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Research Article

CRYSTAL FORMS OF LOMEFLOXACIN: PREPARATION, CHARACTERIZATION AND DISSOLUTION PROFILE

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ABSTRACT

The present work was undertaken with the synthesis of crystal forms of Lomefloxacin from solvents of varying polarity (polar, protic solvents). The purpose of the present investigation was to employ crystallization techniques in order to improve the solubility and dissolution studies of Lomefloxacin. The experimental methods involved the preparation of lomefloxacin crystals by crystallization from single solvent technique. Crystals were prepared from solvents like distilled water, ethanol and methanol. Prepared crystals were undergone various studies in terms of crystal yield, melting point, true density, solubility and *in vitro* drug release study as well as characterized by technique viz: FT-IR, differential scanning calorimetry (DSC) and Powder X-ray Diffractometry(PXRD). Photomicrographs of the crystals shows that the crystals obtained from different solvents existed in different shape. Among all the crystals, LOME-I belongs to Type-1 and LOME–II belongs to Type-2 based on instrumental techniques. Highest crystal yield (88%) and maximum density (1.2021g/mL) was observed with LOME-I. Maximum solubility and dissolution rate was observed in LOME-III followed by LOME-II and LOME-I. However all prepared crystal forms showed higher solubility and dissolution profile when compare with commercial Lomefloxacin. It is concluded that the study has indicated the existence of two polymorphic forms of Lomefloxacin which was having better solubility and *in vitro* release than that of commercial Lomefloxacin.

Key words: Polymorphism, Solubility, Dissolution rate, DSC, FT-IR, PXRD

INTRODUCTION

Many pharmaceutical solids can exist in different crystal forms, such as crystalline, amorphous or glass, and also in solvated or hydrated states. Polymorphism is the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice (Brittain, 1999, Young *et al*., 2008). Different polymorphs may exhibit significantly different morphology, density, habit, melting properties, vapor pressure, shelf-life, physical and chemical properties, mechanical properties, leading to changes in its solubility, stability, dissolution and bioavailability, tabletting and finally, in changes in the efficacy of drugs (Lin, 2007, Prajapati *et al*., 2010, Brittain, 2012). In order to understand and characterize the physical and chemical properties of drugs, excipients and their powder mixtures, and to optimize their use in the solid dosage forms, Pharmaceutical industries are interested in the opportunity to predict crystal morphologies, including crystal habits, particle size, shape flow characteristics, density, hygroscopicity as well as compressibility and compaction (Shariare *et al* ., 2012, Babu *et al.,* 2011).

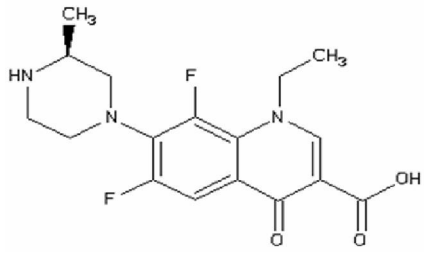

Figure 1. Structure of Lomefloxacin (1).

Fluoroquinolones are broad spectrum antimicrobials which are highly effective in the treatment of a wide variety of clinical

infections. Lomefloxacin is 1-Ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl) 4-oxo-3-quinoline carboxylic acid (Figure 1.), a fluoroquinolone antibiotic, used to treat bacterial infections including bronchitis, urinary tract infections and also used to prevent urinary tract infections prior to surgery (Brittain, 2005, Greici *et al*., 2005). In contrast to the numerous articles on fluoroquinolones such as polymorphism of norfloxacin (Cristina *et al*., 2007), crystal structure of ofloxacin (Petra *et al*., 2006), crystal forms of Pefloxacin (Mange *et al*., 2008), its first time report of Lomefloxacin polymorphism.

Lomefloxacin is white to pale yellow powder with a molecular weight of 387.8. It is slightly soluble in water and sparingly soluble in ethanol and methanol. Lomefloxacin HCl is stable to heat and moisture but is sensitive to light in dilute aqueous solution (Brittain, 2005, Greici *et al*., 2005). In the present work, it was planned to prepare crystal forms of Lomefloxacin to improve the solubility and dissolution profile and to characterize them using instrumental techniques.

MATERIAL AND METHODS

Lomefloxacin (1) was obtained from IPCA laboratories Ltd. Bhopal,India. The solvents used for crystallization were methanol, ethanol and distilled water. These solvents were obtained from S.D Fine Chemicals Ltd. Mumbai, India.

Preparation of crystal forms from diferent solvents

From distilled water (LOME-I)

The drug (0.5g) was dissolved in distilled water (50mL) to check its solubility. To this solution, another weighed amount of Lomefloxacin (2.5g) was added and refluxed with distilled water (250mL), over water bath for 3h. The solution was filtered through whatmann filter paper and the filtrate was kept at room temperature to afford well defined crystals of Lomefloxacin. The crystal Form I, obtained were collected by filtration, dried under vaccum for 24h and stored in well closed container.

From methanol (LOME-II)

The drug (0.5g) was dissolved in methanol (50mL) at its boiling point to check its solubility. To this solution, another weighed amount (2g) of Lomefloxacin was added and refluxed with methanol (230mL) for 90min. The solution was filtered through whatmann filter paper and concentrated by recovery of the solvent to one third of its original volume and kept for crystallization at room temperature to afford well defined crystals of Lomefloxacin. The crystal Form II, obtained were collected by filtration, dried under vacuum for 24h and stored in well closed container.

From ethanol (LOME-III)

The drug (0.5g) was dissolved in ethanol (45mL) at its boiling point to check its solubility. To this solution, another weighed amount (3.5 g) of Lomefloxacin was added and refluxed with ethanol (280mL) for 2.5h. The solution was filtered through whatmann filter paper and the filtrate was concentrated by recovery of the solvent to one third of its original volume and kept at room temperature to afford well-defined crystals of Lomefloxacin. The crystal Form III, obtained were collected by filtration, dried under vaccum at room temperature for 48h and stored in well closed container.

Analytical techniques used for characterization of crystal forms

Optical microscopy

Photomicrographs of crystals were obtained under Olympus microscope (Model BX40). All the crystals so prepared were viewed under optical microscope for their physical characterization. The samples were prepared by placing a small amount of respective crystal powder (previously passed through No.100 sieve) on the slide, dispersed in a drop of mineral oil (liquid paraffin) and covered with cover slip. The slides were visualized by means of binocular polarizing microscope under 10X/0.25 Ph1 and 40X/0.45 Ph 2. When polarized transmitted light was used to illuminate the sample, the background of the image appeared dark and the sample appeared bright. Samples were observed at a magnification of 100 X also. Photomicrographs were taken by using Kodak film roll (Figure 2).

Table I. Crystal shape, crystal yield and true density of crystal forms of Lomefloxacin.

Solvent Used for Crystallization	Crystals Shape	Crystal Yield (%)	True Density (g/mL)
Distilled Water	Rod like	88	1.2021
Ethanol	Thin Pole like	72	1.0415
Methanol	Thin rod and pole	74	1.0674

Fourier transforms infrared spectroscopy (FT-IR)

FT-IR spectra of the crystal forms were obtained on Shimadzu FT-IR-8300 using KBr pellets. Pellets were prepared by slowly grinding the crystals with KBr in a ratio of 2mg of crystals with 100mg of KBr and then applying a pressure of 500psi in a die-punch.

Differential scanning calorimetry (DSC)

The thermographs of different crystalline forms were recorded on Shimadzu DSC-60 apparatus calibrated with 8 mg indium and zinc at a heating rate of 10°C/min. The thermal behavior was studied by heating 5mg of the sample at a scan rate of 10°C/min in a covered sample pan under nitrogen gas flow and the investigations were carried out over the temperature range 30-360°C (Figure4).

Powder X-ray diffractometry (PXRD)

A Powder X-ray Diffractometer (Philips Analytical, MODEL-X' per PRO Model) was used to identify the polymorphs. The samples were exposed to Cu Kα radiation (40KV and 30mA) and were scanned from 5.0 to 45.0°2 theta at step size of 0.01° and 3 s per step. The divergent slit size was 1mm, the receiving slit 1mm, and the detector slit 0.1mm. Data were collected by a Kevex solid-state (SiLi) detector. Data was analyzed using DMax-3 software. Crystals as powdered specimen were packed in a specimen holder made of aluminum. The powders were passed through a 100 mesh sieve and were placed into the sample holder by the side drift technique. The holder consists of central cavity. In order to prepare a sample for analysis, a glass slide was clipped up to the top face of the sample holder so as to form a wall. Each powder was filled into the holder, gently and used for XRD analysis.

Solubility studies

100mg of sieved crystals (previously passed through sieve No.100) of each form was added to 10mL distilled water in a beaker and stirred for 24h at 25±0.5°C under constant vibration with the help of magnetic stirrer at the speed of 60 rpm. The aliquots were filtered through Whatman No. 41 filter paper. The filtrates were diluted appropriately in distilled water and assayed spectrophotometrically at 285nm. The experiment was conducted in triplicate for each sample (Roya *et al*., 2009).

In vitro dissolution studies

The crystal sample (50mg) previously passed through 100 mesh sieve was stirred in 0.1 N HCl (500mL) at 37 ± 2°C in a dissolution apparatus at 50 rpm. Five ml of sample solutions were withdrawn at 10, 20, 30, 40, 50, 60, min. time intervals.The same volume (5mL) of acid buffer was replaced eachtime in order to maintain the sink conditions. Samples were suitably diluted and filtered through syringe filters (Axiva SFCA25X, 0.45µm). The amount of drug released was analyzed spectrophotometrically (Shimadzu 1650PC) at a wavelength of 285nm. All studies were carried out in triplicate.

RESULTS AND DISCUSSION

Photomicrographs of the crystals shows that the crystals obtained from different solvents existed in different shape (Table I and Figure 2). But, shape alone cannot be a criterion for identifying polymorphs because a polymorph can exist in different crystal habits with varying shapes and sizes. Highest crystal yield (88%) and maximum density (1.2/mL) was observed with LOME-I.

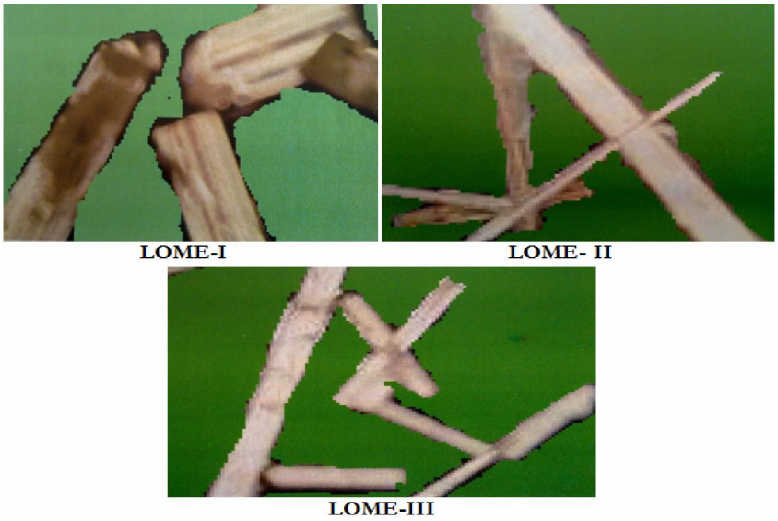

Figure 2. Photomicrographs of LOME- I, LOME-II and LOME-III.

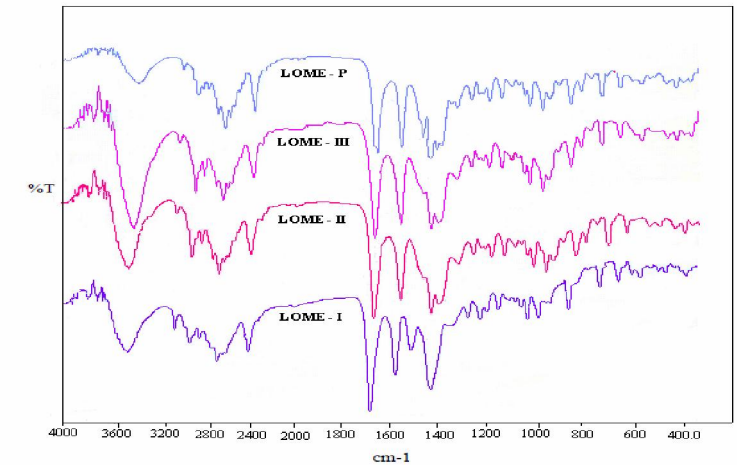

Figure 3. Overlaid FT-IR spectra of LOME-I, LOME-II, LOME-III and LOME-P.

Further analysis was carried out by FT-IR to identify any changes in molecular level. From the spectra (Figure 3) it was observed that characteristic peaks at 1460cm^{-1} and 2700cm^{-1} due to -CH_2 vibration and NH_2 stretching respectively. These characteristic peaks appeared in all FT-IR spectra of crystals indicating no changes in molecular level. On the basis of FT-IR spectroscopy the crystal forms could be categorized into two different forms.

From DSC curve (Figure 4) it was found that LOME-I shows endothermic peak at 318.66°C with heat of fusion 103.4J/g and LOME-II melting was observed at 298.87°C with heat of fusion 149.3J/g. whereas LOME-III shows endothermic peak at 299.61°C with heat of fusion 139.7J/g. From the results it was observed that LOME-I gave higher melting point and Lower heat of fusion when compared to LOME-II and III.

This indicate that it is monotropic with respect to LOME-II and III. Hence it could be categorized as Monotropic (LOME-I) and enantiotropic (LOME-II and III) forms based on DSC results.

Considering Powder X-ray diffraction (PXRD) to be the ideal technique for characterizing polymorphs, and all the crystal

Table II. Powder X-Ray Diffraction Data in Terms of [o2] Theta and Intensities of Various Crystal forms of Lomefloxacin.

LOME - I		LOME - II		LOME - III	
o2 Theta	Intensity	o2 Theta	Intensity	o2 Theta	Intensity
5.20	100.0	5.10	19.2	5.70	19.90
6.10	17.40	6.07	100	7.17	100.0
8.80	26.20	8.08	5.26	8.38	5.160
9.60	14.10	11.8	17.6	12.8	17.90
12.8	36.70	14.3	10.31	13.3	10.71
14.4	51.60	18.3	9.72	19.3	10.70
17.6	22.50	19.4	9.11	19.9	9.310
22.1	18.30	22.2	3.08	21.2	3.080
24.2	11.10	25.9	4.33	26.2	4.450
26.1	20.60	28.6	7.32	29.6	8.320
27.6	14.51	30.7	4.95	31.7	4.250
27.4	9.360	33.6	3.20	33.6	3.900

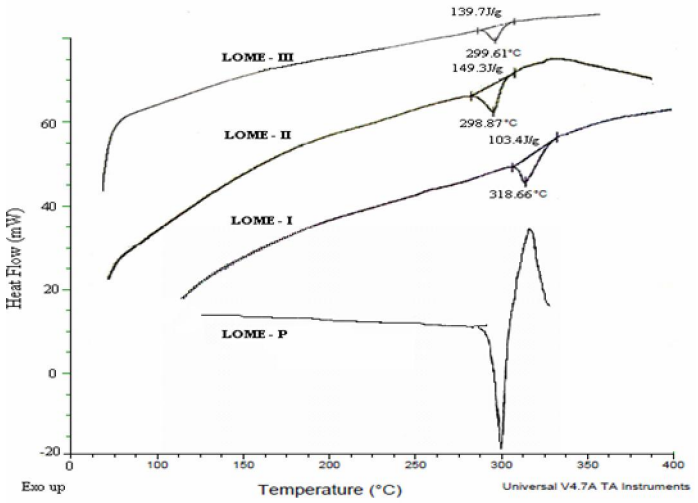

Figure 4. Overlaid DSC thermographs of LOME-I, LOME-II, LOME-III and LOME-P.

forms were submitted for PXRD studies. In PXRD (Figure 5), it was inferred that LOME-II and LOME-III gave exactly superimposable spectra while LOME-I afforded different pattern. Table II gives the PXRD data obtained for all the three crystal forms in terms of lattice spacing and the relative intensities. LOME-I showed characteristic intense lines at 5.2, 6.1, 14.4, 26.1 and 27.6 o2 theta, which were absent in LOME- II and LOME-III. On considering XRD analysis it is the final parameter in deciding the existence of polymorphs in a compound, it could be confirmed that two types of polymorphs were identified in commercial Lomefloxacin. Hence LOME-I belongs to Type- I and LOME-II and LOME-III belongs to Type-II based on instrumental techniques.

Solubility and percentage drug release of Lomefloxacin crystals are shown in figure 6,7. Lomefloxacin crystals showed differences in solubility and dissolution profiles depending on its nature of the crystal forms. Maximum Solubility (27.50mg/mL) and dissolution rate

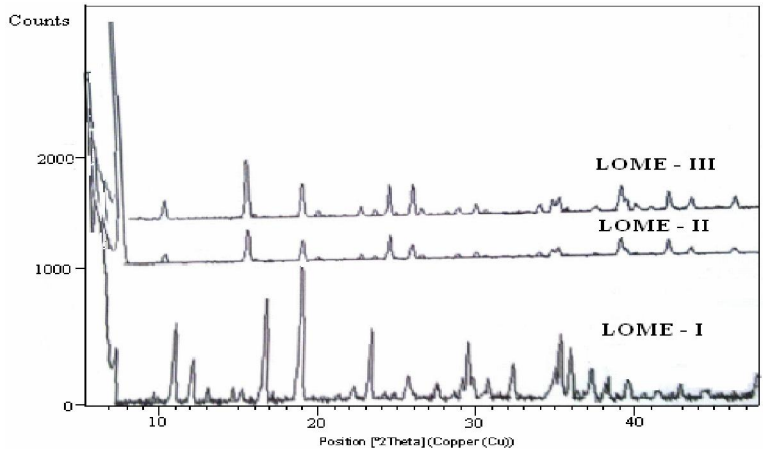

Figure 5. Overlaid PXRD spectra of LOME-I, LOME-II and LOME-III.

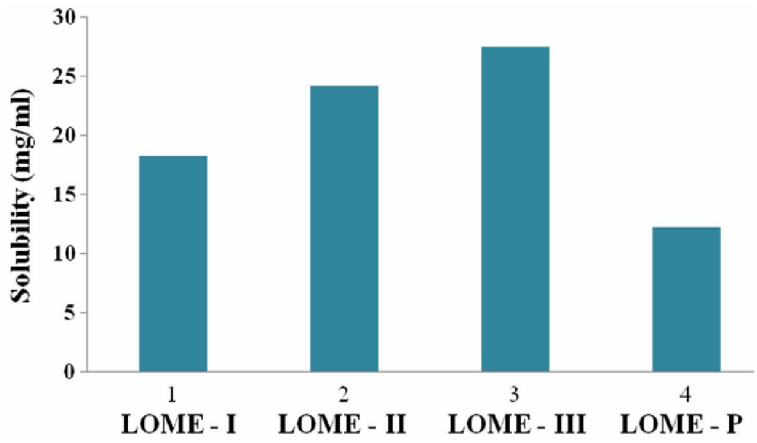

Figure 6. Comparative solubility study of prepared crystal forms of Lomefloxacin.

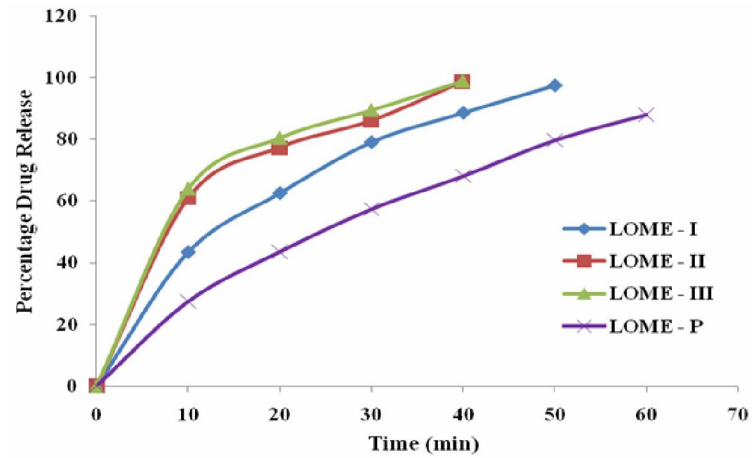

Figure 7. Comparative dissolution study of crystal forms of LOME-I, LOME-II, LOME-III and LOME-P.

(99.0±0.16%) was observed in LOME-III followed by, LOME-II (24.20mg/mL and 98.8±0.16%) and LOME-I (18.30mg/mL and 97.4±0.59%). However all prepared crystal forms were showed better dissolution when compare with commercial Lomefloxacin (12.30mg/mL and 87.9±0.45%).

CONCLUSION

These results indicate that Lomefloxacin exhibits polymorphic modifications that, to our knowledge, not yet reported. Crystallization of Lomefloxacin in different polar, protic solvents offered crystals with different shapes as revealed by photomicrography. All the results obtained from different studies can be very well correlated for all forms prepared. Characterization of the crystal forms were done on the basis of their melting point, FT-IR, DSC and PXRD studies. The DSC curves of all forms showed endothermic peaks corresponding to melting of all the forms. FT-IR and PXRD data confirmed the existence of two polymorphic forms of Lomefloxacin. LOME-I dissolved slowly in comparison to LOME-II and LOME-III. It is concluded finally that the study has indicated the existence of two polymorphic forms of Lomefloxacin. Solubility and dissolution study of the crystals afforded very interesting results. LOME-II and LOME-III offered fastest dissolution.

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