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Original Article

# Evaluation of Topical Anti-Inflammatory Potential of *Mentha piperita* L. Extract by Formulation of Microemulgel

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## ABSTRACT

**Background:** The present study is designed to develop a novel dosage form i.e. microemulgel which will enhance the rate of absorption in the systemic circulation and ultimately enhance the pharmacological effect of the *Mentha piperita* L. extract as anti-inflammatory agent. Its primary components include with constituents including menthol (46.32%), menthofuran (13.18%), menthyl acetate (12.10%), menthone (7.42%) and 1,8-cineole (6.06%). **Materials and Methods:** The research aimed to formulate and assess herbal microemulgel containing *M. piperita* extract, focusing on its *in vitro* anti-inflammatory properties. *M. piperita* herb extraction was carried out using a hydro-alcoholic solvent, followed by phytochemical analysis. Four separate sets of herbal microemulgel were crafted and underwent a series of assessments, encompassing pH levels, spreadability, viscosity, consistency, appearance, color and ease of washing. Additionally, the *in vitro* anti-inflammatory potential of both the extract and the microemulgel formulation was assessed using the HRBC membrane stabilization assay and the protein denaturation assay. **Results:** The findings of this study suggest that the newly developed herbal microemulgel, enriched with *M. Piperita* extract, exhibits promising anti-inflammatory effects. **Conclusion:** The *M. piperita* microemulgel exhibited a remarkable 94.35% drug content with high solubility and compatibility of the drug with the excipients. Permeability studies revealed that the *M. piperita* microemulgel achieved 94% permeability within 48 hr, showcasing enhanced drug permeability facilitated by the microemulsion-based gel system. Moreover, the formulated microemulgel demonstrated significant anti-inflammatory activity. It can be concluded that topical herbal *M. piperita* microemulgel has potential for future applications in this regard.

**Keywords:** *M. piperita*, Menthol, Herbal microemulgel, Anti-inflammatory activity, HRBC membrane, Deodarin.

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## INTRODUCTION

A topical drug delivery system circumvents first-pass metabolism, gastrointestinal degradation and potential irritation linked with oral intake. This method of drug delivery is optimal for targeting skin-related ailments with localized action. The skin serves as a vital barrier for the human body, distinguishing internal biology from the external environment. Nonetheless, it is susceptible to inflammation. Microemulgel represents a dual drug delivery approach achieved by transforming liquid microemulsion into a semi-solid gel.[1] It's regarded as a highly promising novel drug delivery system owing to its dual functionality through both emulsion and gel phases. Furthermore, research has demonstrated that combining emulsion with gel enhances its stability. The selection of the microemulsion system was based on its exceptional ability to solubilize and penetrate the skin effectively, while the gel component ensures sustained drug release, leading to prolonged drug residence time. The microemulgel drug delivery system emerges as the optimal choice for treating skin-related ailments, offering enhanced efficacy with reduced drug dosage.[2]

Treating inflammation often involves the use of plant-based remedies and extracts. Traditionally recognized anti-inflammatory plants include *Curcuma longa* Linn., *Zingiber officinalis* Roscoe, *Borago officinalis* Linn., *Oenothera biennis* Linn. (Evening primrose), *Harpagophytum procumbens* (Devil's claw) and *Mentha piperita* L. for instance, is indigenous to North India, Pakistan, Afghanistan, Tibet and Nepal. Its primary constituents include menthol (46.32%), menthofuran (13.18%), menthyl acetate (12.10%), menthone (7.42%) and 1,8-cineole (6.06%). It is used in the treatment of inflammation, it is used in the treatment of inflammation, *M. piperita* is utilized for a multitude of purposes such as addressing Irritable Bowel Syndrome (IBS), indigestion, bed sores, tension headaches, anxiety, insomnia, memory enhancement and various other applications, the research aims to formulate and evaluate a herbal microemulgel

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infused with *M. piperita* extract, aiming to scrutinize its *in vitro* anti-inflammatory attributes.[3]

While the oral route is commonly preferred for drug administration, both oral and intramuscular injections can lead to severe toxicity and adverse effects in patients. These effects may include gastrointestinal toxicity, renal failure and elevated liver enzymes at high doses. To address the challenges associated with these invasive delivery methods, a novel transdermal drug delivery system has been introduced. This system offers several advantages over oral and intramuscular injections, such as high absorption rates, ease of preparation, thermodynamic stability, avoidance of first-pass metabolism and ease of application. Additionally, its non-invasive nature and local effectiveness make it more patient-compliant compared to conventional delivery systems. The transdermal microemulgel, an enhanced form of emulsion, improves the solubility of poorly water-soluble drugs.[4]

Numerous research endeavors have shed light on the multifaceted biochemical impacts of *M. piperita* through both *in vivo* and *in vitro* investigations. Studies have showcased that various constituents of *M. piperita* exhibit a spectrum of beneficial effects, including anti-inflammatory, analgesic, immunomodulatory, antispasmodic, antihyperglycemic, anticancer, molluscicide, insecticidal, antiapoptotic, antibacterial, anti-sarcoptic, anxiolytic, anticonvulsant, antiulcer and antigastric properties.[5,6]

We can improve drug solubility and skin penetration by creating a microemulgel containing *M. piperita*. This approach facilitates the development of an optimized formulation. Transforming a basic *M. piperita* microemulsion into a microemulgel can address challenges encountered with conventional topical drug delivery systems, offering a larger interfacial area for efficient drug absorption.

## MATERIALS AND METHODS

### Collection and authentication of plant materials

The *M. piperita* herb's raw material was sourced from a nearby local nursery in Nagpur. Authentication of the plant material was carried out with the guidance of Dr. N. M. Dongarwar, associated with the Department of Botany at Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, utilizing a botanical specimen sheet. The authentication number for the *M. piperita* specimen sheet is 10429.

### Processing of the plant material

Following authentication, the *M. piperita* was dried, powdered and subsequently utilized to extract its essence.

### Extraction of plant material

The *M. piperita* powder underwent maceration with 70% alcohol for 48 hr. Afterward, the residue was subjected to extraction with 70% ethanol using a Soxhlet apparatus. The extracts obtained from both processes were then combined, followed by further concentration of the combined extract.

### Formulation of microemulgel

Different batches of microemulgel were formulated by utilizing diverse gelling agents and adjusting their concentrations. 2% *M. piperita* extract was incorporated into the emulsion.[7] The gelling agents were hydrated in water and pH adjustment was carried out by adding triethanolamine. Afterward, the emulsion was incorporated into the gel and thoroughly stirred to form the microemulgel.[8] Isopropyl myristate, Kolliphor PS 80: Transcutol P was gifted by Gattefosse India Pvt. Ltd., Mumbai Tween 80, Isopropyl Myristate (IPM), n-butanol, Carbopol 940 and triethanolamine were also purchased from Sigma-chemicals Nagpur. All solvents and materials employed were of analytical grade. Throughout the formulation processes, distilled water was consistently utilized.[9]

### Construction of microemulsion phase diagrams

Pseudo-ternary phase diagrams were generated employing the aqueous titration method for four weight ratios (1:0, 1:1, 1:2 and 2:1) of Labrafac CC Myristate ($S_{mix}$). Within each phase diagram, *M. piperita* oil and the designated $S_{mix}$ ratio were meticulously combined in varying weight ratios spanning from 1:9 to 9:1, each within separate glass vials. A total of 9 combinations of oil and $S_{mix}$ (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) were prepared to accurately delineate the boundaries of phases formed in the phase diagrams. A gradual titration with the aqueous phase was conducted for every combination of oil and $S_{mix}$, succeeded by visual scrutiny to distinguish transparent and effortlessly flowable Oil-in-Water (O/W) microemulsions.[10]

### Preparation of microemulsion

Phase diagram with $S_{mix}$ 1:1 showed maximum microemulgel region, so from that, concentrations of oil (3-5%w/w) and $S_{mix}$ (85-87%w/w) were pooled and several combinations of ME were formed using Box Behnken Design. To each combination of oil and $S_{mix}$ (1:1), water was added dropwise with stirring at ambient temperature.[11]

### Thermodynamic stability study

**Heating-cooling cycles:** The formulations underwent a series of six heating-cooling cycles, alternating between 4°C and 45°C. Each temperature was maintained for a minimum of 48 hr during storage. The formulations were thoroughly examined at both temperatures for signs of significant instability, such as phase separation, precipitation, cracking and similar issues.[12]

**Centrifugation test:** A centrifugation test was conducted on the formulations at 3,500 rpm for 30 min, with careful observation for any indications of phase separation.

**Freeze-thaw cycle:** The formulations were subjected to three freeze-thaw cycles, oscillating between temperatures of -21°C and +25°C, with each temperature held constant for at least 48 hr during storage.[13]

## Globule size and zeta potential

MEs were characterized for globule size and zeta potential using Zeta Sizer Litesizer DLS 500 Dadasaheb Balpande College of Pharmacy, Besa Nagpur.

## Gravimetric method

0.17 g of monobasic potassium phosphate were accurately weighed and dissolved in 625 mL of distilled water. Similarly, 0.22 g of sodium hydroxide were weighed and dissolved in 2.8 mL of distilled water. The solutions were combined and diluted to a final volume of 25 mL to prepare a buffer solution with a pH of 6.8. An empty test tube was weighed before the experiment. The drug *M. piperita* microemulgel was dissolved in 10 mL of pH 6.8 phosphate buffer until precipitation occurred. The solution was then filtered and the filtrate was subjected to evaporation. After heating, the test tube was weighed again. The difference in weights before and after heating was calculated to determine the drug's solubility in this buffer.[14,15]

## Partition coefficient

The pH 6.8 buffer solution was prepared and 10 mL of it were transferred to a separating funnel. To this, 10 mL of methanol were added. Then, 100 mg of *M. piperita* were accurately weighed and added to the separating funnel. The mixture was vigorously shaken for 30 min and then allowed to stand for 15 min to allow the layers to separate. Both layers were carefully extracted and filtered into separate beakers. The buffer extract was diluted with distilled water, while the methanol extract was diluted with methanol. Subsequently, both extracts were analyzed using a spectrophotometer.[16]

## Organoleptic evaluation

The physical appearance evaluation included analyzing color, homogeneity, consistency and overall appearance. Color assessment was conducted visually, while homogeneity was determined by rubbing the microemulgel between fingers. The appearance of the microemulgel was evaluated visually. Furthermore, the consistency of the microemulgel was tested by applying it to the skin.[17]

## Spreadability

To assess spreadability, the microemulgel was placed between two petri plates and the diameter of the spread microemulgel ring was measured. A gram of microemulgel was weighed and deposited onto a petri plate, with another petri plate positioned on top. A weight of 50 g was applied to the upper petri plate for 60 sec. After this duration, the diameters of the circles formed by the spread microemulgel were measured three times and the average reading was calculated. This average was then utilized in the following formula.[18]

$$S=MxLT$$

Where, S=Spreadability, M=Mass, L=Diameter and T=Time.

## pH

The pH of the formulated microemulgel batches was assessed using a digital pH meter. A quantity of 0.5 g from each batch was dissolved in 10 mL of distilled water. The electrode was then immersed in the solution to measure the pH.[19]

## Stability studies

The stability studies of the microemulgel included storing samples under different conditions: 5°C, 25°C/60% RH, 30°C/65% RH and 40°C/75% RH, for 3 months. Assessments were carried out at 15-day intervals, examining the appearance, pH, viscosity and spreadability of the samples.[20]

## Particle size analysis

The droplet size of the microemulgel was examined using a scanning electron microscope. A calibrated micrometer slide with a droplet of microemulsion was observed under the eyepiece and the droplet size was measured and determined.

## Rheological studies

The viscosity of the microemulgel preparation was measured using the Brookfield Viscometer (DV-E Brookfield Viscometer Model-LVDVE). This viscometer consists of a stationary cup and a rotating spindle. The rotating spindle was immersed in the sample of microemulsion gel. Due to the higher viscosity of the gel preparation, a smaller spindle with a reduced diameter and surface area was employed. The spindle was rotated within the gel preparation until a consistent reading was achieved on the viscometer dial. This procedure was repeated three times to ensure the reproducibility of the results.[21]

## Electrical conductivity

The electrical conductivity test was conducted to ascertain whether the microemulgel was an oil-in-water or water-in-oil microemulsion. The conductivity (σ) of the formulated sample was determined using a conductivity meter, which involved using a probe and meter. Voltage was applied across two electrodes within a probe immersed in the microemulsion. The decrease in voltage, attributed to the microemulsion's resistance, was utilized to calculate the conductivity.

## Differential Scanning Calorimeter (DSC)

Utilizing a DSC-60 calorimeter by SHIMADZU, samples underwent heating under liquid nitrogen from room temperature

to 400°C at a rate of 10°C/min. Approximately 5 mg of the sample in a sealed aluminium pan was subjected to a DSC scan. Pure *M. piperita*, the vehicle and the microemulgel were individually tested and the resulting thermograms were compared.

### Dye solubility test

To ascertain the type of emulsion, whether it was Oil-in-Water (O/W) or Water-in-Oil (W/O), 10 µL of methylene blue, a water-soluble dye, was introduced into the emulsion. This addition aimed to observe whether the dye dispersed uniformly throughout the emulsion or formed clusters.

### Fourier Transform Infrared Spectra analysis (FT-IR)

The functional groups present in the preparation were identified through FT-IR scanning microscopy. As IR rays passed through the sample, the absorbed radiations were transformed into vibrations or stretching, revealing spectrum signals ranging from 4000 $cm^{-1}$ to 400 $cm^{-1}$. These signals provided insights into the functional groups and the types of bonds present, forming a molecular fingerprint of the sample

### Release study

In testing the release rate of *M. piperita* from various microemulgel formulations, a Franz diffusion cell with a rabbit membrane was employed. The abdominal skin of the rabbit was shaved and excised and the integrity of the subcutaneous fat was assessed. The membrane was positioned with the mucous membrane facing upward and the epidermis downward, then secured the Franz diffusion cell facilitated the transfer of substances The transfer took place between the donor and receiver compartments of the cell. The cell was loaded with 9 mL of phosphate buffer at a pH of 6.8 and throughout the experiment, the receptor fluid was continuously stirred using externally driven magnetic bars at a speed of 300 rpm. *M. piperita* microemulgel was introduced into the donor chamber at intervals ranging from 0.5 to 48 hr, 0.5 mL samples were extracted from the receiver chamber for spectrophotometric analysis. These samples were promptly replaced with an equivalent volume of buffer solution. UV-visible spectrophotometry at 354 nm was employed for the analysis of the samples.[22]

### Drug content

A 1 mL aliquot of the emulsion was mixed with 9 mL of buffer solution at pH 6.8 and stirred for 30 min. The mixture was left at room temperature for 24 hr before undergoing an additional 30 min of stirring. Subsequently, the solution underwent centrifugation at 4000 rpm for 30 min to yield a clear supernatant. From this supernatant, 1 mL was extracted and combined with 9 mL of buffer solution. This resulting solution was then analyzed using a UV spectrophotometer at 354 nm and the absorbance was recorded. The concentration of *M. piperita* was determined by referencing a standard calibrated curve of the drug.

### *In vitro* anti-inflammatory Human Red Blood Cell (HRBC) membrane stabilization method

To evaluate the anti-inflammatory potential of the *M. piperita* extract microemulgel, the HRBC membrane stabilization method was utilized. Around 2-3 mL of blood was obtained from a healthy donor and combined in equal proportions with Alsever's solution. Iso-saline was then added, followed by centrifugation for 5 min to isolate the HRBC suspension. Equal amounts of the sample were added to the HRBC suspension, with concentrations of 100, 200 and 300 µg/mL prepared. The mixtures were then incubated at 37°C for 30 min and subsequently centrifuged for 5 min. Negative controls included Alsever's solution and blood, while aspirin served as the standard. UV spectroscopy at a wavelength of 560 nm was utilized to analyze the supernatant for estimation.[23]

### Inhibition of protein denaturation

A volume of approximately 0.02 mL of the sample was measured and combined with 0.2 mL of 1% Bovine Albumin. To this mixture, 4.78 mL of PBS (Phosphate Buffer Saline) was added and thoroughly mixed, followed by an incubation period of 15 min. Afterward, the mixture was heated in a water bath at 70°C for 5 min and then allowed to cool down. UV spectrophotometry was employed to measure the absorbance at a wavelength of 660 nm. Phosphate buffer served as the control, while A was utilized as the standard. Next, the percentage of inhibition of protein denaturation was determined through calculation.[24]

## RESULTS

### Extraction

*M. piperita* was harvested and air-dried before being crushed into coarse powder. One kilogram of this powder was then used for extraction with petroleum ether, employing a Soxhlet apparatus at temperatures ranging from 50°C to 60°C for 72 hr. Following the petroleum ether extraction, the remaining material underwent a second extraction with 95% ethanol in a Soxhlet apparatus, at temperatures between 60°C and 70°C for up to 72 hr. The concentrated alcoholic extract obtained was stored in a desiccator, while the residue was retained for further analysis.

### Pre-formulation studies and formulation of microemulgel

Surfactants and co-surfactants were evaluated based on their emulsification ability and percentage transmittance. For the surfactant screening, 300 mg each of oil and surfactant were heated at 40-45°C for 30 sec. Subsequently, a 50 mg mixture was diluted to 50 mL with distilled water and left to stand for 2 hr. The percentage transmittance was then measured at 688 nm. Labrafac CC was chosen as the surfactant as it gave higher transmittance.

For the screening of Cosurfactant, a mixture of co-surfactant (100 mg), selected surfactant (200 mg) and oil (300 mg) was heated at

40-45°C for 30 sec and proceeded similarly as for the screening of surfactant. Isopropyl myristate was found to be better (Table 1).

## Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were generated utilizing the aqueous titration technique for four different weight ratios (1:0, 1:1, 1:2 and 2:1) of Kolliphor PS 80:Transcutol P ($S_{mix}$) In every phase diagram *M. piperita* oil and a specific $S_{mix}$ ratio were meticulously combined in various weight ratios ranging from 1:9 to 9:1. A total of 9 different combinations of oil and $S_{mix}$ were prepared, including 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 were made to Precisely outlining To delineate the boundaries of the phases formed in the phase diagrams, a gradual titration with the aqueous phase was performed for each oil and $S_{mix}$ combination. Visual inspection was subsequently employed to identify transparent and effortlessly flowable Oil-in-Water (O/W) microemulsions (Figure 1).

*M. piperita* oil-3%-5%, Water- 52%-55%, $S_{mix}$-45%-40% (Kolliphor PS 80: Transcutol P (1:1) (Table 2).

**Table 1: List of surfactant/co-surfactant along with % transmittance.**

| Surfactant/Co-surfactant | % Transmittance |
|---|---|
| Kolliphor PS 20 | 43.74 |
| Kolliphor PS 80 | 44.76 |
| Labrafac CC | 97.23 |
| Isopropyl myristate | 95.35 |

## Thermodynamic stability study of microemulgel

After subjecting the formulations to a series of stability tests, including heating-cooling cycles, centrifugation tests and freeze-thaw cycles, no cracking or phase separation was observed in the microemulgel, indicating its favourable thermodynamic stability.

## Measurement of globule size and zeta potential

This optimum size of *M. piperita* microemulgel showed that surfactant, co-surfactant and oil phases were highly compatible with each other as the surface tension increases the globule size decreases (Figure 2).

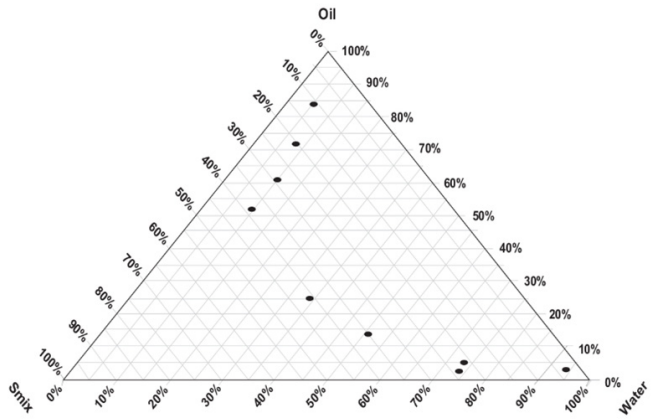


**Figure 1:** Ternary phase diagram of concentration for $S_{mix}$ 1:1.

**Table 2: Composition of microemulgel according to Box Behnken Design and response variables.**

| Run No. | Component (%wt/wt) | | | Responses | | |
|---|---|---|---|---|---|---|
| | *M. piperita* Oil | $S_{mix}$ (1:1) | Water | Mean Diameter* (nm) | PDI | Zeta potential (mV) |
| 1 | 5 | 42.5 | 52 | 287.3 | 0.331 | -27.7±3.7 |
| 2 | 3 | 40 | 53.5 | 247.4 | 0.311 | -25.7±2.3 |
| 3 | 3 | 42.5 | 55 | 252.5 | 0.320 | -26.7±3.7 |
| 4 | 4 | 40 | 52 | 270.3 | 0.340 | -27.9±1.7 |
| 5 | 4 | 45 | 52 | 260.1 | 0.321 | -26.9±2.3 |
| 6 | 3 | 42.5 | 52 | 217.8 | 0.260 | -24.3±1.1 |
| 7† | 4 | 42.5 | 53.5 | 250.3 | 0.321 | -25.7±1.3 |
| 8 | 5 | 40 | 53.5 | 272.2 | 0.341 | -25.3±1.3 |
| 9 | 5 | 45 | 53.5 | 265.35 | 0.325 | -26.9±2.4 |
| 10 | 4 | 40 | 55 | 261.23 | 0.255 | -22.2±1.0 |
| 11† | 4 | 42.5 | 53.5 | 250.8 | 0.312 | -25.2±1.5 |
| 12 | 5 | 42.5 | 55 | 295.5 | 0.381 | -27.2±1.2 |
| 13 | 4 | 45 | 55 | 287.1 | 0.331 | -10.9±1.0 |
| 14 | 3 | 45 | 53.5 | 121.75 | 0.255 | -22.2±1.0 |
| 15† | 4 | 42.5 | 53.5 | 252.5 | 0.326 | -26.2±1.8 |

† Central points* mean of 20 observations±SD.

## Determination of gravimetric method and partition coefficient

The solubility of *M. piperita* microemulgel was assessed across various oils (IPM, Propylene glycol, benzyl alcohol and oleic acid), surfactants (Tween 60, Tween 80 and Span 20) and cosurfactants (n-butanol, polyethylene glycol and diethylene glycol). Among these, the highest solubility was observed in IPM oil, Tween 80 surfactant and n-butanol co-surfactant compared to the other oils and surfactants. Microemulsions formed with IPM, Tween 80 and n-butanol was noted to be clear and stable due to the superior compatibility of these surfactant-co-surfactant pairs with the oil.

## Organoleptic evaluation

The microemulsion of *M. piperita* exhibited a transparent appearance with a light yellow and emitted an aroma reminiscent of alcohol. Notably, the microemulsion remained stable without undergoing phase separation upon the addition of gel to the formulation, confirming its stability.

## Measurement of spreadibility of *M. piperita* microemulgel

Using a drag and slip device, spreadability was assessed. The device was made up of a wooden block with a glass slide installed over it and a pulley fastened to one edge of the block. 2 g of microemulgel was placed over the glass slide over the wooden

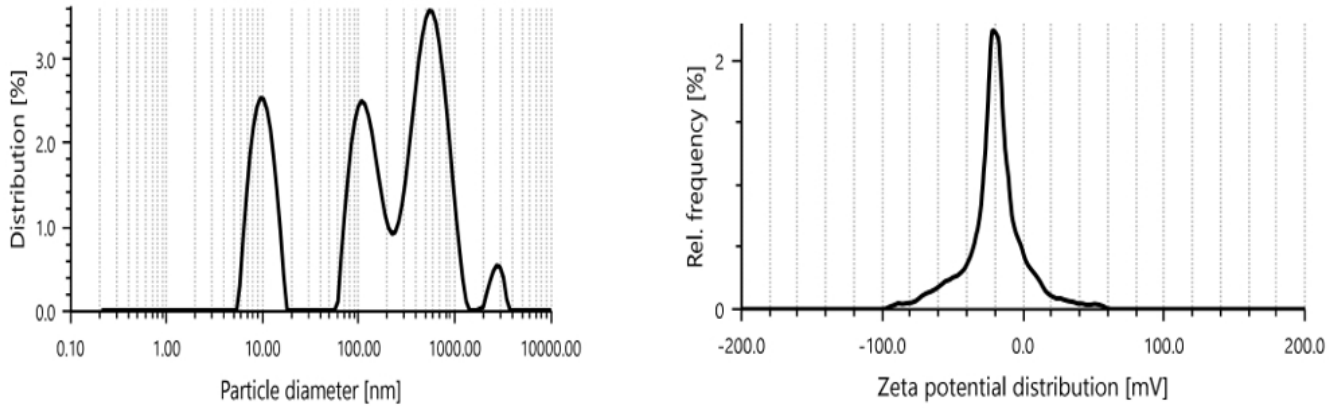


**Figure 2:** Measurement of globule size and zeta potential.

**Table 3: Measurement of spreadibility and viscosity of microemulgel.**

| Sl. No. | *Peppermint* oil %w/w | $S_{mix}$ %w/w | Carbopol %w/w | Viscosity (Poise) | Spreadibility (g. cm/S) * |
|---|---|---|---|---|---|
| 1 | 4 | 40 | 2.5 | 29.89±0.50 | 29.21±0.25 |
| 2 | 4 | 42.5 | 2 | 21.25±0.75 | 32.28±0.15 |
| 3 | 4 | 40 | 1.5 | 11.86±0.63 | 34.43±0.55 |
| 4 | 5 | 42.5 | 2.5 | 47.85±0.25 | 23.72±0.65 |
| 5 | 5 | 42.5 | 1.5 | 35.67±0.35 | 27.21±0.98 |
| 6 | 4 | 45 | 1.5 | 10.20±0.90 | 36.75±0.63 |
| 7 | 4 | 45 | 2.5 | 28.76±0.86 | 29.94±0.35 |
| 8 | 3 | 40 | 2 | 11.85±0.85 | 34.56±0.43 |
| 9 | 3 | 42.5 | 1.5 | 07.50±0.65 | 37.51±0.28 |
| 10 | 4 | 42.5 | 2 | 21.25±0.23 | 32.28±0.87 |
| 11 | 4 | 42.5 | 2 | 21.25±0.46 | 32.28±0.56 |
| 12 | 5 | 45 | 2 | 40.75±0.95 | 25.26±0.45 |
| 13 | 3 | 45 | 2 | 10.95±0.67 | 36.31±0.88 |
| 14 | 3 | 42.5 | 2.5 | 15.5±0.65 | 33.57±0.75 |
| 15 | 5 | 40 | 2 | 42.3±0.55 | 24.54±0.45 |

mean of 20 observations±SD.

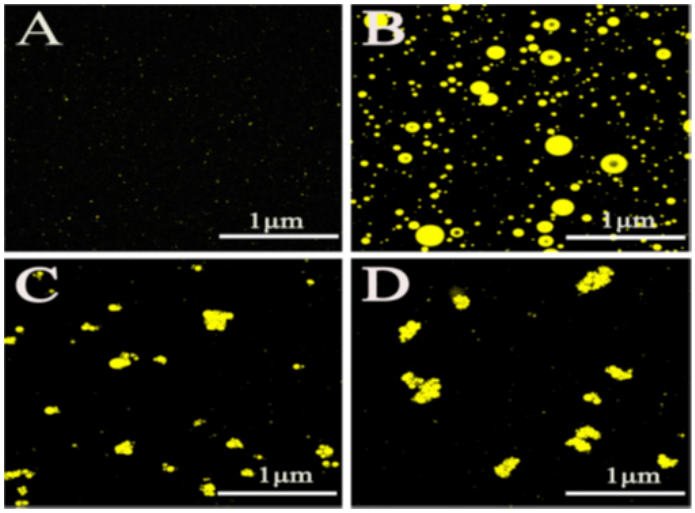


**Figure 3:** Particle Size of *M. piperita* microemulgel (117 nm).

block and another glass slide with the same measurements was placed on top of it. The weight of around 80 g was suspended by attaching a thread to the upper glass slide with the aid of a hook and passed above the pulley. The time in sec taken by the slide to cover 7.5 cm on the fixed glass slide was recorded. The spreadability was then calculated by using the following formula:

$$\text{Spreadability}=(M\times L)/T$$

Where, M=weight tied to upper glass slide, L=length of glass slides and T=time taken to separate the glass slides from each other.

## Measurement of viscosity of *M. piperita* microemulgel

One-gram microemulgel was placed between the plate and cone (no.3) of the viscometer (Brookfield viscometer Cap 2000+) and viscosity was measured at 10 rpm for 30 sec. Viscosity was also measured at increasing shear rate. Measurement was done for 30 sec at each rate of shear.

The viscosity of the *M. piperita* microemulgel measured at 86.7±0.01 cps. The formulation displayed Newtonian flow behaviour, signified by its viscosity showing minimal variation when subjected to external forces such as stirring. This suggests that the microemulgel could endure slight stress and maintain consistent viscosity during both handling and storage (Table 3).

## Measurement of pH of *M. piperita* microemulgel

The pH of the microemulgel was determined to be 6.2, indicating that the elevated S/CO ratio contributed to the increase in pH of the microemulgel.

## Stability studies

The stability study suggested that the formulation was physically and chemically stable when stored at 5°C, 25°C with 60% Relative Humidity (RH), 30°C with 65% RH and 40°C with 75% RH over a period of 3 months.

## Measurement of particle/droplets size distribution

The size of the droplets was 117 nm. This optimum size of *M. piperita* microemulgel showed that surfactant, co-surfactant and oil phases were highly compatible with each other (Figure 3).

A= 117 nm, B=150 nm, C=200 nm and D=250 nm

## Measurement of electrical conductivity of *M. piperita* microemulgel

The conductivity of the *M. piperita* microemulgel registered at 1.4 µS/cm, confirming its classification as Oil in Water (O/W) microemulgel. Notably, pure water typically exhibits a conductivity. The elevated conductivity observed in the microemulsion was attributed to the presence of dissolved salts and oils. As the amount of dissolved salts and oils in the microemulsion increased, so did its conductivity. The higher conductivity of the *M. piperita* microemulgel stemmed from the enhanced solubility of oil, surfactant, co-surfactant and water within the formulation.

## Differential Scanning Calorimeter (DSC)

*M. piperita* exhibited a sharp endothermic peak at around 204.73°C indicating its pure crystalline cubic form.

## Dye solubility test

Since the dye dispersed evenly within the microemulgel, it was inferred that the continuous phase consisted of water. Consequently, the *M. piperita* microemulgel was classified as Oil in Water (o/w) type of microemulsion.

## FT-IR studies

The distinctive peaks or FT-IR bands of pure *M. piperita* were identified at 2955.18 $cm^{-1}$ (C-H) and 1165.99 $cm^{-1}$ (C=O), as depicted in (Figure 4). These quality peaks of *M. piperita* were also evident in the *M. piperita* microemulgel, without any additional auxiliary peaks or significant peak shifts noted. This absence of chemical deterioration or incompatibilities was further confirmed through DSC analysis, which revealed no incompatibilities between *M. piperita* and the various excipients used. Both *M. piperita* and the excipients exhibited peaks in the microemulgel thermograms, affirming their compatibility.

## Release studies

The maximum release of *M. piperita* microemulgel was 94% at 48 hr which indicated that *M. piperita* microemulgel has good topical release properties (Table 4).

## Kinetics studies

The distinct peaks or FT-IR bands characteristic of pure *M. piperita* were detected at 2955.18 $cm^{-1}$ (C-H) and 1165.99 $cm^{-1}$ (C=O). These key peaks of *M. piperita* were similarly observed in the *M. piperita* microemulgel, with no additional auxiliary peaks or significant shifts noted. This absence of chemical deterioration or incompatibilities was further confirmed by DSC analysis, which demonstrated no incompatibilities between *M. piperita* and the various excipients utilized. Both *M. piperita* and the excipients displayed peaks in the microemulgel thermograms, confirming their compatibility.

## Drug content

The *M. piperita* microemulgel exhibited a drug content of 94.35%. This significant drug content indicates that the drug is highly soluble and exhibits compatibility with the excipients used in the formulation.

## *In vitro* anti-inflammatory activity

The anti-inflammatory activity of the extract and the optimized formulation was evaluated using two methods: the HRBC membrane stabilization method and the protein denaturation method (Table 5). Both the extract and the formulated microemulgel demonstrated significant anti-inflammatory activity. The protein denaturation caused by the extract was also examined and presented (Table 6).

**Table 4:** Kinetic release from *M. piperita* microemulgel.

| Values | Zero order | First order | Higuchi model | Korsmeyer peppas | n value |
|---|---|---|---|---|---|
| Rsqr | 0.9701 | 0.9492 | 0.8939 | 0.9742 | 0.697 |
| AIC | 82.4311 | 67.8087 | 82.5439 | 0.9354 | 48 |
| MSC | 3.0476 | 4.1379 | 2.4121 | 0.9453 | 3.9526 |

**Table 5:** Prevention of lysis by *M. piperita* extract and microemulgel (HRBC membrane stabilization).

| Concentration (µg/mL) | Absorbance | | Prevention of lysis. | |
|---|---|---|---|---|
| | *M. piperita* extract | microemulgel | *M. piperita* extract | microemulgel |
| 300 | 0.160 | 0.166 | 36.75 | 34.38 |
| 200 | 0.179 | 0.180 | 29.01 | 28.85 |
| 100 | 0.189 | 0.195 | 25.29 | 22.92 |
| Aspirin | 0.156 | 0.156 | 38.33 | 38.33 |
| Negative Control | 0.253 | 0.253 | 47.23 | 45.34 |

**Table 6:** Protein denaturation by *M. piperita* extract and microemulgel.

| Concentration (µg/mL) | Protein denaturation | | | |
|---|---|---|---|---|
| | *M. piperita* extract | Standard | Microemulgel | Standard |
| 100 | 45.31 | 70.7 | 42.35 | 70.19 |
| 200 | 49.21 | 75.39 | 47.45 | 74.11 |
| 500 | 53.9 | 81.25 | 52.94 | 80.39 |
| 1000 | 58.98 | 86.71 | 57.25 | 85.86 |

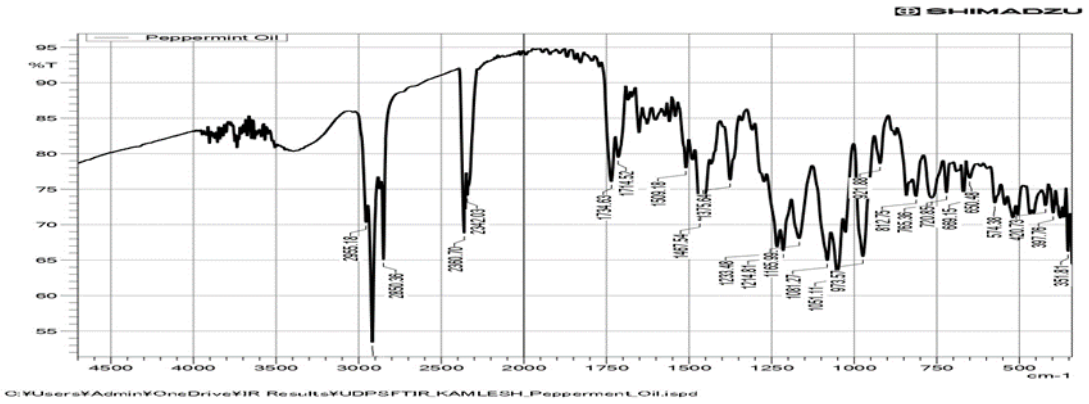


**Figure 4:** FT-IR Measurement of Percentage Transmittance (%T), Corresponding to *M. piperita* Peak at 1509.18 $cm^{-1}$.

## DISCUSSION

In this study, a microemulgel was developed using an herbal extract of *M. piperita* to target inflammation. Through experimentation with various surfactants and co-surfactants in preformulation studies revels that *M. piperita* extract exhibited superior solubility in IPM oil and Tween 80 surfactant, with n-butanol acting as the cosurfactant, surpassing other alternatives. The combination of IPM, Tween 80 and n-butanol yielded a microemulgel that was transparent and remained stable due to their excellent compatibility, which outperformed other surfactant-cosurfactant-oil combinations tested. The formulation demonstrated both physical and thermodynamic stability even under accelerated conditions. The study of Pseudo ternary phase diagram demonstrates that $S_{mix}$ ratio 1:1 provide more stable microemulgel formulation as compared with other $S_{mix}$ ratios. In thermodynamic stability studies, it was observed that there no any separation as well as no cracking of microemulgel. Top of FormBottom of FormThe microemulsion containing *M. piperita* appeared clear and light yellow, emitting a fragrance reminiscent of alcohol. The spreadability of the formulation varied between 27.21±0.98 and 37.51±0.28. Droplet size averaged approximately 117 nm, while viscosity measured at 86.7±0.01 cps. The dye dispersed uniformly throughout the microemulgel.

The microemulgel containing *M. piperita* had a pH of 6.2, indicating that the higher ratio of surfactant to cosurfactant contributed to its elevated pH compared to pure water, which typically exhibits a conductivity of 1.4 µS/cm. In the FT-IR spectra, peaks were observed at 2955.18 $cm^{-1}$ (C-H) and 1165.99 $cm^{-1}$ (C=O). The maximum release of *M. piperita* from the microemulgel reached 94% within 48 hr, demonstrating excellent topical release properties. The *M. piperita* microemulgel showed a drug content of 94.35%. Both the extract and the formulated microemulgel displayed promising anti-inflammatory activity by preventing cell lysis and inhibiting protein denaturation.

## CONCLUSION

In this study, advanced methods is adapted to formulate the microemulgel, this technique can be employed to enhance the solubility and skin permeability. An effective microemulgel was developed using 1% Kolliphor PS 80 as a gelling agent, resulting in sustained release properties and prolonged residence time. The *M. piperita* microemulgel exhibited a remarkable 94.35% drug content, indicating high solubility and compatibility of the drug with the excipients. Permeability studies revealed that the *M. piperita* microemulgel achieved 94% permeability within 48 hr, showcasing enhanced drug permeability facilitated by the microemulsion-based gel system. These findings suggest that this formulation holds promise as a topical delivery vehicle for *M. piperita*. Moreover, the formulated microemulgel demonstrated significant anti-inflammatory activity.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS CONTRIBUTION

Mr. Kamlesh Machewar was involved in conceptualization, data curation, formal analysis, investigation, methodology development and drafting of the original manuscript, as well as editing. Dr. Rajendra Kakde provided supervision, while Dr. Prafulla Sabale contributed to validation, resource management and project administration.

## ABBREVIATIONS

**IPM:** Isopropyl Myristate; **RH:** Relative Humidity; **FT-IR:** Fourier Transform Infrared Spectra Analysis; **gm:** Gram; **HRBC:** Human Red Blood Cell; **%:** Percent; **CM:** Centimetres; **ºC:** Degrees Celsius; **W/O:** Water in oil; **O/W:** Oil in water; **S/CO:** Ratio of surfactant to co-surfactant; **CPS:** Centipoise; **nm:** Nanometre; **mg:** Milligram; **mL:** Millilitre; **rpm:** Revolution per minute; **UV:** Ultra-Voilet; **ME:** Microemulgel.

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