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FORMULATION AND EVALUATION OF MOXIFLOXACIN OCULAR MICROEMULSION GEL

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ABSTRACT

This research was aimed to formulate and characterize micro emolsion systems as an ocular delivery system of Moxifloxacin for preventing corneal infections. In the present work, Micro emulsion (ME) of Moxifloxacin using xanthan gum along with Carbopol934,gellan gum and sodium alginate as gelling agents were formulated to deliver Moxifloxacin via occular route. Since the discovery of micro emulsions by Jack H. Shulman, there have been huge progresses made in applying micro emulsion systems in research and industrial processes. Micro emulsions are clear, stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a cosurfactant. Micro emulsions are optically isotropic and thermodynamically stable liquid solutions of oil, water and amphiphile. To date micro emulsions have been shown to be able to protect labile drug, control drug release, increase drug solubility, increase bioavailability and reduce patient variability. Micro emulsions are readily distinguished from normal emulsions by their transparency, low viscosity and more fundamentally their thermodynamic stability. Micro emulsion(ME) formulations were prepared by mixing of appropriate amount of surfactants including span 60& ethyl oleate, polymer such as Tween, gumming agent such as gellan gum, thickening agent such as xanthan gum also act as stabilizer and chelating agent such a sodium alginate. The prepared MEs were evaluated regarding their Ph, particle size, stability, sterility, ocular irritation studies, and Drug release. The results showed that the Moxifloxacin concentrations delivered by this system remained at therapeutic levels in the cornea. Ocular irritation test revealed good compatibility of the system. Prepared system was effective against most of the gram positive and gram negative microorganisms and any damage to eye tissue was not seen. The prepared system is potential

as ocular drug delivery system as it provided the highest efficiency with chance of forming the desirable viscous pseudo-plastic system after dilution with resident tears. Therefore, it is suggested that this moxifloxacin micro emulsion electrolyte-triggered gelling system might represent an alternative for preventing corneal infections.

KEYWORDS: Micro Emulsion, Ocular Delivery System, Rabbit Cornea, Moxifloxacin.

INTRODUCTION

Ocular diseases are usually treated with topical application of drug solutions (eye drops). These conventional dosage forms account for nearly 90% of the currently available marketed formulations owing to their simplicity and good acceptance by patients. However, one of the major drawbacks associated with topical ocular drug delivery is the rapid and extensive precorneal loss caused by drainage and high tear fluid turnover Typically, less than 5% of the drug applied penetrates the cornea/sclera and reaches the intraocular tissue, with the major fraction of the dose applied often absorbed systemically through the conjunctiva and nasolacrimal duct. This can result in undesirable systemic side effects.[1-5]

Advantage of ocular drug delivery systems

Increased accurate dosing. To overcome the side effects of pulsed dosing produced by conventional systems, To provide sustained and controlled drug delivery,[5-7] To increase the ocular bioavailability of drug by increasing the corneal contact time. This can be achieved by effective adherence to corneal surface.

Limitations of ophthalmic drug delivery

Dosage form cannot be terminated during emergency, Interference with vision, Difficulty in placement and removal, Occasional loss during sleep or while rubbing eyes.[8-11]

Micro emulsion

A micro emulsion is a good candidate for ocular delivery of poorly water-soluble drugs because of its ability to improve drug solubilization. Absorption rate of a drug increases as its thermodynamic activity in the vehicle increases. The thermodynamic activity can be expressed approximately in terms of relative solubility (the ratio of the current concentration of the drug to the concentration in saturated vehicle).[12-15]

Advantages of Micro emulsion Based Systems

Micro emulsions are thermodynamically stable system and allows self-emulsification of the system, Micro emulsions act as super solvents for drug; can solubilise both hydrophilic and lipophilic drugs including drugs that are relatively insoluble in both aqueous and hydrophobic solvents, Having the ability to carry both lipophilic and hydrophilic drugs.[16-20]

Disadvantages of Micro emulsion Based Systems

Require large amount of S/Cs for stabilizing droplets, The surfactant should be nontoxic for use in pharmaceutical applications, The surfactant should be nontoxic for use in pharmaceutical applications.[21]

STRUCTURE OF MICROEMULSION

Microemulsions or Micellar emulsion are dynamic system in which the interface is continuously and spontaneously fluctuating. Structurally, they are divided in to oil in water (o/w), water in oil (w/o) and bi-continuous microemulsions. In w/o micro emulsions, water droplets are dispersed in the continuous oil phase while o/w micro emulsions are formed when oil droplets are dispersed in the continuous aqueous phase. In system where the amounts of water and oil are similar, the bi-continuous micro emulation may result.[22]

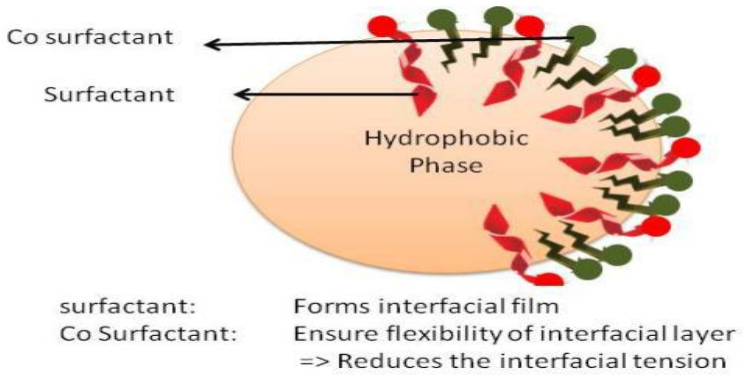

Figure No. 1: Microemulsion Structure.

Types of microemulsion systems:

According to Winsor, there are four types of micro emulsion phases exists in equilibria, these phases are referred as Winsor phases. They are,

Winsor I: With two phases, the lower (o/w) micro emulsion phases in equilibrium with the upper excess oil.

Winsor II: With two phases, the upper micro emulsion phase (w/o) micro emulsion phases in equilibrium with lower excess water.

Winsor III: With three phases, middle micro emulsion phase (o/w plus w/o, called bi-continuous) in equilibrium with upper excess oil and lower excess water.

Winsor IV: In single phase, with oil, water and surfactant homogenously mixed.[23]

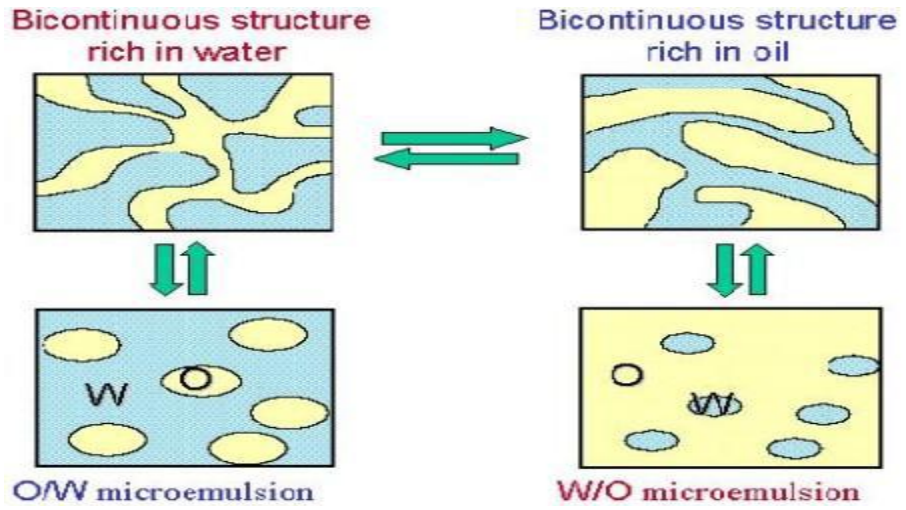

Figure No. 2: Types of emulsions.

METHODOLOGY

Materials

Moxifloxicin is the gifted sample from KP labs, HYD, Ethyl oleate, Tweens, Spans, Gellan gum, Xantham gum, Sodium alginate, Carbapol 940 are the samples obtained from Chaithanya Scientifics private limited, HYD.

Method of preparation

Methods for formulation of microemulsion gel system

Step I

Appropriate amount of Ethyl oleate (oil), surfactant (Tween 80) and co-surfactant sodium alginate were mixed together, and equilibrated with gentle vortex shaking to get the initial concentrate. Then appropriate amount of Moxifloxacin was dissolved in the initial concentrate under ultra-sonication.

Step II

Trials were carried out with various gelling agents individually and in combination to select suitable gelling agent with optimized concentration for prepared microemulsion system.

Step III

The prepared gel system was then added (in place of water in microemulsion composition) in small increments (≤5% v/v) to the mixture of oil (initial concentrate) at room temperature.

Table No. 1: prepared formulations of Moxifloxacin micro emulsion gel.

S.NO.	Ingredients	F1	F2	F3	F4	F5	F6
1	Moxifloxicin (mg)	125	125	125	125	125	125
2	Ethyl oleate	1	1	1	1	1	1
3	Tween	3	3	3	3	3	3
4	Span(mg)	6	6	6	6	6	6
5	Gellan gum (mg)	100	_	_	75	_	_
6	Xanthan gum (mg)	_	100	_	_	75	_
7	Sodium alginate (mg)	_	_	100	_	_	75
8	Carbapol 940 (mg)	_	_	_	25	25	25
9	Water (ml)	15	15	15	15	15	15

Figure No. 3: prepared formulations of Ocular Moxifloxacin micro emulsion gel.

RESULTS AND DISCUSSION

Evaluation studies

FTIR studies

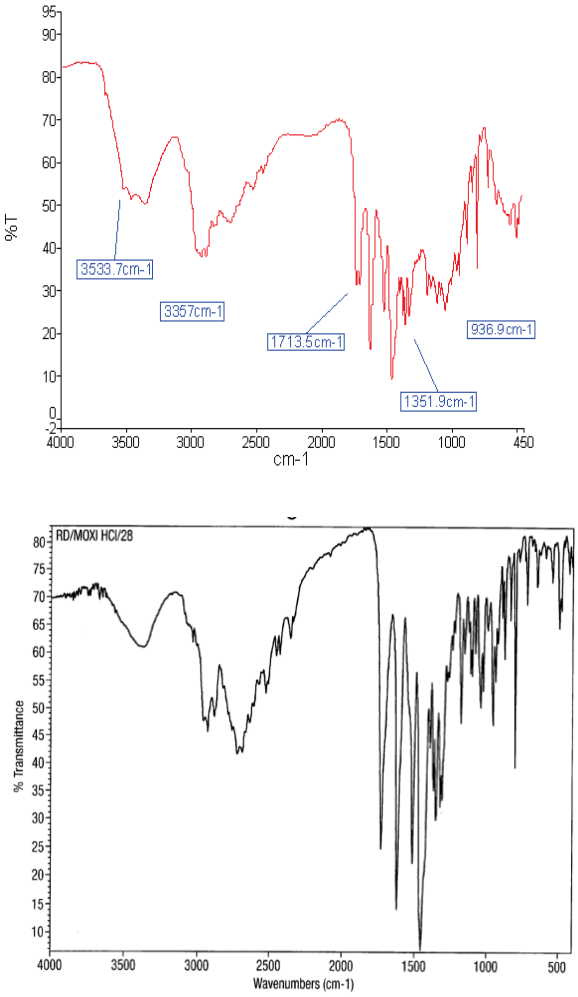

Figure No. 4: FTIR of Moxifloxacin pure drug & FTIR of Optimized formulation.

Drug content

Table No. 2: Drug Content of Prepared formulation of moxifloxacin microemulsion gel (F1 - F6).

S.No.	Formulation code	Drug content
1	F1	99.0±0.01
2	F2	98.3±0.02
3	F3	97.8±0.05
4	F4	99.4±0.03
5	F5	98.6±0.01
6	F6	98.0±0.02

PH

Table No. 3: pH of Prepared formulation of moxifloxacin microemulsion gel (F1 - F6).

S.no	Formulation code	pH
1	F1	5.9
2	F2	6.1
3	F3	6.4
4	F4	7.4
5	F5	5.9
6	F6	6.9

Partical size

The globule size of all the formulations using optical microscope were found to be 78, 97, 91, 60, 89, 73 respectively.

Table No. 4: Particle size of prepared formulation of moxifloxacin microemulsion gel (F1 – F6).

S.no	Formulation code	Particle size
1	F1	78
2	F2	97
3	F3	91
4	F4	60
5	F5	89
6	F6	73

Rheological studies

Table No. 5: Viscosity at (1.0rpm) and Viscosity after with Tear fluid of prepared formulation of moxifloxacin microemulsion gel (F1 – F6).

S.No.	Formulation code	Viscosity (Cps) at 1.0 rpm	Viscosity after dilution) with tear fluid (Cps
1	F1	350	1200
2	F2	250	900
3	F3	475	1350
4	F4	480	1475
5	F5	432	1279
6	F6	590	1610

Antimicrobial efficacy studies

The optimized formulation (F4) was exhibited antimicrobial activity when tested microbiologically by the cup plate technique. Clear zones of inhibition were obtained in the case of optimized formulation and marketed eye drops (Moxifloxacin – Vigamox). The diameter of the zone of inhibitions produced by formulation against both test organisms were

either on part or higher than that produced by marketed eye drops in all the cases as shown in Table and Figure.

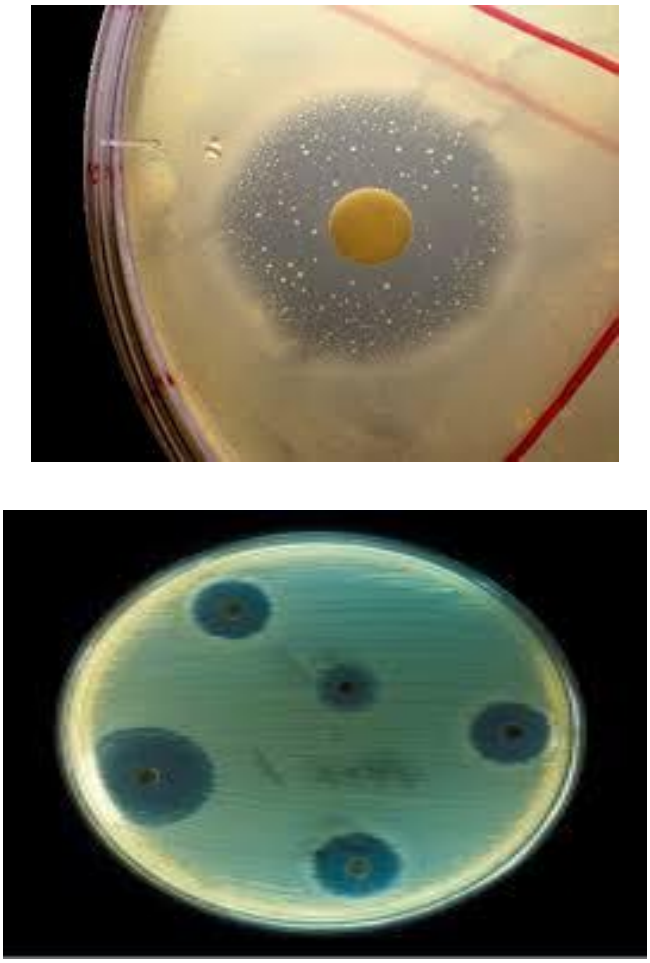

Figure No. 5: Zone of inhibition of F4 against (a) *S. aureus*, (b) *P. aeruginosa.*

STD = standard (marketed eye drops of moxifloxacin); MMG4 = optimized formulation (ME in situ gel system).

Table No. 6: Antimicrobial efficacy of the optimized formulation(F4)against standard drug.

Concentration (µg/ml)	Zone of Inhibition (cm) (% efficiency)	
	STD	MMG4
Vigamox	**s.aureus**	
1	1.6±0.2	1.68±0.2 (105)
10	2.4±0.3	2.68± 0.1(112)
100	3.5±0.2	4.27± 0.2(122)
Moxifloxacin	**P.aeruginosa**	
1	1.8±0.3	1.95±0.1 (108.33)
10	2.7±0.2	3.19±0.2 (118.42)
100	4.0±0.25	5.08± 0.2 (127)

In Vitro drug release study using Goat's cornea

The artificial membranes generally used for *in vitro* drug release studies are not always multilayered. Cellophane membranes, which are usually used in drug release study, are made of cellulose derivatives and they are hydrophilic in nature. They did not match the condition of drug release through the biological membranes. So, instead of cellophane membrane, isolated Goat's cornea was used as a membrane for the *in vitro* drug release studies and drug release profile was determined. in 24 hrs. But, more prolonged release (more than 18 hours) was observed from F4 due to higher viscosity of the formulation (combination of two gelling agents i.e. 0.3% sodium alginate and 0.1% carbapol 940). Higher viscous formulation caused difficulty in vision if remain longer time on surface of the eye as both the polymers sodium alginate and carbomer 940 are bioadhesive in nature. Finally, F4 (microemulsion containing 0.3% w/v gellan gum and 0.1% carbapol 940) was selected as the best formulation and considered for the determination of aqueous penetration of drug.

Table No. 7: *In vitro* drug release data of formulations of moxifloxacin microemulsion gel (F4 & F6) through goat's Cornea.

Time (hrs)	F1	F2	F3	F4	F5	F6
1	1.33±0.42	2.06±0.2	3.16±0.1	10.30±0.5	8.40±0.3	0
2	2.63±0.23	3.03±0.1	5.19±0.2	12.3±0.02	17.50±0.8	3.74±0.02
4	3.94±0.42	4.88±0.7	7.48±0.1	15.88±0.03	28.80±1.3	7.06±0.02
6	6.96±0.99	8.03±1.1	11.0±0.6	21.55±0.01	40.75±0.8	13.52±0.01
8	9.02±0.50	23.0±3.03	38.6±4.9	41.65±0.02	59.51±2.7	20.43±0.03
10	11.86±.18	33.7±3.3	54.4±5.1	58.00±0.03	67.57±2.5	28.81±0.02
12	12.02±0.19	39.8±4.6	59.2±5.3	71.72±0.01	72.56±3.2	36.32±0.03
14	15.18±0.21	42.2±4.7	61.6±5.7	76.73±0.02	73.46±3.6	44.74±0.02
16	24.20±1.28	44.4±5.5	62.8±2.0	85.70±0.01	77.25±3.8	53.34±0.02
18	26.25±1.56	46.6±2.4	63.7±5.5	85.43±0.02	78.12±4.2	55.22±0.01
20	32.93±3.92	48.4±5.5	65.4±1.1	88.65±80.2	80.21±2.3	58.43±0.02
22	35.27±4.61	50.5±4.6	66.6±2.9	90.77±0.01	81.22±2.5	60.35±0.03
24	35.27±4.61	52.9±3.1	67.8±2.3	92.55±0.03	83.41±2.9	62.66±0.03

Ocular irritation studies

The normal average of blinking counts in rabbits was documented to be 2-5 times/min16. The counted blinking rates obtained after instillation of optimized formulation was within the normal range (3-5 blinks/min). Ocular irritation studies indicated that formulation was nonirritant. The formulation was very well tolerated by the eye of rabbit. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible. No signs of redness, watering of the eye and swelling were observed throughout the study with the formulations. The eye irritation in all rabbits (in both eyes) was determined. Irritation was

classified according to four grades: practically non-irritating, score 0–3; slightly irritating, score 4–8; moderately irritating, score 9–12; and severely irritating (or corrosive), score 13–16.

Table No. 8: Observed Eye Irritation in rabbits.

Rabbit number	Observed eye irritation	Observed eye irritation
	Eye drops (vigamox)	formulated moxifloxacin microemulsiongel
1	0	0
2	0	1
3	1	1
4	0	1
5	1	0
6	0	1

score 0–3, practically non-irritating; score 4–8, slightly irritating; score 9–12, moderately irritating; and score 13– 16, severely irritating (or corrosive).

Sterility testing

There was no appearance of turbidity among the prepared formulation moxifloxacin microemulsion gel and hence no evidence of microbial growth when the formulations were incubated for not less than 3 days at 30°C to 35°C in case of fluid thioglycolate medium and at 20°C to 25°C in the case of soya bean-casein digest medium as shown in Figure. The preparations being examined therefore passed the test for sterility.

Table No. 9: Sterility test for prepared formulations of moxifloxacin microemulsion gel.

S.N0.	Formulation code	Turbidity in Soya bean medium	Turbidity in Fluid thioglycolate medium
1	F1	Not Found	Not Found
2	F2	Not Found	Not Found
3	F3	Not Found	Not Found
4	F4	Not Found	Not Found
5	F5	Not Found	Not Found
6	F6	Not Found	Not Found

Comparision of drug release studies between form and marketed formulation.

Table No. 10: Marketed Formulation of Prepared formulation.

Time(hrs)	F4	Marketed formulation
1	10.30	2.60
2	12.3	1.50
4	15.88	4.10
6	21.55	5.10
8	41.65	6.20
10	58.00	10.41

12	71.72	19.18
14	76.73	31.50
16	85.70	49.86
18	85.43	48.65
20	88.65	55.91
22	90.77	60.22
24	92.55	69.15

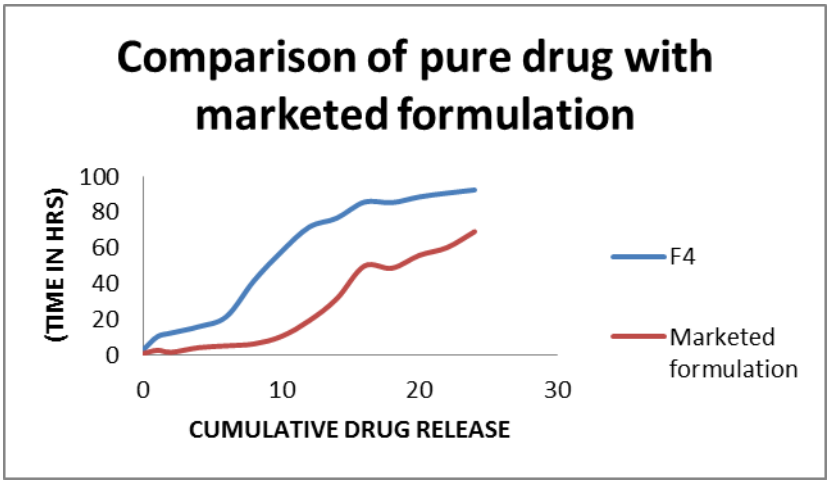

Figure No. 6: Comparison of pure drug with marketed formulation.

Stability

The stability studies were carried out by performing pH, viscosity, drug, release and upon storing the vial (F4) for about 3 months at a temperature of 40±2°C. It was observed that F4 formulation has good stability without altering the pH, viscosity and drug release on centrifugation at 12000rpm for about 30 minutes it was observed that all the formulations are stable and has good stability.Storage at 30 °C.

Table No. 11: Stability test.

S.No	Time period	pH	Units viscosity	*Invitro* drug release	Storage at 40±2 °C for 3 months
1		7.2	480	92.55±1.1	Stable
2		7.1	471	90.00±2.1	Stable
3		7.0	474	91.12±1.2	Stable

CONCLUSION

In this study we investigated the potential of a microemulsion gel for specific delivery of Moxifloxacin to ocular tissue. Comparing all the formulations, the (F4) Moxifloxacin microemulsion gel exhibited better wettability, higher drug levels and prolonged residence in the cornea. Ocular irritation test revealed good compatibility of the system. Prepared system was effective against most of the gram positive and gram negative microorganisms and any

damage to eye tissue was not seen. The prepared system is potential as ocular drug delivery system as it provided the highest efficiency with chance of forming the desirable viscous pseudo plastic system after dilution with resident tears.The pH was found to be good for eye. The partical size is soft for eye. The viscosity of the F4 formulation is has shear thinning and decrease in viscosity and increase in angular velocity. The F4 formulation shows anti microbial activity against s.aureus, P.aeruginosa and non irritant after ocular irritation studies in rabbit eye. Cumulative drug release studies showed that F4 has good formulation through goats cornea with 92.55%.The optimized formulation was compared with marketed formulation and found that because of presence of surfactants and co-surfactants F4 in having higher drug release than marketed formulation. The sterility was found to be positive by using soyabean casein medium fluid, thioglycolate medium. Therefore, it was concluded that this moxifloxacin microemulsion gel represents an alternative for preventing corneal infections.

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