Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 4, Suppl 2, 2011 ISSN - 0974-2441

Research Article

SPECTROSCOPIC INVESTIGATIONS OF A CONTROLLED RELEASE MUCOADHESIVE SUSPENSION

SUBHASHREE SAHOO[1]*, CHANDRA KANTI CHAKRABORTI[1], PRADIPTA KUMAR BEHERA[2], SUBASH CHANDRA MISHRA[3]

[1]Department of Pharmaceutics, Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela, Orissa, India ,[2]School of Chemistry, Sambalpur University, Jyoti Bihar-768019, Orissa, India,[3]Metallurgical and Materials Engg. Dept., National Institute of Technology, Rourkela, Orissa, India, Email: subha77t@yahoo.co.in

Received: 29 July 2011, Revised and Accepted: 14 September2011

ABSTRACT

Norfloxacin, 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 problems, many investigators have prepared different formulations of Norfloxacin. But till now very few formulations are available from which the drug is absorbed uniformly, so that safe and effective blood level of Norfloxacin could be maintained for a prolonged period. To fulfill this requirement, in the present study, a controlled release drug delivery system was designed and chemical interaction between Norfloxacin and a polymer (HPMC), used in the formulation, was studied by FTIR and Raman Spectroscopy. Ultrasonication method was used for preparation of mucoadhesive Norfloxacin formulation in which drug to polymer weight ratio being 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. From the spectral interpretation, it has been found that in formulation, the carboxylic groups of Norfloxacin and hydroxyl groups of HPMC undergo chemical interaction leading to esterification and hydrogen bonding (both intermolecular and polymeric). 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 Norfloxacin may give better controlled release and mucoadhesive action in the gastrointestinal tract. Hence, HPMC could be considered as an effective carrier of Norfloxacin.

Keywords: Norfloxacin, HPMC, Mucoadhesive suspension, FTIR, Raman Spectroscopy.

INTRODUCTION

Norfloxacin (Norflox), 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone carboxylic acid, is a fluoroquinolone antibacterial agent (Fig. 1). Normal dosage regimen varies from 400 to 500 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 6 h. As frequent dosing is required to maintain the therapeutic plasma concentration, it was chosen as a model drug for the controlled release study[1].

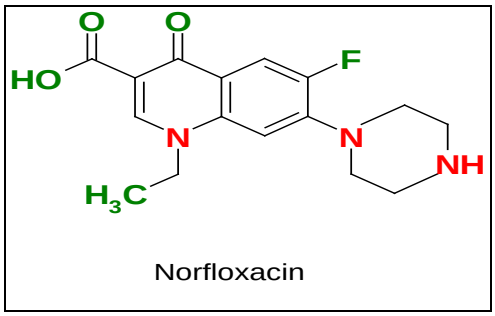


Fig 1: Chemical Structure of Norfloxacin

There are several ways of achieving sustained release, such as by suspending the drug in the suspension (at a concentration exceeding the solubility), by formulating the drug as micro- or nanospheres, by distributing the drugs to the liposome or surfactant aggregates or by utilizing interaction between the drug and the polymer[2].

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[3-7].

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[8]. 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[3].

HPMC is propylene glycol ether of methyl-cellulose. Its chemical structure has been illustrated in Figure 2[8]. 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[3].

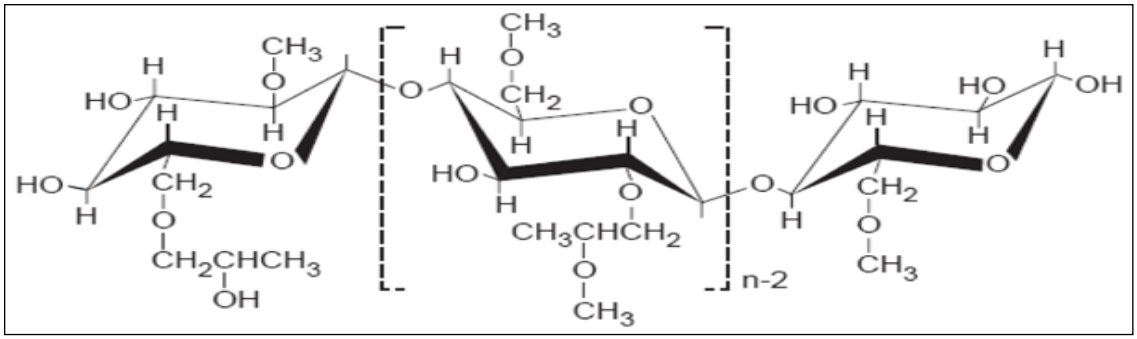
HPMC may form a complex with the low solubility drug like Norfloxacin. The interaction between the Norflox and hydrophilic osmo-polymer HPMC can be determined by several methods such as Fourier Transform Infrared (FTIR) Spectroscopy, Raman Spectroscopy, etc. To know the different functional groups and highly polar bonds of pure Norfloxacin, 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 Norfloxacin and HPMC, both FTIR and Raman analyses were carried out [9, 10].

METHODS

Preparation of Formulation

Preparation of Bulk A

In a beaker 6 ml water was 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.



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.

Preparation of Mucoadhesive Suspension and Ultrasonication

Five millilitre of water was taken in another beaker to which 500 mg of Norflox 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[11-13]. 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}.

RESULTS

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 υ_{O-C-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) [11, 12, 14-16].

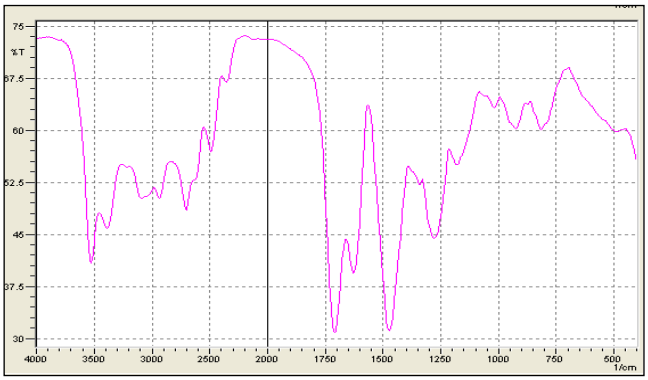


Fig 3: FTIR peaks of Norfloxacin

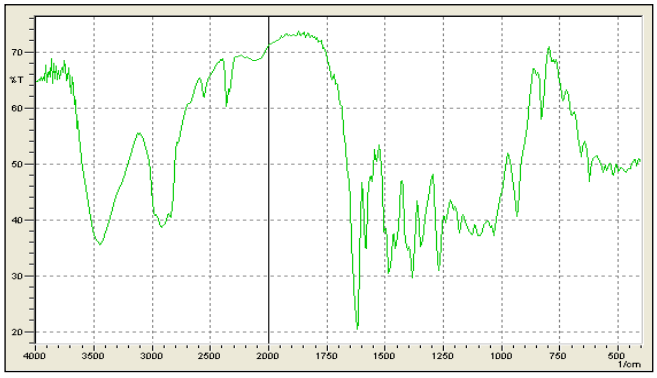


Fig 4: FTIR Spectra of pure HPMC

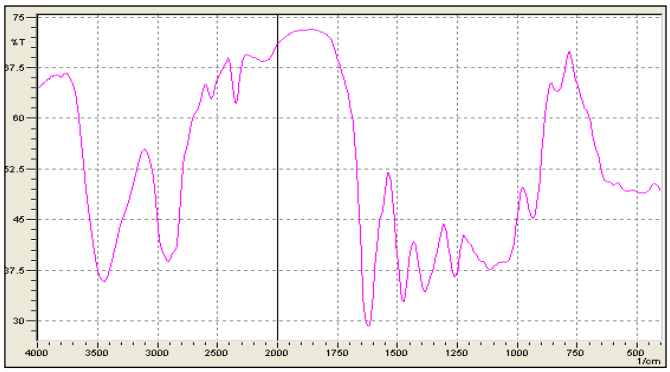


Fig 5: FTIR peaks of Norfloxacin Mucoadhesive Formulation.

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) [11,12]. The symmetric stretching mode of υ_sMe and $\upsilon_s$hydroxypropyl groups was found at 2900 cm^{-1}, in which all the CH bonds extend and contract in phase[12]. The peak at 2550-2500 cm^{-1} was assigned to OH stretching vibration, i.e., υ_{O-H} and intramolecular hydrogen bonding[11, 12]. 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 of 1500-1450 cm^{-1}, the symmetric vibrations were mostly displayed in the range of 1400-1350 cm^{-1} [17,18]. The band between 1400 and 1350 cm^{-1} suggested C-O-C stretching vibration of cyclic anhydrides. The peak at 1300-1250 cm^{-1} was due to C-O-C stretching vibration of 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[19]. The rocking mode of CH_2 was found in the range of 850-800 cm^{-1} [17](Fig. 4 and Table 2). The computed frequencies of HPMC are in a good agreement with experimental frequencies for both carbohydrate region as well as OH and CH region.

In the FTIR spectra of the mucoadhesive suspension, the peak from 3500 to 3400 cm^{-1} was assigned to υ_{O-H} and single bridge hydrogen bonding, the band between 3000 and 2800 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} suggested υ_{C-F} groups[12, 20]. The band at 950-900 cm^{-1} was assigned to υ_{as} of pyranose ring of HPMC[19] (Table 3). Figure 6 shows comparative FTIR spectra of Norflox, HPMC and Norfloxacin mucoadhesive suspension.

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. 7). 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 [21, 22, 23]. The band at 872.7 cm^{-1} represented the symmetric stretching vibration of C-F group [24,25]. 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[26]. 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 [21, 26-29] (Table 4a). 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 $\upsilon_{(C-O-C)}$ stretching vibration of pyranose ring. The peak at 1384.3 cm^{-1} was assigned to C-C stretching vibration (Table 4b) [17, 18, 21, and 24].

The characteristics Raman peaks of mucoadhesive suspension containing both Norflox and HPMC were observed at 343.5, 900-850, 1348.7 and 1800-1700 cm^{-1} (Fig. 9). The band at 343.5 cm^{-1} was assigned to C-C-C out of plane bending of pyranose ring[18]. 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 1348.7 cm^{-1} respesented δCCH and δOCH bending vibration of methoxy group [17]. The Raman shift at 1550-1500 cm^{-1} suggested the asymmetric stretching vibration of O-C-O group. The peak at 1800-1700 cm^{-1} was assigned to C=O stretching vibration of carbonyl groups of esters[18] (Table 4c). Figure 10 shows comparative Raman shifts of Norflox, HPMC and Norfloxacin mucoadhesive suspension

.

DISCUSSION

When FTIR radiation falls on a molecule, it may be absorbed, reflected or transmitted. Absorption leads to the FTIR spectrum, while reflection causes scattering which is utilized in Raman spectroscopy[12].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[11, 12]. In case of FTIR spectra of Norflox, prominent peaks for υ_{C-O} / δ_{O-H} and $\upsilon_{C=O}$ indicated the presence of –CO-, -CHO and -COOH groups (Fig. 3). The presence of above groups can be confirmed by fermi resonance bands for –CHO, υ_{C-O-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} [12, 30, 31]. The band 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 of 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 band at 1050-1000 cm^{-1} indicated the presence of C-F group which takes a major role in its antimicrobial activity (Table 1) [11, 13, 15].

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[17,18, 21,26](Table 2). While comparing the FTIR spectra among pure Norflox and polymer HPMC, and the mucoadhesive suspension containing both Norflox 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 Norflox 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 $\upsilon_{C\text{-}O\text{-}C}$ groups representing esters confirm the esterification between polymeric OH group and COOH group of drug (Norflox). The stretching vibration of C-F group remains more or less unaltered. Another probability of interaction is hydrogen bonding i.e., intermolecular hydrogen bonding due to prominent FTIR peaks between 3500 and 3400 cm^{-1}, and 3000 and 2800 cm^{-1} represent single bridge 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, 3500-2800 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[12]. 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 $\upsilon_{C\text{-}F}$ group of Norfloxacin[11, 12, 20]. The band at 950-900 is due to υ_{as} of pyranose ring of HPMC[19] (Table 1-3).

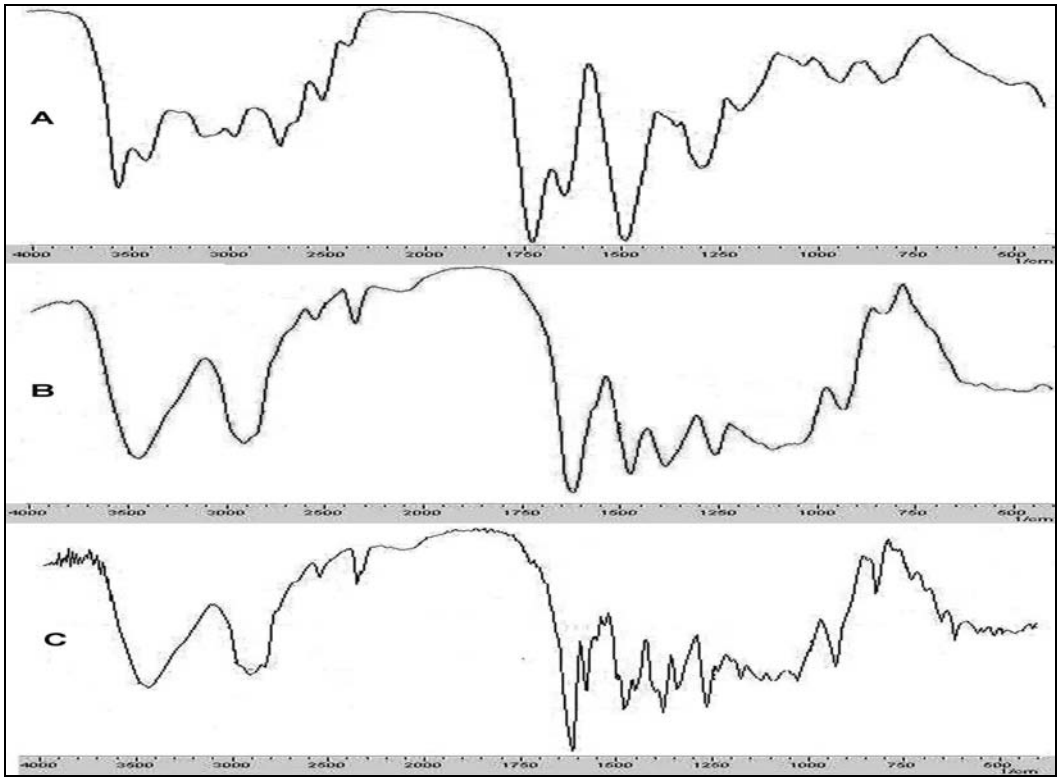


Fig 6: Comparative FTIR peaks of Norflox (A), HPMC (B) and Formulation (C)

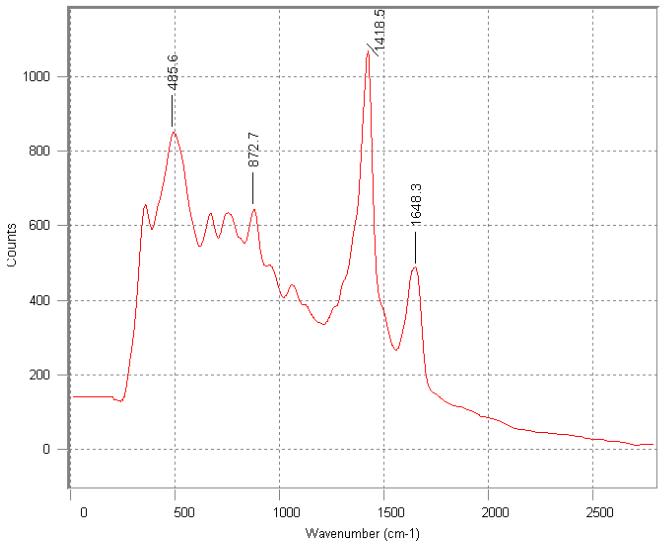


Fig. 7: Raman Shifts of Norfloxacin

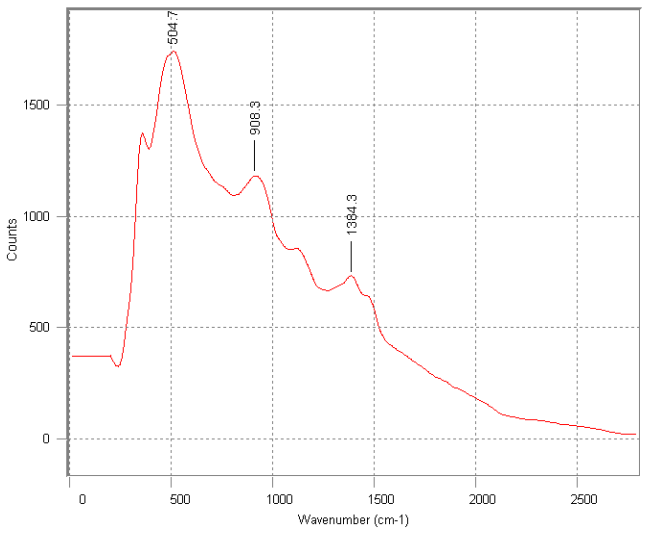


Fig. 8: Raman Shifts of HPMC

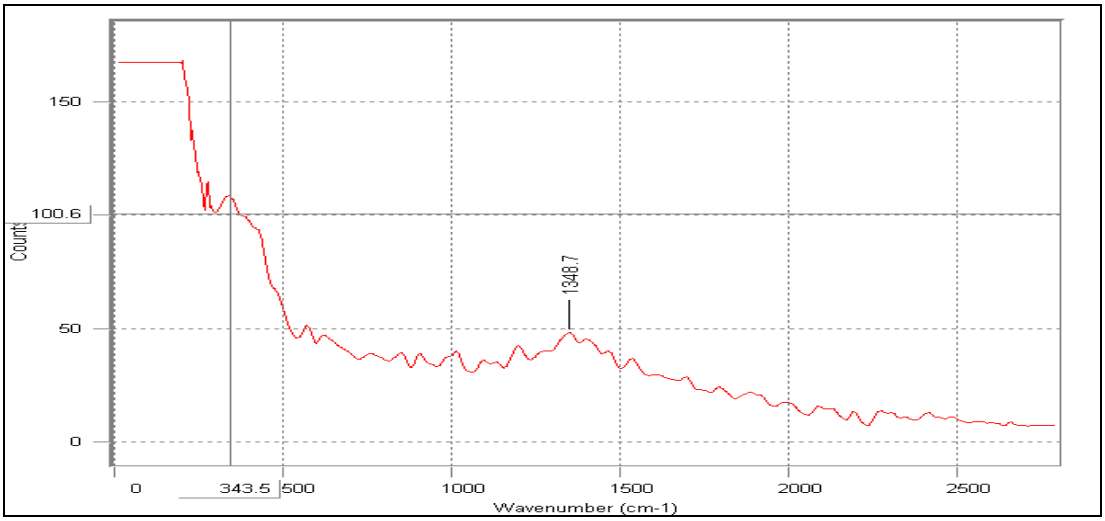


Fig 9: Raman Shifts of Norfloxacin Mucoadhesive Formulation

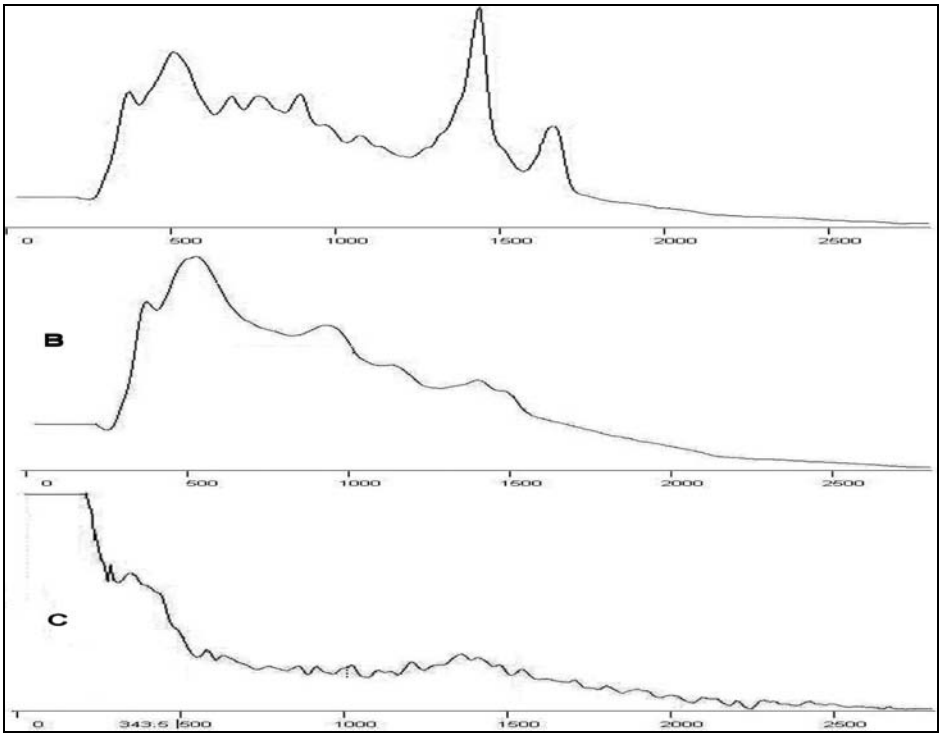


Fig 10: Comparative Raman Shifts of Norflox, HPMC (B) and Formulation (C)

Table 1: Prominent FTIR peaks of Norfloxacin

Peaks(cm-1)	Groups	Peak Assignments
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

Table 2: Prominent FTIR Peaks 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	$\upsilon_{C\text{-}O}$
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

Table 3: Prominent FTIR Peaks of Norfloxacin Formulation.

Peaks (cm^{-1})	Groups	Peak Assignments
3500-3400	Hydroxyl group	O-H stretching vibration, single bridge H-bonded
3000-2800	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, $\upsilon_{C\text{-}O}$ / $\delta_{O\text{-}H}$
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
950-900	Pyranose ring	υ_{as} of pyranose ring of HPMC

Table 4: Raman Shifts of Norfloxacin, HPMC and Norfloxacin 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 piperazinyl 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 Norfloxacin Mucoadhesive Formulation

Raman Shifts(cm^{-1})	Functional Groups / Vibrations
343.5	δ(CC) aliphatic chain
900-850	Symmetric stretching vibration of both C-F group C-O-C group for acrylates and esters
1348.7	υ_sO-C-O
1550-1500	υ_{as}O-C-O
1850-1700	υC=O medium

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 [32]. 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 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 of 3500-2800 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[20]. By comparing the FTIR spectra among the pure drug, HPMC polymer and the mucoadhesive suspension containing both drug and polymer, the FTIR peak of Norflox 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 $\upsilon_{(O\text{-}C\text{-}O)}$ asymmetric and symmetric stretching vibrations, respectively[11,12]. The difference $\Delta[\upsilon_{(CO2)asym} - \upsilon_{(CO2)sym}]$ 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} indicating the deprotonation of the carboxylic acid group and interaction between drug and polymer [33](Table 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 $\upsilon_{O\text{-}C\text{-}O}$ and $\upsilon_{C=O}$ groups vibration at 1418.5 cm^{-1} and 1655.1 cm^{-1}, respectively (Table 4a).

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 [17-21, 25].

By comparing the Raman spectra of pure drug with the drug incorporated in the Norfloxacin mucoadhesive suspension, the peak at 1419.8 cm^{-1} representing symmetric stretching vibration of O-C-O group is not prominent. Moreover, the symmetric stretching vibration of C-O-C group and stretching vibration of C=O group are prominent in our mucoadhesive formulation. From this it is clear that there is esterification reaction between Norflox and HPMC polymer (Table 4). 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.

CONCLUSION

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 Norfloxacin molecule. The main advantage of the present investigation is that higher Norfloxacin drug loading would be possible in dosage forms as compared with alternate formulation strategies, such as conventional solid dispersions. Here, Norfloxacin interacts with the polymer monomerically. The release of the drug from the formulation is very slow because the carboxylic group of Norfloxacin 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.

REFERENCES

1. Sinduri P, Purusotoman M. Formulation and Evaluation of Norfloxacin Microspheres Using Different Polymers. Int J Pharm Ind Res 2011; 1:32-5.
2. Hui HW, Robinsion JR, Lee VHL, Controlled Drug Delivery-Fundamentals and Application. New York : Marcel Dekker, Inc.; 2005.
3. Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev 2001; 48: 139–57.
4. Phaechamud T. Variables Influencing Drug Release from Layered Matrix SystemComprising Hydroxypropyl Methylcellulose, AAPS Pharm Sci Tech 2008; 9(2): 668-74.
5. Talukdar MM, Michoel A, Rombout P, Kinget R. Comparative Study on Xanthan gum and hydroxypropyl methyl cellulose as matrices for controlled drug delivery I. Compaction and In Vitro drug release behavior. Int J Pharm 1996; 129: 233-41.
6. Katiknani PR, Upadrashta SM, Neau SH, Mitra AK. Ethyl Cellulose matrix controlled release tablets of water soluble drug. Int J Pharm 1995; 123: 119-25.
7. Gao P, Skoug JW, Nixon PR, Ju RT, Stemm NL, Sung K. Swelling of Hydroxypropyl Methylcellulose Matrix Tablets. 2. Mechanistic Study of the Influence of Formulation Variables on Matrix Performance and Drug Release. J Pharm Sci 1996; 85(7): 732-40.
8. Fatimi A, Tassin JF, Quillard S, Axelos MAV, Weiss P. The rheological properties of silated hydroxypropylmethylcellulose tissue engineering matrices. Biomater 2008;29(5): 533-43.
9. Venkeirsbilck T, Vercauteren A, Baeyens W, Weken GVD, Verpoort F, Vergote G, et al.Applications of Raman Spectroscopy in pharmaceutical analysis. Trends Anal Chem 2002; 21(12): 869-77.
10. Clarke RH, Londhe S, Premasiri WR, Womble ME. Low-Resolution Raman Spectroscopy: Instrumentation and Application in Chemical Analysis. J Raman Spectrosc 1999; 30: 827-32.
11. Silverstein RM, Webster FX. Spectrometric Identification of Organic Compounds; 6th ed, New York: Jhon Wiley and Sons; 2002.
12. Dani VR. Organic Spectroscopy. 1st ed, New Delhi: Tata McGraw-Hill Publishing Company Limited; 1995.
13. Precautions for Making KBr Pellets; Available from http://www.chemistry.nmsu.edu/Instrumentation/KBr_New.html, accessed on 20.01.2010
14. C.P.S Hsu: Infrared Spectroscopy; Available from http://www.prenhall.com/settle/chapters/ch15.pdf, accessed on 20.01.2010.
15. Sateesha SB, Rajamma AJ, Shekar HS, Mutahar RKM, Jayanthi A. ; Formulation and stability study of palatable norfloxacin dry syrup: comparison among different preparation methods. Asian J Pharma. Sci 2010; 5: 175-84.
16. Al-Mustafa J. Magnesium, Calcium and Barium Perchlorate Complexes of Ciprofloxacin and Norfloxacin. Acta Chim Slov 2002; 49: 457–66.
17. Raj A, Raju K, Varghese HT, Granadeiro CM, Nogueira HIS, Panicker CY. IR,Raman and SERS spectra of 2-(methoxycarbonylmethylsulfanyl)-3,5-dinitrobenzene carboxylic acid. J Braz Chem Soc 2009; 20(3): 549-559.
18. Govindarajan M, Periandy S, Ganesan K. Scaled Quantum FT-IR and FT-Raman Spectral Analysis of 1-Methoxynaphthalene. E-Journal Chem 2010; 7(2): 457-64.
19. Ibrahim M, Alaam M, El-Haes H, Jalbout AF, de Leon A. Analysis of the structure and vibrational spectra of glucose and fructose. Eclet Quím 2006; 31(3): Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100- 46702006000300002&lng=en&nrm=iso&tlng=en
20. Tom RT, Suryanarayana V, Reddy PG, Baskaran S, Pradeep T. Ciprofloxacin Protected gold nanoparticles. Langmuir 2004; 20(5): 1909-14.
21. Raman Data and Analysis; Available from http://www.horiba.com/fileadmin/uploads/scintific/Documents/Raman/bands.pdf, accessed on 20.01.2010.
22. Tua Q, Eisenb J, Changa C: Band Shifts in Surface Enhanced Raman Spectra of Indolic Molecules Adsorbed on Gold Colloids; Available from http://www.icors2010.org/abstractfiles/ICORS20101040.5375VER.5.pdf accessed on 2.01.2010.
23. Xu J, Stangel I, Butler IS, Gilson DFR. An FT-Raman Spectroscopic Investigation of Dentin and Collagen Surfaces Modified by 2- Hydroxyethylmethacrylate. J Dent Res 1997; 76: 596-601.
24. Gruodis A, Alkasa V, Powell DL, Nielsen CJ, Guirgis GA, Durig JR. Vibrational spectroscopic studies, conformations and ab initio calculations of 1, 1, 1 trifluoropropyltrifluorosilane, J Raman Spectrosc 2003; 34: 711-24.
25. Sharts D, Gorelik VS. Method and apparatus for determination of carbon- halogen compounds and applications thereof. United States Patent 6445449; Available from http:/ / www.freepatentsonline.com/ 6307625.html, accessed on 20.01.2011
26. Bright A, Devi TSR, Gunasekaran S. Spectroscopical Vibrational Band Assignment and Qualitative Analysis of Biomedical Compounds with Cardiovascular Activity. Int J Chem Tech Res 2010; 2: 379-88.
27. Skoulika SG, Georgiou CA. Rapid Quantitative Determination of Ciprofloxacin in Pharmaceuticals by Use of Solid-State FT-Raman Spectroscopy. Appl Spectrosc 2001; 55: 1259-65.
28. Lawrence BA, Lei Z, Liling Z, Christopher LE, Andrew RB. Solid-State NMR Analysis of Fluorinated Single - Carbon Nanotubes: Assessing the extent of Fluorination. Chem Mater 2007; 19: 735-44.
29. Agarwal UP, Reiner RS, Pandey AK, Ralpha SA, Hirth KC, Atalla RH: Raman Spectra of Liginin Model Compounds; Available from http://www.treesearch.fs.fed.us/pubs/20194, accessed on 20.01.2010.
30. Pandya SJ, Bhalekar MR, Harinarayana D, Shah SS, Darji D. Preparation and Characterization of Light Sensitive Ofloxacin Complexes under Accelerated Condition. Int J Pharm Res 2010; 2: 28-32.

31. Florence AJ, Kennedy AR, Shankland N, Wright E, Al-Rubayi A. Norfloxacin dehydrates. Acta Cryst 2000; 56: 1372–73.
32. Garrido NJ, Perello L, Ortiz R, Alzuet G, Alvarez MG, Canton E, et al. Antibacterial studies, DNA oxidative cleavage, and crystal structure of Cu(II) complexes with two quinolone family members, ciprofloxacin and enofloxacin. J Inorg Biochem 2005; 99: 677-89.
33. Efthimiadou EK, Psomas G, Sanakis Y, Katsaros N, Karaliota A. Metal complexes with the quinolone antibacterial agent N-propyl-norfloxacin:Synthesis, structure andbioactivity; J Inorg Biochem 2007; 101: 525-35.