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Chitosan nanoparticles: A Novel Vehicle for the Enhanced Ocular Delivery of Moxifloxacin HCl

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ABSTRACT

Poor ocular bioavailability of drugs (<1%) from conventional eye drops (ie, solution, suspension, and ointments) is mainly due to the physiologic barriers of the eye. In general, ocular efficacy is closely related to ocular drug bioavailability, which may be enhanced by increasing corneal drug penetration and prolonging precorneal drug residence time. Hence the present study was aimed to develop and evaluate moxifloxacin containing nanoparticles as potential ophthalmic drug delivery system. Nanoparticles were prepared by a modified spontaneous emulsification method and different process variables were optimized. 0.5 % of chitosan gel and 2.5ml of gluteraldehyde saturated toluene per 25mg of polymer were selected as the optimum concentrations of polymer and cross linking agent. The nanoparticles were characterised by Scanning Electron Microscopy (SEM), Zeta potential analyser and Fourier Transform Infrared (FTIR) Spectroscopy. All the prepared formulations resulted in nano range size partricles (75 - 225 nm) and displayed spherical smooth morphology with Zeta potential (+26.46 mV). The drug incorporation efficiency was 24.8-45.63%. The *in vitro* release profile of moxifloxacin from the nanoparticles showed a sustained release of the drug over a period of 24 hrs. Kinetic release profiles of moxifloxacin from nanoparticles appeared to fit best with first order release kinetics and the mechanism of drug release was non- Fickian anomalous diffusion. Microbiological assay were carried out against Pseudomonas aeruginosa, E.coli and staphylococcus using the cup-plate method. Microbiological assay showed equivalent zone of inhibition compared to marketed formulation. Ocular tolerance was evaluated using hen's egg chorioallantoic membrane (HET-CAM) test. The developed nanoparticle dispersed in phosphate buffer saline pH 7.4 with a score of 0 up to 24 h in HET-CAM assay, showed the nonirritant efficacy of developed formulation. Thus the results suggest that moxifloxacin loaded chitosan nanoparticles appear promising for effective management of ocular conjunctivitis infections.

Keyword: moxifloxacin, Chitosan, Nanoparticles, Ocular delivery

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INTRODUCTION

One of the most attractive areas of research in drug delivery today is the design nanosystems that are able to deliver drugs to the right place, at appropriate times and at the right dosage. These nanocarriers are submicronparticles containing entrapped drugs intended for enteral or parenteral administration, which may prevent or minimise the drug degradation and metabolism as well as cellular efflux. Nanoparticles also have a long shelf-life, have been made of safe materials, including synthetic biodegradable polymers, natural biopolymers, lipids and polysaccharides and have the potential for overcoming important mucosal barriers, such as the intestinal, nasal and ocular barriers. [1,2]

Poor ocular bioavailability of drugs (<1%) from conventional eye drops (ie, solution, suspension, and ointments) is mainly due to the precorneal loss factors that include rapid tear turnover, nonproductive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the drugs to the corneal epithelial membrane. This poor ocular bioavailability imparts the need for frequent instillation to achieve the therapeutic effect, which may sometimes lead to undesirable side effects caused by systemic drug absorption. In general, ocular efficacy is closely related to ocular drug bioavailability, which may be enhanced by increasing corneal drug penetration and prolonging precorneal drug residence time [3]. A variety of ocular drug delivery systems such as inserts and collagen shields and colloidal systems such as liposomes, nanoparticles, and nanocapsules have been designed and investigated for improved ocular bioavailability. The use of nanotechnology-based drug delivery systems such as microemulsions, nanosuspensions, nanoparticles, solid lipid nanoparticles, niosomes, dendrimers, and liposomes has led to the solution of various solubility-related problems of poorly soluble drugs, such as dexamethasone, budesonide, ganciclovir, and so on [4, 5]. Polymeric nanoparticle formulation is one of the strategies currently used to improve drug absorption across biological membranes.

Considering the fact that the cornea and conjunctiva have a negative charge, it was proposed that the use of mucoadhesive polymers, which may interact intimately with these extraocular structures, would increase the concentration and residence time of the associated drug. [6] Based on literature data, the three most commonly used polymers in ophthalmic drug formulations are poly(alkyl cyanoacrylates), polycaprolactone, and poly(lactic acid)/poly(lactic-co-glycolic acid). Other polymers with ocular drug delivery application include chitosan, Eudragit RL/ Eudragit RS, polystyrene, and poly (acrylic acid). Much of the published data suggests that in the case of ophthalmic drug delivery, an appropriate particle size and a narrow size range, ensuring low irritation, adequate bioavailability, and compatibility with ocular tissues, should be sought for every suspended drug. Among the wide variety of mucoadhesive polymers reported in the literature, the cationic polymer chitosan (CS) has been a polymer of choice because of its unique properties including acceptable biodegradability, biocompatibility as well as the ability to increase membrane permeability, both in vitro and in vivo and be degraded by lysozymes in serum. [7]

Moxifloxacin hydrochloride (HCl) is a new 8-methoxy derivate of fluoroquinolones with enhanced activity *in vitro* against gram positive bacteria and maintenance of activity against

gram negative bacteria. It is an anti-infective agent useful in the treatment of eye infection such as bacterial conjunctivitis, keratitis and keratoconjunctivitis. It is presently available as eye drops (0.5%). It is administered at dosing interval of 1 drop in the affected eye 3 times a day for 7 days. The eye drops type dosage form is convenient to use but most of the drug is diluted by tear and rapidly washed out of the sac by constant tear flow which can be avoided by using mucoadhesive nanoparticle by which the therapeutic efficacy of ophthalmic drug can be greatly improved. [8]

Hence, in our current work we develop and evaluate a new colloidal system, that is, chitosan nanoparticles for moxifloxacin ophthalmic delivery, to improve precorneal residence time and ocular bioavailability and also to avoid the drug associated side effects like conjunctivitis, decreased visual acuity, dry eye, keratitis, ocular discomfort, ocular hyperemia, ocular pain, ocular pruritus, subconjunctival hemorrhage, and tearing.

MATERIALS AND METHOD

Materials

Moxifloxacin Hydrochloride was obtained from Chethana Pharmaceuticals, Perinthalmanna. Chitosan was purchased from Yarrow Chem Products, Mumbai. All other chemicals were of analytical grade.

Method

Formulation of chitosan nanoparticles [9]

The chitosan nanoparticles were prepared by modified spontaneous emulsification method. Chitosan gel was prepared as follows: required quantities of chitosan (500mg) and NaCl (2%) were dispersed in required quantities of 3% (v/v) glacial acetic acid and stirred for 2 hours continuously to obtain chitosan gel. Then the solution was kept overnight to obtain clear chitosan gel.

To prepare plain nanoparticles, chitosan gel (5ml) was taken in a beaker and mixed with acetone (5ml) to obtain a clear gel. The polymer gel was added into linseed oil (10ml) and allowed to emulsify under magnetic stirring at room temperature by covering the beaker with a suitable lid. The system was stirred for 30 minutes and then the beaker was kept opened and stirring continued for another 1 hour, which resulted in the precipitation of polymer due to the evaporation of acetone with subsequent formation of smaller spheres suspended in oil phase. To solidify and stabilize the spheres, 2.5 ml of gluteraldehyde saturated toluene containing 10 % Span 80 was selected as a cross linking agent, which was slowly added with a micropipette to the system and stirring was continued for 2 hours.

The suspension obtained was centrifuged at 2500 rpm for 30 minutes, and washed three times each with toluene and acetone. After final washing, the product was suspended in

acetone and poured in to a clean petri dish and dried at room temperature to obtain brown coloured, free flowing, fine powder.

Optimization of process variables

Study on effect of polymer concentration on particle formation

To study the relationship between the concentration of chitosan and particle formation, four different batches were prepared with various concentrations of chitosan gel such as 0.25%, 0.5%, 0.75% and 1%. The morphology of the formed particles was observed through optical microscope and the percentage yield was calculated.

Study on effect of volume of cross linking agent on particle solidification

In order to determine the effect of volume of cross linking agent on particle solidification, six different batches of nanoparticles were prepared using increasing volumes of gluteraldehyde saturated toluene which include 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml, 3ml. The formed particles were observed through optical microscope for their discreteness and uniformity of size.

Fourier Transform Infra-Red spectroscopy analysis

The FT-IR spectra of pure chitosan, pure moxifloxacin Hydrochloride, Moxifloxacin HCl-chitosan physical mixture and the drug loaded nanoparticles were recorded to check drug polymer interaction and stability of drug.

Particle Morphology

The particles were observed through optical microscope to confirm the discreteness and uniformity in size. . The scanning electron microscopy (MODEL JSM 6390) was used to characterize the surface morphology of nanoparticles. The nanoparticles were mounted directly on the scanning electron microscopy (SEM) stub, using double –sided, sticking tape and coated with conductive carbon gold and scanned in a high vacuum chamber with a focused electron beam. Secondary electrons, emitted from the samples were detected and the image formed.

Formulation of the drug loaded nanoparticles

Chitosan gel (5ml) was taken in a beaker and mixed with acetone (5ml) to obtain a clear gel. 5 mg of the drug (moxifloxacin) was added into the gel and dispersed uniformly. The polymer gel containing drug was then added into linseed oil (10ml) and allowed to emulsify under magnetic stirring at room temperature by covering the beaker with a suitable lid. The system was stirred for 30 minutes and then the beaker was kept opened and stirring continued for another 1 hour, which resulted in the precipitation of polymer due to the evaporation of acetone with subsequent formation of smaller spheres suspended in oil phase. To solidify and stabilize the spheres, 2.5 ml of gluteraldehyde saturated toluene containing 10 % Span 80 was

selected as a cross linking agent, which was slowly added with a micropipette to the system and stirring was continued for 2 hours. The suspension obtained was centrifuged at 2500 rpm for 30 minutes, and washed three times each with toluene and acetone. After final washing, the product was suspended in acetone and poured in to a clean petri dish and dried at room temperature. Upon drying, brown coloured, free flowing, fine powder was obtained and was stored in a self sealed cover.

Similarly, four other batches of drug loaded nanoparticles were prepared with different concentrations of drug. The concentration of polymer was kept constant in all the batches.

Nanoparticle recovery (%)

Dried nanoparticle was weighed accurately; and nanoparticle recovery (%) was calculated using Eq. (1):

$$\text{Nanoparticles recovery} = \frac{Mass\ of\ nanoparticles\ \ recovered\ \ X\ 100}{Mass\ of\ polymer\ ;drug\ and\ other\ excipents\ \ used}$$

The individual values for three replicates were determined, and their mean values are reported.

Determination of drug incorporation efficiency [10]

10 mg of drug loaded nanoparticles from each batch was dissolved in 20 ml of 6% acetic acid and stirred for 6 hours. It was then centrifuged at 2500 rpm for 30 minutes and the supernatant was analyzed by UV spectrophotometer at 296 nm. Plain nanoparticle treated in the same manner was used as the blank.

Drug incorporation efficiency was expressed as % drug loading drug; represented by Eq. (2):

$$\%\ \text{Drug Loading} = \frac{\text{Actual drug content X 100}}{\text{Theoretical drug content}}$$

The individual values for three replicates were determined, and their mean values are reported.

Particle size and Zeta potential of nanoparticles [7]

The size and zeta potential of nanoparticles were analyzed by using a Zetasizer (Malvern instrument). The samples were diluted with milli Q water and placed in eletrophoretic cell and measured in the automatic mode.

***In-vitro* Drug Release** [11]

A quantity of nanoparticles, which is theoretically equivalent to 2 mg of drug, was calculated from each drug loaded batch and then it was added to 250ml beaker and to it 100ml of phosphate buffer saline (PBS) pH 7.4 was added. Then the flask was kept in a shaker cum incubator, maintained at 37°C. 2ml of drug release medium was withdrawn at time intervals of

30 minutes, 1,2,3,4,5,6,7,8,9,10,11,12,14,18 and 24 hours while replacing it with fresh 2ml of PBS (maintained at 37°C). The samples were centrifuged and filtered. From the filtered samples 1ml sample were withdrawn and diluted to 10 ml with PBS and the drug content was analyzed by UV spectrophotometer at 289nm. The cumulative % drug release was calculated and a graph was plotted with cumulative % drug release Vs time.

Release Kinetics [12]

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted to various kinetics equations like zero order, first order and Higuchi matrix. In order to define a model which will represent a better fit for the formulation, drug release data were further analyzed by Peppas equation, $Mt/M\infty = kt^n$, where Mt is the amount of drug released at time t and M∞ is the amount released at ∞, Mt/M∞ is the fraction of drug released at time t, K is the kinetic constant and n is the diffusional exponent, a measure of the primary mechanism of drug release. R^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

Microbiological studies [13]

The microbiological studies ascertained the biological activity of the optimized formulation and of the marketed eye drops against microorganisms (E.coli, Staphylococcus and Pseudomonas aeruginosa). A layer of nutrient agar (20mL) seeded with the test microorganism (0.2mL) was allowed to solidify in the Petri plate. Cups were made on the solidified agar layer with the help of a sterile borer at 4 mm diameter. Then, a volume of the formulations (optimized formulation and marketed eye drops) containing equivalent amount of drug was separately poured into two cups. The plates were incubated at 37°C for 24 hours. The zones of inhibition were obtained. The diameter of the zone of inhibition was measured by an antibiotic zone finder. Readings were taken in triplicate.

Ocular tolerance test (HET-CAM test) [7,14]

Maintain the eggs in an incubator, which requires adequate humidity, a 37±0.5°C environment, and rotation every few hours. On the twelth day, a window (2 × 2 cm) was made on the equator of the eggs through which formulations (0.5 mL) were instilled directly onto the CAM surface and left in contact for 5 minutes. The membrane was examined for vascular damage, and the time taken for injury to occur was recorded. A 0.9% NaCl solution was used as a control as it is reported to be practically nonirritant. The scores were recorded according to the scoring schemes.

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Scoring chart for HET-CAM test

Effect	Scores	Inference
No visible hemorrhage	0	Nonirritant
Just visible membrane discoloration	1	Mild irritant
Structures are covered partially due to membrane discoloration or hemorrhage	2	Moderately irritant
Structures are covered totally due to membrane discoloration or hemorrhage	3	Severe irritant

Stability studies [13]

The physical stability of the nanoparticles was evaluated after storage for 3 months. The stability study was carried using the batch F5. A quantity of nanoparticles was stored in closed amber-colored glass vials at 5 ± 2°C (refrigerator) away from direct light. 10mg of the formulation was withdrawn at 1-, 2-, and 3- month time intervals to measure the drug content, as described earlier.

RESULTS AND DISCUSSION

FTIR Spectroscopy

IR studies ruled out the possibility of interaction between the selected polymer chitosan and the drug moxifloxacin. The spectra obtained from IR studies at wavelength from 4000 cm-1 to 400 cm-1 showed that there are neither major shifts nor any loss of functional peaks between the spectra of drug, polymer, physical mixture of drug and polymer and also nanoparticle form of drug.

Peaks for aromatic C-H stretching, secondary N-H stretching, C=O stretching of keto group, OH stretching and OH bending of COOH are present in the region of 2953cm^{-1},3529cm^{-1},1708cm^{-1},2926cm^{-1},1467cm^{-1} in the pure drug. Peaks for N-H stretching of primary amine, OH stretching of primary alcohol and C=O stretching in NH-C=O are present in the region of 3334.92cm^{-1}, 2920cm^{-1}, 1676.14 cm^{-1} in the polymer. The presences of same peak which are characteristic functional group of the drug and polymer are present in the drug polymer physical mixture confirm that there is no incompatibility between the drug and polymer.[8] Figure 1

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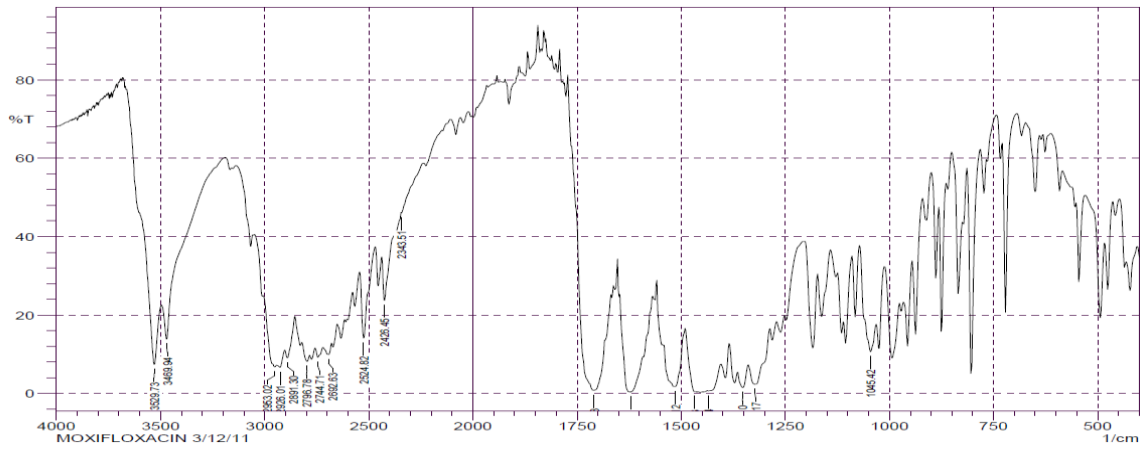

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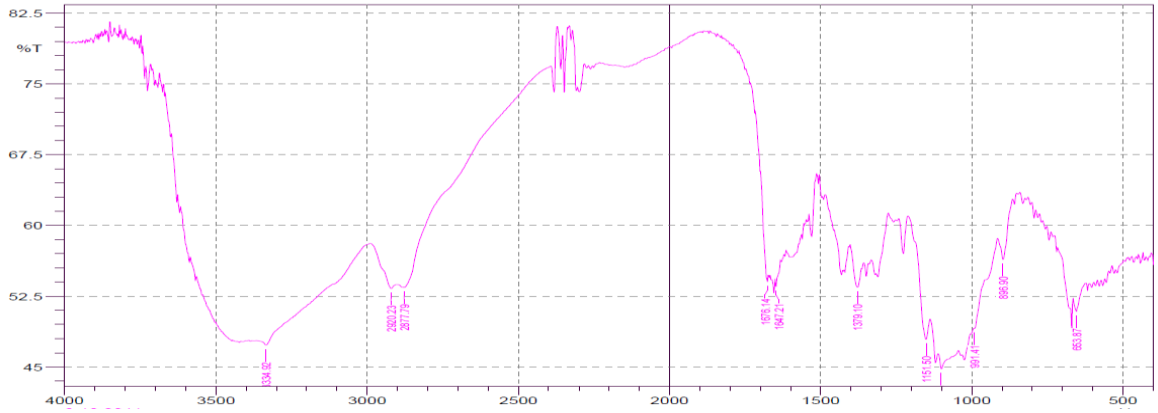

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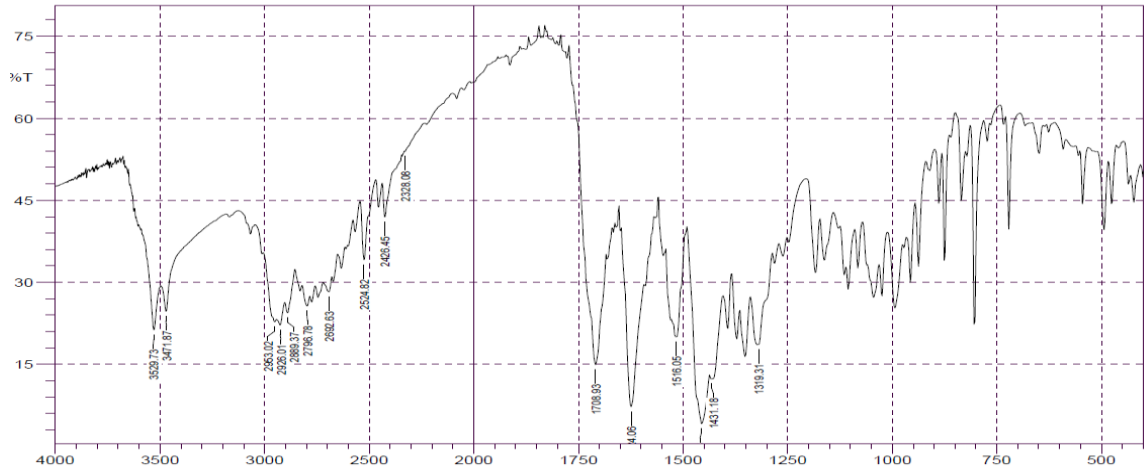

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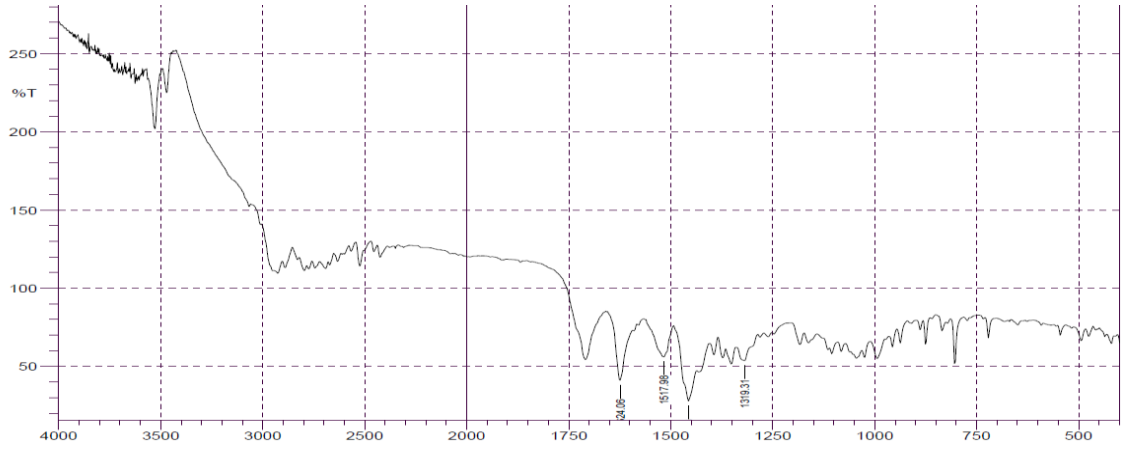

D

Figure1. FTIR Spectroscopy of A) Moxifloxacin HCl B) Chitosan, C) Moxifloxacin Chitosan physical mixture D) Moxifloxacin loaded nanoparticle.

Optimization of process variables

Table 1 Effect of polymer concentration on the properties of chitosan nanoparticles

Batch code	Polymer concentration (%)	% yield	Description of particle formed(observed under optical microscope(100x)
C1	0.25	89	Only few spheres formed
C2	0.5	92	Discrete spheres formed
C3	0.75	87	Less spheres formed and more clumpy particles formed
C4	1	90	Large clumpy masses formed

Table 2 Effect of volume of cross linking agent on particle formation

Batch code	Volume of gluteraldehyde(ml)	Nature of particles formed under optical microscope
G1	0.5	Number of discrete particles few, clumpy observed
G2	1.0	Clumpy masses with few number of spherical particle
G3	1.5	Few clumpy masses with different size of spherical particle
G4	2.0	Less uniform sized discrete spherical particle
G5	2.5	More uniform sized discrete spheres
G6	3.0	Uniform sized spheres

An attempt was made to formulate nanoparticles of discrete nature, good physical stability and distinct particle size and shape. Therefore, a study on the effect of various concentrations of polymer, cross linking agent was carried out. 0.50% chitosan gel and 2.5 ml of gluteraldehyde saturated toluene per 25 mg of polymer were selected as an ideal concentration of polymer and cross linking agent and the nanoparticle recovery was about 92%.Table 1 and table 2

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Scanning electron microscopy

The morphology of the prepared nanoparticles were found to be distinct, sphereical and it was seen in the SEM (Figure2).The size of the nanoparticles was found to be 100-140nm.

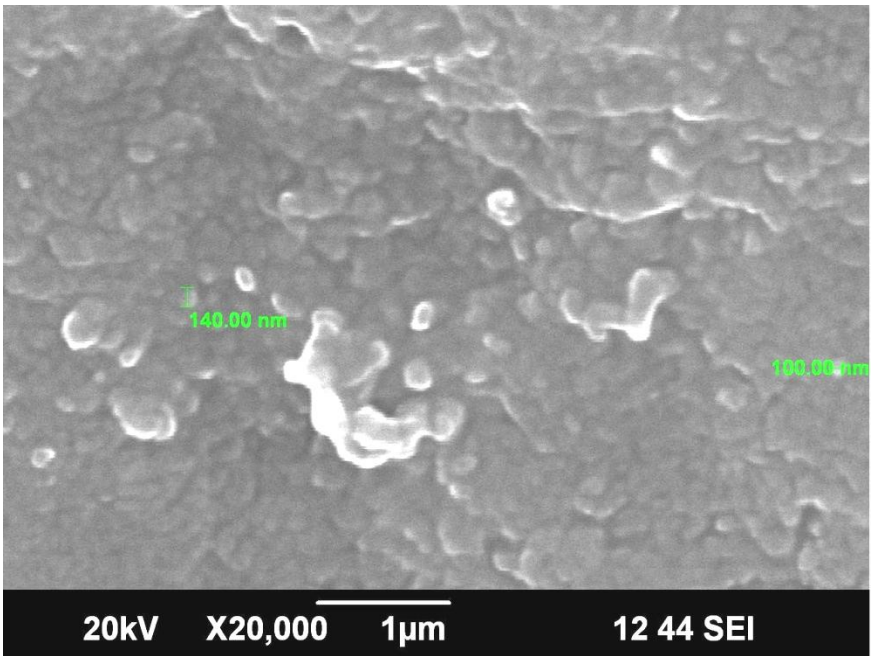

Figure 2 Scanning electron microscopy of prepared nanoparticle

Drug incorporation efficiency

To determine the carrier capacity of chitosan with respect to moxifloxacin HCl, various batches of drug-loaded nanoparticles were prepared with varying concentrations of drug to a constant amount of polymer shows the percentage drug loading of various drug-loaded batches. From table **3**, it can be understood that there is a proportional increase in drug loading capacity with increase in concentration of the drug was noted. This indicates the saturation capacity of the polymer with respect to the selected drug due to saturation of the polymer matrix. From the above data, it has been understood that the saturation capacity of chitosan with respect to moxifloxacin occurs at a concentration of 25 mg/ 25 mg of polymer.

Table 3 % yield and % drug loading of drug loaded nanoparticles

Batch code	Amount of drug added per 25mg of polymer(mg)	% yield	% drug loading
F1	5	88	24.8±0.665
F2	10	92	42.3±0.873
F3	15	89	38.9±0.568
F4	20	91	44.9±0.776
F5	25	90	45.6±0.650

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Particle size and zetapotential of the Moxifloxacin loaded chitosan nanoparticles

Particle size is an important parameter in developing ocular drug delivery. It is also an important parameter to assess the ocular tolerance. Larger particle size will induce rapid tear production which lead to the rapid drainage of the instilled dose and therefore reduced bioavailability. The size of the prepared nanoparticles was confirmed by zetasizer and the particle size was found to be below 250nm. A particle size below 250nm was considered optimum for ocular administration.

Zeta Potential is an important parameter to analyze the long-term stability of the nanoparticles. Generally higher zetapotential values, both (+) or (−) indicate long-term stability because of electrostatic repulsion between particles with same charges avoids aggregation. Zetapotential of developed nanoparticles was found to be around +26.46 mV which is essential for long stable formulation. These positive zeta potential values indicate that the surface of the nanosystems is mostly composed by CS. This net positive charge of the particles is desirable to prevent particle aggregation and promote electrostatic interaction with the overall negative charge of the cell membrane.

***In vitro* Drug Release**

To evaluate the release of drug from all the drug-loaded batches, an *in-vitro* release study was undertaken by centrifugal ultra filtration method. All the drug loaded batches showed a cumulative % release between 70.5% and 87.5%. All the formulation shows bi-phasic release pattern: one initial burst release followed by a second slow-release phase (extended release). An initial burst release is beneficial in terms of antibacterial activity as it helps achieve the therapeutic concentration of drug in minimal time followed by constant release to maintain sustained and controlled release of the drug figure **3**. The initial fast release phase can be due to immediate desorption of drug located in the surface of the nanoparticles. The next slow phase could be due to slow desorption of drug located in the interior of the porous nanoparticles and probably with erosion of the polymer matrix. Among the five batches of nanoparticles, the batch of nanoparticles F5 (containing 25 mg of drug per 25 mg of polymer) showed maximum cumulative % drug release of 87.5%. These batches were selected for further studies

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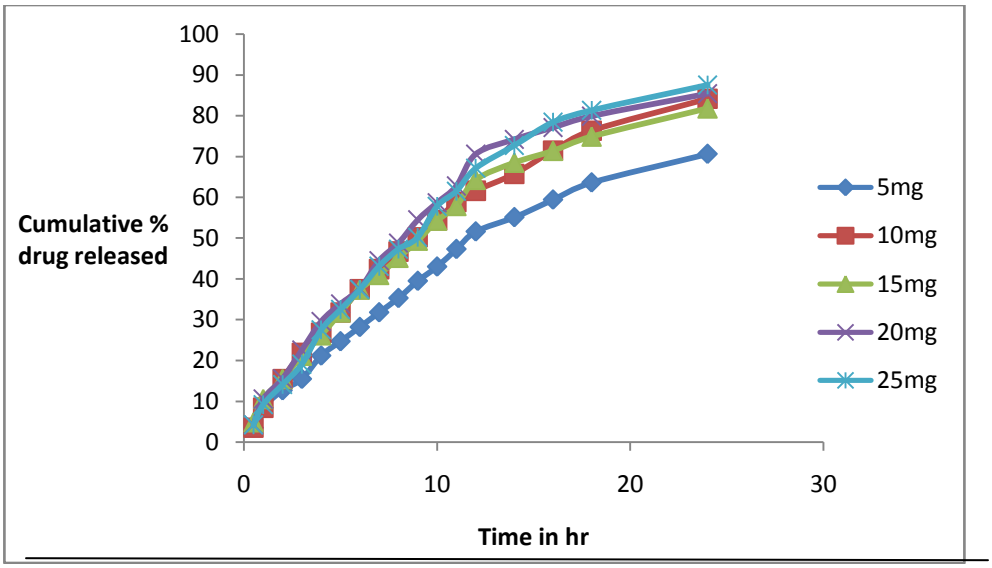

Figure 3. *In-vitro* release profile of drug loaded nanoparticles

Release Kinetics

In order to understand the order and mechanism of drug release, the result of *in-vitro* drug release study of nanoparticles were fitted with various kinetic equations, zero order, first order, Higuchi's model and Korsmeyer- peppas model. 'R^2 'and 'n' values were calculated for the linear curve obtained by regression analysis of the above plots and it is given in (Table **4**). From the table, it is understood that *in vitro* release data is well fitted to Peppa's model. So it was concluded that the mechanism by which drug is being released is a non-Fickian anomalous diffusion mechanism that is drug release is controlled by all diffusion, erosion and swelling mechanism. The *in-vitro* drug release of nanoparticles was best explained by first order kinetics for all formulations.

Table 4. *In-vitro* release kinetics data of chitosan nanoparticles

Batch Code	Zero order regression (R^2)	First order		Higuchi's		Peppa's	
		Regression (R^2)	Rate constant, K^1	Regression (R^2)	Slope (n)	Regression (R^2)	Slope (n)
F1	0.9520	0.9937	0.0529	0.9879	17.311	0.9907	0.7659
F2	0.9320	0.9987	0.0776	0.994	20.688	0.9837	0.8042
F3	0.9255	0.9891	0.0746	0.986	20.309	0.9903	0.7373
F4	0.9070	0.9836	0.0870	0.9789	21.892	0.9832	0.7772
F5	0.9293	0.9929	0.0923	0.9838	22.483	0.9903	0.8066

Microbiological studies

The microbiological assay was carried out as qualitative assessment to compare antibacterial efficacy of developed nanoparticle with marketed formulation. The optimized F5 formulation was tested microbiologically by cup-plate technique. Clear zones of inhibition were obtained for the formulation against the test organisms namely E.coli, Staphylococcus and

Pseudomonas aeruginosa. The results were shown in table **5**. Results reveal that the developed formulation is equivalent to marketed formulation in antibacterial action.

Table 5 Diameter of zone of inhibition for the marketed formulation and the optimized formulation for different organisms

	Diameter zone of inhibition(mm)	
	Formulation	Marketed formulation
E.coli	29±0.351	28±0.750
Staphylococcus	34±0.602	33±0.529
Pseudomonas aeruginosa	24±0.404	22±0.655

Ocular tolerability test (HET-CAM Test)

The eye is a very sensitive organ. Ocular tolerability plays an important role in patient compliance. Ocular tolerance is of utmost importance while formulating ophthalmic delivery systems. Ocular irritation of the developed formulation was checked by HET-CAM test. The HET-CAM is a suitable alternative to animal testing (Draize test). It is based on the direct application of formulation onto the CAM. The CAM of the chick embryo is a complete tissue including veins, arteries, and capillaries and is a borderline case between *in vitro* and *in vivo* systems and does not conflict with the ethical and legal obligations. It responds to injury with a complete inflammatory process, a process similar to that induced in the conjunctival tissue of the rabbit eyes. Developed formulation was tested by this method and result was compared with those obtained using practically nonirritant normal saline, as control. A mean score of 0 was obtained for normal saline. Nanoparticle dispersed in phosphate buffer saline pH 7.4 was found the score 0 up to 24 h (Table 6). With respect to effect of particle size on ocular irritation, only particles of 20 µm will induce problems. Since the developed formulation is in nanometer range, there is no problem. Therefore, the study shows the formulation is nonirritant and is well tolerated.

Table 6 Scores obtained in HET-CAM Test

Formulation	**Scores**								
	Time(in min)								
	0	5	15	30	60	120	240	480	1440
Normal Saline as control	0	0	0	0	0	0	0	0	0
Developed Formulation	0	0	0	0	0	0	0	0	0

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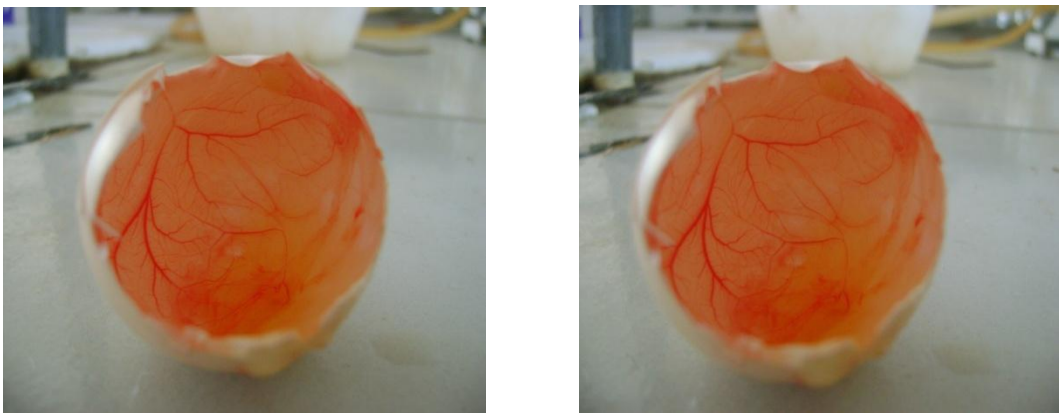

A B

Figure: 4 Photo documentation of HET-CAM test **(A)** Untreated CAM. **(B)** CAM after the application of nanoparticle dispersed in PBS for 5 min.

Stability studies

The stability study data indicated that the drug loaded nanoparticles shows good stability for 90 days, when it is stored in closed amber coloured glass vials at 5±2^{o}C(refrigerator)away from direct light, in terms of no change in percentage drug loading**.** (table7)

Table 7. stability of moxifloxacin nanoparticles during storage at 5 ± 2°C (refrigerator)

Time (days)	Parameters observed	
	% drug loading	Change in morphology(by optical microscopy under magnification 100 X)
1	45.635	Discrete regular spheres
30	45.464	No significant change in morphology
60	45.311	No significant change in morphology
90	45.311	No significant change in morphology

The improved interaction of chitosan loaded nanoparticles with the cornea and the conjunctiva could be found in the mucoadhesive properties of chitosan or it is due to the electrostatic interaction between the positively charged chitosan nanoparticles and the negatively charged corneal and conjunctival cells that is the major force responsible for the prolonged residence of the drug[6,7]. In consistent with these observations and also based on the results of the present study we propose that chitosan nanoparticles may be beneficial in improving the corneal permeation, contact time and bioavailability for the treatment of ocular conjunctivitis infections.

CONCLUSION

The formulated moxifloxacin nanoparticles with chitosan as a carrier was found to be a suitable and potential natural carrier in terms of their particle size, zetapotential, drug loading

capacity, *in vitro* release characteristics and better ocular tolerability. The release profile of moxifloxacin from nanoparticles has shown a sustained release following first order kinetic with non-Fickian anomalous diffusion mechanism. The results demonstrated the effective use of moxifloxacin loaded chitosan nanoparticles as a controlled release preparation for treatment of ocular conjunctivitis infections.

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REFERENCES

[1] Yolanda Diebold, Margarita Calonge. Progress in Retinal and Eye Research 2010; 29: 596-609.
[2] Sanjay K Motwani, Shruti Chopra, Sushma Talegaonkar, Kanchan Kohli, Farhan J. Ahmad, Roop K Khar. Eur J Pharm and Biopharm 2008; 68: 513–525.
[3] Ramesh C Nagarwal, Shri Kant, Singh PN, Maiti P, Pandit JK. J Controlled Release 2009; 136: 2–13.
[4] Sanjeeb K Sahoo, Fahima Dilnawaz and Krishnakumar S. Drug discovery today 2008; 13: 144-151.
[5] Patel Vishal, Agrawal YK. J Global Trends in Pharmaceutical Sciences 2011; 2(2): 131-148.
[6] Azza A Mahmoud, Gina S El-Feky, Rabab Kamel, Ghada EA Awad. Int J Pharm 2011; 413: 229– 236.
[7] Himanshu Gupta, Mohammed Aqil, Roop K Khar, Asgar Ali, Aseem Bhatnagar, Gaurav Mittal. Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery, Nanomedicine: NBM 2010; 6: 324–333.
[8] UL Patel, NP Chotai, CD Nagda. Acta Pharma Sci 2010; 52: 523- 535.
[9] Barnabas Wilson, Malay K Samanta, Kumaraswamy Santhi, KP Sampath Kumar, Muthu Ramasamy, Bhojraj Suresh. Nanomedicine: NBM 2010; 6: 144–152.
[10] Dhanya KP, K Santhi, SA Dhanaraj. Int J Com Pharm 2011; 5(13): 1-5.
[11] S Debnath, D Datta, MN Babu, RS Kumar, V Senthil. Studies on the Preparation and Evaluation of Chitosan Nanoparticles containing Cytarabine, Int J Pharm Sci Nan 2010; 3(2): 957-964.
[12] Hitesh B Gevariya, Suresh Gami, Nitin Patel. Asian J Pharm 2011; 5(2): 114-119.
[13] Himanshu Gupta, M Aqil, RK Khar, Asgar Ali, Aseem Bhatnagar, and Gaurav Mittal. J Drug Target 2011; 19(6): 409–417.
[14] Manuela Curcio, Giuseppe Cirillo, Ortensia Ilaria Parisi, Francesca Iemma et al. J Funct Biomater 2011; 2: 1-17.