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RESEARCH PAPER

Isolation and Characterization of Ciprofloxacin-HCL Crystals

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ABSTRACT

Three crystalline and one amorphous form of Ciprofloxacin-HCL were prepared and characterized by various instrumental techniques. The hygroscopicity, stability, and solubility of all the forms were also determined. Results indicated that all three crystalline and one amorphous form had distinctly different properties. Form I and II were identified as hydrates and Form III as dimethyl formamide (DMF) solvate. Stability determination of various forms under different conditions indicated that Form II was most stable against temperature and Form I against humidity. Among crystal forms, Form I was found to possess maximum aqueous solubility and was expected to exhibit maximum bioavailability.

INTRODUCTION

Most of the drug substances used in pharmacy exist in more than one polymorphic modification. Some of these are stable phases while others are metastable states. These polymorphs differ in their physicochemical properties, hygroscopicity, solubility, and bioavailability (8). Since bioavailability of a pharmacological compound is frequently dependent upon crystal forms, detection of polymorphism and/or pseudopolymorphism is an important feature for the effective clinical use of a drug. A review on "Pharmaceutical application of polymorphism" (1) and "Characterization of habits and crystalline modification of solids and their pharmaceutical applications" (2) have been published.

This report discusses the preparation of three polymorphs of ciprofloxacin-HCL; their characterization by means of x-ray powder diffraction (XRD), IR spectroscopy, differential thermal analysis (DTA), thermogravimetric analysis (TGA), and UV spectrophotometry; and determination of their hygroscopicity, stability in solid state, and solubility.

EXPERIMENTAL

Materials

Ciprofloxacin-HCL (99.07% purity) was provided by Ranbaxy Laboratories, Okhla, New Delhi. All other reagents used in the experiments were of analytical grade or extra pure grade.

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Preparation and Characterization of Various Forms

Crystalline forms were prepared by cooling warm saturated solutions of the drug in different solvents at 25 ± 0.5°C. The crystals were collected by filtration and dried under a stream of air. Amorphous form was prepared by lyophilization (3) and spray drying (4).

Characterization of various forms was done using various techniques (including instrumental) under the following conditions.

XRD patterns were obtained with Philips x-ray diffractometer PM 9920/05 using Ni-filtered Cu K α-radiations at a scan rate of 1°/min from 5° to 50°. IR spectra were recorded as KBr pellets using a Fourier-transform infrared spectrophotometer (FTIR, Nicolet 5DX) from 4000 to 400 cm. DTA and TGA were performed using Rigaku Rotoflex 8150 thermal analyzer from room temperature to 400°C at a scan rate of 10°C/min. The number of solvent molecules (5,6) was determined by measuring the absorbance of aqueous solutions of various crystalline forms (2.71×10^{-2} M). To determine hygoscopicity, samples of various forms were stored in saturated salt solutions of various relative humidities (RH, 35–98%) in dessicators at 25 ± 0.5°C for 3 weeks. Samples were taken out at appropriate intervals and weighed, and maximum amount of water absorbed was calculated from increase in weight. Equilibrium solubility (5) of various forms was measured at 37°C. Density of various forms was measured by liquid displacement method at 25 ± 0.5°C, using benzene.

Stability

Effect of temperature on solid-state stability of various forms stored at 25 ± 0.5°C, 45 ± 0.5°C, and 65 ± 0.5°C for 3 weeks was investigated by analyzing samples at appropriate intervals using UV spectrophotometry and DTA-TGA (7). Effect of moisture on solid-state stability of various forms stored under various RH (37–98%) at 25 ± 0.5°C was investigated by analyzing samples at appropriate intervals using UV spectrophotometry and DTA-TGA.

RESULTS AND DISCUSSION

Conditions during crystallization of various forms are given in Table 1.

Characterization

XRD patterns of crystal Forms I–III and of amorphous form of ciprofloxacin are shown in Fig. 1. XRD patterns are characteristic of each crystal form with distinctly different peak positions from each other. Differences in the XRD patterns suggest different arrangement of ciprofloxacin molecules in the crystal lattice of each form. XRD patterns of amorphous form exhibit no definite diffraction peak. IR spectra of all the forms were found to be different from one another. Percentage transmittance of characteristic peaks were different in different forms and some of the peaks were either shifted to a higher wavenumber or were altogether absent in some forms (Fig. 2). The thermographs obtained by DTA-TGA are shown in Fig. 3 and data are shown in Table 2. All of the polymorphic forms had different melting and decomposition points. Form II had the lowest melting point (312°C). With the least ΔH_f (enthalpy of fusion), Form II requires the least amount of energy to lose its crystal structure and hence shows greater solubility than any other crystalline form. Weight loss percent in TGA was maximum in Form III (16.263%), which indicates solvate formation.

It was found that aqueous solutions of various forms had different absorbance at the same λ_{max} values. This information was utilized in determining the number of

Table 1

Crystallization Data of Various Forms

Solvent	Crystal Form	Crystallization Time	Crystallization Condition	Density (gm/cm^3) in Benzene
Water	Form I	48–54 hr	Cool evaporation	0.796
Water: methanol (2:1)	Form II	48–54 hr	Cool evaporation	0.980
Dimethylformamide	Form III	3–4 days	Cool evaporation	1.042

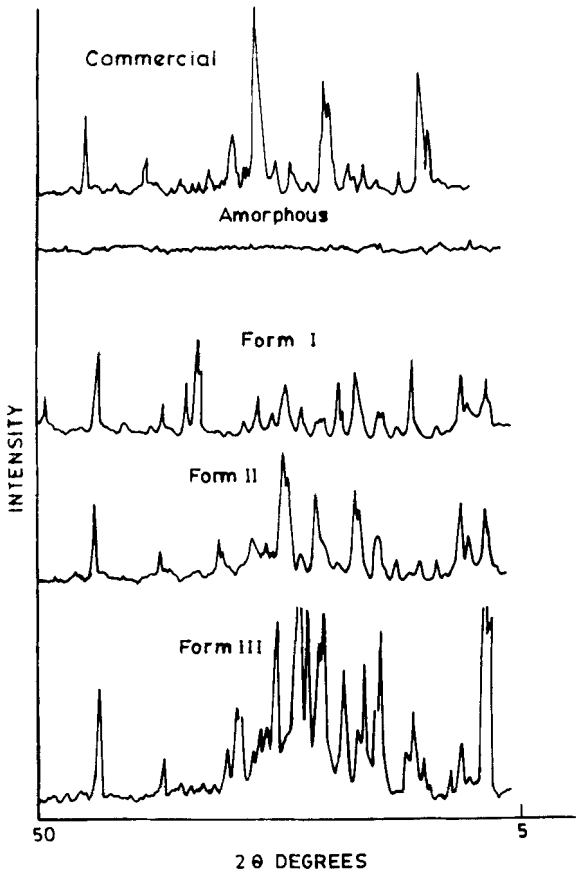

Figure 1. X-ray powder diffraction patterns of various forms of ciprofloxacin-HCL.

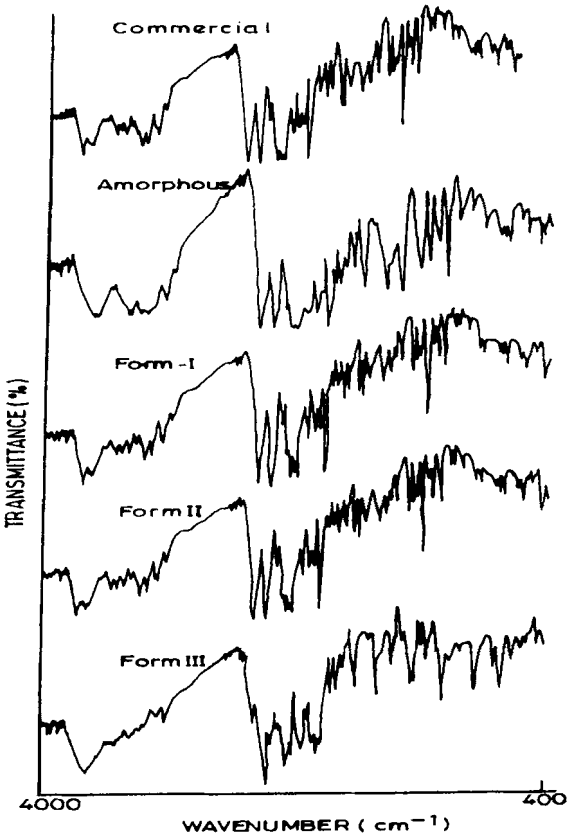

Figure 2. Infrared spectra of various forms of ciprofloxacin-HCL.

solvent molecules. Table 3 shows that all of the forms obtained were partial or complete solvates and hence pseudopolymorphs.

Results of hygroscopicity studies indicate that increase in water content was maximum in amorphous form and minimum in Form I. Various forms in their increasing order of hygroscopicity are Form I < commercial form < Form II < Form III < amorphous form.

The high affinity of amporphous form for water may be explained by considering that amorphous form has a disordered molecular arrangement with more scope for binding water. On the other hand, Form I and commercial form, with very little difference in their mositure

Table 2

DTA and TGA Data of Various Forms

Sample	M.Pt. (°C)	ΔH_f (muv·min/g)	Weight Loss (%)
Commercial	308.9	2439.28	9.079
Amorphous	316.7	969.70	5.048
Form I	313.5	2601.48	10.003
Form II	312.0	2198.23	11.986
Form III	316.3	3267.50	16.263

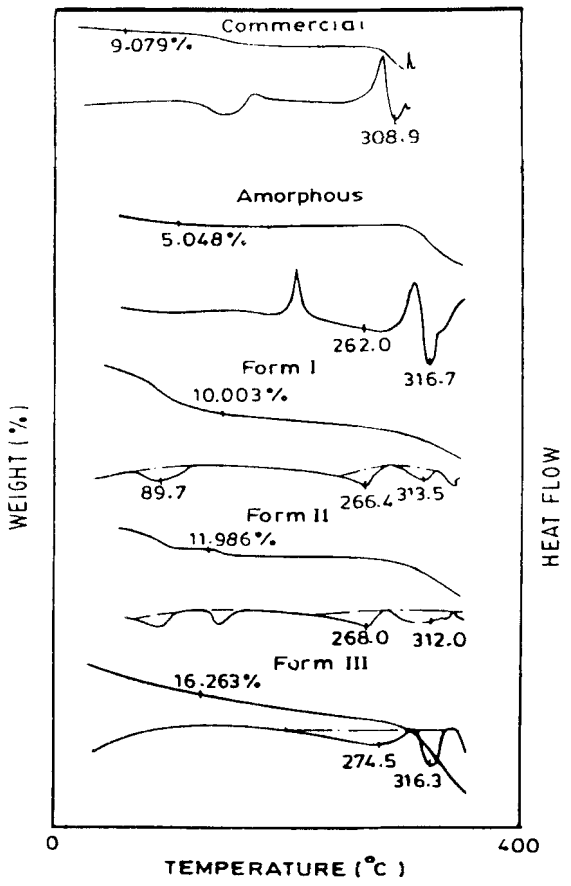

Figure 3. DTA-TGA of various forms of ciprofloxacin-HCL.

absorption, are the most stable. Table 4 indicates the amount of moisture absorbed by various forms at 98% RH, when stored for 3 weeks at 25 ± 0.5°C.

Results of equilibrium solubility measurement show that solubility of amorphous form was higher than the crystalline forms (Table 4). Various crystalline forms in increasing order of solubility at 37 ± 0.5°C are Form III < Form II < commercial form < Form I < amorphous form. This infers that Form III and Form II should be more stable and amorphous form should be least stable. Similar results were obtained in stability studies, except that Form III is not stable. In all other cases, stability decreases as the solubility increases.

Results of density measurement are given in Table 1. Amorphous forms have the lowest density (0.7707 gm/cm^3) which indicates lack of ordered arrangement. Various crystalline forms in increasing order of densities at 25 ± 0.5°C are Form I < Form II < Form III.

Stability

Samples kept at 45°C and 65°C became brown in color. They also exhibited decrease in absorbance due to decomposition of a part of the drug sample. DTA-TGA (Table 5) showed change in melting points of all of the forms (on exposure to 65°C) except for Form II, which showed least change and hence was most stable compared to other forms. Various crystalline forms in increasing order of stability against temperature are amorphous form < Form III < Form I < commercial form < Form II.

Results of UV analyses show that all of the forms decompose above 35% RH. There is also a direct relationship between moisture absorbed and decomposition. DTA-TGA (Table 5) exhibit a marked change in melting point of Form II, III, and amorphous form. Form I and commercial form, however, showed very little change in their melting point and thus were relatively more stable than other forms. Various crystalline forms in increasing order of stability are amorphous form < Form III < Form II < commercial form < Form I.

Table 3

Number of Solvent Molecules in Various Crystal Forms

Sample	Absorbance of 2.71×10^{-2} M Solution	Calculated Absorbance for Equimolar Solution of Crystals	No. of Solvent Molecules/Drug Molecules
Amorphous	1.321	-	-
Form I	1.160	1.152	3
Form II	1.337	1.230	3/2
Form III	1.208	1.202	1/2

Table 4

Hygroscopicity (98% RH, 3 weeks) and Solubility Data of Various Forms

Sample	Amount of Moisture Absorbed (mg)	No. of Water Molecules/ Drug Molecules	Equilibrium Solubility at 37°C mg/ml
Commercial	205	4.18	52
Amorphous	372	7.60	70
Form I	190	3.88	54
Form II	293	5.98	45
Form III	416	8.50	34

Table 5

DTA-TGA Data of Stability Against Moisture and Temperature

	Initial		57% RH		79% RH		65°C	
Samples	M.Pt.°C	Wt. Loss %	M.Pt.°C	Wt. Loss %	M.Pt.°C	Wt. Loss %	M.Pt.°C	Wt. Loss %
Commercial	308.9	9.079	311.8	9.630	313.9	13.751	315.5	5.899
Amorphous	316.7	5.048	265.1	2.156	268.5	6.008	309.7	1.790
Form I	313.5	10.003	307.2	7.386	309.6	10.985	310.7	13.208
Form II	312.0	11.986	303.8	3.261	308.1	10.365	314.2	20.156
Form III	316.3	16.263	301.7	4.980	306.4	6.879	262.5	0.00

CONCLUSION

The crystal forms obtained were pseudopolymorphs. Form I and Form II were hydrates, and Form III was DMF solvate. Form I was most stable against humidity, whereas Form II was most stable against temperature. Among crystal forms, Form I was found to possess maximum aqueous solubility, hence is anticipated to exhibit maximum bioavailability. Further studies are required, however, to perform therapeutic performance studies of various forms in its original (as drug substance) as well as dosage formulations.

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