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[Research article]

Formulation and Evaluation of Moxifloxacin Loaded Alginate Chitosan Nanoparticles

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ABSTRACT

The main objective of this study was to prepare and evaluate Alginate chitosan nanoparticles containing Moxifloxacin by ionic gelation method. The influence of different experimental parameters on the entrapment efficiency (EE), drug loading (DL), rate of recovery, percent drug released etc was evaluated. SEM indicated that nanoparticles have discrete spherical structure without aggregation. The average particle size was found to be 105.2±8nm. The *in vitro* drug release behaviour from all drug loaded batches was found to be sustained release over a period of 96 hours. Nanoparticles were stored at different temperatures and humidity as per ICH guidelines to check the stability.

Key words: Moxifloxacin, Nanoparticles, Tuberculosis, Ionic gelation method, Alginate.

INTRODUCTION

One of the most key areas of research in drug delivery today is the design of nano systems that are able to deliver drugs, to the site of action, at appropriate time in optimum dosage. These nanocarriers are sub micron particles containing entrapped drugs which might prevent or minimize the drug degradation and metabolism as well as cellular efflux[1,2] . Some other applications of nanoparticles include possible recognition of vascular endothelial dysfunction[3]; oral delivery of insulin[4]; brain drug targeting for neurodegenerative disorders such as Alzheimer's disease[5]; topical administration to enhance penetration and distribution in and across the skin barrier[6]; and pH-sensitive nanoparticles to improve oral bioavailability of drugs such as cyclosporine A[7]. Some polymers used in the fabrication of nanoparticles include chitosan, alginate, albumin, gelatin, polyacrylates, polycaprolactones,poly(D, L-lactide-co-glycolide) and poly (D, L-lactide) However, there are concerns about polymeric nanoparticles including cytotoxicity of by-products (although some, such as polyanhydrides, degrade into products that are biocompatible) and scalability.Tuberculosis (TB) caused by the bacterium *Mycobacterium tuberculosis,* is a major infectious burden worldwide and statistical estimates continue to worsen with each passing year. Tuberculosis affects one third of the world population, i.e. nearly 2 billion individuals, also responsible for 3 million death annually. India accounts for 20% of all new TB cases in the world each year[8, 9]. Antimicrobial agents, which are effective against *Mycobacteriumtuberculosis,* are widely available[10]. A fluoroquinolone antibiotic,

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moxifloxacin is active against *M. tuberculosis* being as potent as rifampicin[11]. The present study was performed to determine feasibility of encapsulation of moxifloxacin within alginate chitosan nanoparticles.

MATERIALS AND METHOD

Low molecular weight chitosan with a deacetylated grade of about 75-85% was purchased from Sigma Aldrich. Moxifloxacin were supplied as gift sample by Macleods Pharmaceuticals Ltd, Mumbai, India. Sodium alginate and calcium chloride were purchased from SD Fine Chemicals, Mumbai, India

Preparation of Moxifloxacin Nanoparticles

Moxifloxacin nanoparticles were prepared by the principle involving cation induced controlled gelification method reported by Rajaonarivony et al. Calcium chloride (18 mM) was added dropwise for 60 min under magnetic stirring into beaker containing sodium alginate solution (0.05%) to provide alginate pregel. The different concentration of drug (25 to 100 mg) is added to the alginate pregel solution .Then 25 ml of Chitosan solution (0.06%) was added followed by stirring for 30 min and the mixture was kept at room temperature overnight. The pH of alginate and chitosan solutions was initially set to 4.9 and 4.6, respectively. A colloidal dispersion at pH 4.7 formed upon polycationic chitosan addition, (visible as the Tyndall effect) [12]. Drug loaded nanoparticles were recovered by centrifugation at 15000 rpm for 30-45 min and the obtained sediment then was suspended in water. These two purification steps were repeated twice. The purified particles were lyophilized.

Table: 1 Formulation of Moxifloxacin Nanoparticles.

Formulation	Polymer ratio (alginate:chitosan)	Drug concentration (mg)
MF1	6:5	10
MF2	6:5	25
MF3	6:5	50
MF4	6:5	100

Evaluation of Moxifloxacin Nanoparticles

Nanoparticles were separated from aqueous medium by ultra centrifugation at 35000 rpm for 30 min. Drug loading in nanoparticles and encapsulation efficiency were determined by measuring the leftover drug in the supernatant and washing fluids. The supernatant and the washing fluid were collected in 50 ml plastic tubes. The solution was diluted accordingly and filtered. The amount of drug present in the solution was analyzed by UV spectroscopy[13] . The encapsulation efficiency, drug loading and rate of recovery were determined using the following equation.

Encapsulation Efficiency

(Estimated Drug content/Theoretical drug content) X100

Dug Loading Efficiency

(Estimated drug content/Weight of product) X100

Rate of recovery

(Weight of product/ Weight of drug and polymer) × 100

Physical Characterization of Nanoparticles

The formulations which showed higher encapsulation efficiencies, drug loading and rate of recovery, smaller particle size, poly dispersity index (PDI) lesser than 0.5, better surface charge (zetapotential) was chosen for morphology assessment and *in vitro* release study. The sizes of the nanoparticles were determined by dynamic laser light scattering (DLS) (Nanoserious, Malvern Instruments, UK).The particle size distribution is reported as PDI. . The zeta potential of the nanoparticles was also obtained by zetasizer (Malvern ZS, UK). The morphology of the nanoparticles was observed by scanning electron microscopy.

In vitro Release Studies

In vitro release of Moxifloxacin from alginate-chitosan nanoparticles was determined using, the release media, simulated lung fluid in order to simulate the condition in the lungs. Freeze-dried formulations were taken in the dialysis bag (molecular weight cit off 1000-12000 Da). The

dialysis bag is suspended in 100 ml of the dissolution medium on magnetic stirrer at 37±0.5 C at 100 rpm and the amount of nanoparticles was varied in order to keep constant the amount of drug. A measure of 1 ml samples were withdrawn at appropriate time intervals. Same volume of dissolution medium was replaced to maintain a constant volume. The withdrawn samples were diluted suitably with SLF and absorbance of the resulting solution was measured at 289 nm by UV spectrophotometer. The cumulative amount of drugs was obtained from the calibration curve of Moxifloxacin in Simulated Lung Fluid (SLF) [11]. The test was done in triplicate.

Stability Study

The stability studies were carried using the optimized batch MF3 as per the ICH guidelines. The stability of drug loaded nanoparticles was evaluated in terms of its drug Loading**,** entrapment efficiency, percentage of drug released etc (Aterman, 2007).

RESULT AND DISCUSSION

Moxifloxacin nanoparticles with varying proportions of Moxifloxacin and alginate-chitosan polymer were prepared by ionic gelation method.. The particle size of nanoparticles varied between 105.2 ±8 to 315±4 nm among the different formulations. The results of physiochemical characterization revealed that as the concentration of drug increases Encapsulation Efficiency decreases. The drug loading increases with increase in concentration of drug but after attaining optimum amount (50 mg), it decreases on further increasing the concentration. Maximum drug loading and encapsulation was found in formulation (MF3) having drug concentration of 50 mg. This is due to fact that drug loading is limited and increasing drug concentration led to higher amount of free drug and lower EE. Zeta potential of optimized formulation (MF3) was found to be +36.2 mV. The rate of recovery was highest (76.1±3.2%) in formulation MF 3 shown in the table 2. . The *in-vitro* release profile of all formulation is shown in figure. 2. Among the three formulations, formulation MF3 had a pronounced sustained release of 67.93± 0.1 over a period of four days with burst release in the first four hr was equivalent to 34.67±0.6%. SEM photographs are shown in figure 3.The particles shape was found to be fairly spherical structure without aggregation. The optimized formulation was subjected to stability studies at a room temperature and 40 °C / 75% RH. The optimized formulation was evaluated for zeta potential, particle size and percent drug release. Negligible changes were seen in different physicochemical parameters at a room temperature as well as 40°C/ 75 % RH. There was no significance difference in *in-vitro* release after three-month stability study at both room temperature and accelerated conditions (Table 3).

Table: 2 Physiochemical characterization of Moxifloxacin Nanoparticles

Formulation	Encapsulation Efficiency %	Drug Loading %	Rate of recovery %	Particle Size (nm)	PDI	Zeta Potential (mV)
MF1	86.5±1.3	50.6±3.2	52.4±2.4	256±4.3	0.52	+30
MF2	84.2±2.1	62.4±4.3	76.1±4.2	105.2±3.5	0.42	+36.2
MF3	74.2±3.2	65.2±5.2	70.2±3.2	315±5	0.56	+28

Figure:1 Effect of concentration on Encapsulation Efficiency and Drug Loading

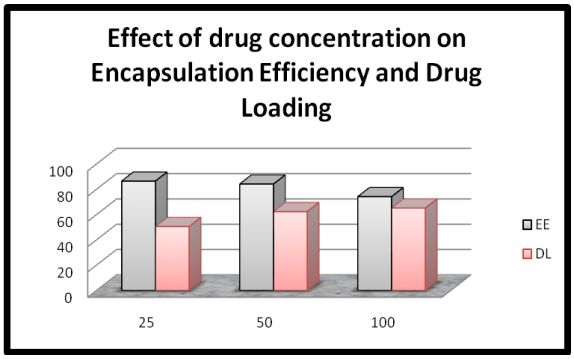

Figure 2 : *In vitro* release profile of optimized formulation(MF3)

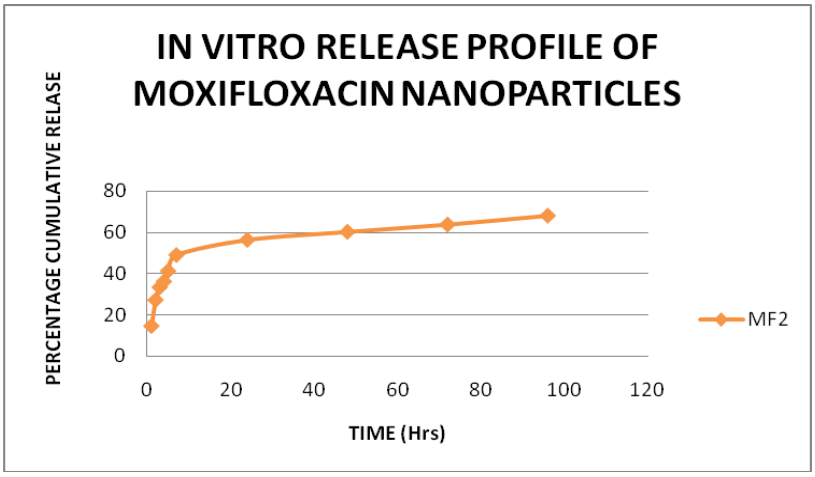

Figure 3:Morphology of Moxifloxacin loaded Nanoparticles

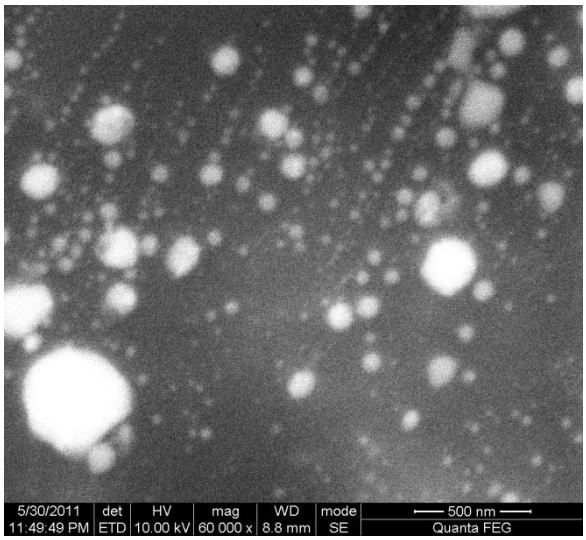

Table 3: Stability studies Physiochemical Characterization of Moxifloxacin Nanoparticles.

Formulation	Encapsulation Efficiency %	Drug Loading %	Rate of recovery %	Particle Size (nm)	PDI	Zeta Potential (mV)	*In vitro releease at 96 hrs (%)*
MF2	82.2±2.3	60.2±1.3	74.1±3.2	108.2±3.5	0.45	+34.4	70.56±2.4

CONCLUSION

Thus from the whole research work, it can be concluded that the objective of proposed research work has been fulfilled and polymeric nanoparticles of Moxifloxacin has been prepared using alginate chitosan- polymer. Formulation MF3 found to have spherical shape, encapsulation efficiency of 84.2±2.1, drug loading of 62.4±4.3, and *in vitro* release of 67.93± 0.1 at the end of 96 hrs. On the bases of experimental data this formulation was considered as optimized formulation.

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