209	0.9906	0.6063

5.4.9   DSC of SA microemulsion gel

Discussion: The temperature peak of the gel formulation was found to be 200ºC and heat flow was found to be15.18 j/g.
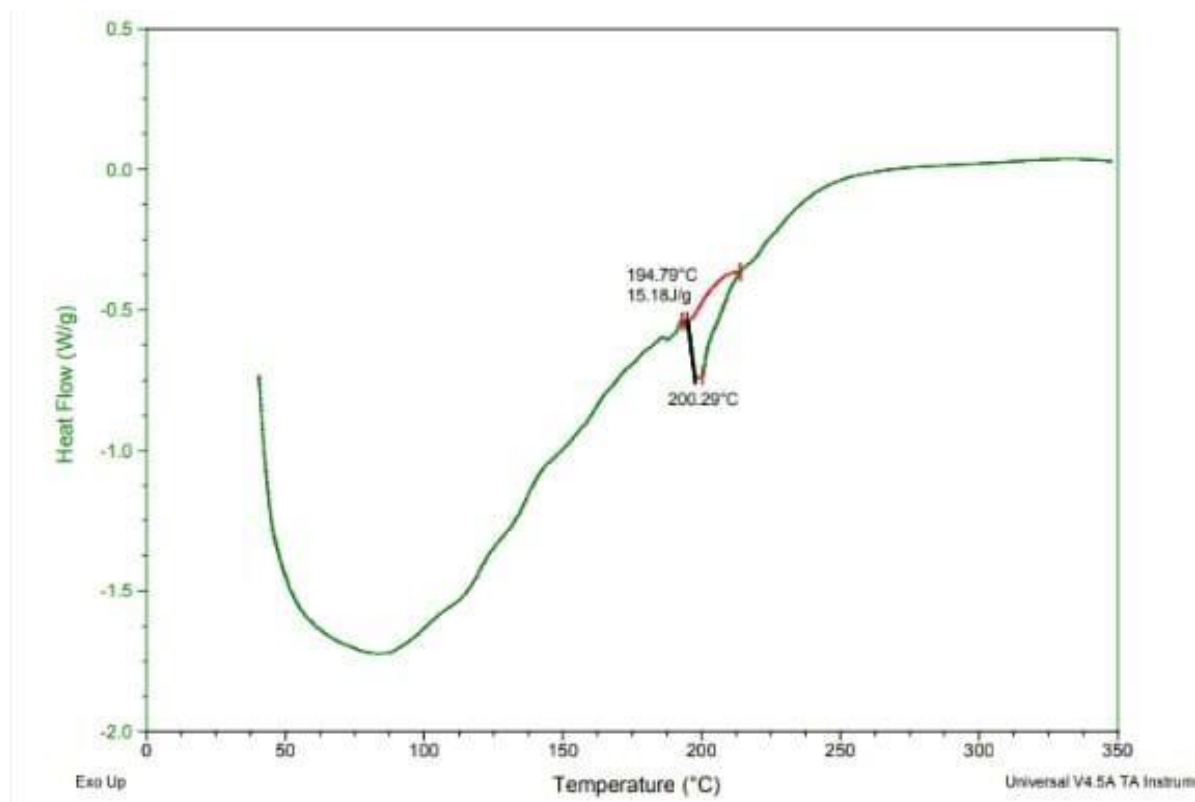

Figure 24: DSC of SA Microemulsion gel

5.4.10   Stability studies:

Discussion: The durability of microemulsion and its gel formulations was examined in regard to temperature stability and centrifugation, microemulsion was centrifuged by high speed at 11,300 x g for 60 minute. There was no phase separation, flocculation or precipitation observed for microemulsion see table no 23. Aside from physical properties, the drug content of M9 and G5 was tested at once in each month for the three-month period. During the observation period, the formulation samples were kept at room temperature in centrifuge tubes to mimic patient usage circumstances. Gel was evaluated for accelerated stability at 37°C. At regular intervals observation, there were no significant changes in the physical appearance, consistency, pH and drug content of the microemulgel formulation at room temperature storage see table no 24.

Table No 23. Stability study of selected micro-emulsion M9 formulation:

Parameters	Initial	24 hours stability studies			After 1-3 Months
		Room temperature	4^{0}C Temperature	40^{0}C temperature	
Appearance(colour)	Light yellow	Light yellow	Light yellow	Light yellow	Lightyellow
Phase separation	No	no	no	No	No
Drug content	99.22%	99.21%			97.12%

Table No 24. Stability studies of micro emulsion gel G5

Parameter	initial	After one month Room temperature	After two months Room Temperature	After three months Room Temperature
Appearance (colour)	Off white	Off white	Off white	Off white
Drug content	96.82	96.80	95.54	94.26

CHAPTER 6
SUMMARY
AND CONCLUSION

Preformulation investigations are important processes for determining the efficacy, safety and stability of a drug dosage form, this displays essential roles in formulating a clinically effective delivery system. The Preformulation studies indicated that salicylic acid is crystal white powder odourless, with acrid taste. The melting point was found to 158^0C. The λmax determination by UV spectrophotometry was measured at 276nm in PBS. The formulation chemicals were selected based on the solubility of salicylic in tween 80, ethanol, water, and propylene glycol. The excipients lecithin, tween 80, propylene glycol, water and ethanol were chosen after analysing the saturation solubility study results and the pseudo ternary phase diagram. The excipients and drug was found to be compatible following the FTIR and DSC determination. Also the excipients belong to the generally recognized as safe (GRA) under FDA regulation.

The microemulsion was prepared by titration method with total of 9 formulations M1, M2, M3, M4, M5 M6, M7, M8, and M9 was prepare using 3%, salicylic acid, 0.1% lecithin and different concentration tween 80, propylene glycol, and ethanol. The formulation M9 procedure 3 having appropriate physicochemical parameters, higher permeation parameters and a good potential to improve solubility and bioavailability of SA was considered as optimized formulation. The optimised formulation M9 was found to be more stable as compared to other formulations after centrifuge and stress testing, other formulation was less stable and shows phase separation. M9 formulation containing 3% salicylic acid, 0.1% lecithin, tween 80 and propylene glycol mixed in the ratio 1.6:1, 16% ethanol and q.s% water was selected and was evaluated for morphological, zeta potential, particle size analysis, drug content, in vitro drug release and further stability investigation.

The M9 optimized salicylic acid-loaded microemulsion formulation was used create microemulsion based gel using different concentration of carbopol 940 (1%, 1.5%, 2%, 2.5%, 3%, and 4%). The gelling agent was prepare separately using 90^0C water, 1% ethanol, 1% tween 80 and triethanolamine was used to adjust the pH to 7. The optimized M9 microemulsion was prepared for in different batches and incorporated one by one in each 6 formulations of the gel phase by dispersion method. Each formulation G1, G2 G3, G4, G5 and G6 was carefully till a semi-solid homogenous mixture was obtained it was stored in a desiccator for 48 hr for hydration

before for physical, viscosity, spreadability, extrudability, drug content, in vitro release study, and storage and stability investigation .

All the 6 formulations were found elegant, consistent, and washable and showed no phase separation G1, G2, and G3 was found to be milky, less viscous and less stable, while G4, G5 was off white, good viscosity and more stable G6 was more solid. The drug content of the gel formulation was found to be G1 91.23%, G2 90.54%, G389.11%, G4 96.29%, G5 99.42% and G6 86.36% indicating that the formulation has a good capacity to retain the medication. G5 was found to have higher drug content in comparison to other gel formulation.

The drug release from the microemulsion-based gel formulations was written in the following order: G5>G4>G1>G2>G3>G6>, with the levels of drug release after 10 hours being 96.82%, 92.73%, 91.28%, 89.36%, 86.95%, and 82.14%, respectively with G5 having maximum drug release rate in cause of 10 hours. As the result of all the investigation above with formulation G5 displaying greater results among other formulations G5 was selected as the best fit.

The cumulative % in vitro drug release data of G5 was fitted in the different kinetic model plots such as zero order, first order, Hugchi matrix and Korsmeyer peppas model, this is done in order obtain the drug release mechanism. The regression coefficient value R^2 of Korsmeyer peppas model was found to be greater than other models with the displayed R^2 of 0.9906, this is to confirm that the drug release of G5 formulation follows Korsmeyer peppas model of kinetics drug release. The formulation M9 microemulsion and G5 microemulsion gel was found to be stable after three months of storage at room temperature.

CONCLUSIONS

The objective of this research was to develop lipid-based carrier of microemulsion gel of salicylic acid to enhance drug solubility and bioavailability while maintaining controlled drug release at the targeted site with minimum or zero side effects. The excipients used have to be pharmaceutically acceptable, non-irritating, and non-sensitizing to the skin, as well as GRAS (generally considered as safe). The microemulsions were prepared by titration method, and incorporated into gel system by dispersion method.

Microemulsion of 9 formulations prepare M9 was selected as the best among others after subjecting them to physical including centrifugation, heating, cooling and freezing testing. The optimized microemulsion M9 composed of 3% salicylic acid, 0.1% lecithin as lipid/oil, tween 80 and propylene glycol in the ratio (1.6:1) as surfactant/co-surfactant as 16% ethanol

was used as co-solvent. Ethanol was used as the dissolving medium for salicylic acid due to its high solubility and penetration rate. M9 have particle size of 91.4nm, zeta potential -0.43, polydispersity index 0.232, refractive index 1.3300, pH 3.7, viscosity 0.8800 cP, drug content 99.83% and in vitro release study 99.22% at 9 hour.

The optimized microemulsion was incorporated with 6 different concentration of carbopol 940 to create gel formulas. In the cause of the evaluation of the gels G5 having better results than G1, G2, G3, G4 and G6 was selected as best formulation. G5 drug content was found to be 99.42% and in vitro drug release of 96.82 at the end of 10 hours interval.

With the outcome of the this study it is concluded that microemulsion gel is good solubilizing formulation for poorly soluble drugs which improve the solubility for better absorption/ permeability and availability of the drug on the skin. It as well minimizes side effects of the medication while sustaining and releasing the drug in a controlled manner with time.

CHAPTER 7
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