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ANALYTICAL CHARACTERIZATION OF A CONTROLLED RELEASE POLYMERIC SUSPENSION

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ABSTRACT

Aims: Qualitative analysis of a mucoadhesive polymeric (Carbopol940) suspension of Norfloxacin was carried out with the aim of developing an oral controlled release gastro-retentive dosage form.

Methods: The characterization of ultrasonicated formulation was performed by Fourier Transform Infrared Spectroscopy (FTIR), Raman Spectroscopy, X-ray powder diffraction (XRD) and Scanning electron microscopy (SEM) analyses. For interpretation, FTIR (400 cm^{-1} to 4000 cm^{-1} region) and Raman (140 to 2400 cm^{-1} region) spectra were used. XRD data of pure drug, polymer and the mucoadhesive polymeric (Carbopol940) suspension were obtained using a powder diffractometer, scanned from a Bragg's angle (2θ) of 10˚ to 70˚. In addition, dispersion of particle was studied using SEM techniques. **Results:** The results from FTIR and Raman Spectroscopic analyses suggested that in the mucoadhesive suspension, carboxylic groups of Norfloxacin and hydroxyl groups of C940 undergo chemical interaction leading to esterification and hydrogen bonding. The XRD data indicated that the retention of crystalline nature of Norfloxacin in the mucoadhesive suspension. Moreover, the SEM image analysis suggested that in the formulation maximum particles exhibited network like structure to produce pseudoplastic flow.

Conclusion: From our analysis, it can be concluded that homogeneous, uniformly dispersed, pharmaceutically stable controlled release Norfloxacin suspension was prepared, which had the property of better bioavailability and penetration capacity.

INTRODUCTION: Due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation, oral controlled release (CR) dosage forms (DFs) have been developed over the past three decades. Incorporation of the drug in a controlled release - gastro retentive dosage forms (CR-GRDF) could remain in the gastric region for prolonged period, which would significantly increase 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. Since the goals of controlled drug delivery are to conserve and maintain effective drug concentration, eliminate night time dosage, improve compliance and decrease side effects, in the present study polymeric bioadhesive delayed gastric emptying devices have been explored [2].

Norfloxacin (Norflox), 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone carboxylic acid, is a second generation fluoroquinolone (**Fig. 1**). It inhibits the enzyme deoxyribonucleic acid (DNA) gyrase preventing DNA and protein synthesis. It requires multiple administration of drug, leading to fluctuation in plasma concentration of the drug [3].

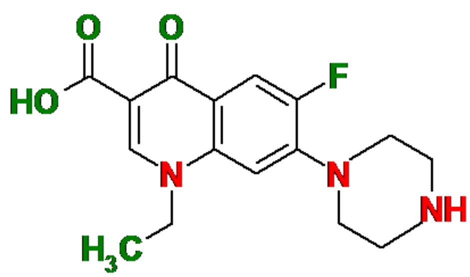

FIG. 1: CHEMICAL STRUCTURE OF NORFLOXACIN

The polymer in the formulation interacts with the mucosal component, increasing the contact time with the mucosa at the site of absorption. The prolonged contact time has been attributed to rheological properties of formulation, which delays its clearance from the mucosa [4]. In the present study, the polymer used is Carbopol940 (C940), which consists of chains of polyacrylic acid [5] (**Fig. 2**).

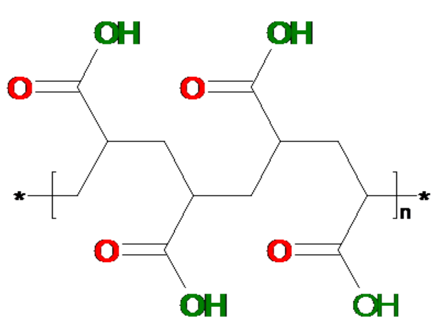

FIG. 2: CHEMICAL STRUCTURE OF CARBOPOL POLYMER

Carbopol polymers are considered as environmentally responsive polymers or "smart gels" which produce pseudo fed state, thereby reducing peristaltic contraction [6, 7] that alters their swelling behavior upon exposure to an external stimulus such as change in pH [8, 9], temperature [10], light, or electric field which provides on-off release [11-14]. In stomach, Carbopol polymer forms hydrogen bonding 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 polymers are very highly swollen [15].

Carbopol940 may form a complex with the low solubility drug like Norfloxacin. The interaction between Norflox 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 Norflox 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 methods, there are band intensity differences between the two techniques. That is why both FTIR and Raman Spectroscopic analyses were conducted. 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 [16]. It is known that increased dissolution rate and delayed release of drug from dosage forms occur with increase in crystallinity [17, 18].

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, could influence a drug's bioavailability [19]. Since, the SEM image analysis gives insight into the rheological properties and pharmaceutical stability of the suspension, SEM image analysis of suspension was carried out [20-22].

Therefore, to obtain more detailed information about chemical interaction between Norfloxacin and C940, FTIR and Raman analyses were carried out [23, 24]. Moreover, considering the bioavailability, stability and degree of dispersion of the particles present in the formulation, XRD and SEM analyses were conducted [9, 16, 20-22]. Considering all the positive aspects of different qualitative analyses, in the present investigation we performed FTIR analysis, Raman Spectroscopy, XRD and SEM studies of the controlled release mucoadhesive suspension.

MATERIALS AND METHODS:

Materials: The following materials were used: Norfloxacin was obtained from Dr. Reddy's Lab, Hyderabad, India, as a gift sample. Carbopol940, Pluronic F68 and Soya lecithin were purchased from Himedia Laboratories Pvt. Ltd., India. Glycerol, Mehtyl praraben, Propyl paraben, Sorbitol solution I.P. and Sucrose were kindly supplied by Cosmo Chem. Laboratory, India. Ultra pure water was obtained from a Millipore Milli-Q UV water filtration system.

Methods:

Formula for Preparation of Mucoadhesive Suspension: Formula for Preparation of Suspension Base-

(Percentage with respect to Norfloxacin)

- 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

Concentration of Norfloxacin used in the Formulation: 500mg/25ml

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 milliliter 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. 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 milliliter of water was taken in another beaker to which 500 mg of Norfloxacin 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.

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. 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 µm-100 µm [25-27]. 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 fiber 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 [28]. 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 [29]. The samples were given a conductive coating (using Pt, of about 600 Å 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.

RESULTS: The infrared spectra are recorded on Fourier Transform Spectrometer in the mid-infrared region (MIR) within the range (400-4500 cm^{-1}) [30]. 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} [25, 26].

In FTIR spectra of Norfloxacin, one prominent characteristic peak was found between 3550 and 3500 cm^{-1}, which was assigned to stretching vibration of OH group and intermolecular hydrogen bonding by single bridge. A band at 3500 to 3300 cm^{-1} suggested the NH stretching vibration of the imino-moiety of piperazinyl groups. The peak at 2750-2700 cm^{-1} indicated the presence ethyl group. The band at 2500 cm^{-1} was due to the υOH group of the carboxylic acid. The peak at 1700 cm^{-1} represented the carbonyl C=O stretching i.e., $\upsilon_{C=O}$. The band at 1650 to 1600 cm^{-1} was assigned to υN-H bending vibration of quinolones. The peaks at 1500 to 1450 cm^{-1} represented $\upsilon_{O\text{-}C\text{-}O}$ of acids and 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 band between 1050 and 1000 cm^{-1} was assigned to C-F group. The peak in the region 950-900 cm^{-1} suggested the δNH bending vibration of amines. The band at 800 cm^{-1} was due to the meta distribution of the aromatic protons (**Fig. 3** and **Table 1**) [26, 31, 32].

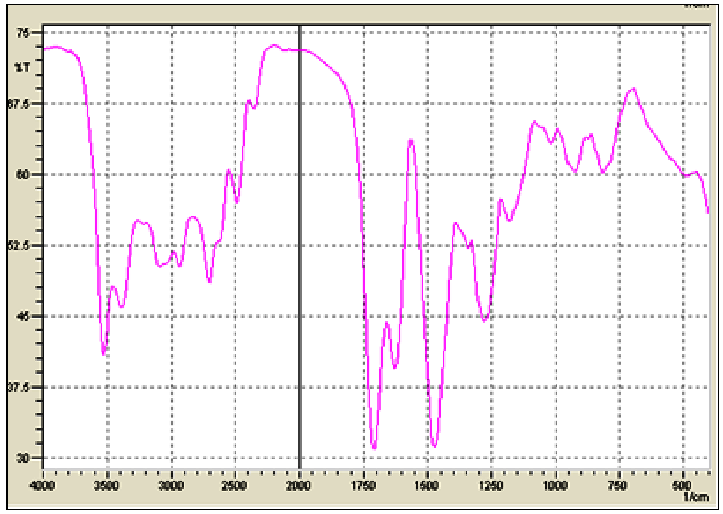

FIG. 3: FTIR PEAKS OF NORFLOXACIN

TABLE 1: PROMINENT FTIR PEAKS OF NORFLOXACIN

PEAKS(cm-1)	GROUPS	PEAK ASSIGNMENT
3550-3500	Hydroxyl group	Intermolecular H -bonding by single bridge
3500-3300	Imino-moiety of Piperazinyl groups	NH stretching vibration
3000-2950	Aromatic, cyclic enes	υ=CH & Ar-H
2750-2700	Ethyl group	υCH_2
2500	Acid group	υOH group
1700	Carbonyl of acids	υC=O stretching vibration
1650-1600	Quinolones	υN-H bending vibration
1500-1450	O-C-O group of acid	υ_s stretching vibration of O-C-O group
1300-1250	Hydroxyl group	δO-H bending vibration
1050-1000	C-F groups	υC-F
950-900	Amines	δNH bending vibration
800	Aromatic m – distribution	δAr-H

In case of C940, the FTIR spectra having peak between 3000 and 2950 cm^{-1} represented OH stretching vibration, i.e., $\upsilon_{O\text{-}H}$ 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} suggested to $\upsilon_{C\text{-}O\text{-}C}$ of acrylates [26, 30]. The band between 850 and 800 cm^{-1} was for out of plane bending of =C-H i.e., $\delta_{=C\text{-}H}$ [25, 30] (**Table 2**).

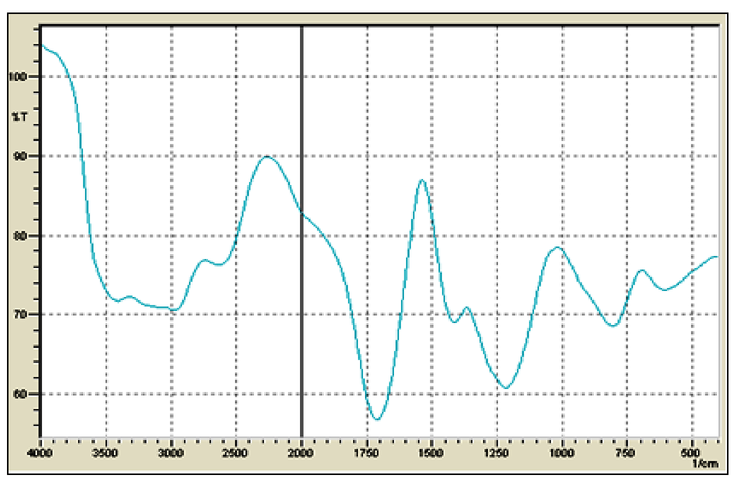

FIG. 4: FTIR PEAKS OF CARBOPOL940

TABLE 2: PROMINENT FTIR PEAKS OF C940

PEAKS (cm-1)	GROUPS	PEAK ASSIGNMENT
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

In the FTIR spectra of formulation containing both Norflox and C940, the prominent band, found between 3550 and 3500 cm^{-1}, was assigned to υ_{O-H} and polymeric hydrogen bonding (**Fig. 5**). The peak at 2600-2500 cm^{-1} represented the υ_{O-H} of carboxylic acid i.e., strong intermolecular 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 1500 - 1450 cm^{-1}(w) was for υ_{C-O} / δ_{O-H}. The band from 1300 to 1250 cm^{-1} was due to υ_{C-O-C} of acrylates. The peak between 1100 and 1000 cm^{-1} represented υ_{C-F} groups. The band at 800 cm^{-1} indicated the meta distribution of δ_{Ar-H} group [25, 26, 30] (**Table 3**).

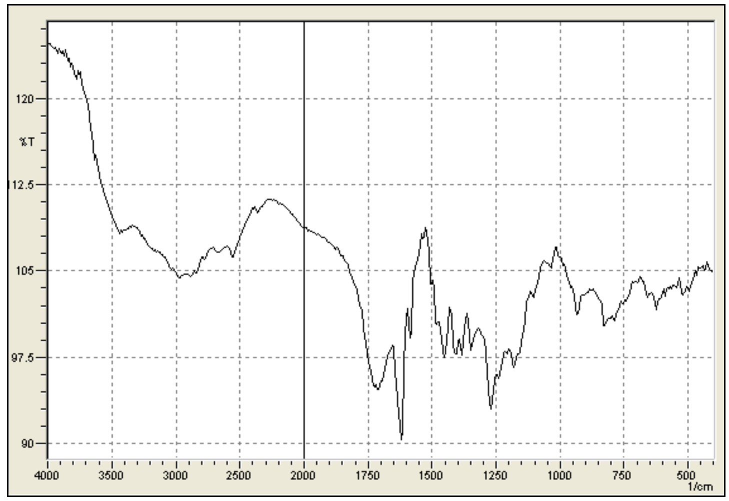

FIG. 5: FTIR PEAKS OF NORFLOXACIN MUCOADHESIVE FORMULATION

TABLE 3: PROMINENT FTIR PEAKS OF MUCOADHESIVE FORMULATION

PEAKS (cm-1)	GROUPS	PEAK ASSIGNMENT
3550-3500	Hydroxyl group	Polymeric H -bonding
2600-2500	Hydroxyl group of carboxylic acid	Strong intermolecular H- bonding
1650-1600	O-C-O group of acid	υ_{as} stretching vibration of O-C-O group
1500-1450	O-C-O group of acid	υ_{s} stretching vibration of O-C-O group
1300-1250	Acrylates & esters	C-O-C stretching vibration
1100-1000	C-F groups	υC-F
800	Aromatic m – distribution	δAr-H

By Raman spectroscopy of Norfloxacin, the prominent Raman shifts have been observed at 485.6, 872.7, 1418.5 and 1655.1 cm^{-1} (**Fig. 6**). The Raman shifts at 485.6 cm^{-1} indicated strong bending vibration of C-C of the aliphatic chain and C-N stretching vibration of piperazinyl group [33-35]. The band at 872.7 cm^{-1} represented the symmetric stretching vibration of C-F group [36].

The peak at 1418.5 cm^{-1} was due to symmetric stretching vibration of O-C-O group of carboxylic acid and methylene deformation mode of the piperazinyl group [37]. A band at 1655.1 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 at 1655.1 cm^{-1}) also indicated the N^+H_2 scissoring of piperazinyl group [33, 37-40] (**Table 4a**).

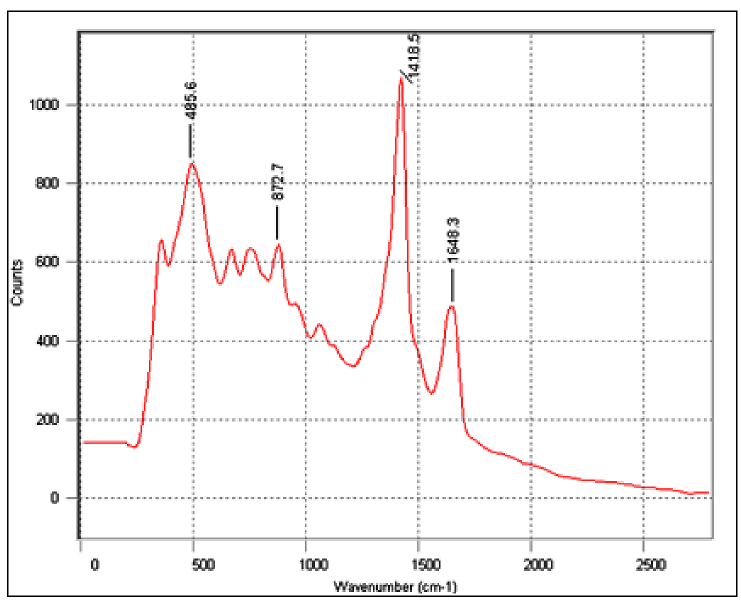

FIG. 6: RAMAN SHIFTS OF NORFLOXACIN

TABLE 4: RAMAN SHIFTS OF PURE DRUG, POLYMER AND FORMULATION

a) Prominent Raman Shifts of Norfloxacin	
Raman Shifts(cm^{-1})	**Functional Groups / Vibrations**
485.6	Strong $\delta_{(CC)}$ aliphatic chain and C-N stretching vibration
872.7	Symmetric vibration of C-F bond
1418.5	$\upsilon_{S\ O\text{-}C\text{-}O}$ and methylene deformation of the piperazinyl group
1655.1	υ_s of C=O group of pyridone moiety and N^+H_2 scissoring of piperzinyl group
b) Prominent Raman Shifts of C940	
Raman Shifts(cm^{-1})	**Functional Groups / Vibrations**
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 Norfloxacin Mucoadhesive Formulation	
Raman Shifts(cm^{-1})	**Functional Groups / Vibrations**
338.8	δ(CC) aliphatic chain
900-800	Symmetric stretching vibration of both C-F group C-O-C group for acrylates and esters
1100-1050	Stretching vibration of CO
1400-1350	υ_sO-C-O
1550	υ_{as}O-C-O
1850-1700	υC=O medium

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 band at 1366.5 cm^{-1} was assigned to symmetric vibration of O-C-O of acids [33] (**Table 4b**).

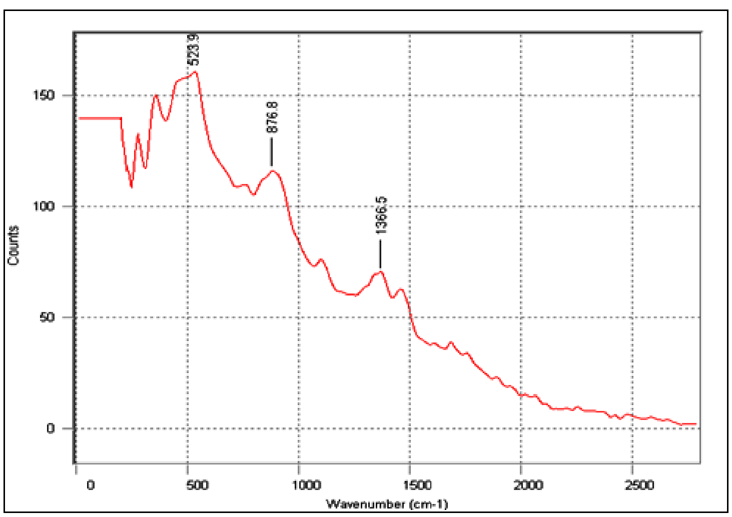

FIG. 7: RAMAN SHIFTS OF CARBOPOL940

In the formulation containing both Norflox and C940, the Raman peak at 338.8 cm^{-1} represented bending vibration of δCC of aliphatic chain (**Fig. 8**).

The band at 900-850 cm^{-1} was assigned to symmetric stretching vibration of both C-F group and C-O-C group for acrylates and esters. The peak at 1100-1050 cm^{-1} represented stretching vibration of carbonyl group. The band at 1400-1350 cm^{-1} suggested for symmetric stretching vibration of O-C-O group. The peak at 1550 cm^{-1} was due to asymmetric vibration of O-C-O group. The band at 1850 to 1700 cm^{-1} was the characteristic of stretching vibration of carbonyl group of esters [33, 40] (**Table 4c**).

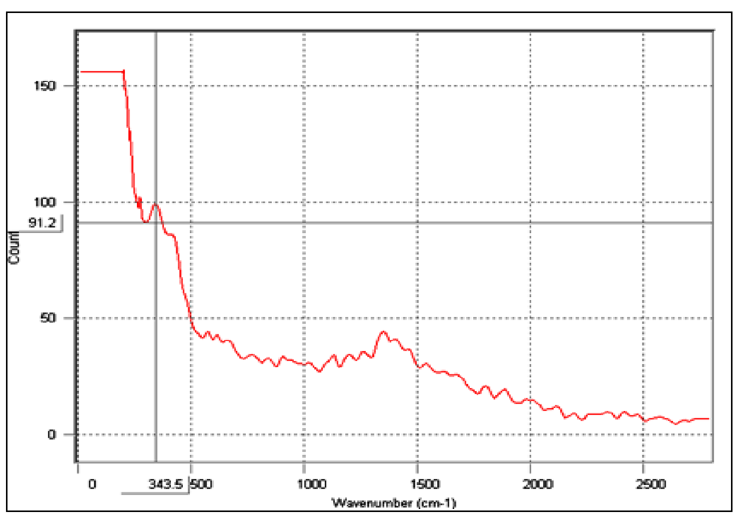

FIG. 8: RAMAN SHIFTS OF NORFLOXACIN MUCOADHESIVE FORMULATION

All the high intensity peaks (relative intensity) observed in the XRD pattern of the pure Norflox were compared with its mucoadhesive polymeric suspension (**Tables 5** and **6**). Both the polymeric suspension and pure Norflox were found to show sharp XRD peaks but their XRD patterns are different (**Figs. 9-11**). Identification of a structure from its powdered diffraction pattern is based upon the position of peaks and their relative intensities.

TABLE 5: THREE STRONGEST PEAKS IN THE XRD PATTERNS UNDER THE HANAWALT SYSTEM

	Norfloxacin				Mucoadhesive Suspension			
Sl. No	2θ	d-spacing	I/I_0	H	2θ	d-spacing	I/I_0	H
01	24.98	3.56	100.00	3397	25.63	3.47	100.00	646
02	13.22	6.69	39.70	1348	23.14	3.84	87.26	564
03	20.72	4.28	33.52	1139	21.72	4.09	46.02	297

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: XRD DATA IN TERMS OF LATTICE SPACING AND RELATIVE INTENSITIES OF NORFLOXACIN AND ITS FORMULATION

Sl. No	Norfloxacin		Mucoadhesive Suspension	
	d-spacing	I/I_0	d-spacing	I/I_0
01	8.39137	15.61	8.33	14.92
02	7.85364	19.22	6.87	24.54
03	6.68947	39.70	5.18	28.97
04	6.07254	0.96	4.64	27.06
05	5.58651	5.23	4.33	35.73
06	4.98324	5.42	4.09	46.02
07	4.64229	11.36	3.84	87.26
08	4.28272	33.52	3.47	100
09	4.13894	17.81	3.18	38.08
10	4.07080	18.53	2.98	21.42
11	3.75223	6.86	2.77	9.87
12	3.56180	100	2.62	6.47
13	3.31130	8.33	2.55	5.47
14	3.11649	3.33	2.27	7.06
15	3.04064	10.94	2.18	3.15
16	2.84944	8.41	1.69	1.79
17	2.76768	16.68	1.66	2.09
18	2.70768	6.19	1.58	2.54
19	2.60958	8.72	1.55	1.66
20	2.53713	2.00	1.49	0.70
21	2.45129	3.55	1.43	1.32
22	2.35204	5.31	1.40	1.29
23	2.29726	3.10		
24	2.22986	4.37		
25	2.16093	2.72		
26	2.13003	8.92		
27	2.01871	4.85		
28	1.97484	4.24		
29	1.87436	2.04		
30	1.85171	2.89		
31	1.80162	1.95		
32	1.77813	1.70		
33	1.74976	1.79		
34	1.63676	1.25		
35	1.58599	1.22		

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

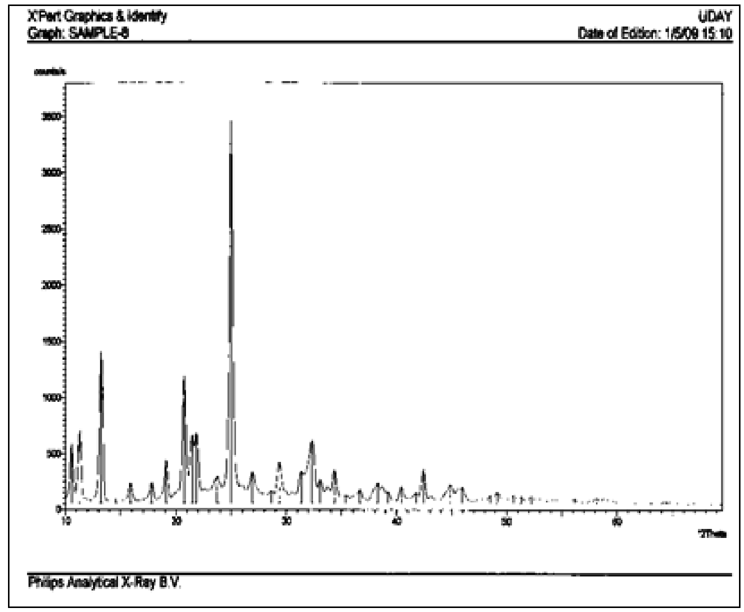

FIG. 9: XRD PATTERNS OF NORFLOXACIN

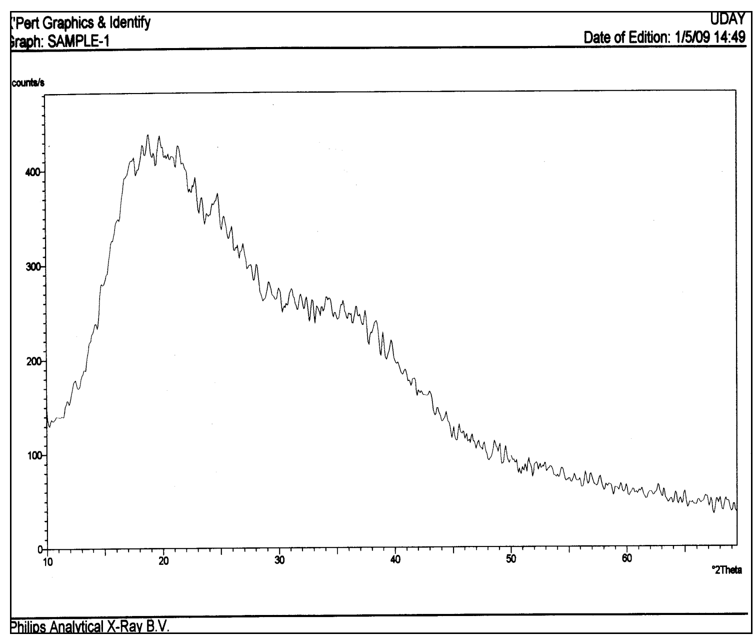

FIG. 10: XRD PATTERNS OF CARBOPOL940

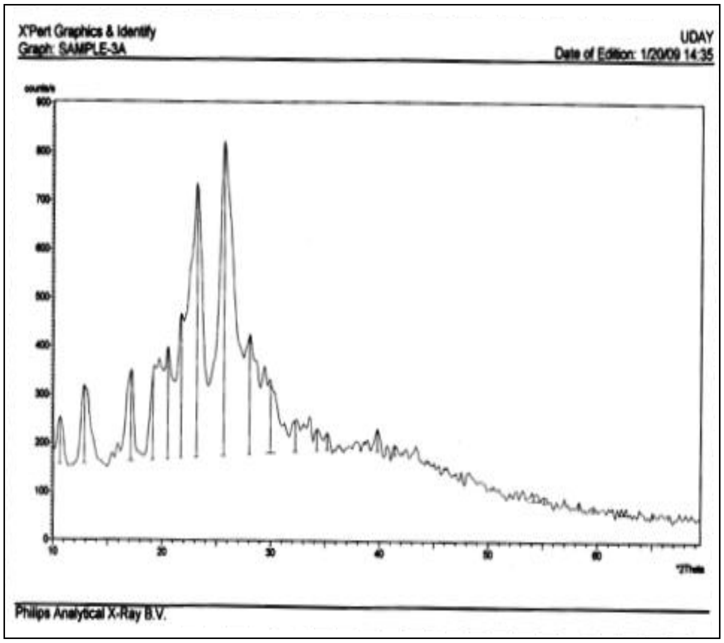

FIG. 11: XRD PATTERNS OF NORFLOXACIN MUCOADHESIVE FORMULATION

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 d-spacings of the prominent XRD peaks of pure Norfloxacin are changed in XRD patterns of formulation. The relative intensities and heights of three prominent peaks of the formulation were less than those of pure Norflox (**Table 5**). Moreover, complete diffraction patterns of both pure Norflox and the formulation can be seen in **Table 6**.

From the SEM image analysis of suspension, it has been found that particles had network like structure (**Fig. 12**). Due to that we could not perform particle size distribution analysis. For the same reason we were unable to determine length/width ratios (aspect ratio) of individual particles satisfactorily.

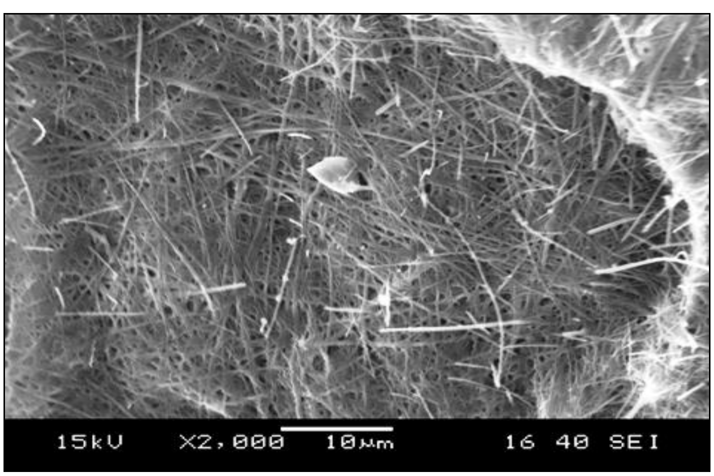

FIG. 12: SEM OF NORFLOXACIN MUCOADHESIVE FORMULATION

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 [25]. 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.

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 [25, 26].

In case of FTIR spectra of Norflox, prominent peaks for $\upsilon_{C\text{-}O}$ / $\delta_{O\text{-}H}$ and $\upsilon_{C=O}$ indicated the presence of –CO-, -CHO and -COOH groups (**Fig. 3**). The presence of above groups could be confirmed by Fermi Resonance bands for –CHO, $\upsilon_{C\text{-}O\text{-}C}$ bands for esters and absence of these two for ketones. This suggested the existence of –COOH group in Norflox molecule (**Table 1**).

Another probability of intermolecular hydrogen bonding may be due to prominent FTIR peaks between 3550 and 3500 cm^{-1}. The band at 3500-3300 cm^{-1} indicated the presence of piperazinyl group. The presence of ethyl group was confirmed by the appearance of a sharp peak at 2750-2700 cm^{-1} [26, 41]. The peak at 1650-1600 cm^{-1} was due to the quinolone moiety of Norfloxacin. The bending vibration of O-H group showed medium to strong band in the region around 1300-1250 cm^{-1}, which confirmed the presence of COOH group. Here, the FTIR peak at 950-800 cm^{-1} suggested the probability of bending of NH group. The peak at 1050-1000 cm^{-1} indicated the presence of C-F group which takes a major role in its antimicrobial activity (**Table 1**) [25, 26, 31].

In case of FTIR spectra of Carbopol940, there were prominent peaks for intramolecular hydrogen bonding, υ_{OH} stretching vibration, carbonylic C=O and C-O stretching vibration and stretching vibration for the C-O-C, which confirmed the presence of acrylates (**Fig. 4**). The peak for out of plane bending vibration of =C-H was found between 850 and 800 cm^{-1} (**Table 2**).

While comparing the FTIR spectra among the pure Norflox and C940, and the formulation containing both Norflox and C940, it is clear that the band position of

C=O group has been affected by esterification and conjugation involving C=O group. Here, the stretching vibration of C=O in pure Norflox was found at 1700 cm^{-1}, which was lowered to 1650-1600 cm^{-1} in this formulation might be due to formation of β-ketoesters (**Fig. 6**). The FTIR peaks assigned to $υ_{C-O}$ and $υ_{C-O-C}$ represented acrylates and esters, which confirmed the esterification between polymeric OH group and –COOH group of Norflox. The stretching vibration of C-F group remained nearly unaltered.

The another probability of interaction is hydrogen bonding i.e., intermolecular hydrogen bonding due to prominent FTIR peaks between 3550 and 3500 cm^{-1}, and 2600 and 2500 cm^{-1} represented polymeric O-H...O-H…O-H and strong carboxylic OH hydrogen bonding, respectively. The hydrogen bonded -OH stretching vibration occurred over a wide range, 3550-2500 cm^{-1}. The bending vibration of O-H group showed medium to strong bands in the region around 1300-1250 cm^{-1}. The FTIR peak at 800 cm^{-1} suggested the probability of out of plane bending of –ene bond and m-substitution of $δ_{Ar-H}$ hydrogen atom (**Table 3**) [25, 26, 30].

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 could be deduced because the corresponding band found from 1650 to 1600 cm^{-1} was due to probability of formation of β-ketoesters [36]. From the above data, it can be inferred that the carboxylic group of Norflox 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 flouroquinolone 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 3550-2500 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 [41].

By comparing the FTIR spectra among the pure drug, Carbopol polymer (C940) and the formulation containing both drug and polymer, the FTIR peak of Norflox at 1700 cm^{-1} was not detected in the mucoadhesive system probably due to interaction with polymer. The missing peak was replaced by two very strong characteristic bands in the range of 1650-1600 cm^{-1} and at 1500-1450 cm^{-1}, which were assigned to $υ_{(O-C-O)}$ asymmetric and symmetric stretching vibrations, respectively [26].

The difference Δ $[υ_{(CO2)asym}-υ_{(CO2)sym}]$ is a useful characteristic for determining the involvement of the carboxylic group of Norflox. 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 [30] (**Tables 1- 3**).

In case of Raman spectra of Norflox, band at 485.6 cm^{-1} was assigned to the stretching vibration of ethyl group. The peak at 872.7 cm^{-1} represented stretching vibration of C-F group. The presence of carboxylic acid group was confirmed by $υ_{O-C-O}$ and $υ_{C=O}$ groups vibration at 1418.5 cm^{-1} and 1655.1 cm^{-1}, respectively (**Table 4a**).

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 $υ_{s\ O-C-O}$, is not prominent. 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 Norflox and Carbopol polymer (**Table 4**).

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.

When an X-ray beam hits a sample and gets diffracted, we can measure the distances between the planes of the atoms that constitute the sample by applying Bragg's Law (**Eqn. 1**).

$n\lambda = 2d\sin\theta$……………………………………………………………(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 (the *d*-spacings), and θ is the angle of incidence of the X-ray beam. Since we know λ and we can measure θ, we can calculate the *d*-spacings. 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 drug present in the polymeric composites [42].

Table 5 and **6** give the data obtained for pure Norfloxacin and its formulation in terms of lattice spacing and relative peak intensities. Most of the characteristic peaks in the diffraction patterns were generally prominent and sharp, so measurement of the angles and hence of d-values was accurate. Proper sample preparation helped attain exact peak positions for qualitative analysis.

From the XRD patterns of both C940, it is clear that the polymer is fully amorphous in nature as there is no sharp prominent peak **(Fig. 10)**. From **Table 5,** it has been confirmed that the three prominent peaks of both pure Norfloxacin and its formulation have different d-spacings corresponding to similar 2θ values. As the d-spacings of the prominent XRD peaks of pure Norfloxacin are changed in XRD patterns of formulation, it can be concluded that the change in crystallinity of Norfloxacin in the formulation is due to rise in atomic densities in that particular plane of crystal lattice.

Moreover, under Hanwalt system of XRD data interpretation, it may be mentioned that the peak heights of three prominent peaks of Norfloxacin in the formulation are lesser than those of pure Norfloxacin actually suggesting a decrease in crystallinity of the drug present in the formulation. From this we may predict that there could be change in orientation of crystal lattice due to incorporation of some extra atoms into it by esterification and hydrogen bonding.

It is known that Carbopol940 polymers are long-chained, high molecular-weight compounds containing active polyacrylic acid groups spaced along their length. This polymer promotes network like flocculation through adsorption of part of the chain on the surface of particle and the remaining part project out into the dispersion medium. Formation of bridge between the drug and projected parts leads to formation of floccules which retards sedimentation.

This results in pseudoplastic flow in suspension that promotes the physical stability of suspension. Since, our SEM image analysis indicated that in the formulation maximum particles exhibited network like structure, this leads to produce pseudoplastic flow which provides supporting evidences for homogeneous, uniformly dispersed, pharmaceutically stable controlled release mucoadhesive Norfloxacin suspension [21].

CONCLUSIONS: On the basis of the above interpretation, it can be concluded that by preparing mucoadhesive suspension of Norfloxacin 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 Norflox 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 Norflox 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. Moreover, from the SEM image analysis, it may be concluded that the formulation containing Norflox and C940 was having maximum particles which exhibited network like structure. This leads to produce pseudoplastic flow which provides supporting evidences for homogeneous, uniformly dispersed, pharmaceutically stable controlled release mucoadhesive Norfloxacin suspension.

The utility of the present work may be improved if the delivery rate, biodegradation and site-specific targeting of such controlled suspension would be properly monitored and controlled.

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