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Research Article

CHARACTERIZATION OF AN OFLOXACIN / CARBOPOL940 MUCOADHESIVE POLYMERIC SUSPENSION

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ABSTRACT

Aim: Mucoadhesive polymeric (Carbopol 940) suspension of Ofloxacin was prepared and optimised with the aim to develop an oral controlled release gastro-retentive dosage form. The qualitative analysis of the formulation was performed by FTIR, Raman Spectroscopy, XRD and SEM analyses.

Methods: Ultrasonication method was used for the preparation of mucoadhesive Ofloxacin suspension. FTIR (400 cm^{-1} to 4000 cm^{-1} region) and Raman (140 to 2400 cm^{-1} region) spectra were used for interpretation. X-ray powder diffraction (XRD) data of pure drug, polymer and the formulation were obtained using a powder diffractometer. The dispersion of particle was observed using Scanning electron microscopy (SEM) technique.

Results: The results from FTIR and Raman Spectroscopic analyses suggested that in formulation, the carboxylic groups of Ofloxacin and hydroxyl groups of C940 undergo chemical interaction leading to esterification and hydrogen bonding (both intermolecular and polymeric). The XRD data indicated that the retention of crystalline nature of Ofloxacin in the formulation. The SEM image analysis suggested that in the formulation maximum particles were having aspect ratio from 2 to 4 and standard deviation was very less. Conclusion: It may be concluded that Ofloxacin is compatible with Carbopol 940. It forms a stable suspension without hampering the C-F bond of the quinolone nucleus, which is responsible for the antibacterial activity of the drug. Decrease in solubility and delayed release of the drug from homogeneous, uniformly dispersed polymeric suspension with better bioavailability and penetration capacity are expected. As a result, this polymer may be considered as effective carrier for Ofloxacin.

Keywords: Ofloxacin, Mucoadhesive suspension, C940, FTIR, Raman Spectroscopy, XRD.

INTRODUCTION

There is a demand for a dosage form that will provide a controlled release action of the drug in solution, particularly in the basic pH conditions of the intestinal lumen over the full dosage period. By achieving constant blood level, drug benefit is maximized while its potential toxicity is minimized.[1,2]

As frequent dosing is required to maintain the therapeutic plasma concentration, Ofloxacin (Oflox) was chosen as a model drug for the controlled release study. Ofloxacin, 9-fluro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperizinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxaine-6-carboxylic acid, is a fluoroquinolone antibacterial agent [Fig. 1].[3]

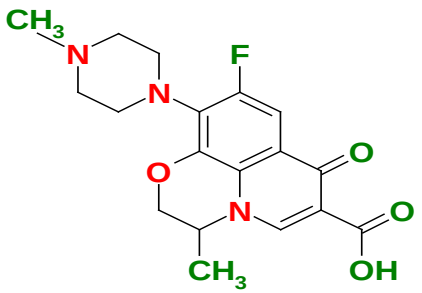
Carbopol polymers form hydrogel that change their swelling behaviour upon exposure to an external stimulus such as change in pH,[4,5] temperature,[6] light, or electric field, and are known as "environmentally responsive polymers" or "smart gels".[7,8] They have recently attracted considerable interest in the field of drug delivery as a means of providing an on-off release by shrinking and swelling in response to the change in pH.[9-12] In stomach, Carbopol polymer forms hydrogen bond with the drug and also with the polysaccharides or proteins of mucosa, which is probably the major mechanism for bioadhesion. In addition, under alkaline condition of the intestine, Carbopol gels are very highly swollen.[13] Carbopol polymer in mucoadhesive formulation may provide a gastric retention system by swelling in the stomach and inducing a pseudofed state, thereby reducing peristaltic contraction. This phenomenon is dependent on viscosity - the higher the viscosity, the lower the contraction.[14] In the present study design, Carbopol940 (C940) is used as a polymer, which consists of chains of polyacrylic acid [Fig. 2].[15] This hydrophilic polymer may form a complex with the low solubility drug like Ofloxacin. Because it is known that the solubility is the crucial factor for drug effectiveness independent of the route of administration.[16]

While the functional groups of the molecules can be determined by FTIR analysis, the backbone structures and symmetric bonds of molecules can be 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. Therefore, to obtain more information in detail about chemical interaction between Ofloxacin and C940, both FTIR and Raman analyses were carried out.[17,18]

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.[19] 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 dissolution rate, release profile and ultimately bioavailability of a drug.[20-22]

Scanning electron microscopic (SEM) analysis of a suspension gives insights even into the stability relating to the modification of mechanical properties, particle-matrix interaction, drug dispersion and the overall structure of the suspension.[23-27]

Considering the importance of FTIR, Raman spectroscopy, XRD and SEM studies, in this article those analyses of the formulation containing Ofloxacin and C940 were carried out.[17-19,22,23,26, 27].



Ofloxacin

Fig. 1: Structure of Ofloxacin

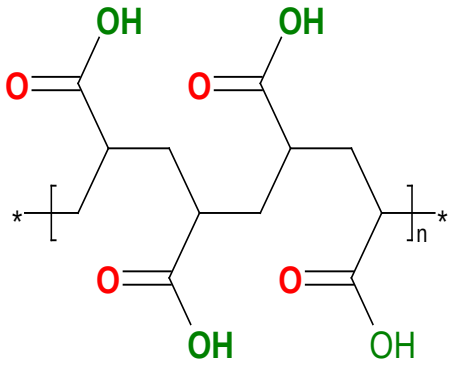


Fig. 2: Structure of Carbopol Polymer (Polyacrylic acid)

MATERIALS AND METHODS

Materials

The following materials were used: Ofloxacin was obtained from Dr. Reddy's Lab, Hyderabad, India, as a gift sample. C940, 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

Praparation of Bulk A

In a beaker, 6 ml water was heated up to 80° C. Sucrose (10 gm) was added under 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.

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

Preparation of Mucoadhesive Suspension and Ultrasonication

Five millilitre of water was taken in another beaker to which 250 mg of Oflox 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 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. The sample was then divided into two parts –one part was for FTIR analysis and the other part was used for Raman spectroscopy.

Fourier Transform Infrared Spectroscopic Analysis

After ultrasonication, the polymeric 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. This powder sample was used for FTIR analysis. The Fourier transform infrared analysis was conducted to verify the possibility of interaction of chemical bonds between drug and polymer. 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. Solid powder samples were oven dried at about 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 µm-100 µm.[28-30] 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 1.5405 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.[31] 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.[32] The samples were given a conductive coating (using Pt, of about 600 A^0 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.[26] Length/width ratios are satisfactorily determined by the aspect ratio value.

RESULTS

The infrared spectra are recorded on Fourier Transform Spectrometer in the mid –infrared region (MIR) within the range (400-4500 cm^{-1}).[33] Due to the complex interaction of atoms within the molecule, IR absorption of the functional groups may vary over a wide range. However, it has been found that many functional groups give characteristic IR absorption at specific narrow frequency range. Multiple functional groups may absorb at one particular frequency range but a functional group often gives rise to several characteristic absorptions. Thus, the spectral interpretations should not be confined to one or two bands only; actually, the whole spectrum should be examined.

While the FTIR bands at 4000-1300 cm^{-1} represented functional group region, the appearance of strong absorption bands in the region of 4000 to 2500 cm^{-1} was due to stretching vibrations between hydrogen and some other atoms with a mass of 19 or less. The O-H and N-H stretching frequencies were in the 3700 to 2500 cm^{-1} region with various intensities. Hydrogen bonding has a significant influence on the peak shape and intensities, generally causing peak broadening and shifts in absorption to lower frequencies. The C-H stretching vibration occurred in the region of 3300 to 2800 cm^{-1}.[28,29]

In FTIR spectra of Oflox, one prominent characteristic peak was found between 3050 and 3000 cm^{-1}, which was assigned to stretching vibration of OH group and intramolecular hydrogen bonding [Fig. 3]. This band also suggested the NH stretching vibration of the imino-moiety of piperazinyl group which was less prominent due to intense OH stretching vibration. The peak at 2700 cm^{-1} was assigned to υCH_3 of methyl group. The band at 1750-1700 cm^{-1} represented the acidic carbonyl C=O stretching i.e., $\upsilon C{=}O$.[34] The peak at 1650 to 1600 cm^{-1} was assigned to υN-H bending vibration of quinolones. The 1550 to 1500 cm^{-1} represented the υCH_2 of the aromatic ring. The band at 1450-1400 cm^{-1} was assigned to the stretching vibration of CH_2 confirming the presence of methylene group in benzoxazine ring. The peak at 1400-1350 cm^{-1} represented the bending vibration of hydroxyl group. The band at 1250 to 1200 cm^{-1} suggested the stretching vibration of oxo group. In addition, a strong absorption peak between 1050 and 1000 cm^{-1} was assigned to C-F group. The band at 900-800 cm^{-1} represented the out of plane bending vibration of double bonded enes or =CH groups [Table 1a].[28,29,35-37]

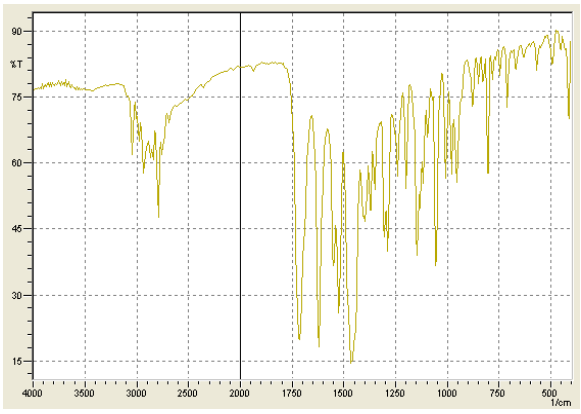


Fig. 3: FTIR Spectra of Ofloxacin

In case of C940, the FTIR spectra having peak between 3000 and 2950 cm^{-1} represented OH stretching vibration, i.e., υO-H and intramolecular hydrogen bonding [Fig. 4]. The prominent band between 1750 and 1700 cm^{-1} was assigned to carbonyl C=O stretching vibration i.e., $\upsilon_{C=O}$. While the peak at 1450 to 1400 cm^{-1} was for $\upsilon_{C\text{-}O}$ / $\delta_{O\text{-}H}$, the band at 1250 to 1200 cm^{-1} was due to $\upsilon_{C\text{-}O\text{-}C}$ of acrylates.[29,33] The peak between 850 and 800 cm^{-1} suggested the out of plane bending of =C-H i.e., δ=C-H [Table 1b].[28,33]

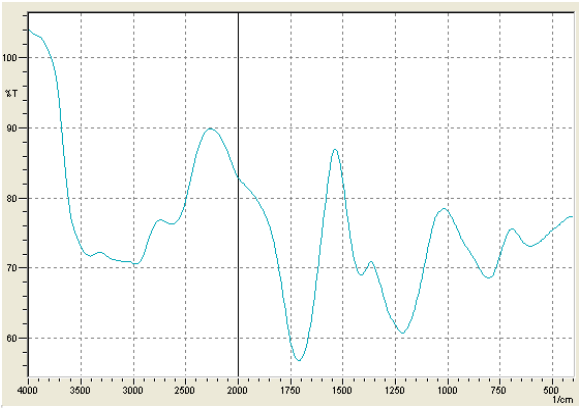


Fig. 4: FTIR Spectra of C940

In case of FTIR spectra of Oflox with C940, the prominent peak found at 3500-3400 cm^{-1} was assigned to polymeric υO-H group [Fig. 5]. The band between 3100 to 3000 cm^{-1} represented υ=C-H (m). While the peak at 2800-2700 cm^{-1} suggested intermolecular hydrogen bonding, the band at 1750-1700 cm^{-1} was assigned to $\upsilon C{=}O$. Moreover, the bands at 1650-1600 cm^{-1} and 1500-1400 cm^{-1} indicated both asymmetric and symmetric stretching vibration of O-C-O group of carboxylic acids, respectively. The peak at 1250-1200 cm^{-1} suggested υC-O-C of acrylates and ethers. In addition, the band at 1050-1000 cm^{-1} was assigned to υC-F and at 800 cm^{-1} was for bending vibration of Ar-H groups [Table 1c].[28,29]

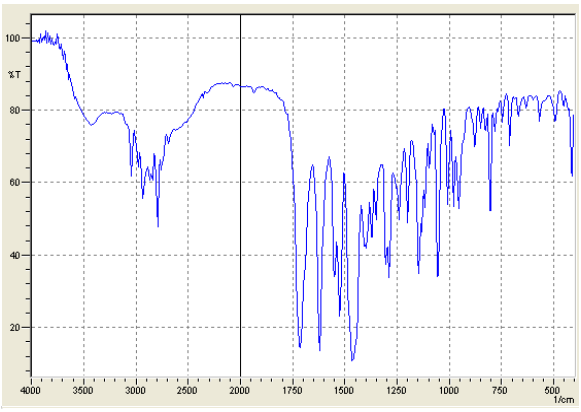


Fig. 5: FTIR Spectra of Ofloxacin Mucoadhesive Formulation

By Raman spectroscopy of Ofloxacin, the prominent Raman shifts were observed at 518.4, 797.5, 1419.8 and 1649.6 cm^{-1} [Fig. 6]. The Raman shift at 518.4 cm^{-1} represented the bending vibration of aliphatic carbon atom, C-N stretching vibration of piperazinyl group and O-H torsional vibration of carboxylic acid.[38-41] The band at 797.5 cm^{-1} suggested the symmetric stretching vibration of C-F group.[42] The peak at 1419.8 cm^{-1} was for the symmetric stretching vibration of O-C-O group of carboxylic acid and methylene deformation mode of the piperazinyl group.[41] A band at 1649.6 cm^{-1} was due to 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 at1649.6cm^{-1}) also indicated the $N^{+}H_2$ scissoring of piperzinyl group [Table 2a].[38,41,43-45]

Table 1: Prominent FTIR peaks of ofloxacin, C940 and formulation

PEAKS(cm-1)	Groups	Peak assignment
a) Prominent FTIR peaks of Ofloxacin		
3050-3000	Hydroxyl group	O-H stretching vibration, intremolecular H-bonded
3000-2950	Aromatic, cyclic enes	υ=CH & Ar-H
2750	Alkyl groups	υCH_3
1750-1700	C=O group of acids	υC=O stretching vibration
1650-1600	Quinolines	δN-H bending vibration
1550-1500	Alkyl groups	υCH_3 and υCH_2
1450-1400	Methylene group in Benzoxazine	stretching vibration of CH_2
1400-1350	Hydroxyl group	δO-H bending vibration
1250-1200	Oxo group	C-O-C stretching vibration
1050-1000	C-F group	C-F stretching
950-800	Aromatics & enes	=C-H out of plane bending vibration
b) Prominent FTIR peaks of C940		
3000-2950	Hydroxyl group	O-H stretching vibration, intramolecular H-bonded
1750-1700	C=O group of acids	$\upsilon_{C=O}$ stretching vibration
1450-1400	Carbonyl group of acids	υ_{C-O}
1250-1200	Acrylates	C-O-C stretching vibration
850-800	Aromatics & enes	=C-H out of plane bending vibration
c) Prominent FTIR Peaks of Ofloxacin Mucoadhesive Formulation		
3500-3400	Hydroxyl group	υ_{O-H}
3100-3000	enes	$\upsilon_{=C-H(m)}$
2800-2700	O-H groups	Intermolecular H-bonded
1750-1700	C=O groups	$\upsilon_{C=O}$
1650-1600	O-C-O group of acid	υ_{as} stretching vibration
1500-1450	O-C-O group of acid	υ_{s} stretching vibration
1300-1250	Hydroxyl group	δ_{O-H}
1250-1200	Acrylates & esters	C-O-C stretching vibration
1050-1000	C-F groups	υ_{C-F}
800	Aromatic & enes	δ_{Ar-H} & $\delta_{=C-H}$

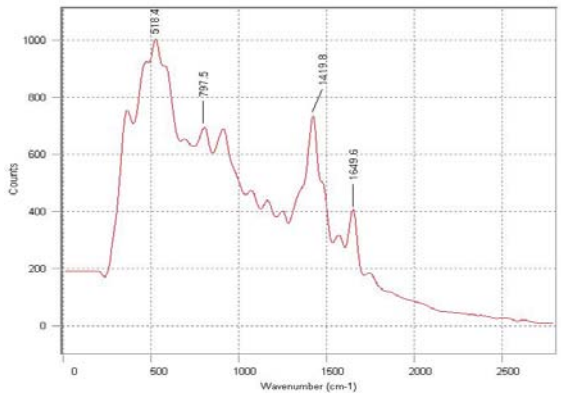


Fig. 6: Raman Shifts of Ofloxacin

The characteristic prominent Raman bands for C940 were observed at 523.9, 876.8 and 1366.5 cm^{-1} [Fig. 7]. The bending vibration of C-C-O group was indicated by the Raman shift at 523.9 cm^{-1}. The band at 876.8 cm^{-1} was due to stretching vibration of C-O-C for acrylates and carboxylic acid. The Raman shift at 1366.5 cm^{-1} was assigned to symmetric vibration of O-C-O of acids [Table 2b].[38]

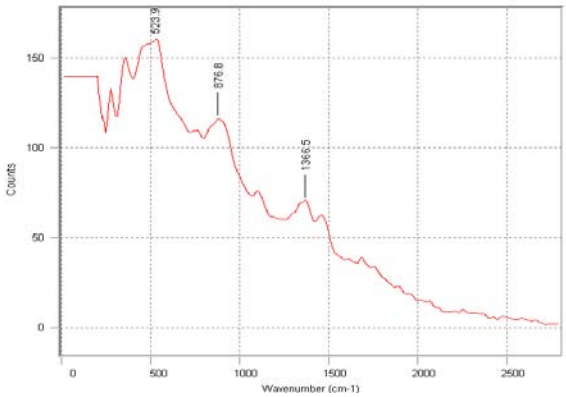


Fig. 7: Raman Shifts of C940

In the formulation containing both Oflox and C940, the Raman peak at 352.9 cm^{-1} represented bending vibration of δCC of aliphatic chain [Fig. 8]. The band at 900 cm^{-1} was assigned to symmetric stretching vibration of both C-F and C-O-C groups for acrylates and esters. The peak at 1050 cm^{-1} represented stretching vibration of carbonyl group. The band at 1250 cm^{-1} suggested symmetric stretching vibration of O-C-O group. The band at 1800 to 1750 cm^{-1} was the characteristic of stretching vibration of carbonyl group of esters [Table 2c].[37,44]

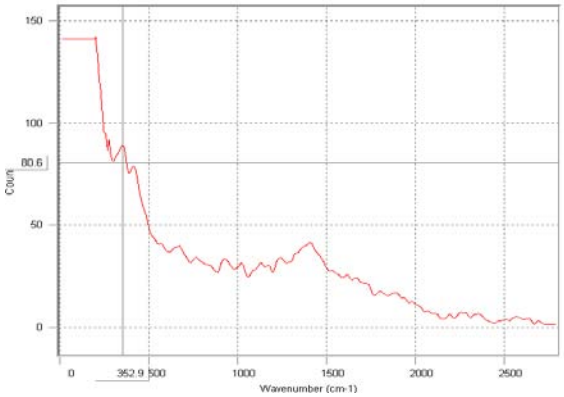


Fig. 8: Raman Shifts of Ofloxacin Mucoadhesive Formulation

Table 2: Raman Shifts of Ofloxacin, C940 and Formulation

Raman Shifts(cm^{-1})	Functional Groups / Vibrations
a) Prominent Raman Shifts of Ofloxacin	
518.4	Strong δ (CC) aliphatic chain, C-N stretching vibration and O-H torsional vibration
797.5	Symmetric vibration of C-F bond
1419.8	υ_S O-C-O and methylene deformation of the piperazinyl group
1649.6	υs of C=O group of pyridone moiety and N^+H_2 scissoring of piperzinyl group
b) Prominent Raman Shifts of C940	
450-300	Strong $\delta_{(CC)}$ aliphatic chain
523.9	C-C-O bending vibration
876.8	$\upsilon_{(C\text{-}O\text{-}C)}$ of acrylates
1366.5	$\delta_{(CH3)}$ medium
c) Prominent Raman Shifts of Ofloxacin Mucoadhesive Formulation	
352.9	δ(CC) aliphatic chain
900	Symmetric stretching vibration of both C-F group C-O-C group for acrylates and esters
1050	Stretching vibration of CO
1250	υ_SO-C-O
1800-1750	υC=O medium

Table 3 gives the XRD data obtained for the pure Oflox, and its polymeric suspension with C940 in terms of the lattice spacing and the relative peak intensities. Identification of the molecular structure from its powdered diffraction pattern is based upon the position of peaks and their relative intensities. Both the polymeric suspension and pure Oflox were found to show similar XRD patterns [Figs. 9-11]. Most of the characteristic peaks in the diffraction patterns were generally prominent and sharp, so measurement of the angles and d-values was accurate. Each XRD pattern was 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. While the relative intensities of drug and formulation were different, their corresponding d-values were more or less identical [Table 3].

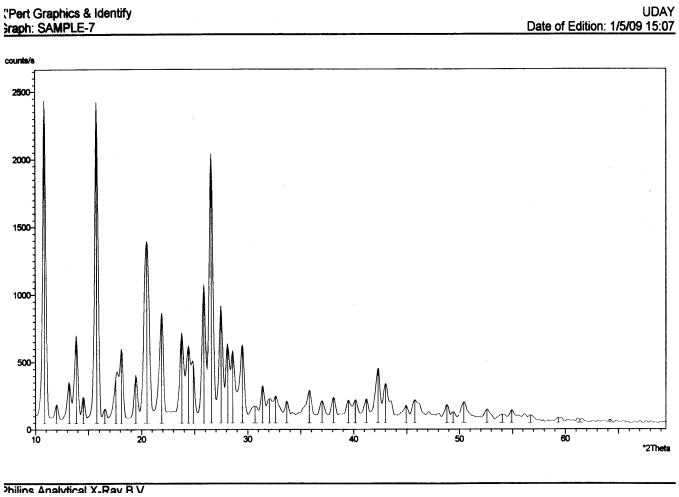


Fig. 9: X-ray diffraction patterns of Ofloxacin

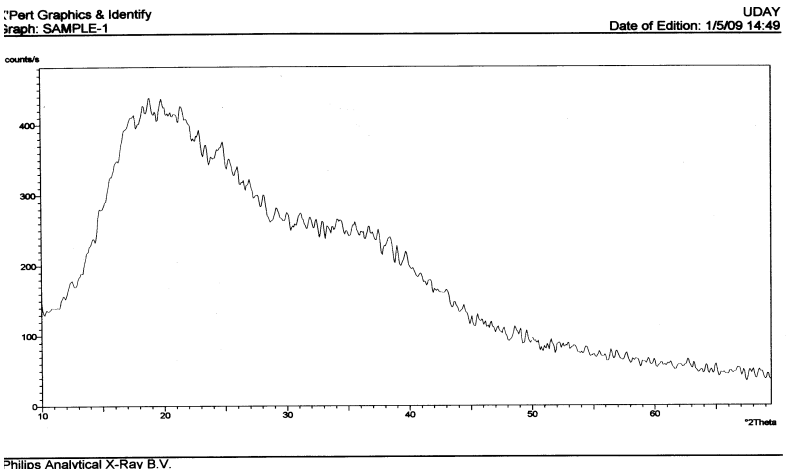


Fig. 10: X-ray diffraction patterns of C940

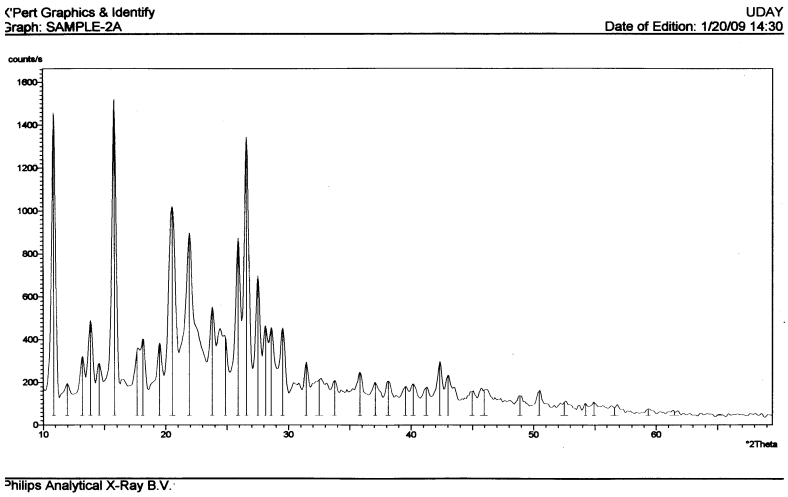


Fig. 11: X-ray diffraction patterns of Mucoadhesive Suspension

Table 3: Lattice spacing (Å) and relative intensities (I/I_0) (based on the Hanawalt System) of the three strongest peaks in the diffractograms of oflox and Mucoadhesive Suspension

Sl. No	Ofloxacin				Mucoadhesive Suspension			
	2θ	Å	I/I_0	H	2θ	Å	I/I_0	H
1	8.14	8.14	100.00	2383	10.86	8.14	95.91	1414
2	15.77	5.61	99.43	2375	15.78	5.61	100	1474
3	26.55	3.35	83.15	1993	26.57	3.35	88.18	1300

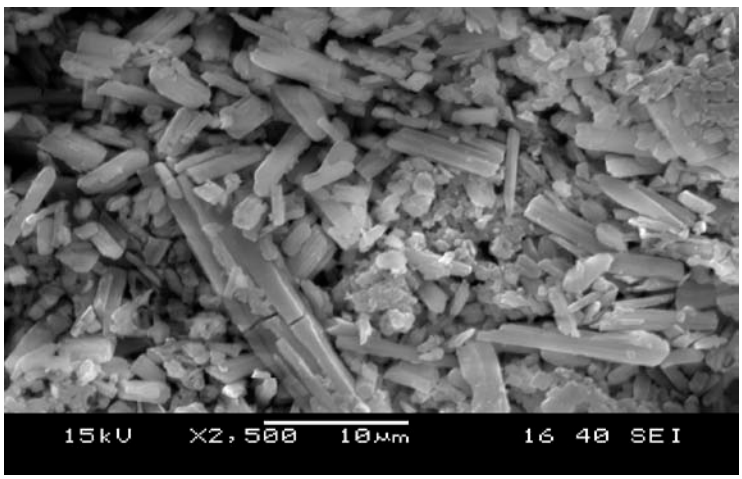


Fig. 12: SEM of Mucoadhesive Ofloxacin suspension

Figure 12 shows SEM image of mucoadhesive suspension. The length/width ratios of individual particles can satisfactorily determine their aspect ratios (ARs). Particle size distribution (PSD) analysis of the formulation showed different ranges of length of particles along with their frequencies. While within 25-30 µm range no particle was found, maximum number of particles was observed within 0-5 µm [Table 4]. In case of formulation, maximum aspect ratio frequency was found to be 2 to 4 [Table 5].

Table 4: Particle Size Distribution of Mucoadhesive Suspension

L (µm)	f	c.f	m	(m –A) / i or (m-7.5)/i=d	fd	Ā	fd^2	σ	C.V.
0-5 5-10 10-15 15-20 20-25	25 20 1 2 2 N=50	25 45 46 48 50	2.5 7.5 12.5 17.5 22.5	-1 0 +1 +2 +3	-25 0 1 4 6 Σfd= -14	6.1	25 0 1 8 18 Σfd^2= 52	4.9	80%

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

Table 5: Aspect Ratio Analysis of Mucoadhesive Suspension

A.R. (L/D)	f	c.f	m	(m–A)/ i or (m-3)/i =d	fd	Ā	fd^2	σ	C.V.
0-2 2-4 4-6 6-8	3 24 15 8 N=56	3 27 42 50	1 3 5 7	-1 0 +1 +2	-3 0 15 16 Σ fd= +28	4.12	3 0 15 32 Σ fd^2= 50	1.66	40%

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

DISCUSSION

From the above mentioned results it is clear that the band position of C=O group in Ofloxacin has been affected by esterification and conjugation in the formulation. The FTIR peaks assigned to $\upsilon_{C\text{-}O}$ and $\upsilon_{C\text{-}O\text{-}C}$ represented acrylates and esters, which confirmed the esterification between polymeric OH and –COOH groups of Oflox. The stretching vibration of C-F group remained nearly unaltered. The C=O group of drug lowers the stretching vibration of C=O frequency in the formulation, 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 could be deduced because the corresponding bands found from 1650 to 1600 cm^{-1} and 1250-1200 cm^{-1} were due to probability of formation of β-ketoesters.[46] From the above data it can be inferred that the carboxylic group of Oflox undergoes the interaction with the polymer, as would be expected chemically. Thus, the nitrogen atoms are not 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, ethyl 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 of 3500-2700 cm^{-1} could 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 in 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.[28,29] In the formulation, the strong characteristic bands in the range of 1650-1600 cm^{-1} and at 1500-1450 cm^{-1}, which were assigned to $\upsilon_{(O\text{-}C\text{-}O)}$ asymmetric and symmetric stretching vibrations, respectively, represented the formation of β-ketoesters (as mentioned earlier).[29,32] The differenc**Δ** [$\upsilon_{(CO2)asym}$-$\upsilon_{(CO2)sym}$] is a useful characteristic for determining the involvement of the carboxylic group of Oflox. The Δ value for the interaction falls in the range of 183 - 250 cm^{-1} indicates the deprotonation of the carboxylic acid group and interaction between drug and polymer [Table 1].[44]

By comparing the Raman spectra of pure drug with the drug incorporated in the Carbopol suspension, the peak at 1418.5 cm^{-1}, assigned to the $\upsilon_{s\ O\text{-}C\text{-}O}$, is not prominent in the formulation. The symmetric stretching vibration of O-C-O group is found in suspension containing C940. Moreover, 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 Oflox and Carbopol polymer [Table 2].

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 bonding are also prominent from the FTIR spectra of the formulation.

From the XRD patterns of C940 it is clear that the polymer is fully amorphous in nature as there are no sharp and prominent peaks [Fig. 10]. The relative intensities of three distinct peaks of our formulation were different from that of pure Oflox. As the d-spacing of the prominent XRD peaks of pure Oflox is not changed in the polymeric suspension, it may be concluded that Ofloxacin shows identity in the suspension inspite of interaction between Oflox and C940

[Figs. 9-11]. The change in relative intensities of the 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.

From our SEM study it has been found that maximum particle size of the formulation is within the pharmaceutically acceptable limit. In cases of PSD and AR, we have found that both the standard deviation and coefficient of variation are small which indicate more consistent, uniform, stable and homogeneous suspension [Tables 4 and 5].[47-49] The mean particle size and AR values of the formulation (6.1µm and 4.12, 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.[50,51] The morphologies and mechanical properties of the formulation impart SEM sectioning and imaging, which can allow direct measurement of PSD and AR 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 containing Oflox and C940 is having better bioavailability and penetration

capacity, as maximum particles are of AR values between 2 to 4.[52] The AR analysis suggests that the formulation is more stable because it has lesser standard deviation. Hence, it indicates that the particles in the formulation are uniformly dispersed.

CONCLUSION

On the basis of the above interpretation, it can be concluded that by preparing mucoadhesive suspension of Ofloxacin 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,[53] by virtue of which a compatible and stable mucoadhesive suspension would be produced. From the XRD data supported by FTIR analysis it appears that the crystalline form of pure Oflox 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 Oflox 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. From the SEM image analysis it may be concluded that the formulation containing Oflox and C940 is stable having uniform dispersion of particles, 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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