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"PREPARATION AND CHARACTERIZATION OF MICROEMUL GEL CONTAINING COMBINE DRUGS AND ITS ANTIMICROBIAL ACTIVITY"

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ABSTRACT

Pre-formulation studies were conducted to evaluate the fundamental characteristics of the drug, including organoleptic properties, solubility, melting point, pH, λ max, and functional groups through FTIR analysis. A calibration curve was developed for precise drug quantification. Based on these findings, a microemulsion containing the drug was successfully formulated and characterized for encapsulation efficiency, zeta potential, particle size distribution, and surface morphology using SEM. Subsequently, a microemulgel formulation was prepared and evaluated for pH, viscosity, spreadability, skin irritation, and in vitro drug release behavior. The release kinetics were analyzed to understand the drug's release profile. Furthermore, antimicrobial activity was assessed using the well diffusion method, confirming the formulation's efficacy against microbial strains. Overall, the results indicated that the formulated microemulgel exhibited desirable physical stability, efficient drug release, and promising antimicrobial potential, making it a suitable candidate for topical drug delivery applications.

KEYWORDS: Pre-formulation studies, microemulsion, Emulgel, FTIR analysis, drug release kinetics, antimicrobial activity, topical formulation.

1. INTRODUCTION

Topical drug delivery systems have gained significant importance in recent years, particularly for the treatment of skin-related disorders such as infections, hyperpigmentation, and inflammatory conditions. Such systems offer several advantages, including localized drug action, avoidance of first-pass metabolism, and improved patient compliance **(Brito *et al.*, 2024).** However, the main challenges associated with topical formulations are poor drug solubility, limited skin permeability, and short drug retention time at the application site. To address these challenges, researchers have developed advanced carrier systems such as *microemulgels*, which combine the advantages of both *microemulsions* and *gels* to improve drug delivery efficiency and stability **(Sharma *et al.*, 2016).**

A *microemulgel* is a biphasic system in which a microemulsion—composed of oil, water, surfactant, and co-surfactant—is incorporated into a gel base **(Kushwah *et al.*, 2021).** Microemulsions provide excellent solubilization capacity for both hydrophilic and lipophilic drugs and enhance their permeation through the skin, while the gel matrix offers desirable viscosity, spreadability, and ease of application. The integration of these two systems results in a stable, non-greasy, and patient-friendly formulation suitable for topical use **(Souto *et al.*, 2022).**

In the present study, *Hydroquinone* was selected as the active pharmaceutical ingredient for incorporation into a microemulgel formulation **(Mahajan *et al.*, 2022).** Hydroquinone (1,4-dihydroxybenzene) is a well-established depigmenting agent commonly used for the

treatment of various hyperpigmentation disorders such as melasma, freckles, solar lentigines, and post-inflammatory hyperpigmentation. It exerts its therapeutic effect by inhibiting the enzyme tyrosinase, which plays a critical role in the biosynthesis of melanin. By suppressing melanin production, Hydroquinone helps in lightening the skin and achieving a more uniform complexion **(Draelos *et al.*, 2020).**

In the present study, *Fusidic acid* was chosen as the active pharmaceutical ingredient for incorporation into a microemulgel formulation. Fusidic acid, a steroidal antibiotic derived from *Fusidium coccineum*, exhibits potent antibacterial activity, particularly against Gram-positive microorganisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Corynebacterium* species **(Fernandes, 2016).** It acts by inhibiting bacterial protein synthesis through interference with elongation factor G (EF-G), thereby preventing bacterial growth and proliferation. Because of its strong antibacterial properties and safety profile, Fusidic acid has been widely used for the treatment of various skin infections, including impetigo, infected dermatitis, folliculitis, and minor wound infections **(Dallo *et al.*, 2023).**

This research aims to develop and characterize a *microemulgel containing Hydroquinone and Fusidic acid* with enhanced physicochemical properties and potent antimicrobial activity. The study focuses on optimizing the formulation to achieve maximum drug stability, improved skin penetration, and sustained drug release, ultimately providing an effective and convenient therapeutic option for the management of hyperpigmentation associated with skin infections.

2. MATERIALS AND METHODS

2.1 Chemicals

2.2 Pre-formulation studies

Pre-formulation studies are an essential phase in the creation of a medicinal product, focusing on understanding the physical, chemical, and mechanical properties of a drug substance before its formulation. The goal is to gather critical data that guides the choice of appropriate formulation strategies, ensuring the drug's effectiveness, safety, and ease of manufacturing. Pre-formulation studies help optimize the drug's delivery mechanism and improve its overall performance **(Soni and Singhai 2013).**

2.2.1 Organoleptic Properties

Organoleptic properties refer to the sensory characteristics of a material that is observed with the senses, particularly through sight, smell, taste, and touch. When assessed through visual inspection, these properties include attributes such as color, shape, texture, and clarity (**Bilous and Kovalevska 2019).**

2.2.2 Solubility study

To perform a solubility study of medications in different solvents by visual observation, first, select various solvents like water, ethanol, or acetone. Accurately weigh 1mg in 1ml of the drug and add it to separate test tube that has each solvent. Stir or shake the mixtures and observe the clarity of the solutions. If the medication dissolves, the solution will be clear; if undissolved particles remain, drug is insoluble or poorly soluble in that solvent. Record the visual results for each solvent, noting the appearance of the solution, which helps determine the drug's solubility profile **(Williams et al., 2012).**

2.2.3 Melting point

To perform a melting point study of Hydroquinone and Fusidic acid using melting point apparatus **(Chaurasia G.2016).**

2.2.4 pH determination

To perform a pH determination study of Hydroquinone and Fusidic acid with the use of a digital pH meter **(Murata et.al., 2003).**

2.2.5 Determination of Lambda max and calibration curve (Kumbhar and Salunkhe 2021, Behera et al., 2012)

- **Preparation of standard stock solution:**

About 5 mg of hydroquinone and 5 mg of fusidic acid were weighed and placed to a 5ml volumetric flask (separately). To generate a solution with a concentration of 1000 µg/ml, the volume was increased to 5ml by adding the appropriate solvent. To create a standard stock solution containing 100 µg/ml of hydroquinone and fusidic acid, 1ml of stock solution was diluted to 10 ml using methanol.

- **Lambda max**

To prepare concentration of 10 µg/ml, transfer 1.0 ml of stock solution (Hydroquinone and Fusidic acid) to a 5 ml volumetric flask (separately) and mark with solvent. The sample was scanned for hydroquinone and fusidic acid with UV-VIS spectrophotometer in the 200-400 nm range, with methanol solvent serving as a blank. The wavelength connected to the highest absorbance (max) was identified.

- **Linearity**

A standard Hydroquinone solution (100 µg/mL) was used to prepare aliquots ranging from 15–55 µg/mL and 5–25 µg/mL, while Fusidic acid working solutions were accurately measured into 5 mL volumetric flasks and diluted with solvent. The absorbance was recorded at 293.5 nm for Hydroquinone and 237.0 nm for Fusidic acid against a blank. Calibration curves were plotted for both drugs, showing linearity within the studied ranges. The regression equations obtained were $y = 0.0322x - 0.1113$ ($R^2 = 0.9997$) for Hydroquinone and $y = 0.0198x - 0.0119$ ($R^2 = 0.9992$) for Fusidic acid, confirming excellent linear response.

2.2.6 Fourier transmission Infra-Red Spectroscopy

The FT-IR readings of hydroquinone and fusidic acid was recorded from 4000 to 400 cm−1 using a KBr pellet technique and an FT-IR spectrophotometer. The KBr disc was made by combining 1 mg of hydroquinone and 1 mg of fusidic acid in 100 mg of spectroscopic grade KBr, which was then dried under an infrared light. To produce a disc, KBr and drug were mixed together and subjected to hydraulic pressure. This disc was placed in the FT-IR chamber. The infrared spectra were collected in the 4000–400 cm-1 range **(Kahar and Bagre 2019).**

2.3 Preparation of micro-emulsion formulation

To determine the solubility of the drugs in different excipients, excess drug was added to 2 mL of selected oils (oleic acid, Capryol 90, olive oil), surfactants (Cremophor RH 40, Tween 80), and the co-surfactant (polyethylene glycol 400) in 5 mL stoppered vials and mixed using a vortex. The components showing maximum solubility were selected for formulation. A microemulsion was then prepared using olive oil as the oil phase, propylene glycol as the surfactant, Tween 80 as the co-surfactant, and distilled water as the aqueous phase. Various surfactant-to-co-surfactant ratios were optimized for each batch, and the mixtures were stirred magnetically until a uniform dispersion was obtained. Double-distilled water was used to prevent contamination by surface-active impurities **(Yadav et al., 2018; Singh & Vingkar, 2018).**

Table 1: Composition of micro emulsion formulation.

Ingredients	FORMULATION CODE				
	MEF1	MEF2	MEF3	MEF4	MEF5
HDQ and FA Drugs (200:100) (mg)	2:1	2:1	2:1	2:1	2:1
Propylene Glycol (ml)	0.1	0.2	0.3	0.4	0.5
Tween 80 (ml)	1.0	1.0	1.0	1.0	1.0
Olive oil (ml)	2.0	2.0	2.0	2.0	2.0
Stirring Time (min.)	30	30	30	30	30
Distilled water	1.5	1.5	1.5	1.5	1.5

2.4 Evaluation of prepared micro-emulsion formulation

2.4.1 Physical properties

The physical characteristics of microemulsion were checked through visual inspection by observing its appearance, transparency, and phase separation **(Froelich et al., 2017).**

2.4.2 Quantitative analysis (Entrapment Efficiency)

Indirect estimation was accustomed to calculate percentage entrapment efficiency. The drug- loaded microemulsion was centrifuged at 1500 rpm for 30 minutes with the REMI Ultra Centrifuge. The non-entrapped drug (free drug) in the supernatant solution was measured using a UV spectrophotometer. The peak area was calculated, and quantity of free medication was

Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc. ×100

2.4.3 Particle size

Particle size is one of the most important factors in describing micro emulsions. A Malvern Zeta sizer (Malvern Instruments) was used to measure the micro emulsion's size **(Singh and Vingkar 2008).**

2.4.4 Zeta potential

Measurements of the zeta potential were made to ascertain particle movement velocity in an electric field along with particle charge being examined using Zeta-sizer Malvern equipment **(Đorđević et al., 2022).**

2.4.5 Scanning Electron Microscopic (SEM)

The drug-loaded micro emulsion's morphological properties were determined using an electron beam from a SEM. A sputter coater was accustomed to apply a thin layer (2-20 nm) of metal(s) such as gold, palladium, or platinum under vacuum. The pre-treatment specimen was then struck with an electron beam, resulting in the development of secondary electrons known as augers. From this interaction between the electron beam and the specimen's atoms, only the electrons dispersed at 90° were picked and further processed based on Rutherford and Kramer's Law to acquire surface topography images **(Ahma et al., 2020).**

2.4.6 *In vitro* drug release study of optimized micro-emulsion formulation

Phosphate buffer with a pH of 6.8 was poured into the diffusion cell's receptor compartment, which has capacity of 25 ml. The membrane, which had a 1 cm2 surface area and was installed between the diffusion cell's donor and receptor compartment, there then trimmed to the necessary size. The assembly was mounted on a hot plate magnetic stirrer, and a magnetic bead was employed to continuously agitate the solution in receptor compartment to maintain a temperature of 37°C. After applying emulgel to the donor compartment membrane, 1ml of the sample was removed at regular intervals. Throughout the experiment, sink conditions must be.

2.5 Formulation of micro-emulsiongel

Carbopol-940 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. To create a stiff gel, 50 milliliters of warm water (B) was combined with carboxymethyl

cellulose and methyl paraben in a different container and constantly agitated. Stirring continuously, mixtures A and B were combined. Following the addition of triethanolamine (dropwise) to balance the pH, the optimum formulation micro- emulsion was mixed into the dispersion to create the hydrogel. Propylene glycol, a permeability enhancer, was introduced at this point. The final dispersion was agitated until smooth gel was formed without lumps**.**

Table 2: Composition of micro-emulsion gel formulation.

Name of Ingredient	Formulation I
Carbopol 940	0.5 gm
Carboxymethyl cellulose	0.5 gm
Propylene glycol	0.25 ml
Methyl paraben	0.1 ml
Microemulsion	5 ml
Triethanolamine	q.s
Water	50 ml

2.6 Characterization of drug loaded microemulgel formulation

2.6.1 Physical appearance

The appearance was assessed to ensure the gel was clear, smooth, and devoid of any visible defects

2.6.2 Measurement of pH

A digital pH meter was used to measure the pH. **(Shankar et al., 2018).**

2.6.3 Determination of Viscosity

Viscosity was tested using the Brookfield viscometer **(Kaur LP 2013).**

2.6.4 Skin irritation test

The test for skin inflammation was carried out on one Wistar rats. One day before the trial began; rat`s back skin shaved for a 5 cm2 region. After 24 hours, rat received optimized emulgel and the ratwas assessed for symptoms of discomfort. Scores were assigned based on observed indicators of discomfort **(Giri et al., 2019, Murthy and Hiremath 2001).**

2.6.5 Spreadability

Spreadability was tested on two glass slides that were 7.5 cm long. 350 mg of microemulgel was carefully weighed and placed on one glass slide. Five centimeters above it was another glass slide. A 5-gram weight was placed on the upper slide, and after 1 minute, the diameter of the circle that was spread was measured in cm. The observed diameter determines the type of gel.[187] The time it took for the gel to travel a certain distance from its original position was recorded. The spread ability was calculated using formula below.

$S = M \times L/T$

Where, S-Spread ability, g.cm/s M-Weight put on upper glass L-Length of glass slide T-Time for spreading gel in sec **(Sandeep DS 2020).**

2.7 Antimicrobial activity

2.7.1 Preparation of Nutrient Agar Media

2.8 grams of Nutrient Media were dissolved in 100 milliliters of purified water. Prior to sterilization, the pH of the media was tested. The media was autoclaved for 15 minutes at 121 degrees Celsius and 15 pounds of pressure. Nutrient media was poured onto plates and placed under laminar air flow until the agar solidified.

2.7.2 Well Diffusion Assay

A standardized *E. coli* suspension (10^8 CFU/mL) was placed in a shaker, and 100 µL of the inoculum was transferred onto sterile agar plates (Mohammadi et al., 2013). The inoculum was evenly spread using a sterile spreader, and four 6 mm wells were made with a sterilized cork borer. The wells were filled with 100 µL each of C1 (control), C2 (0.05 mg/mL), C3 (0.1 mg/mL), and C4 (1.0 mg/mL) formulation solutions. Plates were left at room temperature for 30 minutes to allow diffusion, then incubated at 37°C for 18–24 hours. After incubation, zones of inhibition (ZOI) were observed and measured in millimeters using a ruler placed against the inverted Petri plate on a non-reflective black background **(Manandhar et al., 2019).**

3. RESULT AND DISCUSSION

3.1 Pre-formulation study of drug

3.1.1 Organoleptic properties

Table 3: Organoleptic properties of Hydroquinone.

Drug	Organoleptic properties	Observation
Hydroquinone	Color	White crystals
	Odor	Odorless
	Appearance	Crystalline
	State	Crystalline powderor flakes

Table 4: Organoleptic properties of Fusidic acid.

Drug	Organoleptic properties	Observation
Fusidic acid	Color	White to off-white
	Odor	Odorless
	Appearance	Powder
	State	Crystalline powder

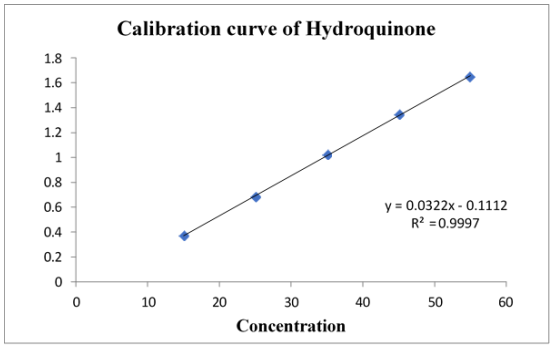
3.1.2 pH determination

Table 5: Melting point, pH of Hydroquinone and Fusidic acid.

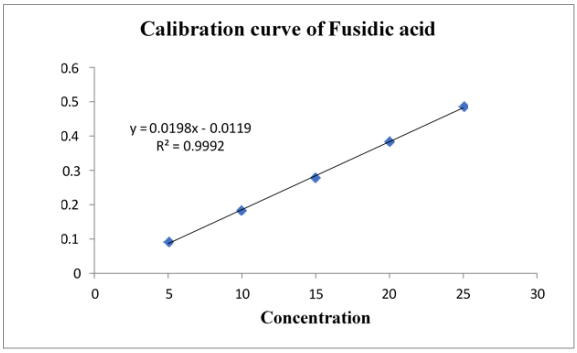
Drugs	Observed (pH)	Observed (Melting Point)	Reference (melting point)
Hydroquinone	4.7	172°C	170°C to 173°C
Fusidic acid	5.3	192.5°C	192°C to 193°C

3.1.3 Calibration curve of both drugs

1. Standard calibration curve of Hydroquinone and Fusidic acid



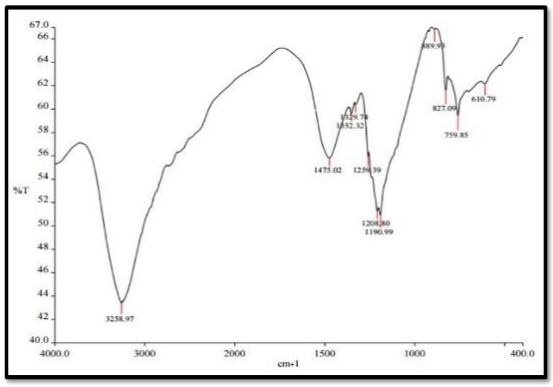
Graph 1: Calibration curve of Hydroquinone.



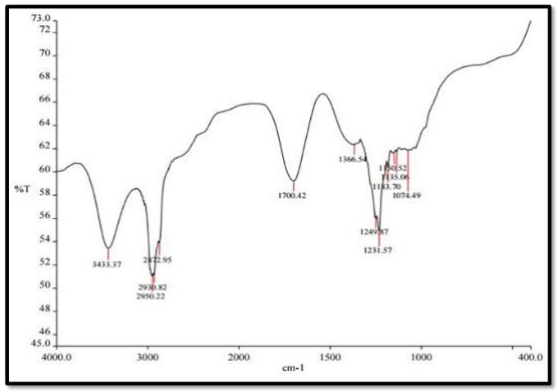
Graph 2: Calibration curve of Fusidic acid.

3.1.3 Functional group identified by Fourier transform infrared (FTIR) study

1. FTIR of Hydroquinone and fusidic acid



Graph 3: FTIR of Hydroquinone,



Graph 4: FTIR of Fusidic acid.

Table 6: Interpretation of IR spectrum of Hydroquinone.

Peak obtained	Reference peak	Functional group	Name of functional group
3258.97	3550-3200	O–H stretching	Alcohols
1352.32	1372-1335	S=O stretching	Sulfonate
1259.39	1275-1200	C-O stretching	Alkylaryl ether
1190.99	1205-1124	C-O stretching	Tertiary alcohol
827.09	840-790	C=C bending	Alkene
759.85	850-550	C-Cl stretching	Halo compound
610.79	690-515	C-Br stretching	Halo compound

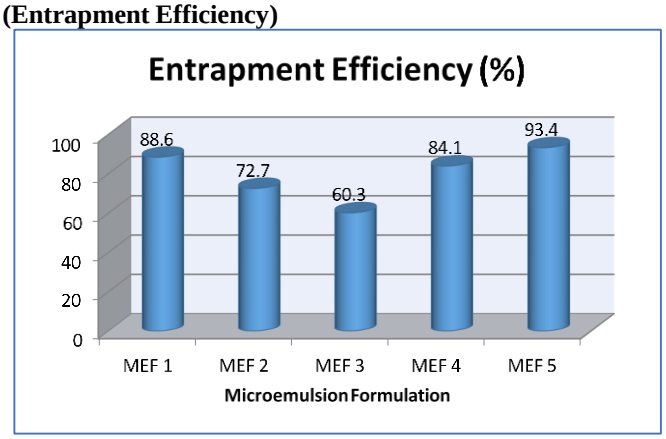
Table 7: Interpretation of IR spectrum of Fusidic acid.

Peak obtained	Reference peak	Functional group	Name of functional group
3433.37	3500- 3400	N-H Stretching	Primary Amine
2950.22	3000-2800	C-H Stretching	Alkane

1700.42	1710-1680	C=O Stretching	Conjugated acid
1366.54	1372-1290	N-O stretching	Nitrocompound

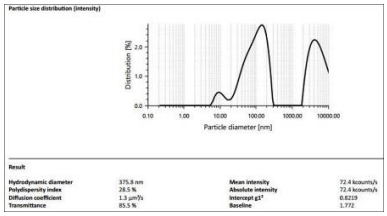
3.2 Characterization of drug loaded microemulsion

3.2.1 Quantitative analysis (Entrapment Efficiency)

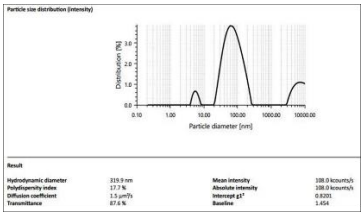


Graph 5: % EE of all formulation.

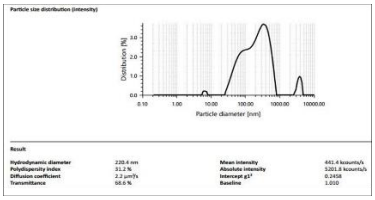
3.2.2 Particle Size



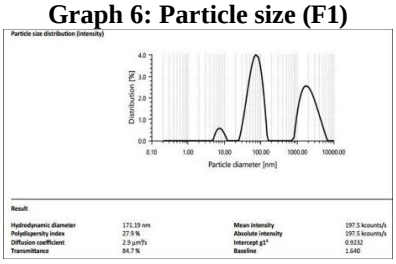
Graph 6: Particle size (F1)



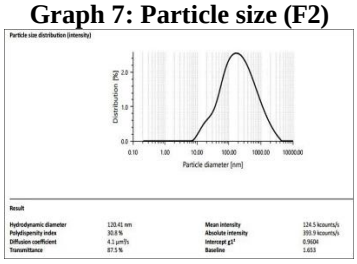
Graph 7: Particle size (F2)



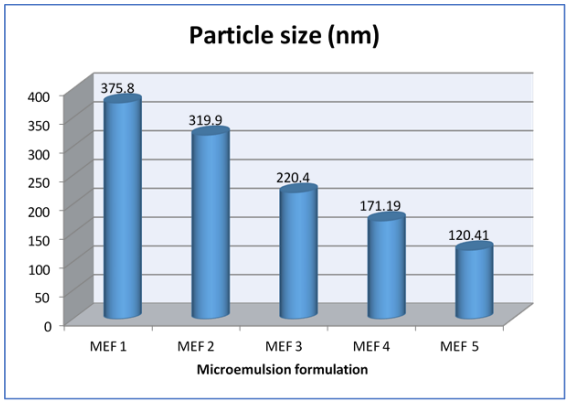
Graph 8: Particle size (F3)



Graph 9: Particle size (F4)

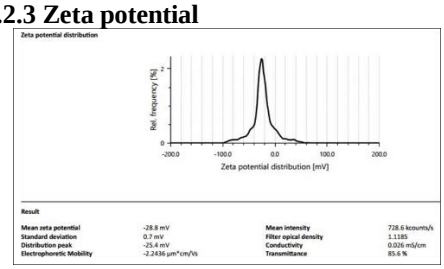


Graph 10: Particle size (F5)

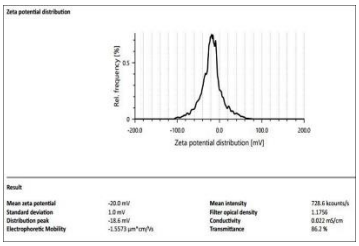


Graph 11: Particle size of all formulation.

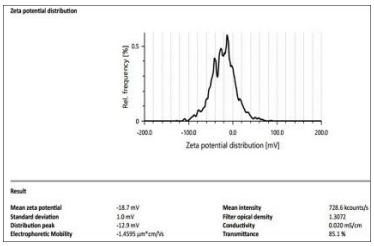
3.2.3 Zeta potential



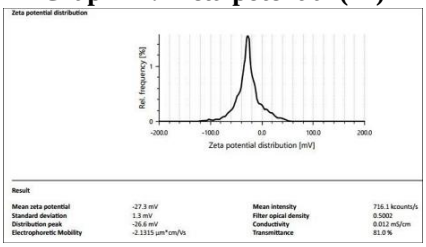
Graph 12: Zeta potential (F1)



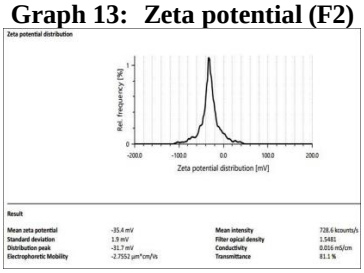
Graph 13: Zeta potential (F2)



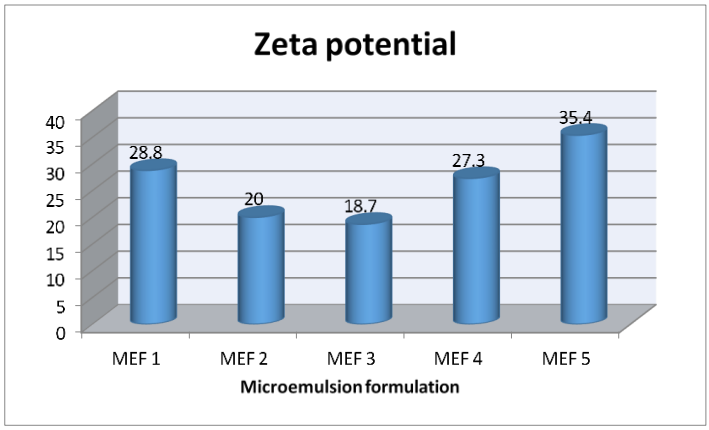
Graph 14: Zeta potential (F3)



Graph 15: Zeta potential (F4)



Graph 16: Zeta potential (F5)



Graph 17: Zeta Potential Of All Formulation.

3.2.4 Scanning electron microscope (SEM)

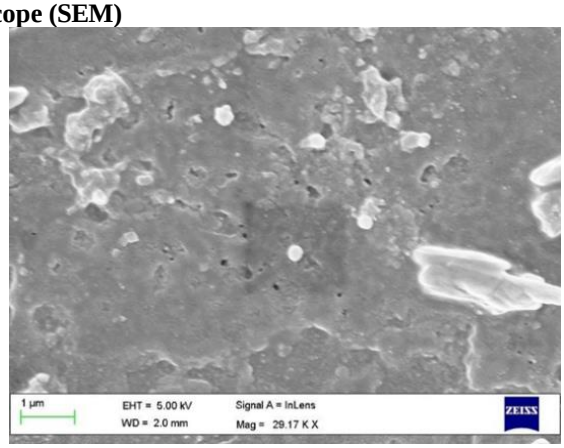
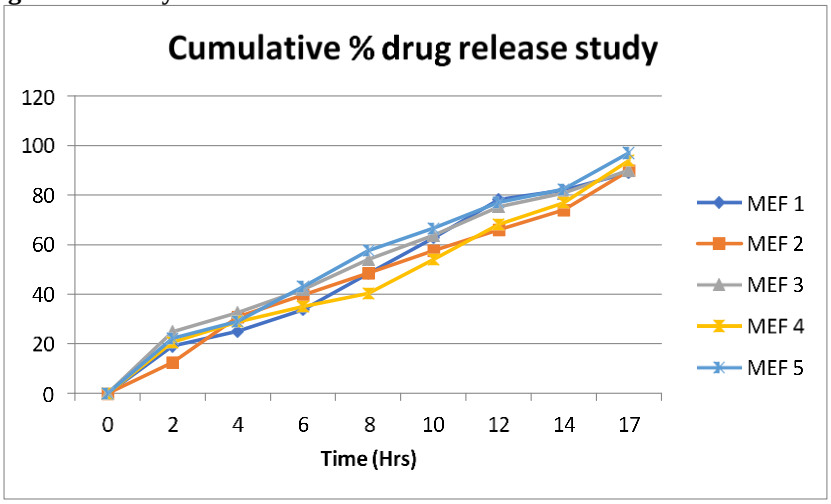


Figure 1: Scanning electron microscope (SEM)

Table 8: *In vitro* drug release study of all formulations.



Graph 18: Drug release study of all formulations.

3.3 Evaluation parameter of micro-emulsion gel formulation

3.3.1 Organoleptic properties

Table 8: Organoleptic properties.

Parameters	**Results**
Physical appearance	Semisolid gel
Colour	White
Homogeneity	Absence of aggregates

3.3.2 Measurement of pH, Viscosity, Skin irritation study and Spreadability

Table 9: Measurement of pH, Viscosity, Skin irritation study and Spreadability.

Formulation	**pH**	**Viscosity**	**Skin irritation study**	Spreadability test
Micro-emulsion gel	6.8	4531±0.89	Not irritant observed	13.09

3.4 Results of antimicrobial activity

3.4.1 Antimicrobial activity of Microemul Gel formulation

Table 10: Antimicrobial activity of Microemul Gel formulation (C1, C2, C3 and C4 Different Concentration)

Sample name	**Zone of Inhibition (mm)**
C1(control)	0.0mm
C2 (0.05mg/ml)	8 mm
C3 (0.1mg/ml)	11 mm
C4 (1.00 mg/ml)	16 mm

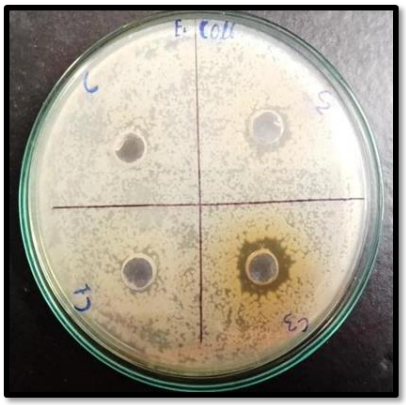
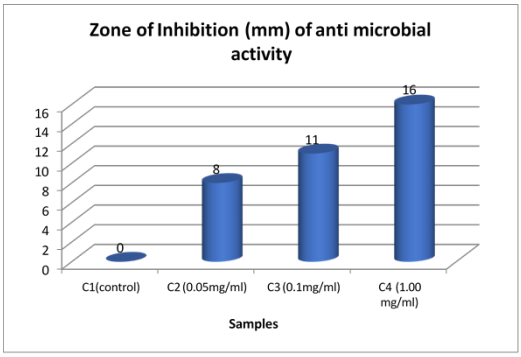


Figure 2: Antimicrobial Activity.



Graph 19: Graphical representation of antimicrobial activity.

DISCUSSION

The formulated drug-loaded microemulsion was thoroughly characterized to assess its physicochemical properties, including encapsulation efficiency, zeta potential, and particle size distribution, which confirmed its stability and uniform dispersion. SEM analysis revealed smooth and uniform droplet morphology, supporting the formation of a stable system. The microemulsion was then converted into a microemulgel and evaluated for pH, viscosity, spreadability, and skin compatibility, all of which indicated suitability for topical application. *In vitro* drug release studies showed a sustained release pattern, and kinetic analysis confirmed a controlled release mechanism. The antimicrobial assay demonstrated significant inhibitory activity against tested microbial strains, validating the formulation's potential as an effective topical therapeutic system.

4. CONCLUSION

The pre-formulation studies provided a comprehensive knowledge of the chemical and physical characteristics of the medication, establishing a solid foundation for its further development. The microemulsion and microemulgel formulations were successfully developed and characterized, with key parameters indicating stability and appropriate characteristics for the delivery of drugs. The antimicrobial activity testing demonstrated the potential effectiveness of the formulation as a treatment against bacterial strains. Overall, the results confirmed that the formulated microemulgel could offer a potential substitute for medication delivery methods, exhibiting suitable properties for effective application in topical treatments.

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