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Formulation and characterization of clarithromycin based nanoparticulate drug delivery system

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Abstract

The aim of the present work is to prepare and evaluate the oral mucoadhesive sustained release nanoparticles of clarithromycin in order to improve its therapeutic effect and reducing dosing frequency and its dose related side effects. Clarithromycin containing chitosan nanoparticles were prepared by ionotropic gelation method. The result showed that this method is reproducible easy and led to the efficient entrapment. Formulation had spherical particles in the particle range from 100 - 1500nm. Some process variables like effect of chitosan concentration, TPP concentration, acetic acid concentration were also evaluated with respect to drug content and encapsulation efficiency. The maximum encapsulation efficiency and drug content were 67.43 % and 6.13. The sustained release behavior of chitosan nanoparticles were evaluated both in phosphate buffer saline and simulated gastric fluid and results revealed that clarithromycin loaded chitosan nanoparticles are most suitable mode of delivery of drug for promising therapeutic action.

Key-Words: Clarithromycin, Chitosan nanoparticles, Mucoadhesion.

Introduction

Clarithromycin is a broad spectrum macrolide antibacterial agent that is effective both *in vitro and in vivo* against major pathogens responsible for pepticulcer by *Helicobacter pylori* and other respiratory tract infections by Clamydia Pneumoniae, and Mycoplasma Pneumomiae. Normal dosage regimen of drug varies from 250-500 mg, and it can be administered twice or thrice a day depending on severity of infection. In severe cases long term therapy may also be required. As biological half life of drug is 3-4 hrs and that is why frequent dosing is require. To overcome with these problems, mucoadhesive nanoparticles of clarithromycin were formulated.[1] Chitosan is a widely used mucoadhesive polymer. It is a multivalent cationic hydrophilic polysaccharide comprising copolymers of glycosamine and N-acetyl glucosamine and can be derived by partial deacetylation of chitin from crustacean shells. This mucoadhesive polymer in nanoparticles formulation prolongs the residence time of dosage form in the gastro intestinal tract and hence more suitable as matrix material for oral controlled release.

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The present research paper describes the preparation and characterization of nanoparticulate drug delivery system of clarithromycin for *Helicobacter pylori* infection.[2-4]

Material and Methods

Clarithromycin was procured as a gift sample from Ind. Swift Labs. Chandigarh (India). Chitosan (purified viscosity grade 80 cps, molecular weight 150 kDa, deacetylation degree 85% was obtained as a gift sample from Central institute of fisheries technology, Cochin. Sodium tripolyphosphate and dialysis membrane (MWCO1400) was purchased from Himedia, Mumbai, India. All other chemicals used were of analytical grade. Distilled water was used throughout the study.

Preparation of Chitosan – Tpp Nanoparticles

Chitosan nanoparticles were prepared with suitable modifications based on the ionotropic gelation with TPP anions. Chitosan (2mg/ml) was dissolved in aqueous acetic acid (1%) solution and TPP was dissolved in distilled water at the concentration of 1mg/ml. Drug (10%) was added to TPP solution (previously dissolved in 100 ml distilled water under magnetic stirring. Finally 1.5 ml of drug containing TPP solution was added to 4ml of chitosan solution through a syringe needle under magnetic stirring at room temperature. The dispersion so formed was sonicated for 15 minutes, then disperse in water and

centrifuge the sample at around 10000 rpm for 30 minutes. The supernatant was discarded and sediment was dissolved in distilled water and lyophilized and used for further characterization.[3] Effect of formulation variables on the particle size are given in (table-1)

Particle size, surface morphology

The nanoparticles were characterized for their size, shape and morphology by Transmission electron microscopy (Joel, Jem1400). A drop of sample solution was placed on to a 300 mesh copper grid 2% w/v uranyl acetate for 10s and excess of solution was drained off with a filter paper to remove excess surface water and air dried and viewed under transmission electron microscope. Particle size, zeta potential and polydispersity index of drug loaded nanoparticles were assessed by photon correlation spectroscopy using zetasizer (Zetasizer 3000HS, Malvern, UK) [5]

Percent encapsulation efficiency

The entrapment efficiency of clarithromycin in nanoparticulate formulation was determined by using following equation.

$$EF\ (\%) = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

About 10 ml of nanoparticulate suspension was digested with minimum amount of ethanolic solution (water: ethanol in 7:3 ratios). The digested homogenates were centrifuged at 15000 rpm for 30 min and supernatant was analysed for drug entrapment. The clarithromycin entrapment was measured at 286 nm using Shimadzu 1601 UV/VIS spectrophotometer.[8]

Fourier transforms infrared spectroscopy

FTIR spectral measurement was performed to assess the coupling of chitosan to drug loaded nanoparticles.[3]

Differential scanning calorimetry studies

DSC study was performed in order to characterize the physical state of clarithromycin in nanoparticles. Thermatograms were obtained using a DSC (Shimadzu, Kyoto). About 5mg of sample was weighed, crimped into an aluminum pan and analysed at a scanning temperature range from 50-300ºC at the heating rate of 10^0 C/min. Baseline was performed before every run.[3, 9]

***In vitro* release study**

In vitro release of clarithromycin was carried out with slight modification .The scheme of using the simulated fluids at different pH was as follows:

The drug release studies were conducted in simulated gastric fluids pH 1.2 and phosphate buffer pH 7.4. Simulated gastric fluid (2gm sodium chloride, 7ml of conc. HCL, 3.2 gm pepsin in 1000 ml distilled water) pH was adjusted to 1.2. Phosphate buffer pH 7.4 was prepared by dissolving (2.38 gm of disodium hydrogen phosphate. 0.19 gm of potassium dihydrogen phosphate and 8 gm of sodium chloride in 1000 ml distilled water).[10-12]

In vitro drug release from the nanoparticle formulation bearing drug was studied using dialysis bag (MWCO 14000 with a pore size of 2.4mm) was placed in a beaker containing 100 ml of simulated fluids at 37 ºC with slow magnetic stirring under perfect sink conditions and fluids were changed according to (Souder and Ellenbogen[3]) scheme at each time point samples (5ml of aliquots) were withdrawn periodically and replaced with the same volume off fresh dissolution media and the amount of drug was quantified spectrophotometrically (UV117)at 286 nm.

Results and Conclusion

Clarithromycin loaded chitosan nanoparticles were prepared by ionotropic gelation method after selection and optimization of various process variables bioactive polymer concentration.

Smaller particles have higher surface area/volume ratio, which makes it easier for the encapsulated drug to be released from the Nanoparticles via diffusion and surface erosion and also have the added advantage for the drug loaded nanoparticles to penetrate into, and permeate through the physiological drug barriers. It is reported in the literature that smaller Nanoparticles will have greater ease of entry and durability in the gastric mucosa. Particle size of Chitosan Nanoparticles is13 ± 0.06 (µg/mg). The polymer concentration was varied from 20mg given in (fig.1). TEM was performed to investigate the morphology of nanoparticles. Clarithromycin loaded chitosan nanoparticles displayed a spherical shape uniform with a smooth surface and no aggregation was observed. No difference was observed in the morphological properties of nanoparticles due to presence of the drug given in fig. 2. Particle size of optimized formulation was 100 nm given in fig.3. The percentage encapsulation efficiency of prepared nanoparticles was found to be 67.44, with a drug content of 6/10ml-40mg/20mlwhile keeping other processing parameters at constant conditions. On increasing the polymer concentration leads to a gradual increase in nanoparticle diameter. It was observed that increase in polymer concentration leads to an increased in the viscous forces resisting droplet break down by stirring and leads to increase in particle size. The viscous force opposes the shear stress and final size and size distribution of particles depends on the net shear stress available for droplet breakdown. The increase in polymer viscosity increases the diffusional resistance of the drug molecules thereby entrapping more drugs. Also the time required for polymer precipitation decreases at higher polymer concentration, so there is less time for drug molecules

to diffuse out of nanoparticles. Effect of polymer concentration is given in (table 1).

The acetic acid concentration was varied from 0.5-2.0% w/v keeping other processing parameters at standard conditions. As the acetic acid concentration increases the chitosan solution was not able to pass through the syringe needle, because of too viscosity. When acetic acid concentration was 0.5%, flake formation occured hence 1% w/v acetic acid solution was suitable for preparing optimized preparation. No particles were obtained in 2% acetic acid concentration because of the precipitation of polymer.

The speed of magnetic stirrer was varied from 1000rpm to 4500 rpm while keeping the other processing parameters at standard conditions. On increasing the speed of magnetic stirrer decrease in particle size and diameter was observed. These trends occur because the eternal energy and thus the shear stress causing droplet breakdown is increased with increasing the stirring speed.

The TPP concentration was varied from 1mg/ml to 3mg /ml while keeping other processing parameters at standard conditions. As the TPP concentration was increased, the particle size of particles was also increased due to the increased viscosity of the aqueous phase; the viscosity increases reduces the net shear stress available for droplet breakdown by stirrer.

The *in vitro* drug release study of prepared nanoparticles was performed in simulated gastric fluid pH 1.2 and phosphate buffer pH 7.4 saline. The cumulative % drug release was shown in fig.4 and 5 mentioned below. The study was carried in triplicate in simulated gastric fluid (pH 1.2) and phosphate buffer saline (pH 7.4).

DSC is very useful in the investigation of the thermal properties of drug delivery carriers, providing both qualitative and quantitative information about the physiochemical state of drug inside the delivery system There in no detectable endotherm if the drug is present in the a molecular dispersion or solid solution state in the polymeric systems loaded with drug in the present investigation. The DSC study of clarithromycin and chitosan is shown in the fig. no. 6, 7& 8 .It was shown that chitosan showing a characteristic peak at indicating that two peaks occurred due to enhanced hydrogen bonding. Chitosan shows a peak at 280 ºC indicates its amorphous nature.

Present work summaries the latest progress in nanoparticle technology and describes the systematic study of preparation, optimization and characterization of clarithromycin loaded nanoparticles with emphasis mainly on particle size and drug content. By varying the formulation parameters the particle size, drug content and other properties could be changed to the requirement of drug delivery system. The observation from present work shows that ionotropic gelation can be used for entrapment. The ionotropic gelation method was found to be a suitable method for the formation of nanoparticles with a mean size of 100 nm.

Different polymer concentrations were investigated to prepare nanoparticles. On increasing the polymer concentration the actual drug content, encapsulation efficiency initially increased and then decreased with gradual increase in particle size. Hence NP4 was selected as the optimized nanoparticulate formulation. The effect of TPP concentration on optimized formulation showed that the particle size was minimum with 1mg/ml of TPP concentration. Also, 1% acetic acid was suitable concentration for preparation of nanoparticles because of ease of solubility of chitosan in acidic solution. It was also found that on increasing the speed of magnetic stirrer decreased the mean diameter and drug content of nanoparticles.

It was concluded that due to process variables the optimized formulation showed the particle size 100 nm, polydispersity index 0.839. Zeta potential -13mV, drug content 6.13 and encapsulation efficiency 67.43%.TEM images showed that the nanoparticles had spherical shape and smooth surface with small particle size without any rough pores. Based on above results it can be concluded that the prepared nanoparticulate drug delivery system of clarithromycin may be considered as a promising delivery system of drug in effective way.

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Table 1: Effect of Formulation Variables on the Particle Size

Batch Code	Polymer conc. %)	Acetic acid conc. (%)	Speed of magnetic stirrer	TPP conc.	Particle size
NP1	20%	0.5%	2000/rpm	1	1463 nm
NP2	30%	0.5%	2000/rpm	1	566 nm
NP3	40%	0.5%	2000/rpm	1	1463 nm
NP4	20%	1%	4500/rpm	1	100 nm
NP5	30%	1%	4500/rpm	2	143.94 nm
NP6	40%	1%	4500/rpm	2	297.06 nm
NP7	20%	2%	1000/rpm	3	216.98 nm
NP8	30%	2%	1000/rpm	3	particles were not found
NP9	40%	2%	1000/rpm	3	particles were not found

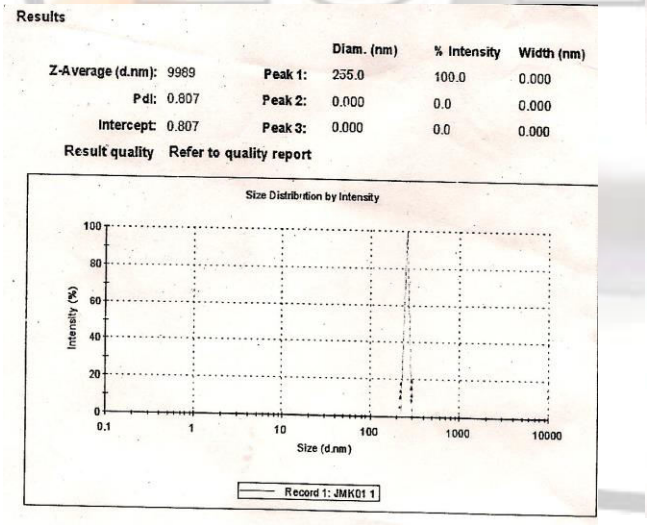

Fig. 1: Particle size of optimized nanoparticles

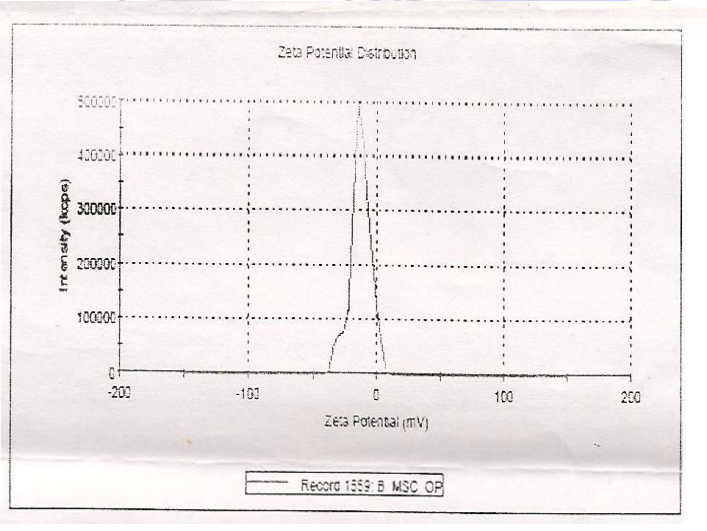

Fig. 2: Zeta potential of optimized nanoparticles

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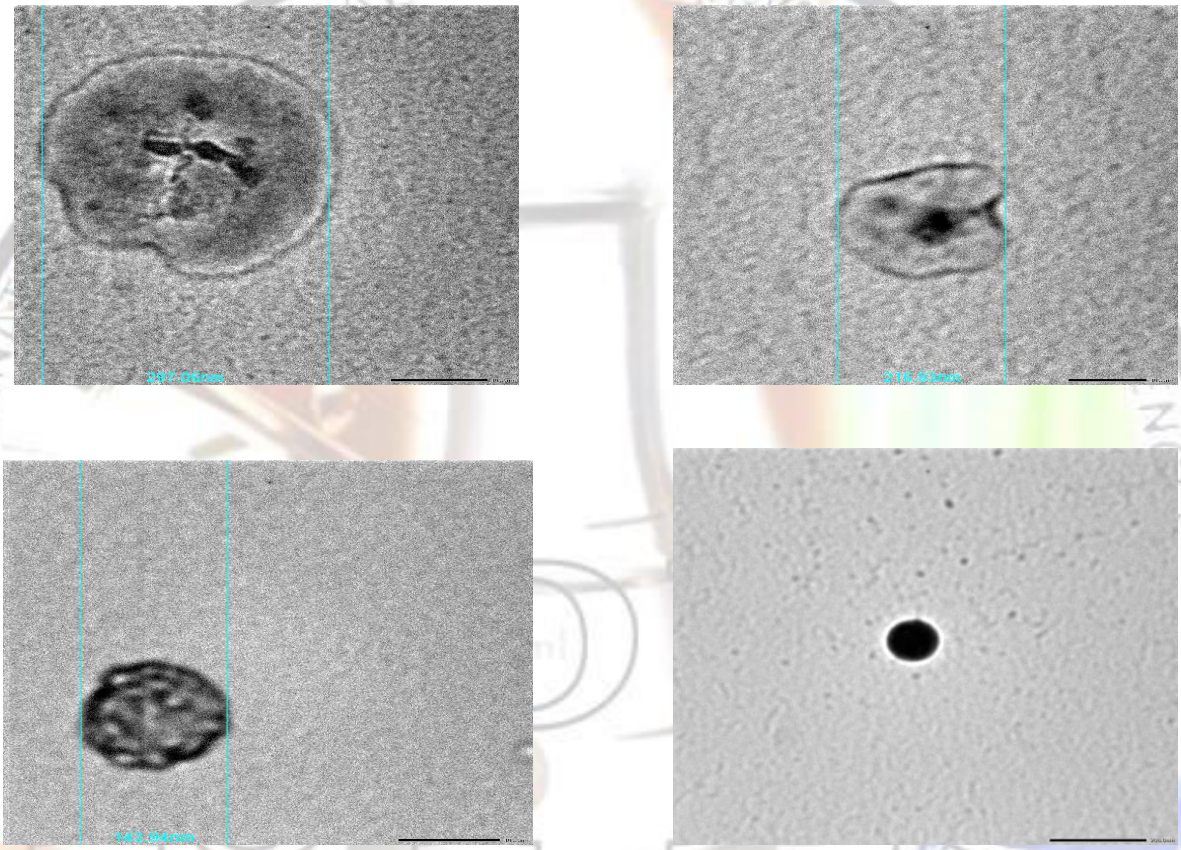

Fig. 3: TEM images of chitosan nanoparticles

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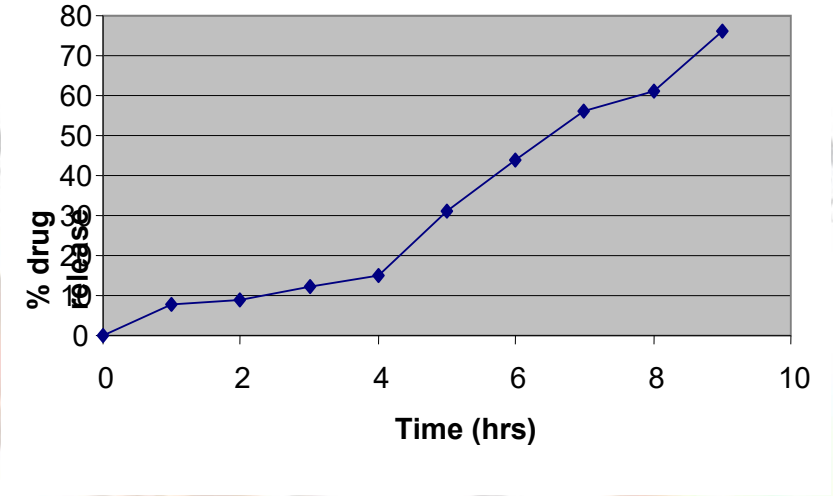

Fig. 5 - in-*vitro* % Cumulative drug release in PBS (pH 7.4)

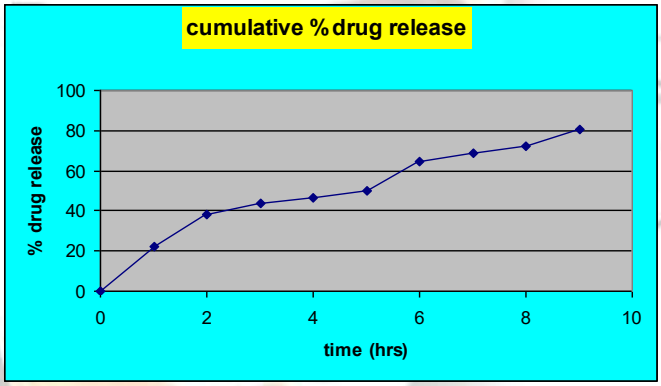

Fig. 4: *In-vitro* % Cumulative drug release in SGF (pH 1.2)

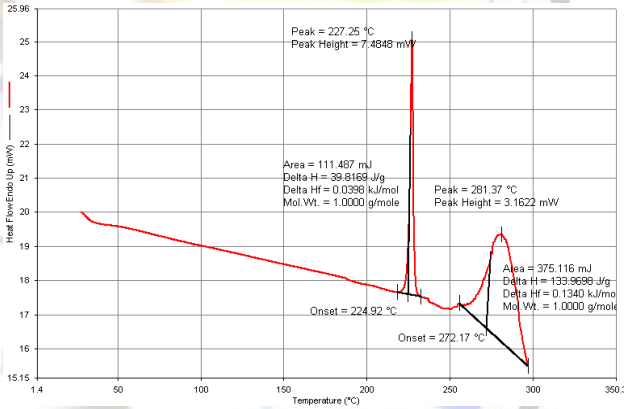

Fig. 6 - DSC of clarithromycin

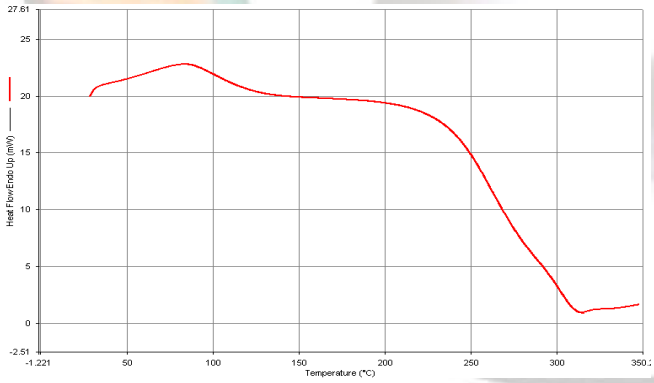

Fig. 7 - DSC of chitosan

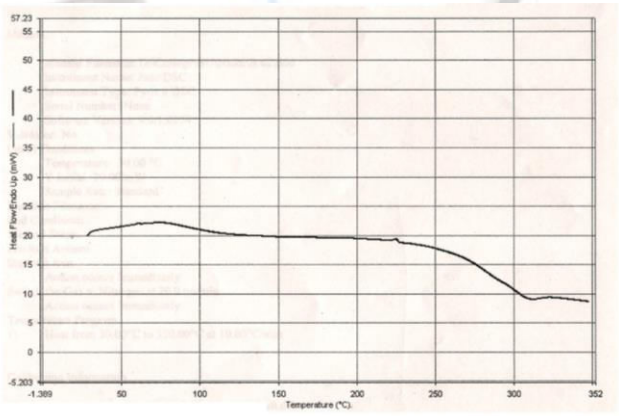

Fig. 8 - DSC of chitosan nanoparticles