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Research Article

APPLICATION OF TAGUCHI ORTHOGONAL ARRAY DESIGN FOR OPTIMIZATION OF CHITOSAN NANOPARTICLES OF HYDROPHOBIC CARDIOVASCULAR DRUGS

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ABSTRACT

Nanotechnology based drug delivery system have shown to improve solubility, rate of dissolution and oral bioavailability of poorly water soluble drugs. Present study is aimed to develop Nebivolol loaded chitosan nanoparticles (NB-CS-NPS) for enhancing its oral bioavailability. The NPs optimized by implementation of Taguchi orthogonal array design. The optimized batch (NB-CS-NPS-1) of NPs exhibited average particle size of 91 ± 45 nm, entrapment efficiency of 70.98% and with zeta potential of $+36.8\text{mV} \pm 2\text{mV}$ and stability at $25 \pm 2^\circ\text{C}/60 \pm 5\%$ RH. The *in vitro* release data studies have shown improved solubility and oral bioavailability of Nebivolol.

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INTRODUCTION:

Poor aqueous solubility of the pharmaceutical entity is one of the major limitations in successful oral drug delivery. Among various approaches available to improve problems related to poor solubility of drugs, nanotechnology based drug delivery system offers advantages to overcome the problems associated with the oral delivery of these drugs. Nanoparticles exhibit increased surface area which results in increased dissolution rate, enable to control pharmacokinetic properties of a dosage form, ease of administration and readily penetrate through capillary and epithelial membrane, which permits an effective oral delivery of poorly soluble drugs. The present study is aimed to develop Chitosan nanoparticles containing poorly soluble antihypertensive drug, Nebivolol, in order to improve its solubility and oral bioavailability.

MATERIAL AND METHODS:

Material

Chitosan (CS) was obtained as gift sample from Central Institute of Fisheries Technology (Cochin, India). Sodium tripolyphosphate (TPP) was procured from Loba Chemie Pvt. Ltd. (Mumbai, India). Nebivolol (NEB) was obtained as a gift sample from Lupin Ltd. (Pitahmpur, Indore, India). All other chemicals and reagents were of analytical grade.

Methods

The NB-loaded NPs were fabricated according to the procedure reported. Chitosan solutions of different concentrations were prepared by dissolving chitosan in 1% aqueous acetic acid solution. Tween 80 (2% v/v) was added as a surfactant to it under constant stirring at room temperature. Subsequently, drug (2.5%) was dissolved in dichloromethane (2.5 mL), and then this oil phase was added drop wise to the aqueous phase. This addition was accompanied by continuous stirring for 5 minutes at different speeds using high speed homogenizer. Finally, 10ml TPP solution of different concentration was added drop wise into o/w emulsion to induce cross-linking of the particles under magnetic stirring at 500 rpm. The stirring was continued to ensure complete evaporation of dichloromethane, it was kept overnight at 40°C. Nanoparticles were collected by centrifugation at 15,000 rpm for 25 minutes at 20°C using cooling centrifuge. The supernatant was subjected for the determination of presence of free Nebivolol by UV spectrophotometer (UV 1700, Shimadzu, Japan).

Optimization of nanoparticles By Taguchi orthogonal array design

The optimization of the NPs formulation was carried out by using Taguchi orthogonal array design. Based on the number of factors and their levels, L_9 (3^4) orthogonal

array was employed. Four factors i.e. polymer concentration (%w/v), TPP concentration (%w/v), CS: TPP ratio (%v/v) and stirring speed (rpm) were selected and assigned three levels i.e. low, medium and high. The optimum conditions with optimal desirability were determined with the minimum possible effect of the noise factor.

Characterization of nanoparticles

Transmission electron microscopy (TEM)

The morphology of nanoparticles was observed under transmission electron microscopy (Morgagni 268D TEM instrument, AIIMS, New Delhi). The diluted and filtered sample was plunged on the 200 mesh carbon coated copper grids and was allowed to dry completely in the air. After drying, sample grid was loaded onto a specimen holder and viewed under a transmission electron microscope.

Drug-excipient compatibility studies by differential scanning calorimeter (DSC)

The nanoparticles and drug powder were subjected to previously calibrated differential scanning calorimeter (DSC-60, Shimadzu Corporation, Japan). The sample was sealed hermetically in an aluminum pan and subjected to nitrogen gas at a flow rate of 50 ml/min. The thermograms were obtained at scanning temperature range of 50-250°C at a heating rate of 10°C/min. DSC thermograms were recorded for CS, NEB and NEB-CS NPs.

Measurement of particle size, polydispersity index (PDI) and zeta potential (ZP) of nanoparticles

Particle size, PDI and ZP of nanoparticles were determined through Dynamic light scattering (DLS) analysis with Malvern Zetasizer Nano S (Malvern, UK). About 100 µL of the prepared nanoparticles dispersion was diluted to 5ml with double distilled water and analyzed with zetasizer. The analysis was performed in triplicate at a temperature of 25 °C.

Determination of entrapment efficiency

The entrapment efficiency of the nanoparticulate formulation was determined in triplicate using *ultra-violet spectrophotometer*. The nanoparticles were separated from the aqueous medium (containing untrapped NEB) by centrifugation at 25000 rpm for 30 min (REMI CPR-24 Plus, Remi Elektrotechnik, India). The supernatant was diluted with an appropriate amount of 0.1 N HCl and analyzed for the amount of untrapped drug by UV-Visible spectrophotometer (Shimadzu 1700, Japan) at 285 nm.

The percentage drug encapsulated was determined by following the formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total drug} - \text{free drug}}{\text{Total drug}} \times 100$$

In vitro drug release studies

The *in-vitro* drug release of nanoparticles was studied by using dialysis membrane (Himedia, India) with a pore size of 2.4nm and molecular weight cut-off between 12,000–14,000 in phosphate buffer saline

(PBS) pH 7.4 at $37 \pm 2^\circ\text{C}$. Dialysis membrane was soaked overnight in double distilled water prior to the release studies. The drug-loaded nanoparticles were placed into a dialysis bag and were suspended in a beaker containing PBS under magnetic stirring while maintaining perfect sink condition. Aliquot samples were withdrawn periodically and replaced with fresh dissolution medium in the same volume. The amount of drug released was analyzed spectrophotometrically at 285 nm for NEB. For comparative purpose, the *In vitro* drug release study was also performed for the marketed formulation using USP paddle type dissolution apparatus.

Accelerated stability studies

Nebivolol loaded nanoparticles were subjected to a stability testing for three months as per International Conference on Harmonisation (ICH) Q1A (R 2) guidelines. Freshly prepared nanoparticles were transferred to 5 ml glass vials sealed with plastic caps and were kept in stability chamber (Remi SC-12 Plus, Remi Instruments, Ltd. Mumbai, India) maintained at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{RH}$ for a period of total 3 months. The formulations were monitored for changes in particle size, zeta potential and entrapment efficiency.

RESULT AND DISCUSSION:

Particle size analysis by transmission electron microscopy (TEM)

The structural morphology of nanoparticles was examined by TEM. TEM image showed that the optimized formulation is nearly spherical in shape and a smooth surface distributed throughout the sample (Figure 1).

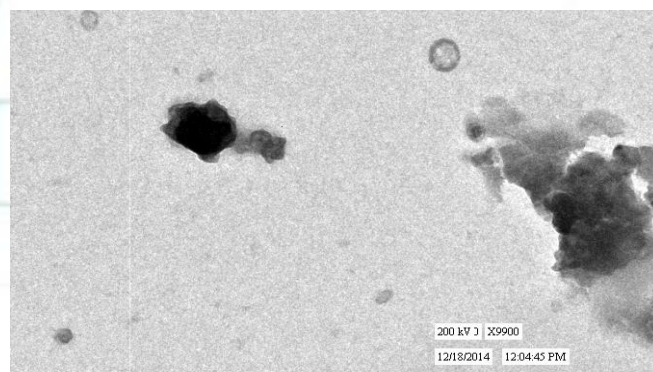


Figure 1: Drug-excipient compatibility studies

The pure drug, Nebivolol, showed a sharp endothermic peak at 221°C corresponding to its melting temperature. Chitosan showed broad endothermic peaks at 102°C corresponding to its glass transition temperature. NEB-CS NPs showed both broad and sharp endothermic peaks at 102°C and 221°C which corresponds to chitosan and Nebivolol respectively which predicts that the drug is homogeneously dispersed in polymer matrix.

Particle size, poly dispersity index (PDI) and zeta potential of nanoparticles

The average particle size of the optimized batch (NB-CS-NPS-1) of nanoparticles was found to be 91 ± 45 nm. Particle size along with zeta potential (ζ) is the

critical factor that affects the biological performance of chitosan nanoparticles. The zeta potential of NB-CS-NPs were found to be $+36.8\text{mV} \pm 2\text{mV}$, which indicate the physical stability of the formulation. The zeta potential also tends to affect particle stability and mucoadhesivity.

Entrapment efficiency

The entrapment efficiency acts as an important factor influencing the drug release, as well as the overall efficacy of the formulation. All the formulations were analyzed for entrapment efficiency by using UV-Visible spectrophotometer (*Shimadzu 1700, Japan*) at 285 nm and. The entrapment efficiency of the optimized batch (NB-CS-NPS-1) of nanoparticles was found to be 70.98.

In vitro drug release

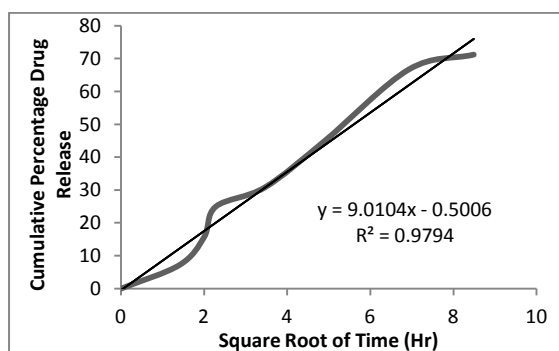


Figure 2: *In vitro* drug release

The *In vitro* drug release studies were carried out for NEB-CS NPs and marketed formulation in PBS 7.4 at $37 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$. The drug release profile of NB-CS-NPs showed biphasic release pattern with an initial burst release in the first 2 h followed by a controlled release over a period of 72 hours and cumulative percentage of drug released was obtained to be 71.24 %.

Accelerated stability studies

Stability studies were conducted in triplicate for optimized formulation which showed slight variations in particle size, zeta potential, and drug entrapment during 3 months of storage. The obtained results indicated no significant change in the particle size, zeta potential, and drug entrapment during 3 months of storage that ensured the stability of nanoparticles.

CONCLUSION:

The major challenge in the formulation development is the poor aqueous solubility of the new chemical entity or existing drug molecules. The formulation of these molecules by the application of conventional approaches is difficult and associated with several pharmacological or therapeutical performance issues. The nanoparticles provide a promising approach for enhancing solubility and oral bioavailability of water insoluble drugs. In conclusion, formulation of chitosan nanoparticles could be an effective strategy for enhancing oral bioavailability of nebigolol and other lipophilic drugs upon further *in vivo* pharmacokinetics and pharmacodynamics studies.

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