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Formulation, Optimization and Evaluation of SMEDDS of Norfloxacin for Treatment of Ocular Infection

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ABSTRACT

In the modern pharmaceutical and clinical fields, ocular therapy has been shown to be the most appealing and practical method of drug application since ancient times. A successful public health care system must overcome several pharmacokinetic and pharmacodynamic challenges, including the overwhelming need for dose-optimization techniques to lower toxic effects, increase drug efficacy in a given formulation, and reduce dosing frequency. The majority of formulations currently on the market still suggest ocular therapy. The present research aimed to formulate, optimize and evaluate SMEDDS of Norfloxacin for treatment of ocular infection which produced sustained in-vitro drug release and supposed to elicit enhanced bioavailability. The drug excipient compatibility was determined by FTIR studies which was further confirmed by TLC studies. Conclusively Norfloxacin was found to be compatible with various excipients incorporated in preparation of SMEDDS formulation. Different physicochemical parameters of prepared SMEDDS were determined; droplet size range (91-182nm), Zeta potential (-0.11)-(-25.07) mV, pH (7.4), viscosity (26.5-35.3cPs) and drug content (91.61–99.96%). All SMEDDS were found thermostable and no phase separation was observed upon centrifugation stress testing. The in-vitro drug release results revealed that % drug release ranged between 72.019% - 94.101% in pH 7.4 phosphate buffer and 95.072%-71.022& in STF at the end of 24 hrs. On the basis of evaluation parameters, formulation B1 was considered as optimized batch. The results obtained with optimized batch were droplet size (91nm), zeta potential (-25.07) & the percentage drug release was found to be 94.101% and 95.072% in pH 7.4 phosphate buffer and STF respectively. Stability study was performed with the optimized formulation (MEF) as per the ICH guideline and the outcomes indicated that formulations was stable and thus complied with dose conformity criterion. All above data satisfactory complied with the characteristic requirements for the formulation of SMEDDS of Norfloxacin for ocular delivery.

Keywords: *SMEDDS, norfloxacin, ocular, infection, bioavailability*

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1. Introduction

The particular architecture and physiology of the eye make treating ocular infections which can be caused by bacteria, viruses, fungi, and parasites very difficult. It is challenging to get therapeutic concentrations of antimicrobial drugs at the infection site because of the blood-retinal barrier and the avascular cornea, which limit medication penetration. In order to improve the ocular bioavailability of medicinal medicines, novel drug delivery methods are desperately needed.[1-2]

A common treatment for many bacterial infections, including those affecting the eyes, is norfloxacin, a broad-spectrum fluoroquinolone antibiotic. By blocking the enzymes topoisomerase IV and bacterial DNA gyrase, which are essential for transcription and replication of bacterial DNA, it works by suppressing these processes. Norfloxacin, like many antibiotics, has drawbacks, including reduced ocular absorption and poor water solubility. Due to quick precorneal clearance and short ocular residence times, traditional formulations like eye drops frequently fall short of providing enough drug concentrations to the intended tissues.[3-4]

The development of Self-Microemulsifying Drug Delivery Systems (SMEDDS) in recent years has shown this to be a viable solution to these problems. When the aqueous environment of the gastrointestinal system or the surface of the eye is slightly disturbed, SMEDDS isotropic mixes of oils, surfactants, and co-surfactants spontaneously create tiny oil-in-water microemulsions. Drugs that are poorly soluble in water can have their solubility and bioavailability greatly increased by these systems. SMEDDS provide improved drug penetration, extended drug retention on the ocular surface, and defense against drug degradation for ocular applications.[5-6]

SMEDDS utilization in ocular medication administration takes advantage of the special properties of the eye to improve therapeutic effectiveness. Better medication absorption through the corneal and conjunctival barriers is made possible by the microemulsion created by SMEDDS, whose tiny

droplet size enhances the surface area for absorption. Furthermore, the SMEDDS's oily phase can serve as a reservoir to deliver a steady release of the medication. This is especially helpful in treating persistent ocular infections, where it's critical to retain therapeutic drug levels for a long time.[7-8]

SMEDDS with norfloxacin are made to get over the drawbacks of traditional formulations. Norfloxacin's solubility in the lipid phase is improved when it is added to a SMEDDS, which enables a larger concentration of the active component to be delivered to the ocular tissues. Increased therapeutic effectiveness, fewer dosage adjustments, and better patient compliance are possible outcomes of this enhanced solubility and bioavailability. Moreover, the drug's release profile may be modulated by the surfactants and co-surfactants included in SMEDDS, thereby providing both short-term and long-term therapeutic benefits.[9-10]

The oil phase, surfactant, and co-surfactant are carefully chosen throughout the creation of Norfloxacin SMEDDS. In order to be biocompatible with the ocular surface, the oil phase has to be able to solubilize a significant quantity of norfloxacin. Medium-chain triglycerides, which have a good solubilizing ability and safety profile, are among the frequently utilized oils. Surfactants, such polysorbates and polyethylene glycol esters, are essential for maintaining the stability of the microemulsion and promoting the absorption of drugs. In addition to increasing the stability of the system overall and lowering the risk of ocular irritation, co-surfactants often alcohols or glycols help lower the surfactant concentration required.[11-13]

In order to create a homogenous preconcentrate, surfactants and co-surfactants are usually added after the drug has been dissolved in the oil phase of the preparation process for norfloxacin-loaded SMEDDS. The next step is to dilute this preconcentrate with an aqueous media, such tear fluid, to create a microemulsion. The microemulsion in the final formulation creates a clear, non-irritating solution that spreads readily over the surface of the eye and may be used as eye drops.[14-15]

The possibility of continuous drug release is one of the main benefits of employing SMEDDS for ocular administration. The mucin layer of the eye might cling to the microemulsion droplets, extending the duration of the drug's residency on the ocular surface. Because of its longer retention period, norfloxacin may be released gradually, which is advantageous when treating chronic or recurrent infections. Furthermore, the drug's resistance to degradation by tear film-resident enzymes may be prevented by encapsulating norfloxacin in the oil phase, which improves the formulation's stability and effectiveness.[16-17]

Numerous preclinical research has looked into the effectiveness of norfloxacin SMEDDS in treating eye infections. In comparison to traditional eye drop formulations, these investigations have shown that SMEDDS can greatly increase the ocular bioavailability of Norfloxacin. By eradicating harmful germs more effectively, the increased medication concentration in the ocular

tissues can shorten the length and severity of illnesses. Additionally, SMEDDS can reduce the frequency of dose, which enhances patient adherence to the prescribed course of action.[18-19]

In conclusion, a potential breakthrough in the management of ocular infections is the creation of SMEDDS for the ocular administration of norfloxacin. The drawbacks of standard formulations are addressed by this novel drug delivery method, which provides increased solubility, prolonged release, and improved bioavailability of the medication. With more study and development in this field, norfloxacin SMEDDS may eventually be used as a regular therapy for ocular infections, providing better therapeutic results and happier patients. Continued research and development of SMEDDS and related advanced formulations might have a significant impact on how ocular drug delivery develops in the future.

2. Materials and Methods

2.1 Materials

Drug sample and chemical reagents used in the formulation of liposomal gel of Atorvastatin were procured from different reputed companies.

2.2 Experimental work

2.2.1 Preparation of standard calibration curve in phosphate buffer (pH 7.4)

2.2.1.1 Preparation of standard stock solution

2.2.1.1.1 Preparation of stock A (1000µg/ml) solution

Accurately weighed quantity of Norfloxacin (100mg) was transferred into 100ml volumetric flask and dissolved in 50ml of pH 7.4 phosphate buffer & finally volume was made up to 100 ml by using the same medium to obtain stock solution of 1000µg/ml.

2.2.1.1.2 Stock-B (100 µg/ml) solution

Using stock solution A, 100µg/ml concentration (Stock B) was prepared by diluting its 10ml in another flask and volume made up to 100ml with pH 7.4 phosphate buffer.

2.2.2 Estimation of λ max

A sample (stock B) was scanned between 200-400nm to access the λ max for Norfloxacin which was reproduced and confirmed by obtaining the overlain U.V spectra of the drug using different concentrations i.e. 2, 4, 6, 8 and 10µg/ml.

2.2.3 Preparation of standard calibration curve of Norfloxacin in pH 7.4 phosphate buffer

Stock B was further diluted to obtain Serial dilutions in the concentration range of 5-25µg/ml and run on UV-vis spectrophotometer at 300nm. The respective absorbances were recorded and a graph of concentration Vs absorbance was plotted to obtain the standard calibration curve.

2.2.4 Preparation of standard calibration curve in Simulated tear fluid

2.2.4.1 Preparation of Simulated Tear Fluid (STF)

STF solution was prepared by using the following composition and its pH was adjusted to 7.4.

Table 1. Composition of STF

S. No.	Ingredients	Quantity
1	Sodium chloride	0.670gm
2	Sodium bicarbonate	0.2gm
3	Calcium chloride dihydrate	8.0mg
4	Distilled water up to	1000ml

2.2.4.2 Preparation of stock C (1000µg/ml) solution of the drug

Accurately weighed quantity of drug (100mg) was transferred into a 100 ml volumetric flask and dissolved in little quantity of STF and made up to the mask with same solvent.

2.2.4.3 Preparation of stock D (100µg/ml) solution

About 10 ml of stock C was diluted up to 100ml using simulated tear fluid (STF) to prepare stock D solution. Different aliquots containing 2, 4, 6, 8 and 10 mcg/ml of Norfloxacin were further prepared by using stock D.

2.2.4.4 Preparation of standard calibration curve of Norfloxacin in STF

The standard calibration curve was obtained with the same sample concentrations as opted in the above process by plotting absorbance V/S. concentration graph.

2.3 Drug- Excipient Compatibility Study

2.3.1 FTIR technique

Drug-excipient compatibility study was carried out by FTIR Spectrophotometry. A fine power of drug and KBr was compressed into disc in the ratio of 1:9 was ground into fine power using mortar pestel and transformed to pellets by 75 kg/cm^2 in a hydroulic pressure, which was scanned 45 time at a resolution of $2cm^{-1}$. The characteristic peaks were recorded. [20]

2.3.2 TLC Method

Compatibility of drug with surfactant, cosurfactant and oil was carried out by densitometric TLC evaluation using (Silica gel F_{254}) glass plates. Mobile phase comprised of Dichloromethane: Methanol : Toluene : Diethylamine : Water in the ratio of 40:40:20:14:8 (v/v) respectively. The separated spots were evaluated at 300nm.[21]

Distance travelled by the solute

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by mobile phase}} \times 100$$

2.4 Construction of Pseudo Ternary Phase Diagram

The phase diagrams were constructed to obtain the appropriate concentration of oil, surfactant & co-surfactant and water as well as to investigate largest existing area of SMEDDS. Ternary phase diagram was prepared by using a constant ratio of surfactant (Tween 80) to co-surfactant (Tween 20) i.e 2:2.

2.5 Formulation Design

Eighteen batches of SMEDDS were prepared using different drug, surfactant & co-surfactant ratio as depicted in the following table:

Table 2: Formulation design (surfactant & co-surfactant Ratio 2:2)

S. No.	Oleic Acid (% v/v)	Water (% v/v)	Tween 80/Tween 20 (2:2) (%v/v)
MEF_1	5.00	75	20.00
MEF_2	5.00	82.80	12.20
MEF_3	5.00	61.50	33.50
MEF_4	5.00	67.30	27.70
MEF_5	5.00	72.90	22.10
MEF_6	5.00	77.60	17.40
MEF_7	5.00	53.70	41.30
MEF_8	5.00	49.30	45.70
MEF_9	5.00	88.00	06.00
MEF_{10}	5.00	57.25	37.75
MEF_{11}	5.00	57.69	37.31
MEF_{12}	5.00	51.16	43.84
MEF_{13}	5.00	60.00	35.00
MEF_{14}	5.00	57.14	37.86
MEF_{15}	5.00	71.42	23.58
MEF_{16}	5.00	65.3 9	29.61
MEF_{17}	5.00	68.30	26.70
MEF_{18}	5.00	73.00	20.00

2.6 Preparation of Norfloxacin SMEDDS

The composition of Oil, Surfactant:Co-surfactant and water thus obtained from phase diagram was further used for SMEDDS preparation. Oleic acid, Tween 80 and tween 20 (2:1) were mixed using a homogenizer maintained at room temperature. It was treated with water till transparency was achieved and stirring was continued for 2hr. Calculated amount of drug was finally added to the above mixture with continuous stirring to obtain SMEDDS.

2.7 Evaluation of SMEDDS

2.7.1 Droplet size

Droplet size of prepared SMEDDS was determined with the help of photomicroscope. The droplet size distribution was determined and the average diameter was calculated for each formulation.

2.7.2 Thermal stability

20ml of prepared SMEDDS was transferred in 25ml transparent borosilicate volumetric flask and volume was made up to the mark and then stored at three different temperatures (4°, 25°and 40°C) in BOD for 2 months. Samples were periodically observed for any physical changes i.e. decrease of clarity, coalescence, turbidity etc.

2.7.3 Centrifugation stress testing

Prepared SMEDDS were centrifuged at different speeds (5000 and 10000 rpm) for 30 minutes and observed for any change in homogeneity like phase separation, phase inversion, aggregation, creaming and cracking of the SMEDDS.

2.7.4 pH

The pH values of different formulations were measured by a digital pH meter.

2.7.5 Rheological study

The viscosity of SMEDDS were determined at 37^0C using a Brookfield viscometer (Brookfield DV-E viscometer). Spindle No 40 was used and rpm was set at 12.

2.7.6 Drug entrapment efficiency

SMEDDS equivalent to 100mg was dissolved in 100ml of glacial acetic acid in a volumetric flask. The solution was filtered and about 1ml was pippetted out and transferred to 100ml volumetric flask and diluted upto the mark with glacial acetic acid. The prepared solution was analyzed spectrophotometrically at 300nm. The concentration of Norfloxacin in SMEDDS was obtained using standard calibration curve of the drug.

2.7.7 Zeta potential

The Zeta potential of all SMEDDS batches was determined by Zeta Sizer.

2.7.8 Surface morphology studies

The surface morphology was studied by SEM in the case of each batch of SMEDDS.

2.7.9 In-vitro release studies

In-vitro release study of SMEDDS formulation was carried out by using Franzs Diffusion cell using pH 7.4 phosphate buffer and STF. The SMEDDS formulation was placed in donor compartment & freshly prepared STF solution in receptor compartments. A dialysis membrane was placed between receptor & donor compartments. The assembly was placed on thermostatic magnetic stirrer. The temperature of the medium was maintained at $37^0C \pm 0.2^0C$. 1ml sample was withdrawn at predetermined time intervals and fresh medium was replaced. The withdrawn samples were suitable diluted with pH 7.4 phosphate buffer & STF and analyzed by UV spectrophotometer at 300nm.

2.7.10 Evaluation parameter of optimized batch

Optimized batches thus obtained was also evaluated for; droplet size, thermal stability, centrifugation stress, pH determination, rheological study, % drug content, zeta potential, in-vitro permeation studies.

2.7.11 Accelerated stability studies

The optimized formulation was placed in amber colored vials & sealed with aluminum foil for the short term accelerated stability study at temperature 40 ± 2^0C & 75 ± 5% RH as per ICH guidelines. The samples were analyzed at predetermined intervals in-vitro dissolution respectively.

3. Results and Discussion

3.1 Experimental Studies

3.1.1 Spectrophotometric scan of Norfloxacin

The stock solution of Norfloxacin (100µg/ml) was prepared using glacial acetic acid and scanned within the range of 200-400nm. The scan concluded λmax of 300nm for Norfloxacin.

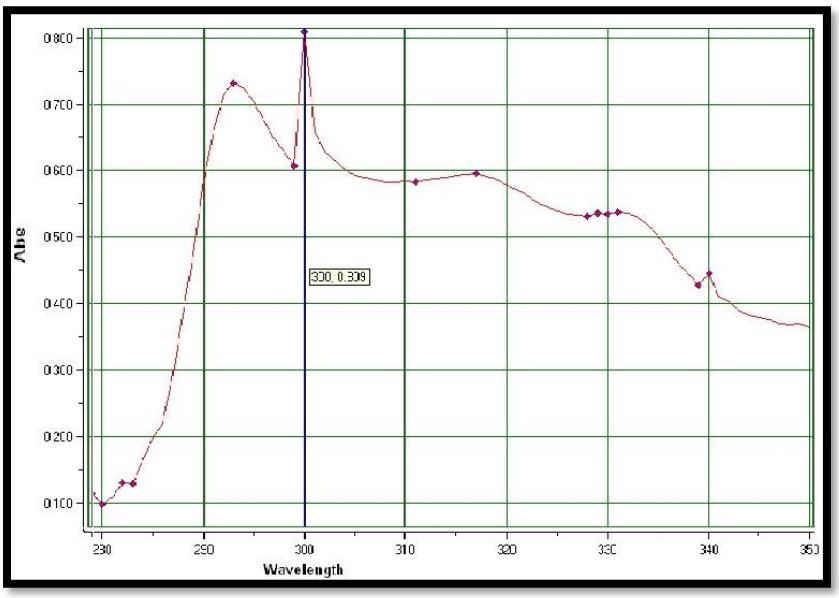

Figure 1. Spectrophotometric scan of Norfloxacin

3.1.2 Validation of λmax

The samples containing different concentrations of the drug (2-10µg/ml) were analyzed and overlain spectra were obtained that confirmed and validated the process.

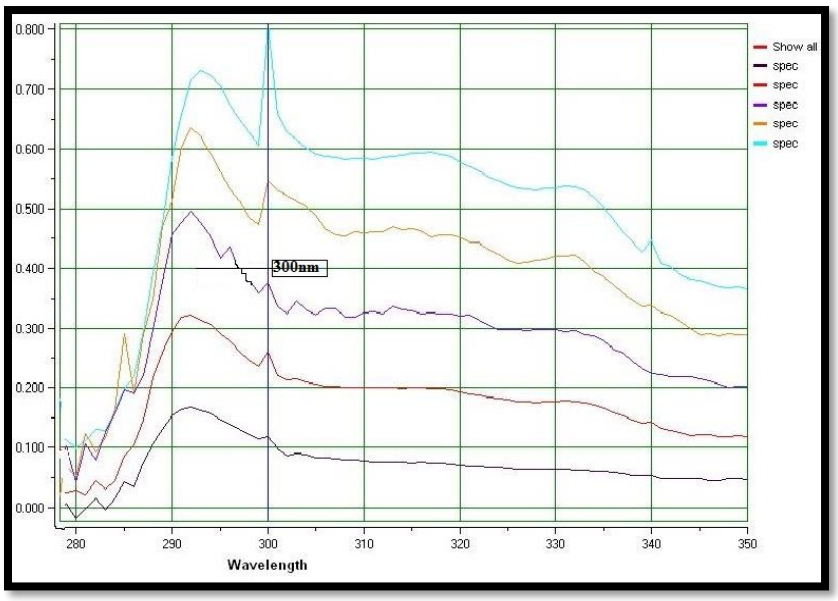

Figure 2. Overlain spectra of Norfloxacin

3.1.3 Preparation of standard curves

3.1.3.1 Preparation of standard curve using pH 7.4 phosphate buffer solution

A standard curve of Norfloxacin was also obtained by measuring absorbance of aliquots in similar concentrations (2-10µg/ml) at 300nm and plotting the graph [concentration (µg/ml) v/s absorbance].

Table 3. Concentration v/s Absorbance data of Norfloxacin

S. No.	Concentration (µg/ml)	Absorbance
1.	2	0.145
2.	4	0.277
3.	6	0.425
4.	8	0.573
5.	10	0.661

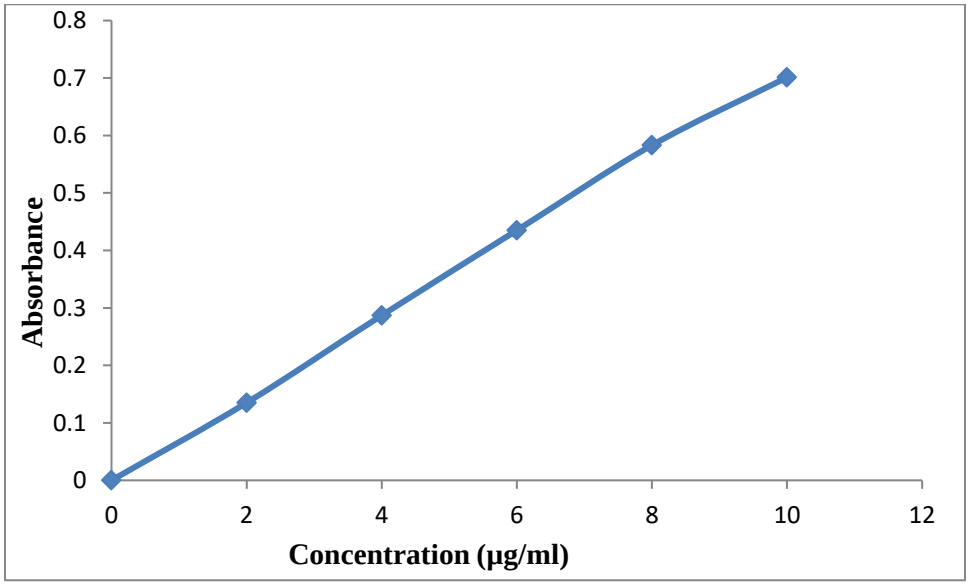

Figure 3. Standard curve of Norfloxacin in pH 7.4 phosphate buffer

The straight line thus obtained revealed that the drug obeyed Beer's Lambert law in the chosen concentration range.

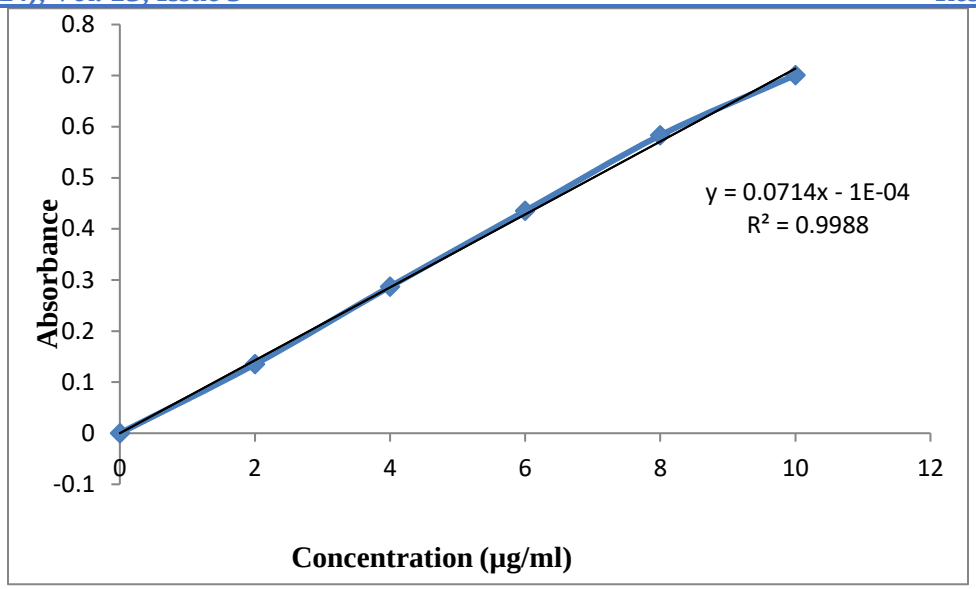

Figure 4. Regression curve of Norfloxacin in pH 7.4 phosphate buffer

3.1.3.2 Preparation of standard curve in Simulated Tear Fluid

A standard curve of Norfloxacin was obtained by measuring absorbance of various aliquots (2-10 µg/ml) at 300nm and plotting the graph [concentration (µg/ml) v/s absorbance].

Table 4. Concentration v/s Absorbance data of Norfloxacin

S. No	Concentration (µg/ml)	Absorbance
1.	2	0.135
2.	4	0.287
3.	6	0.435
4.	8	0.583
5.	10	0.701

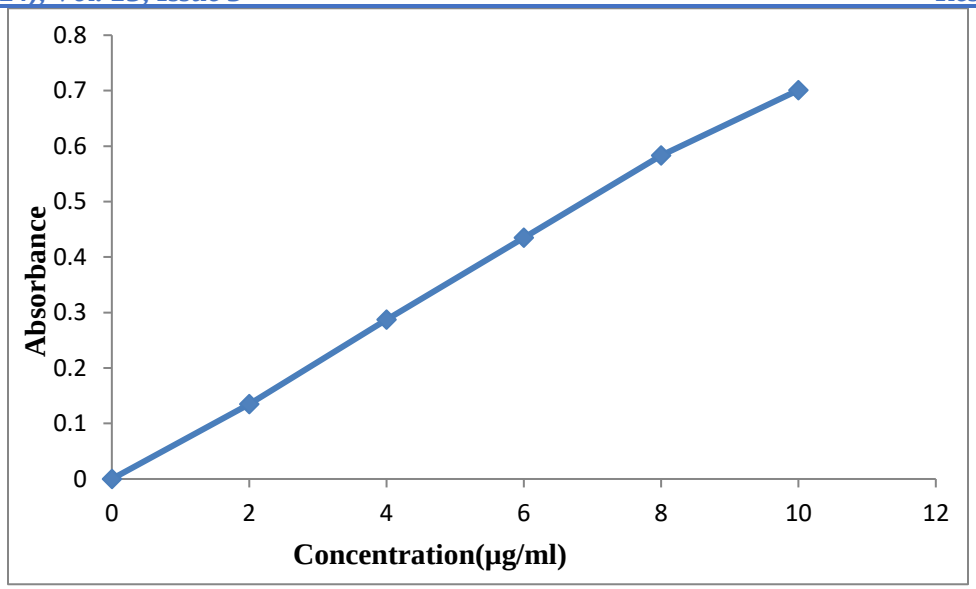

Figure 5. Standard curve of Norfloxacin in Simulated Tear Fluid

The straight line reflected that the drug obeyed Beer's Lambert law in the chosen concentration range.

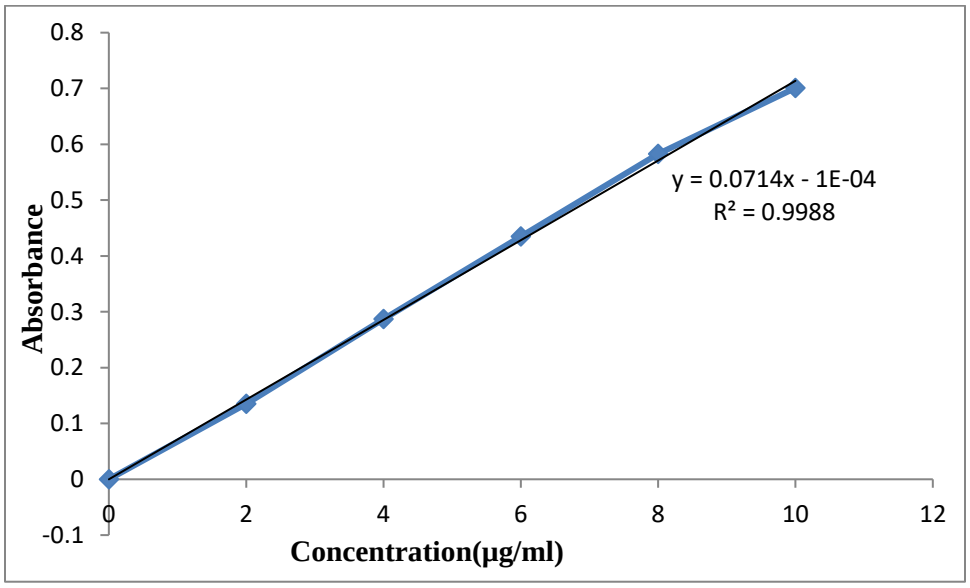

Figure 6. Regression curve of Norfloxacin in Simulated Tear Fluid

3.2 Drug-Excipient Compatibility Studies

3.2.1 FTIR analysis

The IR absorption spectra of Norfloxacin was obtained using KBr pellet technique and peaks obtained were compared with the reference which showed the characteristic peaks of some

functional groups. Similarly, IR spectra of drug and excipients i.e oleic acid, Tween 80 and Tween 20 were obtained.

The retention of characteristic peaks of the pure drug in combination with excipients confirmed compatibility of drug with all excipients incorporated in the formulation.

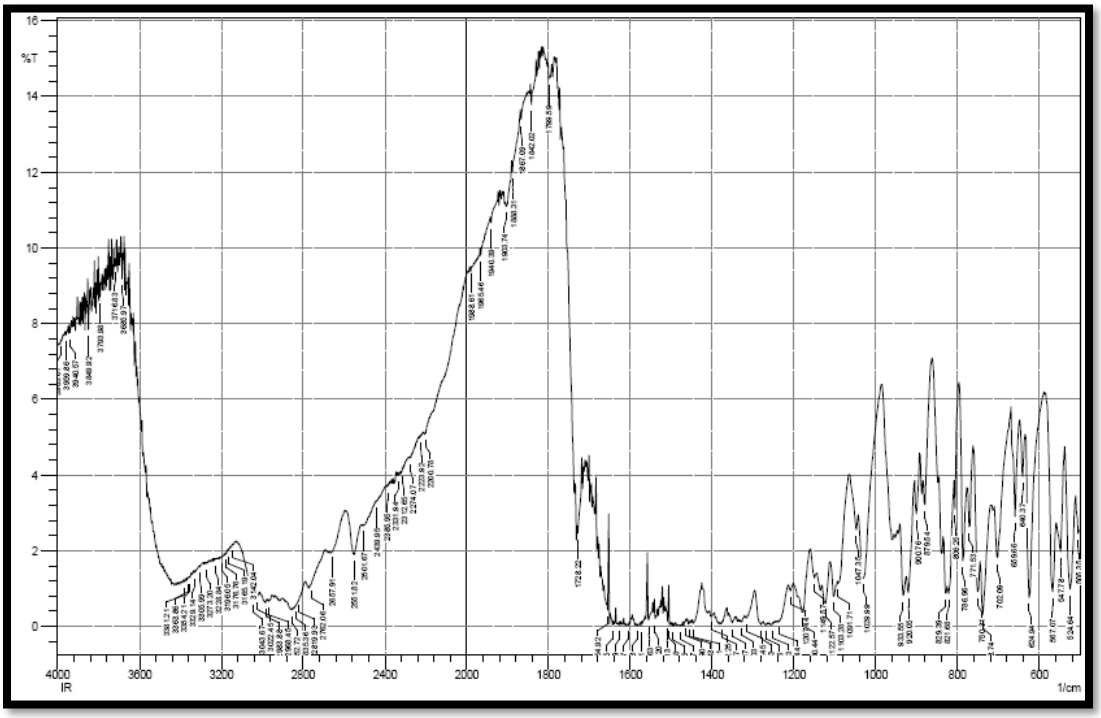

Figure 7. FTIR spectra of Norfloxacin (pure)

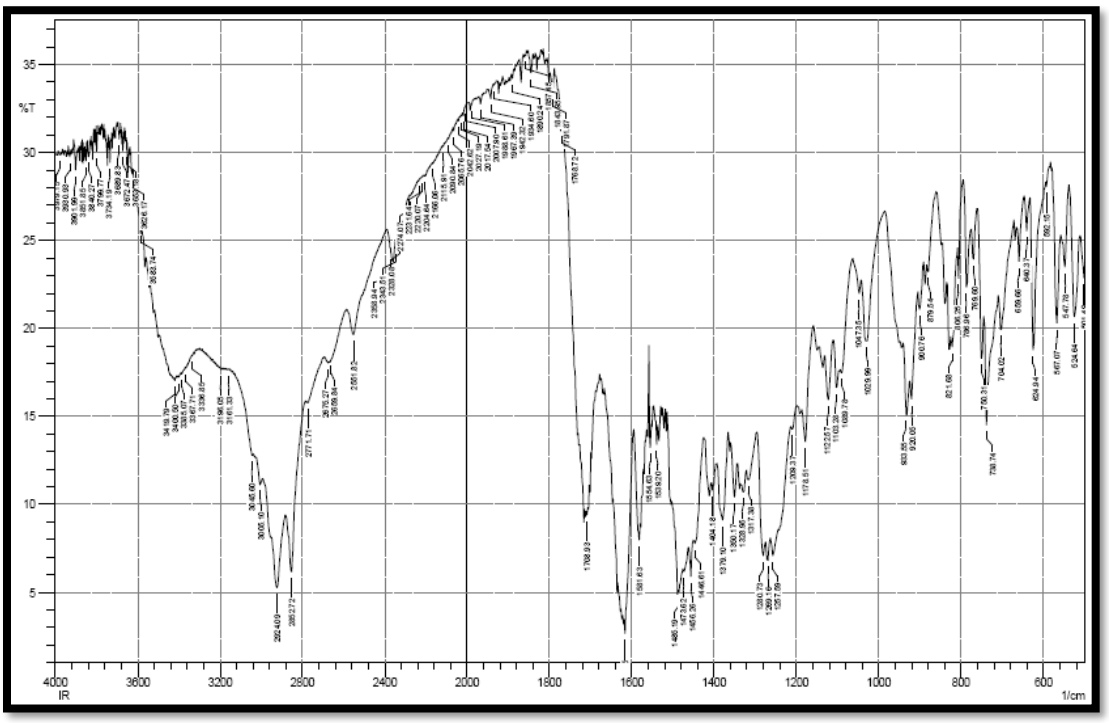

Figure 8. FTIR spectra of Norfloxacin with oleic acid

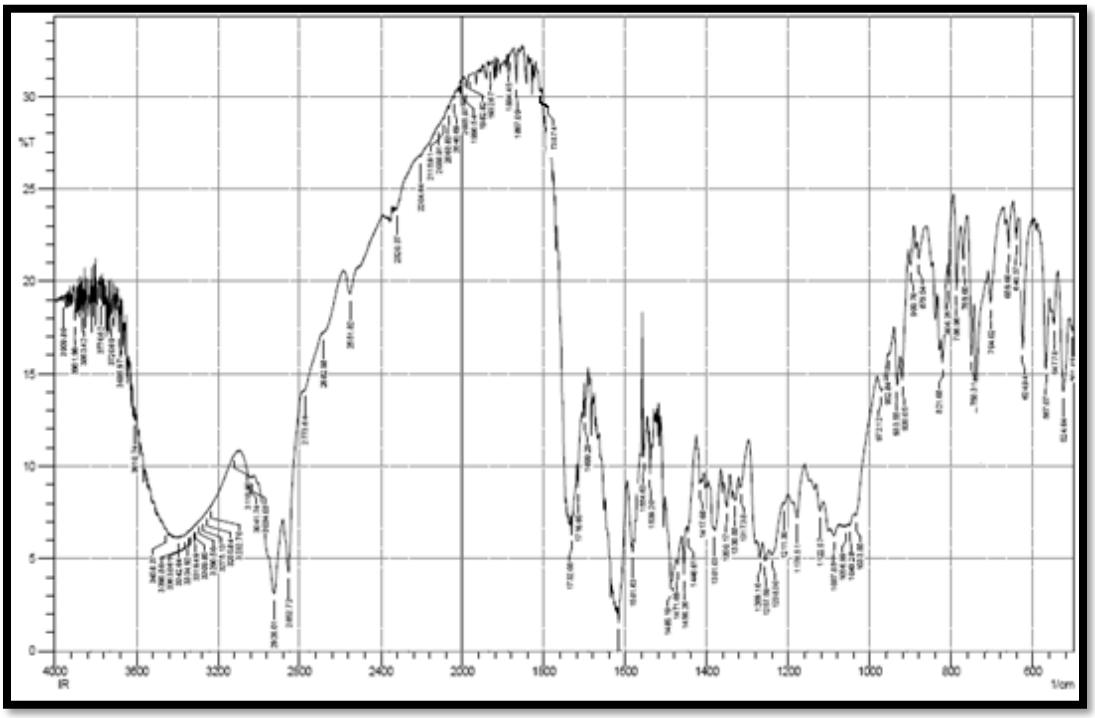

Figure 9. FTIR spectra of Norfloxacin with Tween 80

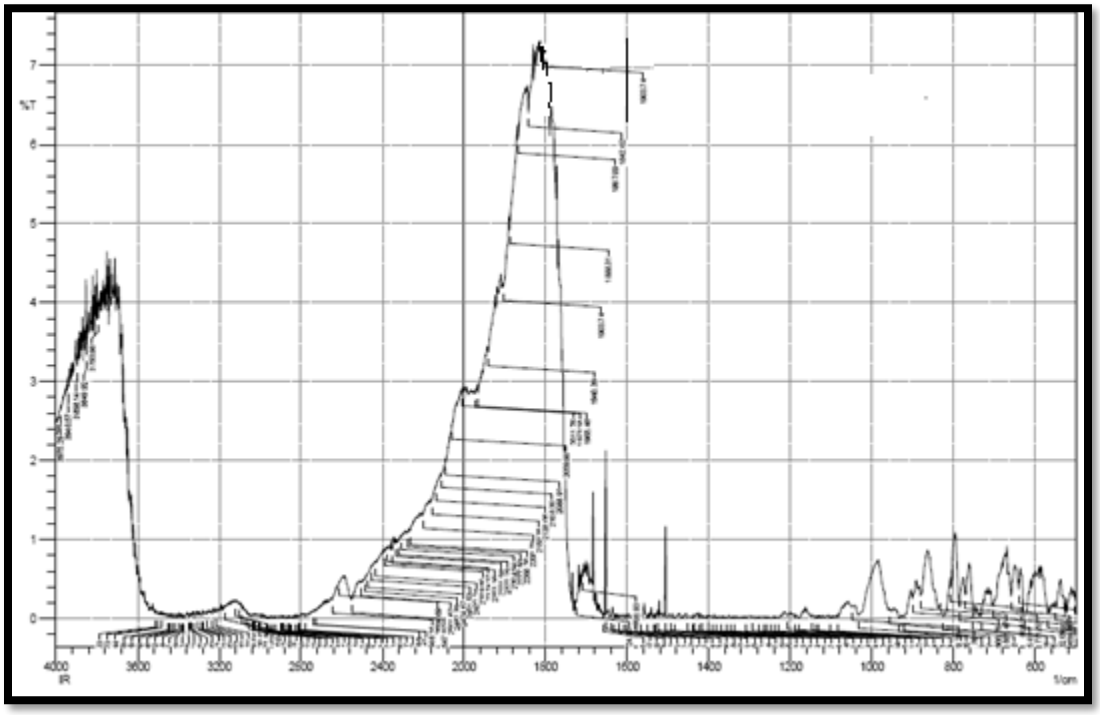

Figure 10. FTIR spectra of Norfloxacin with Tween 20

3.2.2 Thin Layer Chromatography (TLC) method

The R_f values of Norfloxacin with different excipients were compared with the pure drug (0.750) and found nearly similar thus confirmed compatibility between drug and different excipients i.e. Surfactant/co-surfactant and oil.

Table 5. R_f values of different combinations of drug and excipients

Spot No.	Ingredients	R_f value
A	Norfloxacin	0.750
B	Norfloxacin : Oleic acid	0.751
C	Norfloxacin : Tween 80	0.743
D	Norfloxacin : Tween 20	0.740
E	Norfloxacin : Tween 20 : Tween 80 : Oleic acid	0.747

Figure 11. Photographic representation of TLC of Norfloxacin with excipient combination

3.3 Preparation of Norfloxacin SMEDDS

3.3.1 Construction of Pseudo-ternary phase diagram

A pseudo-ternary phase diagram was constructed to determine the composition of an aqueous phase, an oil phase (Oleic acid) and a surfactant:co-surfactant phase (Tween 80; Tween20) that yielded a SMEDDS.

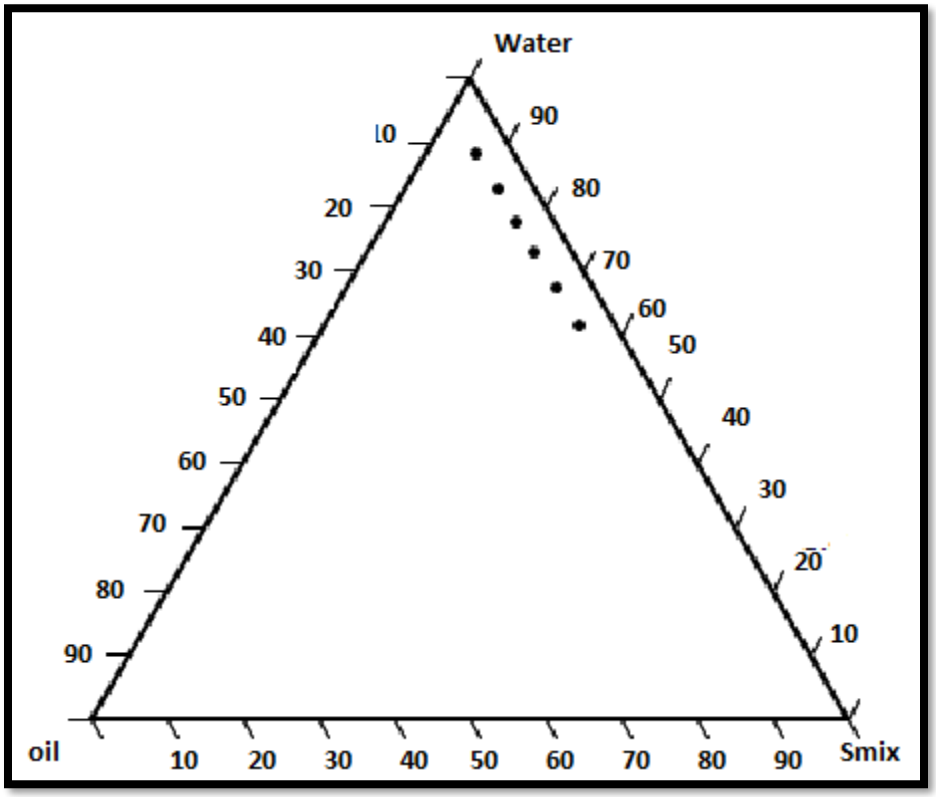

Figure 12. Pseudo ternary phase diagram of Norfloxacin, Oleic acid, Smix (Tween 80 & Tween 20) and water (batches MEF_1-MEF_6)

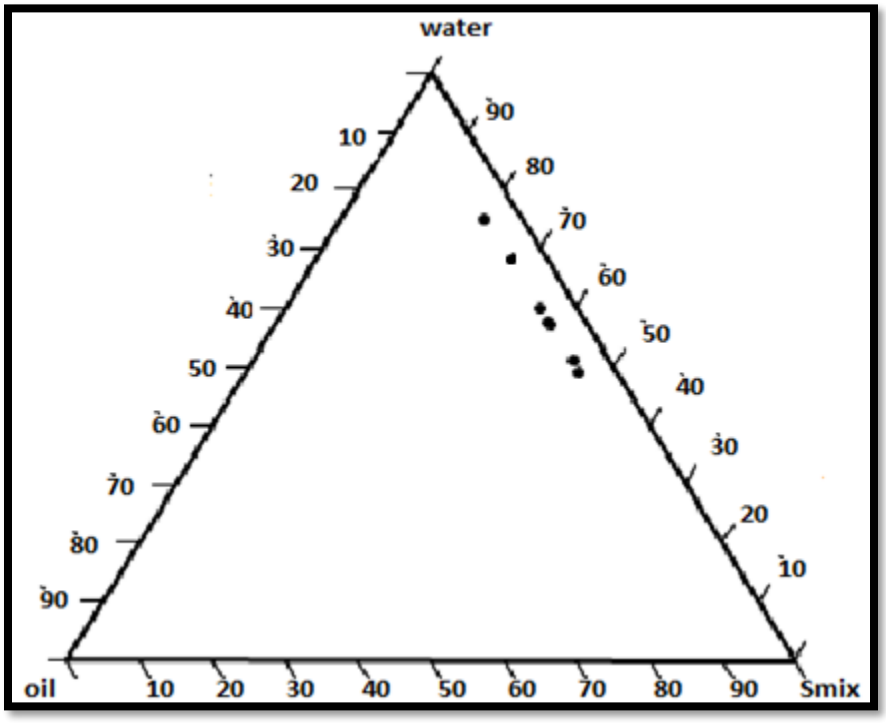

Figure 13. Pseudo ternary phase diagram of Norfloxacin, Oleic acid, Smix (Tween 80 & Tween 20) and water (batches MEF_7-MEF_{12})

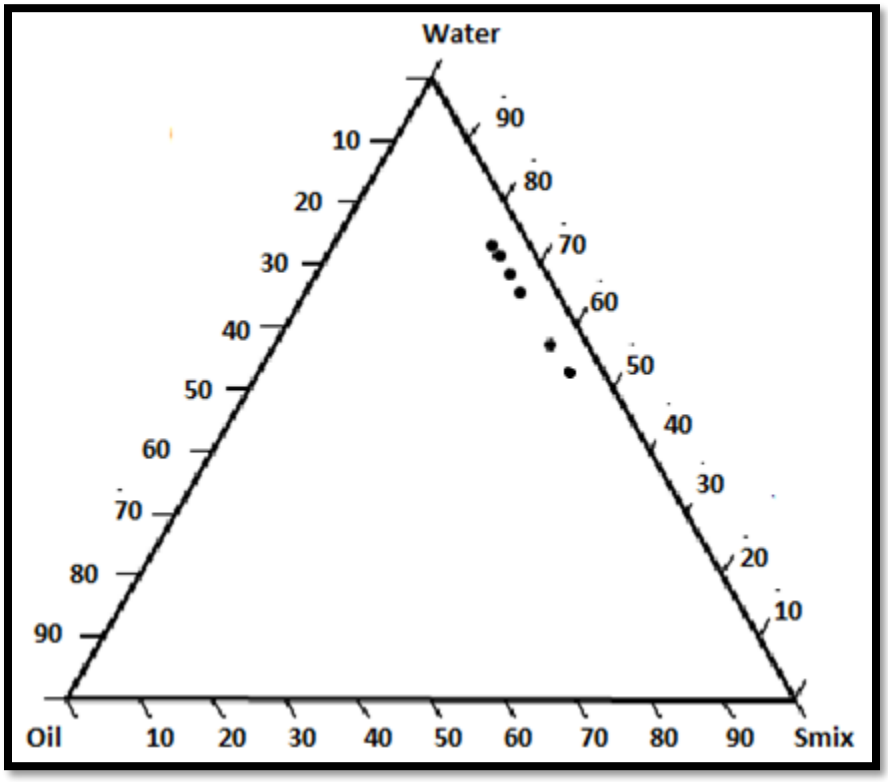

Figure 14. Pseudo ternary phase diagram of Norfloxacin, Oleic acid, Smix (Tween 80 & Tween 20) and water (Batches MEF_{13}-MEF_{18})

3.4 Evaluation Parameters

3.4.1 Droplet Size Determination

Table 6 represented the average droplet size of prepared O/W SMEDDS. The droplet size of the SMEDDS batches (MEF_1-MEF_{18}) ranged between 91-182.

Table 6. Particle size of SMEDDS batches (MEF_1-MEF_{18})

S. No.	Formulation Code	Particle size(nm)
1.	MEF_1	91
2.	MEF_2	103
3.	MEF_3	110
4.	MEF_4	125
5.	MEF_5	113
6.	MEF_6	117

7.	MEF_7	182
8.	MEF_8	124
9.	MEF_9	109
10.	MEF_{10}	181
11.	MEF_{11}	152
12.	MEF_{12}	132
13.	MEF_{13}	148
14.	MEF_{14}	131
15.	MEF_{15}	116
16.	MEF_{16}	129
17.	MEF_{17}	118
18.	MEF_{18}	137

3.4.2 pH determination

The SMEDDS samples were taken into the test tubes and the pH was determined. The pH of different batches was found to be 7.4 nearly similar to lacrimal environment.

Table 7. The pH of SMEDDS batches (MEF_1-MEF_{18})

S. No.	Formulation Code	pH
1.	MEF_1	7.4
2.	MEF_2	7.4
3.	MEF_3	7.4
4.	MEF_4	7.4
5.	MEF_5	7.4
6.	MEF_6	7.4
7.	MEF_7	7.4
8.	MEF_8	7.4
9.	MEF_9	7.4
10.	MEF_{10}	7.4
11.	MEF_{11}	7.4
12.	MEF_{12}	7.4
13.	MEF_{13}	7.4
14.	MEF_{14}	7.4
15.	MEF_{15}	7.4
16.	MEF_{16}	7.4

17.	MEF_{17}	7.4
18.	MEF_{18}	7.4

3.4.3 Rheological study

The viscosity of SMEDDS was determined by Brookfield's viscometer. The viscosity of SMEDDS formulation batches ranged between 26.5-35.3cps.

Table 8. The Rheological studies of SMEDDS batches (MEF_1-MEF_{18})

S. No.	Formulation Code	Viscosity
1.	MEF_1	26.5
2.	MEF_2	20.2
3.	MEF_3	22.7
4.	MEF_4	25.3
5.	MEF_5	35.3
6.	MEF_6	29.5
7.	MEF_7	31.3
8.	MEF_8	20.7
9.	MEF_9	22.9
10.	MEF_{10}	19.8
11.	MEF_{11}	30.6
12.	MEF_{12}	20.3
13.	MEF_{13}	23.7
14.	MEF_{14}	20.9
15.	MEF_{15}	23.9
16.	MEF_{16}	24.9
17.	MEF_{17}	25.4
18.	MEF_{18}	26.9

3.4.4 Drug entrapment efficiency

The maximum drug entrapment efficiency was found to be 99.96% for batch MEF_1 while minimum entrapment of 91.61% was found with MEF_9.

Table 9. Drug entrapment efficiency of SMEDDS batches (MEF_1-MEF_{18})

S. No.	Formulation Code	% Drug content
1.	MEF_1	99.96
2.	MEF_2	93.55
3.	MEF_3	95.33
4.	MEF_4	91.87
5.	MEF_5	96.34
6.	MEF_6	97.20
7.	MEF_7	95.10
8.	MEF_8	94.4
9.	MEF_9	91.61
10.	MEF_{10}	92.98
11.	MEF_{11}	95.76
12.	MEF_{12}	93.89
13.	MEF_{13}	96.67
14.	MEF_{14}	91.91
15.	MEF_{15}	98.09
16.	MEF_{16}	93.57
17.	MEF_{17}	96.45
18.	MEF_{18}	95.78

3.4.5 Zeta potential analysis

The analysis was performed for all eighteen batches by the Malvern zeta sizer. The results were as shown in table no 4.10.

Table 10. Zeta potential analysis of SMEDDS batches (MEF_1 to MEF_{18})

S. No	Formulation Code	Zeta potential mean (mV)
1.	MEF_1	-25.07
2.	MEF_2	-17.23
3.	MEF_3	-13.54
4.	MEF_4	-19.63

5.	MEF_5	-20.38
6.	MEF_6	-11.65
7.	MEF_7	-15.34
8.	MEF_8	-10.56
9.	MEF_9	-21.10
10.	MEF_{10}	-9.45
11	MEF_{11}	-7.34
12.	MEF_{12}	-16.78
13.	MEF_{13}	-20.89
14.	MEF_{14}	-4.12
15.	MEF_{15}	-8.82
16.	MEF_{16}	-3.56
17.	MEF_{17}	-11.34
18.	MEF_{18}	-0.11

3.4.6 In--vitro permeation studies

Table 11. Comparative permeation study of SMEDDS batches (MEF_1- MEF_9) in pH 7.4 phosphate buffer

Time	**% Drug release**								
(Hr)	**MEF_1**	**MEF_2**	**MEF_3**	**MEF_4**	**MEF_5**	**MEF_6**	**MEF_7**	**MEF_8**	**MEF_9**
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	09.645	7.087	8.052	7.872	4.472	3.164	3.695	2.098	2.223
4	16.075	14.391	13.786	13.765	10.883	8.492	8.925	7.116	6.040
6	22.455	19.53	17.553	17.453	14.933	14.233	13.364	10.671	08.377
8	29.683	26.45	24.931	24.343	20.394	18.742	17.806	13.345	11.335
10	35.132	32.48	29.836	29.223	25.936	23.424	19.473	17.223	15.375
12	42.261	39.67	36.407	36.562	31.895	28.471	25.396	23.754	20.256
14	51.660	48.35	45.807	45.871	40.357	37.715	34.567	32.986	30.944
16	60.748	58.43	56.952	54.562	52.778	48.592	46.578	43.677	40.113
18	69.625	67.56	65.314	63.673	61.552	58.735	56.329	53.878	50.322
20	78.627	76.45	73.712	70.874	68.348	65.383	63.114	60.654	58.681
22	85.99	83.45	80.076	78.343	76.966	74.486	72.655	70.682	67.081

24	94.101	90.563	87.881	85.674	83.343	80.076	78.912	75.352	73.751

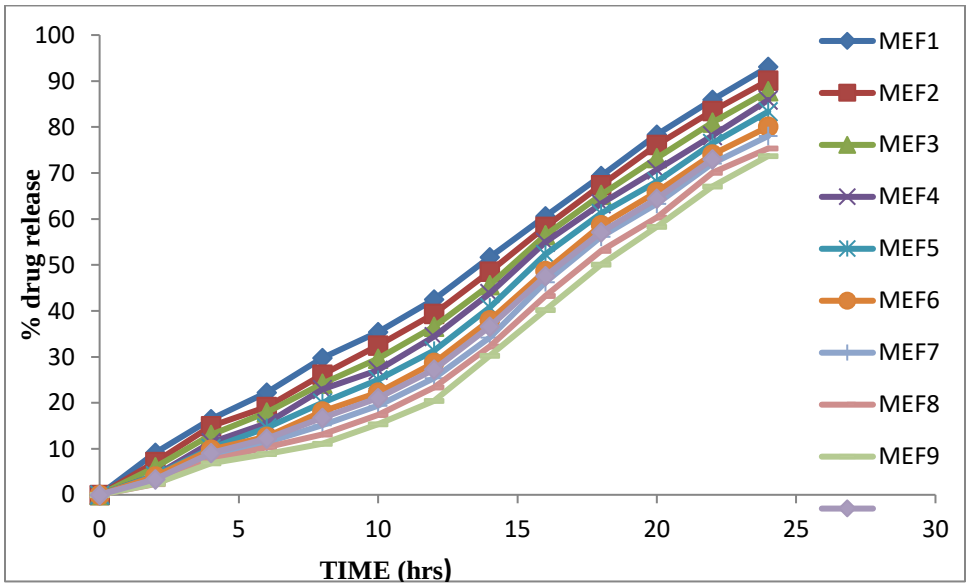

Figure 15. Comparative release profile of SMEDDS batches (MEF_1-MEF_9) in pH 7.4 phosphate buffer

Table 12. Comparative permeation study of SMEDDS batches (MEF_{10}- MEF_{18}) in pH 7.4 phosphate buffer

Time (Hr)	% Drug release								
	MEF_{10}	MEF_{11}	MEF_{12}	MEF_{13}	MEF_{14}	MEF_{15}	MEF_{16}	MEF_{17}	MEF_{18}
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	10.134	9.231	8.221	8.987	6.231	4.321	2.431	2.110	2.101
4	14.075	12.386	11.112	10.867	9.641	7.541	6.871	4.781	4.091
6	17.916	16.551	15.567	14.671	12.371	10.371	8.871	6.981	5.321
8	24.186	22.931	21.012	20.145	18.152	16.132	14.134	12.871	10.931
10	28.636	26.136	25.012	24.223	22.374	20.373	18.373	16.981	14.985
12	35.562	34.407	32.345	31.756	29.371	27.373	25.451	23.091	21.893
14	43.602	41.807	40.781	38.987	36.251	34.251	31.251	29.701	27.781
16	54.485	51.952	49.324	46.679	44.242	42.242	40.242	38.901	36.98
18	65.253	63.311	61.234	59.672	57.112	55.114	53.112	51.981	49.921
20	73.272	70.712	69.123	66.876	64.322	62.322	60.322	58.891	56.981
22	81.994	79.119	77.454	75.007	74.081	72.081	70.081	68.091	66.876
24	89.125	86.881	84.356	82.061	80.345	78.345	75.712	73.812	72.019

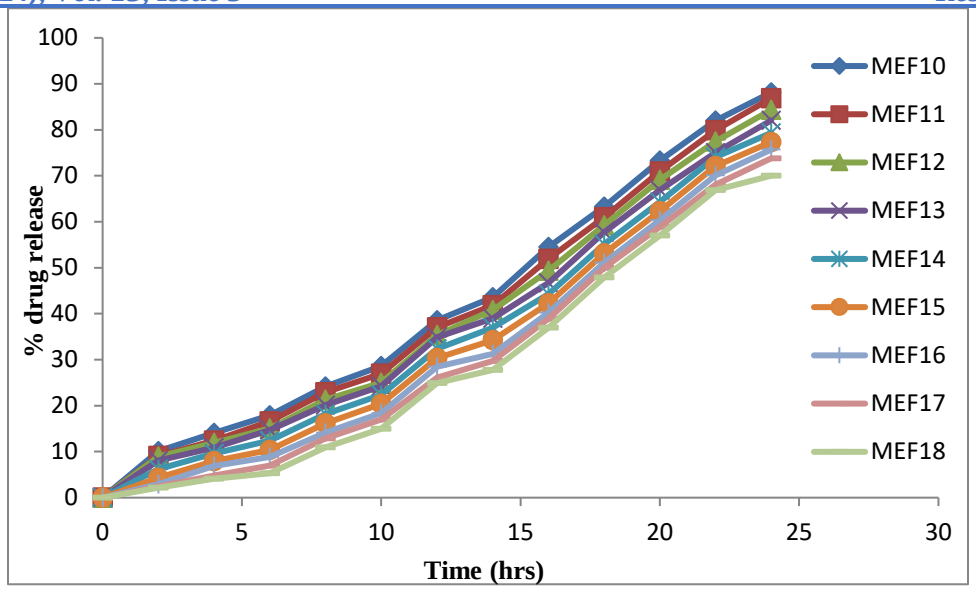

Figure 16. Comparative release profile of SMEDDS batches (MEF_{10}-MEF_{18}) in pH 7.4 phosphate buffer

Table 13. Comparative permeation study of SMEDDS batches (MEF_1-MEF_9) in STF

Time (Hr)	% Drug release								
	MEF_1	**MEF_2**	**MEF_3**	**MEF_4**	**MEF_5**	**MEF_6**	**MEF_7**	**MEF_8**	**MEF_9**
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	8.164	6.115	6.134	4.231	4.221	3.987	3.231	2.321	2.431
4	15.498	13.921	12.075	10.386	10.112	9.867	8.641	7.541	6.871
6	20.231	18.126	15.916	14.551	13.567	12.671	9.371	8.371	6.871
8	25.747	23.138	22.186	20.931	19.012	17.145	14.152	12.132	10.134
10	32.424	29.477	26.636	24.136	23.012	21.223	18.374	17.373	14.373
12	38.479	35.339	33.562	31.407	29.345	27.756	22.371	23.373	20.451
14	47.718	44.568	41.602	40.807	38.781	36.987	43.251	32.251	30.251
16	58.591	54.276	52.485	49.952	47.324	45.679	43.242	41.242	39.242
18	67.381	64.323	62.253	59.311	57.234	54.672	52.112	50.114	47.112
20	75.381	73.118	70.272	68.712	66.123	53.876	61.322	59.322	56.322
22	83.966	81.487	79.994	78.119	75.454	73.007	70.081	68.081	65.081
24	95.072	92.113	90.825	86.881	84.356	82.061	80.345	78.345	75.712

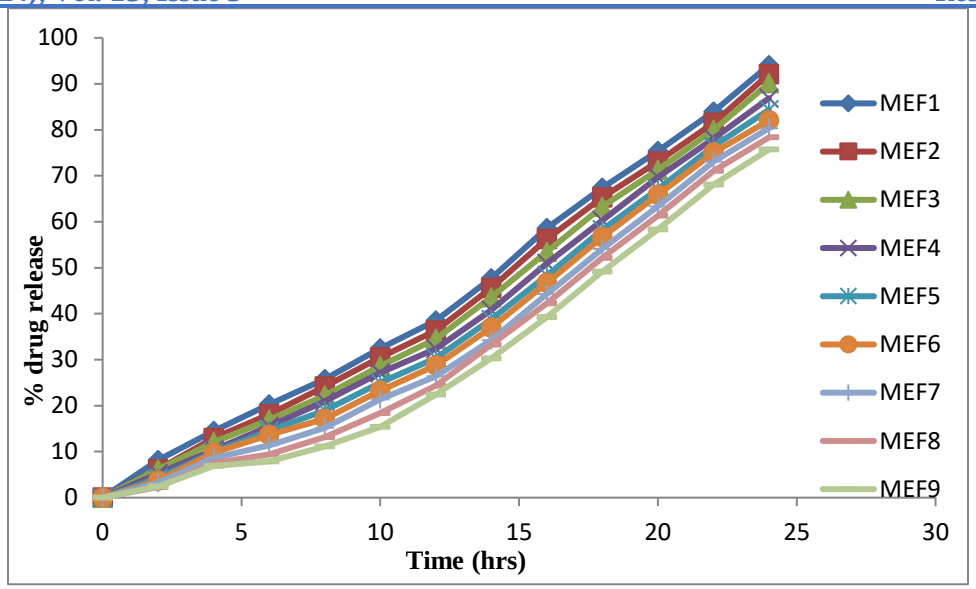

Figure 17. Comparative release profile of SMEDDS batches (MEF_1- MEF_9) in Simulated Tear Fluid

Table 14: Comparative permeation study of SMEDDS batches (MEF_{10}-MEF_{18}) in STF

Time (Hrs)	% Drug release								
	MEF_{10}	MEF_{11}	MEF_{12}	MEF_{13}	MEF_{14}	MEF_{15}	MEF_{16}	MEF_{17}	MEF_{18}
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	10.134	9.231	8.221	8.987	6.231	4.321	2.431	2.110	2.101
4	14.075	12.386	11.112	10.867	9.641	7.541	6.871	4.781	4.091
6	17.916	16.551	15.567	14.671	12.371	10.371	8.871	6.981	5.321
8	24.186	22.931	21.012	20.145	18.152	16.132	14.134	12.871	10.931
10	28.636	26.136	25.012	24.223	22.374	20.373	18.373	16.981	14.985
12	38.562	37.407	35.345	33.756	32.371	30.373	28.451	26.091	24.893
14	43.602	41.807	40.781	38.987	36.251	34.251	31.251	29.701	27.781
16	54.485	51.952	49.324	46.679	44.242	42.242	40.242	38.901	36.98
18	63.253	61.311	59.234	57.672	55.112	53.114	51.112	49.981	47.921
20	73.272	70.712	69.123	66.876	64.322	62.322	60.322	58.891	56.981
22	81.994	79.119	77.454	75.007	74.081	72.081	70.081	68.091	66.876
24	88.225	86.781	84.256	82.161	79.645	77.445	75.412	73.312	71.022

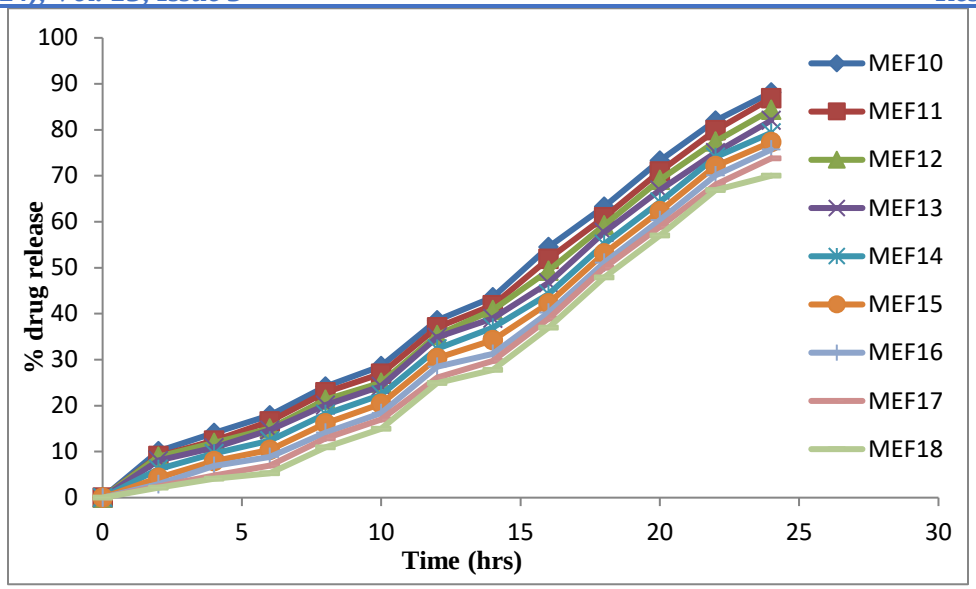

Figure 18. Comparative release profile of SMEDDS batches (MEF_{10}- MEF_{18}) in Simulated Tear Fluid

3.4.7 Zeta Potential analysis

Zeta potential analysis of Norfloxacin SMEDDS belonging to optimized batch i.e. MEF_1 was performed using Malvern zeta sizer.

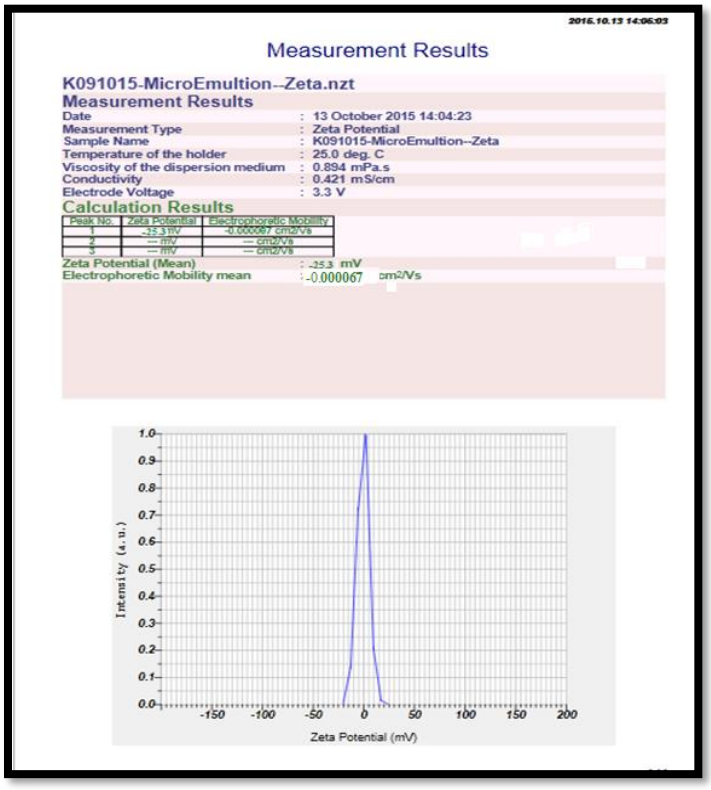

Figure 19. Zeta potential report of SMEDDS batch (MEF_1)

3.4.8 Surface Morphology Studies

The optimized batch of Norfloxacin SMEDDS i.e. MEF_1 was examined by Scanning Electron Microscopy.

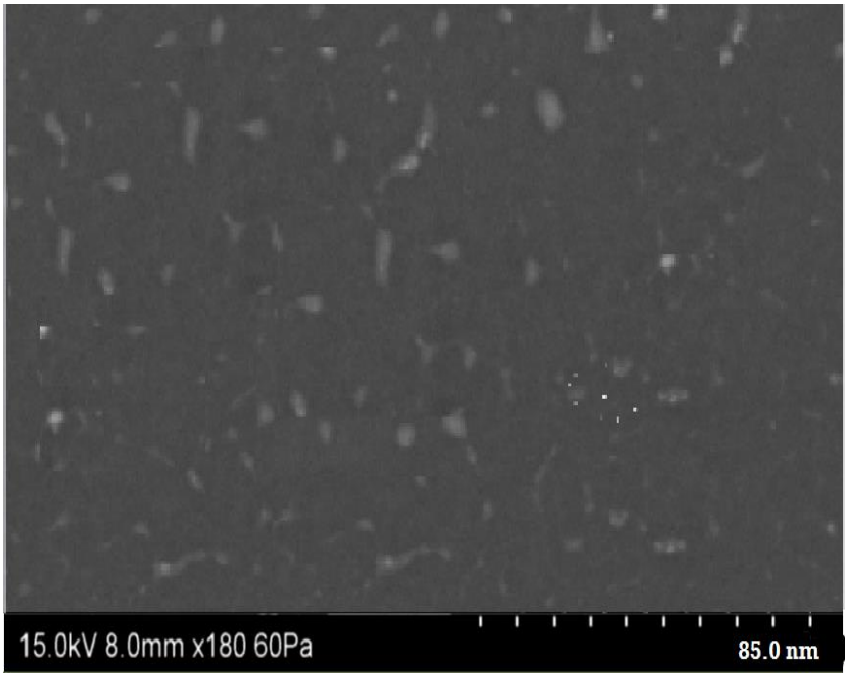

Figure 20. SEM image of optimized SMEDDS batch (MEF_1) (5000X magnification)

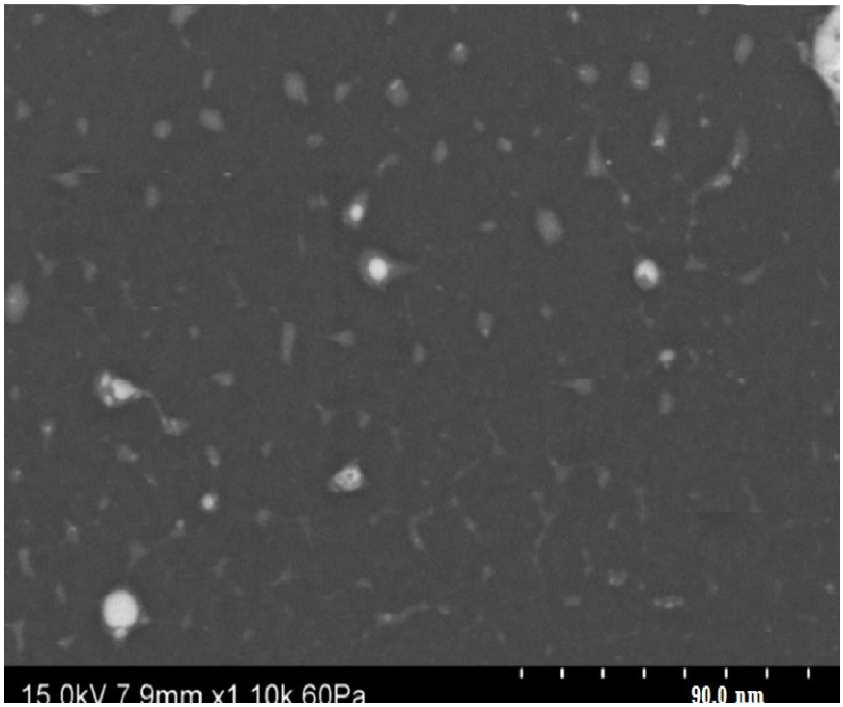

Figure 21. SEM image of optimized SMEDDS batch (MEF_1) (20000X magnification)

3.4.9 Stability study of optimized batch of Norfloxacin SMEDDS

The stability study was evaluated for the optimized SMEDDS formulation batch (MEF_1) as per ICH guidelines at accelerated conditions (40º ±2 º C, 75 % ± 5% RH) and the result showed that the optimized batch was stable for six months.

Table 4.15. Stability study of SMEDDS formulation MEF_1 (at 40º ±2 º C, 75 % ± 5% RH)

Time (hrs)	% Drug Release				
	0 Day	30 Days	60 Days	90 Days	180 Days
0	0.000	0.000	0.000	0.000	0.000
2	9.165	8.695	7.637	6.237	4.587
4	15.497	14.920	13.075	11.786	9.867
6	20.231	18.367	16.368	14.552	12.671
8	25.748	22.816	20.686	18.931	16.345
10	32.424	28.477	26.137	24.838	23.223
12	38.475	35.390	32.262	30.407	28.756
14	46.719	44.567	41.610	39.808	36.987
16	58.591	53.576	49.485	47.952	45.679
18	67.385	63.325	58.254	55.310	53.671
20	75.382	71.118	67.272	65.012	62.876
22	86.966	84.488	80.995	77.119	74.657
24	95.072	92.564	91.725	91.832	90.312

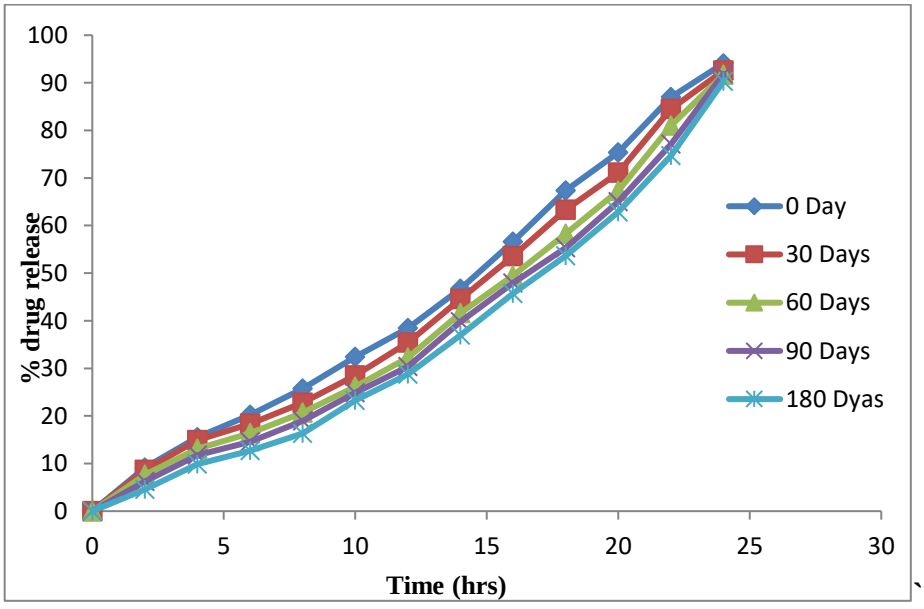

Figure 22. Release profile of optimized microemulsion batch ($MEF_{1)}$ on stability (0-180 days)

4. Conclusion

Since ancient times, eye treatment has shown to be the most enticing and useful way to apply drugs in the realms of clinical and pharmaceutical research. Numerous pharmacokinetic and pharmacodynamic obstacles must be addressed for a public health care system to be effective. These obstacles include the overwhelming need for dose-optimization strategies to decrease adverse effects, boost therapeutic efficacy in a particular formulation, and decrease dosage frequency. Most formulations that are presently available on the market still recommend eye treatment. In order to treat ocular infections, the current study formulated, optimized, and assessed Norfloxacin SMEDDS, which resulted in prolonged in-vitro drug release and was anticipated to yield increased bioavailability. FTIR tests were used to establish the medication excipient compatibility, and TLC experiments were used to validate it. In conclusion, it was discovered that norfloxacin was compatible with the different excipients used to make the SMEDDS formulation. The following physicochemical properties of the produced SMEDDS were measured: pH (7.4), viscosity (26.5-35.3cPs), droplet size range (91-182nm), Zeta potential (-0.11)-(-25.07) mV, and drug content (91.61–99.96%). During centrifugation stress testing, all SMEDDS were confirmed to be thermostable and no phase separation was seen. According to the in-vitro drug release data, at the conclusion of a 24-hour period, the percentage of drug released varied between 72.019% and 94.101% in pH 7.4 phosphate buffer and 95.072%-71.022& in STF. Formulation B1 was regarded as the optimal batch based on the assessment parameters. Droplet size (91 nm), zeta potential (-25.07), and percentage drug release in pH 7.4 phosphate buffer and STF were determined to be 94.101% and 95.072%, respectively, with the optimized batch. According to the ICH guideline, a stability study was conducted using the optimized formulation (MEF), and the results showed that the formulation was stable and met the dosage compliance condition. The aforementioned information fully corresponded with the specifications needed to formulate Norfloxacin SMEDDS for ocular administration.

5. Conflict of interest

The authors have no conflict of interest.

6. Acknowledgement

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