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Formulation and Development of Solid Nano-Emulsifying Drug Delivery System of BCS Class IV Drug Delafloxacin

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Abstract: ***Aqueous solubility of drugs is one of the most important factors that determine their dissolution performance and hence oral absorption and bioavailability. About 70% of new drug substances are poorly water soluble and exhibit slow dissolution rates and often incomplete oral bioavailability. The aim of present investigation is to develop a novel emulsifying drug delivery system (SNEDDS) to enhance the dissolution rate profile by increasing the solubility of Delafloxacin. The oral route is the most commonly used method for administration of drugs, with nearly 80% of the marketed dosage forms being delivered orally. This route of drug administration is the most convenient and non- invasive, leading to better patient compliance. Therefore, different formulation strategies have been investigated for enhancing the solubility of poorly soluble drugs with the aim of improving oral bioavailability. However, the optimized formulation has chosen a suitable emulsifying rate with a quick dissolution rate based on the smaller z-average diameter. The optimized Delafloxacin SNEDDS has exhibited faster dissolution rate as 97±2.07 % drug released which is clearly indicating that developed SNEDDS enhanced the dissolution rate of the drug.***

Keywords: SNEDDS, Emulsifying, Delafloxacin, Bioavailability, Dissolution, Enhancing.

I. INTRODUCTION

Delafloxacin is a fourth generation fluoroquinolone antibiotic which has been used in trials studying the treatment and basic science of Gonorrhea, Hepatic Impairment, Bacterial Skin Diseases, Skin Structure Infections, and Community Acquired Pneumonia, among others.[1,2] Comparing to other FQs, a major structural substitute change on C-7 makes DLF more powerful against gram positive and gram negative bacteria. Due to the lack hydrogen ion substitute in the chemical structure of DLF, it got the distinct property of weakly acidic moiety with enhanced intracellular permeation and potent bactericidal effects. The maximum plasma concentration (Cmax) of DLF was achieved between 1 and 2.5 h in healthy volunteers and the reported absolute bioavailability of DLF is 58.8% only which might be due to its poor solubility profile. Poorly water-soluble drugs frequently display problems of low solubility and slow dissolution rate in gastrointestinal (GI) media which results in low and variable oral absorption. Therefore, different formulation strategies have been investigated for enhancing the solubility of poorly soluble drugs with the aim of improving oral bioavailability.[3,4]

II. MATERIALS AND METHODS

A. Materials

Delafloxacin was obtained as gift sample from Vama Pharma (Nagpur). Capryol 90, Poloxamers 188 Span 20, Tween 20 etc was obtained from Anand agencies Pune. All chemicals used were analytical grade.

B. Methods

1) Preformulation Study

a) Melting Point

The melting point determined by Thieles tube method. The results were reported in results part.

b) Solubility study

Solubility study of Delafloxacin were carried out in different volatile solvent i.e., water, 0.1 N HCl and 6.8 pH phosphate buffer. Saturated solutions were prepared by adding drug in excess amount to the vehicle for 48 hr at 25 ^{o}C under constant Stirring.

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After this period the solution were filtered, diluted with distilled water (at least 1000 times) and analyzed by UV spectrophotometer at a wavelength of 298 nm.

2) UV Spectrophotometric Study

a) Preparation of calibration curve of Delafloxacin

A stock standard solution (1 mg/ml) of Delafloxacin was prepared in Potassium Phosphate buffer 3.6 and diluted with ion-free water to prepare working solutions at concentrations of 0, 10, 20, 30, 40, 50 µg/ml respectively. The absorbance of standard solution was determined using UV/ VIS spectrophotometer at 298 nm.

b) Study of Delafloxacin Solubility in Various Oils, Surfactants and Co-surfactants

In order to find out the right SNEDDS components with good solubilizing capacity for Delafloxacin, saturation solubility was performed on different oils (Capryol 90, Algae oil, Palm oil, and peanut oil), surfactants (Poloxamer 188, Cremophor S9, Labrasol, Tween 20,Span 20) and co-surfactants (Transcutol HP, PEG 400, Propylene glycol and Glycerin) using the shake flask method. All measurements were done in triplicate and the solubility was expressed as the mean value (mg/ml) ±SD.

3) Preparation of Delafloxacin loaded SNEDDS

a) Nine different formulation of Delafloxacin loaded SNEDDS were prepared by composition shown in table.

b) Accurately weighed of surfactant (Tween-20, span-20, Poloxamer 188) and co-surfactant (Transcutol HP) were mixed in a vial on magnetic stirrer. The weighed amount of drug Delafloxacin was dissolved in the selected amount of oil (Capryol 90 oil) in a separate beaker. The oil phase was added drop wise to the surfactant co-surfactant mix and stirring was continued for 1 h to obtain Delafloxacin loaded liquid SNEDDS.

c) S-SNEDDS of Delafloxacin was prepared by adsorption method. Optimized L-SNEDDS preparation was transformed to S-SNEDDS by the adsorption method. The benefit of the adsorption technique is to have more surface area with good physical adsorption, high dissolution efficiency, good uniformity, and reproducibility. In a glass mortar, Delafloxacin loaded L-SNEDDS was added drop wise in 1:1 w/w proportion with adsorbent Aerosil. After every addition, the blend was mixed properly until a uniform solid powder was obtained. The resultant damp mass was collected from the apparatus and processed through the mesh size 250 microns (mesh no 60). solid SNEDDS was placed at 25◦C in desiccators for further characterizations.

Table No. 1: Formulation of Delafloxacin SNEEDS

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Delafloxacin (mg)	800	800	800	800	800	800	800	800	800
Capryol 90 (ml)	1	1	1	1	1	1	1	1	1
Tween 20 (mg)	100	200	300	-	-	-	-	-	-
Poloxamers 188 (mg)	-	-	-	100	200	300	-	-	-
Span 20 (mg)	-	-	-	-	-	-	100	200	300
Transcutol HP (mg)	50	100	150	50	100	150	50	100	150
Ethanol (Co-solvent) (ml)	25	25	25	25	25	25	25	25	25

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III. EVALUATION OF DELAFLOXACIN LOADED S-SNEDDS

1) Percent transmittance

The percent transmittance was measured at 298 nm using UV-Vis spectrophotometer against double distilled water as the blank. The studies were conducted at 50, 100, 250 and 1000 times dilution.

2) Zeta potential

The zeta potential of the diluted SNEDDS formulations was measured using a Malvern zetasizer. The SNEDDS were diluted with a ratio of 1:2500 (v/v) with distilled water and mixed for 1 min using a magnetic stirrer. Zeta potential of each SNEDDS was determined in triplicate.

3) Mean globule size

The formulation, 100 µL was diluted to 250 mL in a beaker and gently mixed using a glass rod. The resultant emulsion was then subjected to particle size analysis using Malvern zetasizer. All studies were repeated for six times, with good agreement being found between measurements.

4) Polydispersity Index

The efficiency and dispersibility of self-emulsification were determined through USP dissolution apparatus 2. Briefly, 1 mL of each formulation was added drop wise into 200 mL of simulated intestinal fluid (pH 6.8, without enzymes), maintained at 37 °C, with gentle stirring using stainless steel paddles rotated at 60 rpm.

5) Viscosity

The viscosity of Delafloxacin SNEDDS was determined with Brookfield viscometer (digital viscometer + Pro) at 20 rpm at room temperature (25°C)

6) Cloud point measurement

The SNEDDS formulations were compared for cloud point value. Each formulation was diluted with water in the ratio of 1:100 and placed in a water bath with gradual increase in temperature. At the cloud point, drop in the percent transmittance of sample from the zero point was measured spectrophotometrically.

7) Micromeritic Properties of S-SNEDDS

The values obtained for the angle of repose of the S-SNEDDS formula F5 was $27.64^{o} \pm 1.03^{o}$, as shown in Table. This value indicate that formula have good Flowability. The bulk density of the formula F5 was found to be 0.48 ± 0.03 g/ml. However, tapped density was 0.57 ± 0.021 g/mL for formula F5. Carr's index of formula F5 was found to be 14.22 ± 1.62 which give an indication about the good Flowability of the S-SNEDDS formula. This was further supported by the values of Hausner's ratio. The results of Hausner's ratio of formula F5 was 1.24 ± 0.10. The improved Flowability of S-SNEDDS formula may be due to good sphericity of particles.

8) Drug Loading Efficiency/Drug content

Samples were prepared in triplicate and absorbance was measured after suitable dilutions at 298 nm using UV–Vis Spectrophotometer. The amount of Delafloxacin present in each formula was calculated from a calibration plot.

9) Scanning Electron Microscopy (SEM)

Scanning electron micrographs for Delafloxacin, prepared S-SNEDDS formulae were taken using Scanning electron microscope operating at 20 kV to study surface topography of S-SNEDDS. The samples were fixed on SEM stub and then coated with thin layer of platinum.

10) Differential Scanning Calorimetry (DSC)

Thermograms of Delafloxacin optimized S-SNEDDS formulae were obtained using differential scanning calorimeter. The thermal behavior was studied by heating nearly 2 mg of samples in sealed aluminum pans under nitrogen gas flow (30 ml/min) over a temperature range of 0 to 250°C and a heating rate of 10°C/min.

11) Fourier Transformed Infrared Spectroscopy (FTIR)

FTIR Spectra of pure Delafloxacin prepared and optimized S-SNEDDS formulae were obtained using Fourier transformed infrared spectrophotometer. Solid samples were mixed with small quantity of IR grade potassium bromide and compressed into discs by applying pressure. The compressed disc was placed in light path and the spectrum was obtained. Each KBr disc was scanned at 4 mm/s at a resolution of 2 cm over a wave number region of 4000-400 cm^{-1}.

12) In Vitro Drug Release Studies

The in vitro drug release of Delafloxacin from the optimized SNEDDS formulation, pure drug and marketed product was performed utilizing USP disintegration apparatus type II. The dissolution medium comprised of 900 mL of newly pre-arranged phosphate buffer pH 3.6 kept up with at 37±0.5°C and the paddle speed was set at 50 rpm.

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Hard gelatin cases, size "000" stacked up with pre concentrate were joined to paddles using Para film spring to hold cases back from floating. Aliquots (5 mL) from the disintegration medium were removed at standard time stretches (5, 10, 15, 30, 45, 60, 90 and 120 min) utilizing an adjusted expendable needle. The examples were then separated through a film channel (0.45 µm, Whatman) and medication fixation was gotten after appropriate weakening by means of UV approved technique at 298 nm using UV-Vis Spectrophotometer.

IV. STABILITY STUDIES

The SNEDDS formulations were filled into empty hard gelatin capsules (size 0) and subjected to stability studies at 25°C/60 % relative humidity (RH) and 40°C/75% RH. Samples were charged in stability chambers with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 3 month.

V. RESULTS AND DISCUSSIONS

1) *Preformulation Study*

Table No. 2: Organoleptic Properties of Delafloxacin

Test	Specification	Observation
Color	White to pinkish crystals	White to pinkish crystals
Odor	Odorless	Odorless

2) *Melting Point*

Table No. 3: Melting Point of Delafloxacin

Drug Name	Standard Melting Point	Observation Melting Point
Delafloxacin	224-227 ^{o}C	227 ^{o}C

3) *Solubility*

The solubility of Delafloxacin in different solvent Water, 0.1 N HCl and Phosphate buffer pH 3.6 was determined.

Table No. 4: Solubility of Delafloxacin in Different solvent

Medium	Delafloxacin (mg/ml)
Water	0.122
0.1 N HCl	8.72
Phosphate buffer pH 3.6	4.31

4) *Calibration Curve of Delafloxacin*

The maximum of Delafloxacin was found at 298 nm. The linear relationship was observed over the range of 0-50 µg/ml. Absorbance were noted at 298 nm against Potassium Phosphate buffer pH 3.6 as a blank. A calibration graph of the absorbance versus drug concentration of the drug was plotted.

Table No. 5: Calibration curve for Delafloxacin

Sr. No.	Concentration(µg/ml)	Absorbance (nm)
1	0	0
2	10	0.215
3	20	0.414
4	30	0.601
5	40	0.732
6	50	0.907
R^2		0.994

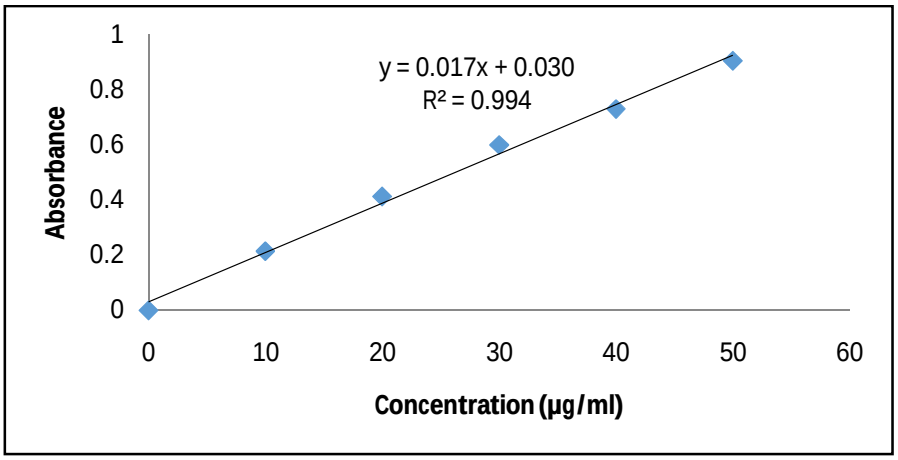


Figure No.1: Calibration curve of Delafloxacin

5) Study of Delafloxacin Solubility in Various Oils, Surfactants and Co-surfactants

The results of solubility studies of drug in various oils, surfactants and co-surfactants are as shown in Table. These highlighted ingredients were selected for further study of emulsification ability and miscibility with other ingredient

Table No 6: Solubility of Delafloxacin Solubility in Various Oils, Surfactants and Co-surfactants

Category	Name of excipient	Solubility (mg/ml)
Oil	Palm oil	102.44± 1.43
	Capryol 90	136.72± 2.21
	Algae oil	98.04 ± 2.01
	Peanut oil	74.36 ± 1.36
Surfactant	Poloxamer 188	307.63±3.18
	Cremophor S9	69.29±3.45
	Labrasol	38.57±3.76
	Tween 20	304.98±3.03
	Span 20	161.34±1.34
Co-surfactants	Transcutol HP	99.09±3.52
	PEG 400	80.72±2.46
	Propylene glycol	53.85±1.45
	Glycerin	44.32±1.23

6) Evaluation of S-SNEDDS

The transmittance values of all formulation above 90%, confirming the self-nano-emulsification efficiency of the SNEDDS.

Table No 7: Effect of dilution on different SNEDDS formulations using double distilled water as a dilution media

Dilution	Percentage of transmittance								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
50 times	79.23±0.0378	77.35±0.078	75.23±0.054	80.23±0.0571	82.23±0.0861	81.82±0.0417	85.23±0.0527	61.89±0.0532	69.23±0.0683
100 times	84.89±0.0235	85.56±0.0124	80.89±0.00137	85.89±0.0582	87.89±0.0196	84.23±0.0937	87.89±0.0462	75.43±0.0237	74.89±0.0163

250 times	88.46±0.0904	89.53±0.0457	84.46±0.0739	88.46±0.0329	94.46±0.0644	86.46±0.0523	91.46±0.0682	89.46±0.0567	89.46±0.0628
1000 times	90.51±0.0670	91.81±0.0781	90.99±0.0235	93.51±0.0523	99.51±0.0824	94.51±0.0682	95.51±0.0435	90.34±0.0473	94.51±0.0241

7) Measurement of Physical Properties of SNEDDS

Physical properties including Z-average diameter, PDI, Zeta potential, viscosity and cloud point of formulation batches F1-F9 were measured.

Table No 8: Interpretation of results of physical properties

Test parameter	SNEDDS formulation								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Z- average diameter	148.29±0.198	182.64±0.157	168.58±0.147	174.23±0.231	124.68±0.145	156.75±0.142	142.11±0.561	161.54±0142	146.51±0.157
PDI	0.195±0.0119	0.173±0.0124	0.254±0.201	0.169±0.0214	0.125±0.0137	0.578±0.0657	0.565±0.0632	0.531±0.0271	0.651±0.0652
Zeta potential (mV)	−4.84	−2.53	−4.18	−3.75	−5.74	−6.57	−4.63	−4.35	−3.46
Viscosity (cPs)	36	59	39	48	24	34	45	54	38
Cloud point (°C)	76-77	75-77	75-76	74-75	77-78	73-74	73-75	75-76	75-77

8) Drug Loading Efficiency/Drug content

The amount of Delafloxacin present in the optimized SNEDDS formulae was found to be within the USP limit. The drug content in SNEDDS was almost identical with the results obtained in SNEDDS, so there was no change of percentage drug content.

Table No. 9: % Drug loading efficiency

Formula	Drug loading efficiency
F1	92.37±0.75
F2	93.30 ±0.86
F3	96.85± 1.23
F4	95.22 ±2.22
F5	99.09± 0.56
F6	96.32±1.88
F7	98.53± 1.45
F8	94.68±1.91
F9	95.76±1.64

9) Scanning Electron Microscopy (SEM)

The surface morphology of pure Delafloxacin powder and S-SNEDDS formulae of Delafloxacin was determined using scanning electron microscope. The Delafloxacin powder appeared with an irregular crystalline shape as irregular and plate-shaped crystals having rough surfaces.

The image of the solid SNEDDS formulae F-5 containing Delafloxacin however, illustrate that the particles had the same outer macroscopic morphology consisting of well separated spherical particles with relatively deep dents and similar diameters.

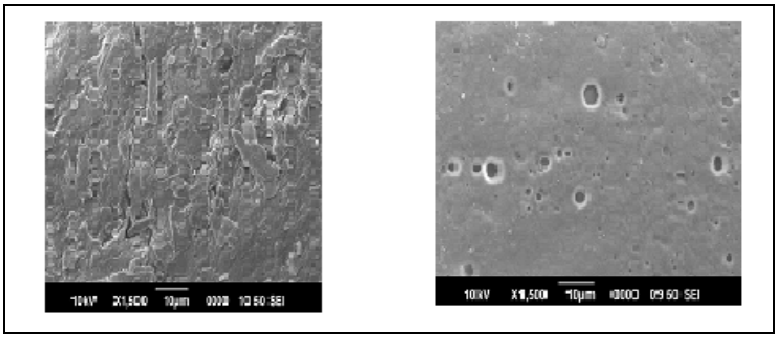


Figure No. 2: Surface morphology of pure Delafloxacin powder

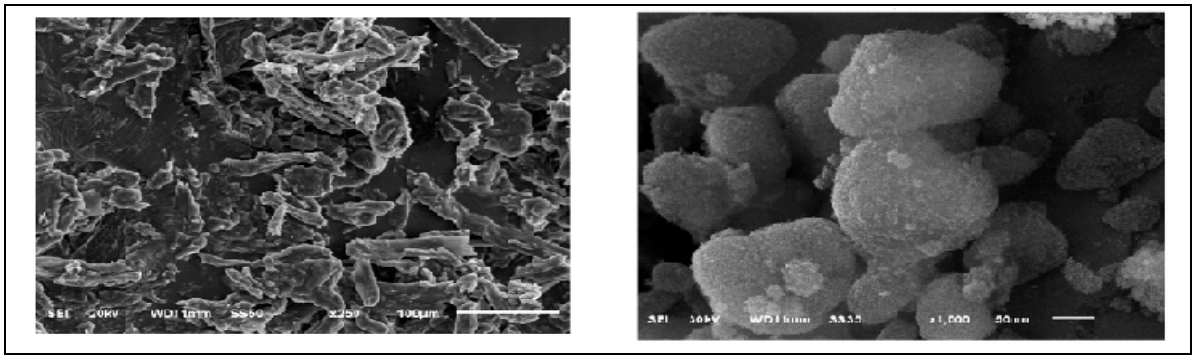


Figure No. 3: Surface morphology of S-SNEDDS formulae

10) Differential Scanning Calorimetry (DSC)

Thermograms of pure Delafloxacin, and prepared optimized S-SNEDDS F-5 were obtained using differential scanning calorimeter. It is known that transforming the physical state of a drug to the amorphous or partially amorphous state leads to a high-energy state and high disorder, resulting in enhanced solubility. As a result, it was expected that the solid particles would also have enhanced solubility.

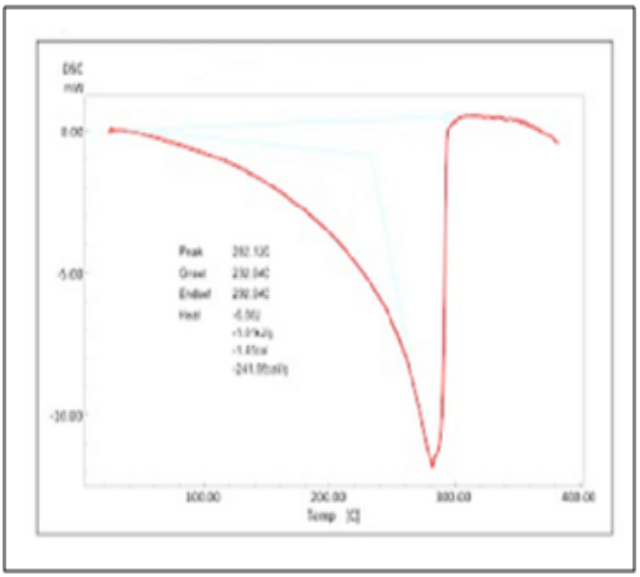


Figure No 4: Thermograms of Pure Delafloxacin

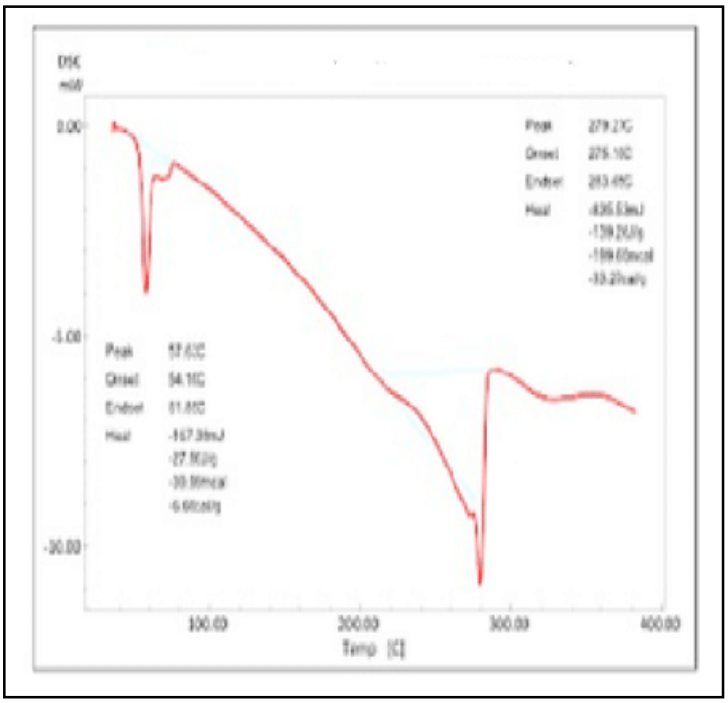


Figure No 5: Thermograms of optimized S-SNEDDS F-5

11) Fourier Transformed Infrared Spectroscopy (FTIR)

FTIR spectra are mainly used to determine interaction between the drug and any of the excipients used. The presence of interaction is detected by the disappearance of the important functional group of the drug.

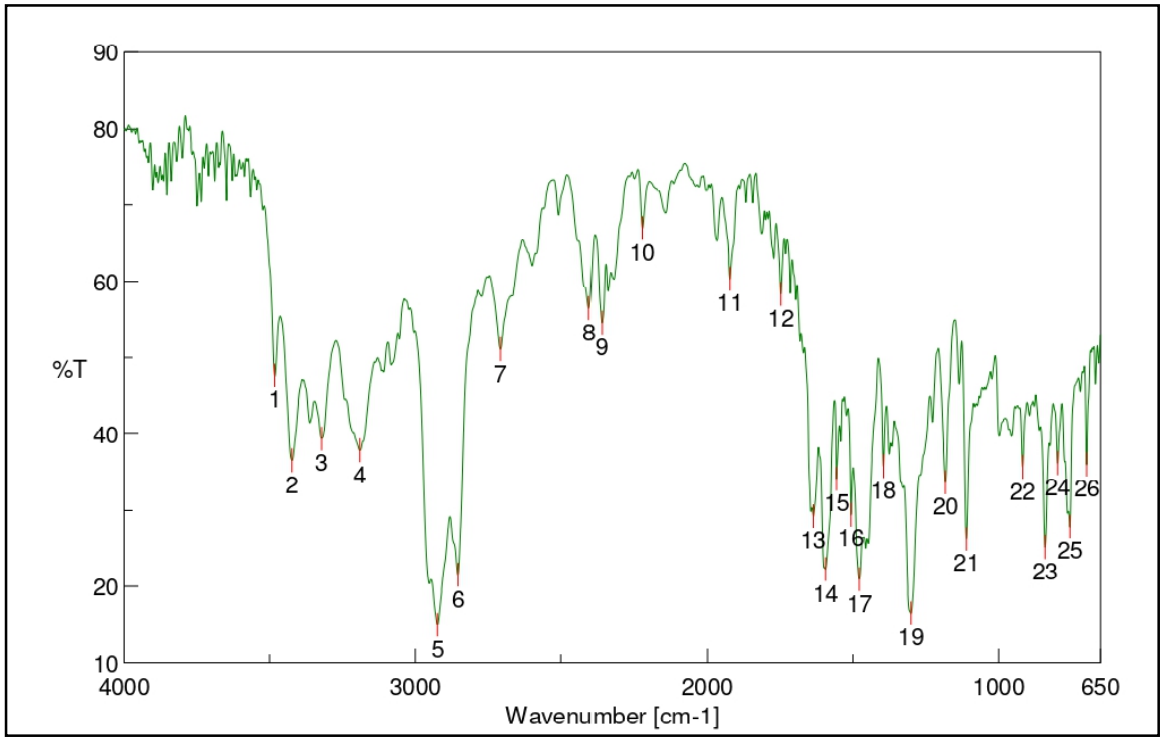


Figure No 6: FTIR spectra of pure drug

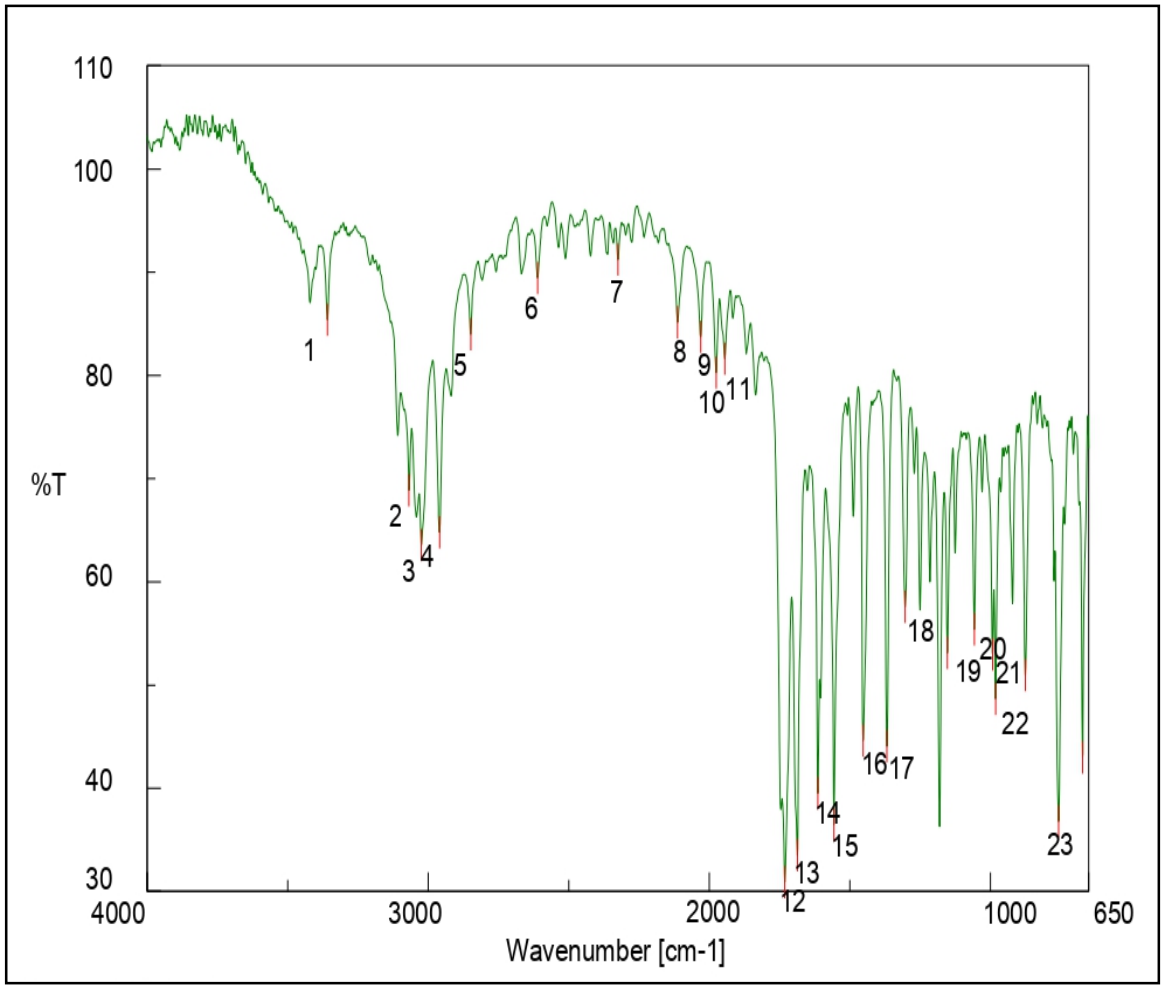


Figure No 7: FTIR spectra of optimized S-SNEDDS F-5

12) In Vitro Drug Release Studies

The percentage drug release from S-SNEDDS was found to be higher than that of pure Delafloxacin and marketed product as shown.

Table No. 10: In Vitro Drug Release

Time (h)	Cumulative%drugrelease								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	11 ±1.07	12 ±2.47	20±2.47	10 ±1.17	10 ±2.97	14±1.63	20±2.54	18±2.35	18±1.55
10	22±3.56	21±3.25	28±2.94	23±1.34	21±2.29	26±3.25	32±2.66	27±2.57	33±2.88
15	31±2.78	32±3.59	33±3.78	32±2.52	33±3.33	39±3.68	43±2.70	36±3.61	44±2.63
30	42±3.38	44±2.80	44±4.58	41±3.16	43±2.71	50±3.67	55±2.37	47±3.95	53±3.85
45	54±4.14	56±3.11	53±3.84	55±3.74	53±4.04	63±2.81	70±3.91	55±3.68	64±4.08
60	65±3.90	68±4.08	62±4.52	64±3.81	64±3.81	78±3.58	78±2.46	67±2.74	75±3.73
90	79±3.14	70±2.04	80±2.28	74±2.24	71±3.50	90±1.74	87±2.77	83±2.37	82±1.88
120	90±1.78	93±2.16	86±2.36	94±1.17	97±2.07	92±1.93	95±1.30	92±1.91	90±2.51

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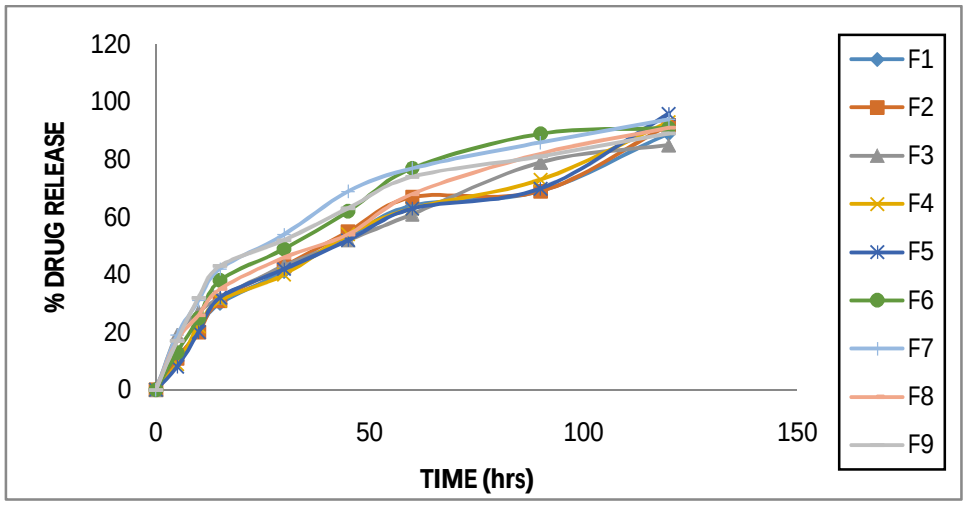


Figure No. 8:Cumulative % drug release

Delafloxacin dissolved and released from S-SNEDDS reached 97.02 % ±2.07 % for formula F-5, within two hour.

13) Mathematical Model Approach

Dissolution data of the formulations were fitted to various mathematical models (zero-order, first-order, Peppas, and Hixon–Crowel) in order to describe the kinetics of drug release. The smallest value of the sum of squared residuals (SSR) and the highest value of the correlation coefficient (r) were taken as criteria for selecting the most appropriate model. It is clear that optimized formulas have R^2 value 0.977, indicating Fickian diffusion-controlled release

Table No 11: In vitro drug release studies (F1-F9)

Formulation Code	KINETIC MODELS				
	Zero order R^2	First order R^2	Higuchi R^2	Korsmeyer R^2	Best Fitted Model (Higuchi) R^2
F1	0.918	0.956	0.986	0.934	0.986
F2	0.910	0.928	0.980	0.932	0.980
F3	0.913	0.989	0.996	0.868	0.996
F4	0.934	0.937	0.987	0.939	0.987
F5	0.932	0.865	0.977	0.944	0.977
F6	0.867	0.979	0.977	0.912	0.977
F7	0.843	0.992	0.980	0.865	0.980
F8	0.912	0.991	0.996	0.887	0.996
F9	0.830	0.980	0.974	0.861	0.974

14) Stability Studies

Table No. 12: Stability Study of Delafloxacin S-SNEDDS

Days	Temp. condition	PDI	% of transmittance	Drug content
0	2-8°C 30±2°C/65±5% RH 40±2°C/75±5% RH	0.125±0.01370.1 25±0.0137 0.125±0.0137	99.51±0.84 99.51±0084 99.51±0.84	99.09± 0.56 99.09± 0.56 99.09± 0.56
30	2-8°C 30±2°C/65±5% RH 40±2°C/75±5% RH	0.142±0.01760.1 67±0.0136 0.179±0.0114	99.79±0.15 99.85±0.81 99.46±0.25	99.55± 0.015 99.29± 0.008 99.15± 0.011
60	2-8°C 30±2°C/65±5% RH 40±2°C/75±5% RH	0.150±0.01870.1 87±0.0127 0.195±0.0125	99.83±0.18 99.89±0.09 99.51±0.13	99.39± 0.010 99.19± 0.18 99.93± 0.20
90	2-8°C 30±2°C/65±5% RH 40±2°C/75±5% RH	0.125±0.01370.1 25±0.0137 0.125±0.0137	99.80±0.015 99.85±0.012 99.69±0.012	99.32± 0.013 99.89± 0.014 99.79± 0.006

Physical appearance of SNEDDS was observed during the stability studies and the SNEDDS remained clear with no signs of precipitation. This indicated that the drug remained solubilized even at accelerated stability conditions (40 ± 2°C/75 ± 5% RH). Also, no significant decrease in the Delafloxacin content was observed indicating that drug remained chemically stable in the SNEDDS. Thus, it can be concluded that the Delafloxacin SNEDDS would remain physicochemical stable at long-term stability conditions (30 ± 2°C/65 ± 5% RH) as well as accelerated conditions (40 ± 2°C/75 ± 5% RH) for 3 months.

VI. CONCLUSION

Delafloxacin is a lipophilic drug as constrained of aqueous solubility, hepatic first-pass metabolism with poor absorption rate thus, formulated to overcome such hindrances. The developed liquid formulations have found appropriate results of thermodynamic stability, emulsifying rate and adequate dissolution rate. Whereas, based on the characterization and evaluation of numerous parameters have revealed that the developed formulations were providing an efficacious therapeutic efficacy of the drug. The optimized Delafloxacin SNEDDS has selected based on the smaller z-average diameter and faster dissolution rate as well as an appropriate emulsification rate as compared with the other formulations. The optimized formulation had exhibited faster dissolution rate as 97±2.07 % drug released which is clearly indicating that developed SNEDDS enhanced the dissolution rate of the drug. Therefore, It reveals that the characterization, evaluation and comparison of various parameters have to reached on the conclusion that S-SNEDDS is a potential drug delivery system of the drug. Finally, we can conclude that S-SNEDDS is a robust and promising nanocarriers to enhance drug bioavailability and therapeutic efficacy.

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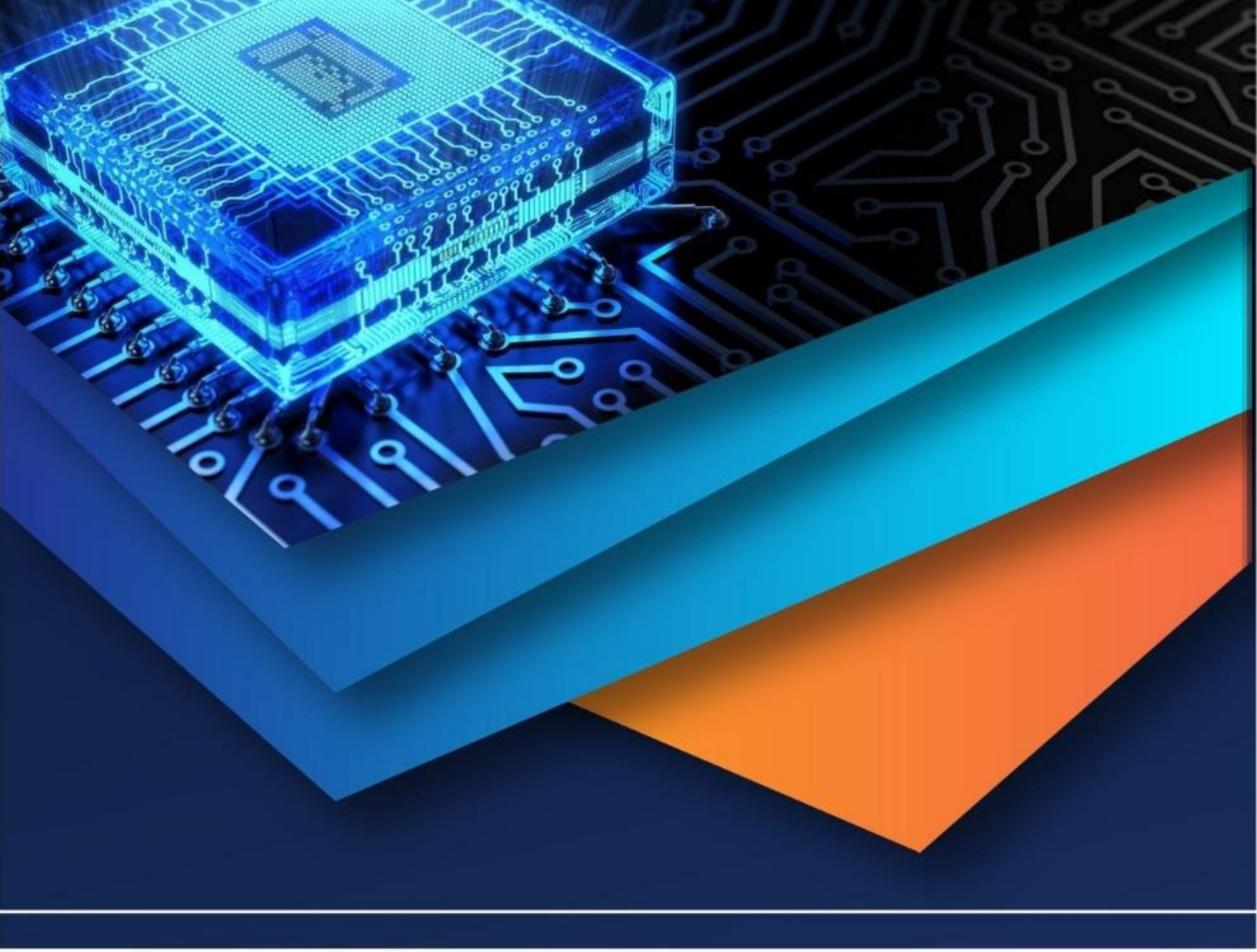
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