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Development and Characterization of Ofloxacin Microemulsions for Ocular Application

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ABSTRACT

The objective of the present investigation was to develop effective delivery vehicle for ocular administration of ofloxacin. The solubility of ofloxacin in oils and surfactants was checked to identify components of the microemulsion. The pseudo ternary phase diagrams were plotted to identify the area of microemulsion existence. The prepared ME's were characterized for pH value, refractive index, viscosity, stability, and surface morphology by SEM analysis, zetapotential, FTIR studies and invitro diffusion studies. They show acceptable physico-chemical behaviour, especially pH value, refractive index, viscosity. A prolonged drug release from the microemulsions was shown in in-vitro experiments. Therefore, the microemulsions were suggested to be a promising drug carrier for ocular and allowing the possibility of decreasing the number of applications of eye drops per day.

Key words: Ofloxacin, Microemulsion, Physicochemical characteristics, In-vitro diffusion studies.

1. INTRODUCTION

Poor aqueous solubility of compounds affects pharmaceutical product development in nearly all therapeutic areas including ophthalmology. Despite the accessibility of the front of the eye, efficient delivery of drug to treat various ocular disorders is a challenge to the formulation scientist in addition to the often low drug solubility. The majority of ophthalmic medications are formulated as eye drops delivered topically to the eye. Due to anatomical constraints, the volume that can be administered is limited to approximately 30 μL. This, together with the efficient clearance system that exists in the front of the eye, makes it difficult to maintain an effec tive pre-ocular drug concentration for a desired length of time[1]. The bioavailability of eye drops is typically less than 5% in spite of frequent instillations. Various formulation strategies have been used to increase aqueous solubility of active pharmaceutical ingredients and pre-ocular retention of eye drops.

The conventional ophthalmic dosage forms are relatively simple: water-soluble drugs arc delivered in aqueous solution and water-insoluble drugs are prepared as suspensions or ointments. However, these delivery systems currently used present very low drug absorption in the cornea, systemic exposure because of nasolachrymal drainage and lack of efficiency in the posterior segment of ocular tissues[2], consequently, after instillation of an eye drop, most of the applied dose does not reach the intraocular tissues. This is particularly due to tbe corneal resistance and rapid loss of the instilled preparation from tbe precorneal area. However, next to the relative safety and convenience, one of the advantages of topical application in ophthalmology over other administration forms is the low risk of systemic side effects. Various systems as carriers to new drug delivery vehicles have been developed to increase ocular absorption of opbthalmic drugs[3]. Most of the formulation strategies aim at maximizing ocular drug permeability through prolongation of the drug residence time in tbe cornea and conjunctival sac, as well as minimizing precorneal drug loss[4]. Recent research efforts have focused on the development of microemulsions, which seems to be an interesting alternative to topical ocular drug delivery. They have been proposed to achieve sustained release of a drug applied to the cornea and higher penetration into the deeper layers of tbe ocular structure and the aqueous humour[5].

Microemulsions were first described by Hoar and Schulman in 1943[6] and they are promising dosage forms for ocular use because they present several advantages regarding

ophthalmic administration[7]. These systems are dispersions of water and oil that require surfactant and cosurfactant agents in order to stabilize the interfacial area. They have a transparent appearance, thermodynamic stability and small droplet size of the dispersed phase (<1.0 μm). Production and sterilization are relatively simple and inexpensive; they do not require much energy or the use of special equipment, and can be sterilized by filtration. These systems offer additional advantages that include; low viscosity, great ability as drug delivery vehicles and increased properties as absorption promoters. Furthermore, the possibility of prolonged release of drugs in microemulsions makes these vehicles very attractive for ocular administration because they can decrease the number of applications, per day, of eye drops[8].

OFL is a pale yellow or bright yellow, crystal-line powder. OFL is antibacterial and is the most commonly used fluroquinolone. It inhibits the enzyme bacterial DNA gyrase, which nicks double stranded DNA, introduces negative supercoils and then reseals the nicked ends. This is necessary to prevent excessive positive supercoiling of the strands when they separate to permit replication or transcription. The bactericidal action probably results from digestion of DNA by exonucleases whose production is signaled by the damaged DNA[9]. OFL has a broad antimicrobial spectrum against gram-positive and gram-negative microorganisms. This drug is routinely used in the many ocular conditions like infections, inflammations, conjunctivitis, blepharitis, iritis, corneal ulcer etc[10, 11]. In the present study, it was aimed to prepare ocular microemulsion containing ofloxacin along with hydrophilic and hydrophobic polymers with better solubility and longer duration of action delivering the drug in zero order kinetics.

2. EXPERIMENTAL MATERIALS

Ofloxacin purchesed from Yarrow chemicals pvt.ltd, Mumbai, India, oleic acid (OA), ethyl oleate (EO), Linseed oil (LO), Olive oil (OL), Castor oil (CO) and Soyabean oil (SO) were purchased from CDH Laboratory Pvt. Ltd Mumbai, India. Propylene glycol, Polyethylene glycol 200 (PEG-200) was purchased from HiMedia Laboratories Pvt. Ltd. Mumbai. India. Span-80 (S-80), Tween-80 (T-80) was purchased form Merck, (Mumbai, India). Other chemicals are of analytical grade.

2.1. Screening of oils and surfactants for microemulsions

The solubility of OFL was determined in various oils and surfactants. The oils employed were OA, EO, LO, OL, CO and SO. The surfactants used were S-80, T-80 respectively. Drug powder of OFL was added in excess to each of the oils and surfactants and then vortexed. After vortexing, the samples were kept for 72 h at ambient temperature for attaining equilibrium. The equilibriated samples were then centrifuged at 5000 rpm for 30 min to remove the undissolved drug. The aliquots of supernatant were filtered through 0.45 μm membrane filters and the solubility of OFL was determined by analyzing the filterate spectrophotometrically after dilution with methanol at 296 nm. Appropriately diluted solutions of oils in methanol were taken as blank.

2.2. Construction of pseudo-ternary phase diagrams

In order to find out the concentration range of components for the existing range of microemulsions, pseudo-ternary phase diagrams were constructed using H_2O titration method at ambient temperature (25°C). Oleic acid was selected as the oil phase. T-80 and PEG-200 were selected as surfactant and co-surfactant, respectively. Distilled water was used as an aqueous phase. Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratios of T-80 to PEG 200, respectively. For each phase diagram at a specific surfactant/co-surfactant weight ratio, the ratios of oil to the mixture of surfactant and co-surfactant were varied as 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5: 5.5, 5:5, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1, 9.5:0.5. The mixtures of oil, surfactant and co-surfactant at certain weight ratios were diluted with H_2O dropwise, under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions, crude emulsions or gels. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous type microemulsions. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after tilted to an angle of 90^0.

2.3. Preparation of OFL loaded microemulsions

After the microemulsion regions in the phase diagrams were identified, the microemulsion formulations were selected at different component ratios as described in Table 1. In order to prepare the drug loaded microemulsions, a stock solution containing OFL was prepared with the mixture of OA and PEG 200. The clear oily phase containing OFL was obtained by diluting the weighed amount of stock solution with OA and PEG 200. T-80 was taken

and solubilized in the distilled water. Then water was added to the clear oily phase drop by drop. The o/w microemulsions containing ofloxacin were obtained under a magnetic stirring at ambient temperature. (S: Surfactant, CoS: Co-Surfactant)

Table No.1: Composition of the microemulsion formulations

	S:CoS-1:1 ratio		S:CoS-2:1 ratio		S:CoS-3:1 ratio	
Components	A	B	C	D	E	F
	300	300	300	300	300	300
OA (ml)	12	12	12	12	12	12
Tween 80 (ml)	10	20	20	38	30	27
PEG200 (ml)	10	20	10	19	10	9
Water	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml

2.4. Determination of Physico-Chemical Parameters

2.4.1. Determination of pH

The pH of the prepared microemulsion was determined using Electronic digital pH meter, standardized using pH 4.0, 7.0, 9.18 standard buffers before use (Elico make).

2.4.2. Droplet size and Size distribution

Droplet size of drug loaded microemulsions were determined by using dynamic light scattering method using melvern zeta master (MAL, 500962, Malvern, India). One ml of formulation was diluted to 10 ml in a test tube and gently mixed using a glass rod. The resultant emulsion was then subjected for particle size analysis[12].

2.4.3. Zeta potential

Zeta potential of formulations was determined by using dynamic light scattering method (MAL, 500962, Malvern, India). One ml of formulation was diluted to 10 ml in a test tube and gently mixed using a glass rod. The resultant emulsion was subjected for zeta potential analysis [13].

2.4.4. SEM Analysis

The surface characteristics of microemulsion were performed on the formulation selected on the basis of particle size the formulation using scanning electron microscopy. The SEM micrographs of the formulation are presented in Figure No.1.

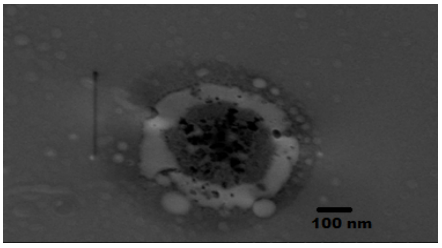


Figure No.1: Typical SEM image of OFL-ME formulation.

2.4.5. Viscosity Measurement

The viscosities of the prepared microemulsion formulations were measured at 25^{0}C using Brookefield Viscometer.

2.4.6. Percentage Transmittance

The % Transmittance of prepared microemulsion formulations were measured by using colorimeter at 570 – 590 nm.

2.4.7. Refractive Index

The refractive index of microemulsion formulations was determined using an Abbe-type refractometer.

2.4.8. Surface Tension

The measurement of surface tension was made with a stalagmometer.

2.4.9. Stability study

Stress conditions normally applied for evaluating the stability of microemulsion includes aging, temperature and centrifugation. The main parameters which are used to determine the stability is phase separation, viscosity and globule size distribution[14]. Selected formulations were kept at three different temperatures i.e. 4^{0}C, 25^{0}C, 45^{0}C and observed for phase separation and particle size for about 45 days. Formulations were also subjected to centrifugation at 3000, 5000, 10,000 rpm for 15 minutes at room temperature and samples were withdrawn after a particular time interval and observed for phase separation and average particle size.

2.4.10. FT-IR analysis

FT-IR spectra of OFL, selected microemulsion formulation and without drug microemulsion formulation were recorded at room temperature in the range of 4000–400 cm−1 by KBr pellet method using a FT-IR spectrophotometer. The FT-IR spectrum graphs are shown in Fig. 2.

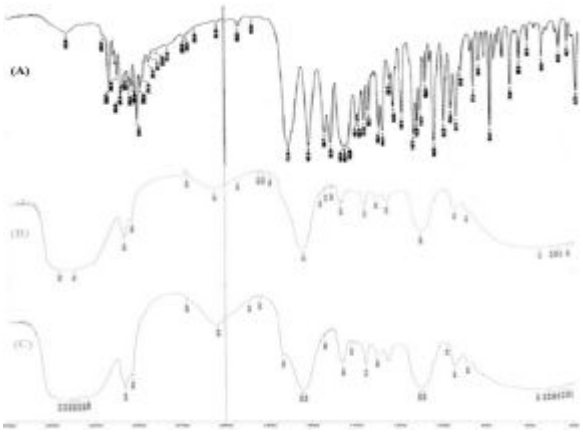


Figure No.2: FT-IR spectra of (A) Drug-OFL (B) without drug microemulsion formulation [Placebo] (C) Drug loaded microemulsion formulation.

2.4.11. In-vitro diffusion studies

Selection of receptor solution used for permeation experiments is critical in case of topical application because they mimic the in vitro situation. Buffer solutions are the most commonly used media for lipophilic compounds. Dialysis membrane (average flat width- 24.26 mm, average diameter- 14.3mm, capacity factor- 1.61ml/cm) was used as an artificial membrane for preliminary in vitro studies because of simplicity, homogeneity and uniformity. This membrane was hydrated in distilled water for 24 hours prior to use. The permeation studies were carried out using a modified Franz Diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 1.2 cm^2 and 22 ml respectively. The temperature of receptor fluid was maintained at 37±1°C. The receptor compartment contained Bicarbonate Ringer solution (pH 7.4). Dialysis membrane was mounted between the donor and receptor compartment. 1 ml microemulsion formulation of drug was applied to the upper side of membrane in donor compartment. Samples were withdrawn through the sampling port of the diffusion cell at predetermined intervals over 24 hours and analyzed spectrophotometrically (15) at 288 nm. An equal volume of fresh Bicarbonate Ringer solution (pH 7.4) maintained at 37±1°C was replaced into the receptor compartment after each sampling. The plots of % drug release versus time were plotted.

3. RESULTS AND DISCUSSION

For development of microemulsion system for topical delivery of OFL, the suitable oil and surfactant have to be choosen. So the solubility of OFL was determined in various oils and surfactants (Table No.2). Among the nonionic surfactants studied T-80 led to the highest solubility of OFL (11.88±2.38 mg/mL). Moreover, T-80 is known to be less affected by pH and ionic strength changes and acts as a solublizing agent [16]. On the other hand, there was no significant difference in the solubility of OFL among the various oils tested except LO, OO, SO which exhibited low solubility as compared to other oils. However, OA, increased the solubility of OFL (24.88±0.24 mg/mL) compared with other oils.

Table No. 2. Solubility of OFL in various oils and surfactants (mean±S.D, n=3)

Vehicle	Solubility (mg/ml)
Oils	
Oleic acid	24.88±0.24
Ethyl oleate	9.23±1.86
Linseed oil	9.48±1.45
Olive oil	10.84±0.18
Castor oil	6.53±0.25
Soyabean oil	5.22±0.74
Surfactants	
Span-80	6.55±1.37
Span-20	2.47±1.78
Tween-80	11.88±2.38
Tween-40	7.31±1.43

In addition, in respect of convenience of formation and use, the oil OA is better choice in comparison with other physiologically tolerable oils. From these solubility results, T-80 and OA were chosen as a surfactant and oil respectively, for the preparation of ME formulations of OFL for further studies.

The aim of the construction of pseudo-ternary phase diagrams was to find out the existence range of microemulsions. The studied systems were composed of OA, T-80, PEG-200 and water. The pseudoternary phase diagrams with various weight ratios of T-80 and PEG-200 were prepared and the result ternary phase diagrams are shown in figure no. 3. The shaded region in the figures represents the microemulsion formation regions.No distinct conversion from oil-in-water (o/w) to water-in-oil (w/o) microemulsion was observed for both. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. The microemulsion region was changed slightly in size with increasing ratio of T-80 to PEG-200.

The formulated microemulsions were subjected to the study of physicochemical characteristics Table No.3. The formulations were optically clear, transparent and elegant in appearance. The pH of the formulations was found to be between 6.50 and 7.20. The average droplet size of 10 and 100 times diluted form of microemulsions were found to be in the range of 32.51±0.1 nm - 47.53±0.2 nm

as shown in the Figure no. 4 (For 10 time's diluted microemulsion).

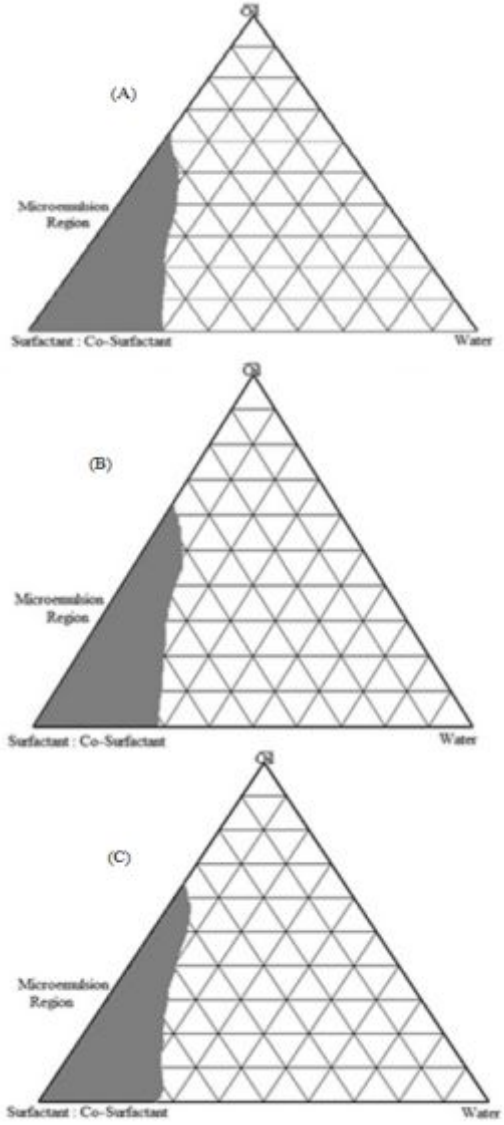


Figure No.3: Pseudo-ternary phase diagrams of the oil-surfactant-water system at (A)1:1, (B) 2:1 and (C) 3:1 weight ratios of Tween 80 to PEG-200 at 25 ^{0}C.

The Polydispersity Index was found to be well below 1.0 which confirms that the optimized microemulsion remains stable upon dilution. Zeta potential of 10 and 100 times diluted microemulsions were found to be in the range of -4.74±0.89 mV and -14.4±0.99 as shown in the Figure no. 5 (For 10 times diluted microemulsion), indicating that dilution does not significantly affect the zeta potential of the microemulsion. SEM imaging of OFL loaded microemulsions revealed that droplets were spherical in shape Figure No.1. Rheological behavior of the microemulsion systems indicated that the systems were non-Newtonian in nature showing decrease in viscosity at the increasing shear rates. As the concentration of the surfactant increases there was significant difference found between the viscosities of prepared microemulsion systems. The % transmittance of the formulations was clear and transparent with the % transmittance range of 98 to 100 %. The formulations had refractive index values ranging from 1.348 to 1.378. The surface tension of the prepared microemulsions ranging from 25 to 33 mN/m. When formulations are subjecting to the centrifugation at 3000, 5000, 10,000 rpm for 15 minutes at room temperature, they did not cause phase separation. The data of the physicochemical characteristics of developed microemulsions is given in Table 3.

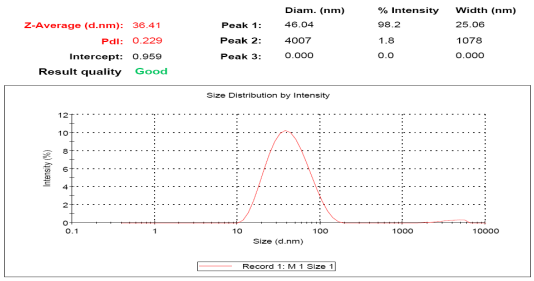
3.1. FTIR Studies

The major peaks (Table No.4) which are present at 2936 cm^{-1}, 1714 cm^{-1}, 1621 cm^{-1}, 1550 cm^{-1}, 1459 cm^{-1}, and 1086 cm^{-1}nm, are also present in the microemulsion formulations thus there is no interaction between the drug and excipients.

Table No.4: Infrared frequencies of different functional groups present in the components of microemulsion.

Frequency(cm−1)	Functional moiety
2936 cm^{-1}	CH_2 Stertching
1714 cm^{-1}	(C=O) Stertching
1621 cm^{-1}	$(C=O)_{ring}$ Stertching, (N-C=N)+(NH2), (NH3) in-plane deformation
1550 cm^{-1}	CC+CF Stertching
1459 cm^{-1}	(CH_3) Stertching
1086 cm^{-1}	(CH)+Ring in-plane deformation

The comparison of IR spectra of drug loaded microemulsion and pure microemulsion (figure No. 2) in the region of 3200 to 3600 cm^{-1}nm. As the concentration of surfactant is more in pure microemulsion formulation (without drug) there will be stronger intraction between water and surfactant which leads to decrease in peak frequency. Where as in drug loaded microemulsion formulation the intraction between due to the replacement of the surfactant molecules by drug molecule there will be increase in the frequency due to weak intraction of water and surfactant[17].Figure 4: Droplet size of 10 times diluted Microemulsion



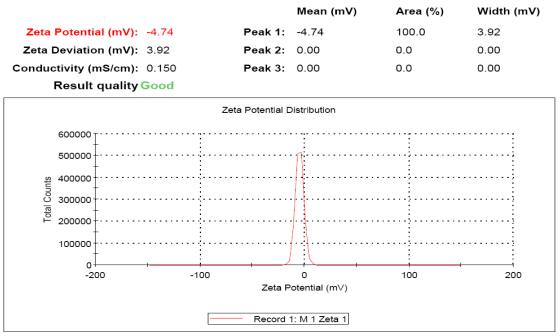


Figure 5: Zeta Potential of 10 times diluted Microemulsion.

3.2. Invitro Diffusion studies

The In-vitro diffusion studies were performed for both marketed OFL eye drops and the prepared ME formulations (Table No.5), in which the prepared formulations shows prolong Invitro % drug release compared to marketed OFL eye drops (Figure No.6).

Table No. 3: Different parameters of OFL loaded Microemulsion formulations (Mean ± SD)

Formulations (Mean ± SD)	pH	%Transmittance	Refractive index	Viscosity (mPa.s)	Surface tension (mN/m)	Droplet sizes (nm)	Zeta potential (mV)
A	6.87±0.02	99.85 ± 0.19%	1.348±0.01	49.21±0.98	32.86±0.01	36.41±0.2	-4.74±0.89
B	6.92±0.02	99.35 ± 0.23%	1.358±0.01	51.02±0.13	31.96±0.21	32.51±0.1	-5.32±0.18
C	6.95±0.02	99.15 ± 0.29%	1.378±0.01	52.12±0.73	29.32±0.16	44.15±0.1	-5.52±0.52
D	7.02±0.02	98.95 ± 0.11%	1.352±0.01	57.62±0.92	27.36±0.31	47.53±0.2	-7.64±0.09
E	7.09±0.02	98.82 ± 0.43%	1.357±0.01	66.83±0.19	27.80±0.20	41.35±0.3	-13.0±0.36
F	7.12±0.02	98.65 ± 0.39%	1.368±0.01	72.62±0.03	25.88±0.98	39.41±0.2	-14.4±0.99

Table No.5: In-vitro percentage drug release of different formulations of OFL-ME

Time in hours	% Drug Release-Eye drops	% Drug Release-A	% Drug Release-B	% Drug Release-C	% Drug Release-D	% Drug Release-E	% Drug Release-F
0	0	0	0	0	0	0	0
1	11.67±0.31	3.46±0.23	5.01±0.01	4.10±0.03	4.83±0.01	3.83±0.95	3.74±0.31
2	32.99±0.23	7.90±0.31	8.52±0.36	8.39±0.74	8.88±0.54	7.28±0.33	8.01±0.45
3	47.50±0.09	12.18±0.09	16.92±0.21	12.96±0.95	15.02±0.87	13.90±0.54	16.39±0.32
4	62.79±0.39	16.90±0.90	25.42±0.32	17.53±0.28	20.05±0.54	18.79±0.34	22.03±0.23
5	79.32±0.43	20.63±0.33	29.43±0.65	21.83±0.39	25.46±0.29	22.97±0.30	28.18±0.45
6	89.01±0.11	25.96±0.21	35.21±0.78	28.12±0.47	30.26±0.39	28.03±0.56	34.46±0.34
7	99.52±0.34	30.48±0.56	41.48±0.47	33.01±0.12	36.51±0.43	32.46±0.12	40.89±0.55
24	37.14±0.01	77.47±0.30	86.44±0.58	80.74±0.31	88.57±0.38	79.61±0.09	82.29±0.22

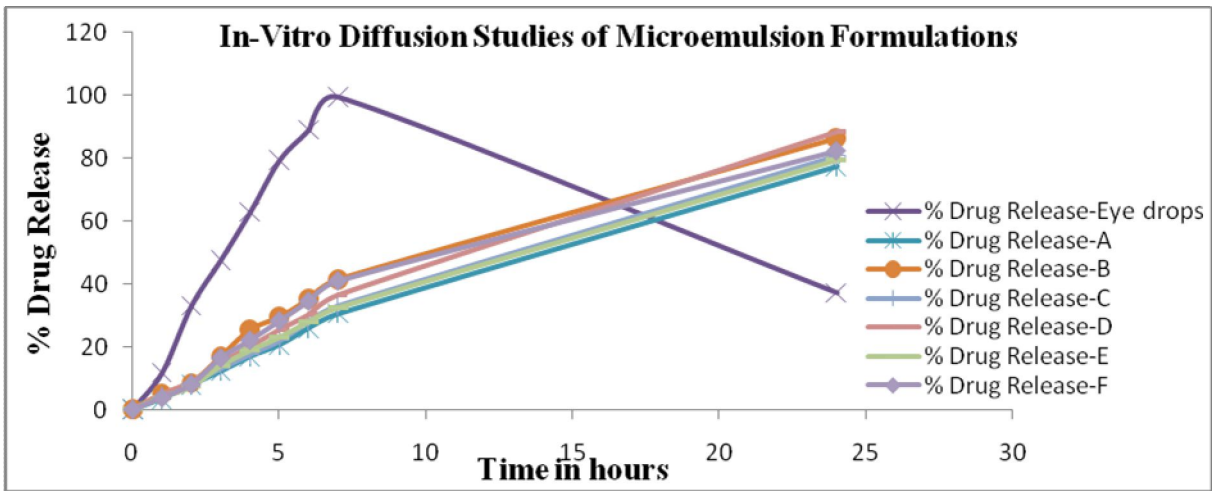


Figure No.6: Graph of In-vitro percentage drug release of different formulations of OFL-ME

4. CONCLUSION

Rational of the present study was to improve the solubility of OFL was significantly improved by ME systems which were optimized using pseudo-ternary phase diagrams. The prepared OFL-ME showed acceptable physico-chemical behaviour and presented good stability for 3 months and the particle size of the formulations is within the range of 10-100 nm. By the invitro diffusion studies, the ME formulation showed prolanged drug release when compared with a conventional OFL eye drops and concluded that the prepared OFL-ME is advantageous for ophthalmic use and viable alternative to conventional eye drops by providing prolong release of medicaments to the eye.

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