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Formulation And Evaluation Of Microemulgel Containing An Nonsteroidal Anti-Inflammatory Drug

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ABSTRACT:

Background: Capsaicin is a non-steroidal anti-inflammatory drug and used in the topical drug delivery system. It treats light pain caused by rheumatoid arthritis, muscle pulls or strains. Microemulgel is a combination of microemulsion and gel and thus is a relatively new topical drug delivery system and has following features and probable application in dermatology. This is such a feature for topical drug delivery which can allow Microemulgel to encapsulate drugs of low solubility (BCS II class) for local administration.

Objectives: The purpose of the present study was to synthesize and assess the efficiency of capsaicin loaded microemulgel for inflammation. The synthesis of microemulgel was done using phase titration, according to the pseudo ternary phase diagram. To the ME different combination of gelling agent are incorporated and studied.

Materials and Method: Capsaicin microemulsion was prepared based on the pseudo ternary phase diagram by phase titration method using various oils: Clove oil, Cinnamon oil, Turpent oil. Surfactant: Tween 80. Co-surfactant: n-Butanol. Microemulgel was prepared by incorporating microemulsion into a different gelling agent: Aerosil, chitosan. Formulations for physical appearance, pH, spreadability, in-vitro release and particle size etc are formulated and tested.

Results: The characterization and evaluation test conducted on the prepared Capsaicin Microemulsions were as follows; globule size of 31.6 nm, polydisperse index, % transmittance and drug content. The present microemulsion system is also physically stable with no phase separation observed in different concentrations. Microemulgel G1 was having higher drug content than microemulgel G2. As seen from the results, chitosan (G1) Microemulgel was optically transparent, exhibited improved spreadability, extrudability, pH and invitro release.

Conclusion: From the performed pseudo ternary phase diagrams with the help of different oils, 3 pseudo ternary phase diagrams were selected on the basis of the study of area of microemulsion for the formulation of microemulsions.

From 3 microemulsions formulation (f1, f2, f3), the latter one shown better result in all the parameters. Therefore it was concluded that among all the oil phases turpentine oil is the most suitable oil for preparation of microemulsion than clove oil and cinnamon oil. In vitro release data also corroborated that drug release from Microemulgel was influenced by type of gelling agents and it was decided that chitosan 3.5% was the most efficient gelling agent.

Key words: Microemulsion, Microemulgel, capsaicin, pseudo ternary diagram, Aerosil, chitosan.

INTRODUCTION:

Topical drug delivery systems (TDDS) are systems which deliver the drug to the particular site of action & do not require the drug to pass through the first-pass metabolism in the liver. This method comprises several application sites including skin, eyes, rectum as well as vaginal mucosa in which drugs can penetrate directly into the tissues. TDDS has a better advantage over systemic delivery since it provides localized effects, low side effects, and high bio availability(1).

The skin is one of the largest organs by which to deliver drugs knowing that it offers a protective membrane as well as the possibility of uptake of administered compounds. Structurally, the skin is composed of 3 main layers,

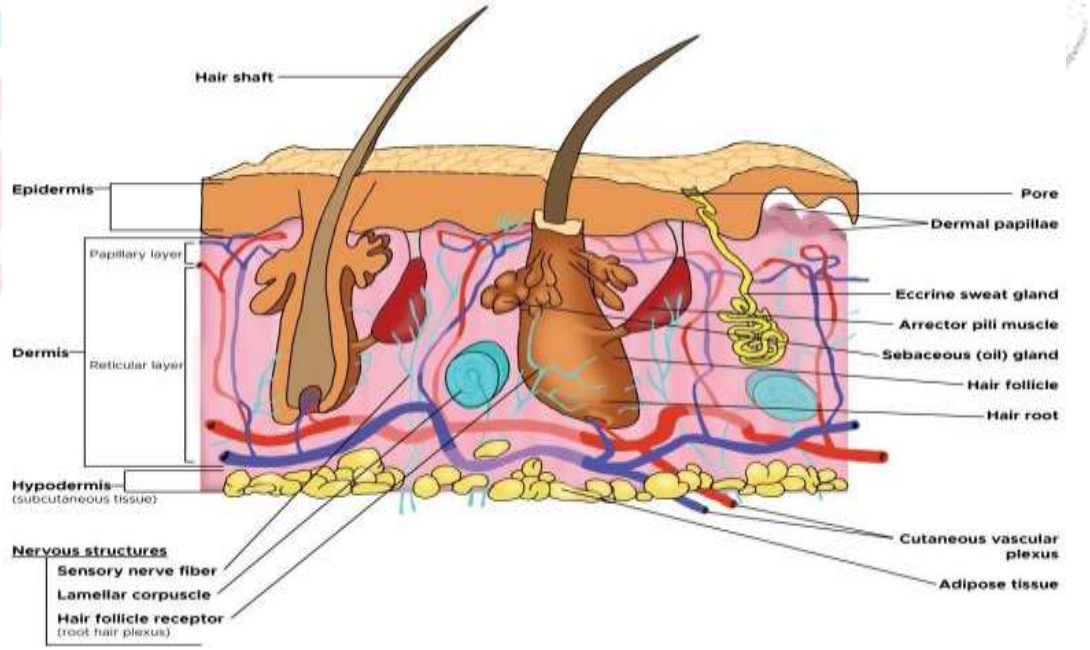

Fig 1: Cross-section of the skin

Epidermis: It has several sub layers among which are the outermost layer Epidermis is the outermost layer of the skin. It doesn't contain any veins and capillaries. Epidermis is composed 4 of sublayers: stratum corneum, stratum granulostum, stratum spinosum, stratum basale.

Stratum Corneum: A layer one cell layer thick pigmented, flat, dead keratinocytes that do not allow water loss and also act as a barrier. This layer is dead keratin cells arranged in a structure that is comprised of a lipid base containing ceramides, fatty acids and cholesterol. To improve skin barrier function, natural moisturizing factors of skin like Sodium PCA, and other Sphingolipids are also incorporated(2,3)

Stratum Granulosum and Stratum Spinosum: These intermediate layers consist of cells called keratinocytes, which itself transform to more outer keratinized form as they reach outward. This process called cornification leads to a strengthening of the skin barrier.

Stratum Basale: The final list contains its following layers that generate new keratinocytes, host melanocytes that synthesize melanin and provide UV shield (4)

Dermis: Present under the epidermis, dermis is another layer of skin densely surrounded by collagen and elastin fibers making the skin to be strong and stretchy. Blood vessels, nerve endings, and sweat glands, including eccrine, apocrine, and sebaceous glands, which shape thermoregulatory processes, and nutrients supply, are located in the dermis as well(50)

Subcutis (Hypodermis): Superficial fascia which consists of adipose tissue provides protection and insulation to the organism. Its thickness depends with the place and the density of the person's body mass and offers limited structural support to the structures below it(6).

All these layered structures have unique functions in targeted delivery of drugs in the topical system. Topical drug molecules can either be absorbed through the stratum corneum, hair follicles or sweat glands. However, drugs are not easily permeable through the skin; most, if not all, experience a barrier in the lipid-rich SC, which allows their diffusion only in the outermost regions of the skin without the aid of formulation enhancement techniques(7)

Emulsions: Emulsion is a drug prepared from oil and water, where the two phases are stabilized by surfactants. Concerning the nature and distribution, the emulsions formed by the surfactants can be of two types: oil in water emulsions with lower HLB values (O/W), or water dispersed in oil emulsions with higher HLB values (W/O). Emulsions are preferred for topical application because the system admits both hydrophilic and lipophilic drugs (8, 9)

Emulgels: Emulgels are the drugs where the emulsion is incorporated in a gel matrix. These factors further improve the coalescing properties to achieve a smooth spreadable and stable formation and finally offers non greasy feel to the applied drug. Emulgels can also contain gelling agents to enhance cohesiveness and stability in subdermal use making emulgels better than emulsions in dermatological utility (10)

Micro emulsions: Micro emulsions have been described as clear, thermodynamically stable and isotropic entities composed of surfactants, oil, water & co-surfactants. Relative to conventional emulsions, micro emulsions offer higher stability, better drug solubilisation, and improved skin permeation that are particularly desirable for lipophilic drugs with poor water solubility. Micro emulsions are not as sensitive to some instability factors like Ostwald ripening that affects Nano- emulsions (11)

Basically, Capsaicin, the chemical compound that causes the hot sensation when one takes chillies is well studied for its pain relieving, anti-inflammatory and potential anti-cancer activity. Capsaicin derives its effects from the interaction of the compound with the TRPV1 channel located on the terminal of the sensory neuron causing temporary analgesia.12 Due to its lipophilic property and a short half-life, capsaicin iontophoresis requires formulation modifications for dermal administration. The solubility and stability of capsaicin are often improved by micro emulsions or microemulgels in order to achieve a better take through skin and longer acting release on site (13)

Capsaicin is known to act as a high affinity selective activator of the TRPV1 receptor. Other substances in the capsaicin family also trigger the 'hotness' through the TRPV1 receptor. Even so some biological activities of capsaicin such as anti-neoplastic activity, cardio protective activity do not work through the TRPV1 receptor.

Advantages (17, 18)

1. Lipophilic drugs are relatively freely soluble.

2. Best system for enhancing the rate of absorption as and the degree of bio availability by minimizing interferences.

3. Micro emulsion may be advantageous in preparing the controlled & sustained release drug system.

4. It decrease the first pass metabolism.

5. Route of administration: oral, parenteral and topical routes for drug administration.

6. Appears to be more appropriate for long-term application since microemulsion is thermodynamically more favorable than the conventional system.

Disadvantages (19, 20)

1. Expensive, if the usage of the excess amount of the surfactant and cosurfactant requires additional use.

2. Since the surfactant is a surface-active material, higher concentration of the same may cause various degrees of mucosal toxicities.

Materials & methods

Materials

Capsaicin was obtained as a free API from Formosa clababies chemicals Chennai, Aerosil 200 was obtained from sunglow pharmaceuticals Puducherry India, n-Butanol obtained from l S d fine-chem limited, Tween 80 obtained from Thomas baker, Clove oil obtained from Yucca enterprises Cinnamon oil obtained from Yucca enterprises, Turpentine oil obtained from Karnataka fine chemand, Chitosan obtained from Loba chemie PVT. LTD. all this chemicals were used without any further chemical modification.

Preformulating studies

Before the drug substance is converted into a dosage form it is required by law that it undergoes chemical and physical testing. This information will be provided by pre-formulation studies so will go a long way to provide the much needed structure for the drug combination with other excipients in the formation of a formulation.

Identification Studies:

1. **Organoleptic properties of Drug:**

   The drug sample identified as Capsaicin was described by its Colour, odour, Taste and appearance.

2. **Determination of melting point:**
   The Open Capillary method was used to determine the melting point of the sample. A small quantity of powdered drug was inserted into the thin capillary tube and closed at one end by melting. Then the capillary was introduced into melting point apparatus. After certain time, at definite temperature, drugs were melted that the temperature was the melting point of drug. The data collected also shows that the melting point of Capsaicin was 62˚ C - 65˚ C (144˚ to 149˚ F).
3. **Solubility analysis:**
   5mg of about 5mg Capsaicin was dissolved in 10ml of different solvent and then it was sonicated for 10minutes, then checked for solubility and compared with standard.

4. **Fourier transform infrared radiation (FTIR)**

Other compatible studies of the drug with the selected polymers were done using the FTIR spectrometer. FTIR spectrometer 1% w/v of the sample was mixed with 3 ml of dried potassium bromide and then pressed into clear smooth pellets. The obtained pellets are then subjected to IR region analysis from 4000 cm-1 to 400cm-1.

5. **Standard calibration curve**

A standard curve was prepared for Capsaicin to obtain the equation needed to determine the penetrated concentration of Capsaicin invitro permeation study. A standard stock solution was prepared by dissolving 10 mg of drug in a 10mL of PBS: M (70: 30 v/v) solution. From the stock solution, taken 1 mL and diluted with a 10 mL of PBS: A and about 36.25 µg of M solution (concentration of the solution was 100 µg /mL). Further, pipette out the 1 mL from the above solution and diluted into PBS: A discriminative stimulus was established for the M solution (concentration was 10 µg/mL). The resulting solution was analyzed using scan spectrophotometrically in wavelengths of 200 nm to 400 nm. The λmax was determined to be 280nm in the solution (Okie et al., 2008, p.21)

6. **Determination of solubility of Capsaicin in different surfactant, oils, and co surfactant.**

Clove Oil, cinnamon oil & turpent oil were used to carry out the solubility studies while n-butanol and tween 80 are used as surfactants and cosurfactant. Higher concentrations of the drug was added to a range of oils & surfactants. A mixture continued to be agitated in the Rotary Shaker for a total of 72hrs at room temperature and after which it was spun in centrifuge at 1000 revolution per minute for 20 minutes. The supernatant liquid was allowed to stand in the side-bore pipettes and an aliquot of 1ml of the filtrate was pipetted into 1000 ml methanol. All the diluted samples were analyzed at wavelength of 280nm in UV spectrophotometer. Sample soluble in various oils & surfactant and cosurfactant was determined concerning standard calibration curve of capsaicin by using methanol (22).

Construction of pseudo ternary Phase diagrams:

There are 2 ways which can be used in construction of pseudo ternary phase diagram, namely as water titration or as oil titration. In this study, titration by water was used in order to establish phase diagram of the subject solution. The ration of surfactant: cosurfactant weight (Smix) ranged from 1:1.2:1 up to 3:1 in water titration: the oil; cosurfactants & surfactants were mixed in the proportions of 9:1; 8:2; 7:3 ;6: 4; 5:5; 4:6: 3:7; 2:8; 1: After each addition of water, the samples in the vials were shaken at an intensity of 3000 rpm for 2-3 minutes, the vials containing the mixtures were left to stand at 25˚ for 30 minutes after which phase separation and transparency of the layers were checked. Any point of the mixture prepared by titration that became turbid was the end point, the titration point before it became turbid was taken as micro emulsion.

Preparation of microemulsion

Determination of preparation was based on water titration technique. Surfactants & cosurfactants are used 3:1 smix ratio used was latex of clove oil mixed with cinnamon oil; while another smix ratio used was of Latex of turpentine oil. Amount of oil, smix & water used for the preparation of microemulsion was taken from pseudo ternary phase diagram. cosurfactant and the surfactant was then stirred for 5minutes to obtain the mix of the two (Smix). Drug that was required to be included in the desired percentage of the total weight of the formulation was accurately weighed and dispersed with the oily phase and was vortexed until the drug formed clear solution. The oil mixture was calculated and added in smix mixture and vortex for 5 minutes. The water in the above mixture was added in the drop wise manner under stirring. This mixture was stirred for 30 min to obtain the required microemulsion until the solution becomes transparent.

Table 1: Formulation of capsaicin microemulsions

INGREDIENTS	F1 %	F2 %	F3 %
Capsaicin	0.4%	0.4%	0.4%
Clove oil	7.7%	_	_
Cinnamon oil	_	7%	_
Turpentine oil			7.2%
Tween 80	27.67%	31.02%	18.15%
n-butanol	9.22%	10.35%	18.15%
Water	55.4%	51.6%	56.5%
Methyl paraben	00.03%	00.03%	0.03%
Propyl paraben	00.1%	0.1%	0.1%

Preparation of capsaicin microemulgel

The gel was synthesized by dissolving the polymer with water & the solution has been stirred at 300 rpm for about 120 minutes & determines the gelling concentration of an each polymer. To the microemulsion mixture 20 various gelling concentrations were used at different concentration. Turpentine Microemulsion (F3) was then incorporated into different formulations of gel that is (G1 and G2) in the ratio of 1:3 with continuous stirring.

Table 2: Composition of Gelling concentration

Gelling agent	**Concentration**
Chitosan (G1)	3.5%
Aerosil 200(G2)	13%

CHARACTERIZATION OF CAPSAICIN MICRO EMULSIONS

1. **Morphology of micro emulsion:**

Identification of the micro emulsion was done using light microscope and photomicrographs of the sample are provided below.

2. **% Transmittance measurement:**

Using of continuous phase, micro-emulsion will be diluted to 100 times. The % transmittance of formulation was determined by diffusion coefficient using UV-visible spectrophotometer at a particular

wavelength using UV Visible spectrophotometer with continuous phase as blank. It is equal to zero for complete opaque solution and 1 when all of it is transmitted.

3. **Drug Content Determination:**
The content of the drug in order to facilitate improved distribution in the micro emulsion formulation was determined. Determination of quantity of the drug in micro emulsion was as follows micro emulsion about 1gm was dissolved in 100 ml phosphate buffer pH 7.4. Then solutions was measured with volumetric flasks and from it, dilutions are made with the buffer solution as described above. These elicited solutions were subsequently filtered using membrane filter users of about 0.45 mm and then tested spectro photographically at 280nm. Drug was first normalised and computed to using a common curve of Capsaicin.
4. **Centrifugation:**
Stability analysis of the prepared micro emulsion was determined by centrifugal test at a speed of 3,000 rpm for two hours. After the centrifugation the sample was visually analyzed for clarity and such features as phase separation or precipitation.
5. **Particle size measurement:**

Particle size analysis of the optimized batch of micro emulsion was done by nano particle analyser. The determination of particle size is accomplished using dynamic light scattering. These dynamic ranges depend on the physical characteristics of the sample and the size and microemulsion of the sample: the range is 0.3 nm to 8 µm and microemulsion is about 10 – 300 nm.

6. **In-vitro release studies:**

An in vitro drug release study was carried out using the help of diffusion cells. By using Egg membrane was interposed between receptor & donor receiving cases. First, an equivalent of 1gm micro emulsion was used in the donor compartment; phosphate buffer at pH 7.4 was used as the receptor compartment. Then, the diffusion cells was set at 37 ± 0.5 °C with continuous stirring at 100 rpm in the entire course of the experiment. At an interval of one, two, three four, five, and six hrs respectively, five ml aliquots were drawn from the receiver compartment through the side tube and analyzed through UV spectrophotometer at wavelength maximum of 280 nm.

EVALUATION OF MICROEMULGEL TOPICAL GEL

1. **Extrudability:**
The synthesised gel was packed in standard aluminum tubes it is capped & then it is sealed at the end. The weights of the aluminium tubes was notes . The tubes were placed between two glass slides & clamped, 500 g was placed over the slides and then the cap was removed. The quantity of the extruded gel was determined and weighed. The percentage of the extruded gel was calculated (>90% extrudability: Excellent, >80% extrudability: Good
2. **Spread ability** :
0.5gm of microemulgel was properly transferred and adjusted within a circle of 1cm diameter marked on a glass plate over which the 2nd plate is placed. Upper glass plate was placed below and on it a weight of 500g was put and left undisturbed for 5 min. The data illustrates that the increase in diameter. Spreadability range 1-8
3. **pH Measurement:**
The pH of the systems was determined directly by immersing the electrode of the pH meter at room temperature. The pH of the microemulgel was determined to vary between pH 5.2 and pH 6.2.
4. **Drug content:**
The gel was taken from different regions of the mixer and analyzed for the drug concentration in order to verify the distribution of the drug within the gel formulations. The drug content of the gel was determined

by accurately weighing about 1 gm of the gel and dissolving it in about 100 ml of phosphate buffer pH 7.4. This process was qualitatively transfer these solutions to volumetric flask & the desired volume required dilution was taken of the same buffer. Again, the solutions of purified by membrane filter using of pore size 0.45mm. Thereafter measure the absorbance solution at 280 nm from UV spectrophotometer. Drug content that resulted from the calibration of graphs that was capsaicin at 23.

6. In-vitro release studies:

Diffusion cell method was considered, and an experiment of in-vitro drug release was carried out. For the purpose of this experiment, egg membrane was placed between the receptor & the donor chambers. Approximately 5 ml of microemulsion gel of equal volume of 1 gm was preserved in the donor chamber, while the receptor chamber was supplemented with the phosphate buffer of pH level 7.4. The diffusion cells were maintained at 37 ± 0.5 ℃, with stirring rate at 100 rpm throughout the experiment period. Receiver compartment was withdrawn with side tube at 60mins, 120mins, 180mins, 4 hours, 5 hours and 6 hours by withdrawing five ml aliquots each time and estimated with UV spectrophotometer at a maximum wavelength of 280 nm.

7. The kinetics /release pattern of selected formulation G1 Microemulgel.

To further elaborate the mechanism of drug release kinetics of the microemulgel G1, lipophilicity coefficients were incorporated into the results, coupled with different kinetics Korsmeyer-Peppas model equations ,zero order, Higuchi model, first order, &. The actual regression coefficient was determined (25).

RESULTS

1. Preformulation studies

The preformulation studies of Capsaicin was carried out on the basis of following test.

a) Organoleptic properties of drug:

The drug was identified on the basis of organoleptic properties. Capsaicin was colourless, highly pungent, Crystalline to waxy solid compound.

b) Determination of melting point

It was determined that the melting point of Capsaicin was 62˚ C.

c) Solubility analysis

Capsaicin sample was not soluble in water, sparingly soluble in ether & very soluble in organic solvents ethanol, n-butanol, and methanol.

d) Fourier transform infrared radiation (FTIR)

The compatibility studies of drug & the polymers was established using Fourier Transform Infra-Red spectrometer. FITR spectra of capsaicin and physical mixture of capsaicin with turpentine oil, tween-80, cinnamon oil, clove oil, n-butanol chitosan & Aerosil-200 are recorded on FITR according to Table 3 below

Table 3: Interaction studies of Capsaicin with excipients through FTIR

Groups	Drug peak (cm^{-1})	Drug +cinnamon oil +tween 80 +clove oil +n-butanol +turpentine oil peaks	Drug +clove oil + cinnamon oil+ tween 80+n-butanol+chitosan + turpentine oil+ aerosol peaks (cm^{-1})	Drug +chitosan+ aerosol peaks (cm^{-1})	Observation

		(cm^{-1})			
NH Stretching	3379	3388	3401	3356	No interaction
OH Stretching	2968	2926	2927	2928	No interaction
CN Stretching	2322.07	2360	2359	2337	No interaction
C=O stretching	1653.76	1677	1677	1627	No interaction
CH Bending	1469.81	1515	1515	1457	No interaction

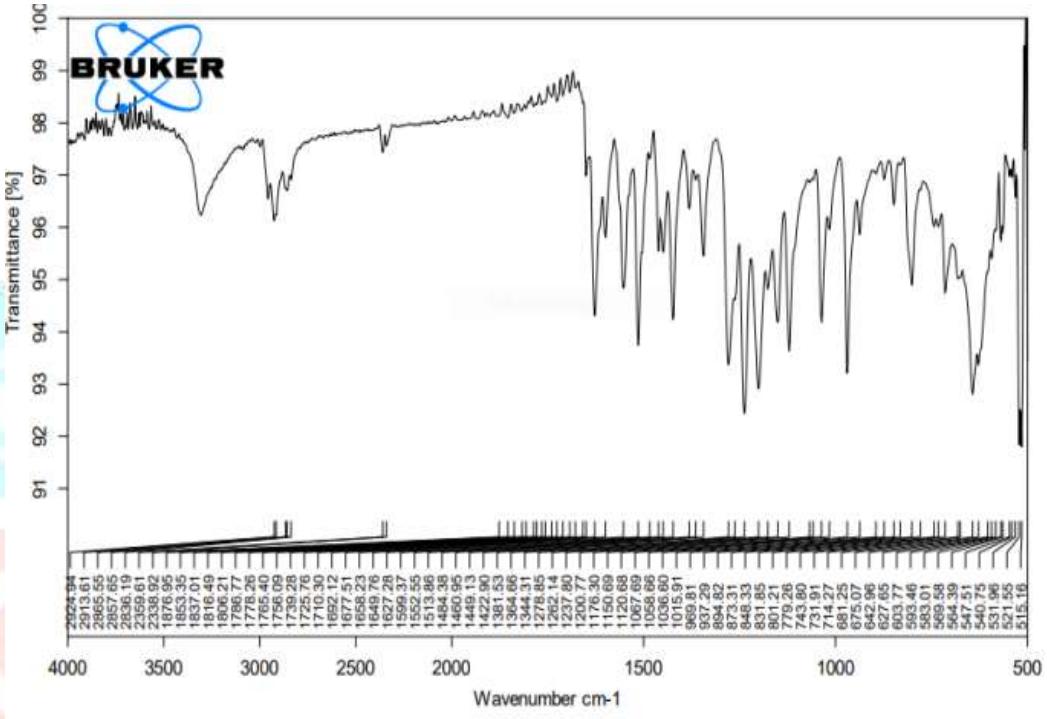

Figure 2: FTIR Spectrum of Capsaicin drug

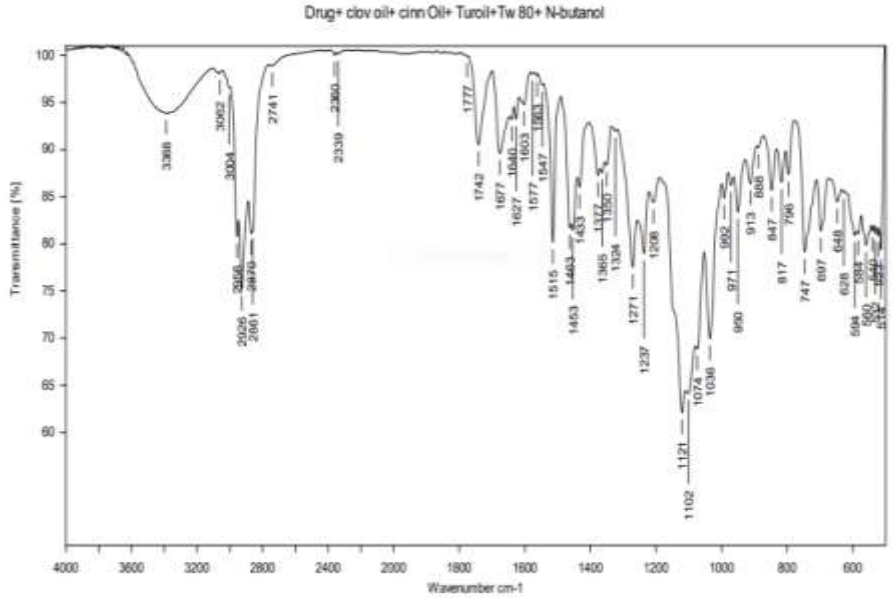

Figure3: FTIR Spectrum of capsaicin with excipients

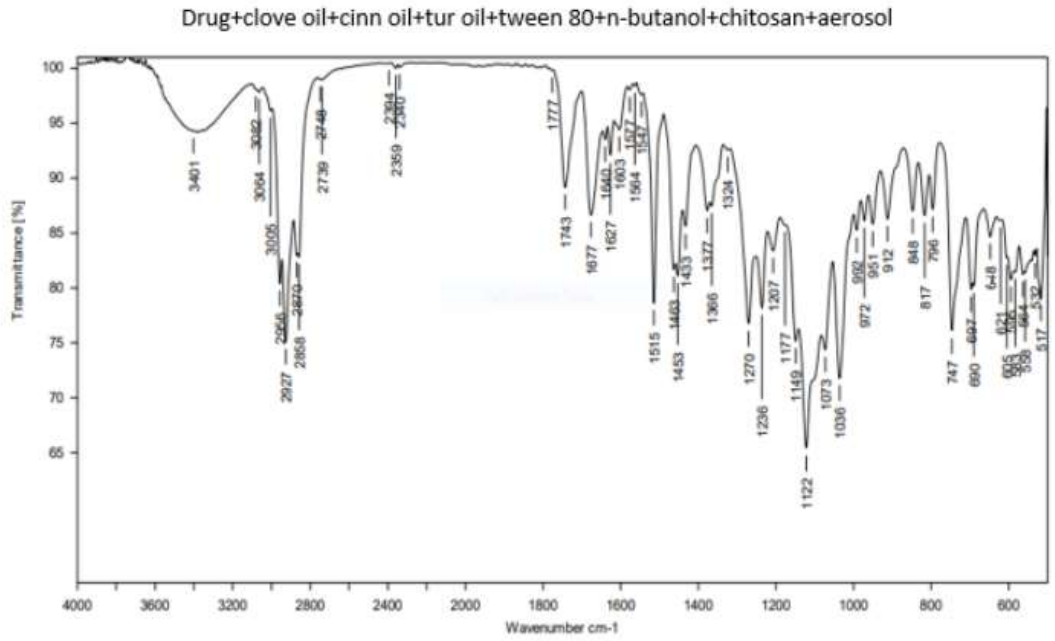

Figure 4: FTIR Spectrum of capsaicin with excipients & polymers

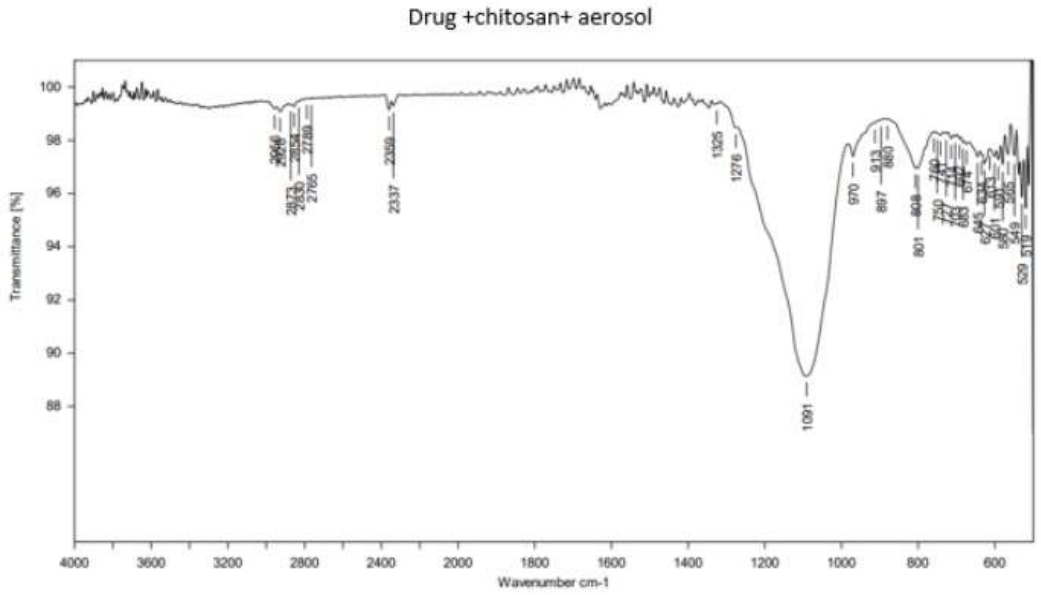

Figure 5: FTIR Spectrum of capsaicin with polymer

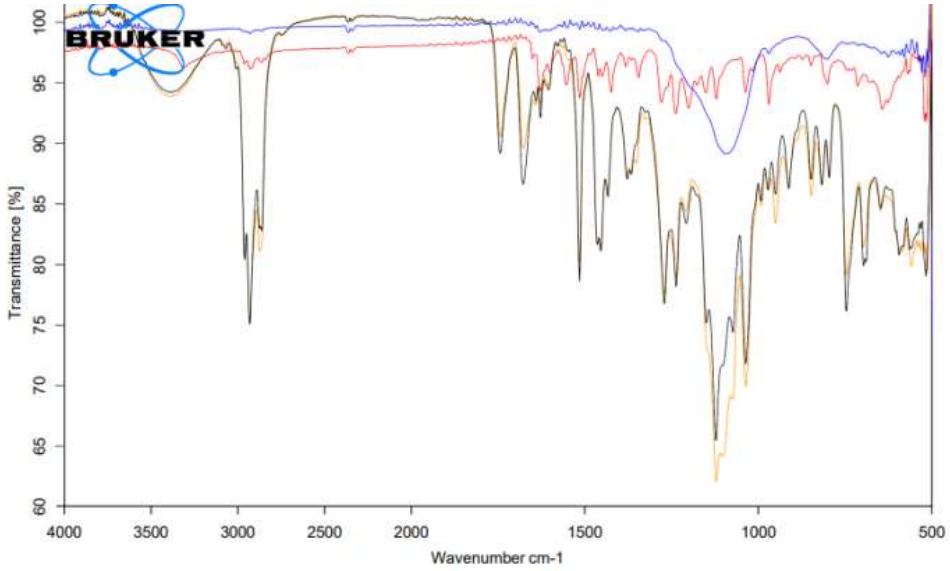

Figure 6: Comparison study of FTIR Spectrum with drug & polymers

The peaks of characteristic groups of capsaicin and similar peaks was detected in samples of capsaicin with smix and oils, capsaicin with oils, smix and polymers & capsaicin with polymers respectively and no shift in the peaks was observed suggesting no interaction of capsaicin and various excipients studied.From the above table we also infer that there was no disappearance of any characteristic peaks. smix, mixture of capsaicin with smix ,polymers, oils, mixture of capsaicin with polymers & there was no interaction between capsaicin & excipients used in the study. From the above table it is also concluded that, there was no disappearance of any characteristic peaks. This indicates that there were no interaction.

Table 4: Standard calibration of Capsaicin using methanol

Sl.no	**Concentration (µg/ml)**	**Absorbance**
0	**0**	**0**
1	2	0.208
2	4	0.4199
3	6	0.6413
4	8	0.7241
5	10	0.9808

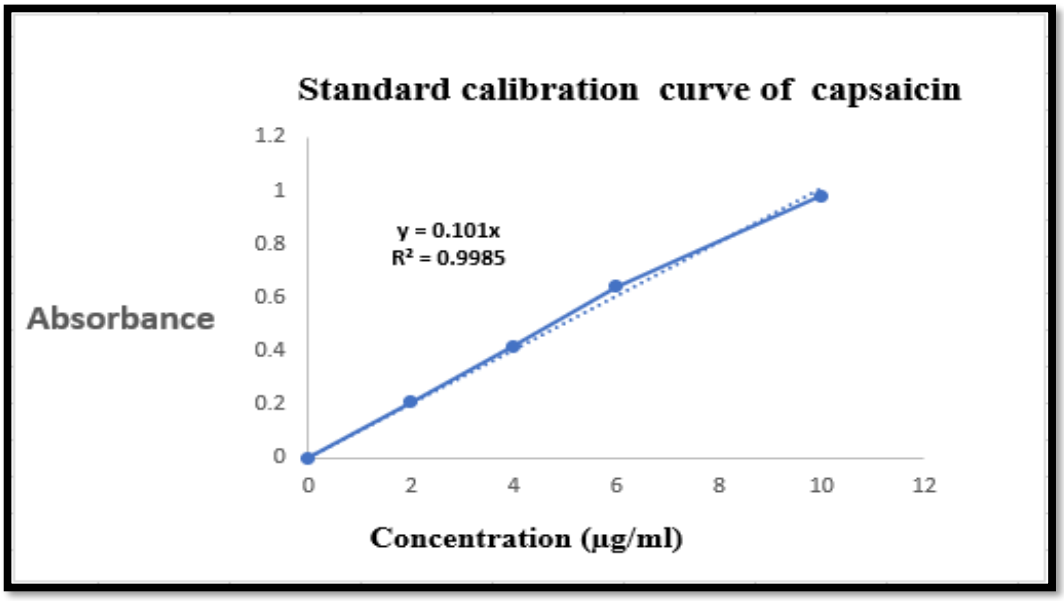

Figure 7: Calibration curve of capsaicin in phosphate buffer solution: methanol (70:30) at 280.6 nm.

The standard calibration curve of capsaicin using phosphate buffer solution: methanol (70:30),λ max was found to be 280.6nm, has good reproducibility and this method used in study. Linearity range is 1-10 µg/ml. the R^2 was found to be 0.9985 as shown as in table: 3 and fig:6

Pseudo ternary plot diagram:

In method, various oils like cinnamon oil and turpentine oil, clove oil used with different ratio of same smix tween80: n-butanol to titrate for the ternary plot diagram of the desired solvents comprising the solvent, isopropyl alcohol & n -butanol.

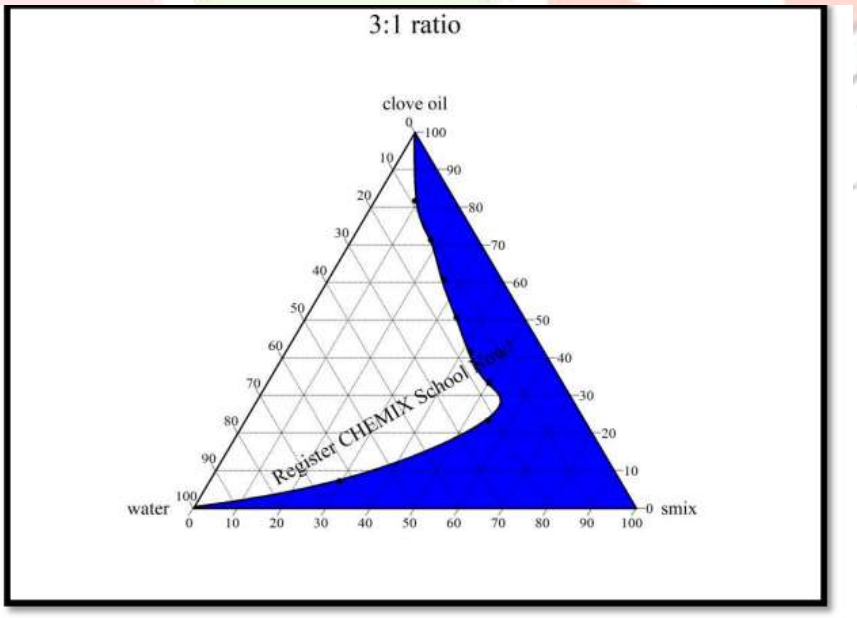

Figure 08: Oil: Clove oil, Smix: tween80: n-butanol (3:1)

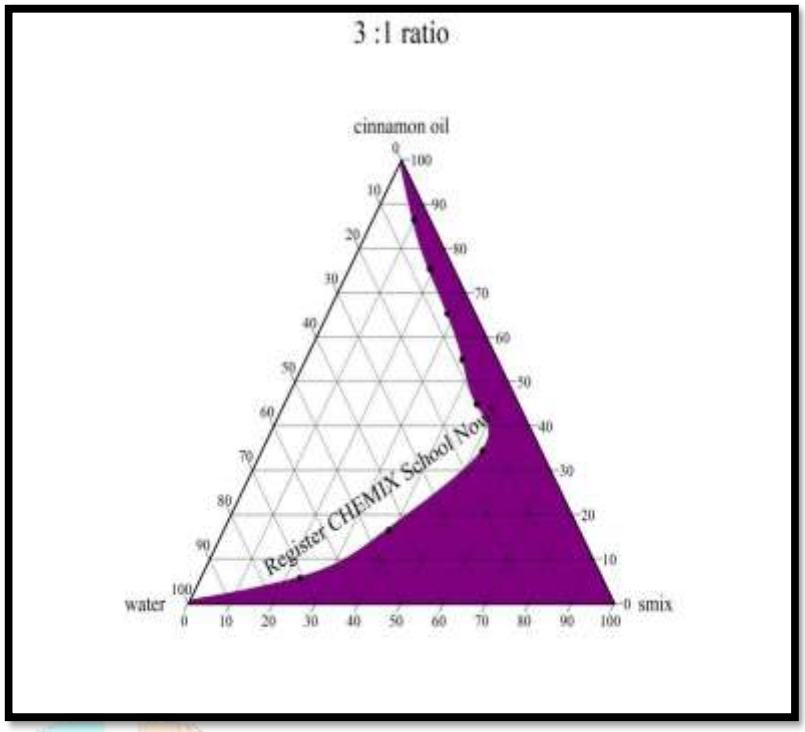

Figure 9: Oil: Cinnamon oil, Smix: tween80: n-butanol (3:1)

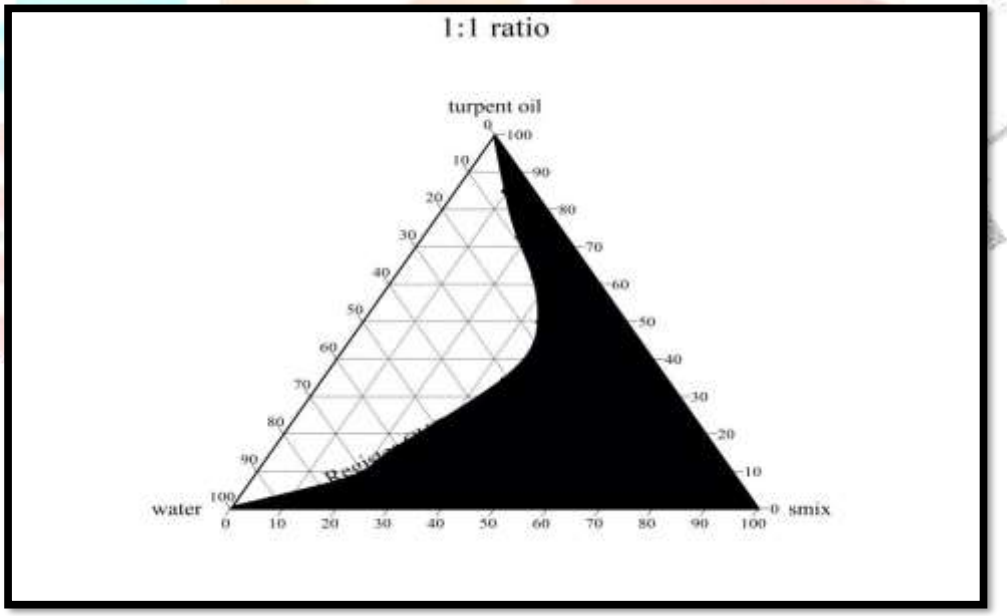

Figure 10: Oil: Turpentine oil, Smix: tween80: n-butanol (1:1)

In this pseudo ternary diagram choice of surfactant, oils & co-surfactant had been diagnosed at the bases of Capsaicin solubility. In approach, numerous oils like cinnamon oil ,clove oil & turpentine oil used with one of a kind ratio of equal smix (tween80: ethyl, isobutyl and n-butanol to have titration for ternary plot diagram. Total 11 titrations were achieved with three one-of-a-kind oils by way of converting ratio of equal smix (tween80: We titrated n-butanol with particular oils and constructed the ternary diagrams for all of the eleven titrations of the look at. Of the 15 ternary plot diagrams 3 ternary plot diagrams changed into selected for the primarily based on the bigger area of microemulsion while in comparison to the

ultimate ternary plot diagrams. Choi and Yoo pronounced that the surfactant, oils & cosurfactant had been selected using pseudo ternary plot diagrams. The larger the location, extra would be the part of microemulsion region. Clove oil with Tween80: This changed into observed to be large for Cinnamon oil with Tween80: n-butanol (3:1), Turpentine oil with Tween80: n-butanol (1:1) respectively. (Fig: These three ternary plot diagrams were carried out to get in addition Emulphor® based microemulsion formula as shown in fig 8, 9, 10.

CHARACTERIZATION OF CAPSAICIN MICROEMULSION

1) Morphology of microemulsion

Fig: 11 Morphology of F3 formulation

The microemulsion was characterized by light microscope and the photomicrographs so obtained are presented in fig. Light microscopic studies reveal that optimised microemulsion dispersion particle size was in nano-size which is in conformity with particle size analysis. The micrographs reveal that the particles are in a micro shape where there is a good dispersion of the oil internal structure as well as from each other.

2) Percentage Transmittance:

Transmittance is the relative percent of light that passes through the sample. F2; the formulation with higher_GE_ has a slightly yellowish colour than F1 and F3 formulations; consequently, most light transmittance is through F3.(Table 3)

2) Particle size measurement formula

Table 5: Evaluation of micro emulsion

Dispersion code	Z average (nm)	PDI	%Transmittance
F1	273.8 nm	1.629	69.58%
F2	-	-	67.76%
F3	31.6 nm	0.441	97.66%

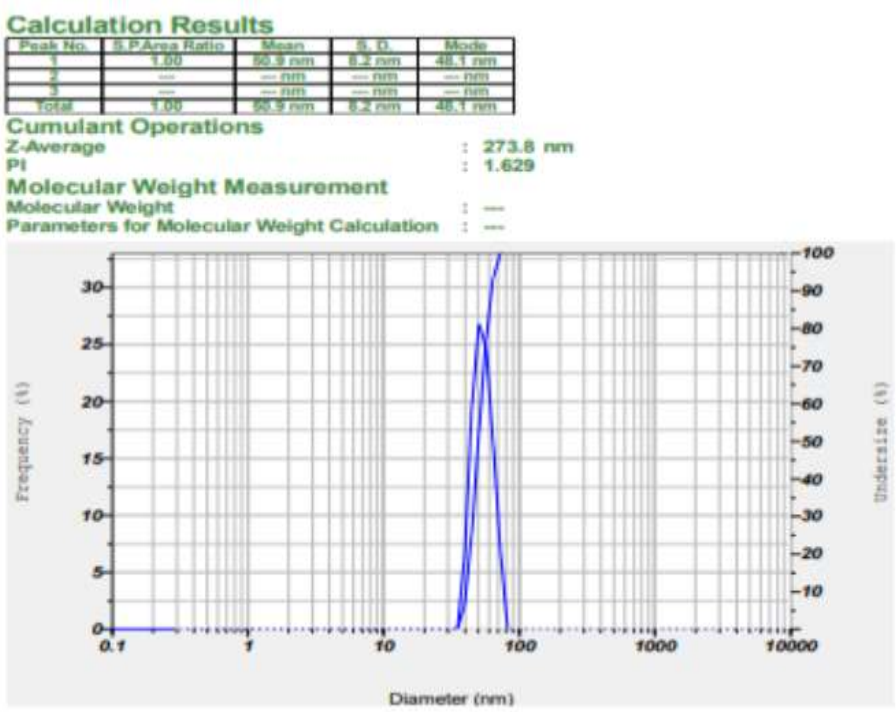

Figure 12: F1 Particle size and PDI

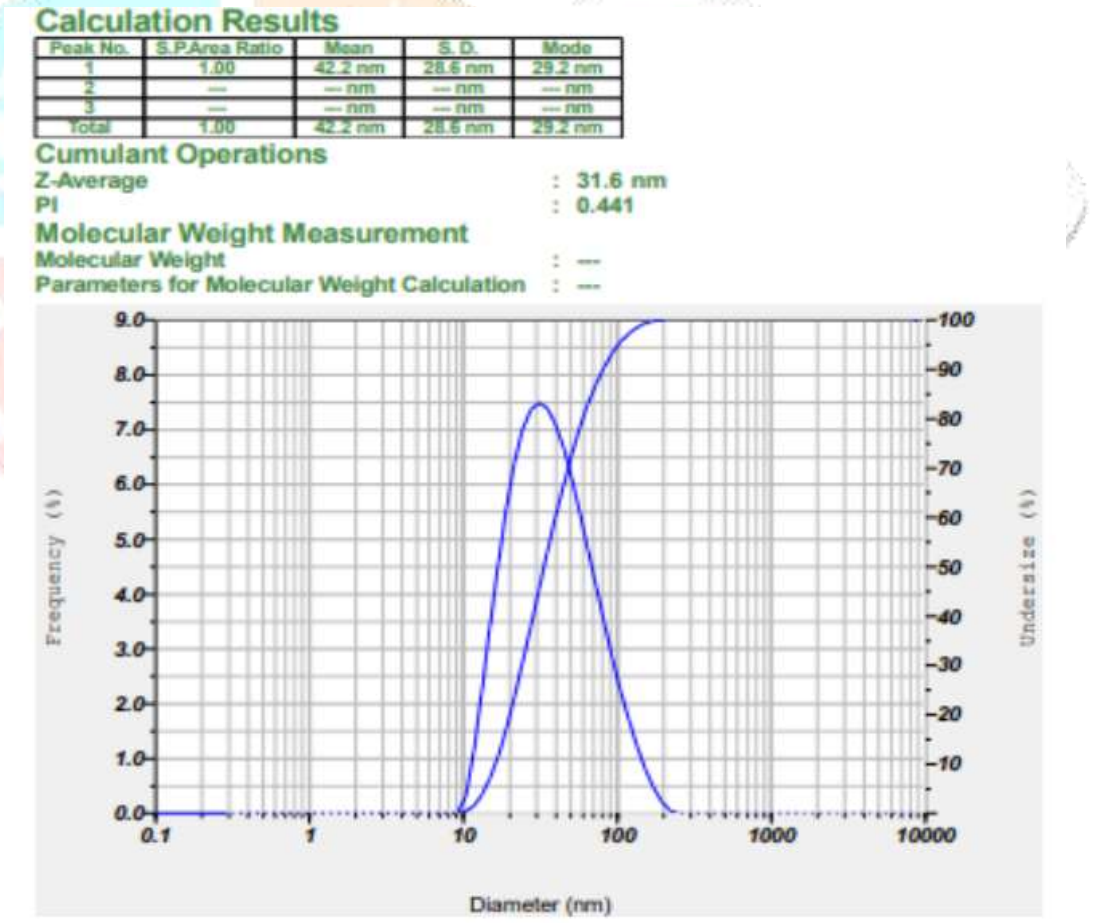

Figure 13: F3 particle size and PDI

To further ascertain the efficiency of the developed microemulsions, particle size determination was carried out. In Table: 3 we found F1 (273.8nm) and F3 (31.6nm) available in the range however F2 was beyond range. The particle size in all 3 formulations was different because density of oils and the particle size also depending on the ratio of smix and the poly dispity index of dispersions should be less than 'one'.5 F3~0.441it has been proved that dispersions were less than one and F1 is greater than 1 In Table: 3 shown that F1 (273.8nm) and F3 (31.6nm) were in range, whereas F2 was out of the range. The particle

size varied in all three formulations due to density of oils & particle size also depends on the ratio of smix and the poly dispersity index of dispersions should be less than 1. In table: 5 F3~0.441shown that dispersions were less than one and F1 is greater than 1. Therefore we concluded that formulation F3 was the best formulation.

3) Drug content

Content of drugs was established to confirm that the added amount of capsaicin in the formulation is present in the microemulsion dispersion.

Table 6: Drug content of Capsaicin micro emulsions

Dispersion code	Drug content in %
F1	87 ± 0.22
F2	73.80 ± 0.21
F3	92.2 ± 0.24

Drug content was calculated to ensure that the increase in the concentration of Capsaicin is incorporated in the microemulsion dispersion. From table 5, microemulsion dispersions show drug content of F3 has 92.20 % of Capsaicin in microemulsion dispersion

4. Centrifugation test

The centrifugation test was also followed by all the 3 formulation and None in all the 3 formulations was observed in terms of phase separation.

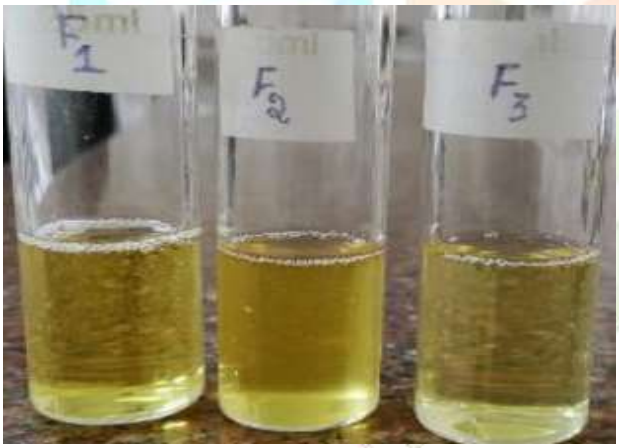

Figure14: Observation of microemulsion after performing centrifugation test

In order to check the physical stability of the microemulsions centrifugation test was performed. As can been seen in Table 3, all the 3 formulations F1, F2, F3 were remained homogenous without any phase separation and therefore it can be concluded that all the 3 formulations are physically stable.

Table 7: Invitro drug release of capsaicin microemulsion

	% CDR		
Time	**F1**	**F2**	**F3**
0	0	0	0
15 min	6.31	2.88	6.89
30 min	12.96	7.05	14.84
60 min	20.35	12.55	24.13
120 min	32.83	25.90	36.355
180 min	48.76	40.43	53.49
240 min	68.00	62.11	72.55
300 min	90.39	86.82	94.07

A Franz diffusion cell was used in invitro drug release for Capasificin microemulsions. From table 5.8 the percentage of drug release of microemulsions was found to be 86-94% at 5 hours. However, F3 formulation exhibited the highest release record of (94%), from the two formulation F1 and F2.

EVALUATION OF MICROEMULGEL

The assessment of formulation Microemulgel derived from F3. Which was optimized formulation added to various gelling agents? Concentrations gelling are added based on the topical gel consistency that has been established. Those are represented as G1 (chitosan 3.5%) and G2 (Aerosil 13%).

Table 8: Evaluation of Microemulgel

Gel code	**G1**	**G2**
Drug content in %	95.5 ± 0.16	85.6 ± 0.1
Ph	5.5 ± 0.07	6.5 ± 0.09
Extrudability	Good	Fair
Spreadability(cm)	7.5	3 ± 0.01

pH

The pH of microemulgel was done for both formulations G1 and G2 and the values obtained were 5.5 and 6.5 respectively. Hence it was concluded that the prepared microemulgel G1 was well within the acceptable limit that is permissible for topical preparation.

Extrudability

In its application and acceptance in patients this aspect of extrusion of the gel from the tube is critical. Higher consistency gels, might not come out of the tube easily, on the other hand, lower viscous gels may flow out very quickly, therefore the gel requires the right consistency to flow out of the tube. All the formulations of G1 were rated to have good extrudability, and the results are presented in the Table 8 as deputed **Spreadability**

Permeation rate is extremely effective when it comes to the ability of a drug to penetrate through the skin. The spreadability of G1and G2 microemulgels was calculated to be 7.5 and 3. G 1 microemulgel was having good spreadability with good transparent appearance of the formulation. Other formulation was translucent or had white colour.

Table 9: Invitro drug release of capsaicin microemulgel

	%CDR	
Time	**G1**	**G2**
0	0	0
1	11.34	8.92
2	17.73	14.92
3	28.15	23.84
4	38.88	34.78
5	48.81	46.65
6	56.22	51.90
7	69.44	65.99
8	71.43	70.86
9	84.06	82.34

The evaluation of the selected microemulsion formula was done and from formulation G1

which was prepared from turpentine oil and chitosan 3.5%, its % release was 82.34 % of the drug after 9 hrs. The drug release of chitosan gelling concentration level G1 was showing good appearance , stability & transparency as compared to the other gel it was good.

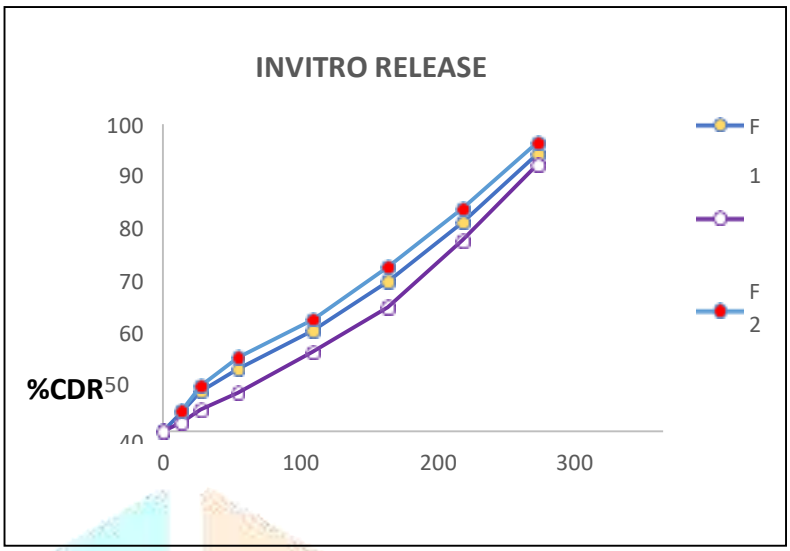

Figure 14: Invitro drug release graph for capsaicin microemulsion F1, F2, F3

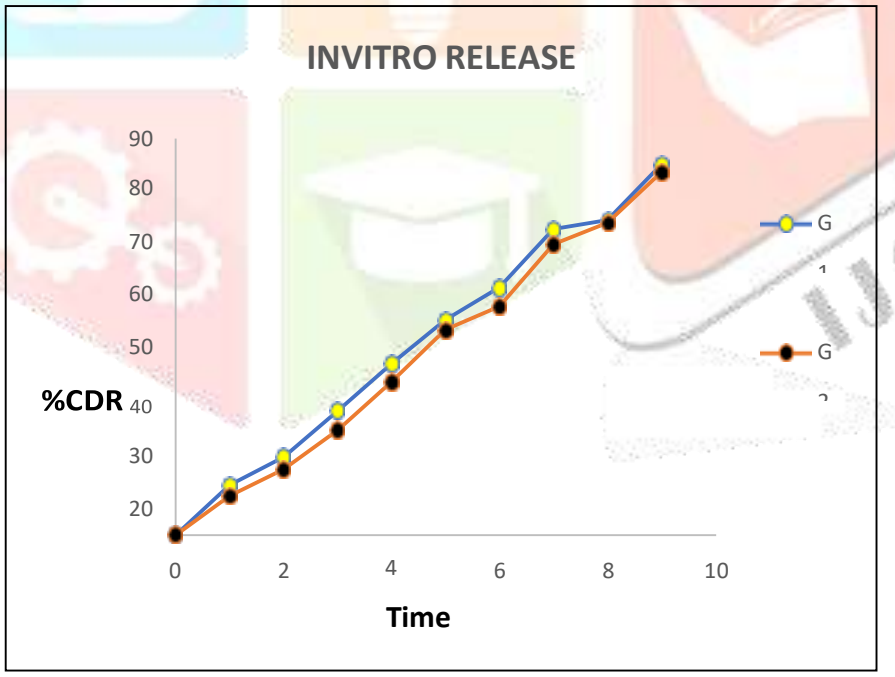

Figure 15: Invitro drug release graph for capsaicin microemulgel G1 and G2

Kinetics / release pattern of selected formulation G1 Microemulgel.

In order to understand the mechanism of drug release kinetics of the microemulgel G1, the data generated was fitted in to zero order kinetics, first order kinetics, Higuchi model and Korsmeyer-Peppas model. There was estimation of the regression coefficient. Figures: Graphs of kinetic models were plotted with suitable data are given in Figures 5.23 to 5.27 graphs of reaction R2 are summarized in Table 10.

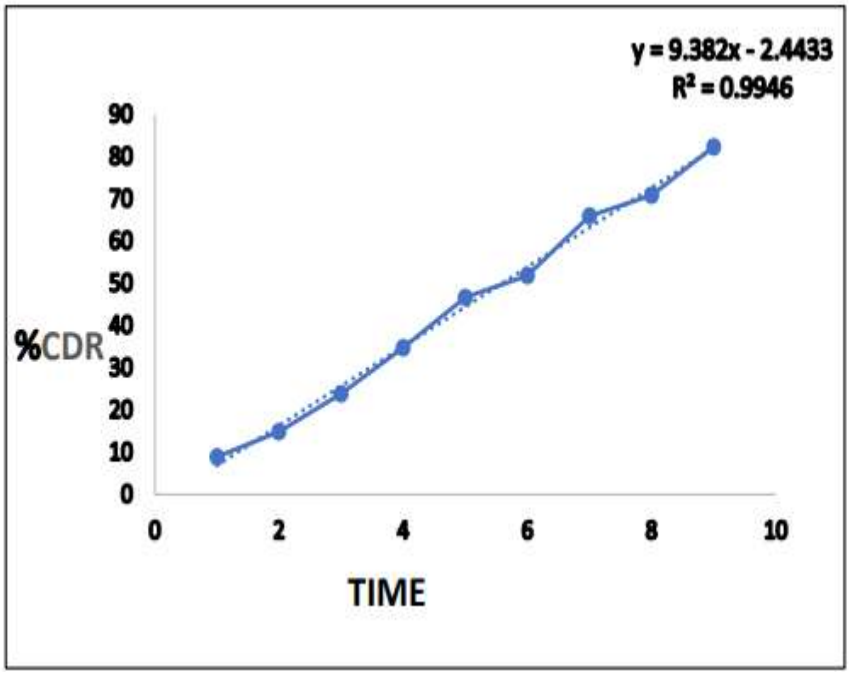

Figure 16: Zero order release of microemulgel, G1

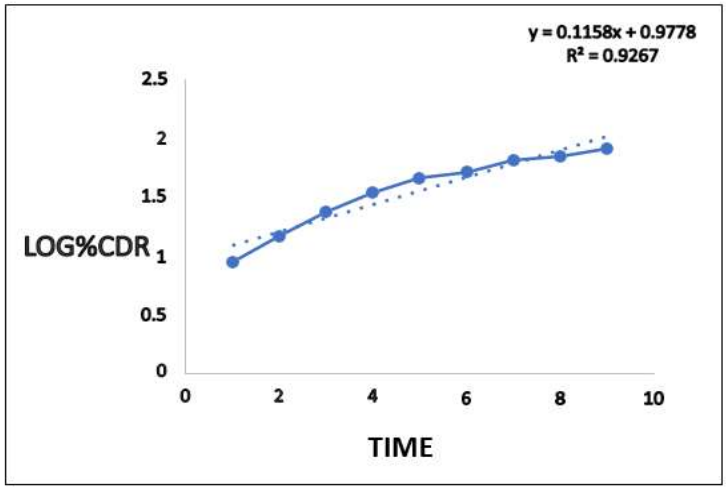

Figure 17: First order release of microemulgel, G1

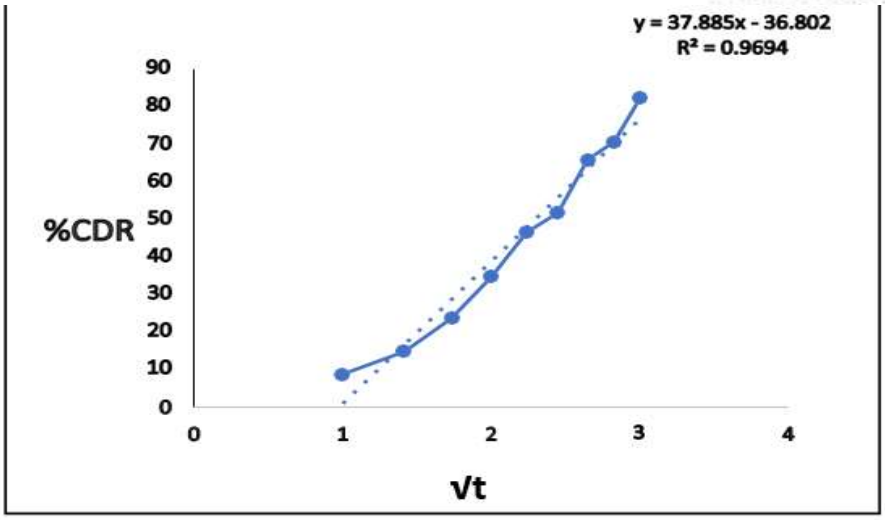

Figure 18: Higuchi release model of microemulgel, G1

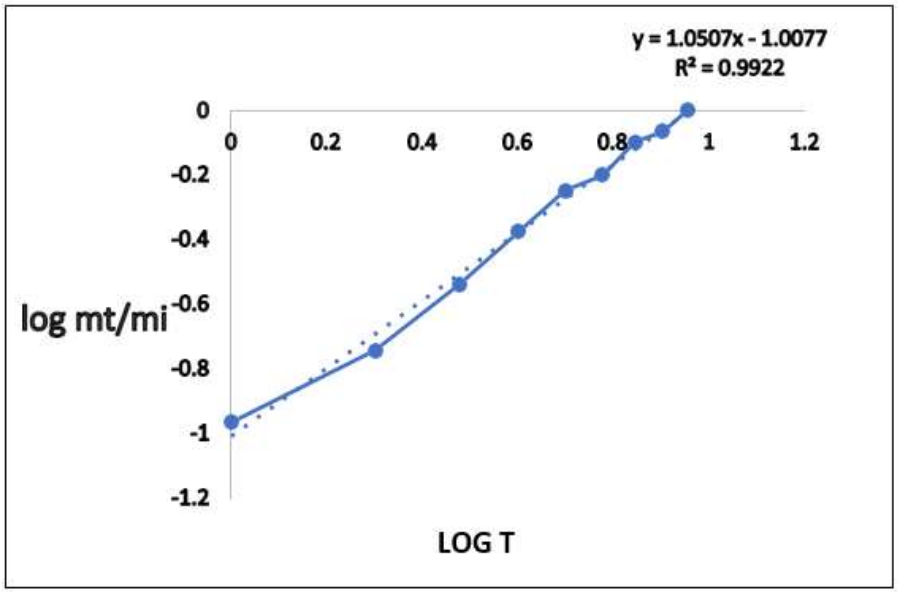

Figure 19: Kors Meyer- Peppas release profile of microemulgel, G1

Table10: Data of regression coefficient of different kinetic models

mulationcode	rder(R^2)	order(R^2)	Higuchi (R^2)	Korsmeyer-Peppas (R^2)
G1	0.9942	0.9267	0.9694	0.9922

Data analysis (curve fitting analysis)

Appropriate linear regression coefficient of each kinetic models was determined and mechanism of drug release from the dose was studied. This places the optimized formulation G1 into the mechanistic model of the reaction as it demonstrates the highest R2 value for the zero order mechanism. The value of set "n" exponent in the optimized batch was identified to be 9.382.

CONCLUSION

Capsaicin was successfully formulated in the form of Microemulgel by using clove, cinnamon and turpentine oil with a chitosan and Aerosol as a gelling agent. Turpentine oil is less solubilizing the capsaicin still it is used because it gives larger micro emulsion region and it actsas a permeation enhancing property, so it helps in the permeation of skin at higher rate.

F3 formulation was as optimized formulation based on % transmittance, drug content, particle size, and invitro drug release.

The compatibility studies using IR spectral studies revealed the absence of interaction betweenthe drug, excipients and the polymers.

The in vitro release data showed that drug release from the microemulgel has been affected bythe type and gelling concentration of Microemulgel. Among 2 formulations i.e., G1 and G2 with different type and concentrations of Chitosan and aerosol respectively, formulation G1 shows more drug release and drug content. Formulation G1 spreadability, extrudability and pH was within the range hence selected as best formulation from above data and it was concluded that G1 is the optimized formulation.

All the formulations (G1, G2) showed good physicochemical properties such as appearance, spreadability, extrudability, pH, drug content. The invitro release data showed that the drug release from the

Microemulgel has been affected by the type of concentration of the gelling agent and it was concluded that chitosan was the best gelling agent compared to Aerosil 200. Among 2 formulations different type and concentrations of G1 got the best result with better gelling consistency and better drug release 84.06% than the G2.

The developed Microemulgel are stable, and had increased drug release good, appearance, spread ability had a good potential for anti- inflammatory treatment. The invitro release data
Shown that the drug release from the Microemulgel has been affected by both type and concentration of the gelling agent and it was concluded that chitosan (3.5%) was the best gelling agent compared to Aerosil 200 (13%).

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