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Formulation development and evaluation of aqueous injection of poorly soluble drug made by novel application of mixed solvency concept

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Abstract

It is commonly recognized in the pharmaceutical industry that on average more than 40% of newly discovered drug candidates are poorly water-soluble. The objective of present research is to explore the application of mixed solvency technique in the injection formulation of poorly soluble drugs and to reduce concentration of individual solubilizers (used for solubility enhancement) to minimize the toxic effects of solubilizers. In the present work poorly soluble drugs Ofloxacin are selected as model drugs. Ofloxacin is an antibiotic drug tried to formulate the aqueous injection by the use of various physiologically compatible solubilizing agent like Lignocaine Hydrochloride, Niacinamide, Sodium benzoate, Sodium citrate, PEG 400, PEG 4000, PVP 40000, Ethanol, and Propylene Glycol. For expected synergistic enhancement effect on solubility of these poorly soluble drugs various blends of solubilizers shall be tried to decrease the amounts of Solubilizer employed for a desired solubility enhancement ratio. The study further opens the chances of preparing dry powders for injection of drug which are not stable in aqueous solution, ready to use injection.

Keywords: Mixed solvency solubilization, Ofloxacin, solubility enhancement, synergistic enhancement effect.

Introduction

Parenteral dosage forms and delivery systems include injectables (i.e., solutions, suspensions, emulsions, and dry powders for reconstitution), intramammary infusions, intravaginal delivery systems, and implants. Maheshwari proposed the concept of mixed solvency. He is of the opinion that all substances whether liquids, gases or solids possess solubilizing power and hence concentrated aqueous solution containing various dissolved substances can also improve the solubility of poorly water soluble drugs. In pharmaceutical science, solubility is commonly related to the bioavailability of the compound of interest, especially for poorly soluble compounds. Slow absorption rate result in an erratic and variable profile of drug level. Administration of a drug in any dosage form, except solution involves a dissolution step. Most co-solvent has hydrogen bond donor and or acceptor groups as well as small hydrocarbon regions. Their Hydrophilic hydrogen bonding groups ensure water miscibility while their hydrophobic hydrocarbon regions interfere with water hydrogen bonding network, reducing the overall intermolecular attraction of water. By disrupting waters self-association, co-solvent reduces water ability to squeeze out non-polar, hydrophobic compounds, thus increasing solubility [1-7].

Materials & Method

Ofloxacin was obtained from Enicar Pvt. Ltd. Bhoisar Niacinamide was obtained from Morden Lab Ltd. Indore, Lignocaine Hydrochloride, Sodium Benzoate, Sodium Citrate, PEG 400, PEG 4000, PVP 40000, Ethanol, Propylene glycol was obtained from S D fine chemicals, Mumbai. [8,9]

Method of Preparation

Preparation of Calibration curve in distilled water
10 mg of Ofloxacin wt. & transferred in to volumetric flask by addition of distilled water and volume made up to 100 ml Distilled water so as obtained solution 100 ppm . Concentration range 2 to 10 ppm. Absorbance of these solutions was measured on double beam UV visible spectrophotometer at 200 nm to 400 nm against distilled water and spectrum is shown in fig no. 1, Linearity graph shown fig no. 2 and absorbance recorded in table no. 1[10,11]

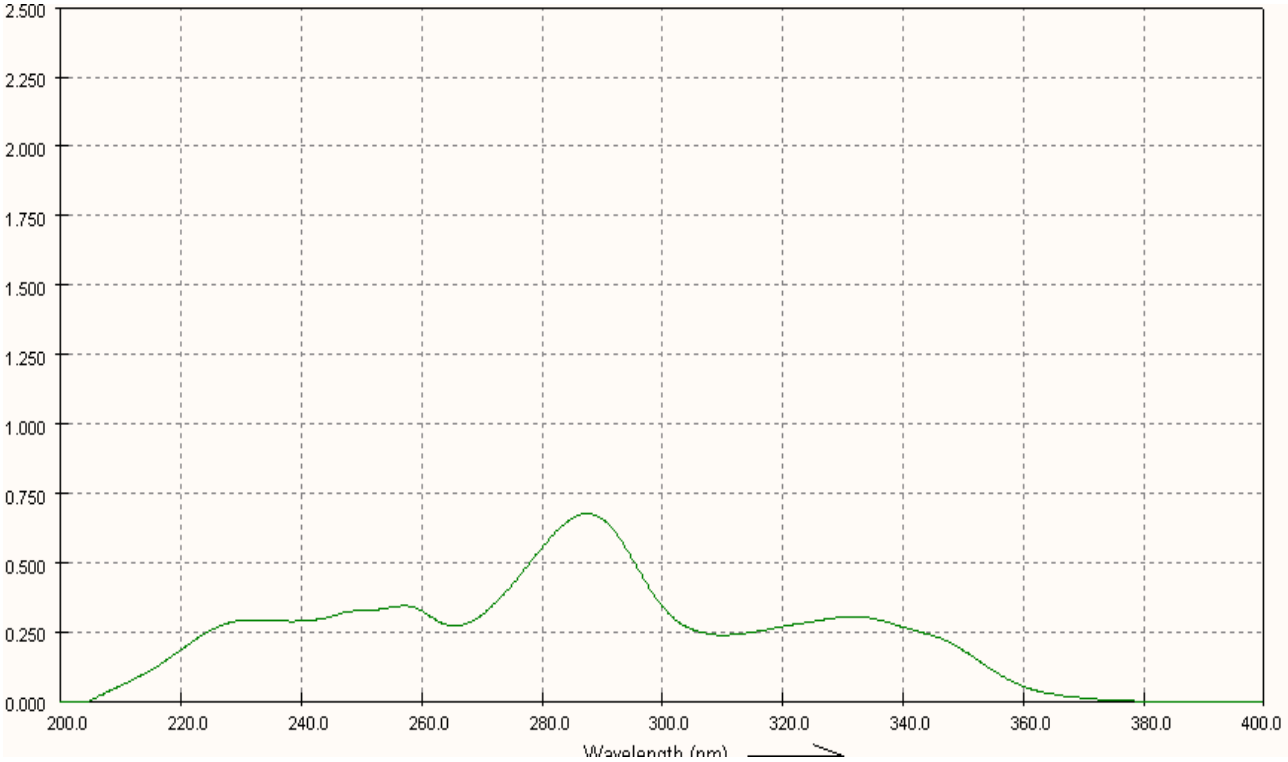

Figure 1: Calibration curve at 287.6nm

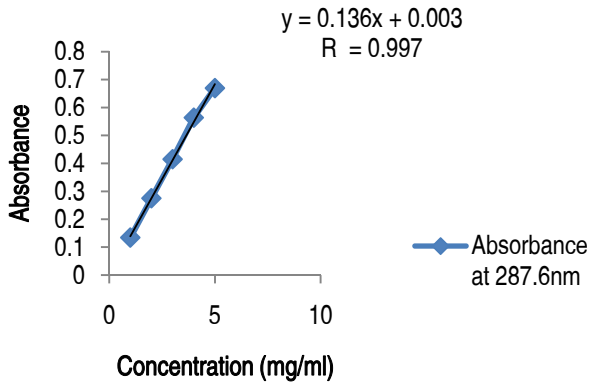

Figure 2: Linearity curve

Table 1 Absorbance recorded

ppm solution	Absorbance at 287.6nm
2 ppm	0.134
4 ppm	0.275
6 ppm	0.415
8 ppm	0.564
10 ppm	0.670

FT-IR Spectroscopy study Drug & Solubilizers

About 1-5 mg of Ofloxacin drug sample was triturated with approximately 300 mg of dry, finely potassium bromide IR and compressed as pellet & spectra was recorded for pure drug shown in fig. no. 3. And Ofloxacin with Solubilizer was combination 1:1 mix then finally powdered KBr IR compressed as pellet and spectra was recorded for drug and Solubilizer shown fig. no. 4 to 9.[12,13]

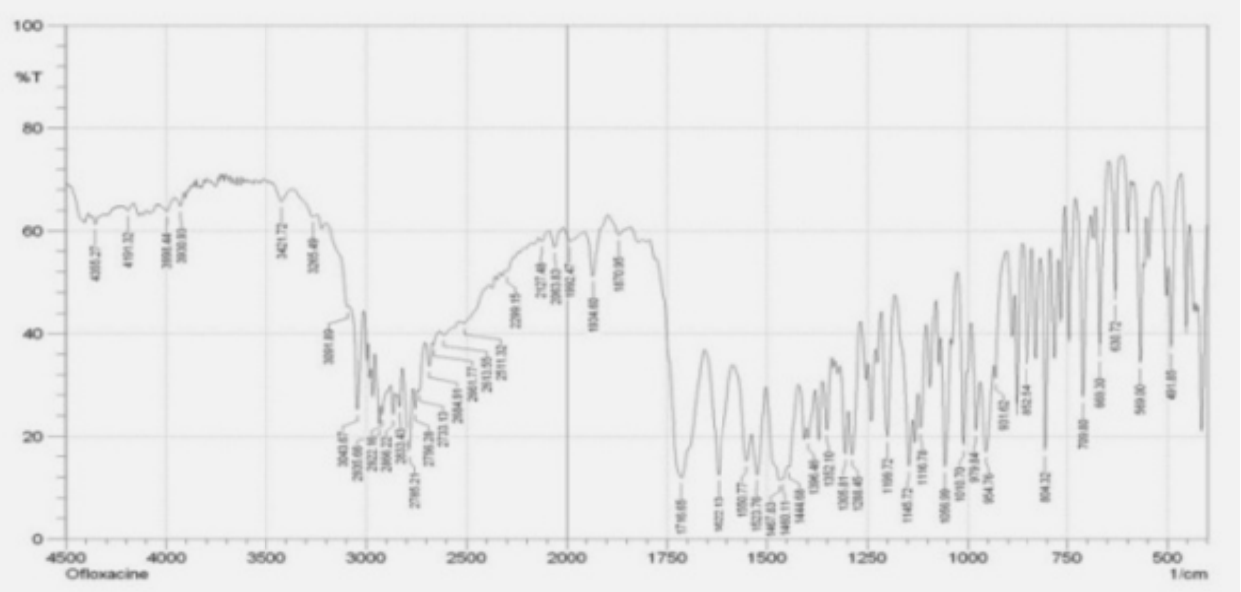

Figure 3: FT-IR Spectrum of Ofloxacin

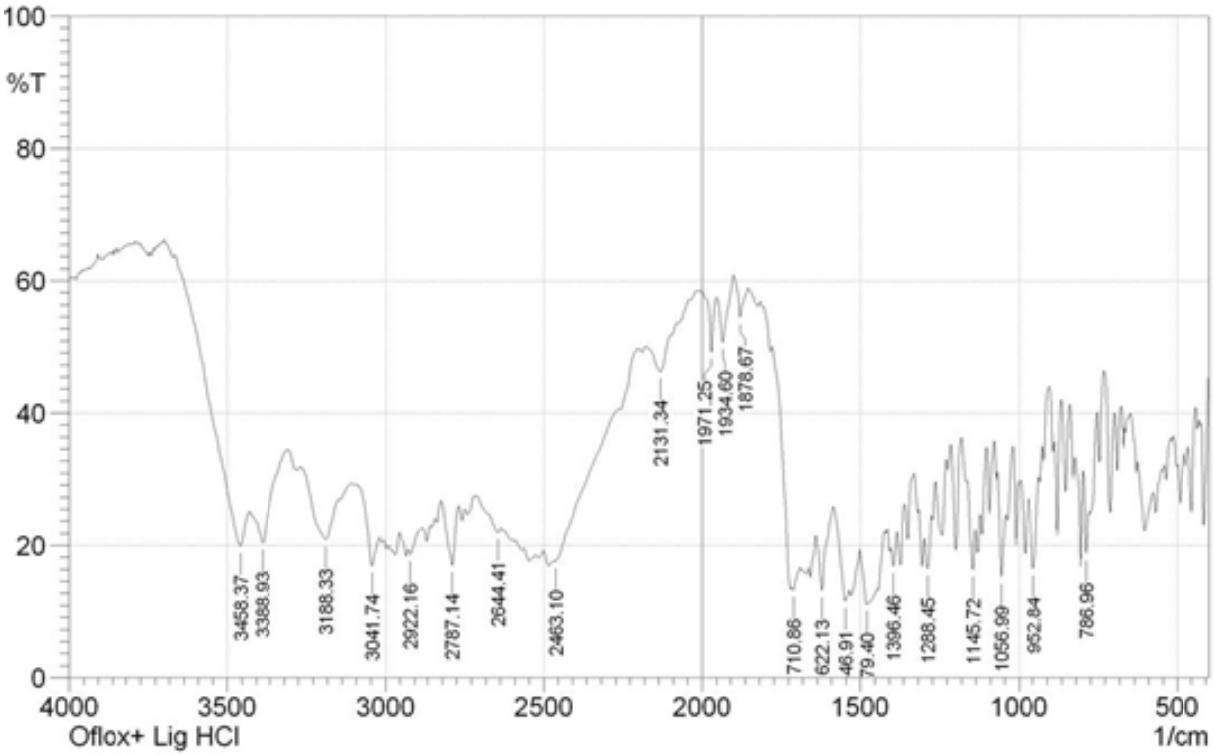

Figure 4: FT-IR Spectrum of Ofloxacin with Lignocaine HCl.

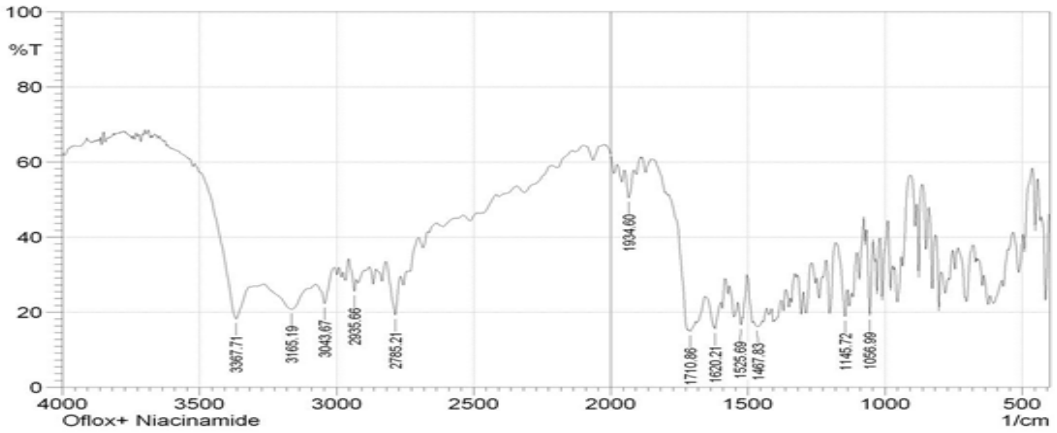

Figure 5: FT-IR Spectrum of Ofloxacin with Niacinamide.

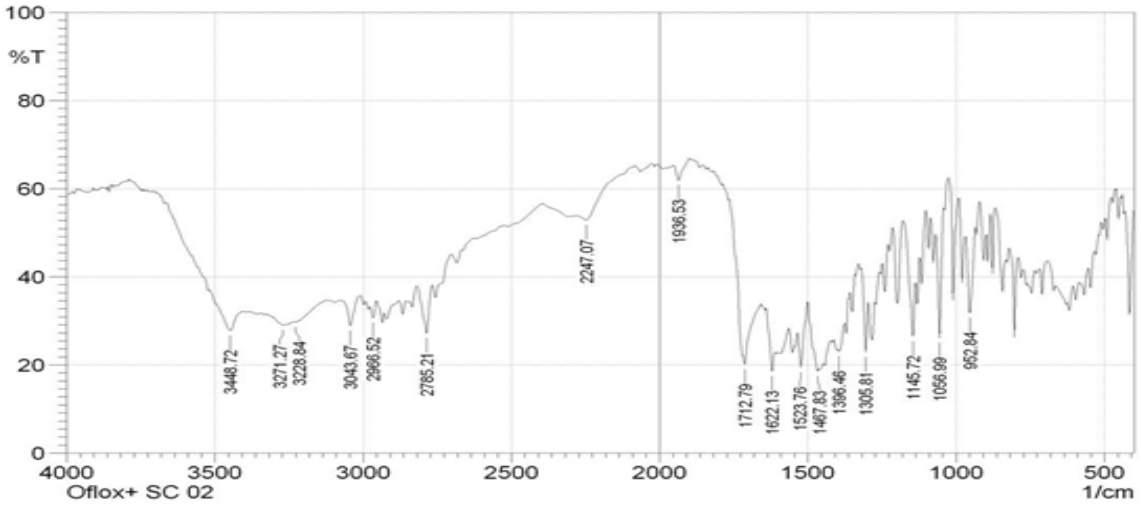

Figure 6: FT-IR Spectrum of Ofloxacin with Sodium Citrate

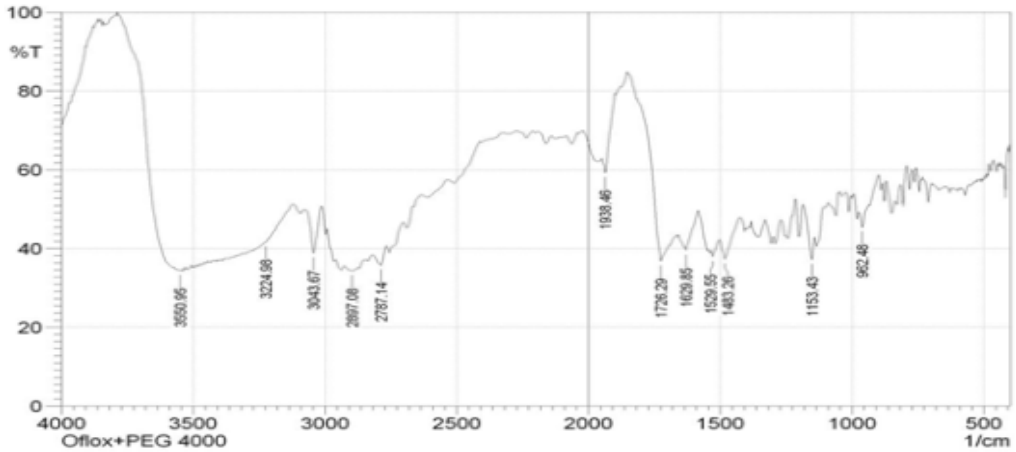

Figure 7: FT-IR Spectrum of Ofloxacin With PEG-4000

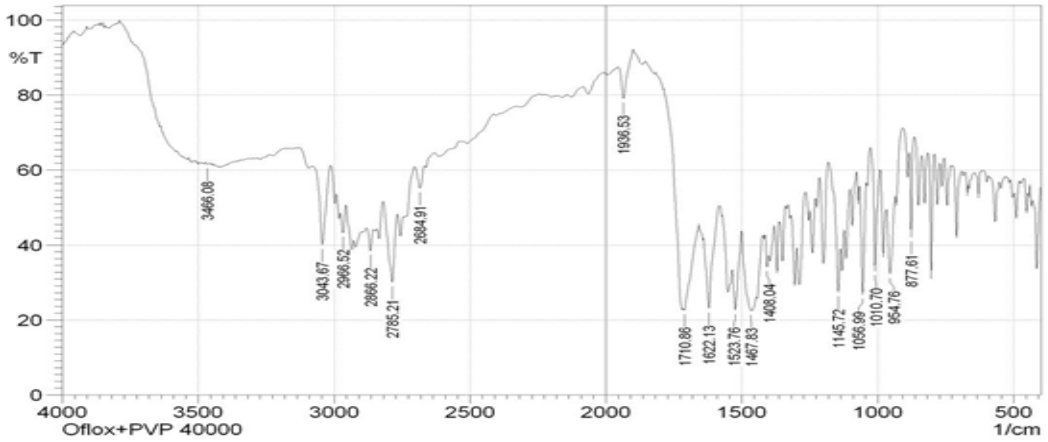

Figure 8: FT-IR Spectrum of Ofloxacin with PVP 40000

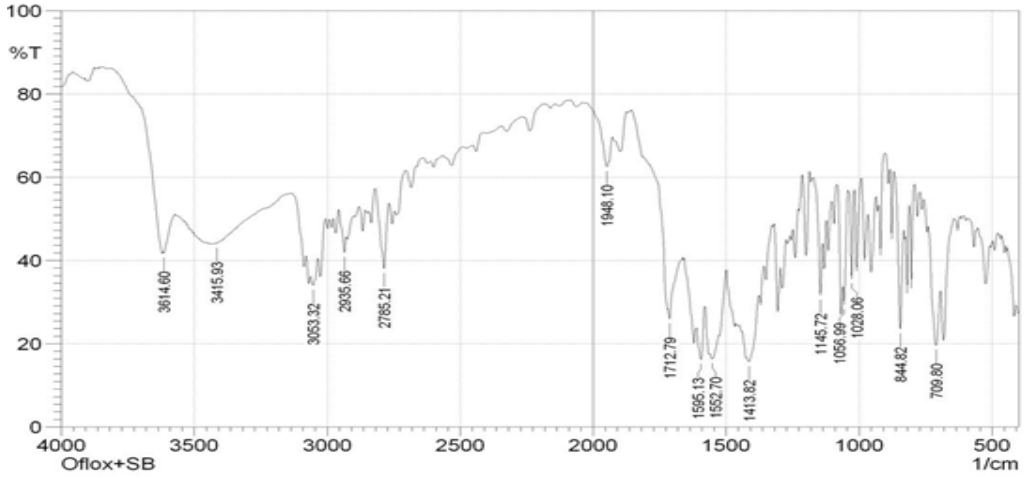

Figure 9: FT-IR Spectrum of Ofloxacin with Sodium Benzoate

DSC of Drug Sample

Sample of 2-5 mg of the pure Ofloxacin or the above-mentioned sample was sealed in Al. Pans at constants heating rate of 10^0C/min. in scanning temp. Range of 30 to 300^0C. The DSC spectrum of Ofloxacin drug sample in fig. no. 10.

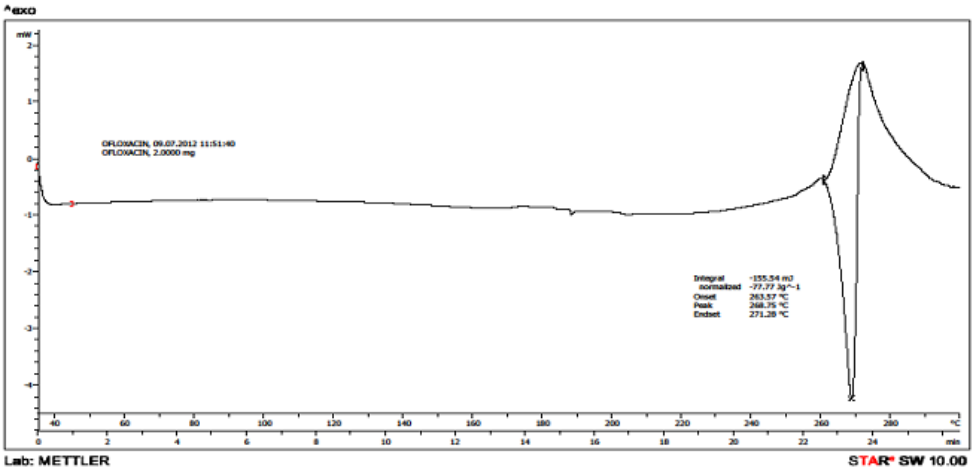

Figure 10: DSC Thermogram of Ofloxacin

Physical Stability of Drug & Solubilizers

The compatibility of drug the solubilizers was assesses by drug-solubilizers interaction studies. The drug mixed with excipients in 1:1 (50 mg) in separate glass vials which were then properly sealed 7 kept undisturbed at different condition storage condition at room temp., 40^{0}C, & in refrigerator for period of one month. The observation was recorded in given table no. 2.[17]

Table 2: Observations of Drug-excipients Interaction Study

Sr. No.	Drug-Excipients Mixture	Initial Appearance	Storage conditions											
			Refrigerator (2-8ºC)				Room Temperature				40ºC			
			Weeks				Weeks				Weeks			
			1	2	3	4	1	2	3	4	1	2	3	4
1	OFLOX.	White powder	N	N	N	N	N	N	N	N	N	N	N	N
2	OFLOX+PEG 4000	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
3	OFLOX+LH	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
4	OFLOX +SB	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
5	OFLOX +SC	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
6	OFLOX +PVP-FT	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
7	OFLOX +NM	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N

OFOLX = Ofloxacin
LH = Lignocaine hydrochloride
SB = Sodium benzoate
SC = Sodium citrate
N = No Change
NM = Niacinamide
PVP-FT = PVP-40000

Solubilization Study in Distilled water and Drug in Aqueous Solution Containing Individual Solubilizers (25% w/v)

Solubility of Ofloxacin in distilled water was determined by shake flask method. About 5 ml distilled water taken in three vials of 10 ml capacity separately, to each vials an excess amount of Ofloxacin was added then 10 min for proper mixing. They were kept in orbital flask shaker maintained at 25^{0}C for 12 hrs. The solution was then allowed to equilibrilate for 24hrs. Then solution containing excesses undissloved drug were transferred in to centrifuge tubes and centrifuges at 2000 rpm for 10 min. & supernatant was filtered through Whatman filter paper # 41. Filtrate was suitably diluted with distilled water and analyzed using double beam UV visible spectrophotometer at fixed wavelength at 287.6 nm against distilled water. And all individual (25% w/v) solubilizers' procedure as same above result recorded in table no. 3 & shown in fig. no. 11[9].

Solubility Determination of Drug in Aqueous Solution Mixed Blend Solubilizers (25% w/v)9

For the preparation of mixed blends (aqueous solutions) containing solubilizers, required amount of individual solubilizers were weighed and transferred to volumetric flask of 10 ml capacity containing seven ml of distilled water, to this the solubilizers were added and flask was shaken vigorously to dissolve to added solubilizers. After complete dissolution of solubilizers the volume was made up to the mark with distilled water, flask was shaken again to get homogenous solution. The prepared blends were filtered and used for further solubilization studies. Mixed Blend shown in table no.4 & 5 and solubility ratio fig no. 12.

Table 3: Solubility's of Ofloxacin in Aqueous Solutions Containing Individual Solubilizers

Aqueous solution of solubilizers (25% w/v)	Equilibrium solubility of Ofloxacin (mg/ml)	Solubility enhancement Ratio
Distilled water	0.11	
PEG 4000	2.55	23.18
PEG 400	1.97	17.90
Tween 20	3.98	36.18
Niacinamide	3.25	29.54
Propylene glycol	2.28	20.72
PVP-40000	1.69	15.36
Ethanol	1.55	14.09
Sodium benzoate	4.75	43.18
Lignocaine hydrochloride	2.66	24.18

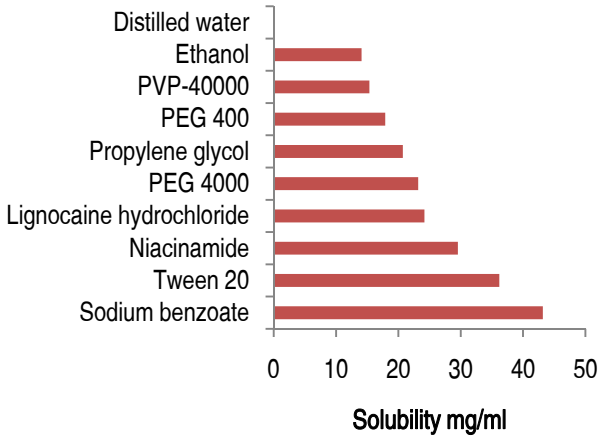

Figure 11: Solubility Profile of Ofloxacin in Aqueous solution of Individual solubilizers 25% w/v

Determination of pH Dependent Solubility of Drug

The USP buffer solution of pH 2.4, 5.6, 6.8, 7.4, & 9 were prepared & pH of this solution was adjusted digital pH meter. About 5 ml each buffer solution was taken in vials separately. Then excess amount of Ofloxacin was added then 10 min. for proper mixing. They kept in orbital flask shaker maintained at 25^{0}C for 12 hrs. The solution was then allowed to equilibrate for 24 hrs. , solution containing excess undissloved drug transferred in to centrifuge tubes and centrifuged at 2000 rpm for 10 min. & supernatant was filtered through Whatman filter paper # 41. Filtrate was suitably diluted using distilled water and analyzed using UV visible spectrophotometer at fixed wavelength at 287.6 nm against respective reagent blanks. Result obtained in table no. 6 & fig. no. 13.[9].

Chromatographic Study of Solubilized Drug product

A plate of silica gel GF 254 was activated at 110 for 1 hr. and used. The Methanolic solution of Ofloxacin alone & drug & solubilizers as Blend 19, 21, 22 & 23 Then, the plate was left in air for 10 min. to dry and transferred to solvent jar saturated with solvent system was allowed to run for about 4.2 cm. finally, The plate was left in air 10 min. to dry and transferred to solvent jar saturated with solvent system composed of mixture of chloroform and methanol 1:1 v/v. Finally plate was transferred to an oven maintained at temp. 80^{0}C for 2 min. & observed in UV chamber at short wavelength for visualization of spots. In order to predict the possible interaction or Complexation between drug and solubilizers the TLC studies were performed. The R_f values were determined & recorded in table no. 7 [14].

Optimization of Blend for preparation of injection

On the basis of result obtained from solubility studies, mixed blends in which solubility of Ofloxacin was more than 4 mg/ml and in all 4 blend contained no Sodium benzoate & Lignocaine HCl. Due presence of Precipitate , such selected blend were B-19, B-21, B-22, B-23. To develop 1 ml of Ofloxacin injection, amount of solubilize and drug that will be administered through each mixed blend was determined. The proposed formulations are shown in table no. 8 to 11.

Table 4: Preparation of Mixed Blend 25% w/v of solubilizers

Blend No.	Lignocaine HCl	Niacinamide	Sodium Benzoate	PEG 400	PEG 4000	PVP 40000	Propylene glycol	Sodium citrate	Tween 20	Ethanol
B-1	6.25	-	6.25	6.25	6.25	-	-	-	-	-
B-2	6.25	6.25	-	6.25	6.25	-	-	-	-	-
B-3	6.25	6.25	6.25	6.25	-	-	-	-	-	-
B-4	6.25	6.25	6.25	6.25	-	-	-	-	-	-
B-5	5	5	5	5	5	-	-	-	-	-
B-6	-	6.25	6.25	6.25	6.25	-	-	-	-	-
B-7	6	6	-	5	5	-	-	3	-	-
B-8	6	-	6	5	5	-	-	3	-	--
B-9	6	5	4	6	2	-	2	-	-	-
B-10	6	6	6	-	5	-	2	-	-	-
B-11	8	2	8	2	-	-	5	-	-	-
B-12	6	-	6	6	-	-	7	-	--	-
B-13	8	-	-	8	-	-	9	-	-	-
B-14	8	8	1	8	-	-	-	-	-	-
B-15	5	6	4	5	-	-	-	-	5	-
B-16	-	5	-	5	5	-	5	-	5	-
B-17	4	5	4	4		-	-	-	4	4
B-18	5	5	3	-	-	3	5	-	-	4
B-19	5	5	-	5	5	-	-	-	-	5
B-20	5	5	5	5	-	-	-	-	-	5
B-21	5	5	-	5	4	3	-	-	-	3
B-22	-	5	5	5	-	5	5	-	-	-
B-23	5	5	-	-	5	4	3	-	-	3
B-24	5	-	5	-	5	5	5	-	-	-

Formulation of Aqueous Injection

i) Preparation of Blend: Every ingredient for each blend as mentioned in table no. 8 to 11 were accurately weighed & transferred in 25 ml volumetric flask. 20 ml of water for injection was added to flask was shaken to dissolve the content. Then volume was made up to 25 ml.

ii) Preparation of Aqueous solution of Ofloxacin: For preparation of aqueous solution of Ofloxacin about 20 ml of Hydrotropic Blend solution was taken in 25 ml volumetric flask. Weighed amount of Ofloxacin was transferred in to volumetric flask. & flask was shaken until complete Dissolution of drug. Volume made up to 25 ml. to Shaken to get Homogeneous solution.

iii) Treatment of packaging material: Glass vials were first washed three times with distilled water. All these vials were dried in an oven and sterilized by dry heating in an oven at 160 C for 2 hrs in inverted position. Rubber closures and aluminum seals used for plugging the vials were first washed several times with distilled water and then autoclaved at 15 lbs pressure (121 C) for 20 minutes and finally dried in oven.

iv) Preparation of aseptic area: The walls and floor of aseptic room were thoroughly washed with filtered tap water followed by 5% phenol solution. The room was fumigated using a mixture of formaldehyde and potassium permanganate. The laminar air flow bench was cleaned ad wiped out with 70% ethanol solution and switched on UV light was for 30 min. prior to filling of injection in to vials.

v) Aseptic filtration: The Aqueous solution of Ofloxacin was prepared as above & sterilized by filtration under the Membrane filter 0.22 µm (Millipore) was used for the filtration. The membrane filtration assembly fitted with the membrane filter was sterilized previously in the autoclave at 121 C and 15 lbs pressure for 20 min.

Evaluation of injection

i) Appearance and colour of formulation15

In which Appearance of Aqueous Injection of Ofloxacin show in Fig. no , and colour of aqueous injection. Result recorded in table no. 12.

ii) Determination of pH of the developed aqueous injection9

The pH of prepared formulations was determined using digital pH meter. The pH so obtain were recorded in table no. 12.

iii) Accelerated stability studies17

As soon as the product is developed, it is subjected to ageing; as a result its physical properties, chemical composition and even its biological availability may be changed. The prepared formulations

Table 5: blends

Blend codes	Solubility (mg/ml)	Solubility enhancement ratio
B-1	1.112	10.10
B-2	1.612	14.65
B-3	2.312	21.01
B-4	2.229	20.26
B-5	1.787	16.24
B-6	1.578	14.34
B-7	1.732	15.74
B-8	1.280	11.63
B-9	1.633	14.84
B-10	1.834	16.67
B-11	1.922	17.47
B-12	2.510	22.81
B-13	3.156	31.96
B-14	4.430	40.27
B-15	4.221	38.37
B-16	3.321	30.19
B-17	4.767	42.33
B-18	2.157	19.60
B-19	**2.589**	**23.53**
B-20	2.540	23.09
B-21	**5.273**	**47.93**
B-22	**4.680**	**42.54**
B-23	**4.178**	**37.98**
B-24	3.341	30.37

Table 6: Solubility's of Ofloxacin in buffer solutions of different pH

Buffer (pH)	Solubility (mg/ml)
2.4	0.94
5.6	0.81
6.8	0.74
7.4	0.66
9	0.52

were subjected to 2-8 0C, 25 0C, 40 0C and 55 0C to observe the stability of medicament in developed formulations. Samples were withdrawn at interval of 7 days, suitably diluted with distilled water and analyzed using UV/Visible spectrophotometer against respective reagent blanks at fixed wavelength 287.6 nm to determine the amount of drug remaining in formulation. The initial drug content in the formulation was taken as 100%. Percent drug remained at definite time intervals were recorded in table no. 13 to 16 & degradation curve shown in fig no. 15 to 18.

iv) Clarity testing of aqueous injection15

Clarity test of Aqueous injection product was performed by visually inspecting the externally clean vial under a good light, baffled against reflection into the eyes, and viewed against black and white background, with the content set in swirling motion. The results so obtained are shown in table no. 12.

v) Leakage test of aqueous formulation15

The methylene blue solution was prepared 1% then all vials emerged in solution for few min. then check leakage test for formulation, the results so obtained are shown in table no. 12.

vi) Viscosity of aqueous injection11

The Brookfield Helipath Stand is designed to slowly lower or raise a Brookfield Viscometer/Rheometer so that its rotating T-bar

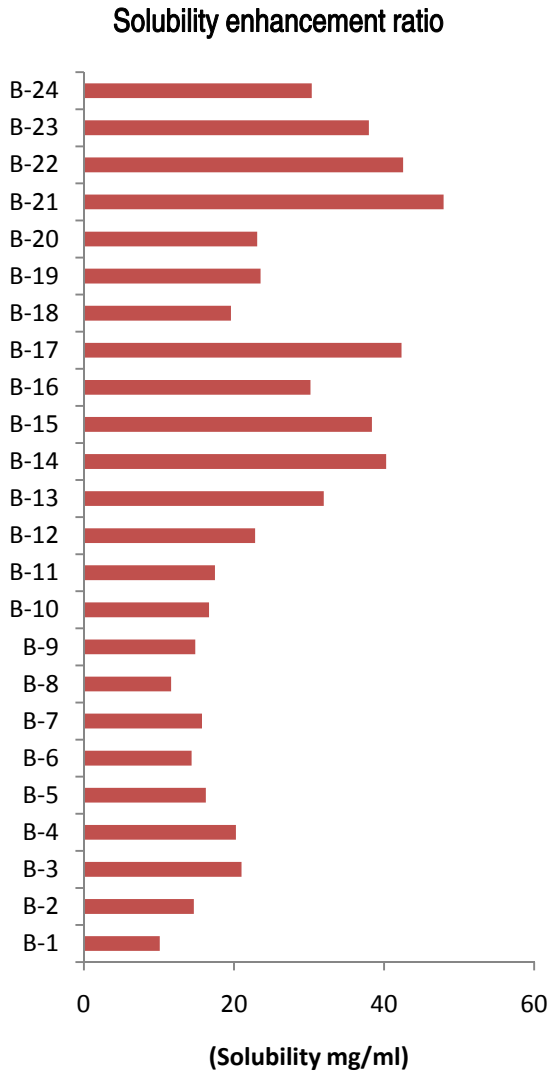

Figure. 12: Equilibrium solubility data of Ofloxacin in various blends

spindle will describe a helical path through the test sample. By always cutting into fresh material, the problem of channeling or separating is eliminated and meaningful viscosity/consistency

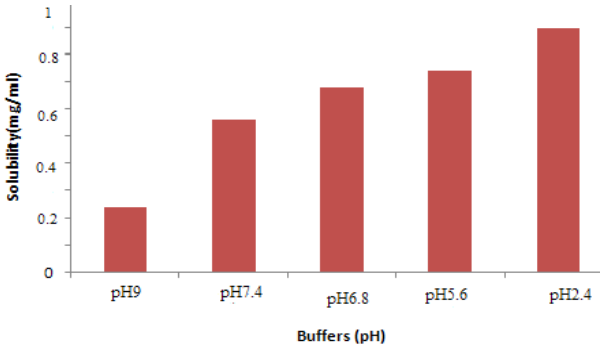

Figure 13: pH Dependent Solubility Profile of Ofloxacin

measurements can be made. The automatic reversing feature of the Helipath Stand allows measurements to be made over a variable period of time.

Table 7: R_F Values of Ofloxacin

Solvent system	Adsorbent	R_F value			
		Ofoxs.	B-21	B-22	B-23
Chloroform: methanol (1:1)	Silica gel GF 254	0.56	0.57	0.54	0.59

Procedure

1) Prepared formulation of Aqueous Injection Ofloxacin of various Blend 19, 21, 22, 23
2) This injection taken in 50 ml beaker.
3) To measure viscosity by using 96 S spindle no. at 5 rpm. and results obtained in table no. 12.
4) Sterility test for aqueous injection as per I.P.15
The test for sterility is intended for detection and presence of viable form of micro-organism is a pharmaceutical preparation the test tube must be carried out under condition designed to avoid accidental contamination during test.

Table 8: Formulation B-21

Sr. No.	Ingredients	Formula for 5.27mg/1ml batch	Formula for 131.75mg/25 ml batch
1	Ofloxacin	5.27mg	0.131g
2	Lignocaine hydrochloride	0.05g	1.25g
3	Ethanol	0.03g	0.75g
4	Niacinamide	0.05g	1.25g
5	PEG 400	0.05g	1.25g
6	PEG 4000	0.04g	1g
7	PVP-40000	0.03g	0.75g
8	Sterile water for injection	Up to 1ml	Up to 25ml

Membrane filtration

Use membrane filters 50 mm in diameter and having a nominal pore size of not greater than 0.45 μm the effectiveness of which in retaining bacteria has been established for the type of preparation under examination. Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation under examination to each of two membrane filters and filter immediately. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as buffered sodium chloride-peptone solution pH 7.0. Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of medium 2 and the other, intended for the enumeration of fungi, to the surface of a plate of medium 3. Incubate the plates for 5 days, unless a more reliable count is obtained in shorter time, at 30 to 35 in the test for bacteria and 20 to 25 in the test for fungi. Count the number of colonies that are informed. Calculate the number of micro-organisms per g or per ml of the preparation under examination, if necessary counting bacteria and fungi separately.

Table 9: Formulation B-19

Sr. No.	Ingredients	Formula for 2.58mg/1ml batch	Formula for 64.5mg/25 ml batch
1	Ofloxacin	2.58mg	0.0645g
2	Lignocaine hydrochloride	0.05g	1.25g
3	Niacinamide	0.05g	1.25g
4	PEG 400	0.05g	1.25g
5	PEG 4000	0.05g	1.25g
6	Ethanol	0.05g	1.25g
7	Sterile water for injection	Up to 1ml	Up to 25ml

Pour-plate method

For bacteria — Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation and about 15 ml of a liquefied casein Soyabean digest agar such as medium 2, at not more than 45º. Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary dilute the pretreated preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate 30 to 35 for 4 days, unless a more reliable count is obtained in a shorter time. Count the number colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

Table 10: Formulation B- 22

Sr. No.	Ingredients	Formula for 4.68mg/1ml batch	Formula for 117mg/25ml batch
1	Ofloxacin	4.68mg	0.117g
2	Propylene glycol	0.05g	1.25g
3	Sodium benzoate	0.05g	1.25g
4	PEG 400	0.05g	1.25g
5	Niacinamide	0.05g	1.25g
6	PVP-40000	0.05g	1.25g
7	Sterile water for injection	Up to 1ml	Up to 25ml

Table 11: Formulation B-23

Sr. No.	Ingredients	Formula for 4.17mg/1ml batch	Formula for 104.25mg/25ml batch
1	Ofloxacin	4.17mg	0.104g
2	Lignocaine hydrochloride	0.05g	1.25g
3	Niacinamide	0.05g	1,25g
4	PEG 4000	0.05g	1.25g
5	Propylene glycol	0.03g	0.75g
6	PVP 40000	0.04g	1g
7	Ethanol	0.03g	0.75g
8	Sterile water for injection	Up to 1ml	Up to 25ml

Figure 14 Photographs of Aqueous Injections

For fungi — Proceed as described in the test for bacteria but use Sabouraud dextrose agar with antibiotics such as medium 3 in place of medium 2 and incubate the plates at 20 to 25 for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies. Result shown in table no. 17 & 18.

Result and Discussion

Calibration curve in distilled water

The UV visible of Ofloxacin showed peak at 287.6 nm, which is same as reported in literature shown fig. no. 1 & absorbance recorded in table no. 1 From the calibration curve equation is given as y = 0.136x + 0.003. The value of R^2 is 0.997. On the basis of obtained result it was concluded that Ofloxacin, Obeyed Beers Lamberts law in the range of 2 µg/ml to10 µg/ml.

Table 12: Evaluation of injection

Sr. No.	Mixed Blend	Appearance	Clarity Test	Leakage Test	pH of Aq. Injection	Viscosity of Aq. Injection (cp)
1	B-19	Clear Transparent	Clear	Passes	5.6	1.52
2	B-21	Clear Transparent	Clear	Passes	6.2	1.72
3	B-22	Clear Transparent	Clear	Passes	6.8	1.67
4	B-23	Clear Transparent	Clear	Passes	7	1.59

FT-IR Spectroscopy study Drug & Solubilizers

The infrared spectrum of Ofloxacin was concordant with the reference spectrum of Ofloxacin and shows all the major peaks as shown in Fig. no. 3 the procured sample is pure and can be used for further studies. And IR spectra's were recorded for Ofloxacin with combination of lignocaine Hydrochloride. Sodium Benzoate, Sodium Citrate, PEG-400, PVP-40000, Niacinamide, To study the compatibility of drug with excipients FTIR spectra of drug in combination with excipients in the form of formulation was studied.

Table 13: Chemical stability B-19

Time (days)	% Drug remaining			
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C	55 ^{0}C
0	100.0	100.0	100.0	100.0
7	92	87	83	79
14	87.3	79	77	63
21	78	72	68	52
28	69	58	-	-

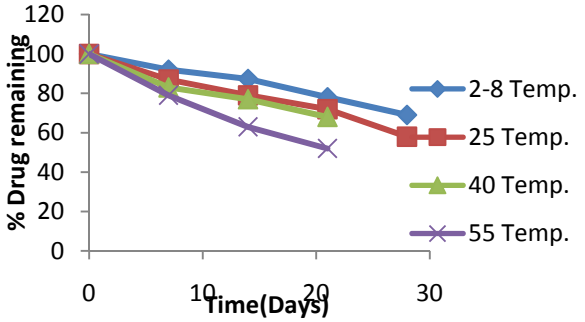

Figure15: Degradation Curve for the Formulation

Table 14: Chemical Stability B-21

Time (days)	% Drug remaining			
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C	55 ^{0}C
0	100.0	100.0	100.0	100.0
7	96.4	94	93	82
14	89.3	85.6	82	69
21	80	78	76	60
28	73	68	-	-

All the above characteristic peaks of drug appear in the spectra of physical mixture at the same wave number indicating no modification and interaction between the drug and Solubilizer. Results of the drug interaction studies suggest that all the studied excipients are compatible with Ofloxacin recorded in fig no. 4 to 9.

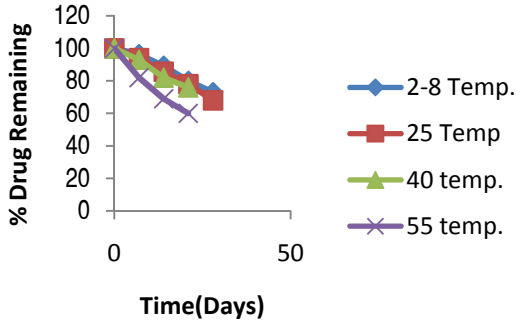

Figure 16: Degradation Curve for the Formulation

Table 15: Chemical Stability B-22

Time (days)	% Drug remaining			
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C	55 ^{0}C
0	100.0	100.0	100.0	100.0
7	93	83	78	67
14	88	77	73	61
21	76	72	61	56
28	71	63	-	-

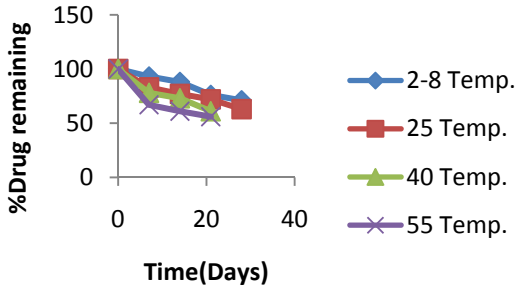

Figure 17: Degradation Curve for the Formulation

Table 16: Chemical Stability B-23

Time (days)	% Drug remaining			
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C	55 ^{0}C
0	100.0	100.0	100.0	100.0
7	91	81	76	67
14	82	74	68	61
21	71	64	55	51
28	63	54	-	-

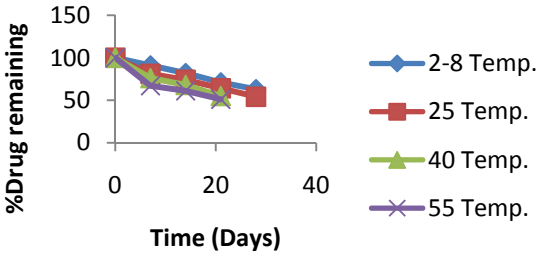

Figure 18: Degradation Curve for the Formulation

DSC of Drug Sample

The thermogram of Ofloxacin showed peak indicating the melting point of the drug is 268.75^{0}C which is identically near to the melting point mentioned in standards. From obtained result it was concluded that drug was in pure form. DSC Curve shown in figure 10.

Physical Stability of Drug & Solubilizers

All Drug & solubilizers were found to be stable & no interaction and result shown in table no. 2.

Solubilization Study in Distilled water and Drug in Aqueous Solution Containing Individual Solubilizers (25% w/v)

The solubility of Ofloxacin was increased by use of various solubilizers. The solubilizing power of different solubilizers could be ranked as Sodium benzoate > Tween 20> Niacinamide> Lignocaine HCl.> PEG 4000 > Propylene glycol > PEG 400> PEG 40000 > ethanol. While the solubility of Ofloxacin was decreased by sodium citrate hence, it is clear that solubilizers may increase or decrease the solubility of drugs. Solubility recorded in table 3 and Solubility profile shown in figure 11.

Table no.17 Observations of Soyabean Casein Digest Media

Sr.no.	Formulation code	Media used	Observation	Inference
1	B-19	Soyabean casein Digest media	No colony form	Tested formulation showed No growth of Bacteria
2	B-21	Soyabean casein Digest media	No colony form	Tested formulation showed No growth of Bacteria
3	B-22	Soyabean casein Digest media	No colony form	Tested formulation showed No growth of Bacteria
4	B-23	Soyabean casein Digest media	No colony form	Tested formulation showed No growth of Bacteria

Table no.18 Observation of Sabouraud-dextrose Agar with Antibiotic

Sr.no.	Formulation code	Media used	Observation	Inference
1	B-19	Sabouraud-dextrose agar	No colony form	Tested formulation showed No growth of Fungi
2	B-21	Sabouraud-dextrose agar	No colony form	Tested formulation showed No growth of Fungi
3	B-22	Sabouraud-dextrose agar	No colony form	Tested formulation showed No growth of Fungi
4	B-23	Sabouraud-dextrose agar	No colony form	Tested formulation showed No growth of Fungi

Solubility Determination of Drug in Aqueous Solution Mixed Blend Solubilizers (25% w/v)

All 24 blend solubility was determined in mg/ml. In Blend no.1, 3,4,5,8,9,10, 12,14,15,17,18,20,24 they are form precipitate (ppt) due presence of Sodium benzoate and lignocaine HCl. In Blend no. 1 shows low solubility & Blend no. 21 shows high solubility. Solubility recorded in table no. 5 & profile shown in fig. no. 12.

Determination of pH Dependent Solubility of Drug

The solubility of Ofloxacin slightly decreased with increase in pH but it did not vary significantly with pH change. The solubility enhancement ratio at pH 2.4 was (as compared to solubility in distilled water) which was less than the enhancement in mixed blends. Thus, enhancement in solubility of Ofloxacin in mixed blends was not due to the effect of pH only. Solubility recorded in table no. 6 & profile shown in figure 13.

Chromatographic Study of Solubilized Drug product

From the table 7, it is evident that all the spots in three blends correspond with the R_F value of standard Ofloxacin drug. From the result of chromatography, it was concluded that there was no Complexation and/or interaction between drug and solubilizers.

Optimization of Blend for preparation of injection

Aqueous Injection of various blends shown in table 8 to 11.

Evaluation of Aqueous Injection

i) Appearance and colour of formulation
In which Appearance of Aqueous Injection of Ofloxacin show in fig. no. 14, and recorded in table 12.
ii) Determination of pH of the developed aqueous injection
The pH of formulation of aqueous injection was measure satisfactory and recorded in table 12.
iii) Accelerated stability studies
From the results shown in tables 13 to 16 it is evident that the developed formulations of aqueous injection of Ofloxacin were not sufficiently stable at room temperature and refrigerated condition in all Blends. The shelf lives of formulations B-19, B-21, B-22 and B-23 were found as 14 days, 21 days, 28 days and 7 days respectively. To overcome the problem of instability of formulation,

it may be formulated as dry powder for injection by using the solid solubilizing agents & Degradation curve shown in fig. no. 15 to 18.

iv) Clarity testing of aqueous injection

The Clarity test of given Aqueous Injection of Ofloxacin was found to be clear. Shown in table no. 12.

v) Leakage test of aqueous formulation

The leakage test of given Aqueous Injection of Ofloxacin was found to be Passes & result shown in table. no. 12.

vi) Viscosity of aqueous injection

The result show in table no.12, that viscosity was found to be satisfactory.

vii) Sterility test for aqueous injection as per I.P.

There is No colony formation on the membrane filter after filtration that shows there is No presence of bacteria and fungi & result shown in table no. 17 to 18.

Summary and Conclusion

The objective of present work was to explore the novel application of mixed solvency technique in the injection formulation of poorly soluble drugs and to reduce concentration of individual solubilizers (used for solubility enhancement) to minimize the toxic effects of solubilizers. In most of the methods of Solubilization, high concentration of an additive (hydrotropic agent/cosolvents/surfactants/etc.) is required to produce an appreciable increase in solubility of a poorly soluble drug. In this case, the solubilizing agent employed to give a desirable solubility for the poorly soluble drug may produce its own toxicity. In the present study, practically water insoluble drug Ofloxacin were selected as model drugs. These drugs were tried to solubilize by employing the combination of physiologically compatible solubilizers in attempt to prepare their parenteral formulations (aqueous injection of Ofloxacin).

In the present work, the procured Ofloxacin drug samples were characterized by various tests. Melting point was determined by open capillary method and DSC the melting points were found to be 253-257ºC for Ofloxacin respectively. Spectrophotometric method was used to analyze the drugs; Ofloxacin showed peak at 287.6 nm was selected for analysis. FTIR spectroscopy was performed for further characterization of drugs, prior to use in the formulation. The procured samples of drugs were found to be in confirmation with the reported literature and were thus, used for further studies.

In preformulation studies, calibration curves of drugs were prepared in various media, viz., ethanol, distilled water and distilled water in presence of solubilizers. The correlation coefficients obtained were very close to one which confirmed that the Beer-Lambert's law was obeyed in concentration range of 2-10 µg/ml and solubilizer range 10-50 mcg/ml for drug solutions in all media for both Ofloxacin. The UV interference studies (in aqueous media for Ofloxacin) showed that none of the selected solubilizers interfere in UV estimation of drugs. Solubility studies of Ofloxacin were performed in various media, viz., Distilled water, 25% aqueous solution containing individual solubilizers (Sodium Benzoate, Niacinamide, PEG 4000, PEG 400, Lignocaine Hydrochloride, Propylene Glycol, Tween 20, PVP- 40000) and aqueous solutions of mixed blends of solubilizers. The Ofloxacin was found to be practically insoluble in water having solubility 0.11mg/ml. There was appreciable enhancement in solubility of Ofloxacin in aqueous solutions containing individual solubilizers and combination of solubilizers. The solubilites in aqueous solutions of individual solubilizers, PEG 4000, PEG 400, niacinamide, Propylene glycol, PVP- 40000, sodium benzoate, lignocaine hydrochloride, Ethanol were found to be 2.55 mg/ml, 1.97 mg/ml, 3.25 mg/ml, 2.28 mg/ml, 1.69 mg/ml, 4.75 mg/ml, 2.66 mg/ml and 1.55 mg/ml respectively. These results showed synergistic enhancement in solubility when the solubilizers were used in mixed blends. To check the influence of pH on the Solubility of Ofloxacin solubility's of Ofloxacin were determined in different pH solution. Ofloxacin solubility slightly of Ofloxacin decreases with increase in pH. From the results of solubility studies, mixed blends in which solubility of Ofloxacin was more than 4 mg/ml were selected to develop the injections, such mixed blends were B-19, B-21, B-22 and B-23. The injection formulations of various strengths were developed based on solubility of Ofloxacin in individual mixed blends. Prepared formulations were sterilized by membrane filtration method flushed with nitrogen and filled in glass vials. The formulations when subjected to accelerated stability studies showed rapid degradation of drug, the shelf lives of formulations were predicted as 14 days, 21 days, 28 days and 7 days for B-19, B-21, B-22 and B-23 respectively. From the results of stability studies and literatures it was concluded that Ofloxacin is sufficiently stable in aqueous media, so it was decided to formulate Ofloxacin as Aqueous injection by using solubilizers. The clarity and leakage test were passes found as per I.P. Standard. Viscosity was measure by Brookfield viscometer various blend B-19, B-21, B-22, B-23 were respectively 1.52, 1.72, 1.67 and 1.59 in centipoises. And Sterility was passes & No fungi or bacteria presence of due to unhygienic condition at small scale production of aqueous injection of Ofloxacin. Thus, above findings supports that by using novel technique of mixed solvency the required solubility can be achieved, solubilizers can be selected in safer range and dosage forms can be developed which are expected to show good stability also. The above research findings showed that, a stable aqueous injection formulation containing Ofloxacin were successfully developed. There is good scope for other poorly water-soluble drugs to develop their aqueous formulation by the use of combination of suitable solubilizers are known to safe hence, toxicities/safety related issues may not rise, suggesting the adoptability for large scale manufacturing . The proposed techniques would be economical convenient and safe. Thus, the study opens the chances of preparing such aqueous formulation of poorly-water soluble drugs.

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References

[1]. Sweetana S, Akers MJ. Solubility principle & practice for parenteral Drug Dosage form development. J. Pharm. Sci. Techno. 1996 ; 50 (5): 330-42

[2]. Liu, R., Water Insoluble Drug Formulation. 2nd ed.; Taylor Francis: London, 2008; p 69-71.

[3]. Jain, N. K., Jain S., Singhai A.k., Enhanced Solubilization and Formulation of an Aqueous Injection of Piroxicam. pharmazie 1997, 52, 942-51.

[4]. Saleh, A.; Khordagui, L., Hydrotropic Agent a New Definition. Int J Pharm 1985, 24, 231-38.

[5]. Maheshwari, R. K., "Mixed-solvency Approach"- Boon for Solubilization of Poorly Water Soluble Drugs. Asian Journal of Pharmaceutics 2010, DOI: 104103/0973-839863981.

[6]. Maheshwari, R. K., Mixed-solvency- A Novel Concept for Solubilization of Poorly Water Soluble Drugs. Journal of Technology & Engineering Sciences 2009, 1, (1), 39-44.

[7]. Maheshwari, R. K., Potentiating of Solvent Character by Mixed-Solvency Concept: A Novel Concept of Solubilization. Journal of Pharmacy Research 2010, 3, (2), 411-13.

[8]. Maheshwari, R. K., Bhawasr N., New Spectrophotometric Estimation of Tinidazole in Tablets using Mixed Solvency Concept. Bulletin of Pharmaceutical Research 2011, 1, (1), 22-25.

[9]. Nikunjn Kalariya, Asija R., Patel C., Formulation and evaluation of Sulphasalazine injection made by mixed solvency solubilization technique. IRJP 2012, 3, (5)

[10]. Laurent Y. G. Clarkes Analysis of Drug and Poisons 3rd ed., 2005.

[11]. Cherng-ju Kim, Advanced pharmaceutics; Physiological principle, CRC press, p 456.

[12]. Withnall, R.; Chowdhry, B., Infrared and Raman Spectra of 3, 5-diamino-6-(o-C_6H_4 X)-1, 2, 4- triazines. Spectrochimi Acta 2002, 58, 1721-29.

[13]. Subhashree S., Chandra K., et.al .FTIR & XRD investigation of some fluoroquinolone Int J Pharm Pharm Sci. Vol.3, 2011, 165-70.

[14]. Shu E. N, , Muka K.N., Ogbaodo S., Detection of Quinolone on TLC plate using Sigma in 1, 4-dioxane, Biomedical research 2007, 18(2): 135-38.

[15]. Indian Pharmacopoeia. Government of India, Ministry of Health and Welfare; The Controller of Publication: Delhi, 2007, Vol.2, Vol. 3, p 54, 681-82, 962-64.

[16]. Rasool AA. Hussain AA., & Dittert LW., Solubility enhancement of some water-insoluble Drug in the presence of nicotinamide & related compound J. Pharm. Sci. 1998; 80 (4): 387-93.

[17]. Subrahmanyam, C. V. S., Textbook of Physical Pharmaceutics. 2nd ed.; Vallabh Prakashan: Delhi, 2000; p 51-75.