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Research Article

Structural Analysis of Ciprofloxacin-Carbopol Polymeric Composites by X-Ray Diffraction and Fourier Transform Infra-Red Spectroscopy

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Abstract

Purpose: *To evaluate physicochemical changes in ciprofloxacin following incorporation in Carbopol polymeric composites.*
Methods: *The ciprofloxacin and Carbopol were mixed in water in a drug:polymer ratio of 1:5 (w/w) and homogenized to produce uniform composites. X-ray powder diffraction analysis of the pure ciprofloxacin and the Carbopol polymeric composites of the drug were obtained using a powder diffractometer. Spectra for the materials were also generated by Fourier transform infrared (FTIR) spectroscope interfaced with an infrared (IR) microscope operated in reflectance mode.*
Results: *Based on the Hanawalt system, three prominent x-ray diffractogram (XRD) peaks of the pure ciprofloxacin and the drug in the polymeric composites exhibited d-spacing at similar 2θ values, but the relative intensity of these peaks was higher in the polymeric composites. FTIR analysis indicates that there were intermolecular hydrogen bonding and esterification between the drug and polymer in the polymeric composites.*
Conclusion: *The changes that occurred in ciprofloxacin indicate increase in stability, decrease in solubility and delayed release of the drug from polymeric composites which could facilitate the formulation of a sustained release form of the drug.*

Keywords: *Ciprofloxacin, X-ray diffraction, Fourier transform infrared, Carbopol, Polymeric composites*

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INTRODUCTION

X-ray powder diffractometry (XRPD) can often be carried out to monitor structural changes in a solid with a high degree of accuracy in relation to conditions such as temperature, pressure or exposure to gaseous atmospheres of varying composition, including relative humidity [1-3]. XRPD is a useful qualitative tool in the pharmaceutical industry for studying the crystal morphology of active ingredients and polymers singly or in a mixture [3,4]. Since the crystalline form of a drug is more rigid and thermodynamically stable than its amorphous form [5], it is usually necessary to study crystalline compounds alone and in composites. Furthermore, delayed release of drug from dosage forms is more pronounced with increase in crystallinity [6,7]. Crystallinity also influences a drug's processing behavior, bioavailability and pharmacological action. Hence, a critical evaluation of crystal habit will aid in maintaining reproducibility of formulation performance as the non-destructive nature of XRPD makes it an ideal tool for systematic investigation of drug-polymer interaction in preformulation studies and formulation development.

Ciprofloxacin (Cipro) is a second generation fluoroquinolone antibacterial. It shows low solubility in aqueous solution and a high rate of absorption from the stomach. It is likely to be precipitated out of solution upon entry into the small intestine where the pH is alkaline. The desire is for a dosage form that will provide a drug at a sustained, constant level in solution in both acidic and basic pH conditions of the GIT over the entire transit period. For this reason, dosage forms that incorporate low solubility drugs provide a major challenge for sustained release developers [8].

Carbopol 934 (C934) and Carbopol 940 (C940) are mucoadhesive, biodegradable and environmentally responsive Carbopol polymers and are considered as 'smart gels' [9,10]. Both consist of chains of polyacrylic acid but differ in the cross linking agents used in their production which are allyl ethers of sucrose (allylsucrose) and pentaerythritol (allylpentaerythritol), respectively [11,12]. They have recently attracted considerable interest in the field of drug delivery as a means of providing an on-off release by shrinking and swelling in response to change in pH [13,14]. The polymer can protect a drug from its physiological environment by improving its stability *in vivo* [15].

Since pure ciprofloxacin is crystalline in nature [3], x-ray diffraction (XRD) and FTIR studies were undertaken in the present work to determine whether the crystallinity of ciprofloxacin was affected when it is incorporated in a composite.

EXPERIMENTAL

Materials

The following major materials were used: ciprofloxacin hydrochloride, obtained from Dr. Reddy's Lab, Hyderabad, India, as a gift; Carbopols 934 and 940, kindly supplied by Cosmo Chem Laboratory, Pune, India. Ultra pure water was generated using a Millipore Milli-Q UV water filtration system.

Sample preparation

1.25 gm of Ciprofloxacin and 0.063 gm of Carbopol were added slowly to water and volume was adjusted up to 25ml. Then the mixture was blended together using an ultrasonic homogenizer (Labsonic M, Sartorius) to produce a uniform composite. Homogenization was carried out for at least 15 min at an operating frequency of 30 kHz. The homogenizer, which incorporates a titanium alloy probe that serves as a λ/2 oscillator, was set to alternately emit sound for 0.8 s and pause for 0.2 s, thus exposing the sample to 100 % amplitude while minimizing heat effect to 80 %. The polymeric composites were sprayed on to an aluminum slip with the aid of an atomizer. The fine droplets were dried overnight at room

temperature and the solid samples were then collected and powdered. The maximum particle size of the powder sample was found to be 10-15 µm.

X-ray diffraction (XRD) study

XRD measurements were made using Philips X'Pert powder diffraction system (Philips Analytical, The Netherlands) equipped with a vertical goniometer in the Bragg-Brentano focusing geometry. The X-ray generator was operated at 40 kV and 50 mA, using the CuKα line at 1.54056 Å as the radiation source.

Each powdered specimen was packed in a specimen holder made of glass. In setting up the specimen and apparatus, coplanarity of the specimen surface with the specimen holder surface, and the setting of the specimen holder at the position of symmetric reflection geometry were ensured. The powders were passed through a 100 mesh sieve and were placed into the sample holder by the side drift technique [16]. The holder consisted of a central cavity. In order to prepare a sample for analysis, a glass slide was clipped to the top face of the sample holder so as to form a wall. The powder sample was filled into the holder, gently tapped and used for XRD measurement.
10 mg of each sample was scanned at 25 ℃ from 10˚ to 70˚ (2θ) and in step size of 0.020 and count time of 2.00 s, using an automatic divergence slit assembly and a proportional detector. Relative intensities were read from the strip charts and corrected to fixed slit values.

Fourier transform infrared (FTIR) spectroscopy

FTIR analysis was performed with an FTIR spectrophotometer interfaced with an infrared (IR) microscope operated in reflectance mode. The microscope was equipped with a video camera, a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector and a computer controlled translation stage, programmable in x and y directions. Each sample (approx. 2 mg) was taken with dry IR-grade KBr (100 mg) and compressed into disc/pellet. Background was first scanned using blank potassium bromide pellet before the samples were scanned. The spectra were obtained in the 400 cm^{-1} to 4000 cm^{-1} region with 8 cm^{-1} resolution, 60 scans and beam spot size of 10 - 100 µm. FTIR imaging was carried out using Perkin Elmer Spectrum RX 1.

RESULTS

X-Ray diffraction (XRD) study

The powder X-ray diffraction patterns for the pure ciprofloxacin, C934, C940 and their polymeric composites are given in Fig 1.

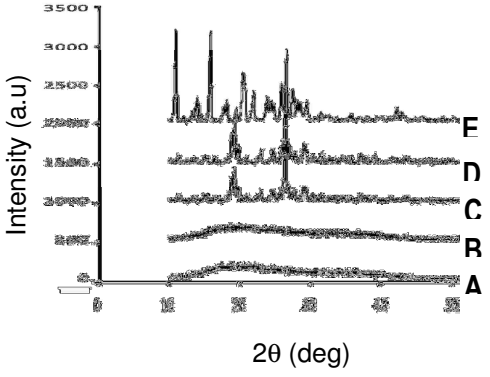

Fig 1: X-ray diffraction patterns of (A) C934, (B) C940, (C) Cipro, (D) Cipro/C934 composite, and (E) Cipro/C940 composite

All the high intensity peaks observed in the diffractograms of the pure ciprofloxacin were compared with its polymeric composite forms, and parameters - lattice spacing (Å) and relative intensities (I/I_0) - were derived. Each diffractogram was characterized by the interplanar d- spacing (Å) and the relative intensities (I/I_0) of the three strongest peaks in the pattern under the Hanawalt system and the data are recorded in Table 1. As this table shows, both polymeric composite types showed similar diffraction patterns. Structure identification in a diffractogram is usually based on the position of peaks and their relative intensities.

Table 1: Lattice spacing (Å) and relative intensities (I/I_0) (based on the Hanawalt System) of the three strongest peaks in the diffractograms of ciprofloxacin and its composites

Sample	Ciprofloxacin			Ciprofloxacin/C934			Ciprofloxacin/C940		
No.	2θ	Å	I/I_0	2θ	Å	I/I_0	2θ	Å	I/I_0
1	19.22	4.61	54.85	9.21	4.62	56.85	9.22	4.62	64.73
2	26.39	3.37	100	6.39	3.38	100	6.38	3.38	100
3	29.16	3.06	28.47	9.16	3.06	29.45	9.13	3.06	33.88

However, entire diffractograms, rather than selected peaks, are still required to distinguish samples. Hence, comprehensive Å and I/I_0 data, presented in Table 2, clearly identify ciprofloxacin even in the polymeric composites

FTIR spectroscopic analysis

The FTIR spectra of ciprofloxacin alone, C934, C940 and ciprofloxacin/Carbopol composites are shown in Fig 2 while their peak assignments are indicated in Table 3.

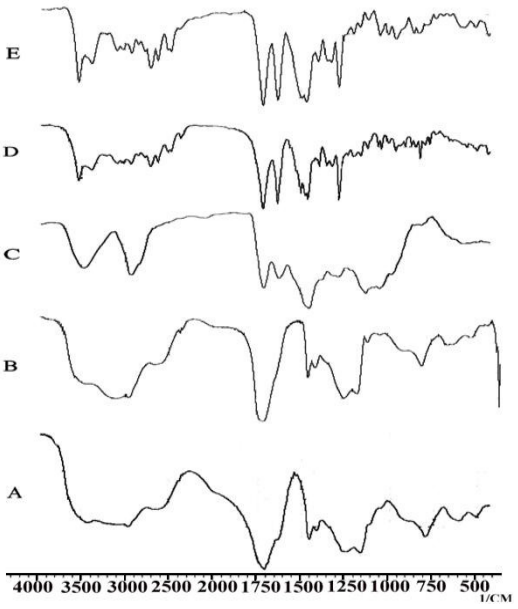

Fig 2: FTIR spectra of (A) Carbopol 934, (B) Carbopol 940, (C) ciprofloxacin, (D) ciprofloxacin/Carbopol 934, and (E) ciprofloxacin/Carbopol 940

In the FTIR spectra of Cipro, one prominent characteristic peak was found between 3500 and 3450 cm^{-1} (Fig 2C), and was assigned to OH stretching vibration (intermolecular hydrogen bonding). Another band at 3000-2950 cm^{-1} represented alkenes and aromatic C-H stretching, mainly $\upsilon_{=C-H}$. The bands at 1750 to 1700 cm^{-1} represented carbonyl C=O stretching, i.e., $\upsilon_{C=O}$ while the peak at 1650 to 1600 cm^{-1} was assigned to quinolones. The bands at the 1450 to 1400 cm^{-1} represented υ_{C-O} and the ones at 1300 to 1250 cm^{-1} suggested bending vibration of O-H group which indicated the presence of carboxylic acid. In addition, a strong absorption peak between 1050 and 1000cm^{-1} was assigned to C-F group [17,18]

For both C934 and C940, their FTIR spectra showed a peak in the 3000 - 2950 cm^{-1} range, representing OH stretching vibration, i.e., υ_{O-H} and intramolecular hydrogen bonding (Fig 2A, B). The prominent peak between 1750 and 1700 cm^{-1} was assigned to carbonyl C=O stretching band i.e., $\upsilon_{C=O}$ while the peak at 1450 to 1400 cm^{-1} was assigned to υ_{C-O} / δ_{O-H}. The band at 1250 to 1200 cm^{-1} was assigned to υ_{C-O-C} of acrylates [17,19]. The ethereal crosslinking, is indicated by the prominent peak at 1160 cm^{-1}, represented a stretching vibration of υ_{C-O-C} group. The band between 850 and 800 cm^{-1} indicated out of plane bending of C=CH, i.e., $\delta_{=C-H}$ [17,20].

In the FTIR spectra of the ciprofloxacin/ Carbopol composites, the prominent band between 3550 and 3500 cm^{-1} was assigned to υ_{O-H} and hydrogen bonding by single bridge (Fig 2D, E) while the peak in

Table 2: Lattice spacing (Å) and relative intensities (I/I_0) of all the identifiable peaks in the diffractograms of ciprofloxacin and its composites

Sample	**Ciprofolxacin**		**Ciprofolxacin/C934**		**Ciprofolxacin/C940**	
No.	Å	I/I_0	Å	I/I_0	Å	I/I_0
01	7.86	9.10	7.87	9.34	7.85	10.93
02	5.50	5.37	6.71	5.42	7.27	3.93
03	5.85	10.98	6.48	5.59	6.50	7.86
04	5.42	5.76	5.85	12.78	5.85	14.05
05	4.71	38.92	5.42	8.28	5.42	9.62
06	4.61	54.85	4.71	41.26	4.71	47.61
07	4.51	22.06	4.62	56.85	4.62	64.73
08	4.23	7.98	4.51	26.25	4.49	22.43
09	3.96	5.97	4.24	10.51	4.24	13.49
10	3.86	18.01	3.86	19.51	3.86	21.51
11	3.60	19.61	3.60	20.74	3.61	26.06
12	3.37	100.00	3.37	100.00	3.38	100.00
13	3.31	27.18	3.20	17.86	3.20	20.36
14	3.26	13.26	3.06	29.45	3.06	33.88
15	3.20	15.74	2.95	12.41	2.95	16.41
16	3.10	28.47	2.83	12.58	2.83	13.97
17	2.95	11.48	2.74	7.35	2.74	9.94
18	2.83	13.70	2.68	6.98	2.68	9.00
19	2.74	6.81	2.58	8.76	2.61	8.36
20	2.68	7.16	2.51	8.71	2.58	11.41
21	2.61	5.69	2.42	11.77	2.51	11.43
22	2.58	8.94	2.37	8.29	2.42	13.91
23	2.51	9.22	2.30	9.66	2.37	10.46
24	2.42	11.24	2.17	7.59	2.30	12.54
25	2.37	7.84	2.13	5.21	2.17	9.95
26	2.30	10.40	2.07	7.75	2.14	6.99
27	2.27	4.49	1.98	5.46	2.08	9.71
28	2.23	2.69	1.94	5.33	1.98	6.81
29	2.17	7.79	1.90	3.84	1.94	6.09
30	2.13	5.80	1.86	3.09	1.90	5.29
31	2.08	7.46	1.83	4.27	1.82	5.04
32	2.06	7.29	1.71	3.23	1.73	3.64
33	1.98	5.70	1.61	2.20	1.71	4.97
34	1.94	5.03	1.50	0.66	1.61	3.08
35	1.90	3.51			1.55	1.31
36	1.82	4.07			1.50	1.42
37	1.78	1.53			1.38	0.25
38	1.76	1.76				
39	1.74	2.86				
40	1.71	3.80				
41	1.66	2.03				

Table 3: Prominent peaks in the FTIR spectra of ciprofloxacin/Carbopol composites

Peak (cm^{-1})	Group	Peak assignment
Cipro and C934		
3550-3500	Hydroxyl group	H –bonding by single bridge
3450-3400	Polymeric OH groups	υ_{O-H}, H-bonding
2650-2600	Strong H-bonding	O-H stretching vibration
1650-1600	O-C-O group of acid	υ_{as} stretching vibration
1450	O-C-O group of acid	υ_{s} stretching vibration
1300-1250	Acrylates & esters	C-O-C stretching vibration
1100-1000	C-F groups	υ_{C-F}
800	Aromatic m - distribution	δ_{Ar-H}
Polymeric composite containing Cipro and C940		
3527.80	Hydroxyl group	υ_{O-H}
3040-3010	Enes	$\upsilon_{=C-H(m)}$
2704.2	Intermolecular H-bonded	O-H stretching vibration
1707	C=O groups	$\upsilon_{C=O}$
1622	O-C-O group of acid	υ_{as} stretching vibration
1463.25	O-C-O group of acid	υ_{s} stretching vibration
1259.16	Acrylates & esters	C-O-C stretching vibration
1050-1000	C-F groups	υ_{C-F}
800	Aromatic & enes	δ_{Ar-H} and $\delta_{=C-H}$

the range 3450 to 3400 cm^{-1} was assigned to polymeric υ_{O-H} and hydrogen bonding; the band between 2650 and 2600 cm^{-1} represented the υ_{O-H}, i.e., strong hydrogen bonding. The band from 1650 to 1600 cm^{-1} was assigned to $\upsilon_{C=O}$ i.e., carbonyl stretching vibration. A prominent peak at 1450 cm^{-1} (w) is for υ_{C-O} / δ_{O-H}. The band from 1300 to 1250 cm^{-1} was assigned to υ_{C-O-C} of acrylates. The peak between 1100 and 1000 cm^{-1} represented υ_{C-F} groups while the band at 800 cm^{-1} indicated the meta distribution of δ_{Ar-H} group [17].

DISCUSSION

When an x-ray beam hits a sample and is diffracted, the distances between the planes of the atoms that constitute the sample can be determined by applying Bragg's Law (Eq 1).

$$n\lambda = 2d\sin\theta \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where the integer *n* is the order of the diffracted beam, λ is the wavelength of the incident x-ray beam, *d* is the distance between adjacent planes of atoms (*d*-spacing), and θ is the angle of incidence of the x-ray beam. Since λ is known and θ can be measured, then *d*-spacings can be calculated. The characteristic set of d-spacings generated in a typical x-ray scan provides a unique "fingerprint" of the drug molecule present in the sample. When properly interpreted, by comparing with pure drug as reference, this "fingerprint" allows for identification and change in crystallinity of the drug present in the polymeric composite [2].

X-ray diffraction

In the present study, most of the characteristic peaks in the diffractograms were generally prominent and sharp, and hence measurement of the angles and therefore, d-values was accurate. Proper sample preparation helped attain exact peak positions for qualitative analysis.

The XRD patterns of both C934 and C940 indicate that these two polymers are fully amorphous in nature as there were no sharp prominent peaks. The three prominent peaks in the diffractograms of both pure ciprofloxacin and its composites had nearly identical d-spacing (difference was not greater than 0.01 Å) at similar 2θ values. Since the d-spacing value of pure ciprofloxacin was remain essentially unchanged from that in the composites, it can

be said that ciprofloxacin exists in a crystalline form in the Carbopol composite. Moreover, since the relative intensities of the peaks increased, the crystallinity of Cipro must have been enhanced in the composites, compared to the pure ciprofloxacin. The increase in the relative intensities of the peaks is probably due to a rise in atomic densities in that particular plane of crystal lattice. Thus, it is likely that there was a small change in the orientation of the crystal lattice due to the incorporation into it of some extra atoms.

FTIR analysis

In the FTIR spectra of the composites, it is obvious that the band position of C=O group was affected by esterification; furthermore, conjugation involving C=O group lowered the $\upsilon_{C=O}$ frequency between 1650 and 1600 cm^{-1} might be due to the formation of β-ketoesters [20]. The FTIR peaks assigned to υ_{C-O} and υ_{C-O-C} representing acrylates and esters confirm the esterification between polymeric OH group and –COOH group of the drug (ciprofloxacin). The stretching vibration of C-F group remained nearly unaltered.

Another probable interaction is hydrogen bonding, i.e., intermolecular hydrogen bonding due to the prominent peaks at 3550 to 3500, 3450 to 3400 and 2650 to 2600 cm^{-1}, representing single bridge O-H…O, polymeric O-H...O-H…O-H and strong hydrogen bonding, respectively. The hydrogen bonded -OH stretching vibration occured over a wide range, 3550 - 2600 cm^{-1}. In FTIR spectra, intramolecular hydrogen bond bands are sharp while intermolecular hydrogen bond bands are broad. Actually, it is less broad than that required for chelation [17]. The bending vibration of O-H group gives medium to strong bands in the region around 1450 cm^{-1}. The FTIR peak at 800 cm^{-1} gives the probability of out of plane bending of –ene bond and m-substitution of δ_{Ar-H} hydrogen atom [17,19]. Both spectra showed prominent peaks for the stretching vibration of O-C-O and C=O groups, which prove the formation of esters between the drug and polymer. Both intermolecular and polymeric hydrogen bonding are also evident from the spectra of the polymeric composites.

Thus, it may be predicted from FTIR analysis that the increase in atomic densities in the particular plane as well as the small change in orientation of the crystal lattice of the composites were due to formation of the esters and intermolecular hydrogen bonding between the carboxylic group of ciprofloxacin and carbopol polymers.

Both XRD and FTIR analyses indicate that although there are intermolecular hydrogen bonding and esterification between ciprofloxacin and carbopol polymers, ciprofloxacin retained its crystallinity in carbopol composites.

CONCLUSION

On the basis of XRD and FTIR results, it can be concluded that the crystalline nature of ciprofloxacin remained essentially altered following its incorporation in carbopol composites. In fact, the crystallinity of ciprofloxacin might have increased in the carbopol composites which could lead to increase in stability, decrease in solubility and delayed release of the drug from polymeric composites. This feature may favour the formulation of ciprofloxacin as sustained release preparations. However, further studies on the release profile and bioavailability of the drug from carbopol composites would require to establish the composites as a suitable sustained release platform.

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