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FTIR AND RAMAN SPECTROSCOPIC INVESTIGATIONS OF CONTROLLED RELEASE OFLOXACIN / HPMC MUCOADHESIVE SUSPENSION

Subhashree Sahoo[1]*, Chandra Kanti Chakraborti[1], Subash Chandra Mishra[2]
[1]Department of Pharmaceutics, Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela-769015, Orissa, India.
[2]Metallurgical and Materials Engg. Dept., National Institute of Technology, Rourkela-769 008, Orissa, India.
Email: subha77t@yahoo.co.in

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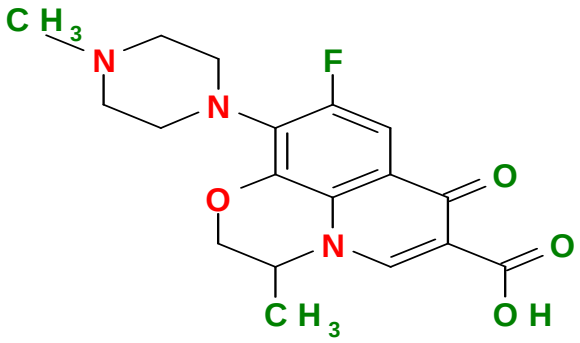
Abstract

Aims: Ofloxacin, an antibacterial agent, is having low solubility in aqueous solution. Moreover, the drug possesses short half-life, so frequent dosing is required. To overcome these difficulties, many researchers have prepared different formulations of Ofloxacin. But till now very few formulations are available from which the drug is absorbed uniformly, so that safe and effective blood level of Ofloxacin can be maintained for a prolonged period. To fulfill this requirement, in the present study, a controlled release drug delivery system has been designed and chemical interaction between Ofloxacin and polymer in formulation has been studied by FTIR and Raman Spectroscopy. **Methods:** Ultrasonication method was used for preparation of mucoadhesive Ofloxacin formulation, taking HPMC polymer with drug to polymer weight ratio 1:5. FTIR (400 cm^{-1} to 4000 cm^{-1} region) and Raman (140 to 2400 cm^{-1} region) Spectroscopic studies were carried out and spectra were used for interpretation. **Results:** From the spectral interpretation, it has been found that in formulation, the carboxylic groups of Ofloxacin and hydroxyl groups of HPMC undergo chemical interaction leading to esterification and hydrogen bonding (both intermolecular and polymeric). **Conclusion:** The formation of micellies due to esterification and hydrogen bonding causes more drug entrapment and formation of a stable formulation. As a result, the mucoadhesive formulation of Ofloxacin gives better controlled release and mucoadhesive action in the gastrointestinal tract. Hence, HPMC can be considered as an effective carrier of Ofloxacin.

Keywords: FTIR, HPMC, Mucoadhesive suspension, Ofloxacin, Raman Spectroscopy

Introduction

Ofloxacin (Oflox), 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)**. Normal dosage regimen varies from 200 to 600 mg administered twice or thrice a day, depending on severity of infection. In severe cases, long-term therapy may also be required. Biological half-life of the drug is from 5 to 6h. As frequent dosing is required to maintain the therapeutic plasma concentration, it was chosen as a model drug for the controlled release study[1].



Ofloxacin

Figure 1: Chemical structure of Ofloxacin.

Hydroxypropyl methylcellulose (HPMC) is one of the most commonly used hydrophilic biodegradable polymers for developing controlled release formulations, because it works as a pH-independent gelling agent. Swelling as well as erosion of it occurs simultaneously inducing a pseudofed state, thereby reducing peristaltic contraction, which contributes to overall drug release. It is a widely accepted pharmaceutical excipient because HPMC is available in a wide range of molecular weights and the effective control of gel viscosity is easily possible[2-6].

HPMC has many pharmaceutical uses, such as a drug carrier, a coating agent, a tabletting agent, and it is also used in ophthalmic solutions and in personal care products, such as KY Jelly[7]. It is the most important hydrophilic carrier material used for the preparation of oral controlled drug delivery systems. One of its most important characteristics is the high swellability, which has a significant effect on the release kinetics of an incorporated drug. Upon contact

with water or biological fluid, the latter diffuses into the device, resulting in polymer chain relaxation with volume expansion. Subsequently, the incorporated drug diffuses out of the system[2].

HPMC is propylene glycol ether of methyl-cellulose. Its chemical structure has been illustrated in **Figure 2**[7]. The physicochemical properties of this polymer are strongly affected by: (i) the methoxy group content; (ii) the hydroxypropoxy group content; and (iii) the molecular weight[2].

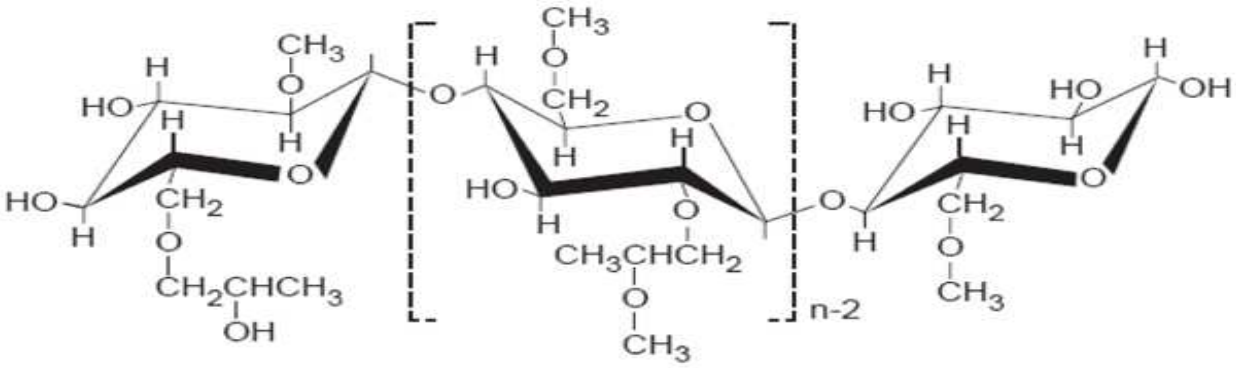


Figure 2: Chemical structure of Hydroxypropyl methylcellulose (HPMC)

The HPMC may form a complex with the low solubility drug like Ofloxacin. The interaction between the Oflox and hydrophilic osmo-polymer HPMC 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 pure ofloxacin, HPMC, and 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. Therefore, to obtain more detailed information about chemical interaction between Ofloxacin and HPMC, both FTIR and Raman analyses were carried out [8,9].

Materials and Methods

Materials:

The following materials were used for the study: Oflox was obtained from Dr. Reddy's Lab, Hyderabad, India, as a gift sample. Hydroxypropyl methylcellulose (HPMC E15 LV Premium) was supplied by Loba Chemie Pvt. Ltd., India. It was having methoxy group (23.8%) and hydroxypropoxy group (8.3%). 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-

1. ***Preparation of Bulk A***

In a beaker 6 ml water was taken and heated up to 80° C. To that water, 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.

2. ***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 properly. To this solution, pluronic F 68 (5%), soya lecithin (1%) and C934 (5%) in w/w of drug were added with continuous stirring.

3. ***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 Spectroscopy-

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 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[10-12]. 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 Spectroscopic Analysis 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}.

Results

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 groups 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., $\upsilon_{C=O}$[13]. 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**)[10,11,14,15].

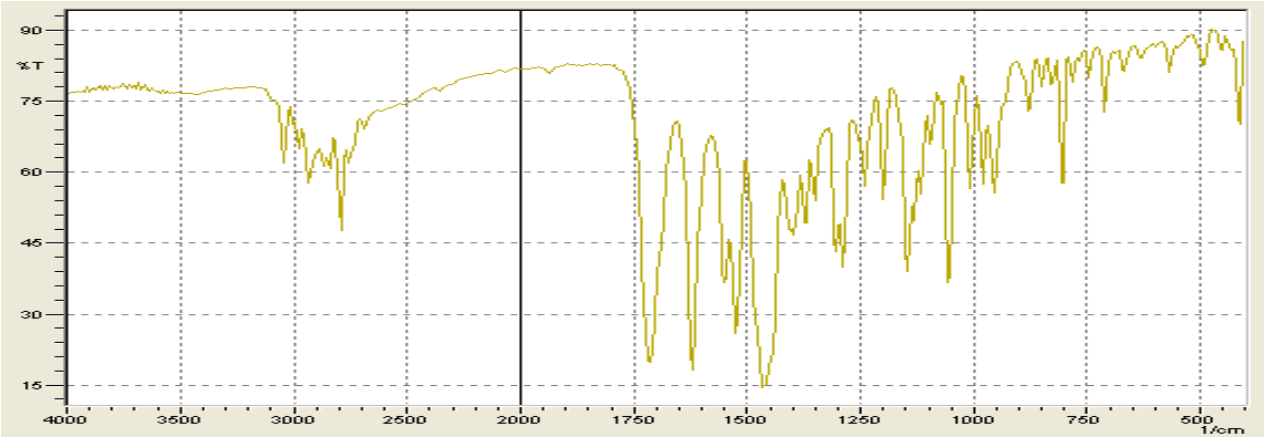


Figure 3: FTIR spectra of pure Ofloxacin

Assignments of FTIR frequencies of HPMC were achieved by comparing the band positions and intensities observed in FTIR spectra with wave numbers and intensities. The peak at 3500 to 3400 cm^{-1} was due to OH vibrational

stretching **(Fig 4)**[10,11]. The symmetric stretching mode of υ_sMe and $\upsilon_s$hydroxypropyl groups was found in the range 2900 cm^{-1} in which all the CH bonds extend and contract in phase[11]. The peak at 2550-2500 cm^{-1} was assigned to OH stretching vibration, i.e., υ_{O-H} and intramolecular hydrogen bonding[10,11]. The band between 1650 and 1600 cm^{-1} indicated the presence of stretching vibration of υ_{C-O} for six membered cyclic rings. Two bending vibrations might occur within a methyl group. The first of these, the symmetric bending vibration of δ_sMe involved the in-phase bending of the C-H bonds. The second, the asymmetric bending mode of δ_{as}Me was due to out-of-phase bending of the C-H bonds. While the asymmetric bending vibrations of the methoxy group normally appeared in the region 1500-1450 cm^{-1}, the symmetric vibrations were mostly displayed in the range 1400-1350 cm^{-1} [16,17]. The band between 1400 and 1350 cm^{-1} suggested υ_{C-O-C} of cyclic anhydrides. The peak at 1300-1250 cm^{-1} was due to υ_{C-O-C} cyclic epoxide. The band at 1100-1000 cm^{-1} was for stretching vibration of ethereal C-O-C groups. The peak at 1000-950 cm^{-1} was due to υ_{as} of pyranose[18]. The rocking mode of CH_2 was found in the range 850-800 cm^{-1} [16] **(Table 1b** and **Fig 4)**. The computed frequencies of HPMC are in a good agreement with experimental frequencies for both carbohydrate region as well as OH and CH region.

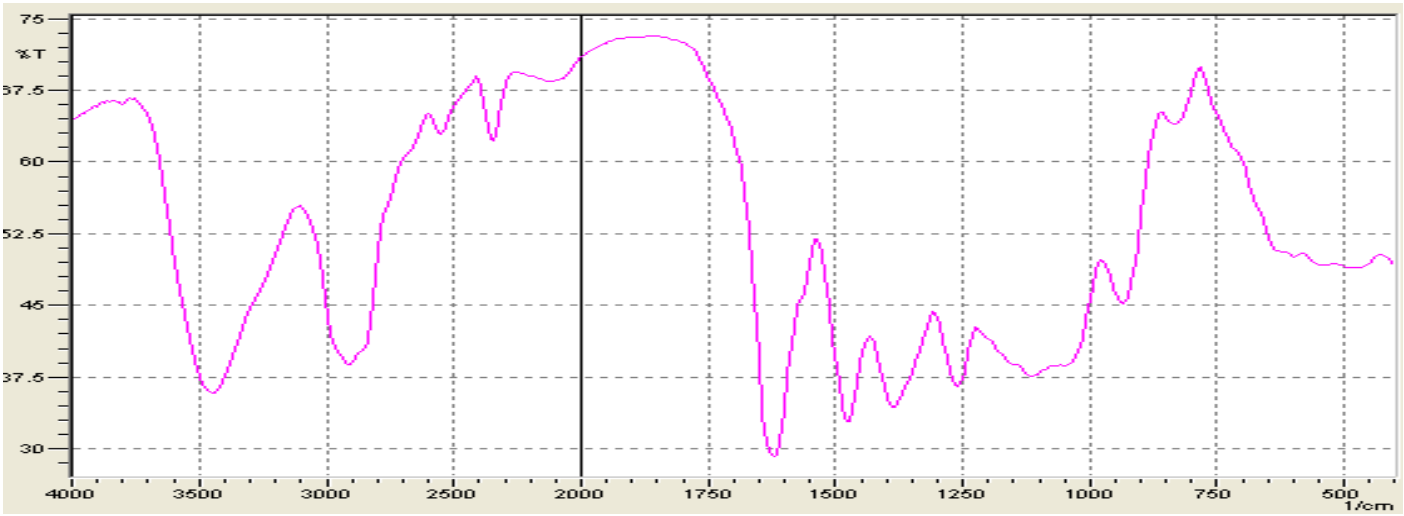


Figure 4: FTIR Spectra of pure HPMC

In the FTIR spectra of the mucoadhesive suspension, the peak from 3100 to 3000 cm^{-1} was assigned to polymeric υ_{O-H} and hydrogen bonding, the band between 3000 and 2600 cm^{-1} represented the stretching vibration of υ_{O-H} i.e.,

strong intermolecular hydrogen bonding **(Fig 5)**. 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 1400-1350 cm^{-1} was assigned to δC-O-C representing esters and symmetric bending of methoxy groups. The peak between 1100 and 1000 cm^{-1} represented υ_{C-F} groups[11,19]. The band at 1000-950 cm^{-1} was assigned to υ_{as} of pyranose ring of HPMC[18] **(Table 1c)**. **Figure 6** shows comparative FTIR spectra of Oflox, HPMC and Ofloxacin mucoadhesive suspension.

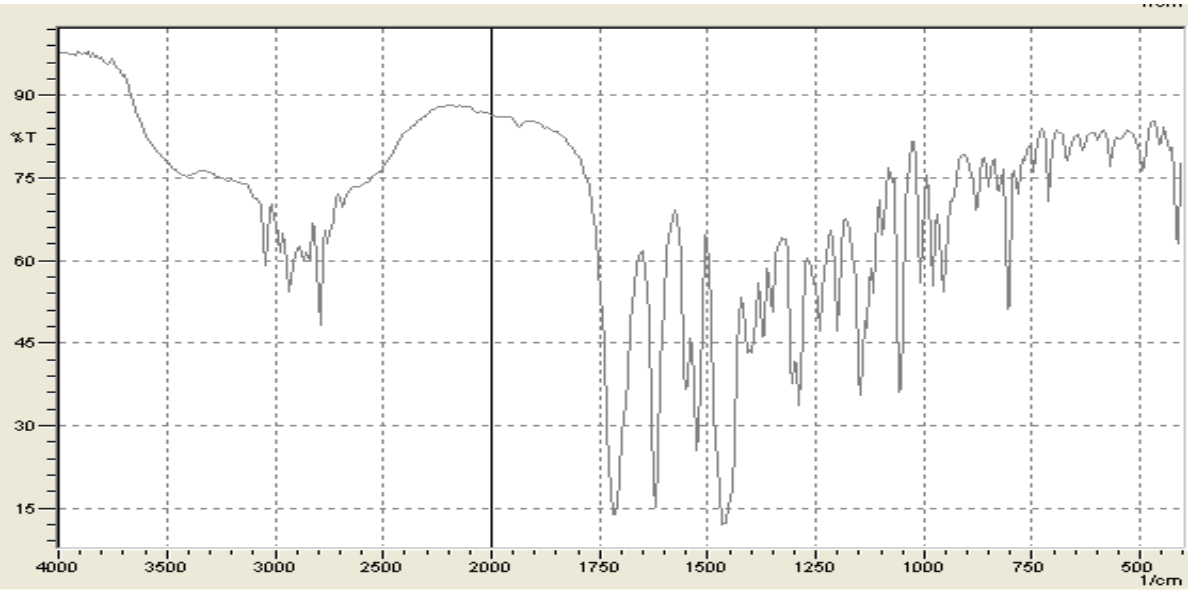


Figure 5: FTIR Spectra of Mucoadhesive suspension containing Ofloxacin and HPMC

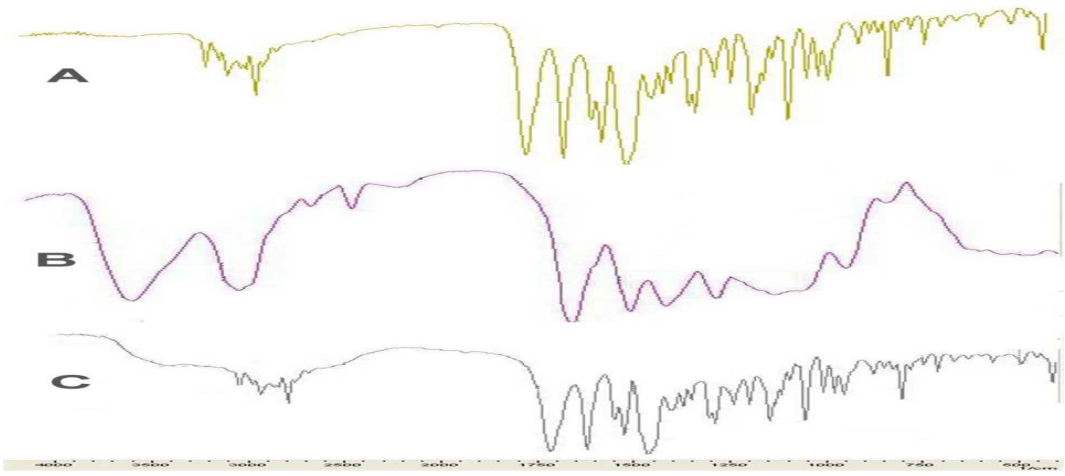


Figure 6: FTIR Spectra of Ofloxacin (A), HPMC (B) and Mucoadhesive suspension (C)

By Raman spectroscopy of Ofloxacin, the prominent Raman shifts were observed at 518.4, 797.5, 1419.8 and 1649.6 cm^{-1} **(Fig 7)**. 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. The band at 797.5 cm^{-1} suggested the symmetric stretching vibration of C-F group[20]. The peak at 1419.8 cm^{-1} was due to symmetric stretching vibration of O-C-O group of carboxylic acid and methylene deformation mode of the piperazinyl group. 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 [21-27] **(Table 2a)**.

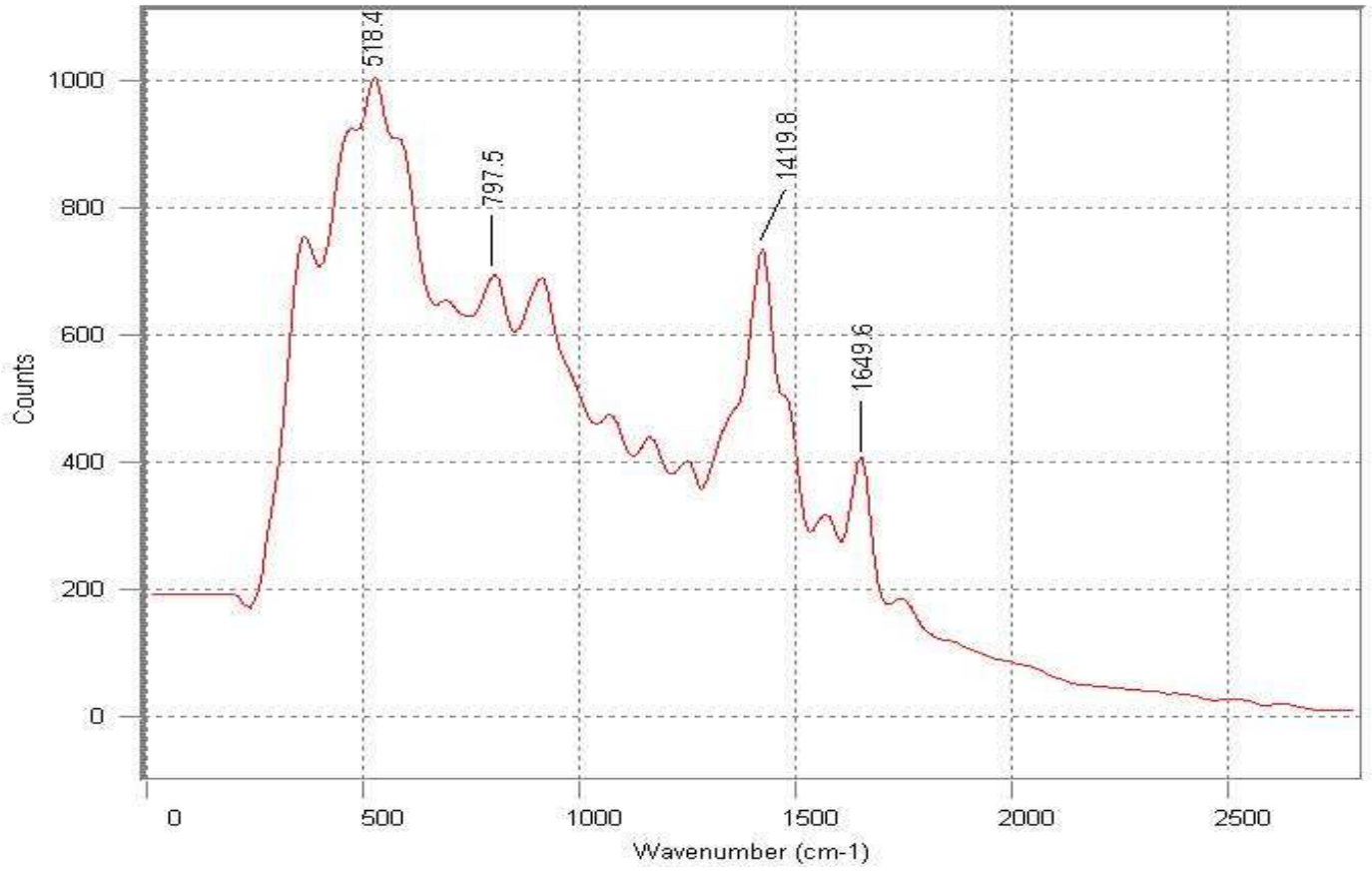


Figure 7: Raman Shifts of pure Ofloxacin

In case of HPMC, the prominent Raman shifts were found at 504.7, 908.3 and 1384.3 cm^{-1}

(Fig 8). The peak at 504.7 cm^{-1} was assigned to C-H out of plane bending vibration and C-C-O bending vibration of β D-glucose monomer of HPMC. The band at 908.3 cm^{-1} was due to C-C-C in-plane bending and $\upsilon_{(C\text{-}O\text{-}C)}$ stretching vibration of pyranose ring. The peak at 1384.3 cm^{-1} was assigned to C-C stretching vibration **(Table 2b)** [16,17,21,25.]

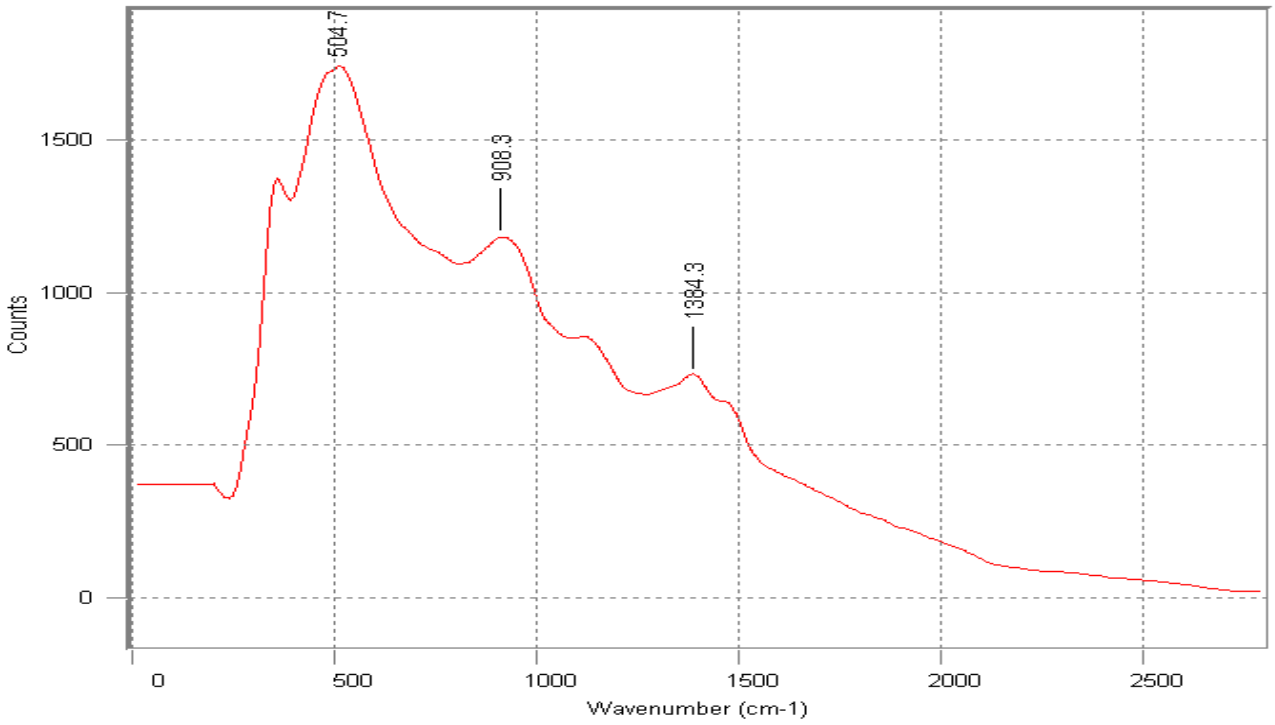


Figure 8: Raman Shifts of pure HPMC

The characteristics Raman peaks of mucoadhesive suspension containing both Oflox and HPMC were observed at 338.8, 900-850, 1340.5 and 1800-1700 cm^{-1} **(Fig 9)**. The band at 338.8 cm^{-1} was assigned to C-C-C out of plane bending of pyranose ring[17]. The peak at 900-850 cm^{-1} was due to symmetric stretching vibration of C-F bond and symmetric COC stretching vibration for esters. The band at 1340.5 cm^{-1} respesented δCCH and δOCH bending vibration of methoxy group[16]. The peak at 1800-1700 cm^{-1} was assigned to C=O stretching vibration of carbonyl groups of esters[17] **(Table 2c)**. **Figure 10** shows comparative Raman shifts of Oflox, HPMC and Ofloxacin mucoadhesive suspension.

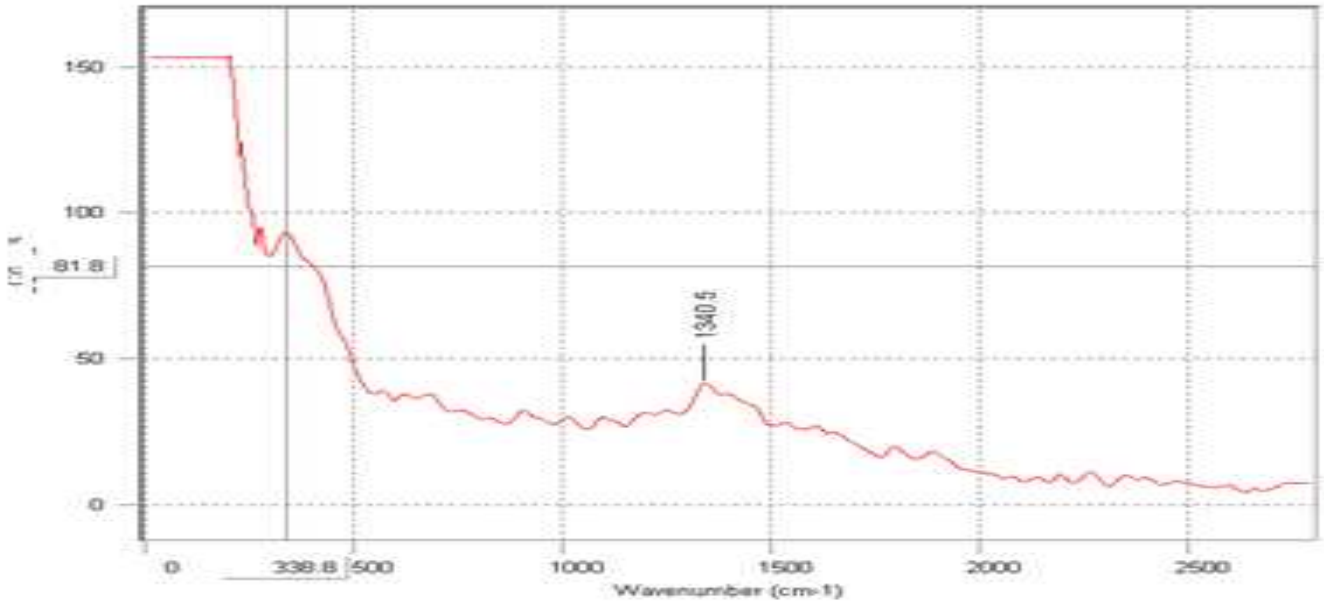


Figure 9: Raman Shifts of Mucoadhesive suspension Containing Ofloxacin and HPMC

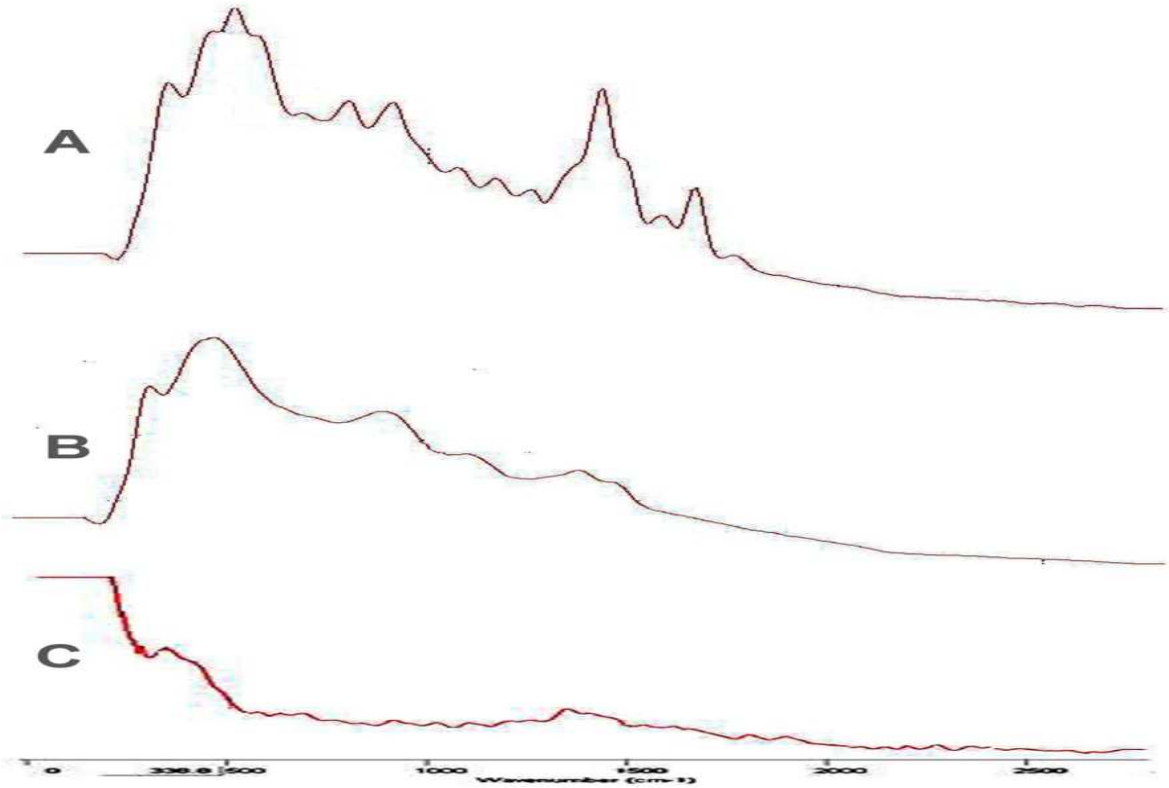


Figure 10: Raman Shifts of Pure Ofloxacin (A), HPMC (B), Mucoadhesive suspension (C)

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[11]. 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[10,11].

In case of FTIR spectra of Oflox, prominent peaks for $\upsilon_{\mathbf{C\text{-}O}}$ / $\delta_{\mathbf{O\text{-}H}}$ and $\upsilon_{C=O}$ indicated the presence of –CO-, -CHO and -COOH groups **(Fig 3)**. The presence of above groups may be confirmed by fermi resonance bands for –CHO; $\upsilon_{\mathbf{C\text{-}O\text{-}C}}$ bands for esters; and absence of these two for ketones. This suggests the existence of –COOH group in Oflox **(Table 1a)**.

From FTIR spectral analysis it has been found that the HPMC shows both intramolecular and intermolecular hydrogen bonding. The presence of pyranose ring of β D-glucose monomers has been confirmed. The stretching vibration of the cyclic anhydride, methoxy and hydroxypropoxy groups along with epoxide helps in the identification of HPMC [16,17,21,25] **(Table 1b)**.

While comparing the FTIR spectra among the pure Oflox and polymer HPMC, and the mucoadhesive suspension containing both Oflox and HPMC, 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 Oflox was found from 1750 to 1700 cm^{-1}, which was lowered to 1650-1600 cm^{-1} in this suspension. This might be due to formation of β-ketoesters **(Figs 3, 4 and 5)**. The FTIR peaks assigned to $\upsilon_{C\text{-}O}$ and $\upsilon_{\mathbf{C\text{-}O\text{-}C}}$ representing esters confirm the esterification between polymeric OH group and COOH group of drug (Oflox). The stretching vibration of C-F group remains more or less unaltered. The another probability of interaction is hydrogen bonding i.e., intermolecular hydrogen bonding due to prominent FTIR peaks between 3100 and 3000 cm^{-1}, and 3000 and 2600 cm^{-1} represent polymeric O-H...O-H…O-H and strong intermolecular hydrogen bonding, respectively. The hydrogen bonded -OH stretching vibration has been found to occur over a wide range, 3100-2600 cm^{-1}. In case of intramolecular hydrogen bonding, FTIR bands are sharp while in intermolecular hydrogen bonding bands are broad. However, it is less broad than which is required

for chelation[11]. The bending vibration of O-H group indicates medium to strong bands in the region around 1450 cm^{-1}. The peak between 1100 and 1000 cm^{-1} represents υ_{C-F} group of Ofloxacin[10,11,19]. The band at 1000-950 is due to υ_{as} of pyranose ring of HPMC[18] **(Table 1).**

Table 1: Prominent FTIR Peaks of Ofloxacin, HPMC and mucoadhesive suspension[10,11,16,18]

(a) FTIR Peaks of Ofloxacin		
Peaks (cm^{-1})	**Groups**	**Peak Assignments**
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) FTIR Peaks of HPMC		
Peaks (cm^{-1})	**Groups**	**Peak Assignments**
3500-3400	Hydroxyl group	O-H stretching vibration, intermolecular H-bonding
2900	Methyl and hydroxypropyl group	$\upsilon_{s\text{-}CH}$ stretching of methyl and propyl group
2550-2500	Hydroxyl group	O-H stretching vibration, intramolecular H-bonding
1650-1600	Six membered cyclic	υ_{C-O}
1500-1450	δCH, δOCH, δCCH	Assymmetric bending vibration of methyl group in CH_3O
1400-1350	Cyclic anhydrides	υC-O-C and symmetric bending of methoxy group
1300-1250	epoxides	υC-O-C cylic
1100-1000	Ethereal C-O-C group	Stretching vibration of C-O-C group
1000-950	Pyranose ring	υ_{as} of pyranose ring
850-800	CH_2 group	rocking mode of CH_2 group
(c) FTIR Peaks of mucoadhesive suspension of Ofloxacin and HPMC		
Peaks (cm^{-1})	**Groups**	**Peak Assignments**

3100-3000	Hydroxyl group	O-H stretching vibration, polymeric H-bonded
3000- 2600	Hydroxyl group	O-H stretching vibration, intremolecular H-bonded
1650-1600	O-C-O group of acids	υ_{as} stretching vibration of acids
1500-1450	O-C-O group of acids	υ_s stretching vibration of acids, υ_{C-O} / δ_{O-H}
1400-1350	Esters and Methoxy groups	δC-O-C symmetric bending of esters and methoxy groups
1100-1000	C-F group	C-F stretching of Ofloxacin
1000-950	Pyranose ring	υ_{as} of pyranose ring of HPMC

The C=O group of drug (present in the formulation) 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 definite conclusion about the keto group in the bonding to the polymer can be deduced because the corresponding band found from 1650 to 1600 cm^{-1} is probably due to the formation of β-ketoesters[28]. 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. Actually, the nitrogen atom of the quinolone ring, 1-ortho to fluorine, is less electron rich due to electron deficient fluoroquinolone ring. In addition, methoxy 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 3100-2600 cm^{-1} can be assigned to the asymmetric and symmetric stretching vibrations of the OH groups present in 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[19]. By comparing the FTIR spectra among the pure drug, HPMC polymer and the mucoadhesive suspension containing both drug and polymer, the FTIR peak of Oflox from 1750 to 1700 cm^{-1} has not been detected in the

formulation, probably due to interaction with the polymer. The missing peak has been replaced by two very strong characteristic bands in the range of 1650-1600 cm^{-1} and at 1450 cm^{-1}. These are assigned to $\upsilon_{(O-C-O)}$ asymmetric and symmetric stretching vibrations, respectively[10,11]. The difference $\Delta[\upsilon_{(CO2)asym}-\upsilon_{(CO2)sym}]$ 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} indicating the deprotonation of the carboxylic acid group and interaction between drug and polymer[29] **(Table 1)**.

In case of Raman spectra of Oflox, the Raman band at 518.4 cm^{-1} is assigned to the stretching vibration of piperazinyl group and O-H torsional vibration of carboxylic acid. While the presence of carboxylic acid group is confirmed by υ_{O-C-O} at 1419.8 cm^{-1}, the stretching vibration of $\upsilon_{C=O}$ groups at 1649.6 cm^{-1} indicates the presence of pyridone moiety **(Table 2a)**.

The C-H out of plane bending vibration and C-C-O bending vibration of β D-glucose monomers have been confirmed from the nondestructive Raman spectroscopic analysis of HPMC. The presence of pyranose ring is also determined by the Raman shift at 908.3 cm^{-1}. The Raman shift for C-C stretching vibration strengthens the FTIR results for the characterization of HPMC polymeric chain [16-21].

By comparing the Raman spectra of pure drug with the drug incorporated in the Ofloxacin mucoadhesive suspension, the peak at 1419.8 cm^{-1} representing $\upsilon_{s\ O-C-O}$ is not prominent. Moreover, the symmetric stretching vibration of C-O-C group and stretching vibration of C=O are prominent in our mucoadhesive formulation. From this it is clear that there is esterification reaction between the Oflox and HPMC polymer **(Table 2)**. The results of both FTIR and Raman spectra indicate that both the spectra show prominent peaks for the stretching vibration of C-O-C 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 suspension.

Table 2: Prominent Raman Shifts of Ofloxacin, HPMC and mucoadhesive suspension[16-27].

a) Prominent Raman Shifts of Ofloxacin	
Raman Shifts(cm^{-1})	**Functional Groups / Vibrations**
518.4	Strong δ (CC) aliphatic chain, C-N stretching vibration of piperazinyl group and O-H torsional vibration of carboxylic acids
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 HPMC	
Raman Shifts(cm^{-1})	**Functional Groups / Vibrations**
504.7	C-H out plane bending and C-C-O bending vibration
908.3	C-C-C in plane bending and stretching vibration of $\upsilon_{(C\text{-}O\text{-}C)}$ in pyranose ring
1384.3	C-C stretching vibration
c) Prominent Raman Shifts of mucoadhesive suspension of Ofloxacin and HPMC	
Raman Shifts(cm^{-1})	**Functional Groups / Vibrations**
338.8	C-C-C out plane bending
900-850	Symmetric vibration of C-F bond, symmetric COC stretching vibration
1340.5	δCCH and δOCH bending vibration
1800-1700	C=O stretching vibration of esters

Conclusions

Due to very good interaction between the carboxylic group of the drug and hydroxyl group of the polymer, esterification and intermolecular hydrogen bonding occur in the formulation, which may lead to a stable controlled release formulation. Moreover, the drug polymer complex may aggregate forming a micelle like structure, which can absorb and solubilize more drugs. As a result of which HPMC polymer may function as a useful carrier for the Ofloxacin molecule. The main advantage of the present investigation is that higher Ofloxacin drug loading would be possible in dosage forms as compared with alternate formulation strategies, such as conventional solid dispersions.

Here, Ofloxacin interacts with the polymer monomerically. The release of the drug from the formulation is very slow because the carboxylic group of Ofloxacin has already interacted with polymeric OH groups. It suggests less active sites of the drug are left for the attack by the water molecules for the hydration and solubilization, which may give controlled release action. In addition, the free polymeric carboxylic groups form hydrogen bonding with the polysaccharides and proteins of mucosa. Due to the presence of HPMC, the formulation is highly swollen and stiffened showing a very good mucoadhesive property in the gastrointestinal mucosa. This may lead to a better bioadhesive and controlled release action. The utility of the present work may be improved, if delivery rate, biodegradation and site-specific targeting of such formulation would be monitored and controlled.

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Corresponding Author:
Subhashree Sahoo*,
E-mail: *subha77t@yahoo.co.in*