ORIGINAL RESEARCH PAPER

Pharmaceutical

FORMULATION AND EVALUATION OF NANOPARTICLES WITH NEURO PROTECTIVE CHEMICALS OF NATURAL AND SYNTHETIC ORIGIN.

KEY WORDS: Nanoparticles, Phytoconstituents, Biodegradable, Targeting

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ABSTRACT

Most of the active phytoconstituents under development are poorly water soluble or have poor bioavailability . Nanotechnology is an approach to overcome the challenges of conventional drug delivery systems and limitations of phytochemicals. Solid Lipid nanoparticles show interesting features concerning therapeutic purposes. The main advantage is that they are prepared with physiologically well-tolerated lipids. Solid Lipid Nanoparticles (SLNs) as novel lipid based nanocarriers with size range between 10 to 1000nm. SLNs were introduced to overcome problems of polymeric nanoparticles. In present research formulation and evaluation of nanoparticles with ethanolic extract of two plants *Celastrus paniculatus* and *Bacopa monnieri* along with Donepezil as a standard drug was undertaken here for the production methods for preparation of SLNs, and pharmaceutical approach of SLNs in drug delivery . The focus of nanoparticle design over the years has evolved toward more complex nanoscopic core–shell architecture using a single delivery system to combine multiple functionalities within nanoparticles which combine the mechanical advantages of biodegradable polymeric nanoparticles and biomimetic advantages of liposomes, have emerged as a robust and promising delivery platform.

Solid liquid nanoparticles having plant extracts were successfully formulated and characterized for their stability.A biodegradable polymeric core is surrounded by a shell composed of layer(s) of phospholipids. This architecture can provide advantages such as controllable particle size, surface functionality, high drug loading, entrapment of multiple therapeutic agents, drug release profile, and good serum stability of phytochemicals.

INTRODUCTION

Solid lipid nanoparticles are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other varied sciences (Thevenot et al, 2007). Due to their unique size-dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could be used for secondary and tertiary levels of drug targeting(Thevenot et al, 2008) . Hence, solid lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and hence have attracted wide attention of researchers. It is a novel approach to prepare SLN, which has following advantages over other production methods like use of pharmacologically acceptable organic solvent, easy handling and fast production process without technically sophisticated equipment. It is based on lipid precipitation from the dissolved lipid in solution. For the production of nanoparticle dispersions by precipitation in o/w emulsions the lipophilic material is dissolved in water-immiscible organic solvent that is emulsified in an aqueous phase (Li et al,2010). Upon evaporation of the solvent nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. cholesterol acetate as model drug and lecithin/sodium glycocholate blend as emulsifier.

MATERIAL AND METHOD

Preparation of solid lipid nano particles using solvent injection method

In this technique the solid lipid was dissolved in water-miscible solvent (eg. ethanol, acetone, isopropanol) or a water miscible solvent mixture. Then this lipid solvent mixture was injected through an injection needle into stirred aqueous phase with or without surfactant.

Formulation of drug loaded solid lipid nanoparticles

Solid lipid nanoparticles were prepared by using solvent injection technique using ethanol as organic solvent for nanoformulation ethanolic extract of *Celastrus paniculatus* and *Bacopa monnieri* was selected and for standard drug Donepezil was selected . Soya lecithin, drug and stearic acid is dissolved in the ethanol in definite ratio and warmed to 70°C. To the phosphate buffer solution (pH 7.4) a definite amount of tween 80 is added to prepare aqueous phase and kept for stirring which is maintain at 70°C. The organic phase was added drop wise with stirring to the pre warmed aqueous solution with the help of hypodermic needle. The mixture was then sonicated (Ultra sonicator, Bath type, Electronic India) for varying time to obtain nanoparticles. The optimum parameters i.e. tween 80 concentrations in definite ratio and maximum sonication time resulted in maximum entrapment efficiency and controlled release were used for the preparation of SLN using similar method. Twelve formulations were prepared by using different concentrations of tween 80 and sonication time to determine the effect of surfactant and sonication time on the potency of the SLNs.

Effect of formulation process variables

The effect of formulation variables such as Amount of Soya lecithin, stearic acid, Tween 80, Sonication time on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations. However, molecular dispersion of the drug in the stearic acid matrix led to a reduction in the crystallinity of stearic acid .

Evaluation of Prepared Solid Lipid nanoparticles

Differential scanning calorimeter

Thermograms were recorded using a differential scanning calorimeter. Samples (5-10mg) were weighed and hermetically sealed in flat bottomed aluminium pans. These samples were heated over a temperature range of 50-400°C in an atmosphere of nitrogen (200ml/min) at a constant rate of 10^{0}C per minute, with alumina being the reference standard.

FTIR Spectra of prepared formulation:

IR spectra of physical mixture of drug and excipients were recorded by KBr method using Fourier Transform Infrared Spectrophotometer. A base line correction was made using dried potassium bromide pellet. The potassium bromide-drug pellet of approximately 1 mm diameter was prepared by grinding 3-5 mg of physical mixture of drug-excipients with

100-150 mg of potassium bromide in pressure compression machine. The sample pellet was mounted in IR compartment and scanned at wavelengths 4000 cm^{-1} to 400 cm^{-1}.

Particle size determination: Particle size and PDI was determined using the particle size analyzer (Malvern Master Sizer, Malvern Instruments Ltd., Malvern, UK) (Ahmed *et al.,* 2010).

Zeta potential

Zeta potential of optimized phytosomes formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK). The electric potential of the phytosomes, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell.

Shape and Surface Morphology:

The shape and surface morphology of the prepered formulations were investigated using scanning electron microscopy (IISER, Bhopal). The solid lipid nanoparticles were fixed on supports with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the Scanning Electron Microscope at 10 kV.

***In-Vitro* drug release**

Dissolution rate studies

In vitro drug release of the sample was done using USP-type II dissolution apparatus (Paddle type). The dissolution medium, 900 ml 0.1 N HCl was set into the dissolution flask maintaining the temperature of 37±0.5°C and rpm of 75. 100mg of prepared SLN was set in every container of dissolution apparatus. The mechanical assembly was permitted to keep running for 72 hours. Sample measuring 5 ml were pulled back after each 1 hour up to 2 hours using 10ml pipette. The new disintegration medium (37°C) was supplanted each time with a similar amount of the sample and takes the area and calculated the percentage drug release (Li et al,2008).

RESULTS AND DISCUSSION

The nano particle size is highly dependent on the molar ratio of membrane lipids (Qian et al,2012). Results showed that the increasing of the polymer molar ratio could reduce the size of prepared nanoparticles . Previous researches showed that the DPPC have excellent biocompatibility to form small nanoliposomes due to the ratio of head group size compared to hydrocarbon tail (Li et al,2008). DSC results of various nanoparticles are given in Fig1-3, indicating the stability and integrity of formulations.The polydispersity index is an important indicator of the physical stability of nanoformulation . The polydispersity index values between 0.1 and 0.25 indicate acceptable uniformity, while values >0.5 are indicative of poor uniformity , present results of nanoparticles shows acceptable results in terms of polydispersity index. FTIR analysis of nanoformulation were given in fig4-6 ,results indicates the entrapment of desired chemical into the nanovesicles. Cryo-TEM analysis showed that the nanovesicles have a fine spherical shape and rough surface (Fig13-15) with a relatively monodispersed size distribution confirming the size distribution measurement studies The particle size(Fig 7-9), zeta-potential (Fig10-12) and polydispersity index of the nano formulations were within the desirable range, which indicates the stability and integrity of nanoparticles .Results indicates the successful formulations of nanoparticles by entrapping desirable therapeutic molecules.

CONCLUSION

Results indicates the stability of these novel formulations which could be a promising approach for *invitro* and *invivo* evaluation of these phytochemicals for their neuroprotective potency.

Future recommendations

The pharmacokinetic and pharmacodynamic (PK/PD) effects of these systems should be critically evaluated. Traditional PK evaluations depend on the availability of the free drug in the biological system to postulate its PD or metabolic fate with nanoformulation is be calculated in future study.

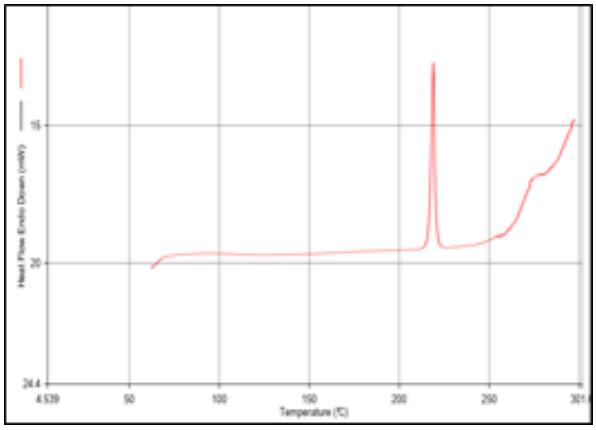

Fig 1 DSC of Solid lipid nanoparticles of *Celastrus paniculatus*

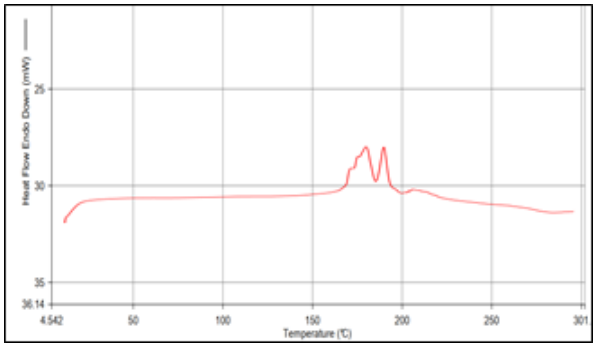

Fig 2 DSC of Solid lipid nanoparticles of Bacopa monnieri

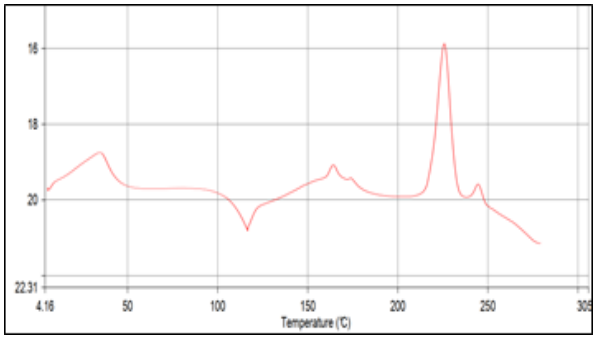

Fig 3 DSC of Solid lipid nanoparticles of Donepezil

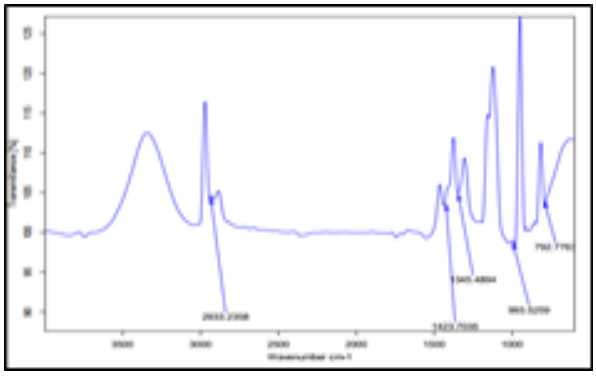

Fig 4 FT-IR of Solid lipid nanoparticles of *Celastrus paniculatus*

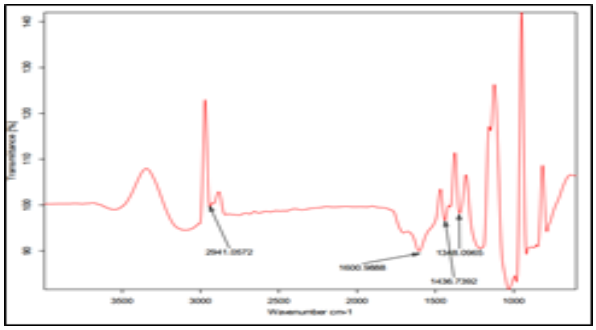

Fig 5 FT-IR of Solid lipid nanoparticles of Bacopa monnieri

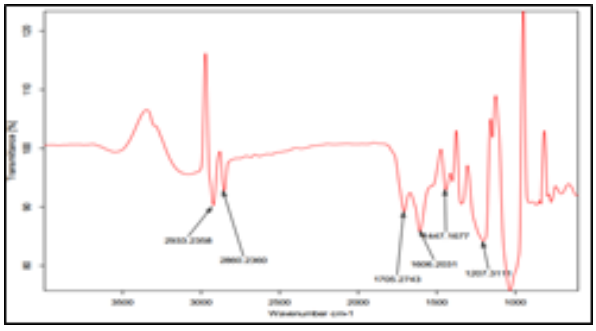

Fig 6 FT-IR of Solid lipid nanoparticles of Donepezil

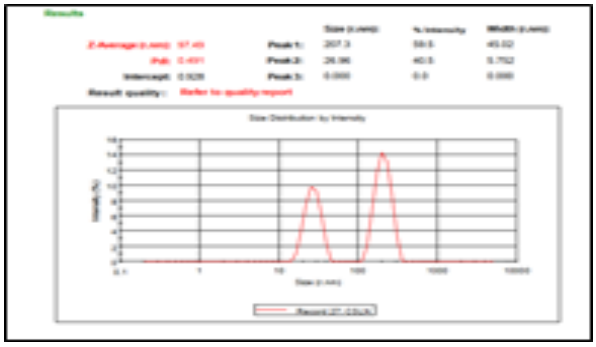

Fig 7: Particle size of Solid lipid nanoparticles of *Celastrus paniculatus*

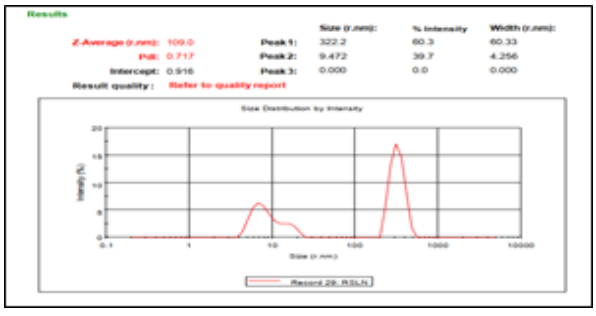

Fig 8: Particle size of Solid lipid nanoparticles of Bacopa monnieri

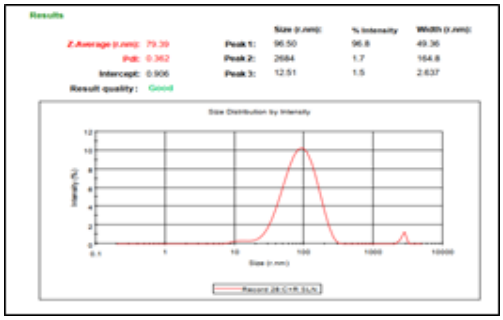

Fig 9: Particle size of Solid lipid nanoparticles of Donepezil

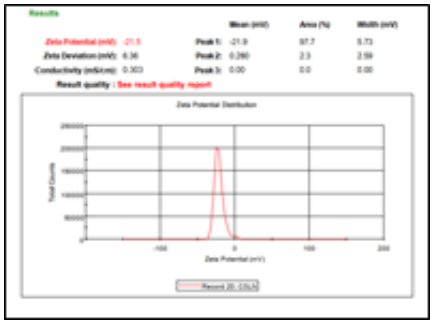

Fig 10 :Zeta Potential of Solid lipid nanoparticles of *Celastrus paniculatus*

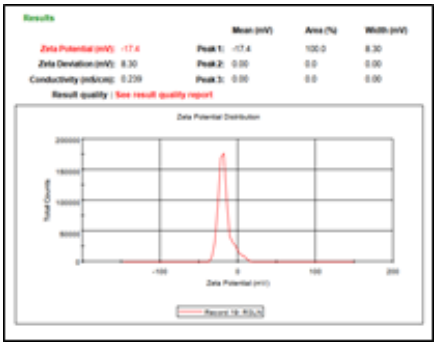

Fig 11:Zeta Potential of Solid lipid nanoparticles of Bacopa monnieri

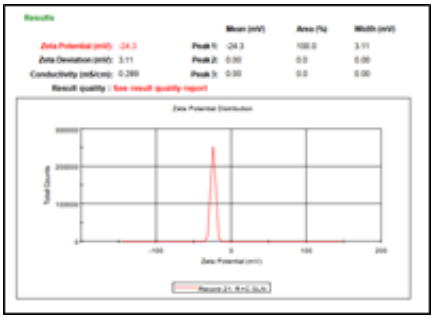

Fig 12 :Zeta Potential of Solid lipid nanoparticles of Donepezil

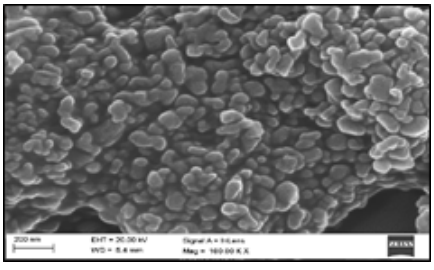

Fig 13: Scanning electron microscopy of Solid lipid nanoparticles of *Celastrus paniculatus*

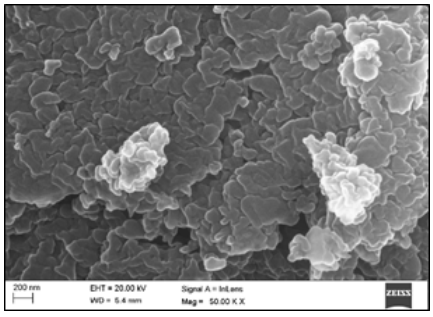

Fig 14: Scanning electron microscopy of Solid lipid nanoparticles of Bacopa monnieri

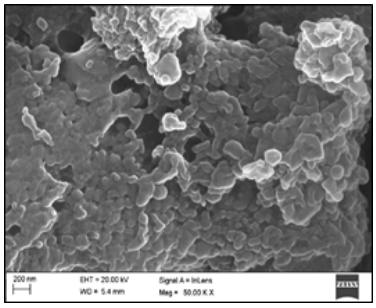

Fig 15: Scanning electron microscopy of Solid lipid nanoparticles of Donepezil

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