**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Spl Issue [4] November 2022 : 300-315

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

**ORIGINAL ARTICLE** **OPEN ACCESS**

# Formulation and Evaluation of Mucoadhesive Nanoparticles Of Moxifloxacin as an Ocular Drug Delivery System

**Nikita Kaushik[1], Ankit kumar[2], Sunaina Arora[3], Parveen Kumari[3], Sushma Maratha[4]***
[1]Jhankar College of Pharmacy, Department of Pharmaceutics Gurugram, Haryana 122503
[2]Baba Mastnath University, AsthalBohar, Rohtak, NH-10, NCR, Haryana, 124021
[3]Starex University, NH-48, Vill-Binola, P.O. Bhorakalan, Gurugram, Hayana 122413
[4]SGT College of Pharmacy, Sgt University, Gurugram
***Corresponding AuthorEmail :**sushma.maratha@sgtuniversity.org

**ABSTRACT**
*Emerging methods for the effective treatment of ocular illnesses include pharmaceutical techniques based on nanotechnologies and the creation of eye drops made of the mucoadhesive polymers chitosan and hyaluronic acid. For the successful development of these novel nanoparticulate systems—which aim to boost drug bioavailability at the ocular surface—the assessment of mucoadhesive ness—the interaction between the ocular delivery system and mucins present in the eye—is crucial. The current study aims to investigate the creation and assessment of mucoadhesive nanoparticles of moxifloxacin as an eye drug delivery mechanism in this setting.*
***Keywords:*** *Mucoadhesion, Nanoparticles, Ocular, Viscosity*

Received 12.10.2022 Revised 23.10.2022 Accepted 21.11.2022

## INTRODUCTION

The organ of vision, the eye, is one of two. The eye is made up of several parts that allow it to take in light impulses from its surroundings and transmit them to the brain as an electrical signal. Numerous different organisms can cause eye infections, which need to be effectively treated to prevent future issues and vision loss. Due to systemic adverse effects and the drug's limited ocular absorption, systemic therapy is not recommended (1). A special organ for medication distribution is the eye. According to the structure and physiology of the eye, only a small portion of prescription medications are really absorbed since they are evacuated by protective systems such tearing, the blinking reflex, and tears flowing from the eyes (2).A substantial portion of the dose is frequently absorbed systemically through the conjunctiva and nasolacrimal duct, with only around 5% of eye medications in the cornea reaching the intraocular tissues (3). Following use, the more topically applied aqueous solution and suspension forms impair vision. However, it is inevitable for some medications to enter the systemic circulation fast and briefly, and the tear layer quickly dilutes this formulation (4). To produce ocular delivery systems with great therapeutic efficacy nowadays, more sensitive diagnostic procedures and cutting-edge therapeutic chemicals are applied. Comparing nanoparticles to conventional delivery systems, they demonstrate a better use (5). The objective of the current study is to create and improve a moxifloxacin mucoadhesive nanoparticulate formulation for ocular administration.

## MATERIAL AND METHODS

### Pre-formulationStudies

The process of creating the drug substance's dosage form starts with pre-formulation investigations. The "Investigation of physical and chemical properties of a drug substance alone and in combination with excipients" is how it is best described. Moxifloxacin, Chitosan, and STPP underwent pre-formulation tests for characterization and compatibility. **(6)**

### Characterization of Drug & Polymer

The procured sample of Moxifloxacin was characterized in terms of its physical description, identification test, melting point, determination of $\lambda_{max}$,solubility studies in various solvents, and FTIR spectral studies**. (7)**

### Physical description

Moxifloxacin was physically characterized in terms of its condition, texture, and color.

**Meltingpoint**

Capillary tube method was used to ascertain the drug's melting point. The drug (Moxifloxacin) was placed inside the capillary tube from one side, which had been fused, and it was then introduced into the melting point device from the other side. By ocular inspection, the temperature at which a solid medication turns into a liquid was documented. To ensure that the Moxifloxacin, Chitosan, and STPP melt at the same temperature, the same technique was carried out three times. The stated value and the observed value were compared.

**Solubility**

Moxifloxacin, Chitosan, and STPP solubility was examined in a range of solvents, including distilled water, alcohol, and buffers. Acetate buffer I.P. pH 4.0, 4.4, 5, 5.5, and 6, citrophosphate buffer B.P. pH 6.0, and phosphate buffer USP pH 5.5, 6, 7.2, and 7.4 were used to assess the solubility of moxifloxacin. Moxifloxacin was dissolved in the buffers at a rate of 0.5% w/v.Use 10 milligrams of a solid or 2 to 3 ml of a liquid. Crush a small bit of the solid with the back of a spatula and place it on a watch glass if it isn't already a fine powder.

Put the proper quantity of your liquid or solid sample in a little test tube. About 2 ml of solvent should be added to the test tube containing the sample. Shake the tube or use a glass stirring rod to mix it. As soon as the solvent is saturated, add fresh samples to it. The amount of a substance that can dissolve completely in a solvent at a specific temperature is known as its solubility. A saturated solution is one such solution. To determine the solubility in g/100g, multiply the solubility in g/100g by the product of the mass of the component divided by the mass of the solvent.**(8-10)**

**Spectralanalysis**

**X-RayCrystallography**

The physical characterization of moxifloxacin was carried out using X-ray diffraction in powder (XRD), a well-liked method for characterizing materials at the nanoscale. Powder XRD study of a sample, in addition to several microscopic and spectroscopic methods, can offer useful details about a sample, including phase identification, sample purity, crystallite size, and, in some cases, morphology. A highly purified substance is crystallized, and the crystals are subsequently exposed to an X-ray beam. After that, information about the crystal packing symmetry and the size of the repeating unit that makes up the crystal can be discovered using the processed diffraction patterns. This is generated from the pattern of diffraction spots.

**Differential Scanning Calorimetry(DSC)**

Moxifloxacin, Chitosan, and STPP DSC curves were produced in a DSC cell utilizing aluminum crucible. A total of 2mg of samples were examined at a heating rate of 10°C/min in the range of 60-300°C in a dynamic N2 environment (flow rate: 50 mL/min).

**Infrared Spectroscopy**

Using the potassium bromide dispersion procedure and FTIR with a diffuse reflectance attachment, the infrared spectrum of each medication was captured (FTIR-8400s, Shimadzu, Japan). The spectra were measured between 400 and 4000 cm 1.

**UV Spectroscopy**

By dissolving 10 mg of each medication in 100 ml of PBS pH-7.4, a stock solution with a concentration of 100 g/ml was created (as it shows better solubility). Each solution was stored in a cuvette with a path length of 10mm after being sufficiently diluted with PBS pH 7.4, and the UV spectrum was recorded using a double beam UV-Vis spectrophotometer in the wavelength range of 200-400nm while using PBS pH 7.4 as a reference.

**CompatibilityStudies**

To determine if pharmaceuticals and polymers were compatible, X-ray crystallography, differential scanning calorimetry research, and FTIR spectroscopy were used. Physical mixes' X-ray diffraction patterns, IR spectra, and DSC thermograms were compared to those of the pure medication.

**Standard CalibrationCurve**

To make the stock solution of moxifloxacin, 10 mg of the antibiotic was dissolved in 100 ml of PBS/methanol, yielding a solution with a concentration of 100 g/ml. Aliquots of 10, 20, 30, 40, 50, 60, 70, and 80 ml were taken from this stock solution and diluted to a final volume of 50 ml in PBS/methanol to provide a range of concentrations of 10, 20, 30, 40, 50, 60, 70, and 80 g/ml, respectively. To obtain a standard calibration curve for the medication, a graph of concentration vs. absorbance was generated**. (11)**

**Preparation of Nanoparticles**

- Chitosan was dissolved in 1% (w/v) acetic acid solution under mixing for the duration of the process at room temperature to create chitosan solution (CS).
- Chitosan was dissolved in 1% (w/v) acetic acid solution under mixing for the duration of the

process at room temperature to create chitosan solution (CS).

- In order to create blank nanoparticles, TPP aqueous solution (0.1, 0.2, 0.3%) was added dropwise to the CS solution and agitated (1000 rpm) for two hours at room temperature.
- The moxifloxacin solution 0.5% was added gradually to the CS solution while stirring gently (1000 rpm) at room temperature to create moxifloxacin-loaded CS-TPP nanoparticles.
- And then TPP solution was added dropwise to the mixture with gentle stirring (1000 rpm) for 2hr.
- Now 5% mannitol (cryoprotectant) is added to the prepared nanoparticles & the resultant solution islyophilized**. (12)**

**Composition of the formulations**

| Formulation | Moxifloxacin (%) | Chitosan (%) | TPP (%) | Ratio (Chitosan:TPP) |
|---|---|---|---|---|
| **B1** | **-** | 0.1 | 0.1 | 1:1 |
| **B2** | **-** | 0.2 | 0.2 | 1:1 |
| **B3** | **-** | 0.3 | 0.3 | 1:1 |
| **F1** | 0.5 | 0.1 | 0.1 | 1:1 |
| **F2** | 0.5 | 0.1 | 0.2 | 1:2 |
| **F3** | 0.5 | 0.1 | 0.3 | 1:3 |
| **F4** | 0.5 | 0.2 | 0.1 | 2:1 |
| **F5** | 0.5 | 0.2 | 0.2 | 2:2 |
| **F6** | 0.5 | 0.2 | 0.3 | 2:3 |
| **F7** | 0.5 | 0.3 | 0.1 | 3:1 |
| **F8** | 0.5 | 0.3 | 0.2 | 3:2 |
| **F9** | 0.5 | 0.3 | 0.3 | 3:3 |

**Where, B = Blank F= Formulation**

**Evaluation**

**Appearance andClarity**

All the produced formulations had their colors and, if any, suspended particle matter, if present, checked. By observing the solutions on a black and white background, the solutions' clarity was further evaluated. The following grades were assigned to the formulations: (-) turbid, (+) somewhat turbid, (++) clear solution, and (+++) clear and transparent. **(13-15)**

**pH**

The ophthalmic formulation's pH should be set so that it will remain stable at that level and, at the same time, cause no irritation to the patient when administered. The pH range for ophthalmic solutions should be 6.5 to 8.5. The formulation's pH was measured using a pH meter (Tectonics 511, Agrawal Electronics, Mumbai), which was dipped into the beaker containing the formulation so that the electrode of the pH meter contacted the formulation solution. The formulation's pH is then stabilized using a pH meter. **(16)**

**% EntrapmentEfficiency**

To ascertain whether Moxifloxacin was entrapped in nanoparticles, 1 ml of freshly made nanoparticle solution was obtained and diluted with the proper STF (pH-7.4). Aliquots were then centrifuged for an additional 30 minutes at a low temperature and 15,000 rpm using a centrifuge. A double beam UV spectrophotometer was used to determine the moxifloxacin content of the resultant solutions, and the following equation was used to get the entrapment efficiency (% EE).

$$\% EE = \frac{\text{Total amount of drug} - \text{Free dissolved drug}}{\text{The total amount of drug}} \times 100$$

**Loading Capacity**

Loading capacity (%LC) helps to deal with nanoparticles after their separation from the medium & to know their drug content

% LC = [Entrapped drug/nanoparticles weight] x 100

**PercentageYield**

The lyophilized nanoparticles from each formulation were weighed and the respective percentage yield was calculated using the following formula.

$$Percentage Yield = \frac{\text{Weight of nanoparticles Obtained}}{\text{Weight of drug, polymer, and other excipientsused}} \times 100$$

**Polydispersityindex**

A parameter to describe the particle size distribution of nanoparticles derived from a particle size analyzer is the polydispersity index. A measure of variance or dispersion within the particle size distribution is the PDI. Lower PDI values are seen in monodisperse samples, while higher PDI values

signify polydispersity and a larger particle size distribution.The usual range of PDI values is<0.1 (monodisperse standard), 0.1 to <0.5 (nearly monodisperse), 0.5 to <1.0 (mid-range polydispersity), and >1.0 (highly polydisperse).

**Particle Size Distribution & ZetaPotential**

The Zeta potential and particle size distribution of the nanoparticles were examined at 25 °C by Zetasizer utilizing the Dynamic Light Scattering (DLS) method. One of the most used techniques for determining the size of nanoparticles is the DLS technique, sometimes referred to as photon correlation spectroscopy. This method assumes that every particle in the solution is in Brownian motion and that every particle is tiny and spherical. When light strikes particles, it causes scattering (often from a laser). Based on the scattered light's physical characteristics, including its angular distribution, frequency shift, polarization, and intensity, the particle size can be calculated. **(17)**

**Transmission ElectronMicroscopy**

Transmission electron microscopy is a type of microscopy in which an image is created by passing an electron beam through a specimen. On a grid, the suspension is positioned. As the beam passes through the specimen, a picture is created as a result of the electrons' interactions with it. An imaging device, such as a fluorescent screen, a sheet of photographic film, or a sensor like a scintillator linked to a charge-coupled device, is then used to magnify and focus the image.

**Surfacemorphology**

To study the particle surface morphology and form, a scanning electron microscope was employed. A slab was covered with a concentrated aqueous suspension, which was then dried in a vacuum. The sample was exposed to a 45-mA current for 5 seconds in an argon gas atmosphere in a cathodic evaporator with a gold layer 20 nm thick. A 15 kV scanning electron microscope was used to take the pictures.

**In-vitro drug releasestudies**

All medicated formulations underwent in vitro drug release. Phosphate buffer, newly produced, was the medium (pH 7.4). A cellulose membrane that had been pre-soaked was fastened to one end of the cylinder. The tubes were filled with the precise amount of the formulation. The cylinders were then fastened to the metal shaft and submerged in 100 ccs of the medium that was kept at 34°C for the duration of the investigation (24 hours). Magnetic bars spun the medium at a rate of 50 rpm. To maintain sink conditions, samples were taken out and replaced every hour.

**Release Kinetics**

To analyze the mechanism for the release and release rate kinetics of the dosage form, the in vitro release data obtained were fitted into a zero-order, First order, Higuchi matrix, Korsmeyer-Peppas, Hixson-Crowell models. The best fit model was selected after analyzing data to determine correlation coefficient ($R^2$) & release kinetics using various mathematical models: **(18-21)**

**Different Models with their Equations**

| Model | Equation |
|---|---|
| Zero ordermodel | $Q = Q_0 + kt$ |
| First ordermodel | $Q = Q_0 \times e^{kt}$ |
| Higuchimodel | $Q = k \times t^{0.5}$ |
| Hixson-Crowellmodel | $Q^{1/3} = kt \times Q_0^{1/3}$ |
| Korsmeyer-Peppasmodel | $Q = k \times t^n$ |

Where Q represents the quantity of drug released in time t, $Q_0$ represents the value of Q at zero-time, k represents the rate constant, n represents the diffusional exponent, a represents the time constant & b represents the shape constant parameter. The correlation coefficient ($R^2$) & the order of release pattern were calculated in each case**. (18-21)**

**Ex-vivo Study**

Utilizing excised goat cornea with a little modification, the trans-corneal permeation capability of moxifloxacin through the CS-TPP nanoparticulate system was assessed. At a nearby abattoir, goat eyes from recently butchered animals were used to isolate the cornea. In a Franz diffusion chamber, the study was conducted. The Franz diffusion cell's donor compartment was towards the epithelial surface while the excised goat cornea was positioned between the donor and receiver compartments.6 mg of a lyophilized prepared formulation containing 0.5% of the medication content suspended in 1 ml STF were placed in the donor compartment. Freshly made STF was placed inside the receiver chamber. The trial was conducted at 32 0.5 °C. Samples were periodically collected for up to 4 hours and submitted to a UV-VIS spectrophotometer for moxifloxacin measurement. **(22)**

**Microbiological efficacy**

The microbiological studies were carried out to ascertain the antimicrobial activity of the prepared formulations and to compare them with the marketed eye drop, against *P. aeruginosa* (ATCC 6580) and *S. aureus* (NCTC 6749) (obtained for Codon BioTech, Sector-63, Noida). A loop of each organism from lab-maintained cultures was transferred into 100 ml of sterilized nutrient broth to create a subculture, which was then cultured for 24 hours at 37 °C. 40 ml of the inoculation medium was added to each Petri plate and allowed to harden after being inoculated with the subculture (20 ml subculture/100 ml of Müller-Hinton-Agar). With the use of a stainless-steel borer (8 mm in diameter), three wells were aseptically created in each plate, ensuring that they were evenly spaced apart.Before being transferred into wells, the weighed amounts of each formulation were taken and suspended individually in a normal saline solution (0.5% w/v). Then, under aseptic conditions, 100 l of each of the test solutions and the commercial eye drops were added to separate Petri plate bores. A negative control (Petri plate without microbe) and positive control (Petri plate with microorganism but placed in normal saline) were also made.

## RESULT AND DISCUSSION

**Pre-formulationStudies**

Pre-formulation studies were performed for Moxifloxacin, Chitosan & STPP for characterization and compatibility studies.

**Characterization of Drug &Polymer**

The drug was evaluated based on certain physicochemical parameters and the findings of these studies help in formulating a stable drug delivery system.

**Physical Description and Organoleptic Properties**

Moxifloxacin was characterized for various parameters and the results are shown in Table 1. All these parameters were found to agree with the literature findings.

**Table 1 Characterization of Moxifloxacin**

| Sr. No. | Properties | Specifications |
|---|---|---|
| 1 | Colour | Almost white to light yellow powder |
| 2 | Physical form | Crystalline powder |
| 3 | Taste | Bitter |
| 4 | Odor | Fruity breath odor |

**Meltingpoint**

The melting point of Moxifloxacin, Chitosan & STPP was determined by the capillary method. Observed values are:

**Table 2 Melting point of Moxifloxacin, Chitosan & STPP**

| Ingredient Name | Observed | Standard |
|---|---|---|
| Moxifloxacin | 242 °C | 242-245°C |
| Chitosan | 88 °C | 88 °C |
| STPP | 622 °C | 622 °C |

Solubility

The amount of a substance that can dissolve completely in a solvent at a specific temperature is known as its solubility. To test the solubility of Moxifloxacin, Chitosan, and STPP in various solvents, 2-3 ml of a liquid or around 10 mg of a solid were used. Crush a small bit of the solid with the back of a spatula and place it on a watch glass if it isn't already a fine powder. Put the proper quantity of your liquid or solid sample in a little test tube. About 2 ml of solvent should be added to the test tube containing the sample. Shake the tube or use a glass stirring rod to mix it. As soon as the solvent is saturated, add fresh samples to it. A saturated solution is one such solution.Divide the mass of the compound by the mass of the solvent and then multiply by100g to calculate the solubility/100g.

**Table 3 Solubility of Moxifloxacin, Chitosan & STPP**

| **Solubility of Moxifloxacin** | | | |
|---|---|---|---|
| **S. No.** | **Solvent** | **Observations** | **Value of solubility** |
| 1 | Water | Readily Soluble | 2.4 g/ 100 ml |
| 2 | Ethanol | Freely soluble | (<1mg/mlat25°C) |
| 3 | Phosphate Buffer | | (1.2mg/ml) |
| 4 | Methanol | Readily soluble | 0.055 mg/ml |
| 5 | DMSO | Readily soluble | 88 mg/ml at 25 °C |
| **Solubility of Chitosan** | | | |
| 1 | Water | Sparingly soluble | |
| 2 | Ethanol (95%) | Practically insoluble | |
| 3 | Organic solvents | Practically insoluble | |
| 4 | Diluted & concentrated solution of acid and alkali | Readily soluble | |
| **Solubility of STPP** | | | |
| 1 | Water | Freely soluble | 14.5 g/100 mL (25 °C) |
| 2 | Alcohol | Insoluble | |

**X-RayCrystallography**

Moxifloxacin's XRD patterns show a typical crystalline diffraction pattern and a substantial increase in the intensity of the peaks at 27, 32, and 47° 2, which helps to cause the crystalline form transition by inducing the loss of integrated water. Chitosan's XRD patterns show a semi-crystalline diffraction pattern with two distinctive crystalline peaks at 10 and 20 degrees two.

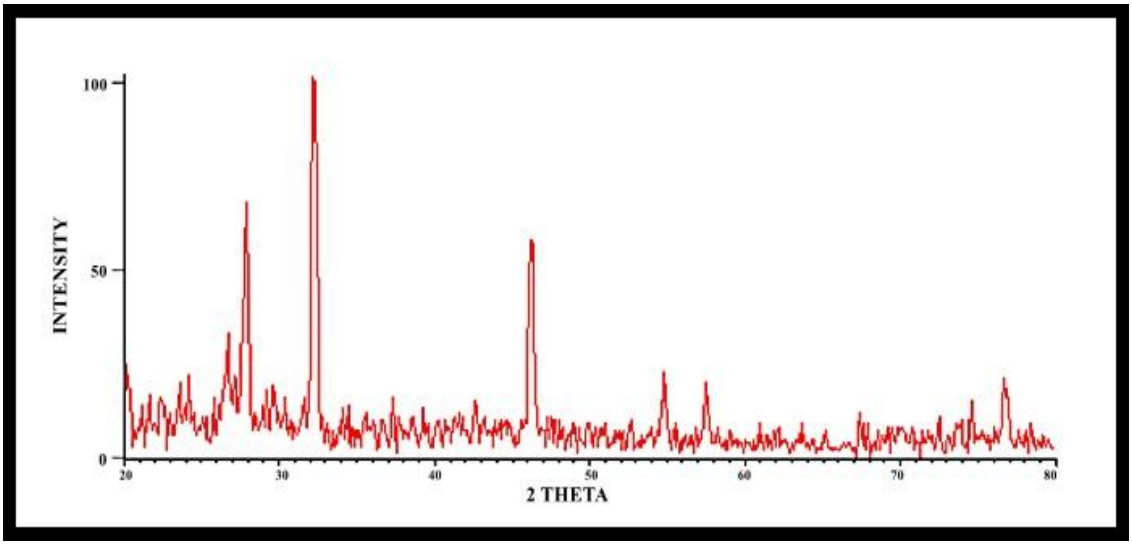



**Figure 1: XRD Pattern of Moxifloxacin**

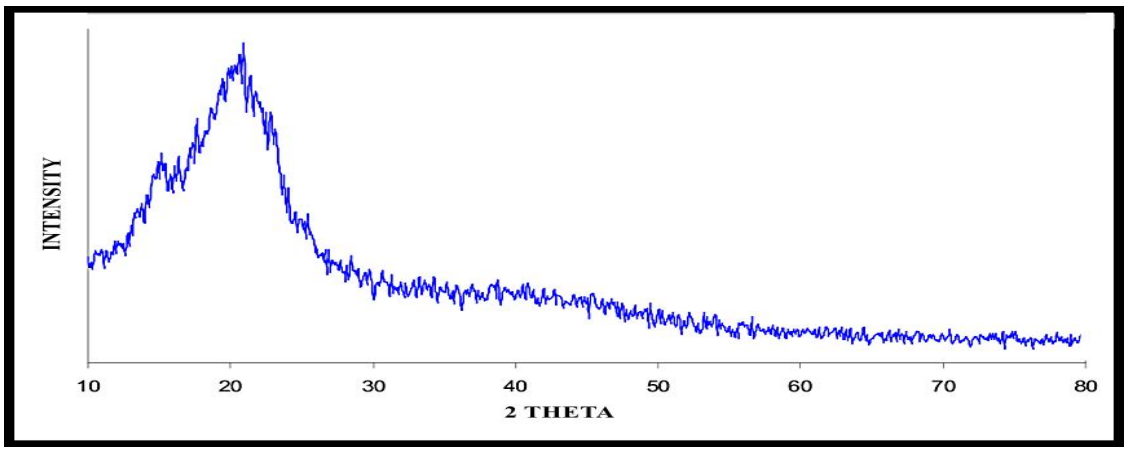



**Figure 2: XRD Pattern of Chitosan**

**Differential Scanning Calorimetry(DSC)**

The DSC curve of moxifloxacin appears to be more thermally stable, and its decomposition, linked to weight loss, begins just after reaching the melting point temperature of 242°C and a quick rise of a jiggered baseline with minor spikes. Chitosan's DSC curve reveals that the peak was centered at about 100°C and that the area of the endothermic peak expanded, indicating that the sample had an amorphous structure.

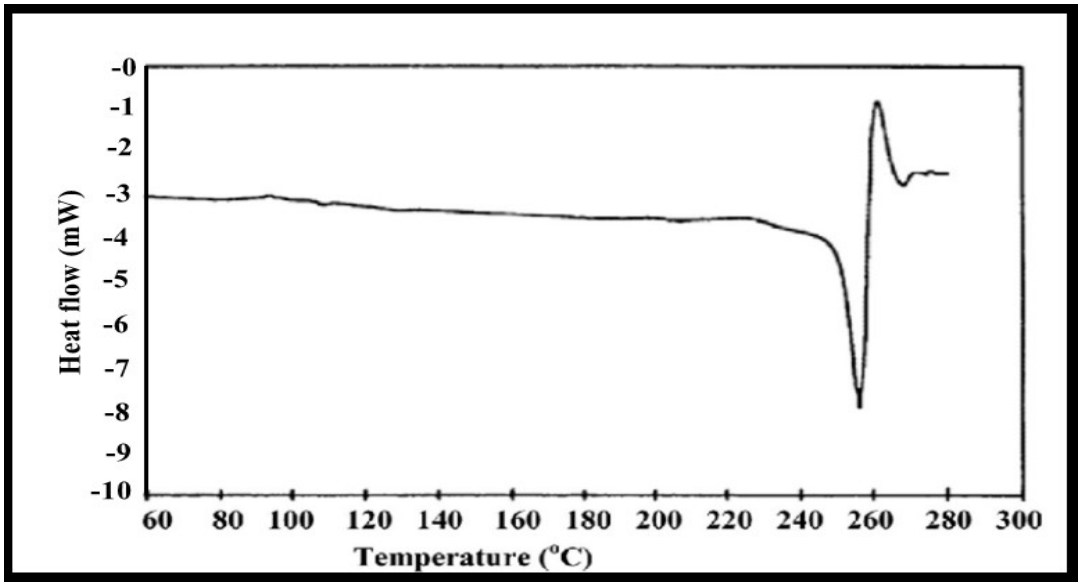



**Figure 3: DSC of Moxifloxacin**

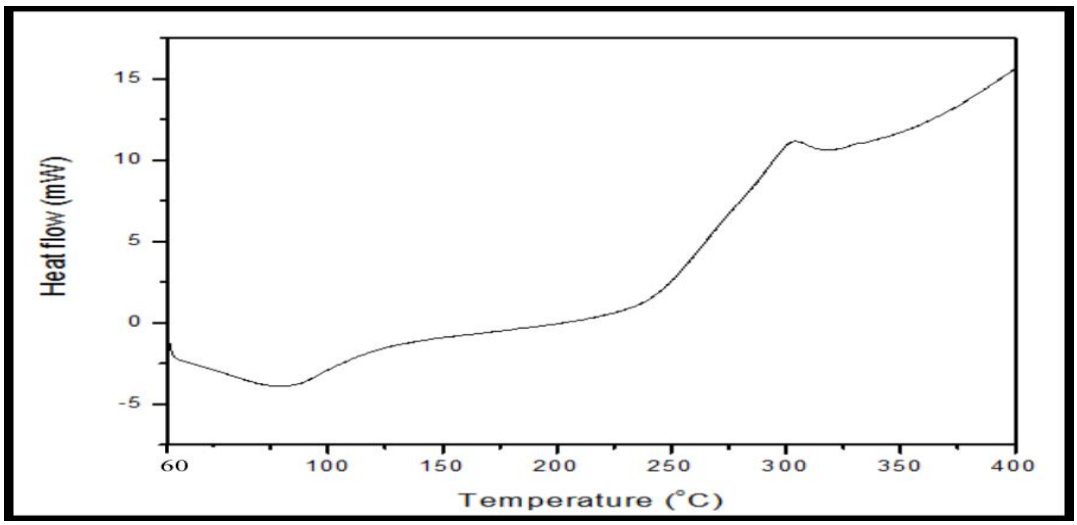



**Figure 4: DSC of Chitosan**

**SpectralAnalysis**

**Infrared Spectroscopy** The infrared spectrum of Moxifloxacin showed characteristic peaks at 1706 $cm^{-1}$ due to carboxylicacidC=Ostretching,C–Nstretchingat1320$cm^{-1}$,and aromaticC=Cstretchingat1622 $cm^{-1}$, 1518 cm,$^{-1,}$ and 1451 $cm^{-1}$, and C–H bending for the substituted benzene at 1875 $cm^{-1}$.

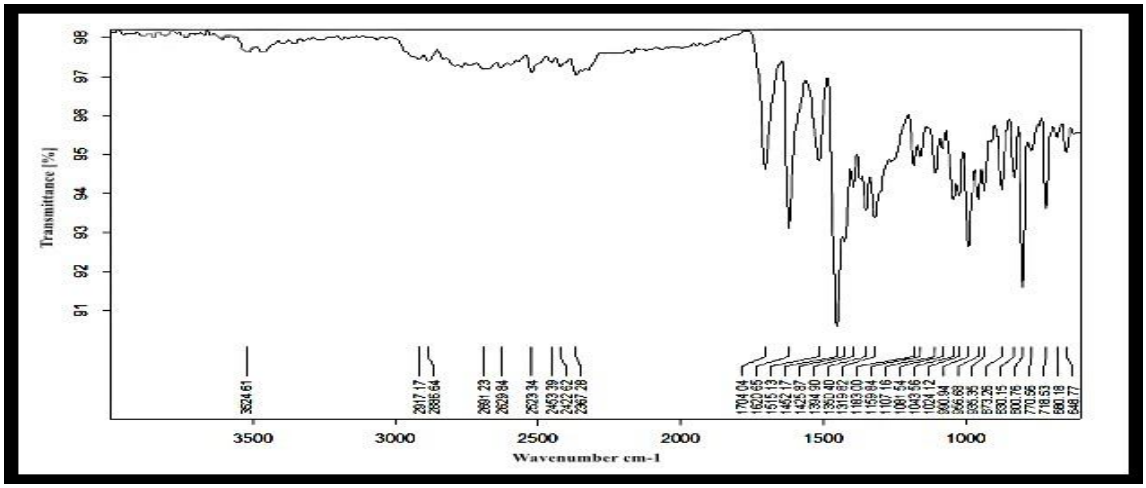



**Figure 5: IR Spectra of Moxifloxacin**

**Table 4: Characteristics peaks of Moxifloxacin**

| IR Spectrum | Observed Peaks Value $cm^{-1}$ | Groups | Stretching/ deformation |
|---|---|---|---|
| Moxifloxacin | 1706 $cm^{-1}$ | C=O | Stretching |
| | 1320$cm^{-1}$ | C–N | Stretching |
| | 1622 $cm^{-1}$<br>1518 $cm^{-1}$<br>1451 $cm^{-1}$ | C=C | Stretching |
| | 1875 $cm^{-1}$ | C–H | Bending |

The infrared spectrum of Chitosan shows a strong band in the region 3291–3361 $cm^{-1}$ correspondingtoN-HandO-Hstretching,aswellastheintramolecularhydrogenbonds.The absorption bands at around 2921 and 2877 $cm^{-1}$ can be attributed to C-H symmetric and

Stretching that is not symmetrical. Other polysaccharide spectra also contain these bands, which are features of polysaccharides. The bands at roughly 1645 cm-1 (C=O stretching of amide I) and 1325 cm-1 (C-N stretching of amide III), respectively, provided evidence of the presence of residual N-acetyl groups. The tiny band at 1550 cm-1 that corresponds to the N-H bending of amide II was not discovered by us. This is the third band that distinguishes conventional N-acetyl groups, and other bands most likely overlapped it.The primary amine's N-H bending is shown by a band at 1589 cm 1. The existence of bands at about 1423 and 1375 cm-1, respectively, confirmed the CH2 bending and CH3 symmetrical deformations. The C-O-C bridge's asymmetric stretching is responsible for the absorption band at 1153 cm1. According to C-O stretching, the bands at 1066 and 1028 cm1 are present. For IR Spectra of Chitosan-TPP-MOX complex (Figure 2.7):The spectral band appears 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 $CH_3$ stretching vibration attributed to MFX and pyranose ring of CH). A band at 2344 $cm^{-1}$ refers to C-N asymmetricband stretching, Abroad,strong $NH_3$ stretching band in the3100-2600$cm^{-1}$region and multiple combinations 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 andtorsionaloscillationo faminesaltNH$_3^+$).One more strong symmetric bending near 1562 $cm^{-1}$ is the characteristic of $NH_3^+$ absorption. 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 MOX), 943-623 $cm^{-1}$ (mono and di-substituted benzenering).

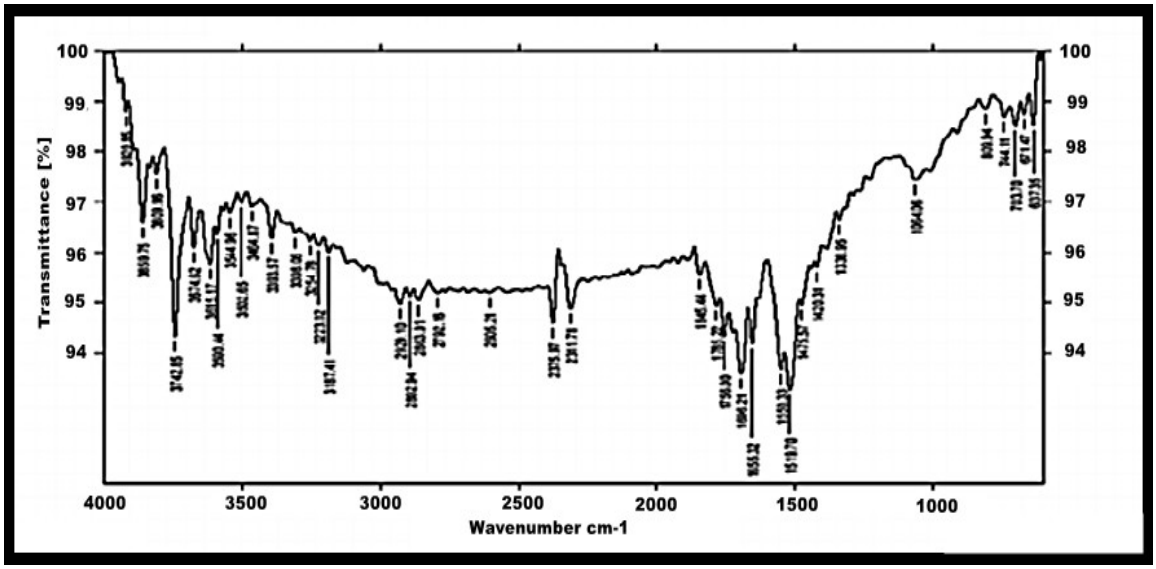



**Figure 6: IR Spectra of Chitosan, TPP & Moxifloxacin physical mixture**

**UV Spectroscopy**

Moxifloxacin has strong UV absorption and good PBS solubility, hence PBS pH 7.4 was chosen as the solvent for the current analytical technique. The maximum absorbance of the resultant solution was found to be 293 nm after the absorbance of the solution was measured at various wavelengths as shown in Fig 7.

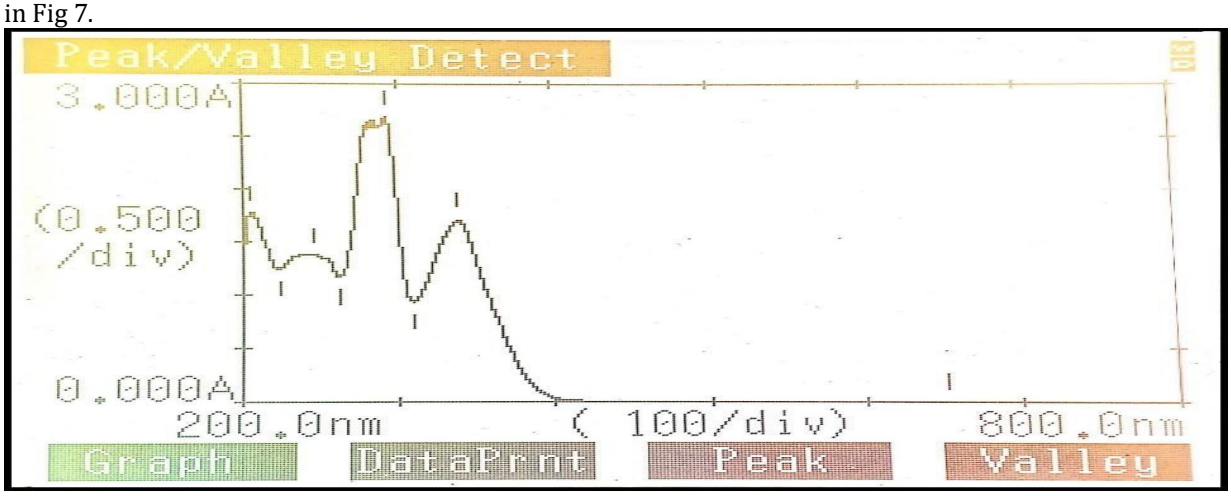
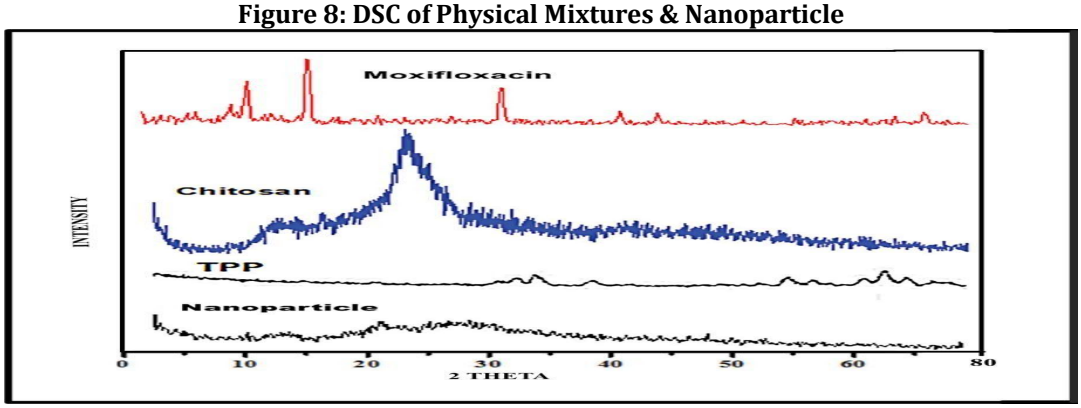



**Figure 7: UV graph of Moxifloxacin**

**Compatibility Studies**

The DSC thermograms of the Chitosan-Moxifloxacin physical mixture exhibit a peak at 248 ºC due to the structural arrangement and the degree of substitutional changes in the polysaccharide. There is no exothermic peak was observed in the Moxifloxacin – Chitosan (MOX-CS) nanoparticle due to excess dilution of MOX.

**Figure 8: DSC of Physical Mixtures & Nanoparticle**



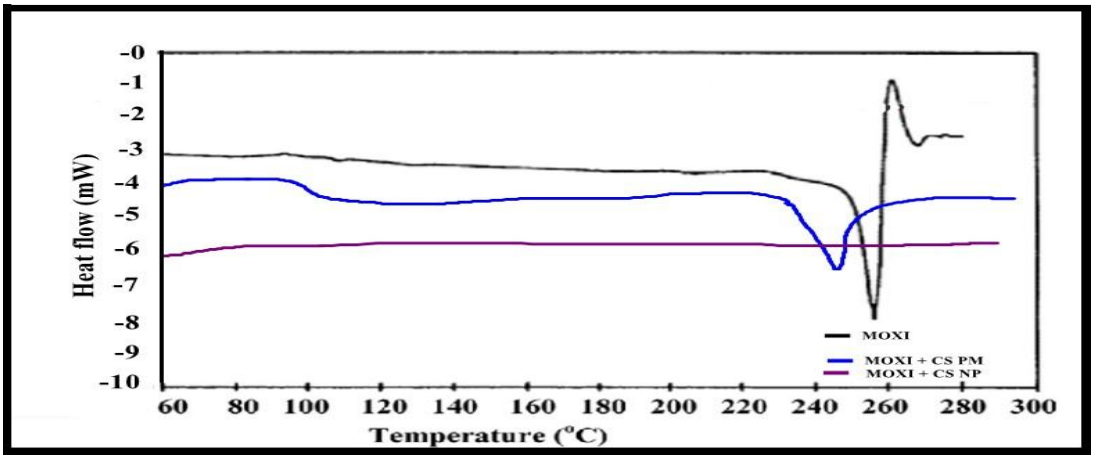



**Figure 9: XRD Patterns of Moxifloxacin, Chitosan & TPP Nanoparticle**

TheX-ray diffracto grams of puredrug intensity peaks were identified, MOX showed intensive peaks at 2θ scattered angles of 27, 32 & 47°, while Chitosan shows at 10 & 20°, however, nanoparticle gave peaks at 12, 18 & 25° 2θ. The XRD patterns indicate that the chitosan contains also an amorphous form due to the presence of OH and $NH_2$ groups which form the inter molecular hydrogenbond.Thus,this distribution will have some regularity to build easily the crystalline regions.

**Standard CalibrationCurve**

Moxifloxacin was analyzed by the proposed UVspectrophotometric method.The calibration curve showed linearity over a concentration range from 10.0 to 80.0 µg/ml. The linearity can be defined by the following equation, y= 90.23x + 71.67(Figure 7.11), where *y* and *x* are Moxifloxacin absorbance and concentration, respectively. The correlation coefficients of the curve obtained with the linear regression method were 0.999.

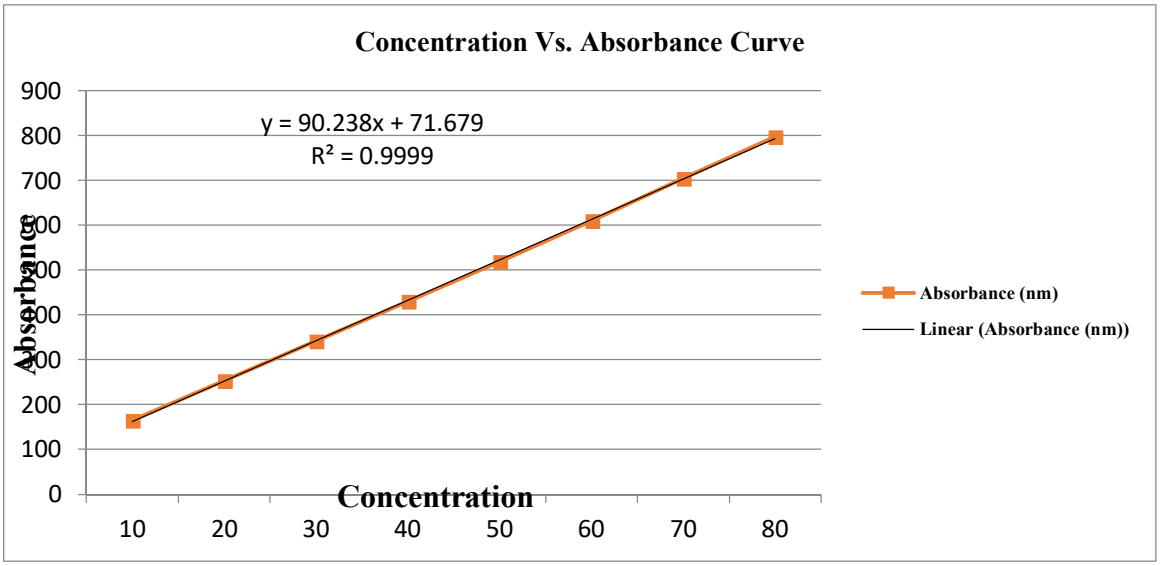



**Figure 2.10: UV Calibration Curve of Moxifloxacin**

**Evaluation**

**Appearance, Clarity &pH**

All the developed formulations were observed cautiously for color and presence of suspended particulate matter assuming any.The clarity of solutions was further assessed & found that F7, F8 & F9 formulations were not clear whereas the rest were a low cloudy appearance. The pH of all ophthalmic formulations was found as shown in table 5.

**Table 5: Appearance, Clarity & pH**

| Formulation | Appearance | Clarity | Ph |
|---|---|---|---|
| F1 | Translucent | + | 6.9 |
| F2 | Translucent | + | 7.1 |
| F3 | Translucent | + | 7.2 |
| F4 | Translucent | + | 6.8 |
| F5 | Translucent | + | 6.9 |
| F6 | Translucent | + | 7.0 |
| F7 | Cloudy | - | 6.5 |
| F8 | Cloudy | - | 6.7 |
| F9 | Cloudy | - | 6.8 |

**Particle Size, Polydispersity Index & ZetaPotential**

All the prepared formulations were characterized for the vesicle size (Z-average) and size distribution using a computerized inspection system (Malvern Zetasizer, Nano-ZSP). ThePDI was a dimension less measure of the width of size distribution calculated from the cumulant analysis ranging from 0to1.A small value of PDI indicates a monodispersed population,while a large PDI indicates a polydisperse or broader distribution of droplet size. The PDI of the formulations varied from 0.137 to 0.512. The particle size of the formulation varies from 183.7 nm to 962.1 μm as shown in table7.8. Zeta potential on the particles determines their physical stability (high zeta potential leads to more stable colloid particles). The zeta potential of the formulations varied from 14.2 to 27.5 as shown in Table 2.8

**Entrapment Efficiency (EE) & Loading Capacity(LC)**

The values of drug entrapment efficiency are shown in table 7.8. The percent Entrapment efficiency of all formulations was found to be in the range of 39.80 to 94.24. The higher entrapment of Moxifloxacin in nanoparticles could be contributed to the greater retentivity of Moxifloxacin in the Chitosan-TPP matrix. The percent loading capacity of all formulations was found to be in the range of 3.32 to 7.87 as shown in table 2.8.

**Table 6: Evaluation of formulation**

| Formulation | Entrapment Efficiency (%) | Loading Capacity (%) | Particle Size (nm) | PDI | Zeta Potential (mV) |
|---|---|---|---|---|---|
| **F1** | 75.02 | 6.27 | 257.5 | 0.290 | 25.2 |
| **F2** | 84.63 | 7.07 | 183.7 | 0.428 | 27.5 |
| **F3** | 65.42 | 5.46 | 341.9 | 0.350 | 26.1 |
| **F4** | 59.01 | 4.93 | 378.3 | 0.512 | 19.5 |
| **F5** | 52.61 | 4.39 | 457.8 | 0.430 | 17.6 |
| **F6** | 39.80 | 3.32 | 489.2 | 0.137 | 21.3 |
| **F7** | 91.03 | 7.60 | 750.2 | 0.380 | 15.5 |
| **F8** | 94.24 | 7.87 | 962.1 | 0.346 | 14.2 |

**In-VitroStudies**

The in vitro drug release investigations are one of the essential components of formulation development. Researchers conducted these experiments to make an educated guess regarding the bioequivalence and bioavailability of the experimental formulation. As a function of a few characteristics that are trustworthy to the pharmaceutical dosage form, the results of drug release are used to build a drug release curve. The quantitative interpretation of drug release study responses and the creation of an equation that can quantitatively establish the relationship between some parameters connected to the dosage form.Theoreticalanalysis can also help in deducing the equation. The medium used was freshlyprepared Phosphate buffer (pH 7.4). At time intervals of 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr& 24 hr respectively, samples were withdrawn and replaced to maintain sink conditions.The in-vitrorelease pattern of different Moxifloxacin-loaded Chitosan-TPP nanoparticles is shown figure12.

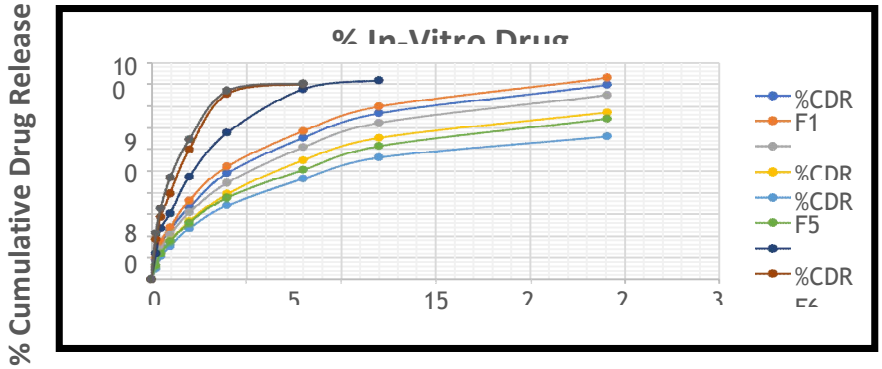



**Figure 12: In-Vitro Cumulative Drug Release of different formulations**

**Determination of Drug ReleaseKinetics**

The release from nanoparticle formulation after being subjected to different model-dependent kinetics (zero-order, first-order, Higuchi model, Korsmeyer-Peppas model & Hixon-Crowell model)was evaluated for their $R^2$ value(Table 6).Hence, theformulation shows a different release model.

**Table 6: Correlation Coefficients ($R^2$) of different nanoparticle formulation**

| Formulation | Zero-order | First order | Higuchi | KorsmeyerPeppas | Hixon-Crowell |
|---|---|---|---|---|---|
| **F1** | 0.8236 | 0.9766 | 0.9635 | 0.9739 | 0.9772 |
| **F2** | 0.8144 | 0.9855 | 0.9561 | 0.9761 | 0.9772 |
| **F3** | 0.8295 | 0.9655 | 0.9664 | 0.9795 | 0.9764 |
| **F4** | 0.8338 | 0.9441 | 0.9683 | 0.9775 | 0.9764 |
| **F5** | 0.8348 | 0.9186 | 0.9686 | 0.9798 | 0.9751 |
| **F6** | 0.8525 | 0.9516 | 0.9766 | 0.9868 | 0.9751 |
| **F7** | 0.9034 | 0.9985 | 0.9850 | 0.9914 | 1 |
| **F8** | 0.8720 | 0.9951 | 0.9694 | 1 | 0.9537 |
| **F9** | 0.8465 | 0.9982 | 0.9586 | 1 | 0.9564 |

**Ex vivo Trans-cornealpermeability**

The inclusion of MOX in the colloidal system significantly enhanced the drug's rate of penetration across the cornea, according to the results. The degree of ionization of the permeant molecules evaluated, as well as their chemical composition, size, and conformation, as well as other factors, affect a substance's capacity to diffuse past epithelial barriers. Chitosan-TPP nanoparticles loaded with MOX demonstrated a noticeably enhanced drug penetration capability. The aggregation of nanoparticles in the conjunctival sac, which creates a depot from which the medication is gradually transported to the precorneal area, may be responsible for the favorable penetration of MOX through the cornea.

**Table 7: Ex-vivo Trans-corneal permeation data of different formulation**

| **Time (hr)** | **Drug Permeation (%)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** |
| **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **0.5** | 3.11 | 6.23 | 3.33 | 2.63 | 2.33 | 2.1 | 1.87 | 1.64 | 1.41 |
| **1** | 11.29 | 14.41 | 11.51 | 10.81 | 10.51 | 10.28 | 10.05 | 9.82 | 9.59 |
| **2** | 19.64 | 22.76 | 19.86 | 19.16 | 18.86 | 18.63 | 18.4 | 18.17 | 17.94 |
| **3** | 25.46 | 31.83 | 23.53 | 22.73 | 21.98 | 21.75 | 18.52 | 18.27 | 18.19 |
| **4** | 38.15 | 44.52 | 36.22 | 35.42 | 34.67 | 34.44 | 18.79 | 18.43 | 18.31 |

**Optimization**

To find the most optimized formulation, characterizing characteristics of all the created vesicular formulations were examined. Based on the parameters of achieving the lowest value of particle size, PDI, highest value of zeta potential, EE%, LC%, In-vitro cumulative drug release, Release kinetics, and Ex-vivo trans-corneal permeability, the best formulation was chosen. The 183.7 nm particle sizes in the optimized formulation (F2) are suitable for nano-formulation, and the PDI indicates that it is polydisperse. The

+27.5 mV Zeta potential of the improved formulation stabilizes the formulation. F2 was determined to have the highest level of entrapment efficiency (84.63%) and a relatively high loading capacity (7.07%), both of which were stable and had nanoscale particle sizes.Some other formulation has better entrapment efficiency, but they exceed the nano range of particle size (F7, F8 & F9) because of that they are not suitable for ophthalmic preparation.

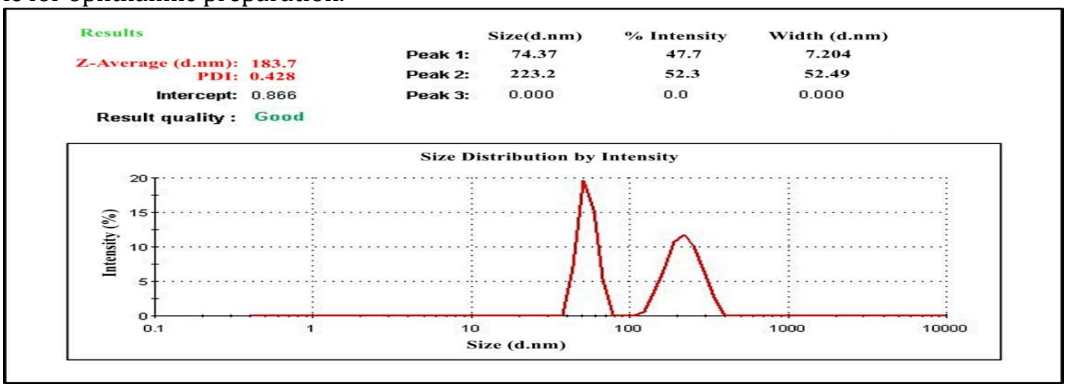



**Figure 14: Particle size of optimized formulation (F2)**

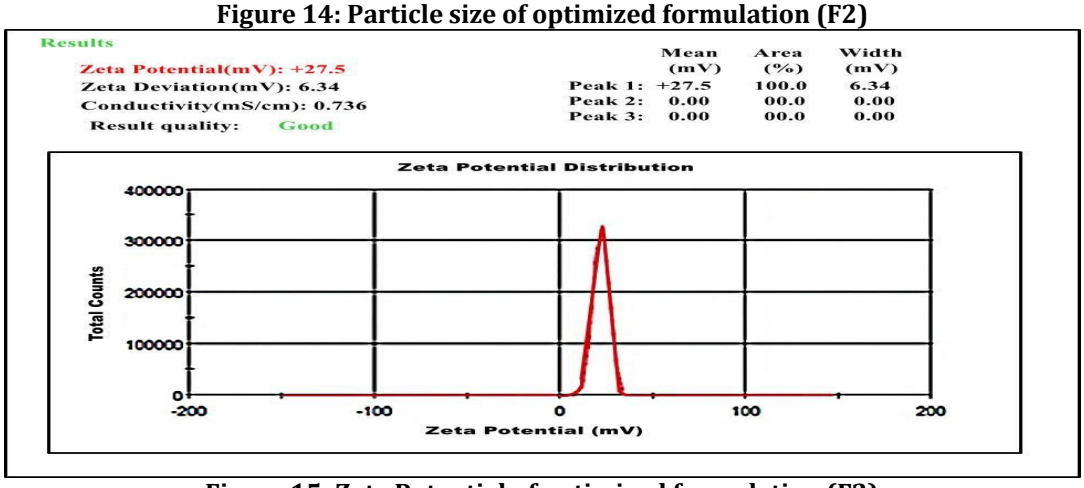



**Figure 15: Zeta Potential of optimized formulation (F2)**

It was discovered that the improved formulation's (F2) release profile obeyed the first order model's release kinetics for formulations (Table 7.10). As a result, the improved formulation (F2) adheres to the first order release model, which states that the drug release rate is concentration-dependent.

First Order Release System is used in the formulation of matrix dissolution-controlled release, matrix diffusion-controlled release, solutions, and sustained release.

**Surface Morphology**

Nanoparticle surface morphology & shape were visualized using SEM using a magnification of 15000 to 20000 X for taking photographs. The drug-loaded nanoparticles of formulation F2 were found to be spherical with a smooth surface (figure 16).

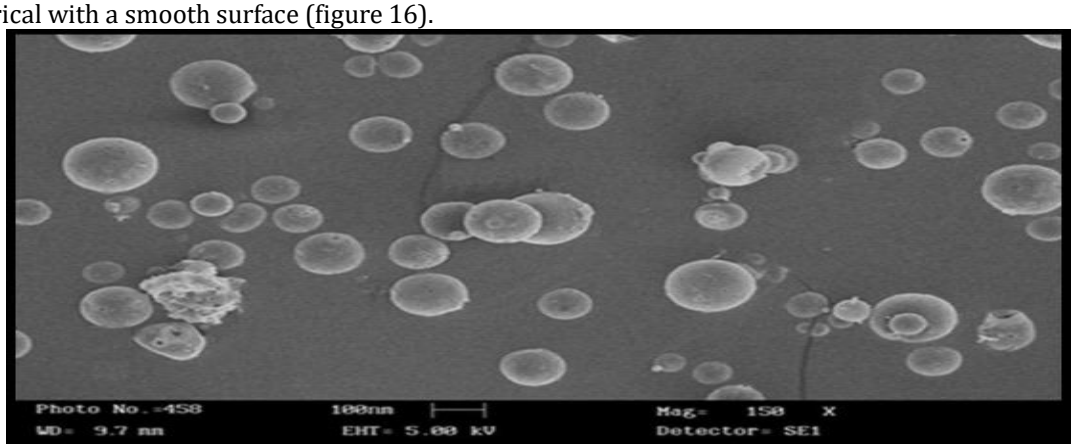



**Figure17: SEM of optimized formulation (F2)**

**Transmission Electron Microscopy(TEM)**

The optimized formulation was characterized for its shape and surface morphology by TEM. Representative TEM image of nanoparticles demonstrates spherical and discrete vesicles of < 300 nm of formulation. The TEM image of optimized nanoparticles is shown in figure 2.18.

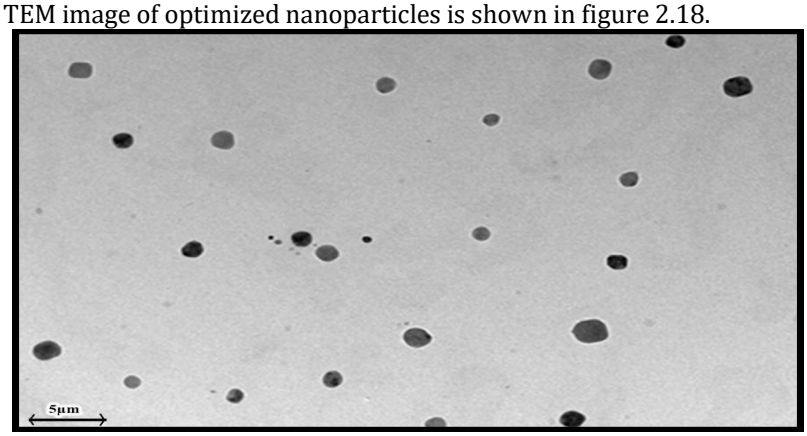



**Figure 18: TEM of optimized formulation (F2)**

**MicrobiologicalStudy**

Optimized formulation(F2) & marketed eye drops of Moxifloxacin HCl were evaluated for antimicrobial activity by the cup-plate method. Formulation (F2) gave a clear zone of inhibition compared with the zone of inhibition given by marketed eye drops. Results showed that the formulation has better antimicrobial efficacy compared with the marketed eye drops. Results obtained were compared with the control (without drug) as shown in Figures 19 & 20.

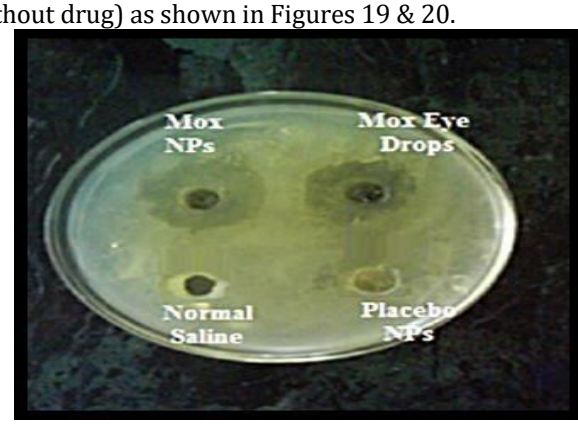



**Figure 2.19: Antimicrobial activity against *Staphylococcus aureus***

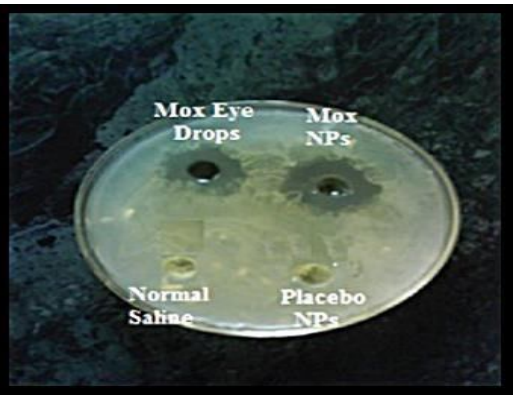



**Figure 2.20: Antimicrobial activity against *Pseudomonas aeruginosa***

**DISCUSSION**

The following findings were drawn from the current research on "Formulation and Evaluation of Mucoadhesive Nanoparticles of Moxifloxacin as Ocular Drug Delivery System":Studies involving FTIR, X-ray crystallography, and differential scanning calorimetry were conducted. The typical peaks of the pure drug were compared to those that were achieved with drug-excipient combinations that stayed quite close to one another. In conclusion, it was discovered that Moxifloxacin was compatible with the chitosan and STPP employed in the ocular drug delivery system. Moxifloxacin has been chosen as the solvent for the current analytical approach because it is well soluble in PBS and exhibits good UV absorption.At various wavelengths, the solution's absorbance was measured (). 293 nm was discovered to be the max. By using a suggested UV spectrophotometric approach, moxifloxacin was examined. Over a concentration range of 10.0 to 80.0 g/ml, the calibration curve demonstrated linearity. Equation y= 90.23x + 71 can be used to define linearity. The curve generated using the linear regression approach had correlation values of 0.999. Further evaluation of the solutions' clarity revealed that F7, F8, and F9 formulations lacked clarity while the others had a low-cloudy appearance. The formulation batches have a pH between 6.5 and 7.2.The formulations' PDI ranged between 0.137 and 0.512. The formulation's particle sizes range from 183.7 nm to 962.1 nm. The particles' physical stability is determined by their zeta potential (high zeta potential leads to more stable colloid particles). The zeta potential of the formulations was changed from 14.2 to 27.5. All formulations' percent entrapment efficiency was found to be between 39.80 and 94.24. The enhanced retentivity of moxifloxacin in the chitosan-TPP matrix may be related to the higher entrapment of moxifloxacin in nanoparticles.All formulations' % loading capacities were discovered to be

between 3.32 and 7.87. The media utilized for in-vitro investigations was freshly made phosphate buffer (pH 7.4). To keep sink conditions, samples were taken out and replaced at intervals of 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, and 24 hours, respectively. The 183.7 nm particle sizes in the optimized formulation (F2) are suitable for nano-formulation, and the PDI indicates that it is polydisperse. The +27.5 mV Zeta potential of the improved formulation stabilizes the formulation.F2 was determined to have the highest level of entrapment efficiency (84.63%) and a relatively high loading capacity (7.07%), both of which were stable and had nanoscale particle sizes. Other formulations have higher entrapment effectiveness, but they are unsuitable for ophthalmic preparation because their particle sizes (F7, F8, and F9) surpass the nano range. The first-order model release kinetics for formulations was discovered to be obeyed by the optimal formulation (F2) release profile exhibiting the maximum R2 value. As a result, the improved formulation (F2) adheres to the first order release model, which states that the drug release rate is concentration-dependent.Moxifloxacin HCl eye drops with improved formulation (F2) and commercial availability had their antibacterial activity assessed using the cup-plate method. A distinct zone of inhibition equal to the zone of inhibition provided by commercial eye drops was produced by formulation (F2). Results revealed that the formulation's antibacterial activity was superior to that of commercial eye drops.

**CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for profit sectors.

**REFERENCES**

1. Duxfield L, Sultana R, Wang R, Englebretsen V, Deo S, Rupenthal ID, Al-Kassas R. Ocular delivery systems for topical application of anti-infective agents. Drug Dev Ind Pharm. 2016;42(1):1-11
2. Thakur RR, Kashiv M. Modern delivery systems for ocular drug formulations: a comparative overview WRT conventional dosage form. Int J Res Pharm Biomed Sci. 2011;2:8-18
3. Lang JC. Ocular drug delivery conventional ocular formulations. Adv Drug Deliv Rev. 1995;16(1):39-43
4. Nagarwal RC, Kant S, Singh PN, Maiti P, Pandit JK. Polymeric nanoparticulate system: a potential approach for ocular drug delivery. J Control Release. 2009;136(1):2-13
5. Das S, Suresh, PK. Drug delivery to eye: Special reference to nanoparticles. Int J Drug Deliv. 2010;2(1):12-21
6. Garima Verma . Pharmaceutical Preformulation Studies in Formulation and Development of New Dosage Form: A Review. International Journal of Pharma Research & Review, Oct2016;5(10):12-20
7. Sadeek S.A. Synthesis, characterization and antimicrobial investigation of some moxifloxacin metal complexes,Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy ,2011, 84(1):99-110
8. Yin L. Drug Permeability and Mucoadhesion Properties of Thiolated Trimethyl Chitosan Nanoparticles in Oral Insulin Delivery: Biomaterials, 2009; 30:5691-5700.
9. Dube A. Chitosan Nanoparticles Enhance the Intestinal Absorption of the Green Tea Catechins (+) Catechin and (−) Epigallocatechin Gallate: European Journal of Pharmaceutical Sciences, 2010;41(2): 219-225.
10. Amidi M. Preparation and Characterization of Protein-Loaded n-trimethyl Chitosan Nanoparticles as Nasal Delivery System, Journal of Controlled Release,2006; 111(1): 107- 116.
11. Katas, H . Development and Characterisation of Chitosan Nanoparticles for RNA Delivery,Journal of controlled release, 2006;115(2):216-225.
12. Wang X. Preparation of Estradiol Chitosan Nanoparticles for Improving Nasal Absorption and Brain Targeting, European journal of pharmaceutics and biopharmaceutics, 2008 ;70(3): 735-740.
13. Wang X. Recent Advances of Chitosan Nanoparticles as DrugCarriers. International Journal of Nanomedicine, 2011; 6:765.
14. Katas H. Physicochemical Effects of Chitosan-TPP Nanoparticles on Antibacterial Activity Against Gram-Positive & Gram-Negative Bacteria. Journal of Medical Sciences,2011; 11(4):192-197.
15. Basu S. K., Kavitha, K. Evaluation of Ketorolac Tromethamine Microspheres by Chitosan/Gelatin B Complex Coacervation. *Scientia Pharrnaceutica*, 2010; 78(1): 79-92.
16. Win P. P., Shin-ya, Y. Effect of Proteolytic Enzymes in Gastrointestinal Fluids on Drug Release from Polyelectrolyte Complex Microspheres Based on Partially Phosphorylated Chitosan. Polymer International, 2005; 54(3):533--536.
17. Berne B. J., and Pecora, R. Dynamic Light Scattering. The British Polymer Journal, John Wiley & Sons, New York, 1976; 9 (2):376.
18. Narayanan D., AnithaA.Synthesis, Characterization and Preliminary In Vitro Evaluation of PTH 1-34 Loaded Chitosan Nanoparticles for Osteoporosis. Journal of Biomedical Nanotechnology, 2012;8(1) :98-106.
19. Pawar P. K, Majumdar, D. K. Effect of Formulation Factors on In Vitro Permeation of Moxifloxacin from Aqueous Drops Through Excised Goat, Sheep and Buffalo Corneas, AAPS PharmSciTech,2006; **7**(1):E89.
20. Kim D. H., Stark, W. J. Ocular Penetration of Moxifloxacin0.5% and Gatifloxacin 0.3% Ophthalmic Solutions into

the Aqueous Humor Following Topical Administration Prior to Routine Cataract Surgery, Current Medical Research &Opinion, 2005;21(1):93 -94.

21. RobertsonS.M.,Curtis,M.A. OcularPharmacokineticsofMoxifloxacinAfterTopicalTreatmentofAnimals and Humans, Survey of Ophthalmology, 2005;50: S32-S45.
22. Charoo V, Ali A. Preparation of In Situ-Forming Ophthalmic Gels of Ciprofloxacin Hydrochloride for the Treatment of Bacterial Conjunctivitis: In Vitro & In Vivo Studies, Journal of Pharmaceutical Sciences,2003; 92(2):407-413.

**CITATION OF THIS ARTICLE**

N Kaushik, Ankit kumar, Sunaina Arora, P Kumari, S Maratha, Formulation And Evaluation Of Mucoadhesive Nanoparticles Of Moxifloxacin As An Ocular Drug Delivery System,. Bull. Env.Pharmacol. Life Sci., Spl Issue [4]: 2022: 300-315