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Preparation and Characterization of Gatifloxacin Encapsulated Chitosan Nanoparticles for Ocular Drug Delivery

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ABSTRACT: The science of chemical bondings and compositions has spread its advantages in different fields, including the area of drug delivery. The change in the interaction caused due to miniaturisation in size has lead to outstanding achievements in the field of drug delivery. Chitosan nanoparticles have gained more attention as drug carriers because of their better stability, simple preparation and versatile routes of administration. The ophthalmic antibiotic Gatifloxacin have several disadvantages such as rapid tear turnover, resulting in pre corneal loss and lacrimal drainage. But, encapsulation of the same within chitosan nanoparticles has been encouraged in order to increase their half life period and decreasing the dosage of the drug administered. The preparation method for synthesis of chitosan nanoparticles cross linked with TPP sodium tripolyphosphate was optimized and characterization was carried to check the particle size distribution and surface morphology and topography. The AFM image showed 56 nm sized Chitosan nanoparticles while the average size of the chitosan drug loaded particles was found to be around 150-180nm. The drug loaded chitosan nanoparticles showed increase in sensitivity towards Staphylococcus aureus and Staphylococcus epidermidis with satisfactory Drug Entrapment Efficiency of 84.9%.

KEYWORDS: Chitosan, Gatifloxacin, ocular, drug delivery.

I. INTRODUCTION

Nanotechnology is the study, construction and utilization of functional structures with characteristic dimension in nanometers [1]. Nanomaterials and systems can be designed to exhibit novel and significantly improved materials with change in their properties, phenomena and processes is becoming increasingly important in fields like agriculture, microelectronics and health care. These nano-scale materials can be potential candidates of future medicine because of their effective routes of administration, better penetration ability, lower toxicity and better interaction at cellular level. Nano encapsulation of the therapeutic molecules provides a media for better drug delivery stability and targeted drug application. Nanobiologist in drug delivery aim at specific, targeted, safe, Biocompatible with reduced toxicity in drug delivery [2]. The main technologies for behind successful nano therapeutics are PEGylation, active targeting and enhanced permeation and retention effect (EPR) [3-5]. Natural and synthetic degradable polymers are ideal carrier molecules [6]. The drug can be incorporated into the polymer where the release depends on either their gradual diffusion from the polymeric matrix, erosion of the matrix, or release from the surface of the matrix [7]. Chitosan is a notable natural polymer for the delivery of therapeutic agents because it is nontoxic, biocompatible, biodegradable, and has mucoadhesive properties [8, 9]. To be named “chitosan”, the deacetylated chitin should contain at least 60% of D-glucosamine residues. By incorporating drug molecules in chitosan nanoparticles the clearance can be decreased and the circulation half-life of the drug extended [10]. Gatifloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1 piperazinyl)-4-oxo-3-quinolinecarboxylic acid, sesquihydrate] is an antibiotic of the fourth-generation-

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Fluoroquinolones family which acts by inhibiting the enzymes involved in bacterial DNA system. Presently Gatifloxacin is available in the form of ophthalmic solution. Commercial dosage of eye drops has several disadvantages such as rapid tear turnover precorneal loss, induction of tear and lacrimal drainage. The main aim of this research was to prepare, develop and characterize Gatifloxacin loaded chitosan nanoparticles for ocular drug delivery applications.

II. MATERIALS AND METHODS

Low molecular weight Chitosan of 0.5% (W/V) was dissolved in Acetic acid – 1% (V/V). It's left to Magnetic stirring for 1 hour and ultrasonicated for 30 minutes. The pH 4-5 was adjusted by NaOH. Gatifloxacin was dissolved in 0.1%Acetic acid solution. Drug solution was added to the chitosan solution and stirred continuously, TPP and Tween-80, which is a nonionic surfactant was added as a solubilizing agent to prevent the agglomeration of nanoparticles, was added on subsequent manner to the stirring solution. This process was repeated to optimize the ratio of drug and polymer added to synthesis, chitosan encapsulated Gatifloxacin nanoparticles with various concentrations. Dialysis was done to obtain uniform particle size.

The size of the nanoparticle was determined using Malvern Zetasizer Nano S. It is also known to be dynamic light scattering with working principle as time-dependent oscillations of the coherent light causing scattering by the suspended particles. UV spectroscopy studies were done in a UV-1800 SHIMADZU UV Spectrophotometer where the wavelengths ranged from 200nm to 1100nm. Scanning electron microscopy (SEM) images were obtained on a SEM vega3 tescan with an applied voltage of 10 kV. For the measurement the samples were thin coated with carbon under the vacuum of 0.01 Torr to make the surface conducting specimen. The drug particles are encapsulated inside Chitosan nanoparticles were visualized under BIO TEM. The sample is diluted and placed on a copper grid andn observed under the microscope. The image was taken under .2 µm range under 100 k scales. The surface morphology of the drug loaded nanoparticles was characterized using AFM. The Atomic Force Microscope (AFM) is one type of scanning probe microscopes, which is used to image surface structures and to measure surface forces. Drug entrapment efficiency was determined by the amount of freeze-dried formulated nanoparticles was digested with minimum amount of ethanolic solution (water/ethanol in 7:3 ratios). The digested homogenates were centrifuged at 15,000 rpm for 30 min and supernatant was analyzed for drug entrapment. The drug entrapment was measured at 287 nm using UV-1800 SHIMADZU UV Spectrophotometer. The percentage drug entrapment was determined using following equation.

$$\text{Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Amount of unbound drug}}{\text{Total amount of drug}} \times 100$$

The sensitivity of the drug loaded nanoparticle was teated on Muller Hilton Agar with Control(C- Water), Chitosan loaded drug nanoparticles (CS – Dr NP), Gatifloxacin Formulation 1 (G1), Gatifloxacin Formulation 3 (G2), Gatifloxacin Formulation 5 (G3). The interpretation was made based on CLSI 2010 [11].

III. RESULTS AND DISCUSSIONS

The preparation of chitosan nano systems, involved mixing the two aqueous phases at room temperature due to electrostatic interaction between amine group of chitosan and negatively charged group of tripolyphosphate (TPP) as to allow the formation of turbid solution. The final concentration range selected for optimisation study was 0.10–0.30% w/v and 0.125% for TPP, respectively. Chitosan nanoparticles have the ability to improve bioavailability which prolongs contact time of the drug. The nanoparticle size enables the drug uptake through the cell membrane [12, 13]. Gatifloxacin loaded chitosan nanoparticles were produced in various formulations in different concentrations of chitosan as shown in table 1

Particle Size Analyzer works on the principle of Dynamic light scattering to characterize the approximate range of particles dispersed in the solution. The size and characterization of the chitosan nanoparticles is important for the

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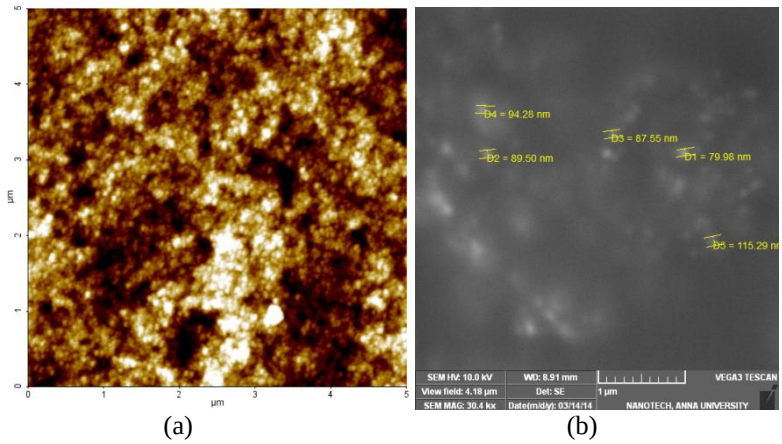
delivery of Gatifloxacin. The average size of the chitosan loaded Gatifloxacin nanomaterials was found to be around 120-160nm, which is well under the nanometer scale. Hence the nanoparticles may exhibit unique properties like high surface to volume ratio, stability of the drugs etc. Their small size favours easy up take by the biological membranes and entry into cells, tissue and organs than their larger counterparts [2, 13].

Table 1 – Different formulations of chitosan encapsulated gatifloxacin

	DRUG	Formulation (%W/V)	Chitosan (%W/V)	TPP	pH	Particle size (nm)
A	Gatifloxacin 1	0.3 g	0.1	0.125 %	5	153
B	Gatifloxacin 2	0.3g	0.15	0.125 %	5	160
C	Gatifloxacin 3	0.3g	0.2	0.125 %	5	98
D	Gatifloxacin 4	0.3g	0.25	0.125 %	5	120
E	Gatifloxacin 5	0.3g	0.3	0.125 %	5	140

The diameter of the smallest blood capillary is roughly 4 µm therefore nanoparticles size needs to be in the ideal range for ocular administration [13]. It is hypothesized that the formulation of gatifloxacin into chitosan nanoparticles would protect gatifloxacin from physical and degradation barriers thus decrease the drug degradation upon administration thereby its increasing half-life, drug stability, circulation time and improve its release characteristics.

The surface morphology of the drug loaded nanoparticle was characterized using AFM, SEM and BIOTEM. It is also important to control the morphology of the nanoparticles in order to enhance the degree of internalization.AFM exhibited smooth spherical nanoparticles as shown in fig. 1(a). Particles that are spherical in shape are more easily internalized than those that are rod or tubular shaped [14]. Nanoparticles with irregular surfaces promote protein adhesion or absorption [15]. Electron Microscopes are instruments that use a beam of highly energetic electrons to examine objects on a very fine scale yielding information about the morphology and crystallography. The TEM and SEM analysis showed results that coincided with the Particle Size analysis as depicted in fig. 1(b) and (c). The average particle size was calculated to be 90nm - 120nm with distinct, solid dense spherical particles and smooth surface which coincides with the results produced by Mohammadpour Dounighi N et al. in 2012 [16]. Consequently, it is ideal to have smooth, spherical nanoparticles. This supports our synthesized nanoparticles to be apt for ocular administration.



(a) (b)

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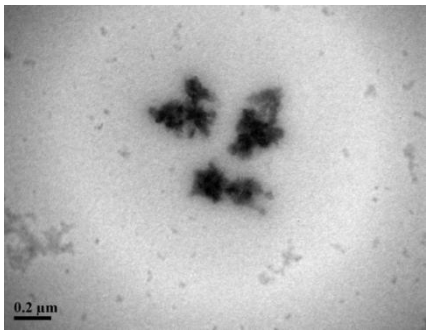

(c)

Fig. 1 (a) Atomic force microscopy image, (b) SEM images and (c) TEM images revealing smooth, spherical Gatifloxacin loaded chitosan nanoparticles

Gatifloxacin showed characteristic absorption peak at 287nm. Gatifloxacin loaded chitosan nanoparticles exhibited absorption at 275 nm as shown in fig. 2. This shift may be due to the loading of the drug in the polymer. These results were justified previously by K. Venugopal et al. in 2005 [17].

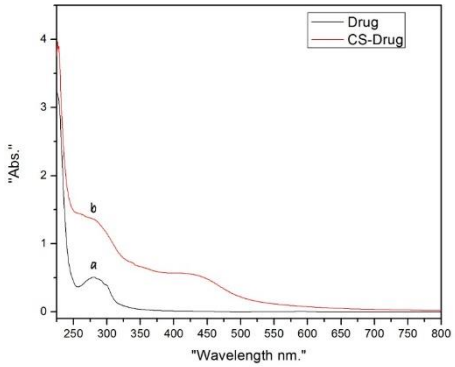

Fig. 2 – shows the absorbance peak of Gatifloxacin and Gatifloxacin loaded chitosan nanoparticles

The entrapment efficiency of the gatifloxacin entrapped chitosan nanoparticles in various concentrations were calculated and tabulated as shown in table 2. The average Drug Entrapment Efficiency was found to be 84.9% with the highest value of 87.77% for G3 Formulation. It was observed that the encapsulation of gatifloxacin into chitosan nanoparticles was highest when the polymer was used in intermediate concentrations.

Table 2: The entrapment efficiency of Gatifloxacin nanoparticles

Formulations	G1(%)	G2(%)	G3(%)	G4(%)	G5(%)
Gatifloxacin Loaded Chitosan nanoparticles	83.90	84.22	87.77	83.74	84.89

The encapsulation efficiency is affected mostly by the chitosan concentration. Lower concentration, the encapsulation efficiency increases with increase in concentrations up to an intermediate concentration. Very high concentrations lead

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to aggregates rather than nanoparticles which may be due to bulkier polymer matrix and less volume for drug encapsulation [18].

Antimicrobial activity of the synthesized drug loaded nanoparticle was checked to see its activity against potent pathogens. The drug exhibited higher sensitivity towards *Staphylococcus aureus* and *Staphylococcus epidermidis*, the zone of inhibition was found to be 5mm against *Staphylococcus aureus* and 3mm against *Staphylococcus epidermidis*.

Fig. 3 – Antibacterial studies against *Staphylococcus aureus* (right) and *Staphylococcus epidermidis* (left)

When compared to its conjugate with sodium alginate these nanoparticles exhibit the same efficiency and morphology [18]. Hence we would like to suggest that chitosan singularly can be used to prepare nanoparticles containing drugs to show all the properties at par to its conjugated forms with others.

VI. CONCLUSION

The chitosan nanoparticles were prepared in different formulations and were used to encapsulate Gatifloxacin antibiotic. The antibiotic fluoroquinolone drug was encapsulated successfully, confirmed by the UV- Vis spectroscopy and BIO-TEM analysis. The morphology of the synthesized nanoparticles were as per desired for an occular administration, which were confirmed by SEM and AFM analysis. These nanoparticles showed high antibacterial activity against gram positive species and appropriate drug loading efficiency. Hence chitosan can be suggested as a potential nanocarrier for ocular delivery of drugs without using any other reactive copolymers and reactants.

REFERENCES

[1] Christian P, Von Der Kammer F, Baalousha M, and Hofmann T, "Nanoparticles: structure, properties, preparation and behaviour in environmental media", Ecotoxicology, Vol. 17, no. 5, pp. 326-343, 2008.
[2] Meena K P, Dangi J S, Samal P K, and Kumar M, "Nanoparticles technology and recent advances in novel drug delivery systems", International Journal of Research in Drug Delivery, Vol. 1, no. 1, pp. 1-5, 2011.
[3] Jin S and Ye K,"Nanoparticle-mediated drug delivery and gene therapy", Biotechnology, Vol. 23, no. 1, pp. 32-41, 2008.
[4] Hoffman A, "The origins and evolution of "controlled" drug delivery systems", Journal of Controlled Release, Vol. 132, no. 3, pp. 153-163, 2008.
[5] Wright J and Hoffman A,"Overview of long acting injections and implants", In J. Wright, & D. Iurgess (Eds.), Long acting injections and implants , New York: Springer, pp 11-22, 2012.
[6] Uchegbu I F, and Schatzlein A G, "Polymers in drug delivery" Florida: Taylor & francis group, pp. 161-243, 2006.
[7] Siepmann J, Siegel R and Rathbone M,"Fundamentals and applications of controlled release drug delivery", 2012.
[8] Sunil A. Agnihotri, Nadagouda N. Mallikarjuna, Tejraj M. Aminabhavi, "Recent advances on chitosan-based micro- and nanoparticles in drug delivery", Journal of Controlled Release, Vol. 100, pp. 5-28, 2004.
[9] Islam M, Firdous J, Choi Y, Yun C and Cho C,"Design and application of chitosan microspheres as oral and nasal vaccine carriers: an updated review", International Journal of Nanomedicine, vol. 7, pp. 6077-6093, 2012.
[10] Kadam R, Bourne D and Kompella U, "Nano-advantage in enhanced drug delivery with biodegradable nanoparticles: contribution of reduced clearance", Drug Metabolism and Disposition, Vol. 40, no. 7, pp. 1380-1388, 2012.

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[11] CLSI, Performance Standards for Antimicrobial Susceptibility Testing;Twentieth Informational Supplement, M100-S20, Vol. 30, no. 1, 2010.
[12] Bowman K and Leong K, "Chitosan nanoparticles for oral drug and gene delivery", International Journal of Nanomedicine, Vol. 1, no. 2, pp. 117-128, 2006.
[13] Tiyabooncha W, "Chistosan: A promising system for drug delivery", Naresuan University Journal, Vol. 11, no. 3, pp. 51-66, 2003.
[14] Quiqnard S, Masse S, Coradin T and A Prokop, "Intracellular delivery: Fundamentals and applications", New York: Springer, pp 333-362, 2011.
[15] K.Venugopal and Ranendra N Saha, "New, simple and validated UV-spectrophotometric methods for the estimation of gatifloxacin in bulk and formulations", Il Farmaco, Vol. 60, pp. 906-912, 2005.
[16] Shi K, Yang R, Han X, Cheng G, Shim C and Cui F, "Cationic formulation of paclitaxel-loaded poly D,L-lactic-co-glycolic acid (PLGA) nanoparticles using an emulsion-solvent diffusion method", Asian Journal of Pharmaceutical Sciences, Vol. 4, no. 2, pp. 89- 95, 2009.
[17] Mohammadpour Dounighi N, Eskandari R, Avadi MR, Zolfagharian H, Mir Mohammad Sadeghi A and Rezayat M, "Preparation and In Vitro Characterization of Chitosan Nanoparticles containing Mesobuthus Eupeus Scorpion Venom as an Antigen Delivery System", The Journal Of Venomous Animals and Toxins including Tropical Diseases(1678-9199), Vol. 18, no. 1, pp. 44-52, 2012.
[18] Sanjay K. Motwani , Shruti Chopra, Sushma Talegaonkar, Kanchan Kohli,Farhan J. Ahmad and Roop K. Khar, "Chitosan–sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimization and in vitro characterization", European Journal of Pharmaceutics and Biopharmaceutics, vol. 68, pp. 513–525, 2008.