INTRODUCTION
MFX is a fourth-generation synthetic fluoroquinolone derivative which has broad antimicrobial activity and is effective after oral administration for the treatment of a wide variety of infectious diseases. MFX, a fluoroquinolone, is available as the monohydrochloride salt of 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aS,7aS)-octahydropyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydro quinoline-3-carboxylic acid. It is a slightly yellow to yellow crystalline substance with a molecular weight of 437.9. Its empirical formula is C_{21}H_{24}FN_{3}O_{4}\cdot\text{HCl} (1). It is marketed worldwide (as the Moxifloxacin hydrochloride) under the brand names Avelox, Avalox, and Avelon. In most countries, the drug is also available in par-

ABSTRACT
OBJECTIVE: In recent years, excipient development has become major area of research in pharmaceutical drug delivery because it influences the formulation development and drug delivery process in various ways. In modern pharmaceutical science biopolymers are choice of research as excipient because of their low toxicity, biocompatibility, biodegradability, stability and renewable nature. Chitosan, a biodegradable polysaccharide derived from chitin and found widely in nature, possesses properties making it particularly suitable as a carrier, including its high viscosity, charge distribution and release mechanisms. Our present research work includes preparation and characterization of chitosan-moxifloxacin conjugates designed in accordance with specific biomedical requirements.

METHODS: In this research work chitosan based chitosan-moxifloxacin conjugates have been successfully formulated by using distilled water and glacial acetic acid mixture and the film was made by solution casting method. Formulated films were characterized by various analytical methods such as UV, IR and DSC as well as biological methods.

RESULTS: The antibacterial activity of chitosan-moxifloxacin conjugates was tested against various microorganisms viz, gram positive bacteria’s Streptococcus pneumoniae and Staphylococcus aureus and the results indicated that the antibacterial activity of chitosan-moxifloxacin conjugates was several time greater than that of parent drug moxifloxacin. This may be due to the favorable pharmacokinetics, pharmacodynamics, excellent bacterial susceptibility and good stability of the drug conjugates.

CONCLUSION: This proposed combination may result in the enhancement of the separate activities of chitosan and moxifloxacin.

KEYWORDS: MFX (Moxifloxacin), CH (Chitosan), conjugate, prodrug, electrostatic-interaction, antimicrobial etc.
enteral form for intravenous infusion. MFX is also sold in an ophthalmic solution (eye drops) under the brand names Vigamox, Moxeza for the treatment of conjunctivitis (pink eye) (2). The quinolone antibiotics target bacterial DNA gyrase and topoisomerase IV (3). For many gram-positive bacteria (such as S. aureus), topoisomerase IV is the primary activity inhibited by the quinolones. In contrast, for many gram-negative bacteria (such as E. coli), DNA gyrase is the primary quinolone target (4). MFX is a broad-spectrum antibiotic that is active against both gram-positive and gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV (3), enzymes necessary to separate bacterial DNA, thereby inhibiting cell replication.

Despite much progress in the field of antibacterial drug discovery, antibacterial drugs in current clinical use generally suffer from a series of deficiencies, including excessive organ toxicity, lack of specificity, short circulation half-lives, and a pronounced tendency to induce resistance in the target cells.

Polymer systems for drug release have been widely used in medicine, since they enable the slow and gradual release of the active ingredient, with better targeting within the body, such as towards areas of inflammation or tumors. CH, a biodegradable polysaccharide derived from chitin and found widely in nature, possesses properties making it particularly suitable as a carrier, including its high viscosity, charge distribution and release mechanisms. Research into the chemical properties of CH has demonstrated its suitability for the preparation of enzymatic biosensors for the analysis of metallic elements, proteins and lipids. Pharmaceuticals possessing antibiotic properties have been explored as good candidates for the preparation of formulations based on polymeric controlled release systems. Carrier materials such as CH have been employed to protect the pharmaceutical agent so that it may be released under optimal absorption conditions, or to adjust the timing of release of different pharmaceutical agents administered simultaneously. The potential application of CH is hindered by its limited solubility in aqueous media (5). CH is a biopolymer which is well tolerated (biocompatible); it also approved to be an efficient carrier for the controlled delivery of drugs. It is well known that CH from the ocean resources has been found to have pronounced tendency to induce resistance in the target cells.

In this work the degree of interaction between CH and MFX is investigated using various analytical techniques, envisaging possible applications in new formulations based on optimization of the therapeutic dose in order to improve the quality of life of patients.

### Experimental section

#### Material and methods

CH with 79% degree of deacetylation (DD) was sponsored by Central Institute of Fisheries Technology (CIFT, Cochin, India). MFX was received as a gift sample from Cosmas Pharmaceuticals Pvt. Ltd. Vill. Kotla Solan Baddi, India. The bacterial test strains Streptococcus pneumoniae and Staphylococcus aureus were purchased from IMTECH, Chandigarh, India. All materials, solvents and solutions were of analytical grade.

#### Preparation of chitosan-moxifloxacin conjugates

1. **Preparation of chitosan solution**

CH is a weak base which is insoluble in water and other organic solvents however, it has a unique property to become soluble in dilute aqueous acidic solution. To synthesize CH-MFX conjugates, 1% CH solution was prepared with the help of glacial acetic acid (1% w/v) and distilled water.

The following mixture of CH was kept at room temperature (20°C) for 24 hours with continuous stirring. The mixture was stirred magnetically until the polymer was completely dissolved and a viscous solution is formed. The solution was filtered through glass-wool to remove undissolved particles of CH.

2. **Preparation of drug solution**

MFX is completely soluble in aqueous solvents. To prepare the drug solution about 200mg of MFX drug was dissolved in minimum amount of distilled water; the solution was stirred until a homogenous mixture is formed.

3. **Drug loading**

MFX solution was gradually added to the above solution over 2 min with continuous stirring. The mixture was then stirred for further 24 hours at room temperature (20°C). To concentrate the resulting solution, it was kept for 48 hrs in an incubator at 37°C. A highly viscous solution was obtained which was then successfully used for further process.

4. **Preparation of films**

CH-MFX prodrug conjugate was prepared by solution casting method (19). Films were prepared by pouring and spreading the CH-MFX mixture on a glass plate, and kept in microwave oven for 30 minutes at 37°C. After microwave treatment the resulting film was kept at room temperature (20°C). After two days films were collected and used for further characterization and antibacterial activity.

#### Measurements/characterization techniques

**UV-Vis spectroscopy**

UV/Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions highly conjugated organic compounds, and biological macromolecules. Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. UV-Vis spectra of the films were scanned in the wavelength range 200–800 nm using UV-Vis spectrophotometer (1800 PC UV spectrometer, Shimadzu).
In this research work which crystallization can occur (21) it is desirable to process the drug at temperatures below those at which crystallization can occur. If it is necessary to deliver a drug in the amorphous form, it is possible to process the drug at temperatures below those at which crystallization can occur. One approach is to use physical methods to soften the drug in order to define processing parameters. For instance, in the pharmaceutical field it is necessary to have well-characterized drug compounds in order to define processing parameters. For instance, if it is necessary to deliver a drug in the amorphous form, it is desirable to process the drug at temperatures below those at which crystallization can occur.

In this research work differential scanning calorimetry was used to examine the thermal properties of CH-MFX conjugate with a constant heating rate of 10°C/min under a nitrogen flow in thermetically sealed aluminium pans. The weight of the sample ranged between 2-3 mg. The results were recorded and analyzed.

**Swelling test**

In order to check the swelling ability of the MFX loaded CH conjugates (CH-MFX films) 50 ML phosphate buffer solution of pH 7.4 was used. Phosphate buffer of required pH was prepared by dissolving 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient water to produce 1000 ml. pH of the buffer solution was adjusted to 7.4. The CH-MFX films (1.5×1.5 cm²) were placed in 50 ml of phosphate buffer solution and incubated at 37°C. At the time intervals of 2, 4, 6 and 8 hours, the films were taken out and excess water was removed from the film carefully using filter paper and films were weighed immediately (22).

Percentage swelling of CH-MFX prodrug at equilibrium was calculated from the following formula.

\[
DS = \frac{(W_w - W_d)}{W_d}
\]

Where, \(W_w\) and \(W_d\) are weights of wet and dry film respectively.

**Antimicrobial activity tests and biological relevance (22)**

Microbiological efficiency of antimicrobial CH-MFX conjugates and conjugates forming solution were evaluated by using agar diffusion method.

**Preparation of microorganisms**

Bacteria was cultured in nutrient broth and incubated overnight at 37°C for 24 hrs. The bacterial cultures obtained were diluted with autoclaved nutrient. This culture served as the inoculums for the antimicrobial experiments.

Preparation of agar plates for antimicrobial activity

Nutrient agar plates were prepared by mixing nutrient agar (28 gm) in 1000 ml distilled water boiled to dissolve the medium completely. Nutrient agar solution was sterilized by autoclaving at 121°C for 15 min at 15 lb pressure. After cooling (45°C), agar solution (25 ml) were poured into sterilized petri dishes and left to solidify. Agar plates were inoculated with an overnight bacterial culture, using spread plate method after appropriate serial dilutions. Nutrient agar plates were then incubated at 37°C for 24 hrs. The diameter of inhibitory zone surrounding film disc and antimicrobial CH based conjugate forming solution was measured after 24 hrs. Two cross sectional points and the average was taken as the inhibition zone and the size of the zone diameter was measured. The plates were then photographed individually.

The inhibition zone diameter of the MFX was compared with the zone diameter of CH-MFX conjugate.

**RESULTS AND DISCUSSION**

**Proposed reaction scheme**

When MFX drug added to the CH solution, in the presence of glacial acetic acid, the acidic group (-COOH) of MFX converts into anion COO⁻ and amine (-NH₂) group of CH converts into cation NH₃⁺. CH-MFX complex forms due to the electrostatic interaction between NH₂ group of CH and COOH group of MFX drug [Reaction Scheme 1]. Furthermore the prepared complex was characterized by various analytical methods.

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Characterization

Ultra violet spectroscopy

Because of its transparency it’s hard to characterize CH by UV spectroscopy methods. However, we can overcome this natural handicap by borrowing chromophores from extrinsic molecule. The acid group moieties play the role of reporter molecules for the spectroscopy study, from which the structural and antibacterial activity is deduced (19).

UV spectra for all the synthesized compounds were recorded, and are included in the appendix. CH solution does not give any sharp peak in its UV spectrum, however a low intensity peak is observed at 278 nm (Figure 1(a)). MFX in aqueous medium showed characteristic absorption band around 285.50 nm (Figure 1(b)), where UV spectrum band of CH-MFX complex showed absorption at 286 nm (Figure 1(c)).

Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectra for all the prepared compounds were recorded, and are included in the appendix. For CH spectrum (Figure 2 (a)): 3422 cm⁻¹ (which is assigned to the N-H and hydrogen bonded O-H stretch vibrational frequencies), while a sharp (shoulder) peak at 3610 cm⁻¹ is that of free O-H bond stretch of glucopyranose units, 2921 and 2867 cm⁻¹ (C-H stretch), 2364 cm⁻¹ (C-N asymmetric band stretching), 1653 cm⁻¹ (amide II band, C=O stretch of acetyl group), 1592 cm⁻¹ (amide II band, N-H stretch) 1375 cm⁻¹ (asymmetric C-H bending of CH₂ group) and 1071 cm⁻¹ (skeletal vibration involving the bridge C=O stretch) of glucosamine residue.

For CH-MFX complex (Figure 2 (b)): the spectral band appear at 3422 cm⁻¹ (axial O-H group of CH). Absorption arising from C-H stretching in the alkanes occurs in the general region of 2949 cm⁻¹ – 2840 cm⁻¹ (symmetric or asymmetric CH₃ stretching vibration attributed to MFX and pyranose ring of CH). A band at 2344 cm⁻¹ refers to C-N asymmetric band stretching. A broad, strong NH₃ stretching band in the 3100-2600 cm⁻¹ region and multiple combination and overtone bands extend the absorption to about 2000 cm⁻¹, this overtone region usually contains a prominent band near 2222-2000 cm⁻¹ (bending vibration and torsional oscillation of amine salt NH₃⁺). One more fairly strong symmetric bending near 1562 cm⁻¹ is the characteristic of NH₃⁺ absorption. A strong absorption of carboxylate ion occurred at 1413 cm⁻¹, while a week absorption at around 1400 cm⁻¹. 1400-1032 cm⁻¹ (C-F band stretching of MFX), 943-623 cm⁻¹ (mono and di-substituted benzene ring).

When IR spectrum of CH-MFX compared with CH it showed purely electrostatic nature of the interaction between CH (NH₃⁺) and MFX (COO⁻) is the new signals at 1562 and 1413 cm⁻¹. This result suggested that the NH₂ group on CH chains were protonated by the H⁺ supplied while the carboxylic group of MFX is deprotonated.

The intensity of these bands totally depends on the concentration, type and bulkiness of the acid.
Differential Scanning Calorimetry (DSC)
The DSC thermograms for CH and CH-MFX complex were recorded, and are included in the Figure 3 (a), (b). CH showed two broad endothermic peaks at 92.3 °C and 212 °C. The former peak may be due to the water vapor present in CH while the latter may be attributed to the molecular arrangement of CH chains. The CH-MFX complex exhibit a sharp endothermic peak at 120°C due to structural arrangement and its degree of substitutional changes in the polysaccharide. There is a sharp exothermic peak at 238°C is due to the thermal decomposition of MFX. The results indicated that the structure of CH chains have been modified due to the introduction of MFX unit.

**FIGURE 3(A).** DSC thermograph of CH

**FIGURE 3(B).** DSC thermograph of CH-MFX complex

Swelling test
It is well known that the sample with the highest degree of swelling will have the highest surface area/volume ratio. The hydrophilic nature of CH material may be a major factor that influences the extent of swelling in the formulations (CH-MFX).

The calculated equilibrium swelling ratio for prepared CH-MFX complex is 1330%. Swelling ratio of CH-MFX complex is demonstrated in Graph 1.

**GRAPH 1.** Swelling ratio of CH-MFX complex films in phosphate buffer solution of pH 7.4

Antimicrobial activity tests and biological relevance
Antibacterial activity of the CH-MFX complex film and its solution against *Streptococcus pneumoniae* and *Staphylococcus aureus* were measured by agar diffusion method. After 24 hrs incubation at 37 °C, the CH-MFX complex film and its solutions showed effective antibacterial effect on gram positive bacteria’s *Streptococcus pneumoniae* and *Staphylococcus aureus*. The ability of the films and its solutions to inhibit growth of the tested strains is listed in Table 1. The inhibitory activity was measured based on the diameter of the clear inhibition zone. In terms surrounding clearing zone, they both showed a very clear inhibitory effect against gram positive bacteria. Control (acetic acid, 0.1% v/v) yielded no antimicrobial activity against all tested microorganisms. From the comparative study in between drug and complex it is clearly evident that CH-MFX complex is more effective than MFX drug (Graph 2).

The present investigation therefore indicates that the complexes were effective for inactivating bacteria with the enhancement in the total activity. This is possibly due to the synergistic effect of both the CH and MFX in the composite. The results suggest that the antimicrobial activity of MFX can be enhanced by its conjugation with the CH in the form of films.

**TABLE 1.** Antibacterial activity of MFX and CH-MFX complex

<table>
<thead>
<tr>
<th>Test cultures</th>
<th>Diameter (mm) of inhibitory zone in aq. Acetic acid (0.1%) solution of the control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MFX (Drug)</td>
</tr>
<tr>
<td>Gram positive</td>
<td></td>
</tr>
<tr>
<td>(a). <em>S. aureus</em></td>
<td>25</td>
</tr>
<tr>
<td>(b). <em>S. pneumoniae</em></td>
<td>21.3</td>
</tr>
</tbody>
</table>

**GRAPH 2.** Comparative antibacterial study of MFX and CH-MFX complex forming solution
SUMMARY AND CONCLUSION
CH-MFX prodrug conjugates have been successfully synthesized by using distilled water and glacial acetic acid mixture and the film was made by solution casting method. CH is soluble only in acidic medium, where in the alkaline medium it is usually precipitated out. Glacial acetic acid is a week acid which is used to maintain the acidic pH of the entire solution so that a homogeneous mixture can be prepared.

CH makes the films more flexible and allows the film wall to enlarge its surface and to swell to compensate for osmotic differences between interior and exterior of the films. The Fourier transform spectrum of the films showed that CH-MFX conjugate exhibits an interaction between NH₂ group of CH and COO⁻ group of MFX, which is a type of electrostatic interaction. Intermolecular bonding of electrostatic type forms a stronger bond because of the stronger attraction forces in between opposite ions. These biocompatible conjugate systems (CH-MFX) can bypass the acidity of gastric fluid without liberating substantial amounts of the loaded compound. The above findings open new prospects and promise a quality material which undoubtedly widens the scope of application of chitosan based material in pharmaceuticals applications.

REFERENCES
8. Kumar P, Pradyumna SP, Padma KUMAR SA, Ramandeep A. MFX (Moksifloksazin), CH (kitozan), konjugat, ön ilaç olarak değerlendirilir mi? Farmasötikler için kitozan moksifloksazin ön ilaçlar hazırlanması, karakterizasyonu ve biyolojik olarak değerlendirilir mi? ÖZET
METOD: Bu çalışmada distille su ve glisyal asetik asit karışımları kullanılarak kitozan-moksifloksazin konjugatları başarı olarak formül edilmiş ve gözele şekillendirme yöntemi ile film oluşturulmuştur. Oluşan filmler UV, IR ve DSC gibi analitik yöntemlerin yanı sıra biyolijik yöntemlerle de karakterize edilmiştir.


SONUÇ: Bu önerilen kombinasyon, kitozan ve moksifloksazinin ayrı ayrı aktivitelerinin artırılması ile sonuçlanabilir.
ANAHTAR KELİMELER: MFX (Moksifloksazin), CH (kitozan), konjugat, ön ilaç, elektrosatik etkileşme, antimikrobiyel vb.


