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FORMULATION DEVELOPMENT AND EVALUATIONS OF AN AQUEOUS INJECTION OF GATIFLOXACIN BY NOVEL MIXED SOLVENCY TECHNIQUE

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ABSTRACT

In the present investigation, mixed-solvency approach has been applied for the enhancement of aqueous solubility of a poorly water soluble drug, gatifloxacin(selected as a model drug). Gatifloxacin is a synthetic fluroquinolone broad spectrum antimicrobial agent used in the treatment of bacterial infection. Aqueous injection of gatifloxacin was prepared by making various blends (keeping total concentrations 30% w/v, constant) of selected water-soluble solubilizer such as hydrotropes (nicotinamide, sodium benzoate, sodium citrate,) and co-solvents (propylene glycol, PEG-400, ethanol). Aqueous solubility of drug in case of selected blends (18 blends) ranged from 22.79 mg/ml–82.05mg/ml (as compared to the solubility in dist.water 1.19 mg/ml).The enhancement in the solubility of drug in a mixed solvent containing hydrtropes(8% nicotinamide, 8% sodium benzoate, 4% sodium citrate) and co-solvents (4% PEG 400, 3% propylene glycol, 3% ethanol) was more than 68.95 fold. This proved a synergistic enhancement in solubility of a poorly water-soluble drug due to mixed co-solvent effect. Each solubilized product was characterized by ultraviolet and infrared techniques. Various physical properties of solution such as pH, viscosity, specific gravity and surface tension were studied. The developed formulation was studied for physical and chemical stability. This mixed solvency shall prove definitely a boon for pharmaceutical industries for the development of dosage form of poorly water soluble drugs.

KEYWORDS: Fluroquinolones, hydrotropes, co-solvents, solubility and stability.

INTRODUCTION

Maheshwari[1-4]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 solutions containing various dissolved substances can also improve the solubility of poorly water soluble drugs. Melted (temperature about 60 ºC), PEG 400,PEG 6000 and PEG 8000 dissolves diclofenac sodium (melting point: 283 ºC). This also shows that melted PEGs act as solvent for diclofenac sodium. Melted urea (M.P.: 132-135ºC) dissolves diclofenac sodium (M.P.: 283ºC).This also shows that melted urea act as solvent for diclofenac sodium. Melted ibuprofen (M.P.:78ºC) dissolves diclofenac sodium (M.P.:283ºC), salicylic acid (M.P.:159ºC) and niacinamide (M.P.:132ºC), which again shows that melted ibuprofen act as solvent for diclofenac sodium, salicylic acid and niacinamide respectively. In supercritical fluid technology liquefied carbon dioxide acts as solvent for many insoluble substances. These indicate that all substances possess some solvent character. Mixed solvency is the phenomenon basically to increase the solubility of poorly soluble drugs, using blends of solubilizers. This technique can provide additive or synergistic enhancement effect on solubility of poorly soluble drugs. Utilization of this method in the formulation of dosage forms made of insoluble drugs can also reduce the concentration of individual solubilizing agents, in order to minimize the side effects (in place of using a large concentration of one solubilizer, a blend of several solubilizers can be employed in much smaller acceptable concentrations, reducing their individual toxicities). All weaker solvents can be made strong solvent by proper choice of solubilizer.

Hydrotropy is another type of cosolvency[5]. Hydrotropic agents are also a type of solubilizers which increase the solubility of poorly water soluble drugs. Mixed hydrotropy is also a type of mixed solvency. Hydrotropy[6-10] and mixed hydrotropy[11-14] have also been used to enhance the aqueous solubility of a large number of poorly soluble drugs.

The objective of present research is to explore the application of mixed solvency technique in the injection formulation of poorly water soluble drug and to reduce concentration of individual solubilizer (used for solubility enhancement) to minimize the toxic effects of solubilizers. In the present work, gatifloxacin, a poorly water soluble drug was selected as a model drug and attempts were made to formulate an aqueous injection of this drug using various model solubilizing agents. The formulation was also studies for physical and chemical stability.

MATERIALS AND METHODS

Materials

Gatifloxacin was obtained as gift sample from Wockhardt Ltd, Aurangabad. Nicotinamide, sodium benzoate and sodium citrate were purchased from Rankem Pvt. Ltd. Mumbai and ethanol was obtained from Merck Chemical Ltd. Mumbai.

METHODOLOGY

Estimation of gatifloxacin

In the present investigation, UV spectrophotometric method was used for the estimation of gatifloxacin. The calibration curve of gatifloxacin was prepared in distilled water and various concentrations of water soluble solubilizers (hydrotropic agents and co-solvents) at 332.5 nm using double-beam UV spectrophotometer (Shimadzu-1800)[7,8,11]

Solubility determination

Solubility of gatifloxacin in various solubilizers solutions was determined by equilibrium solubility method. Sufficient excess amount of gatifloxacin was added to 10mL screw-capped glass vials containing 5 ml of aqueous solution of individual solubilizer and different mixed blend of solubilizers (30% w/v) (Tables 2 & 3). The vials were shaken mechanically for 12 h on mechanical shaker (Lab Hosp, Mumbai, India) at 37 ± 2 ∘C. The solutions were allowed to equilibrate for the next 24 hr. The supernatants of each vial were filtered through 0.45 μ membrane filter and analyzed for drug content by UV visible spectrophotometer Shimadzu-1800) at 332.5 nm after appropriate dilutions.[3,12-14] Solubility enhancement ratio was determined by using following formula: Enhancement ratio= solubility in mixed blend/solubility in water (Table 2 & 3)

Method of determination for additive/synergistic effect on solubility in blends

An equilibrium solubility method was used to determine the additive or synergistic effect on solubility. The total strength of all solubilizers was 30% w/v(constant) in all aqueous mixed solvent systems (Table 3).The solubility of gatifloxacin was determined in these systems.[3]

Properties of mixed solvent solutions

Various properties of the solution such as pH, viscosity, specific gravity, and surface tension were studied using digital pH meter, Ostwald viscometer, Pychnometer and Stalagmometer (Table 4)[15,16,17]

Chromatographic study of solubilized drug product

In order to predict the possible interaction and/or complexation between drug and solubilizers the TLC studies were performed. A plate of silica gel GF 254 (Merck) was activated at 110^{o}C for 1 hour and used. The methanolic solution of gatifloxacin alone and the solution of solubilizers along with gatifloxacin was prepared (amount of drug and solubilizers as in blend B-14 and B-18). Methanolic solution drug and solution of solubilizer along with drug were spotted on the base line with the aid of capilary. Then, the plate was left in air for 10 min to dry and transferred to a solvent jar saturated with solvent system composed of mixture of n-butanol, methanol and liquid ammonia (6M) solution (5: 1: 2 v/v/v).

The solvent system was allowed to run for about 7.4 cm. Finally, the plate was transferred to an oven maintained at temperature 80^{o}C for 2 min and observed in UV chamber at short wavelength for visualization of spots. The respective R_F values were determined and recorded (Table 5)

Drug excipient compatibility studies

UV Spectral studies

To interpret the probable mechanism of solubilization, UV spectral studies of gatifloxacin was performed in different mixed solvent solutions to study the possible spectroscopic changes in the structure of gatifloxacin in the presence of different hydrotropes and cosolvents (Figures 3 to 13)[15,16,17]

Fourier Transform Infrared (FTIR) spectral studies

FTIR spectra were obtained by means of an FTIR spectrophotometer (IR Affinity). The samples were prepared by mixing of drug and potassium bromide in 1:1 ratio and measurements were attempted over the range of 400 –4000 cm-1 (Figures 14 and 15 and Table 6)[15]

Formulation of aqueous injection[15,16,18]

Various steps involved in formulation of aqueous injection of gatifloxacin are as follows:

Treatment of packaging material

Amber color glass vials were first washed three times with water.Then finally rinsed with distilled water. All these vials were sterilized by dry heat in an oven at 180°C for 2 hours in inverted position.Rubber closures and aluminium seals used for plugging the vials were first

washed in an 0.2% liquid detergent solution for 2 hr. then washed several times with distilled water to remove detergent residue and finally sterilised by autoclaving (Labline Pvt. Ltd., Delhi, India) at 15 lbs pressure (121°C) for 15 minutes and finally, the stoppers were rinsed with freshly prepared distilled water and dried in oven under aseptic condition.

Preparation of aseptic area

The walls and floor of asceptic room were thoroughly washed with water then disinfected by mopping with 5% phenol solution. The bench was cleaned with 70% v/v isopropyl alcohol as well as sprayed in the atmosphere. The UV lights were switched on for 30 min prior to formulation of injections and the filling of injections into vial.

Preparation of aqueous injection of gatifloxacin

Initially, the appropriate weighed amounts (required for 50 ml) of solubilizer were transferred to volumetric flask of 50 ml capacity containing 35 ml sterile water for injection. The flask was shaken to dissolve the solubilizers. The volume was made upto the mark with same sterile water for injection. To prepare aqueous injection of drug, the calculated quantity of gatifloxacin was transferred to another flask and prepared blend solution was added to dissolve the drug and sonicated for 30 min. to assure complete dissolution of drug. After complete dissolution of drug, volume was made up to the mark with same prepared blend and shaken to get homogenous solution. Other excipients like chelating agent, buffering agent, antioxidants were not added as they may upset the basic solubility enhancement ratio.

Aseptic filtration

The aqueous injection of gatifloxacin was sterilised by filtration through 0.22 µm disposable membrane filter. The membrane filtration assembly fitted with the membrane filter was sterilized previously in the autoclave (Labline Pvt. Ltd., Delhi, India) at 121°C and 15 lbs pressure for 15 minutes.

Aseptic filling and packing

After filtration the preparations were filled with 4ml volume in vials and packed by sterilised air tight rubber closure and sealed with sterile aluminium caps. The final packed vials were terminally sterilised by autoclaving at 121°C and 15 lbs pressure for 15 minutes.

Table 1:Compositions of prepared formulations

Ingredients	Composition of formulations					
	F1(B7)	F2(B8)	F3(B9)	F4(B14)	F5(B16)	F6(18)
Gatifloxacin	0.20gm	0.20gm	0.20gm	0.20gm	0.20gm	0.20gm
Nicotinamide	0.20gm	0.20gm	0.20gm	0.20gm	0.24gm	0.32gm
Sodium benzoate	0.20gm	0.20gm	0.20gm	0.12gm	0.24gm	0.32gm
Urea	0.20gm	0.20gm	0.20gm	0.12gm	-	-
Sodium citrate	0.20gm	0.20gm	0.20gm	-	0.12gm	0.16gm
HP-β-cyclodextrin	0.20gm	-	0.20gm	0.16gm	0.16gm	-
Ethanol	0.20ml	0.20ml	-	-	0.12gml	0.12gm
Propylene glycol	-	0.20ml	0.20ml	0.12ml	-	0.12gm
PEG400	-	-		0.12ml	-	0.16ml
Glycerine	-	-		0.12ml	0.12ml	-
PEG 4000	-	-		-	0.12ml	-
Tween 80	-	-		-	0.08ml	-
SWFI	q.s to 4 ml	q.s to 4 ml	q.s to 4 ml	q.s to 4 ml	q.s to 4 ml	q.s to 4 ml

Characterisation of aqueous injection

Physical stability studies

The sealed or packed vials of the aqueous injections were visually inspected every day for 4 weeks against black and white backgrounds to see the changes occurring, if any, in physical appearance of aqueous injection like color, crystal growth, turbidity, particulate matter and pH, and so forth (Table 6), on storage at 2–8∘C in a refrigerator, room temperature and 40∘C/75%RH. And results were recorded (Table 7 to Table 14)[19-23]

On the basis of the results simultaneous physical stability testing the promising formulation were further subjected to chemical stability testing for period of 4 week.

Chemical stability studies

As soon as the product is developed, it is subjected to ageing, as a result its chemical composition and even its biological availability may be changed. The prepared formulations were subjected to 2-8^{o}C, 25^{o}C and 40^{o}C to observe the stability of medicament in developed formulations. Samples were withdrawn at interval of 7 days, suitably diluted with demineralised water and analysed using UV/Visible spectrophotometer (Shimadzu 1800) against respective reagent blanks at 332.5 nm to determine the amount of drug remaining in formulation. Percent drug remained at definite time intervals were recorded.From the

chemical stability data, the K values at at 25±1°C were determined.Shelf life of formulations were calculated (Table 15-18).[15-17,24]

Sterility testing

Direct inoculation method

Aliquots of the sample were transferred aseptically into fluid thioglycolate medium (FTM) and soyabbean casein digest medium (SCDM). The inoculated thioglycolate medium was incubated at 32°C and soyabean casein digest medium at 22°C for 14 days.Likewise negative and positive controls are prepared. Results are shown in table (Table 19) [25]

Dilution study

Series of dilutions were done by diluting aqueous injection of gatifloxacin (Formulation F4 and F6) with different diluents, normal saline (0.9% NaCl) and 5% dextrose solution. The diluted products were observed for any precipitation up to 24 hours. The observations were recorded (Table 20 and 21) [24]

RESULTS AND DISCUSSION

Solubility determination

Table 2: Equilibrium solubility data of gatifloxacin in individual solubilizer

Aqueous solution of solubilizers (30% w/v)	Equilibrium solubility of gatifloxacin (mg/ml)	Solubility enhancement ratio
Demineralized water	1.19	0
Sodium benzoate	135.282	113.68
Nicotinamide	49.461	41.56
Urea	27.48	23.09
Propylene glycol	4.492	3.77
HP-β-cyclodextrin	4.941	4.15
Ethanol	5.128	4.3
Sodium citrate	3.856	3.24
PEG 400	5.356	4.5
PEG 4000	5.264	4.42
Tween 80	4.546	3.82
Glycerine	4.933	4.14

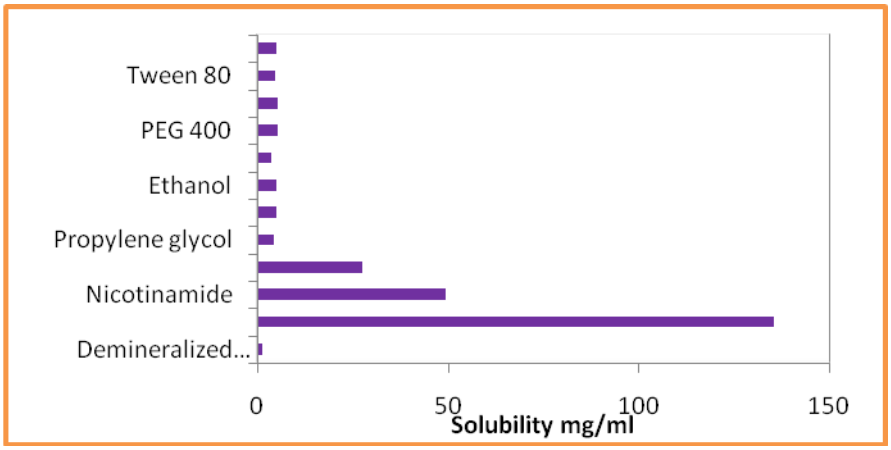

Fig.1: Solubility profile of gatifloxacin in aqueous solution of individual solubilizers (30% w/v)

It is evident from the results that the solubility of gatifloxacin was increased by use of various solubilizers. The solubilizing power of different solubilizers could be ranked as Sodium benzoate >nicotinamide> urea > PEG 400 > PEG 4000 > ethanol > HP-β-cyclodextrin>glycerine> tween 80 > propylene glycol > sodium citrate. So it was further explored for synergistic solubilization effect of two or more solubilizers in combination to reduce individual concentration.

Method of determination for additive/synergistic effect on solubility in blends

Table 3: Equilibrium solubility data of gatifloxacin in various mixed blends containing solubilizers

Blend codes	Solubility (mg/ml)	Solubility enhancement ratio
B-1	35.38	29.73
B-2	33.07	27.78
B-3	22.79	19.15
B-4	36.41	30.59
B-5	35.01	29.42
B-6	49.58	46.66
B-7	51.43	43.21
B-8	63.94	53.73
B-9	58.35	49.03
B-10	49.46	41.56
B-11	27.23	22.88
B-12	47.66	40.05
B-13	49.33	41.45
B-14	78.4	65.88
B-15	49.69	41.75
B-16	53.38	44.85
B-17	31.53	26.49
B-18	82.05	68.95

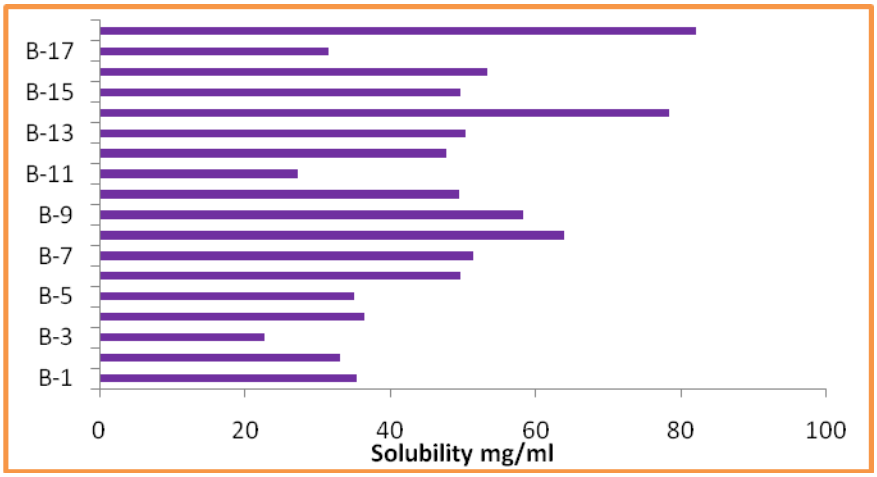

Fig.2: Equilibrium solubilities of gatifloxacin in various mixed blend containing solubilizers

The results showed that solubility of gatifloxacin in different mixed blends was increased significantly. The maximum solubility was observed in B-18 which showed 68.95 folds enhancement. From the Table 2 & 3, it is evident that many blends showed addative/synergistic enhancement in solubilities of gatifloxacin. The total strength of all solubilizers was 30% w/v (constant) in all aqueous systems containing single solubilizers or combinations of solubilizers.

Blends in which solubility was more than 50 mg/ml were further selected for formulation of injections.

Properties of mixed solvent solutions

Table 4: Properties of optimised blends

Experimental blends	pH	Viscosity (cps)	Surface tension (dynes/cm)	Specific gravity
B7	7.41	3.186	57.32	1.046
B8	7.68	3.385	60.14	1.081
B9	7.52	3.488	60.30	1.085
B14	7.77	3.130	59.64	1.073
B16	7.46	3.553	58.74	1.087
B18	7.93	2.959	55.37	1.036

From the Table 4, it is evident that slight changes in physical properties of optimised blends as small deviation in their concentrations

Chromatographic study of solubilized drug product

Table 5: R_F values of gatifloxacin

Solvent system	Adsorbent	R_F value		
n-butanol: methanol: ammonia(6M) 5: 1: 2 (v/v/v)	Silica gel GF 254	Gatifloxacin	B-14	B-18
		0.24	0.24	0.24

From the Table 5, it is evident that all the spots in two blends correspond with the R_F value of standard gatifloxacin drug. From the result of chromatography, it was concluded that there was no complexation and/or interaction between drug and solubilizer.

Drug excipient compatibility studies

UV spectral studies

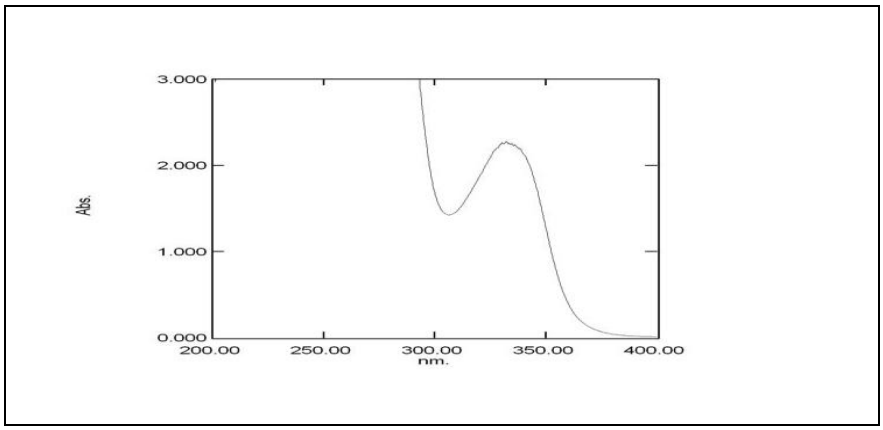

Fig. 3: UV spectra of gatifloxacin in nicotinmide

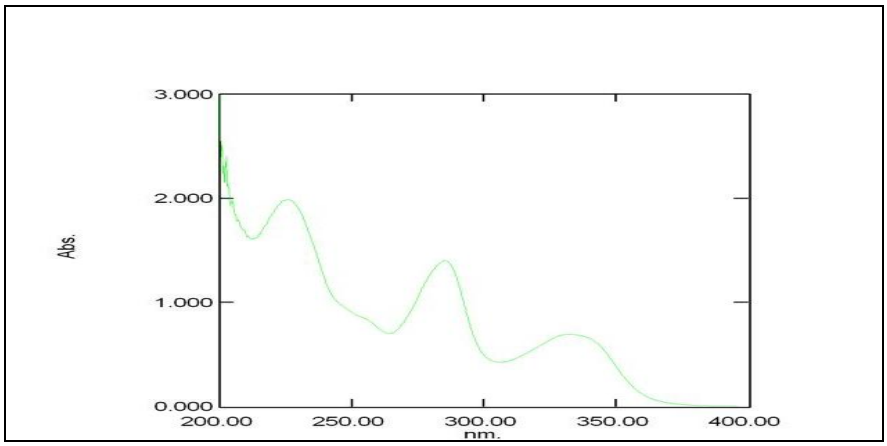

Fig. 4: UV spectra of gatifloxacin in sodium benzoate

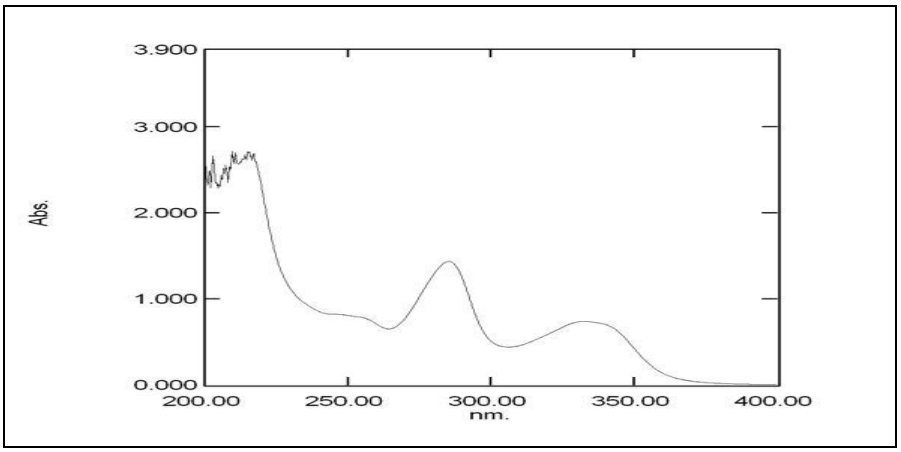

Fig. 5: UV spectra of gatifloxacin in sodium citrate

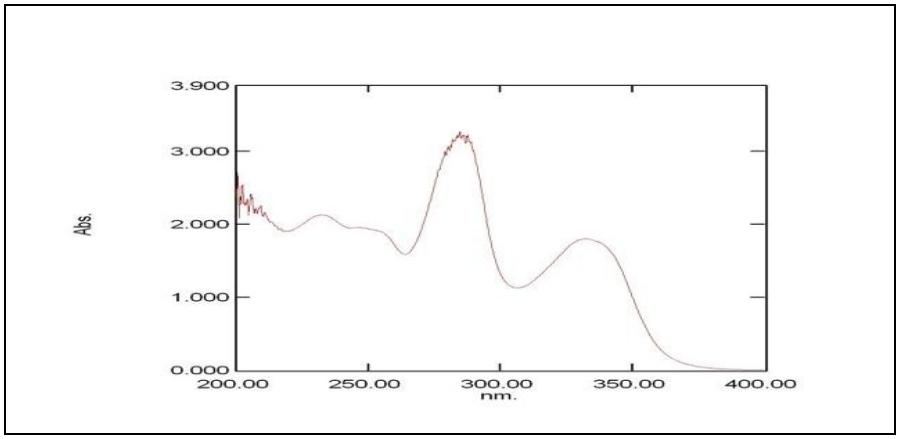

Fig. 6: UV spectra of gatifloxacin in propylene glycol

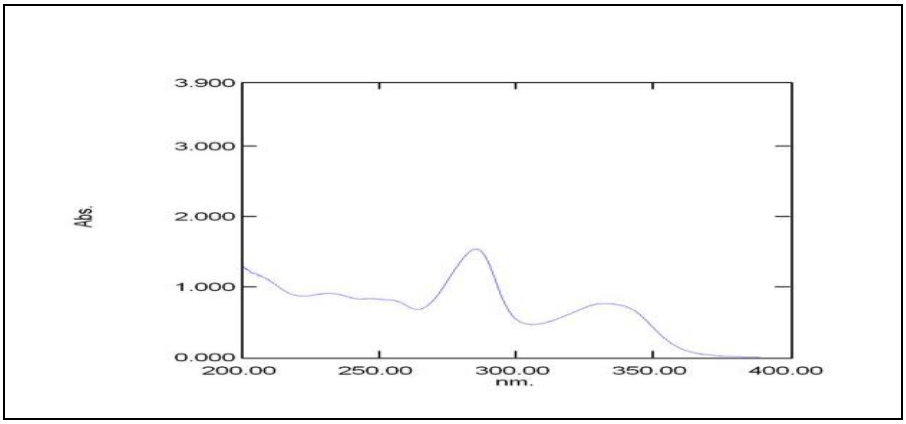

Fig. 7: UV spectra of gatifloxacin in PEG 400

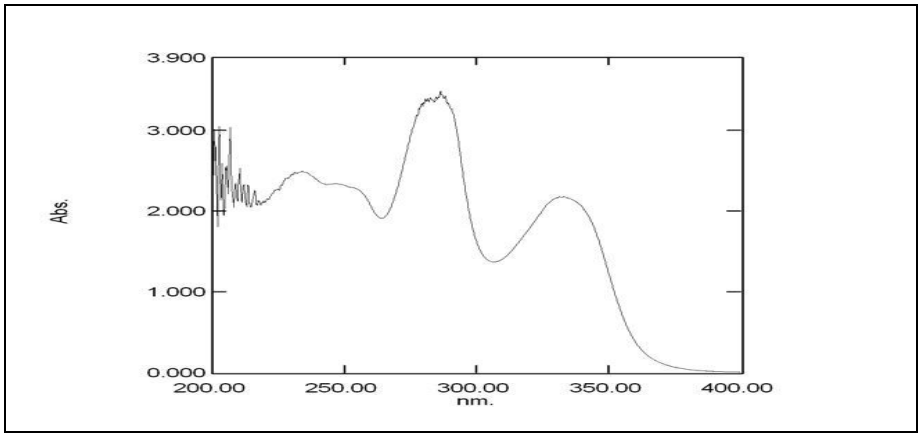

Fig. 8: UV spectra of gatifloxacin in ethanol

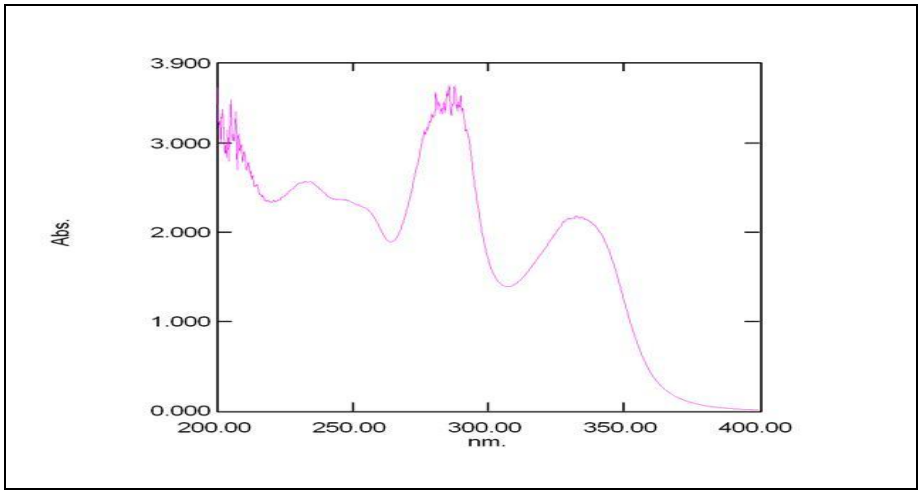

Fig. 9: UV spectra of gatifloxacin in urea

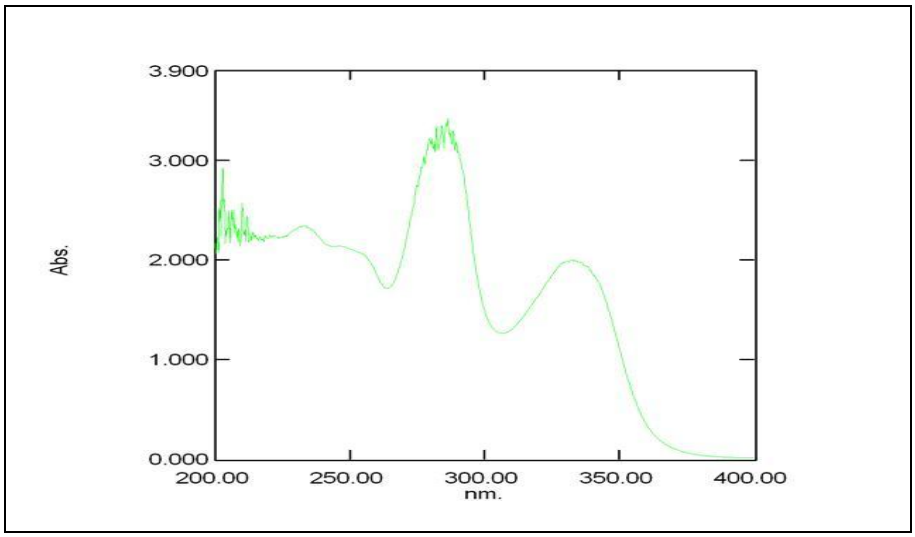

Fig. 10: UV spectra of gatifloxacin in HP-β-cyclodextrin

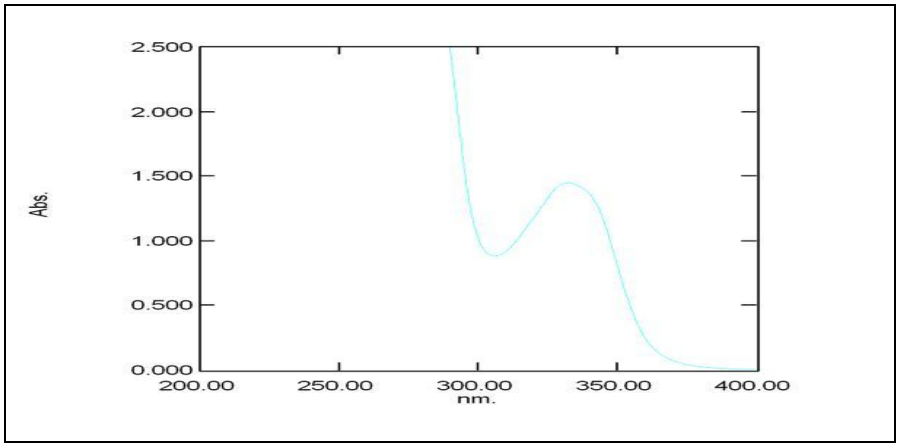

Fig. 11: UV spectra of gatifloxacin in glycerine

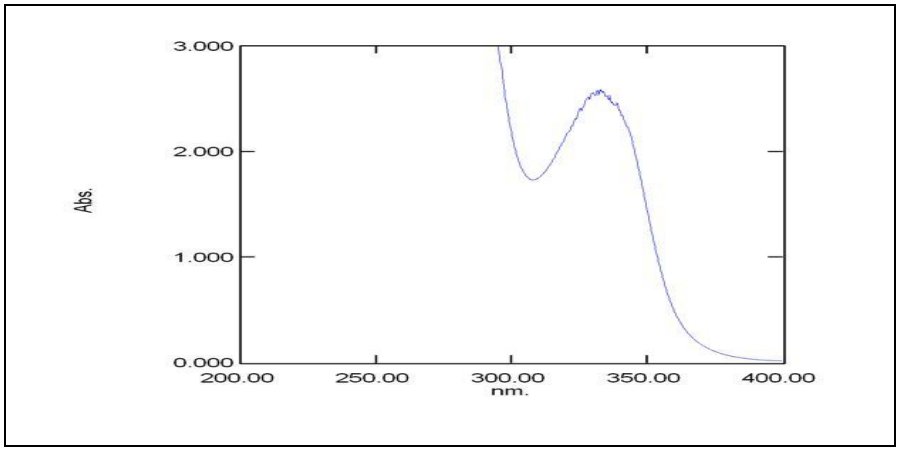

Fig. 12: UV spectra of gatifloxacin in B14

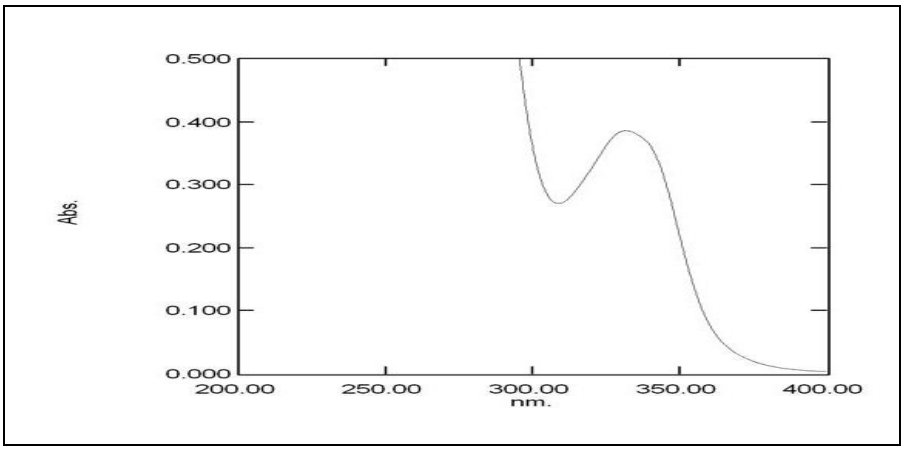

Fig. 13: UV spectra of gatifloxacin in B18

UV absorption spectra gatifloxacin in various individual solubilizers and mixed solubilizers solutions showed slight shift in λ_{max}(333±1 nm), which can be due to minor electronic changes in structure of drug molecule.Small additional peaks of solubilizer and cosolvent

were also observed in fig of UV spectra which indicates that there is no interferance of λ_{max} of the drug.

Fourier Transform Infrared (FTIR) Spectral studies

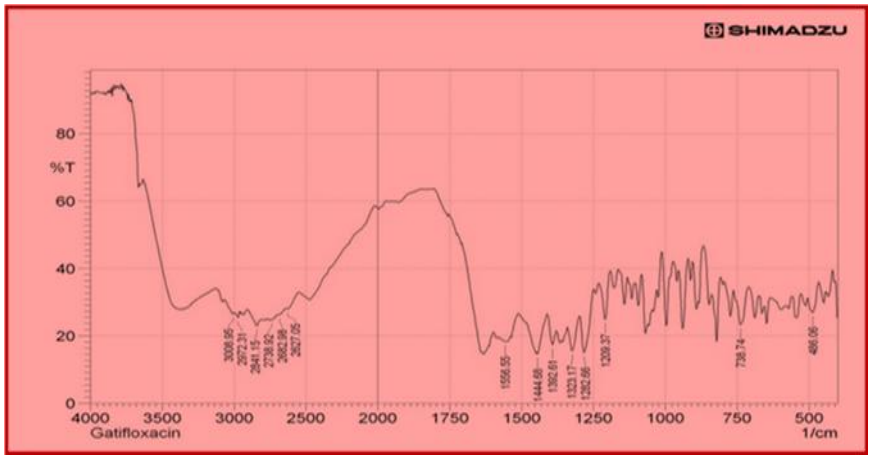

Fig. 14: FT-IR spectrum of gatifloxacin

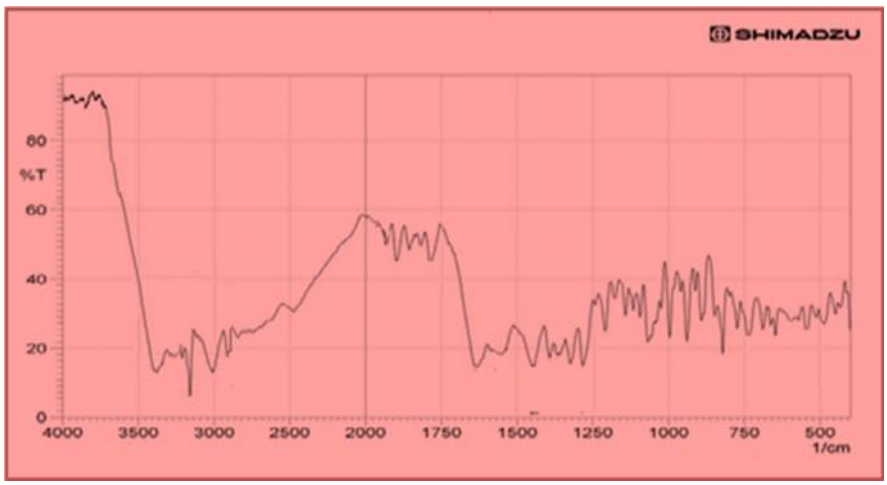

Fig. 15: FTIR spectra ofgatifloxacin with all solubilizer in blend 18.

Table 6: Charecteristic IR absorption of functional groups

Drug Wave no.(cm^{-1})	Drug with solubilisers Wave no. (cm^{-1})	Functional group
1209.37	1209.37	-C-O (Stretch)
1444.68	1444.68	$-CH_3$ (Strech)
1628	1628	-C=O
3008.95	3008.95	-C-H (Aromatic)
1556.55	1556.55	-C=C (Aromatic)
1282.66	1282.66	-C-F
738.74	738.74	-N-H (Out of plane)

The spectra obtained from FTIR studies at wavelength 4000 cm^{-1} to 400 cm^{-1}showed that there are neither major shifts nor any loss of functional peak between the spectra of drug and solubilizer. IR of the drug with all solubilizers was compared with that of pure drug to see whether there was any change in structure or interaction with solubilizers, and results of FTIR spectral analysis showed no evidenced of strong complex formation and intigrity of the drug is not affected.

Characterisation of aqueous injections

Physical stability studies

Determination of colour

Table 7: Effect of different temperature on colour change

Formulation	2-8°C	25°C	40°C
F1	-	+	+
F2	-	-	+
F3	-	-	+
F4	-	-	-
F5	-	+	+
F6	-	-	-

(+) Colour change, (-) No colour change

In the formulation F4 and F6 no colour change were developed after two weeks, so F4 and F6 are stable at temperature studied.

Determination of crystal growth

Table 8: Effect of different temperature on crystal growth

Formulation	2-8°C	25°C	40°C
F1	+++	++	+
F2	+	-	-
F3	+	-	-
F4	-	-	-
F5	++	+	-
F6	-	-	-

(+) Crystal growth, (-) No crystal growth

In the formulation F4 and F6 no colour change were developed after two weeks, so F4 and F6 are stable at temperature studied.

Determination of turbidity

Table 9: Effect of different temperature on turbidity

Formulation	2-8°C	25°C	40°C
F1	-	-	+
F2	-	-	+
F3	+	-	-
F4	-	-	-
F5	-	-	+
F6	-	-	-

(+) Turbidity, (-) No turbidity

In the formulation F4 and F6 no turbidity were developed after two weeks,so F4 and F6 are stable at temperature studied.

On the basis of the result data obtained from physical stability study it is evident that formulation F4 and F6 shows no colour change,no crystal growth and any sign of turbidity,so the formulation F4 and F6 are selected for scale up and further subjected to physical stability and exhaustive chemical stability test and sterility testing for the period of 4 week.

Evaluation of scale up batches

Physical stability

Determination of colour

Table 10: Effect of different temperature on colour change

Formulation	Withdrawal Week	2-8°C	25^0C	40°C
F4	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
F6	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-

(+) Colour change, (-) No colour change

Determination of crystal growth

Table 11: Effect of different temperature on crystal growth

Formulation	Withdrawal Week	2-8°C	25^{0}C	40°C
F4	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
F6	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-

(+) Crystal growth, (-) No crystal growth

Determination of turbidity

Table 12: Effect of different temperature on turbidity

Formulation	Withdrawal Week	2-8°C	25^{0}C	40°C
F4	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
F6	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-

(+) Turbidity, (-) No turbidity

Determination of pH

Table 13: Effect of different temperature on pH

Formulation	Withdrawal Week	2-8°C	25^{0}C	40°C
F4	0	7.82	7.82	7.82
	1	7.84	7.85	7.86
	2	7.85	7.87	7.88
	3	7.87	7.91	7.90
	4	7.91	7.94	7.96

F6	0	7.97	7.97	7.97
	1	8.10	8.13	8.19
	2	8.12	8.14	8.20
	3	8.15	8.19	8.23
	4	8.18	8.22	8.28

Determination of clarity (particulate matter)

Table 14: Effect of different temperature on particulate matter

Formulation	Withdrawal Week	2-8°C	25^{0}C	40°C
F4	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
F6	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-

Chemical stability studies

Table 15: Chemical stability data of gatifloxacin in formulation F4

Time (days)	% Drug remaining		
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C
0	101.13	101.13	101.13
7	98.46	97.54	96.67
14	96.31	95.18	93.79
21	94.41	92.87	91.64
28	92.56	90.82	89.38

Table 16: Chemical stability data of gatifloxacin in formulation F6

Time (days)	% Drug remaining		
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C
0	101.38	101.38	101.38
7	98.92	98.51	97.59
14	96.97	96.26	94.72
21	95.23	93.85	92.61
28	93.74	92.05	90.67

Table 17: Kinetic data of gatifloxacin aqueous injection formulations

Temperature	k (day^{-1})	
	F-4	F-6
2-8 ^{0}C	0.002575	0.002107
25 ^{0}C	0.003522	0.002721
40 ^{0}C	0.004408	0.003633

Table 18. Shelf lives of gatifloxacin aquesous injection formulations

Formulation	Shelf life (days)
F4	30
F6	38

From the results shown in Tables 15 to 16, it is evident that the developed formulations of aqueous injection of gatifloxacin were not sufficiently stable at room temperature and refrigerated condition. From Table 17 & 18 the shelf lives of formulations F4 and F6 were found as 30 days and 38 days respectively. To overcome the problem of instability of formulation, it may opens the challenges for formulation as dry powder for injection by using the solid solubilizing agents.

Sterility testing

Direct inoculation method

Table 19: Sterility study in different medium

Formulation	Fluid Thioglycolate Medium(FTM)	Soyabean-Casein Digest Medium (SCDM)
F4	–	–
F6	–	–

From above result it was found that none of the formulation showed turbidity or signs of microbial growth (except the positive control) at the end of incubation period, indicating all the formulations were sterile

Dilution study

Table 20: Dilution profile of formulation (F4)

Dilution	Time (hrs.)											
	Normal saline solution						5% dextrose solution					
	1	2	4	6	8	24	1	2	4	6	8	24
1:1	-	-	-	-	-	-	-	-	-	-	-	-
1:5	-	-	-	-	-	-	-	-	-	-	-	-
1:10	-	-	-	-	-	-	-	-	-	-	-	-
1:20	-	-	-	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-	-	-	-	-
1:100	-	-	-	-	-	-	-	-	-	-	-	-
1:500	-	-	-	-	-	-	-	-	-	-	-	-

(-) No precipitation, (+) Precipitation

Table 21: Dilution profile of formulation (F6)

Dilution	Time (hrs.)											
	Normal saline solution						5% dextrose solution					
	1	2	4	6	8	24	1	2	4	6	8	24
1:1	-	-	-	-	-	-	-	-	-	-	-	-
1:5	-	-	-	-	-	-	-	-	-	-	-	-
1:10	-	-	-	-	-	-	-	-	-	-	-	-
1:20	-	-	-	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-	-	-	-	-
1:100	-	-	-	-	-	-	-	-	-	-	-	-
1:500	-	-	-	-	-	-	-	-	-	-	-	-

(-) No precipitation, (+) Precipitation

The above results indicate that the formulations (F4 and F6) were observed to have stability (up to 24 hours) towards precipitate formation in normal saline solution and 5% dextrose solution.

4. CONCLUSIONS

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 solubilizer (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/cyclodextrins etc.) is required to produce an appreciable increase in solubility of a poorly soluble drug.The results of the present

investigation showed the possibility of aqueous injection of poorly water-soluble drugs using combination of various solubilizers and hydrotropic agents which act synergistically at very low individual concentrations.Hence, toxicity and safety related issues may not raise concern and would suggest their adoptability for large-scale manufacturing. The proposed techniques would be economical, convenient, and safe. Thus, this study opens the chance of preparing aqueous formulations of poorly water-soluble drugs, if chemical stability of the drug remains unaffected. Mixed solvency approach produces a physical stable formulation which results in the administration of low level of co-solvents to the patient, thus reducing or eliminating the effect of co-solvent toxicity and erythrocyte damage.Thus it can be concluded that, with the carefully designed experimental technique, solubility of poorly water-soluble drug can be improved by using the "mixed solvency"approach. The application of the mixed solvency approach in the development of formulations shall prove to be a boon for pharmaceutical industries because the quantities of water soluble solubilizers present in the blends can be selected at safe level (well below their toxic levels) for a modest increase in solubility of a water-insoluble drug.

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