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Crystal investigation and characterization of the pseudopolymorphic forms of gemifloxacin mesylate

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ABSTRACT
Main objective of this present study was to prepare and investigate the different pseudo polymorphic forms of gemifloxacin using Powder X-Ray Diffraction analysis (PXRD), Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectrometry (FTIR) and dissolution studies. For this purpose, crystals of gemifloxacin (G-1 to G-4) were obtained by solvent evaporation method using various solvents like isopropanol, chloroform, dichloromethane and benzene and characterized. Pseudo polymorph obtained from isopropanol (G-I) showed highest solubility and percentage drug release than others. The existence of different polymorphic forms of gemifloxacin has been proved by appearance and disappearance of peaks on the PXRD graph and melting points of each at different temperature. It was concluded that the gemifloxacin existed four pseudo polymorphic forms and showed highest percentage drug release than the amorphous form.
KEYWORDS: Gemifloxacin meyslate, Pseudo polymorph, FTIR, PXRD, SEM.

INTRODUCTION
Gemifloxacin (Fig. 1) chemically known as 7-[(42)-3-(amino methyl)-4-methoxyimino-Pyrrolidin-1-yl]-1-cyclo propyl-6-fluoro-4-oxo-1, 8-napthyridine-3-Carboxylic acid, has its molecular formula and weight as $C_{18}H_{20}FN_5O_4$, 389.38. The U.S. Food and Drug Administration approved for the treatment of the upper respiratory and urinary tract infection [1]. Polymorphism is defined as the ability of a substance to exist as two or more crystalline phases or forms that have different arrangements and/or conformations of the molecule in crystal lattice. Crystalline polymorphs have same chemical composition but different crystal structures. Solvates also known as pseudo polymorphs are crystalline solid adducts containing solvent molecules in the crystal structure, stoichiometric or non-stoichiometric proportions, giving rise to unique difference in the physical and chemical properties of drug. About 70% barbiturates, 60% sulfonamides and 23% of steroids existed different polymorphic forms and pseudo polymorphic forms. Indapamide, a non- thiazide sulphonamide, a diuretic drug is primarily used in the treatment of hypertension as well as edema caused by congestive heart failure. The existance of polymorphic and psudopolymorphic forms were confirmed by x–ray powder diffraction (XRPD), diffuse reflectance infrared fourier transform (DRIFT) spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), nuclear magnetic resonace (NMR) and

head space gas chromotograpic (HS-GC) analysis [2]. Polymorphs and hydrates differ in internal solid-state structure and therefore, possess different physical properties, including melting point, crystal habit, packing, colour, density, compressibility, thermodynamic, spectroscopic, kinetic, interfacial, and flow properties [3]. Linezolid is a synthetic anti-bacterial agent of the oxazolidinone class used for the treatment of severe infections. Two crystal modifications of linezolid have been obtained through recrystallization process using various organic solvents. The two polymorphic forms were fully characterized by XRPD, DSC, FTIR, Hot stage polarizing optical microscopy methods [4]. Nimesulide crystals were recrystallized from various solvents like methanol, ethanol and DMSO solvent under different conditions (cooling rate, crystallization temperature, ultrasonication techniques). It was confirmed by various analytical techniques like SEM, XRPD, FT-IR, and DSC [5]. There are three polymorphic modifications of fexofenadine hydrochloride, which are deignated as Form A1, Form B1 and Form C1 have been obtained by recrystallization with organic solvents under variable conditions. It was confirmed by analytical methods like DSC, PXRD, and TGA [6]. The present work describes the preparation of various polymorphic forms of gemifloxacin and the investigation and characterization of these obtained pseudo polymorphic forms (solvate), using various analytical techniques.

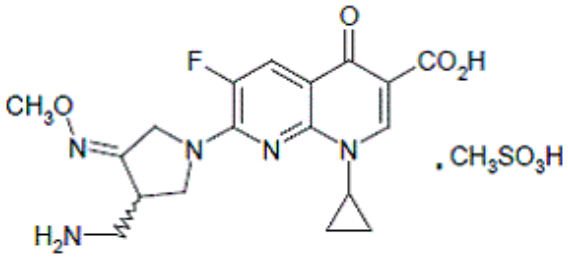

Fig. 1: Chemical structure for gemifloxacin mesylate

MATERIAL AND METHODS

MATERIALS

Gemifloxacin mesylate was obtained from Yarrow Chem. Products, Mumbai. The solvent used for crystallization such as isopropanol, chloroform, dichloromethane, benzene were purchased from S.D.Fine Chemicals Ltd, Mumbai, India

PREPARATION OF SOLVATES

The small quantity of gemifloxacin meyslate was placed in 250ml flask. The hot solvent of isopropanol was added, under constant stirring, the solution was saturated and poured into the petri dish. Then the solution was kept at room temperature for about one hour to collect the crystal (G-1). The other crystalline solids of gemifloxacin meyslate were collected by using various solvents like chloroform, dichloromethane, benzene and labeled them as G-2, G-3, G-4, respectively. The crystals were transferred immediately to vaccum desiccators and dried using silica gel under vaccum for at least 24 hours.

CHARACTERIZATION OF CRYSTAL FORMS

SOLUBILITY STUDIES

Each prepared crystal was weighed individually about 100 mg and placed in a 100 ml Erlenmeyer flask with a stopper. Phosphate buffer pH 7.0 was added in each flask and mechanically shaken at a rate of 80 strokes min^{-1} for 20 h. An aliquot (1 ml) of each solution was withdrawn and filtered through a 0.45 m Millipore filter. The solubility of each pseudo polymorphic form was determined by measurement of the absorbance at 271 nm using UV spectrophotometer.

FOURIER TRANSFORM INFRARED SPECTROMETRY (FTIR)

The FT-IR spectrum of each solvate form was obtained on FT-IR spectrometer, mode spectrum RX1, (Shimadzu, England) over the range 400-4000cm^{-1}. Dry KBr (50 mg) was finely ground in an agate mortar and sample of the drug or the solvate (1-2 mg) was subsequently added and mixed gently. A manual press was used to form the pellet. The resultant pellets were mounted in a solid sample older and full range IR spectrum of all polymorphic forms were recorded from 4000 cm^{-1} to 400 cm^{-1}.

SCANNING ELECTRON MICROSCOPY (SEM)

A Jeol JSM-6100 Scanning electron microscope was used to obtain photomicrographs of crystals of gemifloxacin meyslate. Samples were mounted on a metal stub with an adhesive and coated under vaccum with gold.

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC thermo gram was obtained using a NETZCH STA 449F3 and the temperature range of scan was set from room temperature to 400°C at a rate of 10°C/min. The sample (50-100 mg) was purged under a flow of nitrogen at a flow rate of 50 mL/min. The exact peak temperature, melting point and heat of fusion were determined.

POWDER X-RAY DIFFRACTION (PXRD)

The powder x-ray diffraction patterns of the samples were recorded using a Ricnaku Miniflex 2C. The operating condition were as follows: target,cu, voltage 40kV, current 30mA, receiving slit,0.3mm, preset time, 0.60 sec, scan speed 10 (deg/min), sampling pitch 0.1° . The divergent slit and scatter slit 1° and auto slit was not used.

DISSOLUTION STUDY

The *in-vitro* dissolution studies were carried out using eight stations of LAB INDIA DISSO 2000 dissolution test apparatus. The samples of crystals (100mg) were prepared by direct compression and placed in dissolution medium of phosphate buffer (pH 7.0) at 37±2°C with a constant speed of agitation at 50 rpm using USP type II (paddle). The fixed volume (1 ml) of sample was withdrawn (with replacement) at 15, 30, 45, 60, 90, 120 and 240 min time intervals and diluted appropriately. The percentage drug release was determined by VU spectroscopy method using 271 nm as the λ max of gemifloxacin.

RESULT & DISCUSSION

SOLUBILITY STUDIES

The solubility of crystals of gemifloxacin meyslate such as G-1, G-2, G-4 G-3, were 0.87, 0.75, 0.69, 0.65 mg/ml respectively. The four crystals have different solubility, and this study revealed that G-1 form has more solubility.

FOURIER TRANSFORM INFRARED SPECTROMETRY (FTIR)

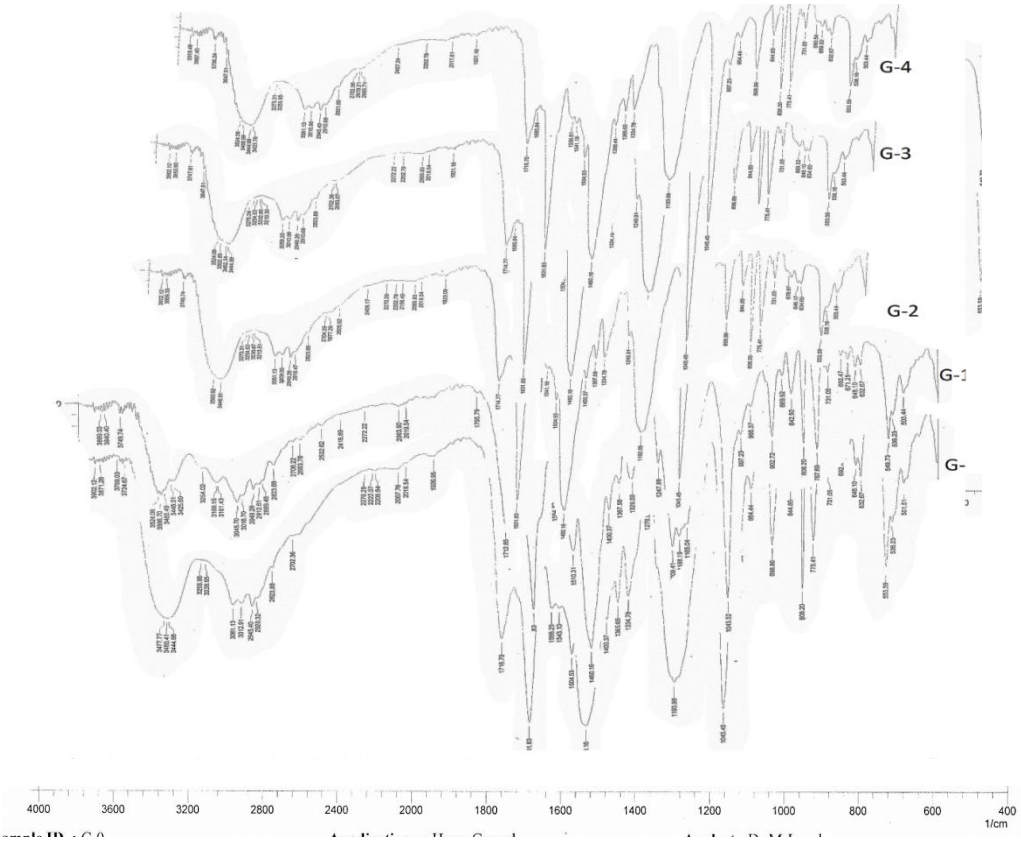

Fig. 2: Comparison of IR spectrum of crystals of gemifloxacin (G-1 to G-4) with amorphous form G-0.

From IR spectrum (Fig.2) of amorphous and polymorphs, it revealed that there was no alteration in the characteristic peaks of functional groups (shown in Table 1) which indicated that there was no interaction between the drug and solvents during the crystallization process.

Table 1: comparison of absorption bands for crystals of gemifloxacin

Absorption Band	Wave number (cm^{-1})	Frequency of band (cm^{-1})			
		G-I	G-II	G-III	G-IV
C-H stretching	2700-3300	2706	2704	2702	2702
C-F stretching	1000-1400	1329	1400	1400	1365
N-H bending	1500-1700	1510	1504	1631	1631
O-H stretching	3000-3700	3254	3213	3219	3010
C-C stretching	1600-1700	1631	1631	1631	1631

SCANNING ELECTRON MICROSCOPY (SEM)

SEM photographs of amorphous and polymorphic forms represented the various shapes of each form with different size. G-I has thick rod shaped crystals and G-II showed crystals of rectangular shape with smaller size. Plate like crystals and bricks like crystals were obtained for form III & IV. The regularly shaped particles of each forms indicated that the formation of different polymorphs.

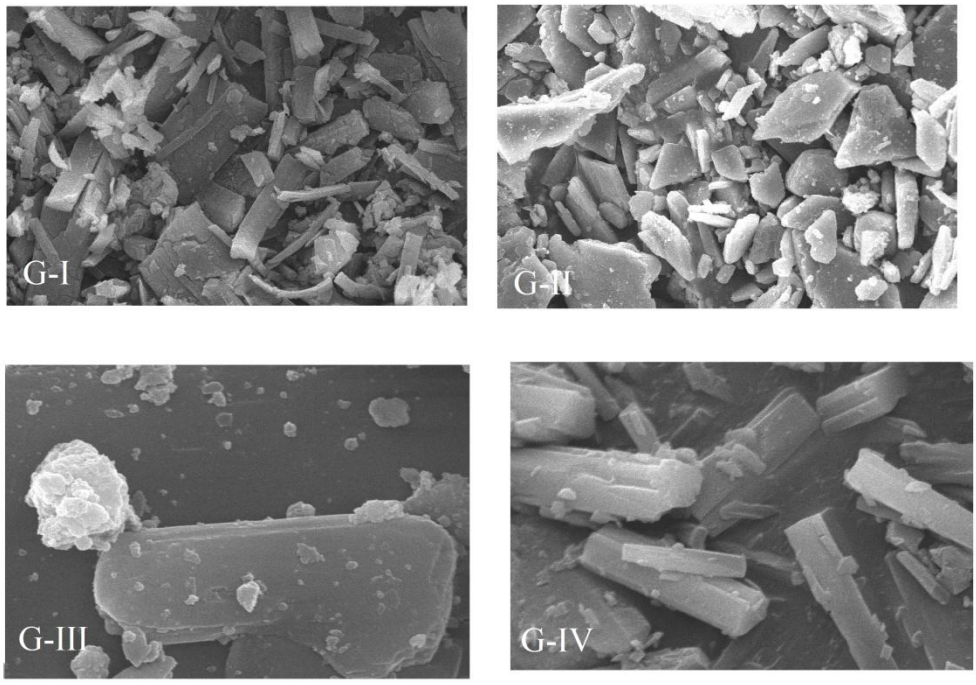

Fig 3: SEM photographs of gemifloxacin crystals

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC curves (Fig. 4) of amorphous form of gemifloxacin meyslate and its crystalline forms showed endothermic peaks at 212.8^0C, 206.6^0C, 208.2^0C, 209.8^0C, 214.2^0C which described their different melting points. Among all these crystals, G-I has the lowest melting point which indicated that it may be meta stable polymorph.

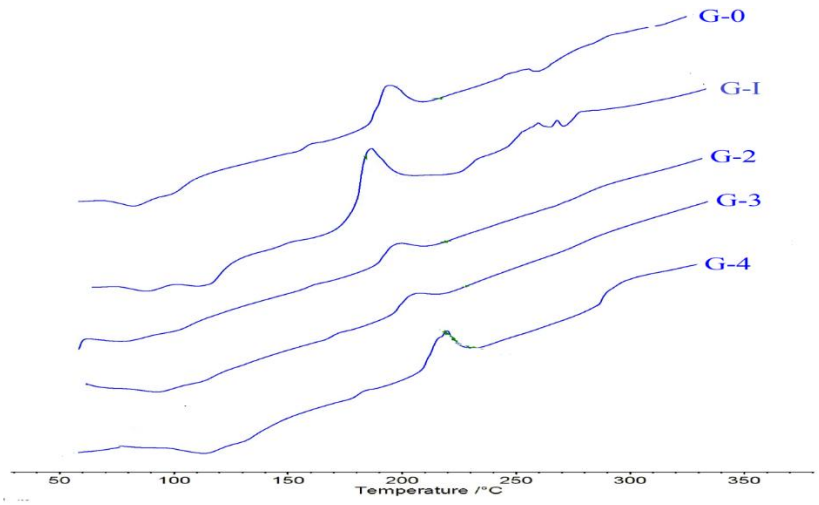

Fig. 4: DSC graphs for gemifloxacin meyslate (G-0) and its polymorphs (G-I, G-II, G-III, G-IV)

X-RAY POWDER DIFFRACTION (PXRD)

The difference in the position of 2θ values of all the pseudo polymorphic forms of gemifloxacin mesylate were confirmed by well resolved diffraction patterns with their corresponding peaks which was displayed on table 2. Appearance and disappearance of some peaks on the XRD spectrum (Fig. 5(a) – 5(e) was clearly indicating the formation of different crystal lattice for all the forms.

Table 2: X-ray diffraction data for gemifloxacin (G-0) and its polymorphic forms (G-I, G-II, G-III, G-IV)

G-0		G-I		G-II		G-III		G-IV	
2θ	I%	2θ	I%	2θ	I%	2θ	I%	2θ	I%
10	45	10	19	10	4	10	28	10	22
10.05	23	10.05	22	10.05	10	10.05	20	10.05	15
10.1	20	10.1	21	10.1	17	10.1	20	10.1	20
10.85	54	10.85	29	10.85	16	10.85	18	10.85	29
11	70	11	35	11	12	11	31	11	21
11.3	48	11.3	32	11.3	19	11.3	26	11.3	26
11.95	28	11.95	62	11.95	21	11.95	73	11.95	30
12	49	12	66	12	23	12	116	12	22
12.9	36	12.9	35	12.9	24	12.9	35	12.9	25
13.4	19	13.4	25	13.4	13	13.4	15	13.4	22
14	71	14	74	14	22	14	31	14	51
14.5	57	14.5	81	14.5	22	14.5	85	14.5	51
14.75	87	14.75	68	14.75	17	14.75	45	14.75	38
15	160	15	69	15	24	15	49	15	47
16.3	186	16.3	128	16.3	33	16.3	68	16.3	67
17.05	72	17.05	69	17.05	15	17.05	64	17.05	30
17.9	80	17.9	128	17.9	20	17.9	98	17.9	56
18.3	107	18.3	65	18.3	23	18.3	56	18.3	45
19.1	122	19.1	99	19.1	19	19.1	75	19.1	53
20	59	20	67	20	26	20	47	20	39
21.1	67	21.1	59	21.1	15	21.1	39	21.1	42
22	113	22	98	22	24	22	64	22	63

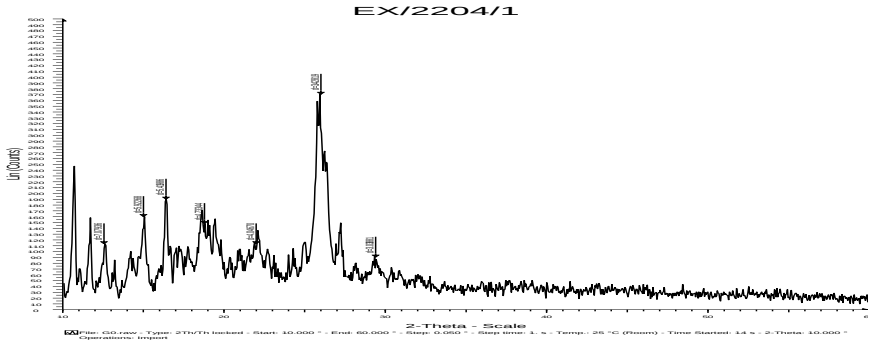

Fig 5(a): XRD graph for gemifloxacin meyslate amorphous form (G-0)

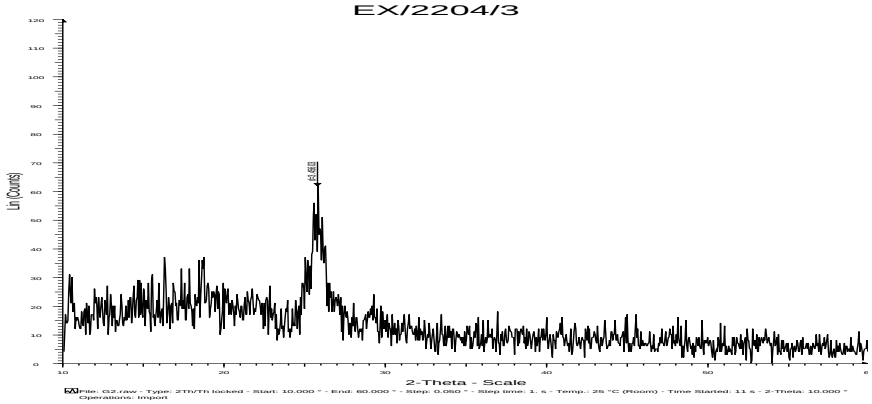

Fig 5(b): XRD graph for gemifloxacin meyslate pseudo polymorphic form (G-I)

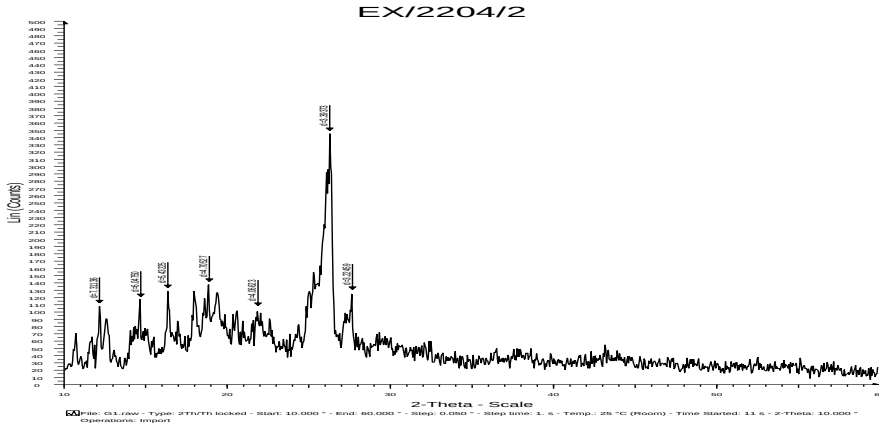

Fig 5(c): XRD graph for gemifloxacin meyslate pseudo polymorphic form (GII)

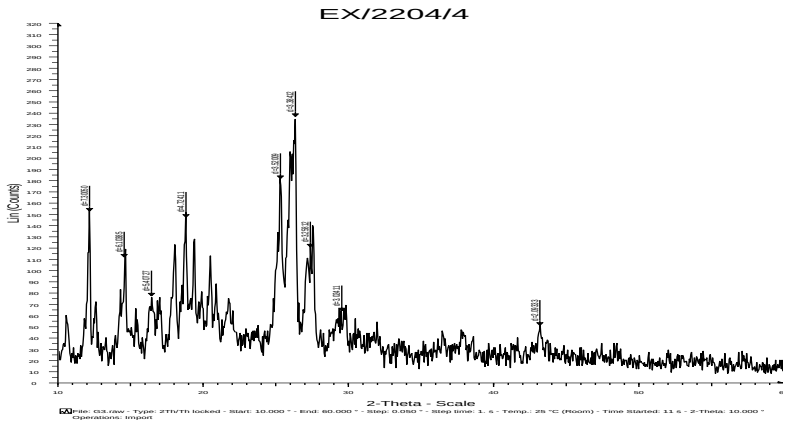

Fig 5(d): XRD graph for gemifloxacin meyslate pseudo polymorphic form (G-III)

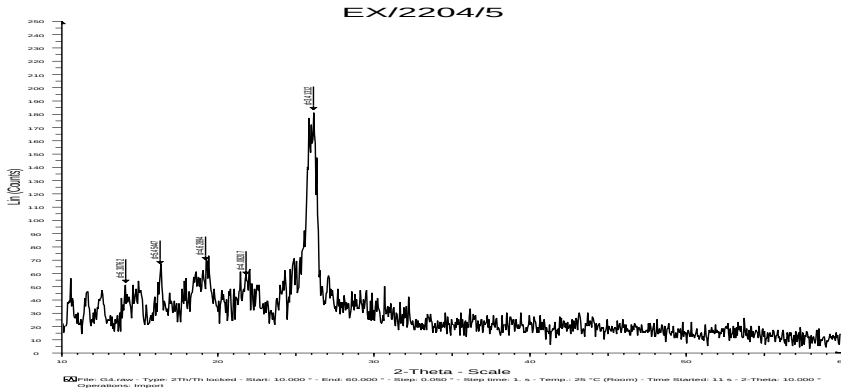

Fig 5(e): XRD graph for gemifloxacin meyslate pseudo polymorphic form (G-IV)

DISSOLUTION STUDIES

The in-vitro dissolution study of amorphous and crystalline forms were performed using phosphate buffer (pH 7.0) and the cumulative percentage drug release of each crystals was calculated by the use of standard calibration graph obtained at 271nm (Fig. 6). Increasing order of drug release was as follows as G-I, G-II, Amorphous, G-IV and G-III, the value of percentage drug release were as 95.85%, 88.40%, 84.78%, 75.0% and 72.6% respectively (Fig 7). Due to more solubility of G-I form; it showed highest percentage drug release. Hence, from the solubility and dissolution studies, crystal (G-I) obtained from Isopropanol having highest cumulative percentage drug release, when compared to other forms.

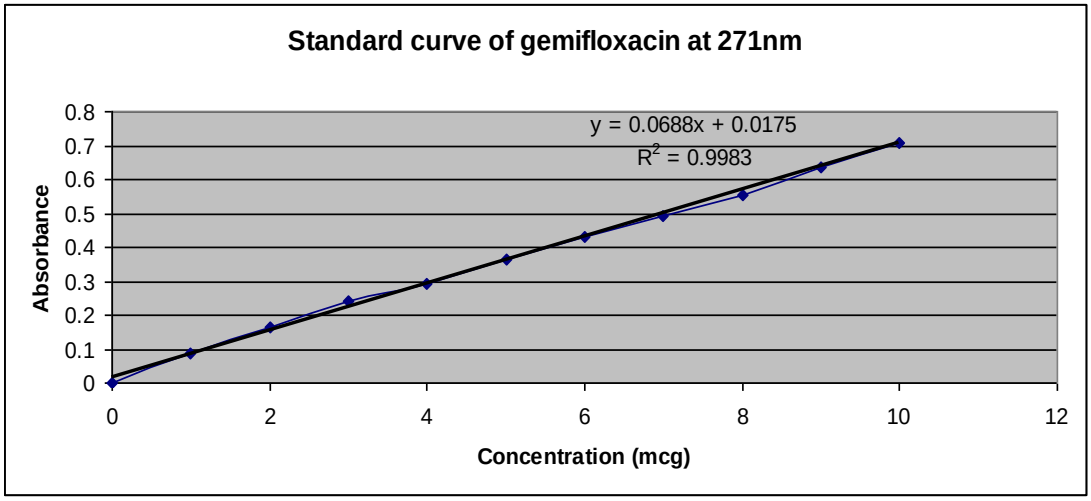

Fig. 6: Standard graph for gemifloxacin mesylate (λ max at 271 nm)

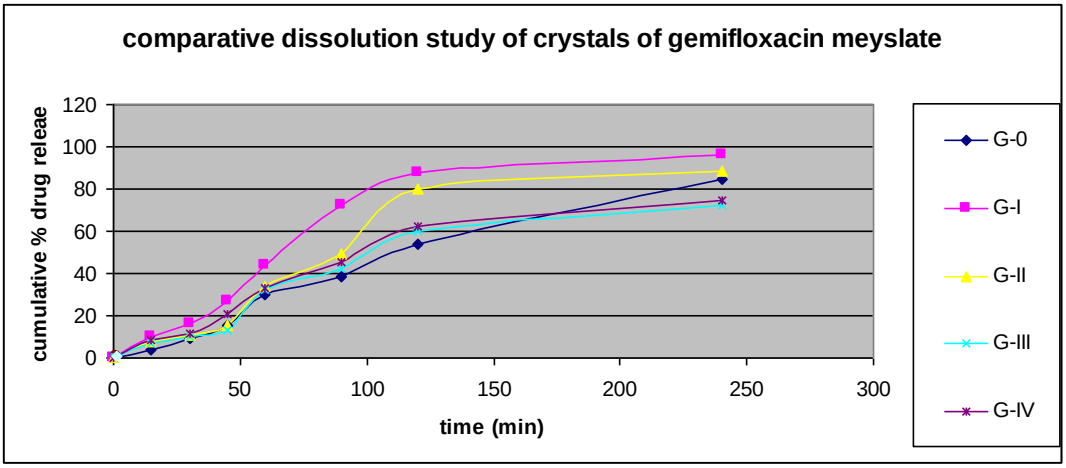

Fig. 7: Graph for comparative dissolution study data

(G-0=Amorphous form, G-I=Polymorph 1, G-II=Polymorph 2, G-III=Polymorph 3, G-IV=Polymorph 4) (*n=4).

CONCLUSION

Pseudo polymorphic forms of gemifloxacin meyslate were obtained from various solvents like isopropanol (G-I), chloroform (G-II), dichloromethane (G-III), benzene (G-IV) and they were characterized and confirmed by various analytical methods like solubility, FT-IR, SEM, DSC, PXRD and dissolution studies. The DSC curve shows that the G-I form has the lowest melting point (206.6^{0}C) which may be the meta stable polymorph, hence confirmed by dissolution study (95.85% drug release). It was concluded that the gemifloxacin existed four pseudo polymorphic forms and they showed highest solubility and percentage drug release. Hence it maybe leads to enhance the absorption and bioavailability of a drug.

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