Advances in Bioresearch
Adv. Biores., Vol 16 (1) January 2025: 310-314

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.1.310314

Advances in Bioresearch

ORIGINAL ARTICLE

Formulation and *In Vitro* Evaluation of Ozenoxacin Loaded Topical Microemulsion

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ABSTRACT
The aim of the current study is to formulate and characterize the Ozenoxacin 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 co surfactant ratio. The developed ME formulation was characterized by various parameters like % Transmittance, viscosity, pH, drug content, surface morphology, zeta potential, and in-vitro drug release study. It was concluded that the microemulsion system studied is a promising tool for the topical delivery and Ozenoxacin be formulated as microemulsion with good release and consistency.
Keywords: *Ozenoxacin, Eucalyptus oil, Microemulsion (ME), Microemulgel (MEG), Pseudo ternary phase diagram, antimicrobial effect.*

Received 24.11.2024 Revised 01.12.2024 Accepted 11.12.2024

How to cite this article:
Gupta Ashish, Jain Sachin Kumar. Formulation and *In Vitro* Evaluation of Ozenoxacin Loaded Topical Microemulsion. Adv. Biores. Vol 16 (1) January 2025. 310-314

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].

MATERIAL AND METHODS

Materials

Ozenoxacin drug was acquired as a gift sample from precise pharma. Ltd. Mumbai. Tween 20, Tween 80 and Propylene glycol was purchased from Loba chemicals, Eucalyptus oil was purchased from local ayurvedic store, Indore MP.

Method of preparation

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 whilemixing 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 mixtureof 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 waterwas calculated and then plotted on triangular coordinate to construct the pseudo ternary phasediagram.

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

Formulation code	S mix ratio	Surfactants	Oils	Percent w/w component in formulation			
				Oil %	S mix %	Water %	Drug %
ME1	1:1	**Tween 80**	**Eucalyptusoil**	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**	**Eucalyptusoil**	40	40	20	0.5
ME5	2:1			20	65	15	0.5
ME6	3:1			25	50	25	0.5

FORMULATIOB OF OZENOXACIN 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 preparedMicroemulsion was evaluated for various parameters.

EVALUATION OF OZENOXACIN MICROEMULSION [8-10]

The prepared microemulsion formulation were characterized for parameters like Drug content, pH, Particle size analysis, Determination of viscosity, Surface morphology, FT-IR analysis, *In vitro* drug release.

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 and the pseudo-ternary stage graph where microemulsion regions are noticed by using Ternary plot.com software.

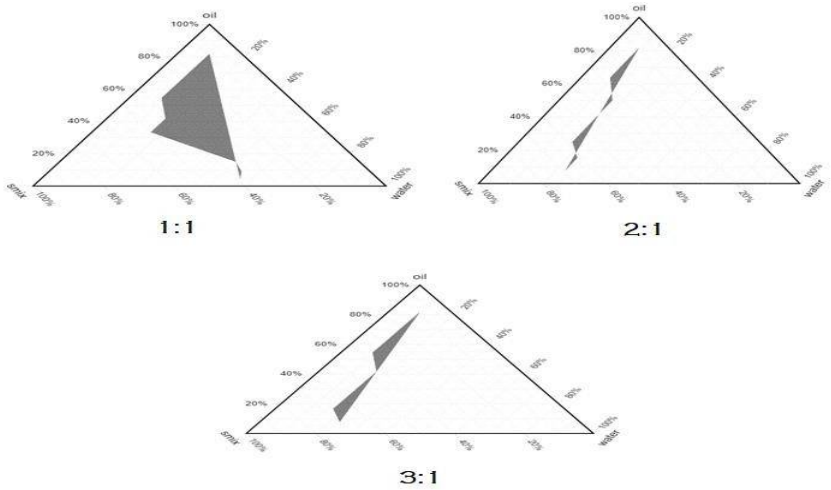

Figure 1: Pseudo ternary phase diagram

The solubility of Ozenoxacin was found to be in Eucalyptus Oil (80 ± 0.075mg/ml). Furthermore, the maximum solubility of Ozenoxacin 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 Ozenoxacin

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, percentage transmittance, viscosity and pH of all the formulations of Ozenoxacin 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 Ozenoxacin microemulsion gel ME3-G was subjected to drug content uniformity. The *In-vitro* diffusion study of all the prepared batches of Eucalyptus oil microemulsion is shown inTable 4.

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

Formulation Code	Transmittance	Viscosity cps	pH	% drugcontent
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

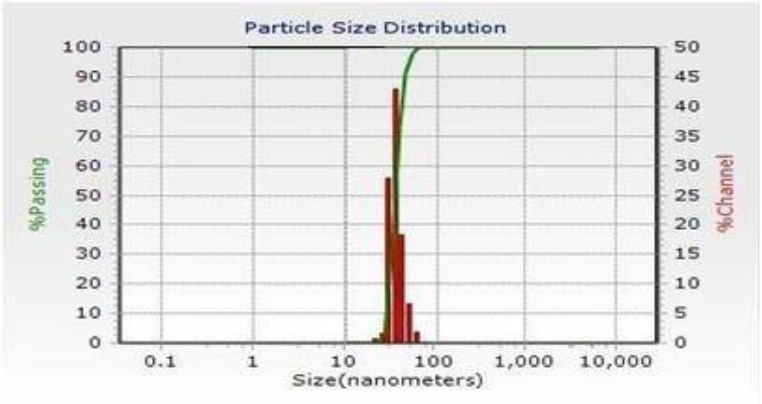

Figure 2: Result of particle size of the formulation ME3.

Table 4: *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	21.738	15.830	16.69	13.81	12.53	12.782
2	26.303	21.898	29.73	39.93	33.70	39.922
3	44.390	25.502	54.38	52.29	52.82	60.526
4	51.55	65.232	60.29	60.27	57.023	70.632
5	58.850	69.79	75.48	77.40	67.215	84.763
6	80.39	74.39	95.93	82.703	75.296	87.702

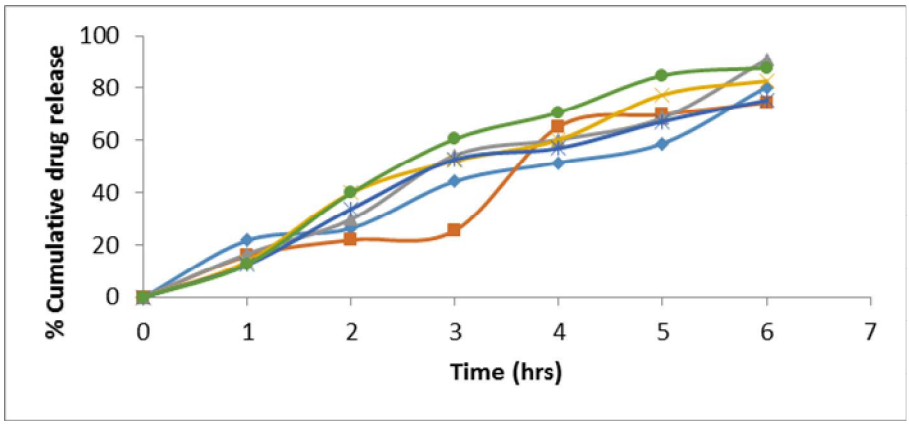

Figure 3: Comparison of % cumulative drug release of ME1-ME6

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. The viscosity of the gels of microemulsion formulations ME3-G wasdetermined and 6827.92 ± 77.14cps.

CONCLUSION

A topical microemulsion was formulated using Ozenoxacin 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 teststo obtain the best optimized formulation. Ozenoxacin 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 Ozenoxacin drug by microemulsion formulation with deeper skin penetration effect. All result showed satisfactory results. Further, clinical study to be done before exploits it into the market.

ACKNOWLEDGEMENT
The authors are thankful to precise pharmaceuticals for providing drug as gift sample and also thankful to Dean Research & Supervisor, Oriental university Indore for their support and encouragement in writing this research.

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