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Research Article

DEVELOPMENT OF LEVOFLOXACIN LOADED MICROEMULSION FORMULATED WITH MUSTARD OIL

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ABSTRACT

The aim of the current study is to develop Levofloxacin loaded microemulsion formulated with mustard oil for better skin penetration effect and to obtain combined effect of both the oil and the drug to improve the efficacy with a reduced dose. Primarily we developed a pseudo ternary phase diagrams to find out the region of microemulsion formation zone by using oil (mustard oil), surfactant (Tween 20 and Tween 80), and co-surfactant (propylene glycol) by water titration method. We formulated six different formulations (MM1-MM6) by varying the concentrations of the oil water and surfactant and cosurfactant ratio. The developed microemulsion formulation was characterized for various parameters % Transmittance, viscosity, pH, drug content, surface morphology, zeta potential, and in-vitro drug release study. The selected microemulsion formulation is further converted in to microemulgel by dispersing the ME into 2%w/$_W$ Carbopol gel (MM-G) and various parameters are evaluated for the gel. The antimicrobial efficacy was carried out for ME and Microemulgel by well diffusion method against *Staphylococcus aureus* (MTCC: 737) compared with the standard streptomycin which showed that MM3 and MM-G have a better antimicrobial effect than standard proved that the drug levofloxacin and the mustard oil shows the synergistic effect in the Microemulsion formulation with better skin penetration effect.

KEYWORDS: Levofloxacin, mustard oil, Microemulsion (ME), Microemulgel, Pseudo ternary phase diagram, antimicrobial effect.

INTRODUCTION

Topical delivery has become an important means of drug delivery. Delivery of drugs to skin for systemic and local effect is called topical delivery. Topical delivery involves in the availability of drug molecules continuously from the surface, through its layers, and maintain a constant concentration [1]. These systems are generally used for local infections like fungal, microbial infections or when the other route of administration fails. Topical route favours safe and effective delivery of drug molecules with lower doses as compared to the conventional system [2].

Emulsions are viscid, multiphase frameworks in which at least one fluid are scattered all through another immiscible fluid as little drops. At the point when oil is the scattered stage and a fluid arrangement is the consistent stage, the framework is assigned as an oil-in-water emulsion. Alternately, when water or a watery arrangement is the scattered stage and oil or oleaginous material is the nonstop stage, the framework is assigned as a water-in-oil emulsion. Emulsions are settled by emulsifying agents, the converging of little beads into bigger drops, and eventually, into a solitary isolated stage. Emulsifying specialists (surfactants) act by aggregating at the interface between the immiscible fluids, in this way giving an actual boundary that lessens the inclination for blend. Surfactants additionally lessen the interfacial pressure between the stages, working with the development of little drops after blending.[3]

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 in individual patients 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 with a massive spectrum closer to Gram-negative and Gram-positive microorganisms and respiratory pathogens. It active against every penicillin-inclined and penicillin-resistant *Streptococcus pneumoniae*. 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.[4] Essential oils (EOs) which are concentrated natural bioactive compounds with strong smell have been greatly appraised for same. Furthermore, aromatherapeutic literature has identified a number of Essential oils used against dermatological infections, thus highlighting the efficacy of Essential oils against pathogens responsible for infections. Moreover, due to the presence of a wide range of compounds, the antimicrobial activity of Essential oils can be attributed to multiple mechanisms rather than a single one. For instance, involving different biochemical and structural mechanisms at various sites on the cell surface as well as within the cell). Interestingly, several investigations have also confirmed the effectiveness of EOs against multidrug resistant bacteria [5]. The antibacterial activity of mustard Essential oil is probably due to the ability of its components to disrupt the membranes of bacterial cells, causing lysing of the cell. According to the Essential oil could inhibit bacteria by disintegration outer membrane and by the release of outer membrane-associated materials from the cells to the external medium. Mustard oil is also used to be in the cosmetic treatment.[6]

In this study, we tried to develop a new formulation of levofloxacin in microemulsion formulated with mustard oil for topical application which may lead to improvement in patient compliance. Microemulsions containing levofloxacin were formulated and examined for in-vitro drug release and antimicrobial studies.

MATERIALS AND METHOD

levofloxacin was acquired as a blessing gift from Ontop Pharma Pvt Ltd, Bengaluru. Tween 20, Tween 80 and Propylene glycol (SD-Fine substance ltd, Mumbai), mustard oil (Sunil Herbal stores, Mysuru).

Development of pseudo ternary stage outline

The pseudo ternary stage graph was utilized to discover the present scope of microemulsions, and stage outlines were built utilizing the water titration strategy at encompassing temperature (25 ºC). Given the accessible dissolvability profile of the medication. The mustard oil was chosen as an oil stage; Tween 20, Tween 80 were utilized as a surfactant, and Propylene glycol was utilized as a co-surfactant. The Smix (surfactant + Co-surfactant) proportions were chosen to be 1:1, 2:1, and 3:1 w/w and utilized. For each stage chart at explicit Smix focus and mustard oil was added from a scope of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 (%w/w) and the blend were weakened with refined water by the consecutive option of 0.1ml of water. The water was added drop by drop while blending on an attractive stirrer at room temperature, and the examples were set apart as being optically clear or turbid. The microemulsion areas were recognized as straightforward and isotropic combinations. The level of three unique stages oil, water, and a combination of surfactant and co-surfactant were determined (Table 1). From the endpoint synthesis of titrated tests, the mass percent piece of the segments like oil, Smix, and water was determined and afterward plotted on a three-sided organize to build the pseudo ternary stage diagram.[7]

The Solubility of levofloxacin

The Solubility was performed for the oil, surfactants, and co-surfactant for forming microemulsion shown in Table2. 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.[8]

Formulation levofloxacin microemulsion

Levofloxacin microemulsion was formulated using water titration method. Predetermined amounts of the drug were dissolved in the required quantity of oil. Surfactant and co-surfactant were blended in a fixed proportion and add the water dropwise shown in Table 1. which resulted in the formulation of a transparent and homogenous microemulsion. Then the prepared microemulsion formulation incorporated to 2% w/w carbopol 934. And evaluated the parameters like pH, viscosity, % drug content particle size and zeta potential, Surface morphology, compatibility studies, centrifugation [9].

EVALUATION OF LEVOFLOXACIN MICROEMULSION

The prepared microemulsion formulation was characterized for parameters like Drug content, Particle size analysis, melting point, viscosity, Surface morphology, FT-IR analysis, Zeta potential. In-vitro drug release and In-vitro antibacterial activity. The optimized formula was incorporated into 2% carbopol 934 gel and evaluated for spreadability, rheological property, pH and In-vitro drug release, and In-vitro antibacterial activity.

a) Drug content
For determination of drug content about one ml of each microemulsion formulation was weighed in a 10 ml volumetric flask and dissolved in methanol. It was diluted appropriately and analyzed spectrophotometrically at 288 nm.

b) pH Determination
The pH value of microemulsions was determined at 25˚C by pH meter (Digital pH meter, Model-112).[10]

c) Percentage transmittance
Transparency of the microemulsion was determined by measuring the percentage transmittance at 650nm against distilled water as blank using UV-Visible spectrophotometer (UV, 1700, Shimadzu, Japan).[11]

d) Viscosity measurements
Rheological behaviour of the formulation was evaluated using an Ostwald viscometer at a room temperature.[12]

e) Measurement of particle size
The average droplet size and zeta potential of the microemulsions were measured using a Microtrac instrument. The measurement was done at 25°C. [13]

f) Surface morphology
The surface characteristics of microemulsion were performed on the formulation selected on the basis of particle size the formulation using scanning electron microscopy.[14]

g) Centrifugation test
In this, the samples were centrifuged at 5000 rpm for 30 minutes and then were examined for whether the system is monophasic or biphasic. (Remi RM-12-C India).[15]

h) In-vitro release studies
An in-vitro drug release study was performed using diffusion cells. Egg membrane was placed between receptor and donor compartments. Microemulsion gel equivalent to 1 gm was placed in the donor compartment and the receptor compartment was filled with phosphate buffer pH 7.4. The diffusion cells were maintained at 37 ± 0.5 °C with stirring at 100 rpm throughout the experiment. At a fixed time, interval, 5 ml of samples were withdrawn for every 1, 2, 3, 4, 5, and 6 hrs from the receiver compartment through the side tube and analyzed by UV spectrophotometer at λ max 288 nm [16].

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 make well (about 6mm diameter) and add different

concentration of the given samples, 50ug/ml drug, 80ug/ml gel and 80 ul of formulations. 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.[17]

RESULT AND DISCUSSION

The pseudo ternary stage charts of different proportions of surfactants (Tween 20, Tween 80)/Co-surfactant (Propylene glycol) were utilized to develop. The Smix weight proportions [1:1, 2:1, 3:1] are addressed in Fig.1 to Fig.2 and Table 1, in pseudo-ternary stage graph where microemulsion regions are noticed by using Ternary plot.com software.

The optimized microemulsion MM3 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.

The levofloxacin melting point was found to be 224 ℃ by Thiel's method and 227 ℃ by DSC (Fig.2) method which complied with IP standards, thus indicating the purity of the drug.

The greatest solvency of levofloxacin was found in mustard oil (40 ± 0.270) Tween 80 (90.55 ± 0.053mg/ml), Tween 20 (88.73 ± 0.520mg/ml) and co-surfactant propylene glycol (89.25 ± 0.283mg/ml) and furthermore dissolvable in pH 7.4 phosphate buffer (110 ± 0.029mg/ml). Shown in Table 3.

The drug content of all the formulations of levofloxacin microemulsion is shown in Table 3. MM3 was exhibited 97.62±0.024 higher drug content than other formulations. The microemulsion drug content of all formulations was found to be within the range of 89-99% which was within the limits of USP specifications. The prepared levofloxacin microemulsion gel MM3-G was subjected to drug content uniformity. The microemulsion gel was in the permissible range of 93.85 ± 0.85% it indicated the drug was uniformly dispersed throughout the formulation. Shown in Table 6.

All the prepared formulations were checked for their pH. All the formulations were showing pH in the range of 5.86 to 6.70 as shown in Table 3. This is well in the range for topical administered formulation. The pH value of optimized microemulsion formulation MM3 was 6.2±0.285 (Table 2) 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 MM3-G gel was found to be 6.6 ± 0.85 (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 MM3 formulation showed 96.54 ± 0.21% compare to other formulations.

The viscosity of microemulsion formulation was determined as shown in Table 3, all samples exhibited Newtonian flow behaviour and formulation MM3 showed 13.12 ±0.25 cps shows less viscous compared to other microemulsion formulations. And the optimized gel MM3-G viscosity was found to be 7270.57 ± 56.16cps. (Table 6).

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 Fig. 4, for MM3. This can have the ability to form a microemulsion.

The particle size and zeta potential were measured by a Marwin zeta analyzer and it was Found that 70 nm for MM3. Confirmed that microemulsions are within the required size ranges confirmed. The Zeta potential of microemulsion MM3 was found to be 24.5 Mv (Fig.5) which shows that they are adequate to be stable.

FTIR Spectrum of levofloxacin was obtained by scanning the drug in the range of 4000 to 400. Major peaks observed were as 3265.45, & 3471.98 cm-1 (COOH), 1724.42, & 1743.71 cm-1 (C=O),2933.83 & 2924.18 cm-1 (C=C), 1446.66, & 1460.16 cm-1 (C-F) and 2847.03, & 2852.81 cm-1 (CH2), and, 1464.66 & 1541.18 cm-1 (C-N) whose presence resembled the structure of levofloxacin. Observed FTIR spectra and standard values were as depicted in Fig. 6.1, 6.2, 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 drugs and other components.

The cumulative drug content permeated from the membrane for all microemulsion formulations was calculated. In-vitro release profiles of levofloxacin across the membrane from the microemulsion system were carried out by diffusion method for 6 hrs and results are depicted in Table 5, for mustard oil-based levofloxacin microemulsion. From the results, we observed that 0 - 21% of the drug was released in 1 hr and more than 50% drug release in 3 hrs, and more than 80% of drug released in 6 hrs for all the formulations. It was observed that higher drug release of 93.08% for MM3 formulation and a very lower release of 74.22 % for MM4 formulation (Fig.7). 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 MM3-G was found to be 72.08 %, respectively as shown in Fig. 8.

The spreadability is an important property of topical formulation from a patient compliance point of view. The increase in the diameter due to spreading of the formulation g MM3-G was 7.2 ± 004. Which is good for topical application Shown in Table 6.

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 80 µg/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. 9. The zone of inhibition was to be for drug 2.8 cm (50µg/ml), standard 1.6 cm (40 µl) mustard oil 2.2 cm (20 µl), Microemulsion MM3 3.8cm (80 µl), Microemulsion gel MM3 2.6cm (80 µg/ µl) shown in (Table 7). The, MM3 and MM3-G shows grater antimicrobial effect compare to standard (Fig 9).

Table 1: Formulation development of mustard 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 %
M111	1:1	**Tween 80**	**Mustard oil**	20	70	10	0.5
M121	2:1			12	78	10	0.5
M131	3:1			15	80	5	0.5
M311	1:1	**Tween 20**	**Mustard oil**	50	35	15	0.5
M321	2:1			30	60	10	0.5
M331	3:1			30	60	10	0.5

Table 2: Solubility of levofloxacin in different excipient solvents

Phase type	Excipient	Solubility mg/ml
Aqueous	Water	1.44±0.285
Oil	Eucalyptus Oil	80 ± 0.075
	mustard Oil	40 ± 0.270
Surfactant	Tween 20	88.73 ± 0.520
	Tween 40	85.23 ± 0.438
	Tween 80	90.55 ± 0.053
Co-Surfactant	Propylene glycol	89.25 ± 0.283
	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

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

Formulation code	Transmittance	Viscosity cps	pH	% drug content
MM1	93.47 ± 0.160	13.828±0.651	6.8±0.185	93.34±0.241
MM2	93.793 ±0.241	15.317±0.783	6.433±0.232	89.76±0.185
MM3	96.546 ± 0.219	13.121±0.258	6.2±0.285	97.62±0.246
MM4	92.32 ± 0.18	16.593±0.851	6.466±0.363	90.36±0.325
MM5	90.56 ± 0.137	20.841±0.696	6.7±0.841	94.85±0.856
MM6	91.546 ± 0.275	22.061±0.454	6.566±0.565	91.29±0.235

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

Functional group	Wave number (cm^{-1}) of pure drug	Wave number (cm^{-1}) of MM3 formulation
COOH	3265.45	3471.98
C=O	1724.42	1743.71
CH3	2933.83	2924.18
C-F	1446.66	1460.16
CH2	2847.03	2852.81
C-N	1464.66	1541.18

Table 5: *In-vitro* diffusion study of MUSTARD OIL microemulsion

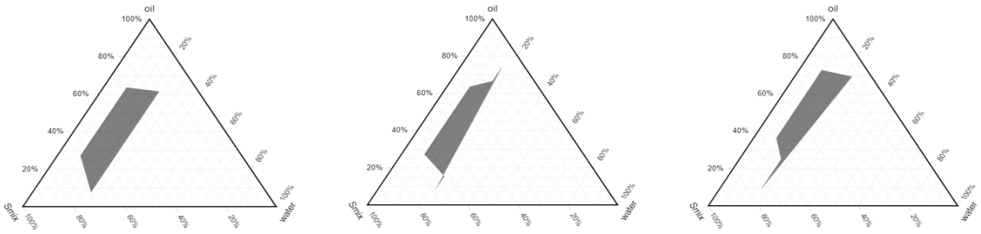
Time in hours	% Cumulative drug release					
	MM1	MM2	MM3	MM4	MM5	MM6
0	0	0	0	0	0	0
1	21.739	13.814	15.561	11.652	13.652	10.782
2	26.304	39.931	33.763	21.507	25.266	33.673
3	44.391	52.288	54.100	42.243	44.041	57.139
4	51.569	70.573	70.564	59.387	56.873	59.642
5	58.856	84.724	81.596	65.985	67.87	66.341
6	80.382	89.164	93.084	74.221	77.492	76.132

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

Formulation code	Spreadability	Viscosity	pH	% drug content
MM3-G	7.2 ± 0.04	7270.57 ± 56.16	6.6 ± 0.28	93.85 ± 0.85

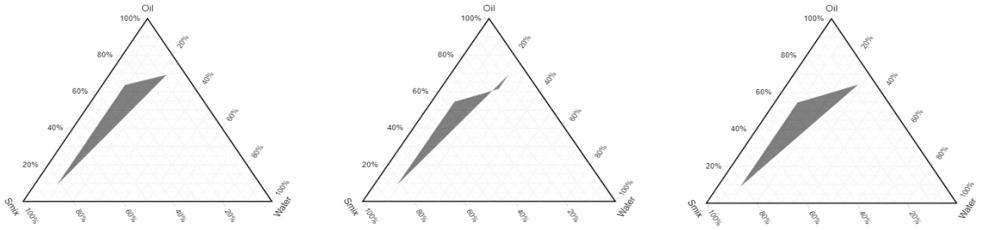
Table 7: Antimicrobial effect of microemulsion formulation and oils against *Staphylococcus aureus*

Components	Quantity	Zone of inhibition in cm
Drug (levofloxacin)	50µg/ml	2.8
Standard (Streptomycin)	40 µl	1.6
Mustard oil	20 µl	2.2
Microemulsion MM3	80 µl/ml	3.8
Microemulsion gel MM3-G	80 µg/ml	2.6



1 :1 **2:1** **3:1**

Figure 1: Pseudo ternary phase diagram using Mustard 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)**



1.1 **2.1** **3.1**

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

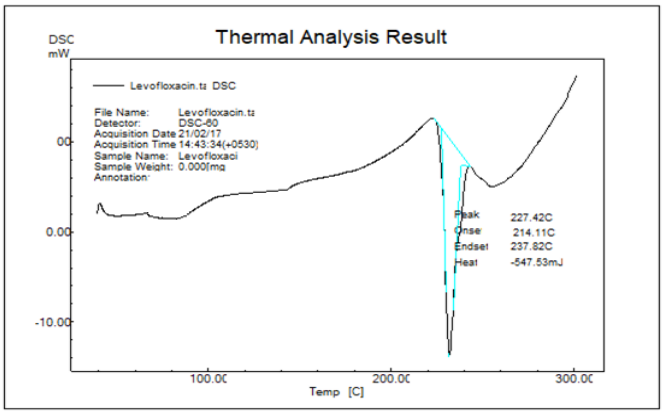


Figure 3: DSC Thermograph of LEVOFLOXACIN

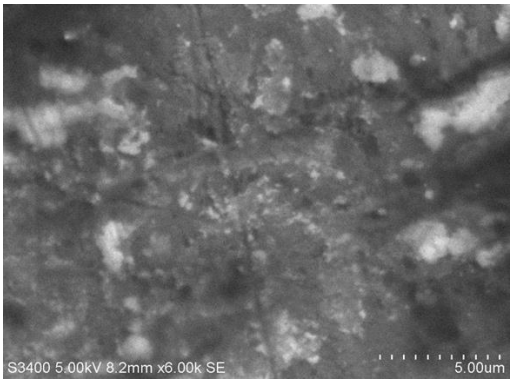


Figure 4: SEM image of MM3

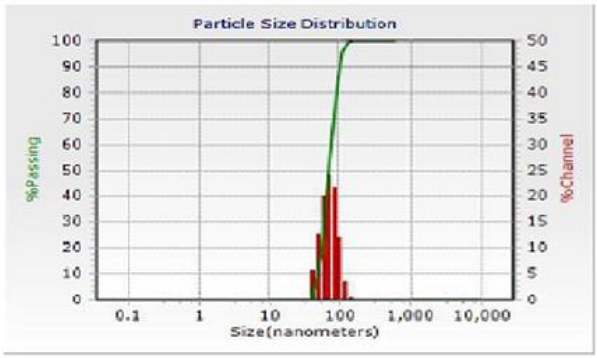


Figure 5: Result of particle size of the formulation MM3

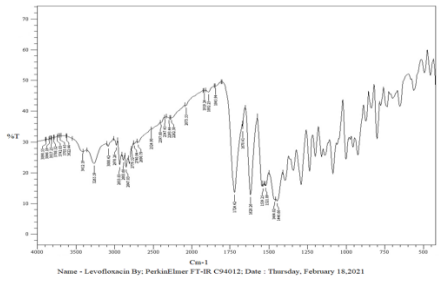


Figure 6.1: FTIR spectra of LEVOFLOXACIN

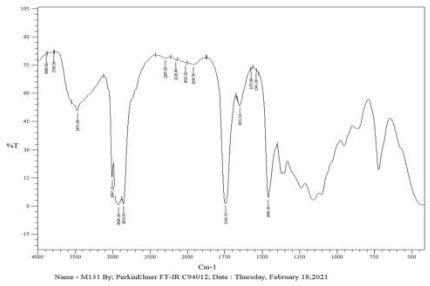


Figure 6.2: FTIR spectra of LM3 formulation

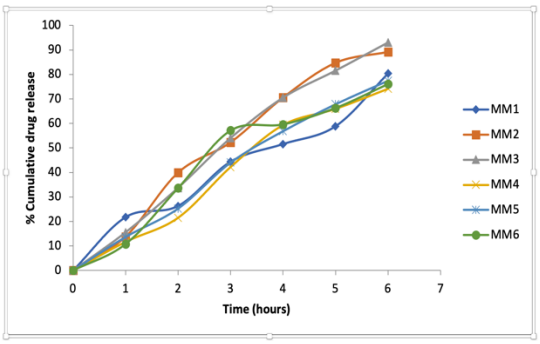


Figure 7: Comparison of % cumulative drug release of MM1-MM6

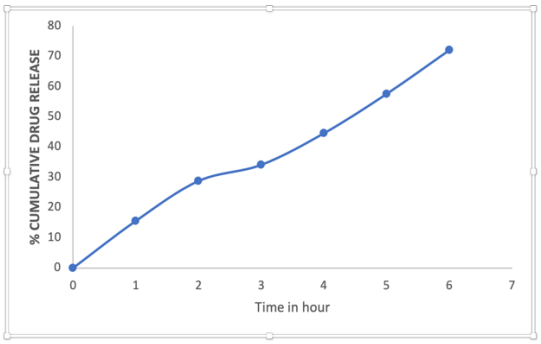


Figure 8: % cumulative drug release of MM3-G

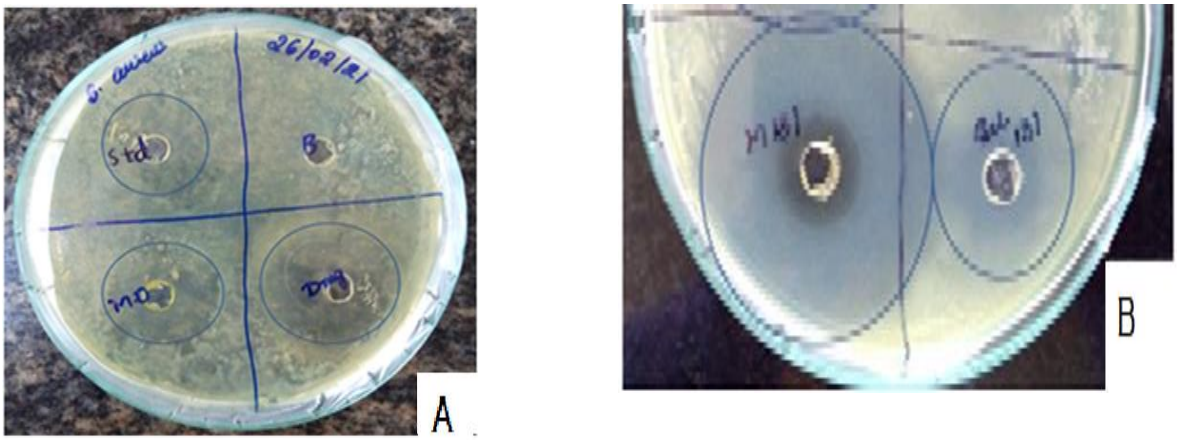


Figure 9: The Antibacterial activity of (A) mustard oil with drug (B) Microemulsion MM3 and gel MM3-G against *Staphylococcus aureus* using well-diffusion method.

CONCLUSION

In this study we observed that optimized formulationMM3 microemulsion and microemulgel MM3-G showed better antimicrobial effect in the combination of levofloxacin and the mustard oil rather than their individual performance. Compared to other microemulsion and microemulgel formulations MM3 and MM3-G shows better antimicrobial effect than the standard, this proved that the synergistic effect could be achieved by formulating microemulsion by using levofloxacin and mustard oil with deeper skin penetration effect.

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