Formulation and Evaluation of Micro Emulsions Containing Ciprofloxacin for Treating Skin Infections

Bysu Sujatha*[1], E. Himabindu[2], Dr. Sowjanya Battu[3], Dr. Konde Abbulu[4]

[1,2,3,4] Department of Pharmaceutics, CMR College of pharmacy,

Kandlakoya Village, Medchal, Hyderabad, Telangana-501401, India.

ABSTRACT

The main aim of the present study is to formulate micro emulsions-based drug delivery and its in vitro evaluation. Solubility studies are performed for the drug in oil Phase of oleic acid, Surfactant Tween 80, and Co-surfactant Propylene glycol. Formulations are developed by the ternary phase diagram to consider the smix and oil ratios. From the pseudo ternary phase diagram 8 formulations are developed and they were characterized. From the evaluation studies F3 Formulation showed good properties of drug release at 10th hour, Drug content, pH, Viscosity, Surface tension and Particle size. The results revealed ciprofloxacin as a best suited drug to formulate into micro emulsion to improve its solubility and bioavailability.

Keywords: Microemulsions, Formulations, Characterization, Pseudo ternary phase diagram.

INTRODUCTION

Skin is the largest organ of the human body and its primary function is to protect the body from infections but sometimes the skin also gets affected by the microorganisms like bacteria and fungi. Various bacterial skin infections include cellulitis, impetigo, boils, and leprosy. Viral infections include shingles, chicken pox, Molluscum contagiosum, warts, Measles, hand foot and mouth diseases. Fungal infections mainly occur in the moist areas of the body and they include athletes' foot, yeast infection, ringworm, fungus on the nails, oral candidiasis and rashes caused by the use of diapers. Parasitic infections are the type of infections that are caused due to parasites. This type of infection may also spread into body by entering through the blood stream. Infection caused by the parasites is not a dreadful one, but it may cause discomfort to the patient. Various Parasitic infections include lices, bedbugs, scabies, and cutaneous larva migrans[1].

Ciprofloxacin is a broad-spectrum antibiotic of the fluoroquinolone class. It is active Against some gram positive and many gram -negative bacteria. Its functions to inhibiting DNA gyrase, and necessary to separate bacterial DNA, there by inhibiting the cell division.

Microemulsions are the dosage forms which are clear physically stable, and isotropic mixtures containing water, oil and surfactant and sometimes inclusion of co surfactant in its formulation. Salts and other ingredients can be constituted as an aqueous phase here as oil phase consists of combination of hydrocarbons and olefins. When compared with the ordinary emulsions micro emulsions are formulated by mixing at high conditions of shear[2].

Mainly there are two basic types of emulsions direct which constitute oil in water and reverse containing water in oil. Particle size of micro emulsion ranges from 10nm to 300nm and they are clear and translucent in appearance due to their small particle size[3].

Micro emulsions are the ternary systems containing two phases' oil and water which are immiscible, and these two phases are made miscible with each other by the inclusion of surfactant. Surfactant molecules create a monolayer at interface of water and oil phase and make them miscible with each other. Surfactant molecule contains hydrophobic tail and hydrophilic head which helps them to dissolve in the oil phase[4].

Micro emulsions are the dosage forms which help to deliver the drug which have low solubility and permeability; it can be used to deliver the drug through various routes whereas transdermal route is having more successful applications. The important three factors that determine the delivery of drug through micro emulsions is permeation of drug, the liberation drug from the vehicle and the release of drug into the skin significantly to stratum corneum. When compared with the other formulations they have more thermodynamic stability and the explanation for this is the stabilization of oil and water phase by the addition of surfactant and this inclusion enhances the elastic properties of the formulation this can be mainly achieved due to the curvature and rigidity of the film[5].

MATERIALS AND METHODS

Materials

Ciprofloxacin (drug), Oleic Acid, Tween 80, Propylene Glycol (Excipients) all are obtained as gift sample for study from Aurobindo pharma company, Hyderabad.

Experimental Methods

Solubility Studies

Solubility of a substance is a chemical property which refers to the ability of a substance to get dissolved in a given solvent. Drug ciprofloxacin is taken in extra amounts and dissolved in a given solvent to get a saturated solution. The resultant saturated solution is sonicated, and it as kept at an optimum temperature. After it attains the equilibrium its solubility is checked with the assistance of UV spectrophotometer.

Construction of Pseudo Ternary Phase Diagrams

In the current formulation Oleic acid, Teen 80 and Propylene glycol are taken as oil phase, Surfactant, and co-surfactant, respectively. To know the amounts of components to be considered in the formulation pseudo ternary phase diagram as constructed by utilizing the titration method with water. 2:1, 4:1 and 6:1 ratio of surfactant and co-surfactant are taken. Oil phase and Smix mixture is taken in the ratios of 1:9 to 9:1 by using the ternary phase diagram. From the pseudo ternary phase diagram totally eight formulations are considered by taking the ratios of 4:1 And 6:1 ratio of surfactant and co-surfactant. Oil is taken at a percentage of 20 and 40, water 5%, and 10 %.

Formulation Development

Table 1: Formulation Table

Ciprofloxacin Drug – 500mg

Formulation	S/C	%Oil	%S+C	%Water
ME-1	6:1	40	50	10
ME-2	6:1	40	55	5
ME-3	6:1	20	70	10
ME-4	6:1	20	74	5
ME-5	4:1	20	75	5
ME-6	4:1	20	70	10
ME-7	4:1	40	55	5
ME-8	4:1	40	50	10

Preparation of Microemulsions

- The Components of oil phase, surfactant and co-surfactant are taken in required quantities in a dried beaker and they are subjected to mixing with the assistance of a magnetic bead.
- 500mg of Ciprofloxacin drug is taken and it is dissolved in 7.4pH buffer with a constant stirring at a rpm of 400 for a time of 15minutes.
- The resultant emulsion is subjected to sonication process in order to obtain a micro emulsion.

Determination of pH

The pH of the prepared micro emulsions mainly based on the Excipients that were taken in the formulation. Any alterations in pH may result in the unstability of the formulations as they show their effect on the zeta potential values. pH of the prepared formulation was analyzed by using the digital pH meter. pH of each formulation as taken for three times and the average of the three values was taken as the final value.

Viscosity Measurement

The Viscosity of the micro emulsion also determines its stability because as the viscosity decreases phase inversion or cracking may occur which results in the instability of the formulation. Viscosity of the formulated micro emulsion is determined by using the Brookfield viscometer. Readings are taken three times and the average of the three readings is taken as the final viscosity value of the formulation.

Surface tension

Surface tension of the formulated microemulsion is measured at a temperature range of 25^0c using Torsion balance equipment.

Drug Content

Content of drug present in the micro emulsion can be measured by the assistance of UV spectroscopy. By using 7.4 pH phosphate buffer as the solvent 2µg/ml sample of microemulsion is prepared and the sample is analyzed at a wavelength range of 275nm.

Globule Size Analysis

100mg equivalent of ciprofloxacin drug is taken and diluted with 20ml of double distilled water in a 50ml volumetric flask. The resultant solution is mixed by shaking the beaker, the obtained solution is analyzed using the Electron microscopy equipment.

In vitro Diffusion Studies

In vitro diffusion studies are carried in a Franz diffusion cell. It consists of two compartments an upper donor and lower receptor compartment. Cellophane membrane is paced in between the both compartments. In the receptor compartment 130ml of 7.4pH phosphate buffer is taken and a magnetic bead is placed. In the donor compartment micro emulsion is placed and the whole setup is made to stir by placing on a magnetic stirrer and stirred continuously at 300rpm for about 10hrs. samples were taken for 1,2,3,4,5,6,7,8,9 and 10hrs by maintaining the sink conditions. The samples collected were analyzed by using a UV spectrophotometer at a wavelength range of 275nm. The obtained results were plotted by taking time on x-axis and %cumulative drug release on y-axis.

Stability Studies

Stability studies were carried out for the optimized formulation according to ICH guidelines by storing the formulation at a temperature of 40±0.5^{0}C and at a humidity of 75±5% for a time period of 3months. The samples were checked for all the evaluation tests at the end of 1st, 2nd and 3rd month respectively. All the reading was taken three times and the average of 3values is taken as the final one.

RESULTS AND DISCUSSIONS

Solubility Studies

Table 2: Solubility Studies Table

S. No	Component	Solubility (mg/ml)
1	Oil (Oleic acid)	13.697±0.53
2	Surfactant (Teen 80)	31.5±0.21
3	Co-surfactant (Propylene glycol)	14.120±0.31

Discussion: Solubility studies were performed for ciprofloxacin in oil, surfactant and co-surfactant and from the results it was proved that oleic acid, Tween 80 and propylene glycol can be considered as oil, surfactant and co-surfactant for the formulation as they are showing good solubility properties for ciprofloxacin.

Construction of Ternary Phase Diagram

Tween 80+ Propylene Glycol 6:1

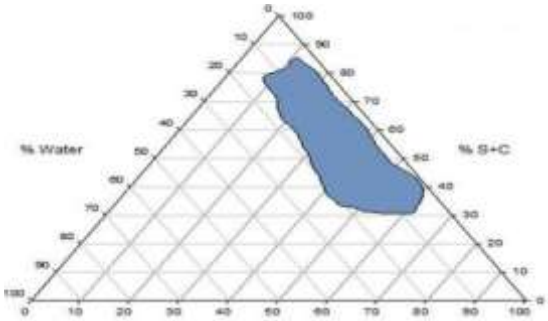

Fig 1: Ternary phase of Tween 80+Propylene Glycol 6:1

Tween 80+ Propylene Glycol 4:1

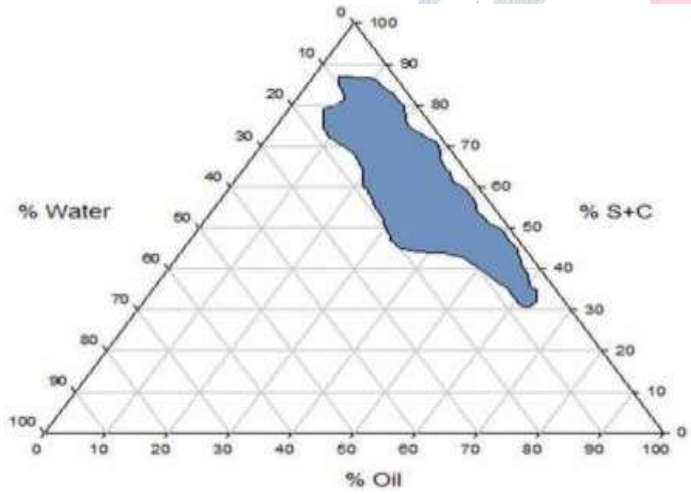

Fig 2: Ternary phase of Tween 80+Propylene Glycol 4:1

Tween 80+ Propylene Glycol 2:1

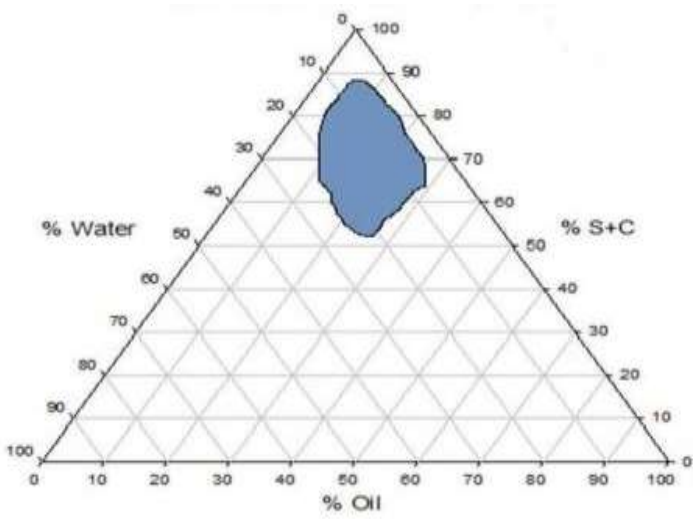

Fig 3: Ternary phase of Tween 80+Propylene Glycol 2:1

Discussion: According to phase diagrams that were plotted from Tween 80+ Propylene Glycol 6:1, 4:1, 2:1compositions were found to produce good Microemulsion formulation. Among them Tween 80+Propylene Glycol 6:1 & 4:1 of phase diagrams found higher area values that there is a greater chance of producing a stable Microemulsions with these compositions.

Determination of pH

Table 3: pH values of Microemulsions

S. No	Formulation	pH
1	ME1	6.23 ± 0.05
2	ME2	6.72 ± 0.08
3	ME3	6.65 ± 0.12
4	**ME4**	**6.0± 0.09**
5	ME5	6.70 ±0.06
6	ME6	6.70 ±0.09
7	ME7	6.89 ± 0.07
8	ME8	6.78 ± 0.10

Discussions: From the above results it can be observed that pH of the formulations ranges from 6.0 to 6.89. ME4 formulation showed a pH range of 6.0 which is best suited for topical administration.

Viscosity Measurements

Table 4: Viscosity Values of Microemulsions

S. No	Formulation	Viscosity (cps)
1	ME1	282.90 ± 1.91
2	ME2	253.73 ± 1.88
3	**ME3**	**802.63 ± 1.66**
4	ME4	681.13 ± 1.98
5	ME5	445.60 ± 1.15
6	ME6	580.83 ± 1.92
7	ME7	364.17 ± 1.60
8	ME8	457.90 ± 1.83

Discussion: Viscosity of the formulation's ranges from 253.73±1.88cps to 802.63±1.66cps. Formulation 3 Exhibits high viscosity.

Surface Tension

Table 5: Surface Tension Values of Microemulsions

S. No	Formulation	Surface tension (dynes/cm)
1	ME1	44.17 ± 1.46
2	ME2	47.67 ± 0.85
3	ME3	46.00 ±1.06
4	ME4	47.67 ± 1.44
5	ME5	47.33 ± 0.76
6	ME6	49.33 ± 1.36
7	**ME7**	**52.17 ± 1.25**
8	ME8	48.50 ± 1.42

Discussion: surface tension of the formulation ranges from 44.17±1.46 to 52.17±1.25dynes/cm.

The values of surface tension indicate that the micro emulsions are water in oil type.

Globules Size Analysis

Table 6: Globule size analysis values of Microemulsions

S. No	Formulation	Globule size (nm)
1	ME1	37.03±0.2nm
2	ME2	79.8±0.7nm
3	**ME3**	**15.03±0.5 nm**
4	ME4	20.3±1.2 nm
5	ME5	21.9±0.6 nm
6	ME6	34.5±1.3 nm
7	ME7	20.3±0.5 nm
8	ME8	31.6±0.3 nm

Discussion:

From the table it can be known that the particle size of the formulation ranges from 15nm to 79nm. And from the results one can observe that the ME3 formulation yielded small particle size i.e. 15nm.

Drug content

Table 7: Drug content values of Microemulsions

S. No	Formulation	Drug Content (%)
1	ME1	94.7 ± 0.29
2	ME2	93.9 ± 0.64
3	**ME3**	**98.5 ± 0.41**
4	ME4	96.7 ± 0.21

5	ME5	97.6 ± 0.61
6	ME6	97.5 ± 0.41
7	ME7	97.3 ± 0.71
8	ME8	97.9 ± 0.41

Discussion: The drug content of the formulation's ranges from 93.9 to 98.5.

Cumulative % Drug Release at 10th Hour

Table 8: Cumulative % Drug Release at 10th Hour

Time (hrs)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
0	0	0	0	0	0	0	0	0
0.5	12.4	7.5	17	16.5	15.5	14.5	17.5	19.1
1	21.7	11.5	25	27.7	24	22	25.5	27
2	27.5	22.5	29	34.25	31	29.1	30	31.5
3	43.2	30.5	36	37.1	43.5	37.2	39.5	41.05
4	58.7	41	42.5	42.1	51.05	47.1	42.5	46.15
5	81.9	54.2	61.05	51.7	59.1	62.05	48.5	53.55
6	92.7	61.5	69.35	57.6	62.3	69.8	59.5	59.65
7	95	68.2	76.05	66.1	73.3	79.1	64.15	62.8
8	95.6	77.05	83.5	77.2	77.5	82.95	73.75	71.6
9	96.1	88.3	91.6	83.1	88.5	91.6	84.65	84.2
10	96.2	96.5	**97.65**	91.15	94.5	95.5	92.7	94.75

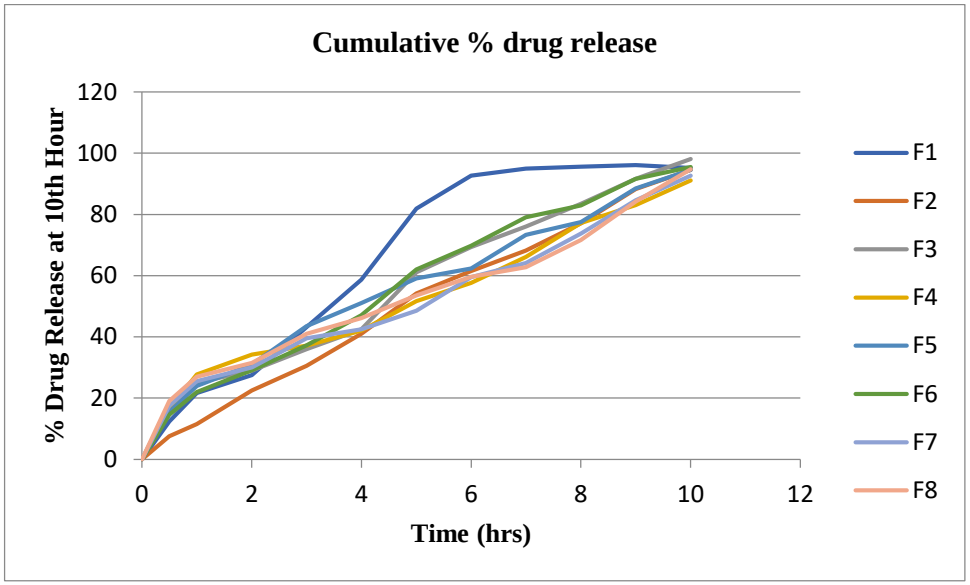

Fig 4: Cumulative % Drug Release Graph

Stability Studies:

After performing all the Evaluation parameters ME3 formulation is considered as the optimized formulation due to its small particle size, more drug content and drug release. Stability studies

are conducted for F3 formulation as per ICH guidelines and the results are tabulated as below

Table 9: Stability Studies Values of Microemulsions

S.no	Evaluation Parameters	Initial Value	1st Month	2nd Month	3rd Month
1	Viscosity	802.63 ± 1.66	801.45± 1.43	801.45± 1.43	801.45± 1.43
2	pH	6.0 ± 0.09	6.0 ± 0.07	6.0 ± 0.07	6.0 ± 0.07
3	Surface Tension	46.00 ±1.06	46.00 ±1.06	46.00 ±1.06	46.00 ±1.06
4	Globule Size Analysis	15.03±0.5 nm	15.03±0.5 nm	15.03±0.5 nm	15.03±0.5 nm
5	Drug Content	98.5 ± 0.41	98.2 ± 0.41	98.2 ± 0.41	98.2 ± 0.41

6	Cumulative Drug release at 10^{th} hour	97.65±0.032	97.63±0.032	97.63±0.032	97.63±0.032

CONCLUSION

The Current study revealed that micro emulsions can improve the solubility and bioavailability of drug there by improves the release properties of drug. In the present work eight formulations were prepared in which F3 formulation is the best formulation as it is showing good release at 10^{th} hour, viscosity and optimum pH that is suitable for skin and surface tension.

Micro emulsions are the promising drug delivery systems which can give prominent results and better therapeutic efficiency.

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