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Formulation development and characterization of Simvastatin loaded nanoparticles

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ABSTRACT: The present study was to formulate nanoparticles (NPs) containing simvastatin (SV) prepared with Poly (D, L Lactide-co-Glycolide) by nano-precipitation-solvent displacement method to achieve a better release profile suitable for per oral administration with enhanced efficacy. The formulations were fabricated according to a 32 full factorial design, allowing the simultaneous evaluation of two formulation independent variables and their interaction. The dependent variables that were selected for study were particle size and % drug entrapment. The influence of various formulation factors (drug: polymer ratio and concentration of surfactants) on particle size, size distribution, zeta potential, drug loading and encapsulation efficiency were investigated. Encapsulation efficiency and drug loading capacity were found to be increased as drug concentration increases with respect to polymer. Addition of surfactants showed a promising result in decreasing particle size of NPs. Dissolution study revealed increased release of SV from NPs.

KEYWORDS: Formulation, Characterization, Nanoparticles

INTRODUCTION

For decades, various pharmaceutical dosage forms such as tablets, capsules, liquids, suppositories, creams, ointments, injections, aerosols, etc. have been used as drug delivery systems for treatments of acute and chronic diseases. Colloidal drug delivery systems namely oil-in-water emulsions, liposomes, micelles, micro particles and nanoparticles opened a new frontier for targeting drugs and pharmaceuticals. Nanoparticles are solid colloidal particles in which the active principles are dissolved, entrapped, and or to which the active principle is adsorbed or attached. Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles. The colloidal carriers based on biodegradable and biocompatible polymeric systems have largely influenced the controlled and targeted drug delivery concepts. Nanoparticles are sub-nanosized colloidal structures composed of synthetic or semi synthetic polymers. Nanotechnology represents an important technological revolution in 21st century. Nanotechnology is the study of nanoparticles, nanosuspension, nanoemulsion etc. Nanoparticle is a collective name for nanospheres and nanocapsule. Nanospheres have a matrix type structure. Drugs may be adsorbed at their surface, entrapped in the particle or dissolved in it.

PREPARATION OF NANOPARTICLE USING POLYMER PRECIPITATION METHOD:

The hydrophobic polymer and hydrophobic drug is dissolved in a particular organic solvent followed by its dispersion in a continuous aqueous phase, in which the polymer is insoluble. Precipitation of the polymer produces nanoparticles with drug loaded in it. The external phase also contains the stabilizer. Depending upon solvent miscibility techniques they are designated as solvent/evaporation method.

Polymer precipitation can be brought out by increasing the solubility of the organic solvent in the external medium by adding an alcohol

By incorporating additional amount of water into the ultra-emulsion

By evaporation of organic solvent at room temperature or at accelerated temperatures or by using vacuum. Using an organic solvent is completely soluble in the continuous aqueous phase – nanoprecipitation.

EVALUATION OF NANOPARTICLES

The prepared nanoparticles are evaluated for various parameters they are:

1. Yield
2. Drug loading
3. Entrapment efficiency
4. Size and morphology
5. In vitro drug release studies
6. Stability testing

METHODOLOGY

POLYMER PROFILE

Bovine serum albumin

Bovine serum albumin (BSA) is a serum albumin protein derived from cows. It is often used as a protein concentration standard in lab experiments. The nickname "Fraction V" refers to albumin being the fifth fraction of the original Edwin Cohn purification methodology that made use of differential solubility characteristics of plasma proteins. By manipulating solvent concentrations, pH, salt levels, and temperature, Cohn was able to pull out successive "fractions" of blood plasma. The process was first commercialized with human albumin for medical use and later adopted for production of BSA.

Properties

The full-length BSA precursor polypeptide is 607 amino acids (AAs) in length. An N- terminal 18-residue signal peptide is cut off from the precursor protein upon secretion; hence the initial protein product contains 589 amino acid residues. An additional six amino acids are cleaved to yield the mature BSA protein that contains 583 amino acids. BSA has three homologous but structurally different domains. The domains, named I, II, and III, are broken down into two sub-domains, A and B.

EXPERIMENTAL INVESTIGATIONS:

CONSTRUCTION OF STANDARD CURVE FOR SIMVASTATIN

By UV Spectroscopy Method

Simvastatin is estimated spectrophotometrically at 238 nm and it obeys Beer- Lambert's Law in the range of 2 – 20 □g/ml.

Determination of Absorbance maximum (λmax)

Simvastatin was dissolved in phosphate buffer saline pH 6.8. Solution with 20 µg/ml concentration was prepared by suitable dilution. The solution was scanned in UV spectrophotometer at 200 to 400 nm using phosphate buffer saline pH 6.8 as blank. Absorbance maximum was determined as 238 nm.

Preparation of Calibration curve

From the stock solution (1 mg/ml) aliquots concentration ranges from 2 to 20 µg per ml. Absorbance of the solution was measured at 238 nm UV spectrophotometrically against drug free PBS pH 6.8 media as blank

PREFORMULATION STUDY

DRUG AND POLYMER COMPATABLITY STUDY BY FTIR

Infrared spectrum of any compound or drug gives information about the groups present in that particular compound. The IR absorption spectra of the pure drug and physical admixtures of drug with various excipients were taken in the range of 4000-400 cm-1 using KBr disc method (Schimadzu IR- Prestige-21) and observed for characteristic peaks of drug.

S.No	Wave number cm-1	Wave range cm-1	Assignment
1	1633.81	1690 - 1630	C=N stretching
2	1579.16	1650 - 1550	C=C stretching
3	3372.79	3400 - 3250	CH3 angular

METHOD OF PREPARATION OF SIMAVASTATIN NANOPARTICLES:

Ionic Gelation Method

Simvastatin (SS) nanoparticles were synthesized using the ion-gelation technique. To begin, specific concentrations of the drug and an aqueous solution of Sodium tripolyphosphate (STP) were prepared. This STP solution was then carefully introduced, drop by drop, into a polymer solution that had been dissolved in 1% acetic acid (v/v) while being stirred at a speed of 400 to 600 rpm. Various drug-to-polymer ratios were tested to identify the formulation with the most desirable characteristics.

EVALUATION OF NANOPARTICLES DRUG ENTRAPMENT STUDY

The entrapment efficiency study was determined by free drug content in the supernatant which is obtained after centrifuging the solid lipid suspension at (15,000rpm for 20 min at zero degree using ultra centrifuge) The absorbance was measured at 238 nm by UV spectrophotometrically.

IN-VITRO DRUG RELEASE STUDIES

The in vitro drug release study was carried out by using the diffusion membrane technique. The nanoparticles preparation

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was placed in a dialysis membrane and it is dropped in to a beaker containing 200 ml of diffusion medium (phosphate buffer saline pH 6.8) the medium was maintained at 370 C under magnetic stirring at constant speed. At fixed time interval 1ml of sample was taken from the diffusion medium for every 1 hour and it was replaced by 1ml fresh medium. This process was carried out for 24 hours. The sample was measured UV spectrophotometrically at 238 nm. The percentage of drug released at various time intervals was calculated from calibration graph.

MORPOLOGY OF NANOPARTICLES BY SIMPLE MICROSCOPY

The optimized formulation was morphologically characterized by microscopy. The small amount sample was placed in a glass slide and investigated in microscopy.

SCANNING ELECTRON MICROSCOPY

The optimized formulation was morphologically characterized by scanning electron microscopy (SEM). The sample for SEM analysis was mounted in the specimen using an adhesive small sample was mounted directly in scotsch double adhesive tape. The sample was analyzed in Hitachi scanning electron microscope operated at 15 kv and photograph was taken.

SURFACE CHARGE (ZETA POTENTIAL) DETERMINATION

The zeta potential of prepared Nanosuspension was measured using Malvern Zetasizer (Nano ZS90, Malvern instruments) at 25°C. The samples were measured by zeta dip cell kept in polystyrene cuvette.

PROCEDURE:

Vials containing Simvastatin-loaded nanoparticles were carefully opened under aseptic conditions provided by a laminar airflow workstation. Every precaution was taken to prevent contamination, both by the process and by the analyst. The drug solution was filtered through a sterile membrane mounted on a membrane holder assembly. After filtration, the membrane was rinsed three the prepared media were then subjected to incubation at different temperatures. The SCDM was incubated at 22.5°C ± 2.5°C, while the FTM was incubated at 32.5°C ± 2.5°C. These containers were monitored over a 14-day period for any signs of turbidity or microbial growth. To ensure the sterility testing procedure was valid; both positive and negative control tests were performed. Times with 100 ml of sterile peptone solution (Fluid A). The membrane was then cut in half using sterile scissors. One half was placed into a container with Soybean Casein Digest Medium (SCDM), and the other half into a container with Fluid Thioglycollate Medium (FTM).

RESULTS AND DISCUSSION

DEVELOPMENT OF SIMVASTATIN NANOPARTICLES

In this study, Simvastatin loaded nanoparticles were prepared by Ionic gelation method using BSA and Chitosan, used at the ratios. Formulations with different ratios of polymer were prepared. Several physicochemical characteristics of nanoparticles such as morphology, particle size determination, drug release profile were investigated and stability of optimized formulation at various temperatures was evaluated.

Table: 1: Entrapment efficiency formulations with Drug and polymer:

S.NO	Formulation Code	Entrapment Efficiency (%)
1	FS1	53±0.13
2	FS2	58±0.14
3	FS3	61±0.02
4	FS4	62±0.15
5	FS5	63±0.02
6	FS6	65± 0.14
7	FS7	67±0.15
8	FS8	72±0.13
9	FS9	75±0.02
10	FS10	78±0.05

In formulation (FS1-FS3) BSA polymer concentration (20-40 mg) was increased and the entrapment efficiency was increased from 53 to 61%. Formulation FS4, FS5, FS6, carried out by same process as like first three formulation but changes in polymer concentration 20 mg, 30 mg, 40 mg of Chitosan is taken. The entrapment efficiency was 62% for F4, 63% for F5, 65% for F6.

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Formulation FS7-FS10 was carried out by combination of both polymers and entrapment efficiency was increased in great number FS7- 67% to FS10-78%.

STABILITY STUDIES OF SIMVASTATIN NANOPARTICLES

The stability studies of the optimized nanoparticle formulation FS10 was carried out for 3 months. The test was performed in three condition 40C, room temperature and 450C/70%RH. At the time interval of one month the nanoparticle formulation were evaluated for entrapment efficiency. The stability of nanoparticle formulation was more stable in refrigerator (4°C) when compared to room temperature and at (450C/70%RH).

SUMMARY AND CONCLUSION

The present study Simvastatin nanoparticles aimed to develop a nanoparticulate drug delivery system of antiviral drug Simvastatin using biodegradable polymer SAG and Chitosan.

The polymer enhances the binding of Simvastatin nanoparticles in specific or targeted site with sustained release of drug increasing therapeutic efficacy. These nanoparticles may also reduce the dose & dose frequency with desired therapeutic response. The pre-formulation studies were performed by using FTIR. The spectra of pure drug, pure polymer and nanoparticle formulation were examined. The study revealed the absence of significant interactions between drug and polymer. All batches of nanoparticles (F1-F10) were prepared by ionic gelation method, formulation was subjected to evaluation involving following tests they are; the entrapment efficiency of the optimized formulation (FS10) was 78% and in- vitro drug release was 95.18% after 24 hours. It also obeys the zero order, follows diffusion and erosion mechanism of release. Particle size determination by Scanning Electron Microscope shows the best formulation containing size of about 100 nm. The stability test performed revealed that the formulation was good. The best formulation was examined for zeta potential determinations. The formulation (FS10) showed maximum deviation of -27mV which demonstrated that the particles are separate and highly repelling. This repelling property found to be more useful in decreasing opsonization and favors target specificity.

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