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Nanomedicine & Nanotechnology Open Access
ISSN: 2574-187X

Preparation & Characterization of Bosentan Loaded Ethyl cellulose Nanoparticles by Solvent Evaporation Technique

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Research Article

Volume 3 Issue 2

Received Date: June 12, 2018

Published Date: June 25, 2018

DOI: 10.23880/nnoa-16000141

Abstract

The aim of this study was to prepare Bosentan loaded nanoparticles by Solvent Evaporation (SE) technique. Bosentan belongs to a class of drugs known as endothelin receptor antagonists (ERAs). It is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension (PAH). Ethyl cellulose (EC) was used as a polymer. Bosentan and ethyl cellulose were dissolved in Ethanol at various drug polymer ratios i.e. 1:1, 1:2, 1:3. Among the three formulations 1:3 formulation was considered as the best formulation with better drug content (97.43%) and entrapment efficiency (95.57%).

Keywords: Nanoparticles; Solvent Evaporation; Bosentan Monohydrate; Ethyl cellulose

Introduction

Definition

The word 'Nano' is derived from the Greek word "nanos", which means dwarf or extremely small. A nanometer is a billionth of a meter or 10^{-9} m. Nanoparticles may be defined as particulate dispersions or solid particles within the size range of 10-1000nm.They are composed of polymers and the active ingredients which may be dissolved, entrapped, encapsulated or adsorbed within the polymer matrix. Depending upon the method of preparation, nanoparticles, nanospheres and nanocapsules are obtained.

Materials such as synthetic polymers, proteins or other natural macromolecules are used in the preparation of nanoparticles. Drug nanoparticles have potential applications in the administration of therapeutic molecules such as tissue targeting in cancer therapy, controlled release, carrier action for the delivery of peptides and increase in the solubility of drug [1,2]

Techniques for the Preparation of Polymeric Nanoparticles

Various techniques can be used to produce polymer nanoparticles, such as solvent evaporation, salting-out, dialysis, supercritical fluid technology, micro-emulsion, mini-emulsion, surfactant-free emulsion, and interfacial

polymerization Desolvation technique, pH induced aggregation [3-6].

Phase Separation in Aqueous Medium

The protein or polysaccharide from an aqueous phase can be desolvated by pH change or change in temperature or by adding appropriate counter ions. Cross-linking may be affected simultaneously or subsequent to the desolvation step. It contains three steps. Protein dissolution, protein aggregation and protein deaggregation. The appropriate levels of desolvation and resolvation, the aggregate size could be maintained and finally these aggregated nanoparticles are cross linked using glutaraldehyde. Sodium sulphate is the main desolvating agent. Alcohol, Ehanol, isopropanol are added as desolvating agents. The addition can be optimized turbidometrically using nephelometer. Only desolvation gives the final product as nanosphere. Desolvation deaggregates the protein and turns the suspension colloidal and hence milky in appearance. Both lipophilic and hydrophilic drugs can be entrapped in nanoparticles using this technique.

H Induced aggregation: The protein phase also be separated through pH change. The pH –induced aggregation to prepare nanoparticle has been extensively used for the preparation of nanoparticles.

Counter Ion Induced aggregation: The aggregation of dispersed phase can occur by adding appropriate counter ions. It can be propagated by adding secondary species of counter ions followed by rigidization step.

Solvent Evaporation Technique

This is one of the most frequently used methods for the preparation of nanoparticles. Emulsification-solvent evaporation involves two steps. The first step requires emulsification of the polymer solution into an aqueous phase. During the second step polymer solvent is evaporated, inducing polymer precipitation as nanospheres. The nanoparticles are collected by ultracentrifugation and washed with distilled water to remove stabilizer residue or any free drug and lyophilized for storage (Song et al., 1997) [7-11].

Bosentan belongs to a class of drugs known as endothelin receptor antagonists (ERAs). It is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension (PAH). Bosentan is used to treat pulmonary hypertension by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure. Patients with PAH have elevated levels of endothelin, a potent blood vessel constrictor, in their plasma and lung tissue. Bosentan blocks the binding of endothelin to its receptors, thereby negating endothelin's deleterious effects.

Mechanism of action: Endothelin-1 (ET-1) is a neurohormone, the effects of which are mediated by binding to ETA and ETB receptors in the endothelium and vascular smooth muscle.ET-1 concentrations are elevated in plasma and lung tissue of patients with pulmonary arterial hypertension, suggesting a pathogenic role for ET-1 in this disease. Bosentan is a specific and competitive antagonist at endothelin receptor types ETA and ETB. Bosentan has a slightly higher affinity for ETA receptors than for ETB receptors (Rubin et al., 2002) [12-15]

Materials and Methods

Materials

Drug: Bosentan (DIVIS Laboratories limited) Polymer: Ethyl cellulose (Hi Media Laboratories Pvt. Ltd., Mumbai) Solvent: Ethanol (SD fine-chem Limited, Mumbai)

Method

Emulsion Solvent Evaporation

Experimental Methodology

Bosentan loaded Ethyl cellulose nanoparticles were formulated by employing Emulsion Solvent Evaporation technique. Ethyl cellulose was used as a polymer and ethanol was used as solvent [16-21].

Emulsion Solvent Evaporation Method

Bosentan and ethylcellulose were dissolved in Ethanol at various drug-polymer ratios (1:1, 1:2, 1:3) and sonicated for 5-10 mins. Then this organic dispersion was emulsified by mixing at 700 rpm with a REMI LAB overhead mechanical stirrer provided with a 3-bladed paddle steel rotor, into an external phase containing Tween-20 (0.25%) at room temperature. The organic phase to the aqueous phase ratio was maintained as 1:10.

The organic phase was added drop wise to the aqueous phase at a constant rate. Stirring of the oil in water emulsion was continued until ethanol was

evaporated. The resultant dispersion was collected by Rotary Vacuum evaporator and kept for drying.

Evaluation

The prepared formulations of Bosentan loaded EC nanoparticles were evaluated for Drug Content, Entrapment Efficiency, Loading Capacity and Invitro Dissolution studies.

Drug Content: Drug content determination was done by transferring accurately weighed 50mg of the drug equivalent to formulation into a 100ml beaker containing 50ml of methanol. Magnetic stirring of this solution was done at 700 rpm for 3 hrs. The resultant solution was then filtered and the drug content was determined by using UV-Spectrophotometer at a wavelength of 272 nm.

$$DrugContent(\%) = \left[\frac{Drugweightinnanoparticles}{TotalWeightofnanoparticles}\right] \times 100$$

Entrapment Efficiency: Entrapment Efficiency determines the amount of drug incorporated into the formulation. The drug incorporation with nanoparticles can either be in the form of entrapment in the matrix and/or adsorption onto the surface.

Entrapment efficiency was determined by using a High-Speed Ultracentrifuge and centrifuging the samples containing 40mg of the drug equivalent to formulation in 40ml of pH 6.8 phosphate buffer at 17000 rpm at a temperature of -4ºC for 40 mins.

$$\text{Entrapment Efficiency (\%)} = \left[\frac{Totaldrugadded - Freenonentrappeddrug}{Totaldrugadded}\right] \times 100$$

Loading Capacity: Loading capacity is the amount of drug loaded per unit weight of the nanoparticle, indicating the percentage of mass of the nanoparticle that is due to the encapsulated drug.

$$\text{Loading capacity (\%)} = \left[\frac{TheAmountoftotalentrappeddrug}{TheTotalnanoparticleweight}\right] \times 100$$

Invitro Drug Release Studies: Drug release kinetics were determined by performing invitro dissolution studies using an Orbitary shaker. Accurately weighed 50mg of each formulation was taken in 250ml conical flask along with 50ml of pH 6.8 phosphate buffer and placed in the orbitarys haker at 100 rpm and the temperature was maintained at 37ºC. 2ml samples were withdrawn at predefined intervals of time and replaced with fresh buffer of the same volume. The amount of drug release was determined by taking the absorbance values at 272 nm using Elico UV-Spectrophotometer.

Results and Discussion

The prepared formulations were evaluated for the above mentioned parameters and the results are discussed below:

Evaluation of Bosentan loaded Ethyl cellulose Nanoparticles

Product Yield: The prepared nanoparticles were dried and the percentage yield of all the three formulations was calculated. The highest % yield (89.84± 2 %) was found to be of 1:3 formulation among the three formulations (Figure 1).

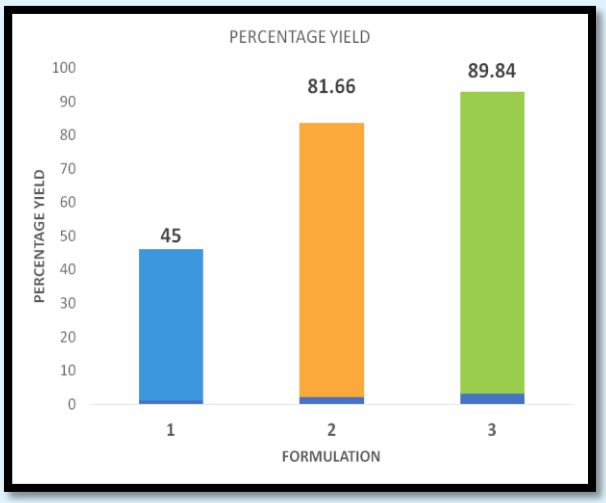

Figure 1: Comparison of % yield of the three formulations 1:1, 1:2 & 1:3.

Drug Content: The prepared nanoparticles were evaluated for their drug content. Among the three

formulations the highest drug content was observed in 1:3 formulation (97.43 ± 3 %) (Figure 2).

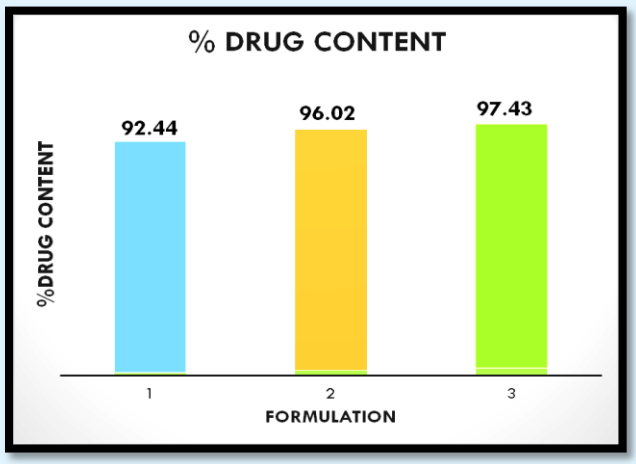

Figure 2: Comparison of drug content of the three formulations 1:1, 1:2 & 1:3.

Entrapment Efficiency: The efficiency of drug entrapment was determined in all the three formulations and it was observed that the drug had a better entrapment efficiency in 1:3 formulation (95.57± 2 %) (Figure 3).

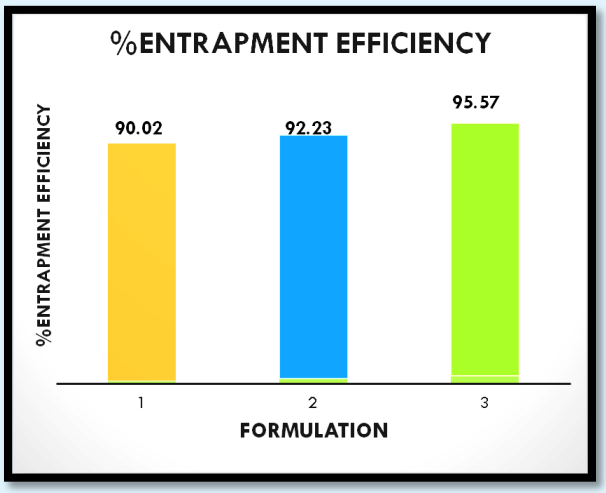

Figure 3: Comparison of entrapment efficiency of the three formulations 1:1, 1:2 & 1:3.

Loading Capacity: It indicates the capacity of the polymer to load a drug. From the results it was found that among the three formulations, 1:3 formulation showed highest loading capacity (Figure 4,5).

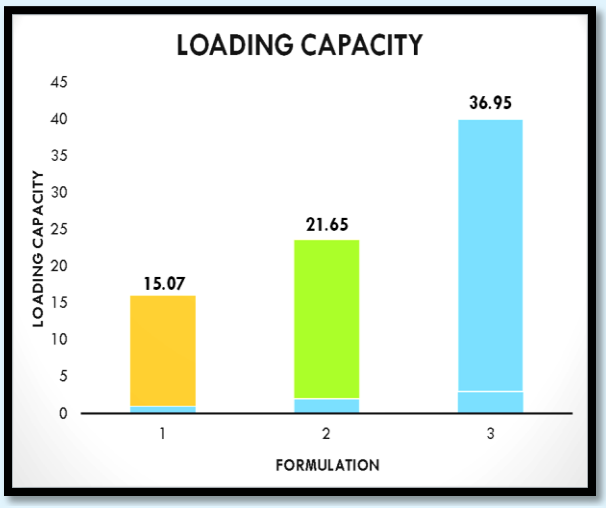

Figure 4: Comparison of loading capacity of the three formulations 1:1, 1:2 & 1:3.

In vitro drug release studies: Invitro drug release studies were done using orbitary shaker for a time period of 6 hrs. The maximum drug release was found to be of 1:3 formulation (24.55 ± 2%) as observed from the Figure 4.

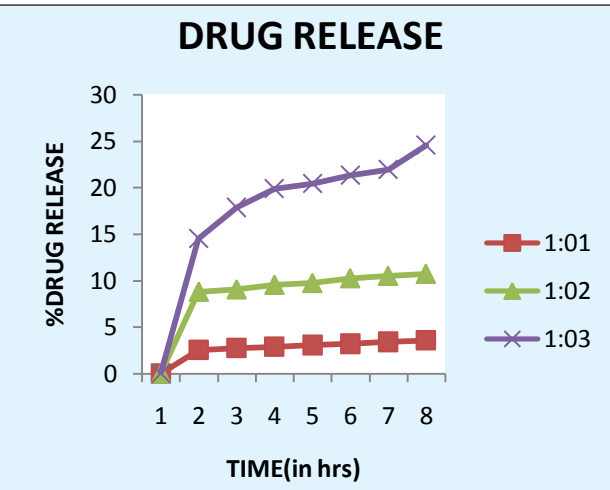

Figure 5: Comparison of % cumulative drug release of the three formulations 1:1, 1:2 & 1:3.

Discussion

In this study, Bosentan loaded ethyl cellulose nanoparticles were prepared by varying drug to polymer ratios i.e. 1:1, 1:2 & 1:3. The formulation 1:3 was found to be the best formulation with better characteristics when compared with the other two i.e. high percentage yield, drug content, loading capacity and better entrapment of the drug with better drug release.

Conclusion

In the present study Bosentan loaded ethyl cellulose nanoparticles were prepared by solvent evaporation technique. Among all the three formulation 1:3 formulation was considered as the best formulation with better results.

Acknowledgment

The authors sincerely thank Dr. M. Sumakanth, The Principal, RBVRR Women's college of Pharmacy for providing access for library and databases. We also thank Mrs. Suvarna and Mrs. Sumalatha, for providing technical assistance.

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