IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2021 Vol.:21, Issue:2

Formulation and Evaluation of Levofloxacin Loaded Topical Microemulsion with Eucalyptus Oil

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Submitted: 22 April 2021
Accepted: 29 April 2021
Published: 30 May 2021

www.ijppr.humanjournals.com

Keywords: Levofloxacin, Eucalyptus oil, Microemulsion (ME), Microemulgel (MEG), Pseudo ternary phase diagram, antimicrobial effect

ABSTRACT

The aim of the current study is to formulate the levofloxacin loaded microemulsion (ME) using eucalyptus oil. Pseudo ternary phase diagram was constructed by water titration method using oil, surfactant mixture and water to find out the region of microemulsion. We formulated six different formulations (ME1 – ME6) by changing the oil/surfactant and cosurfactant ratio. The developed microemulsion formulation was characterized by various parameters like % Transmittance, viscosity, pH, drug content, surface morphology, zeta potential, and in-vitro drug release study. The optimized formulation is further converted into Microemulgel by dispersing the ME into 2% w/w Carbopol gel (MEG) and further evaluated for various parameters. The antimicrobial efficacy was carried out for ME and Microemulgel by well diffusion method against *Staphylococcus aureus* (MTCC: 737) and compared with streptomycin which showed ME and MEG have a better antimicrobial effect than standard streptomycin. It was concluded that the microemulsion system studied is a promising tool for the topical delivery and levofloxacin be formulated as microemulsion with good release and consistency.

INTRODUCTION

Emulsions are viscid, multiphase systems in which a liquid is dispersed throughout another liquid in the form of small droplets. Microemulsions are thermodynamically stable isotropic systems in which two immiscible liquids (water and oil) are mixed to form a single phase by means of an appropriate surfactant or its mixture. The short to medium chain alcohols are generally considered as co-surfactants in the microemulsion system. The presence of surfactant and co-surfactant in the system makes the interfacial tension very low. Therefore, microemulsions form spontaneously, with an average droplet diameter of 10 to 140 nm. Microemulsions have the ability to deliver larger amounts of water and topically applied agents into the skin than water alone or other traditional vehicles such as lotions or creams because they act as a better reservoir for a poorly soluble drug through their capacity for enhanced solubilization [1].

Topical drug delivery can be defined as application of drug via skin to treat the skin disorders. Transdermal drug delivery systems (TDDS) are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation. A transdermal patch or skin patch is a medicated adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and into the bloodstream. These systems are generally used for local skin infection like fungal and microbial infection or in place where other routes of the drug administration fail. These preparations are applied onto the skin surface for providing local or systemic effects. Topical route is safe and effective route to deliver the drug molecules with lower doses as compared to the conventional system. [2].

Levofloxacin is a broad-spectrum, 3rd generation fluoroquinolone antibiotic and optically lively L-isomer of ofloxacin with antibacterial activity. Levofloxacin diffuses through the bacterial cell wall and acts by way of inhibiting DNA gyrase (bacterial topoisomerase II), an enzyme required for DNA replication, RNA transcription, and restoration of bacterial DNA. Inhibition of DNA gyrase hobby ends in blockage of bacterial cell boom.

Levofloxacin is indicated for the treatment of easy pores and skin shape infections (mild to mild) together with abscesses, cellulitis, furuncles, impetigo, pyoderma, wound infections, because of methicillin-willing Staphylococcus aureus, or Streptococcus pyrogens. Levofloxacin is a fluoroquinolone antibacterial agent which shows a massive spectrum of activity against Gram-negative and Gram-positive microorganisms and respiratory pathogens.

Continuous oral administration of Levofloxacin causes severe side effects to overcome this bother, various dose systems inclusive of lip gels, amphiphilo gels, hydrogels, emulsions, microemulsions, emulgels, microemulsion gels, and liposomal gels have been used [3].

Plant essential oils and their predominant chemical components are potential candidates as antibacterial dealers. several varieties of essential oils and their main chemical ingredients from various medicinal aromatic plants had been said to possess a huge variety of bacterial inhibitory potentials. The plant's bloom leaf is utilized for each improvement and from which the fundamental oil is separated. The attributes of its fundamental oil are its unpredictable nature and stable aromatic scents. In this way, the eucalyptus essential oil has been normally utilized in fragrance-based treatment and beautifying agents. it's been likewise utilized as a feature sedative in dentistry. moreover, eucalyptus oil changed into compelling towards Mycobacterium tuberculosis and methicillin-resistant Staphylococcus aureus (MRSA), viruses, and fungi including Candida [4] Eucalyptus oil is available as an important oil that is used as a medicinal drug to treat a diffusion of not unusual sicknesses and conditions inclusive of nasal congestion, asthma, and as a tick repellent. Diluted eucalyptus oil may also be carried out to the skin as a remedy for health problems inclusive of arthritis and pores and skin ulcer [5].

MATERIALS AND METHODS

Materials

Levofloxacin was acquired as a gift sample from ontop pharma. Ltd. Bangalore. Tween 20, Tween 80 and Propylene glycol (SD-Fine substance ltd, Mumbai), Eucalyptus oil (Sunil Herbal stores, Mysuru).

Method of preparation

a) Development of pseudo ternary stage outline:

To find out the existence range of microemulsions, pseudo ternary phase diagrams were constructed using water titration method at ambient temperature (25 ºC). Based upon on the available solubility profile of the drug, Eucalyptus oil was selected as an oil phase; tween 20, tween 80 and propylene glycol were used as surfactant and co-surfactant respectively. The smix (surfactant + Co-surfactant) ratios were selected to be 1:1, 2:1 and 3:1 w/w and used. For each phase diagram at specific smix concentration of the Eucalyptus oil added from the

range of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 (%w/w) and the mixture were diluted with distilled water by sequential addition of 0.1 ml of water. Water was added drop by drop while mixing on a magnetic stirrer at room temperature, and the samples were marked as being optically clear or turbid. The microemulsion regions were identified as transparent and isotropic mixtures. The percentage of three different phases, that is oil, water, and the mixture of surfactant and co-surfactant were calculated (Table 1). From the endpoint compositions of titrated samples, the mass percent composition of the components like oil, smix and water was calculated and then plotted on triangular coordinate to construct the pseudo ternary phase diagram.

Table 1: Formulation development of Eucalyptus oil-based Levofloxacin microemulsion with selected percentages of Oil, Smix, and Water from the Pseudo ternary Phase.

Formulation code	Smix ratio	Surfactants	Oils	Percent w/w component in formulation			
				Oil %	Smix %	Water%	Drug %
ME1	1:1	**Tween 80**	**Eucalyptus oil**	18	62	20	0.5
ME2	2:1			25	65	10	0.5
ME3	3:1			22	68	10	0.5
ME4	1:1	**Tween 20**	**Eucalyptus oil**	40	40	20	0.5
ME5	2:1			20	65	15	0.5
ME6	3:1			25	50	25	0.5

b) The Solubility of Levofloxacin:

The Solubility was performed for the oil, surfactants, and co-surfactant for forming microemulsion. The solvency of the Levofloxacin in oil is a fundamental advance for the microemulsion plan. So before building the stage outline one should need to choose the oil, surfactant, and co-surfactant in which the medication shows the most extreme solvency, to be in the ideal dissolvability range, which is fundamental for the detailing of a microemulsion drug conveyance framework. The powder medication of Levofloxacin has included overabundance to every one of the oils, surfactants (S), cosurfactant (CoS), and afterward vortexed for blending. After vertexing, the samples were saved for 72 hours at the surrounding temperature for achieving harmony. The equilibrated tests were then centrifuged

at 5000 rpm for 30 minutes to eliminate the undissolved medication. The supernatant was taken and weakened with methanol and saw by UV spectrophotometric strategy at 288 nm [6].

c) Formulation Levofloxacin microemulsion:

Microemulsion (ME1- ME6) was prepared by high-energy emulsification method by high-pressure homogenization technique. eucalyptus oil, Surfactant, and co-surfactant were mixed thoroughly by vortex mixture. To the uniform mixer required quantity of water, added and homogenized by high pressure homogenize for 10min at 6,000rpm. The prepared Microemulsion will be stored properly and the optimized formulation will be incorporated in a suitable gel base (Carbopol 2 % w/w). Both ME and MEG were evaluated for various parameters [7].

EVALUATION OF LEVOFLOXACIN MICROEMULSION [8-13]

The prepared microemulsion formulation were characterized for parameters like Drug content, Particle size analysis, Determination of viscosity, Surface morphology, FT-IR analysis, Zeta potential. *In vitro* drug release and *in vitro* antimicrobial activity. The optimized formula was incorporated into 2% Carbopol gel and evaluated for spreadability, rheological property, pH and *In vitro* drug release, and *in vitro* antimicrobial activity.

***In-vitro* release studies**

An invitro diffusion study carried out by using egg membrane as a diffusion layer which is placed between the donor and receptor compartment. Microemulsion gel equivalent to 1gm was placed in the donor compartment and the receptor compartment is filled with the phosphate buffer of 7.4pH. The diffusion cells were maintained at 37±0.5°C with the stirring 100rpm throughout the experiment. At the specific time interval 5ml of sample to be withdrawn every 1, 2, 3, 4, 5 and 6hrs from the receiver compartment through side tube and analysed by UV spectrophotometer at λ max 288nm [14].

***In-vitro* antibacterial studies:**

Preparation of samples: Given samples were dissolved in 100% DFM.

Preparation of inoculums

Bacterial strain *Staphylococcus aureus* (MTCC: 737) was transferred from stock solution to LB agar and incubated overnight at *37°C*.Single colony from plate was transferred to the LB

broth and incubated at 37°C, for 24 h, and used as inoculums. The turbidity of the bacterial suspension was adjusted spectrophotometrically (range of 0.5–1.0) to the McFarland 0.5 turbidity standard (1.5×10^8 CFU/mL).

Antimicrobial activity by well diffusion method

Antimicrobial activity of given samples was investigated using well diffusion method. Test plates (diameter 10 cm) were prepared with 20 mL of LB agar (LBA). After media get solidified, 100 µl of 24 h bacterial culture (1.5×10^8 CFU/mL) was added and uniformly spread over plates using L shaped loop. Then well was drilled about 6mm in diameter and in those wells add 50 µL different concentration of the given samples 50ug/ml drug, 80ug/ml gel and 80 ul of formulations are added. The wells loaded with sterile media considered as Blank 20ug in 40 ul streptomycin was used as a standard. After loading plates were kept in sterile condition until complete absorption of the test compounds. Plates were incubated at 37°C in an appropriate gaseous condition for 24 hrs. Zones of inhibition of microbial growth around the well were measured and recorded after the incubation time. The inhibitory zone was considered the shortest distance (cm) from the outside margin of the samples to the initial point of the microbial growth [15].

RESULT AND DISCUSSION

Different proportions of surfactants (Tween 20, Tween 80)/Co-surfactant (Propylene glycol) were utilized to develop the pseudo ternary stage charts. The smix weight proportions [1:1, 2:1, 3:1] are addressed in Figure 1 to Figure 2 in pseudo-ternary stage graph where microemulsion regions are noticed by using Ternary plot.com software. The optimized microemulsion ME3 was formulated into a gel by the use of Carbopol 934 gels containing 2% w/w gel was found to be suitable for gelling the microemulsion because of desirable consistency. And the optimized formulation was further evaluated for spreadability, viscosity, pH, and percentage assay as shown in Table 6.

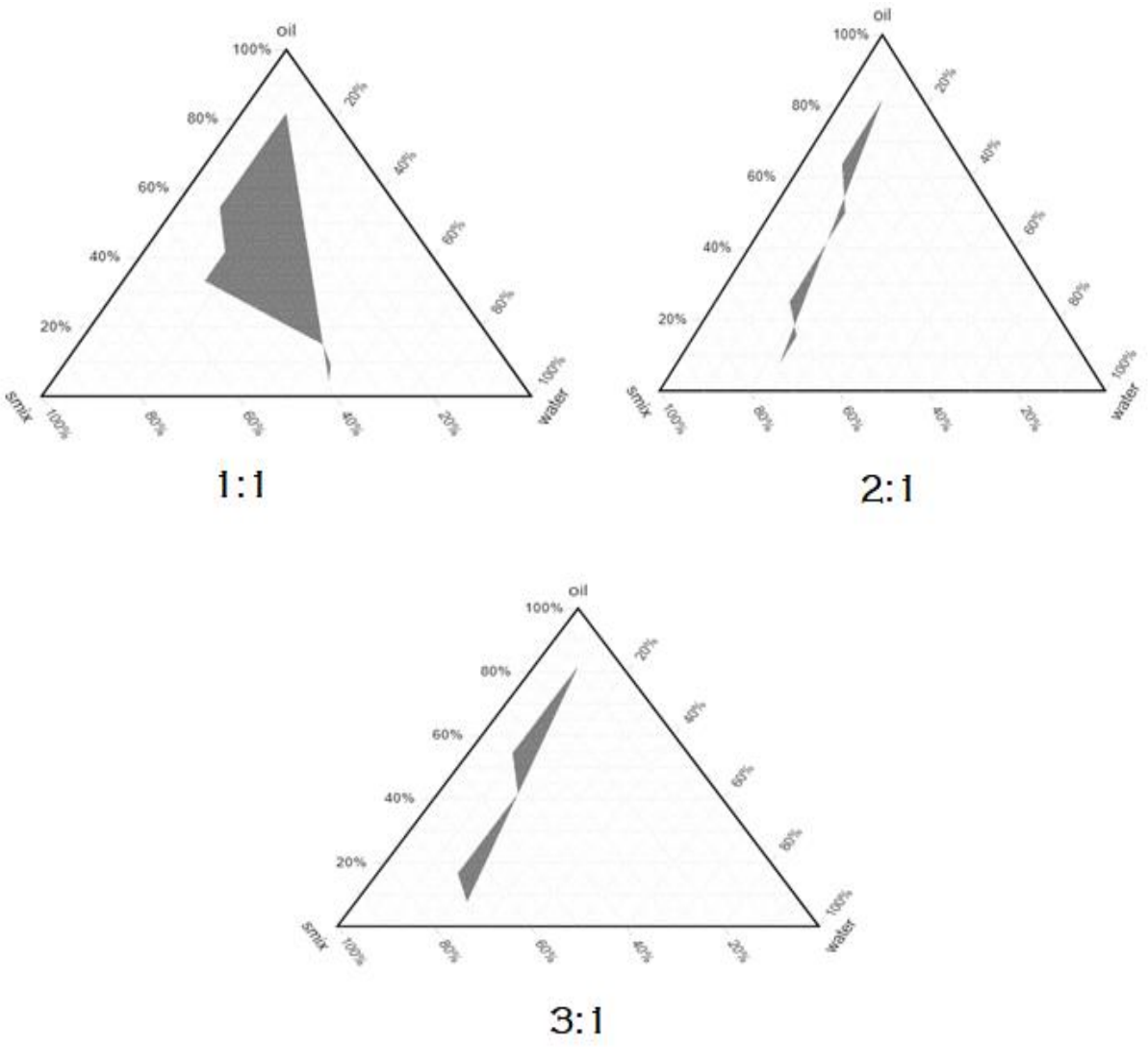

Figure 1: Pseudo ternary phase diagram using Eucalyptus oil as oil, Tween 80 as surfactant, propylene glycol as co-surfactant and water (Tween 80: Propylene glycol = 1:1, 2:1 and 3:1).

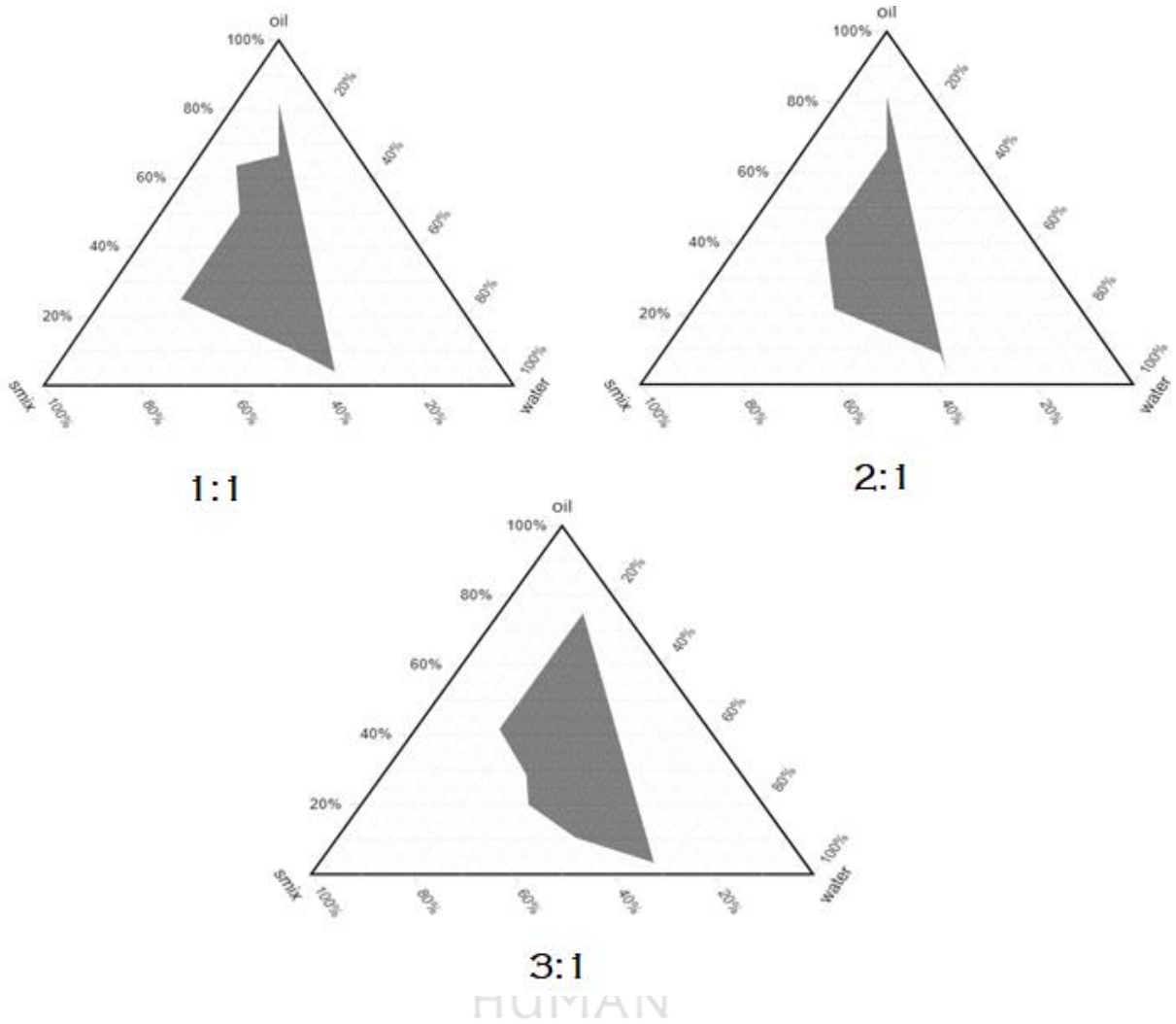

Figure 2: Pseudo ternary phase diagram using Eucalyptus oil, Tween 20 as surfactant, propylene glycol as co-surfactant and water (Tween 20: Propylene glycol = 1:1, 2:1 and 3:1).

The solubility of Levofloxacin was found to be in Eucalyptus Oil (80 ± 0.075mg/ml). Furthermore, the maximum solubility of Levofloxacin in surfactants was found in Tween 80 (90.55 ± 0.279mg/ml), Tween 20 (88.73 ± 0.370 mg/ml) and co-surfactant propylene glycol (89.25 ± 0.083 mg/ml) and also soluble in pH 7.4 phosphate buffer (110 ± 0.029 mg/ml) as shown in Table 2.

Table 2: Solubility analysis of Levofloxacin

Phase type	Excipient	Solubility mg/ml
Aqueous	Water	1.44 ±0.065
Oil	Eucalyptus Oil	80 ± 0.075
	mustard Oil	40 ± 0.270
Surfactant	Tween 20	88.73 ± 0.370
	Tween 40	85.23 ± 0.438
	Tween 80	90.55 ± 0.279
Co-Surfactant	Propylene glycol	89.25 ± 0.083
	PEG 400	78.23 ± 0.177
Phosphate Buffer	pH1.2	12 ± 0.317
	pH 4.4	67.00 ± 0.150
	pH 6.8	90 ± 0.191
	pH 7.4	110 ± 0.029

The drug content of all the formulations of Levofloxacin microemulsion is shown in Table 3. ME3 was exhibited 98.52±0.396% higher drug content than other formulations. The microemulsion drug content of all formulations was found to be within the range of 88.94 to 98.52% which was within the limits of USP specifications. The prepared Levofloxacin microemulsion gel ME3-G was subjected to drug content uniformity. The microemulsion gel was in the permissible range of 94.54 % it indicated the drug uniformly dispersed throughout the formulation. (Table 6).

Table 3: Determination of % transmittance, viscosity and pH, and % drug content of the microemulsion formulation

Formulation code	Transmittance	Viscosity cps	pH	% drug content
ME1	95.7 ±0.441	16.274±0.121	5.733±0.503	91.23±0.121
ME2	93.196 ±0.867	15.309±0.236	6.566±0.441	92.04±0.236
ME3	98.6 ±0.503	11.583±0.327	6.433±0.386	98.52±0.396
ME4	94.333 ± 0.853	16.764±0.955	6.8±0.55	89.32±0.442
ME5	93.74 ± 0.38	18.016±0.546	6.566±0.445	93.56±0.952
ME6	92.213 ±0.883	20.444±0.852	6.1±0.417	88.94±0.546

All the prepared formulations were checked for their pH. All the formulations were showing pH in the range of 5.7 to 6.8 as shown in Table 3. This is well in the range for topical administered formulation. The pH value of optimized microemulsion formulation ME3 was 6.433 ± 0.386(Table 3) and is suitable for topical as well as a transdermal application because of the pH of the skin in the range of 5.5 to 7.0. The pH of microemulsion gel ME3-G gel was found to be 6.4 ± 0.19. (Table 6) and is suitable for topical as well as transdermal application.

The clarity of the microemulsion formulation was checked by % transmittance. All formulations of transmittance values are above 90% as shown in Table 3, which indicates that the microemulsions were transparent which is considered as the primary property of a microemulsion. The ME3 formulation showed 98.6 ±0.50% compare to other formulations.

The viscosity of microemulsion formulation was determined as shown in Table 3, all samples exhibited Newtonian flow behaviour and formulation ME3 showed 11.58±0.37cps shows less viscous compared to other microemulsion formulations. And the optimized gel ME3-G viscosity was found to be 6827.92 ± 77.14cps.

The surface morphology was studied by SEM for the optimized formulations which were confirmed that the particles are globular with globule size in the nanometre scale with a smooth surface as shown in Figure 3, for ME3. This can have ability to form microemulsion.

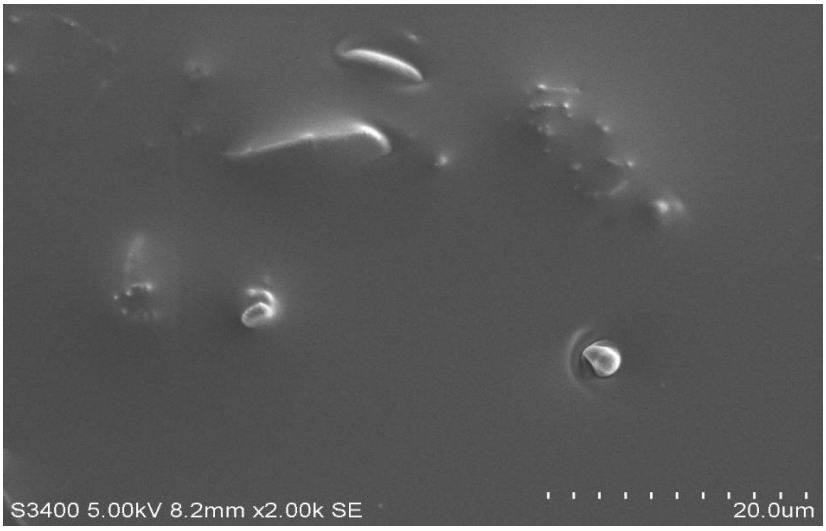

Figure 3: SEM image of ME3

The particle size and zeta potential were measured by a Marwin zeta analyzer and it was Found that 46.45nm for ME3. Confirmed that ME are within the required size ranges confirmed formation of ME. The Zeta potential of microemulsion ME3 was found to be 31.07 Mv (Fig 4) which shows that they are adequate to be stable.

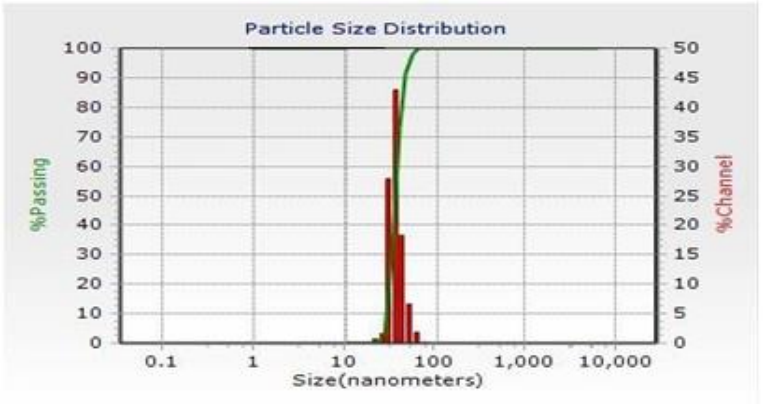

Figure 4: Result of particle size of the formulation ME3.

FTIR Spectrum of Levofloxacin was obtained by scanning the drug in the range of 4000 to 400. Major peaks observed were as 3265.45/3273.31cm^{-1}(COOH) ,1724.42/1735.99 cm^{-1} (C=O), 2933.83/2926.11 cm^{-1}(CH3) ,1446.66/1410.01cm^{-1}(C-F), 2847.03/2872.10cm^{-1}(CH2), 1464.66/1460.16 cm^{-1}(C-N) whose presence resembled the structure of Levofloxacin. Observed FTIR spectra and standard values were as depicted in Fig. 5.1,5.2 and Table 4. The observed value was within the range or very close to the characteristic peaks of standard value confirming the drug as Levofloxacin. And there is no interaction between drug and other components.

Table 4: FTIR comparison of characteristic peak of pure drug and formulation

Functional group	Wave number (cm^{-1}) of pure drug	Wave number (cm^{-1}) of
COOH	3265.45	3273.31
C=O	1724.42	1735.99
CH3	2933.83	2926.11
C-F	1446.66	1410.01
CH2	2847.03	2872.10
C-N	1464.66	1460.16

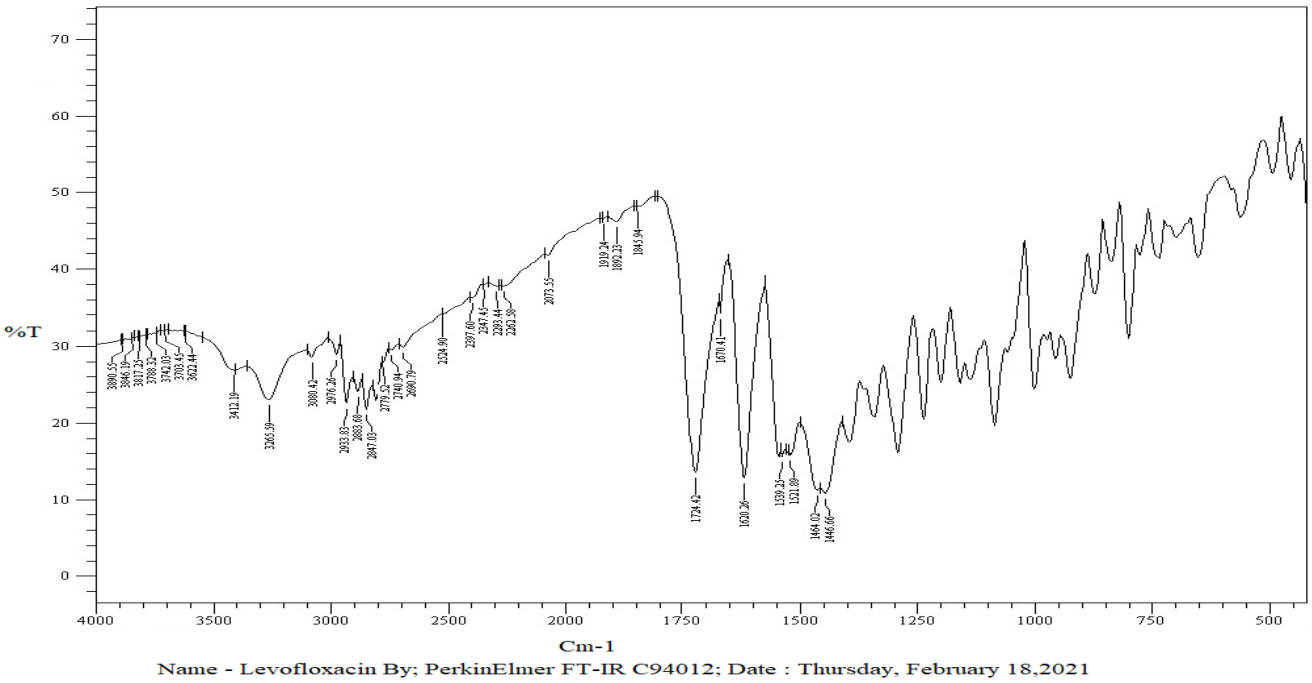

Figure 5.1: FTIR spectra of Levofloxacin

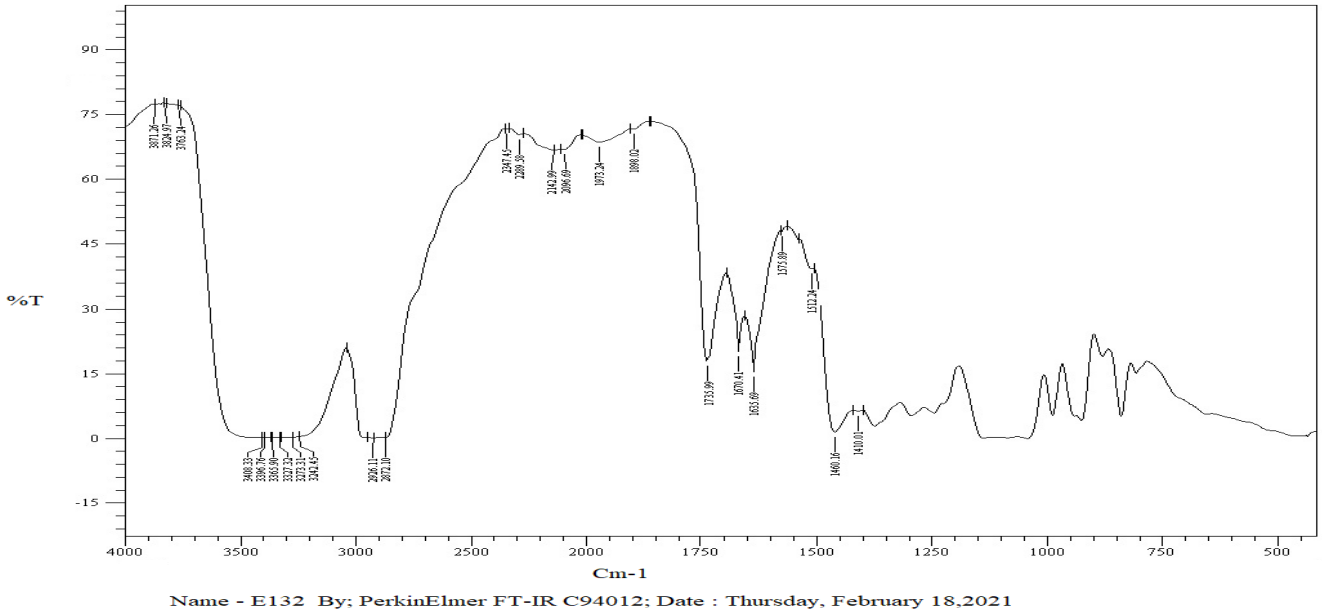

Figure 5.2: FTIR spectra of ME3 formulation

From the *in vitro* release studies, we observed that 0 - 20% of the drug was delivered in 1hrs and over half drug released in 3 hrs, and more than 80% of the drug realised in 6 hrs. The formulation of ME3 showed 95.93% (Figure 6) And it has shown a higher % of medication discharge when compared with other formulation (Table 5). The result of the in-vitro release of Levofloxacin from the gel formulation. However, the results clearly show that the gels can retain the drug for prolonged periods. The % CDR of microemulsion gel formulation ME3-G was found to be 70.93%, respectively as shown in Figure 7.

Table 5: *In-vitro* diffusion study of Eucalyptus oil microemulsion

Time in hrs	% Cumulative drug release					
	ME1	ME2	ME3	ME4	ME5	ME6
0	0	0	0	0	0	0
1	21.739	15.826	16.695	13.814	12.521	12.783
2	26.304	21.897	29.732	39.931	33.69	39.921
3	44.391	25.506	54.375	52.288	52.808	60.525
4	51.569	65.223	60.299	60.263	57.022	70.635
5	58.856	69.789	75.476	77.405	67.211	84.765
6	80.382	74.395	95.935	82.701	75.295	87.701

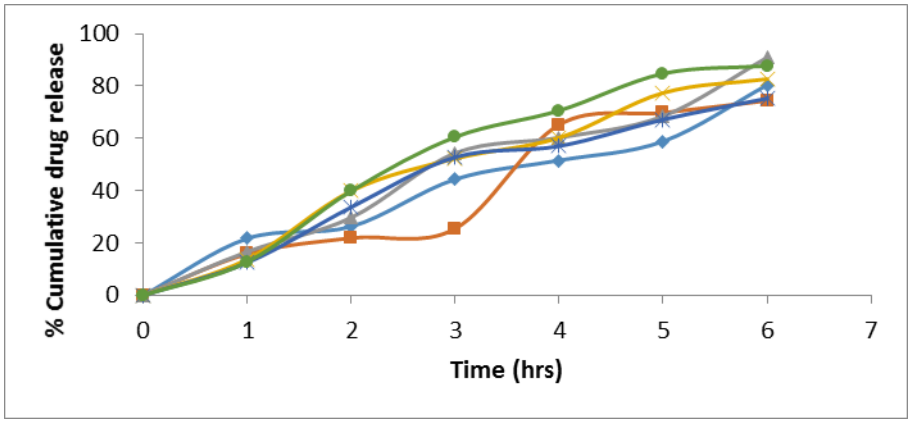

Figure 6: Comparison of % cumulative drug release of ME1-ME6

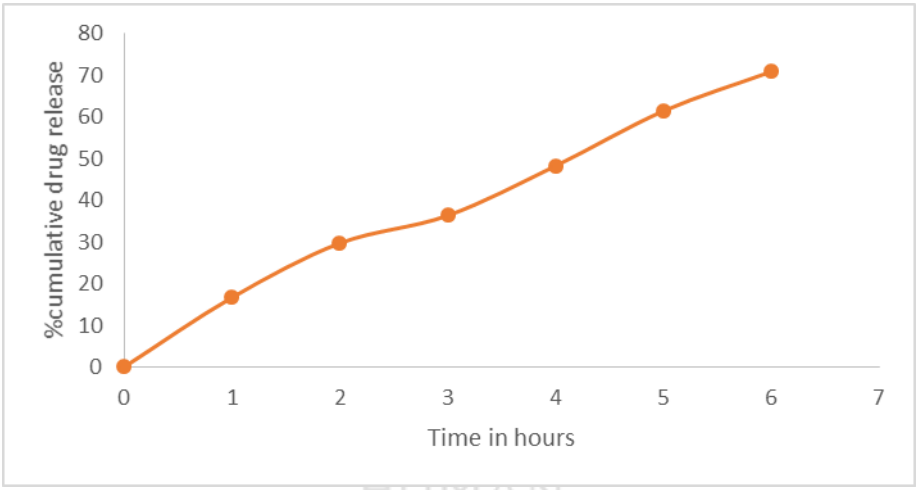

Figure7: % cumulative drug release of ME3-G1

The spreadability is an important property of topical formulation from a patient compliance point of view. Increase in the diameter due to spreading of the formulation ME3-G was 7.4 ± 0.05. (Table 6). The viscosity of the gels of microemulsion formulations ME3-G was determined and 6827.92 ± 77.14cps. (Table 6).

Table 6: Viscosity, pH and % drug content of microemulsion gel

Formulation code	Spreadability	Viscosity	pH	% drug content
ME3-G	7.4 ± 0.05	6827.92 ± 77.14	6.4 ± 0.19	94.54 ± 0.23

In-vitro antimicrobial effect to evaluate the efficacy of optimized formulations, oils, and drugs against antimicrobial evaluation was carried out using bacterial strain *Staphylococcus aureus (MTCC: 737).* The antimicrobial activity by the well-diffusion method was performed

at a concentration of 80µg/ml gels and sterile media as blank, streptomycin as standard placed in well and measured zone of inhibition. *Staphylococcus aureus* was used as a standard bacterium that shown in Fig. 8. The zone of inhibition was to be for drug 2.8 cm (50µg/ml), standard 1.6cm (40 µl) eucalyptus oil 1.8cm (20 µl), Microemulsion ME3 4.2cm(80 µl), Microemulsion gel ME3 3.8cm (80 µg/ µl) shown in (Table 7). The, ME3 and ME3-G shows greater antimicrobial effect compare to standard (Fig 8).

Table 7: Antimicrobial effect of microemulsion formulation and oils against *Staphylococcus aureus*

Sample	**Components**	**quantity**	**Zone of inhibition in cm**
Drug	Levofloxacin	50ug/ml	2.8
Standard	Streptomycin	40 ul	1.6
Oil	Eucalyptus	20 ul	1.8
	Mustard	20 ul	2.2
Microemulsion	ME3	80 ul/ml	4.2
	M131	80 ul/ml	3.8
Microemulsion gel	ME3	80ug/ml	3.8
	M131	80ug/ml	2.6

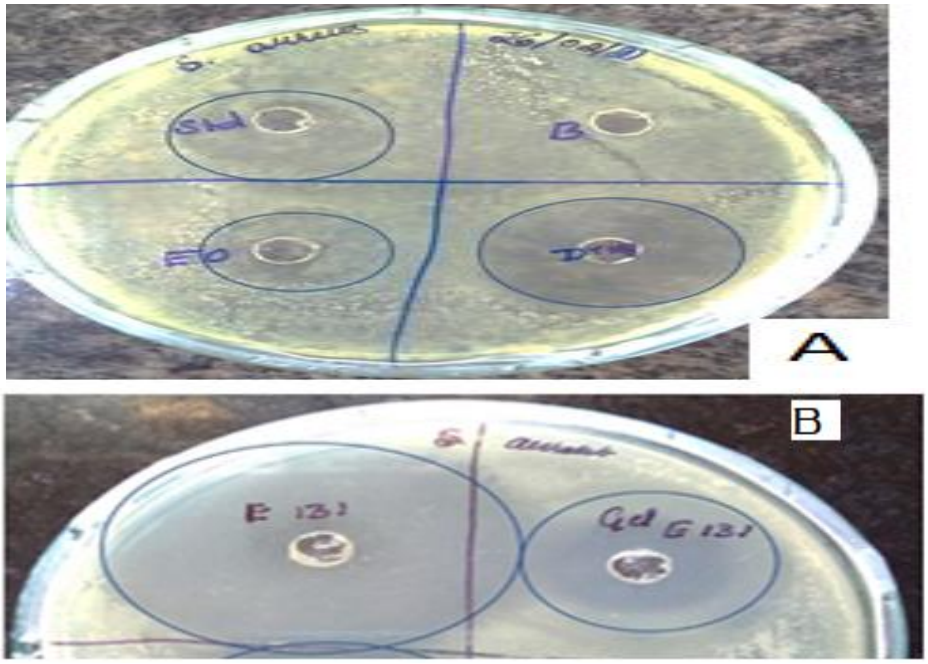

Figure 8: The Antibacterial activity of (A) Eucalyptus oil with drug (B) Microemulsion ME3 and gel ME3-G against *Staphylococcus aureus* using well-diffusion method.

CONCLUSION

A topical microemulsion was formulated using levofloxacin and eucalyptus oil by high-energy emulsification method high-pressure homogenization technique the components of microemulsion and their concentration ranges were obtained by construction of pseudo ternary phase diagrams. The formulated microemulsions were undergone few evaluation tests to obtain the best optimized formulation to formulate microemulgel by incorporating the optimized formulation in 2%w/w Carbopol gel. The formulated microemulsion and micromeulgel exhibited the better antibacterial activity against *staphylococcus aureus* than standard. Levofloxacin shows better antibacterial effect in the presence of eucalyptus oil. Hence, we concluded that the synergistic effect could be achieved by both eucalyptus oil and levofloxacin drug by microemulsion formulation with deeper skin penetration effect.

ACKNOWLEDGEMENT

The author is thankful to the Dr. Parthiban S. HOD Department of pharmaceutics and Bharathi College of Pharmacy, Bharathinagara, Karnataka, for their support and encouragement in writing this research.

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