Qualitative Analysis of a Controlled Release Mucoadhesive Suspension

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ABSTRACT

Objective:Mucoadhesive polymeric (Carbopol940) suspension of Ciprofloxacin was prepared and optimised with the aim of developing an oral controlled release gastro-retentive dosage form. The qualitative analysis of the formulation was performed by FTIR, Raman Spectroscopy, XRD and SEM analyses. Ultrasonication method was used for the preparation of mucoadhesive Ciprofloxacin suspension. **Methods**: FTIR (400 cm^{-1} to 4000cm^{-1} region) and Raman (140 to 2400 cm^{-1}region) Spectroscopic studies were carried out and spectra were used for interpretation. X-ray powder diffraction data of pure drug, polymer and the formulation were obtained using a powder diffractometer, scanned from a Bragg's angle(2θ) of 10° to 70° . The dispersion of particle was observed using Scanning electron microscopy techniques. The particle size distribution (PSD) and aspect ratio (AR) of particles in the polymeric suspension were obtained from SEM image analysis. **Results**:The results from FTIR and Raman Spectroscopic analyses suggested that in formulation, the carboxylic groups of Ciprofloxacin and hydroxyl groups of C940 undergo chemical interaction leading to esterification and hydrogen bonding (both intermolecuar and polymeric). The XRD data suggested that the retention of crystalline nature of Ciprofloxacin in the formulation would lead to increase in stability and drug loading; decrease in solubility; and delay in release of the drug from polymeric suspension with better bioavailability and penetration capacity. The SEM image analysis indicated that in the formulation, maximum particles were having aspect ratio from 2 to 4 and standard deviation was very less. **Conclusion**:Our results provide supporting evidences for homogeneous, uniformly dispersed, stable controlled release Ciprofloxacin suspension which would be pharmaceutically acceptable.

INTRODUCTION

Oral controlled release (CR) dosage forms (DFs) have been developed over the past three decades due to their considerable therapeutic advantages, such as ease of administration, patient compliance and flexibility in formulation. Incorporation of the drug in a controlled release - gastro retentive dosage forms (CR-GRDF) can remain in the gastric region for several hours, which would significantly prolong the gastric residence time of drugs and improve their bioavailability, reduce drug wastage and enhance the solubility of drugs [1]. Several approaches are currently used to prolong gastric retention time. The goals of controlled drug delivery are to conserve and maintain effective drug concentration, eliminate night time dosage, improve compliance and decrease side effects [2]. In the present study, polymeric bioadhesive delayed gastric emptying devices have been explored. Ciprofloxacin (Cipro) is a second generation fluoroquinolone antibacterial (Fig

1). 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 [3].

Carbopol940 (C940) is a mucoadhesive, biodegradable and environmentally responsive Carbopol polymer and is considered as 'smart gels' [4, 5]. It consists of chains of polyacrylic acid having cross linking agent allyl ethers of Pentaerythritol (allylpentaerythritol) (Fig 2) [6]. It has 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 [7, 8]. The polymer can protect a drug from its physiological environment by improving it stability *in vivo* [9, 10].

Carbopol940 may form a complex with the low solubility drug like Ciprofloxacin. The interaction between Cipro and C940 can be determined by several methods such as Fourier Transform Infrared (FTIR) Spectroscopy, Raman Spectroscopy, etc. To know the different functional groups and highly polar bonds of both pure Cipro and C940, and their chemical interactions in the mucoadhesive suspension, FTIR analysis was conducted. However, their backbone structures and symmetric bonds were checked by Raman spectroscopy. Although it is known that Raman and FTIR are complementary vibrational spectroscopic techniques, there are band intensity differences between the two techniques. That is why both FTIR and Raman Spectrscopic analyses were conducted.

The X-ray diffraction (XRD) method has become one of the most useful tools for qualitative characterization of crystalline compounds both in formulation and in pure form of the drug. It is known that increased dissolution rate and delayed release of drug from dosage forms occur with increase in crystallinity [11, 12]. XRD study is important because any change in the morphology of polymers, or in the crystalline state of active ingredients in the final product, resulting from the manufacturing process, can influence a drug's bioavailability [13].The particle size distribution (PSD) and aspect ratio (AR) of particles in the suspension are obtained from Scanning electron microscopic (SEM) analysis [14-16]. The PSD and AR distribution and degree of dispersion in the suspension give insights even into the stability relating to the modification of mechanical properties, particle-matrix interaction, polymer and drug crystallinity and the overall structure of the suspension [14, 17, 18, 1]Therefore, to obtain more detailed information about chemical interaction between Ciprofloxacin and C940, FTIR and Raman analyses were carried out [20]. Moreover, considering the bioavailability, stability and degree of dispersion of the particles present in the formulation, XRD and SEM analyses were conducted [5, 7, 8, 17, 18]. Considering all the positive aspects of different qualitative analyses, in the present investigation we performed FTIR analysis, Raman Spectroscopy, XRD and SEM studies.

MATERIALS AND METHODS

Materials

The following materials were used for the study: Ciprofloxacin was obtained from Dr. Reddy's Lab, Hyderabad, India, as a gift sample. Carbopol940, Pluronic F 68 and Soya lecithin were purchased from Himedia Laboratories Pvt. Ltd., India. Glycerol, Methyl praraben sodium, Propyl paraben sodium, Sorbitol solution I.P. and Sucrose were supplied by Cosmo Chem. Laboratory, Pune, India. Ultra pure water was obtained from a Millipore Milli-Q UV water filtration system.

METHODS

Preparation of Formulation

Preparation of Bulk A

In a beaker 6 ml water was taken and it was heated up to 80° C. Sucrose (10 gm) was added to that water with continuous stirring. The temperature was monitored in such a way so that it should not fall below 70°C, till the sucrose was completely dissolved. The prepared syrup was cooled properly at room temperature and kept overnight. Syrup was filtered using 120 mesh nylon cloth.

Preparation of Bulk B

Five millilitre of Ultra pure water was taken in a beaker to which 1.8 ml of sorbitol solution and 0.2 ml glycerin were added. The mixture was stirred

TABLE: 1 formula for preparation of Mcoadhesive Suspension

INGREDIENTS	QUANTITY REQUIRED
Ciprofloxacin	6%
Carbopol940	5%
Pluronic F 68	5%
Soya lecithin	1%
Sorbitol Solution (80%)	7.2%
Glycerin	0.8%
Methyl paraben sodium	0.015%
Propyl paraben sodium	0.08%
Simple Syrup IP	40%
Purified water qs up to	100ml

properly. Pluronic F 68 (5%), soya lecithin (1%) and C940 (5%) in w/w of drug were added to this solution with continuous stirring.

Preparation of Mucoadhesive Suspension and Ultrasonication

Five millilitre of water was taken in another beaker to which 1.25gm of Ciprofloxacin was added. To the drug suspension, the bulk B and bulk A were added with continuous stirring. Methyl paraben sodium (0.015%w/v) and Propyl paraben sodium (0.08%w/v) were added as preservatives. The volume was made up to 25 ml by Ultra pure water. The pH was adjusted to 5.5. Homogenization was carried out for at least 20 min by ULTRASONIC HOMOZENIZER LABSONIC^{R} M (SARTORIUS), having operating frequency 30 KHZ and line voltage 230 V/50 HZ, using the probe made up of Titanium of diameter 7 mm and length 80 mm. The setting knob "cycle" was adjusted to 0.8, indicating sound was emitted for 0.8 s and paused for 0.2 s. In this manner, we could expose our sample with 100% amplitude, while reducing the heating effect to 80%. This LABSONIC^{R}M generates longitudinal mechanical vibrations with a frequency of
30,000 oscillations / s (30 KHZ). The probes bolted to the sound transducer were made of high-strength Titanium alloys, built as built as λ /2 oscillators. It amplified the vertical oscillation, and transferred the ultrasonic energy via its front surface with extremely high power density into the sample that was to be subjected to ultrasonic waves. In our study, stress applied was sound wave and in addition, mild rise in temperature of the sample occurred during ultrasonication which helped in the homogenization of the suspension. Some portion of the homogenized suspension was kept for Raman Spectroscopic analysis and SEM study. The remaining portion of the suspension was 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 sample was then divided into two parts one part was for FTIR analysis, and the other part was used for XRD study.

Fourier Transform Infrared Spectroscopy

FTIR analysis was performed by FTIR Spectrophotometer interfaced with 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 the x and y directions.in the x and y directions. Solid powder samples were oven dried at around 30°C, finely crushed, mixed with potassium bromide (1:100 ratio by weight) and pressed at 15000 psig (using a Carver Laboratory Press, Model C, Fred S.

carver Inc., WIS 53051) to form disc. The detector was purged carefully using clean dry nitrogen gas to increase the signal level and reduce moisture. The spectra were collected in the 400 cm^{-1} to 4000 cm^{-1} region with 8 cm^{-1} resolution, 60 scans and beam spot size of 10 ìm-100 ìm [21-23]. The FTIR imaging in the present investigation was carried out using a Perkin Elmer Spectrum RX.

Raman Spectroscopic Analysis

The Raman system R-3000 instrument (Raman systems INC.USA), a low resolution portable Raman Spectrometer using a 785 nm solid state diode laser, was adjusted to deliver 250 mw to the sample having spectral resolution 10 cm^{-1} and 12 v dc/5A power supplies and USB connectivity. The solid powder samples i.e., both pure drug and polymers were enclosed in plastic poly bags and tested directly. For our study the fibre optic sampling probe was directly dipped into the formulation (prepared as per the above mentioned procedure) to collect the spectra at room temperature. The interference of the outside light was also prohibited to prevent photon shot noise. The spectra were collected over the wave number range from 140 to 2400 cm^{-1}.

X-Ray Diffractometry

XRD measurements were obtained using the Philips X'Pert on 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. The powdered specimen was packed and prepared in a specimen holder made of glass. In setting up the specimen and apparatus, co-planarity of the specimen surface with the specimen holder surface and the setting of the specimen holder at the position of symmetric reflection geometry were assured. The powders were passed through a 100 mesh sieve and were placed into the sample holder by the side drift technique [24]. 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 and tapped gently. Each sample was scanned from 10° to 70° (2θ) and in stage sizes of 0.020; count time of 2.00 s, using an automatic divergence slit assembly and a proportional detector. The samples were scanned at 25° C. Relative intensities were read from the strip charts and corrected to fix slit values.

Scanning Electron Microscopy

In order to examine the particle surface morphology and shape, SEM was used. The mucoadhesive suspension (as mentioned above) was sprayed on to an aluminum slip with the aid of an atomizer. The fine droplets were dried overnight and it was used for SEM analysis. [25] The samples were given a conductive coating (using Pt, of about 600 A° thick), using sputter ion coater and examined with SEM (JEOL JSM-6480LV) equipped with a backscattered electron detector for imaging and EDXA for elemental analysis. In this method, a focused electron beam is scanned over the sample in parallel lines. The electrons interact with the sample, producing an array of secondary effects, such as back-scattering, that can be detected and converted into an image. The image can then be digitalized and presented to an image analyzer, which uses complex algorithms to identify individual particles and to record detailed information about their morphology. Then particle size can be determined with a programme such as Image Tool or annotate either automatically or manually. Here, manual determination is preferred, because sometimes the particle boundaries are indistinct, and the software may interpret them incorrectly. The PSDs reflect the statistical result from all sections for each sample. As these are rod like particles, the aspect ratios of rod-like particles are evaluated by comparing the particle size distribution data derived from SEM analysis following the techniques described by Jennings and Parslow [17, 18]. Length/width ratios are satisfactorily determined by the aspect ratio value.

RESULTS

FTIR Analysis

In the FTIR spectra of Cipro, one prominent characteristic peak was found between 3500 and 3450 cm^{-1} (Fig 3A), and was assigned to an 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} indicated 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 peaks 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 (Table 1a) [21,26].

In case of FTIR spectrum of C940, band at 2960.73 cm^{-1} suggested υ_{O-H} i.e., intramolecular hydrogen bonding (Fig 3B). While the peak at 1712.79 cm^{-1} represented $\upsilon_{C=O,}$ the bands at 1452.79 cm^{-1} and 1246.02 cm^{-1} were assigned to υ_{C-O} / δ_{O-H} and υ_{C-O-C} (for acrylates), respectively [22, 26]. The ethereal cross linking, proved by prominent peak at 1172.72 cm^{-1}, indicated stretching vibration of υ_{C-O-C} group and finally the band at 800.46 cm^{-1} represented $\delta_{=C-H}$ i.e., out of plane bending of =C-H group (Table 1b) [21,26].

In case of FTIR spectrum of the formulation the prominent peak found at 3527.80 cm^{-1} was assigned to polymeric υ_{O-H} group (Fig 3C). The band between 3040 and 3010 cm^{-1} was due to $\upsilon_{=C-H}$ (m). While the peak at 2704.2 cm^{-1} represented intermolecular hydrogen bonding, the bands at 1707 cm^{-1} was assigned to for $\upsilon_{=C-O.}$ Moreover, the bands at 1622 cm^{-1} and 1463.25 cm^{-1} were due to both asymmetric and symmetric stretching vibration of O-C-O group of carboxylic acids, respectively. The peak at 1259.16 cm^{-1} represented υ_{C-O-C} of acrylates and ethers. While the band at 1050-1000 cm^{-1} was assigned to $\upsilon_{C-F,}$ the peak at 800 cm^{-1} was due to bending vibration of Ar-H groups (Table 2) [21,22,26].

Raman spectroscopy

In case of Ciprofloxacin, the prominent Raman shifts were observed at 484.22, 771.47, 1411.63 and 1655.11 cm^{-1} (Fig 4A). The Raman shifts at 484.22 cm^{-1} indicated strong bending vibration of C-C of the aliphatic chain of cyclopropyl group and C-N stretching vibration of piperazinyl group [27-29]. The band at 771.47 cm^{-1} represented the symmetric stretching vibration of C-F group [30]. The peak at 1411.63 cm^{-1} was due to symmetric stretching vibration of O-C-O group of carboxylic acid and methylene deformation mode of the piperazinyl group [31]. A band at 1655.11 cm^{-1} was for symmetric stretching of the carbonyl group $\upsilon_{C=O}$ of the pyridone moiety, the stretching vibration of (C-C) aromatic ring chain. In addition, it (peak at1655.11 cm^{-1}) also indicated the $N^{+}H_2$ scissoring of piperzinyl group (Table 3a) [25,1-35]

In case of C940, the prominent bands were found at 337, 523.89, 876.80, 1366.5 and 1687.5 cm^{-1} (Fig 4B). The band at 876.80 cm^{-1} was assigned to stretching vibration of (C-O-C) of acrylates. The Raman shifts at 1366.5 cm^{-1} and 1687.5 cm^{-1} were the characteristics for the symmetric stretching vibration of O-C-O and carboxylic group (C=O) of acids (Table 3b) [28,32].

In case of Raman spectrum of mucoadhesive suspension containing Cipro with C940, the Raman peaks were observed at 179.2, 359.38, 862.5, 1402.4 and 1680 cm^{-1} (Fig 4C). The Raman band at 179.2 cm^{-1} was due to lattice vibration in Cipro crystal structure. The Raman shift at 862.5 cm^{-1} was the characterstics of stretching vibration of acrylates and esters. The Raman peak at 1402.4 cm^{-1} was assigned to symmetric stretching vibration of O-C-O of esters. The Raman peak at 1680 cm^{-1} represented symmetric stretching vibration of carbonyl group of esters (Table 3c) [28,36,37].

XRD Study

All the high intensity peaks (relative intensity) observed in the XRD pattern of the pure Cipro were compared with its mucoadhesive polymeric suspension (Tables 4 and 5). Both the polymeric suspension and pure Cipro were found to show similar XRD patterns (Fig 5). Identification of the molecular structure from its powdered diffraction pattern is based upon the position of peaks and their relative intensities. Each XRD pattern is characterized by the interplanar d-spacing and the relative intensities (I/I_0) of the three strongest peaks in the pattern under the Hanawalt system. The relative intensities and heights of three prominent peaks of our formulation were less than those of pure Cipro (Table 4). The entire diffractograms, rather than selected peaks, are still required to distinguish samples. Hence, comprehensive Å and I/I_0 data clearly identify Ciprofloxacin even in the polymeric composites (Table 5).

Table 2: FTIR Peaks of both Ciprofloxacin and C940 [21, 22, 26]

(a) Prominent FTIR Peaks of pure Ciprofloxacin		
PEAKS(cm-1)	**GROUPS**	**PEAK ASSIGNMENT**
3500-3450	Hydroxyl group	O-H stretching vibration, intermolecular H-bonded
3000-2950	Aromatic,cyclic enes	u=CH & Ar-H
2900	Cyclopropyl group	C-H stretching vibration
1750-1700	CO group of acid	C=O stretching vibration
1650-1600	Quinolines	δN-H bending vibration
1450-1400	Carbonyl group	uC-O
1300-1250	Hydroxyl group	δO-H bending vibration
1050-1000	Fluorine group	C-F stretching

(b) Prominent FTIR Peaks of pure C940		
PEAKS(cm-1)	**GROUPS**	**PEAK ASSIGNMENT**
2960.73	Hydroxyl group	O-H stretching vibration, intramolecular H-bonded
1712.79	C=O group of acids	$u_{C=O}$ stretching vibration
1452.40	Carbonyl group of acids	u_{C-O}
1246.02	Acrylates	C-O-C stretching vibration
1172.72	Ethereal C-O-C group	Stretching vibration of C-O-C group
800.46	Aromatics & enes	=C-H out of plane bending vibration

Table 3: Prominent FTIR Peaks of Mucoadhesive Suspension [21, 22, 26]

PEAKS(cm-1)	GROUPS	PEAK ASSIGNMENT
3527.80	Hydroxyl group	H -bonding by single bridge
3040-3010	enes	$\upsilon_{=C-H(m)}$
2704.2	Strong H- bonding	O-H stretching vibration
1707	C=O groups	$\upsilon_{C=O}$
1622	O-C-O group of acid	υ_{as} stretching vibration of O-C-O group
1463.25	O-C-O group of acid	υ_{s} stretching vibration of O-C-O group
1259.16	Acrylates & esters	C-O-C stretching vibration
1050-1000	C-F groups	υC-F
800	Aromatic m – distribution	δAr-H

SEM Analysis

The length/width ratios of individual particles can satisfactorily determine their aspect ratios. PSD analysis of the formulation showed different ranges of length of particles along with their frequencies (Table 6, Fig 6). While within 20-30 µm range no particle was found, maximum number of particles was observed within 5-10 µm. In case of formulation, maximum aspect ratio (AR) frequency was found from 2 to 4 (Table 7).

DISCUSSION

When FTIR radiation falls on a molecule, it may be absorbed, reflected or transmitted. Absorption leads to the FTIR spectrum, while reflection leads to scattering which is utilized in Raman Spectroscopy [21]. In addition, Infra red (IR) absorption of the functional groups may vary over a wide range. However, it has been found that many functional groups give characteristics IR absorption at specific narrow frequency range [21,22].

Infra red (IR) absorption of the functional groups may vary over a wide range. However, it has been found that many functional groups give characteristics IR absorption at specific narrow frequency range [21,22].

In case of FTIR spectra of Cipro, prominent peaks for υ_{C-O} / δ_{O-H} and $\upsilon_{C=C}$ indicated the presence of CO-, -CHO and -COOH groups (Fig 3A). The presence of above groups can be confirmed by fermi resonance bands for CHO, $\tilde{o}_{C-O-C}$ bands for esters and absence of these two for ketones. This suggested the existence of COOH group in Cipro molecule (Table 1a).

In case of FTIR spectra of Carbopol940, there were prominent peaks for intramolecular hydrogen bonding, υ_{O-H} stretching vibration, carboxylic C=O and C-O stretching vibration, and stretching vibration for the C-O-C, which confirmed the presence of acrylates (Fig 3b). The peak for out of plane bending vibration of =C-H was found between 850 and 800 cm^{-1} (Table 1b).

While comparing the FTIR spectra among the pure Cipro and C940, and the formulation containing both Cipro and C940, it is clear that the band position of =O group has been affected by esterification and conjugation involving C=O group. Here, the stretching vibration of C=O in pure Cipro was found from 1750 to 1700 cm^{-1}, which was lowered to 1622 cm^{-1} in this formulation might be due to formation of â-ketoesters (Fig 3). 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 drug (Cipro). The stretching vibration of C-F group remains nearly unaltered. The another probability of interaction is hydrogen bonding i.e., intermolecular hydrogen bonding due to prominent FTIR peaks at 3527.80 cm^{-1} and 2704.2 cm^{-1}. In case of intramolecular hydrogen bonding, FTIR bands are sharp while

TABLE 4: Comparative Raman Peak Assignments [27, 36]

(a) Prominent Raman Shifts of Ciprofloxacin

RAMAN SHIFTS(cm-1)	FUNCTIONALGROUPS / VIBRATION
484.22	Strong δ(CC) aliphatic chain
771.47	υ(CC) Alicyclic chain vibration
1411.63	υ_sO-C-O
1655.11	pyridone moiety

(b) Prominent Raman Shifts of C940

RAMAN SHIFTS(cm-1)	FUNCTIONAL GROUPS / VIBRATION
337	Strong δ(CC) aliphatic chain
523.89	C-C-O bending vibration
876.80	υ(C-O-C) of acrylates
1366.5	υ_sO-C-O of acids
1687.5	υ_s of C=O group of acids

(c) Prominent Raman Shifts of Mucoadhesive Suspension

RAMAN SHIFTS(cm-1)	FUNCTIONAL GROUPS / VIBRATION
179.2	Lattice vibration in crystals
359.38	Strong δ(CC)aliphatic chain
862.5	υ(C-O-C) of acrylates/esters
1402.4	υ_s (O-C-O) symmetric
1680	$\upsilon_{C=O}$ medium

in intermolecular hydrogen bonding bands are broad. However, it is less broad than which is required for chelation. The FTIR peak at 800 cm^{-1} gave the probability of out of plane bending of ene bond and m-substitution of $ä_{Ar-H}$ hydrogen atom [21, 22, 26] (Table 2).

The C=O group of drug lowers the stretching vibration of C=O frequency indicating deprotonation and probably interaction of the said carboxylic C=O moiety with the polymer. However, a definitive conclusion about the keto group in the bonding to the polymer can be deduced because the corresponding band found from 1707 to 1622 cm^{-1} is due to probability of formation of β-ketoesters [30]. From the above data it can be inferred that the carboxylic group of Cipro undergoes the interaction with the polymer, as would be expected chemically. Thus the nitrogen atoms aren't likely to be involved in binding or the interaction. The nitrogen atom of the quinolone ring, 1-ortho to fluorine, is less electron rich due to electron deficient fluoroquinolone ring. In addition, cyclopropyl and piperazinyl groups sterically hinder the reaction. The possibility of involvement of imino moiety of the piperazinyl group is also less prominent due to intense OH stretching vibration. The bands in the region 3500-2700 cm^{-1} can be assigned to the asymmetric and symmetric stretching vibrations; of the OH groups of the inner and outer sphere of polymer. The shift in the characteristic bands of the FTIR spectra suggests change of their intensity leading to the appearance of several absorbance bands of the asymmetric and symmetric stretching vibrations and overtone of the deformation vibrations. This indicates the confirmation of the hydrogen bonding [22]. By comparing the FTIR spectra among the pure drug, Carbopol polymer (C940) and the formulation containing both drug and polymer, the FTIR peak of Cipro from 1750 to 1700 cm^{-1} was not detected in the mucoadhesive system probably due to interaction with polymer. The missing peak has been replaced with two very strong characteristic bands, in the range of 1622 cm^{-1} and at 1463.25 cm^{-1}, are assigned to $υ_{(O-C-O)}$ asymmetric and symmetric stretching vibrations respectively [21,22,26]. The difference ? $[υ_{(CO2)asym}-υ_{(CO2)sym}]$ is a useful characteristic for

Table 5: 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 Formulation

Sl. No	Ciprofloxacin				Mucoadhesive Suspension			
	2θ	Å	I/I_0	H	2θ	Å	I/I_0	H
1	19.22	4.61	54.85	900	19.22	4.62	64.73	773
2	26.39	3.37	100	1642	26.38	3.38	100	1194
3	29.16	3.06	28.47	467	29.13	3.06	33.88	405

2θ - angle of incidence of the X-ray beam; d - distance between adjacent planes of atoms; I/I_0 - relative intensities; H – peak height

Table 6: Lattice spacing (Å) and relative intensities (I/I_0) of all the identifiable peaks in the diffractograms of Ciprofloxacin and its Formulation

Sl. No	Ciprofloxacin		Mucoadhesive Suspension	
	Å	I/I_0	Å	I/I_0
01	7.86	9.10	7.85	10.93
02	5.50	5.37	7.27	3.93
03	5.85	10.98	6.50	7.86
04	5.42	5.76	5.85	14.05
05	4.71	38.92	5.42	9.62
06	4.61	54.85	4.71	47.61
07	4.51	22.06	4.62	64.73
08	4.23	7.98	4.49	22.43
09	3.96	5.97	4.24	13.49
10	3.86	18.01	3.86	21.51
11	3.60	19.61	3.61	26.06
12	3.37	100.00	3.38	100.00
13	3.31	27.18	3.20	20.36
14	3.26	13.26	3.06	33.88
15	3.20	15.74	2.95	16.41
16	3.10	28.47	2.83	13.97
17	2.95	11.48	2.74	9.94
18	2.83	13.70	2.68	9.00
19	2.74	6.81	2.61	8.36
20	2.68	7.16	2.58	11.41
21	2.61	5.69	2.51	11.43
22	2.58	8.94	2.42	13.91
23	2.51	9.22	2.37	10.46
24	2.42	11.24	2.30	12.54
25	2.37	7.84	2.17	9.95
26	2.30	10.40	2.14	6.99
27	2.27	4.49	2.08	9.71
28	2.23	2.69	1.98	6.81
29	2.17	7.79	1.94	6.09
30	2.13	5.80	1.90	5.29
31	2.08	7.46	1.82	5.04
32	2.06	7.29	1.73	3.64
33	1.98	5.70	1.71	4.97
34	1.94	5.03	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

2θ - angle of incidence of the X-ray beam; d - distance between adjacent planes of atoms; I/I_0 - relative intensities

determining the involvement of the carboxylic group of Cipro. The ?value for the interaction falls in the range of 183 - 250 cm^{-1} indicating the deprotonation of the carboxylic acid group and interaction between drug and polymer [36] (Tables 1 and 2).

In case of Raman spectra of Cipro, the Raman band at 771.47 cm^{-1} is assigned to the stretching vibration of cyclopropyl group. The presence of carboxylic acid group is confirmed by $\upsilon_{O\text{-}C\text{-}O}$ and $\upsilon_{C=O}$ groups vibration at 1411.63 cm^{-1} and 1655.11 cm^{-1}, respectively (Table 3a).

By comparing the Raman spectra of pure drug with the drug incorporated in the Carbopol suspension, the peak at 1402.4 cm^{-1} was assigned to the$\upsilon_{s\ O\text{-}C\text{-}O}$ is not prominent. While both symmetric and asymmetric stretching vibrations of O-C-O group are found in suspension containing C940. The Raman peak for stretching vibration of C=O is prominent in the suspension. From this it is clear that there is esterification reaction between the Cipro and Carbopol polymer (Table 3). The results of both FTIR and Raman spectra indicate that both the spectra show prominent peaks for the stretching vibration of O-C-O and C=O groups, which prove the formation of the esters between the drug and polymer. Moreover, both the intermolecular and polymeric hydrogen bondings are also prominent from the FTIR spectra of the formulation.Tables 4 and 5 give the XRD data obtained for the pure Cipro, and its polymeric suspension with C940 in terms of the lattice spacing and the relative peak intensities. Most of the characteristic peaks in the diffraction patterns are generally prominent and sharp, so measurement of the angles and d-values is accurate.

From the XRD patterns of C940, it is clear that the polymer is fully crystalline in nature as there are sharp and prominent peaks (Fig 5). Table 4 confirms that the three prominent peaks of pure Cipro and its mucoadhesive suspension do not have similar d-spacing corresponding to identical 2è values. As the d-spacing of the prominent XRD peaks of pure Cipro is changed in the polymeric composites, it may be concluded that there is interaction between Cipro and C940 (Fig 5). However, Cipro can be easily distinguished even in the formulation. Since relative intensities of the peaks are decreased in formulation, crystallinity is also reduced in the composites as compared with pure Cipro. This decrease in relative intensities of these peaks appears to be due to change in atomic densities in that particular plane of crystal lattice. From this we may predict that there is a little bit change in the orientation of crystal lattice due to incorporation of some extra atoms into it, which may be due to hydrogen bonding and esterification.

As we know the standard deviation measures the absolute dispersion (or variability of a distribution), a small standard deviation indicates a high degree of uniformity of the observations as well as homogeneity of a series. The series, in which co-efficient of variation is less, is said to be less variable, and more consistent, uniform, stable and homogeneous. From PSD study it has been found that maximum particle size of the formulation is within the pharmaceutically acceptable limit (Table 6) [38]. From Table 6, it is clear that the maximum particle size range for formulation containing Cipro and C940 is between 5 and 10 µm. As these are rod like particles, A.R. has been calculated. From the statistical interpretation, it has been found that aspect ratios in the formulation containing Cipro and C940 are homogeneous, consistent and stable with lesser standard deviation (Table 7).

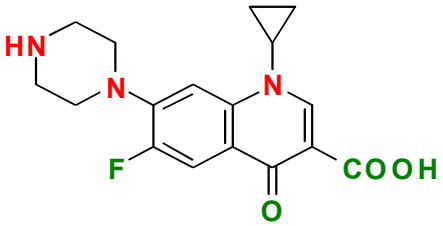
The mean particle size and A.R. values of the formulation (9.95 µm and 4.76, respectively) show a correlation between the particle size, particle shape and stability properties, giving confidence in the usefulness of SEM for characterizing such type of formulations [39,40,41].

The morphologies and mechanical properties of the formulation impart SEM sectioning and imaging, which can allow direct measurement of PSD and A.R. of particles embedded in polymeric suspension. The SEM-derived information correlated well with the mechanical properties of the present formulation. From the above SEM image analysis, it is expected that the formulation

Table 6: Particle Size Distribution of Mucoadhesive Suspension

L (µm)	f	c.f	m	(m–A)/ i or (m-12.5)/i =d	fd	Â	fd²	ó	C.V.
0-5	1	1	2.5	-2	-2		4		
5-10	28	29	7.5	-1	-28		28		
10-15	18	47	12.5	0	0		0		
15-20	4	51	17.5	1	4	9.95	4	3.34	33.56 %
20-25	0	51	22.5	2	0		0		
25-30	0	51	27.5	3	0		0		
	N= 51				Ó fd= -26		Ó fd²= 36		

L – Length of each particle; f – frequency; c.f – cumulative frequency; m.p (m) – midpoint;
A – assumed mean; i – class interval; d – deviation of midpoint from assumed mean;
Ā – actual mean; σ – standard deviation; C.V. – coefficient of variation;
N – total number of particles taken into consideration



Ciprofloxacin

Figure 1: Chemical Structure of Ciprofloxacin

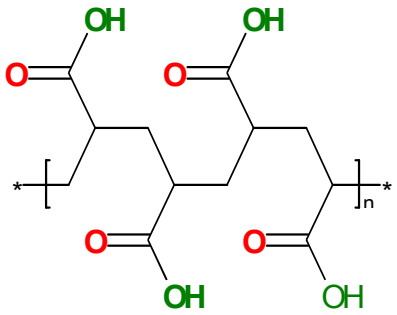


Figure 2: Chemical Structure of Carbopol Polymer

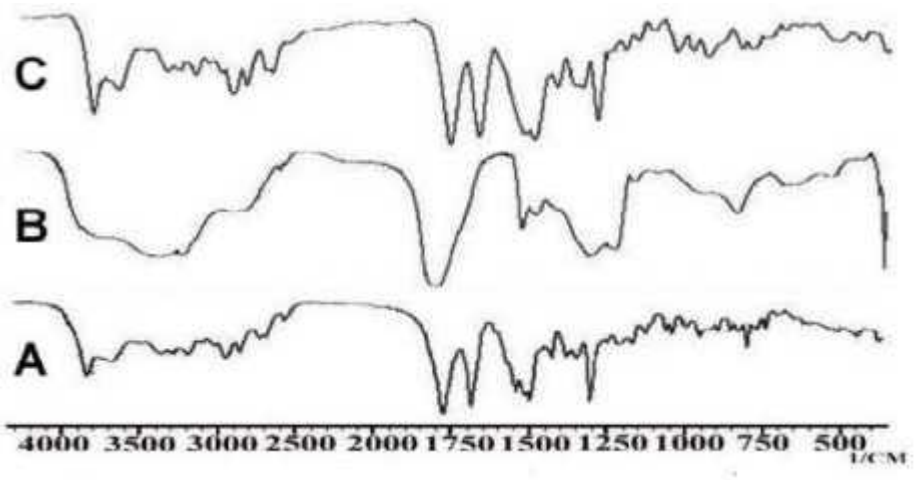


Figure 3: FTIR spectra of (A) Ciprofloxacin, (B) Carbopol 940 and (C) Mucoadhesive Suspension

Table 8: Aspect Ratio Analysis of Mucoadhesive Suspension

L (µm)	f	c.f	m	(m–A)/ i or (m-12.5)/i =d	fd	Â	fd^2	ó	C.V.
0-5	1	1	2.5	-2	-2		4		
5-10	28	29	7.5	-1	-28		28		
10-15	18	47	12.5	0	0		0		
15-20	4	51	17.5	1	4	9.95	4	3.34	33.56 %
20-25	0	51	22.5	2	0		0		
25-30	0	51	27.5	3	0		0		
	N= 51				Ófd= -26		$Ófd^2$= 36		

L – Length of each particle; f – frequency; c.f – cumulative frequency; m.p (m) – midpoint; A – assumed mean; i – class interval; d – deviation of midpoint from assumed mean; Ā – actual mean; σ – standard deviation; C.V. – coefficient of variation; N – total number of particles taken into consideration; D – width of each particle

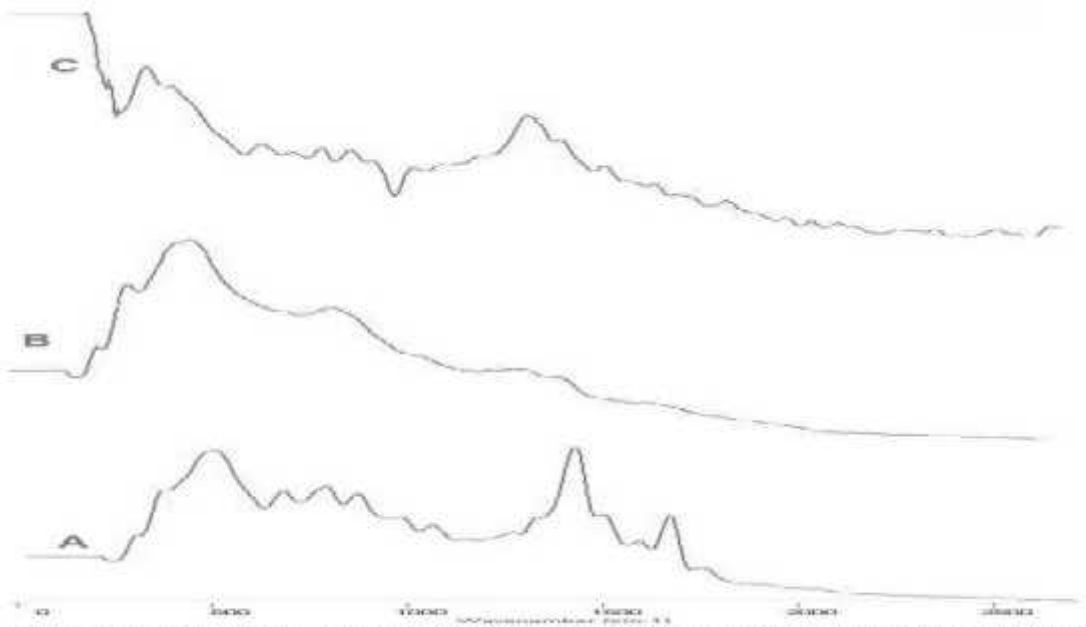


Figure 4: Raman Shifts of (A) Ciprofloxacin, (B) Carbopol 940 and (C) Mucoadhesive Suspension

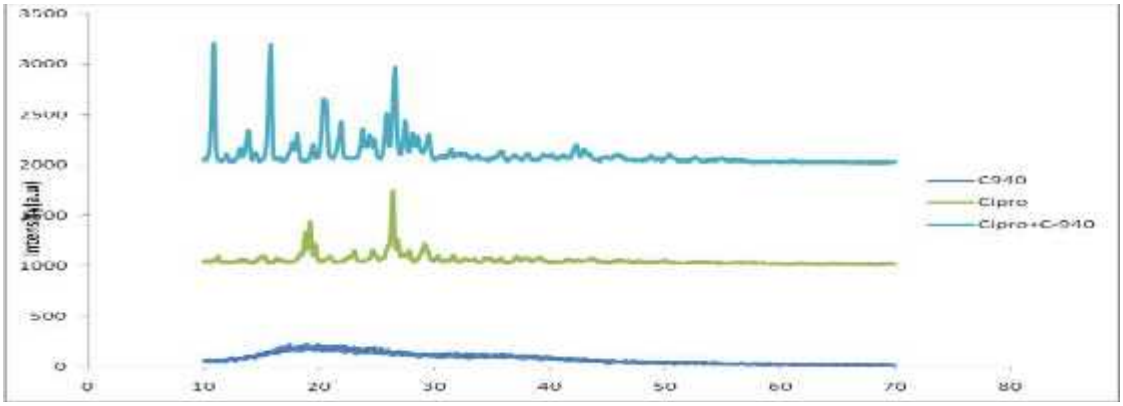


Figure 5: X-ray diffraction patterns of C940, Cipro and Mucoadhesive Suspension

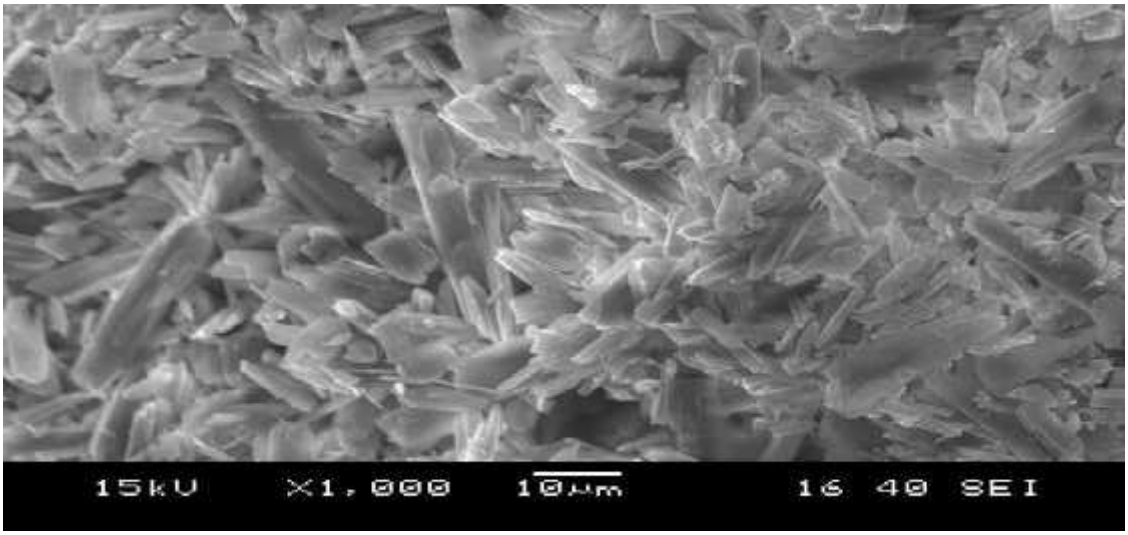


Figure 6: SEM of Mucoadhesive Suspension

CONCLUSION

On the basis of the above interpretation, it can be concluded that by preparing mucoadhesive suspension of Ciprofloxacin with C940 following a novel method of ultrasonication, there is a very good interaction between the carboxylic group of drug and hydroxyl group of polymer. This leads to esterification and intermolecular hydrogen bonding, by virtue of which a stable mucoadhesive suspension would be produced. From the XRD data supported by FTIR analysis, it appears that the crystalline form of pure Cipro under the experimental conditions resulted in little change in crystal habit of the drug. Moreover, size of the crystals was significantly influenced by intermolecular hydrogen bonding and esterification between Cipro and C940. The retention of crystallinity nature of the drug in the formulation may lead to increase in stability, decrease in solubility and delay in release of the drug from polymeric suspension. This may result in controlled release action of the formulation. From the SEM image analysis, it may be concluded that the formulation containing Cipro and C940 is having uniform dispersion of particles and stability, which may lead to better bioavailability and penetration capacity than conventional dosage forms.The utility of the present work may be improved if the delivery rate, biodegradation and site-specific targeting of such mucoadhesive suspension would be properly monitored and controlled.

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