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Research Article

Development of Nano-structured Lipid Carriers Loaded with Corosolic Acid: An Efficient Carrier for Anti-diabetic Effects

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ABSTRACT

The aim of the present study was to develop a stable Nano-structured Lipid Carriers (NLCs) of Corosolic acid to improve its oral bioavailability. Corosolic acid is water insoluble, lipophilic, and highly permeable resulting in its incomplete and variable bioavability. Thus, a suitable formulation is highly desired to enhance the aqueous solubility and dissolution rate of corosolic acid to obtain faster onset of action, minimize the variability in absorption and improves its overall oral bioavailability. NLCs of Corosolic acid were formulated by solvent diffusion method technique after lipid screening tests by using central composite design. The formulated NLCs were characterized for Mean Particle size, Transmission electron microscopy, *in-vitro* drug release study. It was revealed that the average size of NLCs was found 201.4±1.2nm, TEM was found 200nm. *In-vitro* release determined by dialysis bag diffusion technique was found 70% at the end of 6 hr. The result of the studies was concluded that Corosolic acid was successfully in corporate into NLCs by Solvent diffusion method with high entrapment efficiency, so NLCs can be demonstrated as a potential carrier to improve oral bioavailability of Corosolic acid.

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Introduction

Diabetes mellitus is a group of metabolic diseases in which a person experiences high blood glucose levels either because the body produces insulin or the body cells do not respond properly to the insulin produced by the body. NLCs were introduced at the end of 1990s in order to overcome the potential difficulties with SLNs mainly to increase drug loading and to prevent drug expulsion by blending of different lipid molecules i.e. solid lipids with liquid lipids (oils) which enhances the imperfection of the crystal lattice and thus provide more room for accommodation of guest molecule (Patidar, *et al*., 2010). NLCs combine controlled drug release characteristics with some advantages over SLNs. Drugs having higher solubility in oils than solid lipids can be dissolved in the oil and yet be

protected from degradation by the surrounding solid lipids. Depending on the way of the production and the composition of the lipid blend, different types of NLCs are obtained. The resulting matrix of the lipid particles show a melting point depression compared to the original solid lipid but the matrix is still at body temperature (Singhal, *et al*., 2011).

Corosolic acid is penta-cyclic tri-terpine acid, may act as an insulin sensitizer enhancing insulin receptor B phosphorylation indirectly by inhibiting certain non-receptor protein tyrosin phosphateses. NLCs have key advantage of higher drug loading capacity as compared to SLNs. In SLNs, the crystal is more perfect densely packed, so that fewer drug can be incorporated. Corosolic acid may improve the insulin pathway. The action of insulin is mediated by tyrosine phosphorylation and initiated by the binding of insulin receptor. According to biopharmaceutical classification scheme corosolic acid is water insoluble, lipophilic, and highly permeable as a class II compound, resulting in its incomplete and variable bioavability. Corosolic acid is poorly soluble and aqueous solubility is reported to be less than 1mg/ml. Rapid onset of action is desirable to provide fast relief in the treatment of diabetes. Therefore, it is necessary to enhance the aqueous solubility and dissolution rate of corosolic acid to obtain faster onset of action, minimize the variability in absorption and improves its overall oral bioavailability. Corosolic acid conventional tablet, capsule dosage form is available in the market, which is unable to have sufficient oral bioavability. Therefore, alternative drug delivery systems and dosage forms are needed to improve its therapeutic efficacy. Thus, coroslic acid is ideal candidate to formulate NLCs to improve its oral bioavailability as it has high log P value and low solubility. The main objective of this present study was to investigate the formulation, optimization and evaluation of corosolic acid loaded nano structured lipid carriers to improve its oral bioavailability (Gref, *et al*., 1994).

Materials and Methods

Chemicals

Corosolic acid was obtained from Sigma-ALDRICH (Merck group) Country- USA. Capmul mcm Obtained as a gift sample from Abitex limited. Glyceryl mono-stearate was obtained from Alpha Chemicals Pvt Ltd Mumbai. Tween 80 was obtained from Sisco Research Laboratories Pvt Ltd. (Mumbai, India).

Procedure

Selection of solid lipid was based on the study of partitioning of drug in to melted lipid with respect to water phase. Selection of the components for Nano-structured lipid carrier system was based on Corosolic acid solubilizing capacity of the excipients and stability of the formulation. In this study the solvent diffusion method was used for the preparation of NLCs. The solvent chosen for the study was ethanol. The surfactant used for the study was Tween 80 in 1 % concentration. The solid (GMS) to liquid (Capmul MCM) lipid ratio was kept at 4:1 for the study keeping the external medium volume fixed to100ml (Muller, *et al.*, 2002).

Preparation of blank NLC

100 ml of distilled water was taken in a 250ml beaker and heated to 70˚C on a magnetic stirrer with hotplate. 1% Tween 80 was accurately weighed and added to it. It was stirred with the help of magnetic bead. In another 25ml beaker, 200mg of lipid (solid to liquid lipid ratio 3:1 by weight) was taken and to it 6 ml of the IPA/ THF was added. The organic phase was heated to 70˚C and added drop-wise with the help of syringe in to the aqueous phase with continuous stirring (Mehnert, *et al*., 2001). The dispersion was transferred to a round bottom flask and solvents were evaporated under reduced pressure using Rota evaporator at 70˚C. The dispersion obtained at the end of each run was filtered through Whatman filter paper to remove any visible large particle or aggregates formed. The particle size and poly dispersity index were measured by Zeta sizer nano series (Jahnke, *et al.*, 1998).

Preparation of drug loaded NLC

In 10ml beaker Corosolic acid/ Glyceryl mono state and Capmul MCM acid were taken in different proportions. To it, 3ml of ethanol was added. The organic phase was heated to 60-

70^0C till the entire lipid dissolved in solvent. The organic phase was added drop wise with the help of syringe in to the aqueous phase with continuous stirring. The stirring speed was kept at 1000rpm.The dispersion was transferred to a round bottom flask and solvent was evaporated under reduced pressure using Rota evaporator at80^0C. After evaporation of solvent the final volume was made up to 30ml. The dispersion was centrifuged at 10,000 RPM for 1hr at 6^0C using high speed centrifuge. The supernatant was carefully decanted. The pellet obtained was redispersed in 9ml of distilled water. Accurately weighed amount of Mannitol was added to the dispersion as cryo-protectant and kept in freezer for 24hrs.Then it was lyophilized (Abdelwahed, *et al*., 2006).

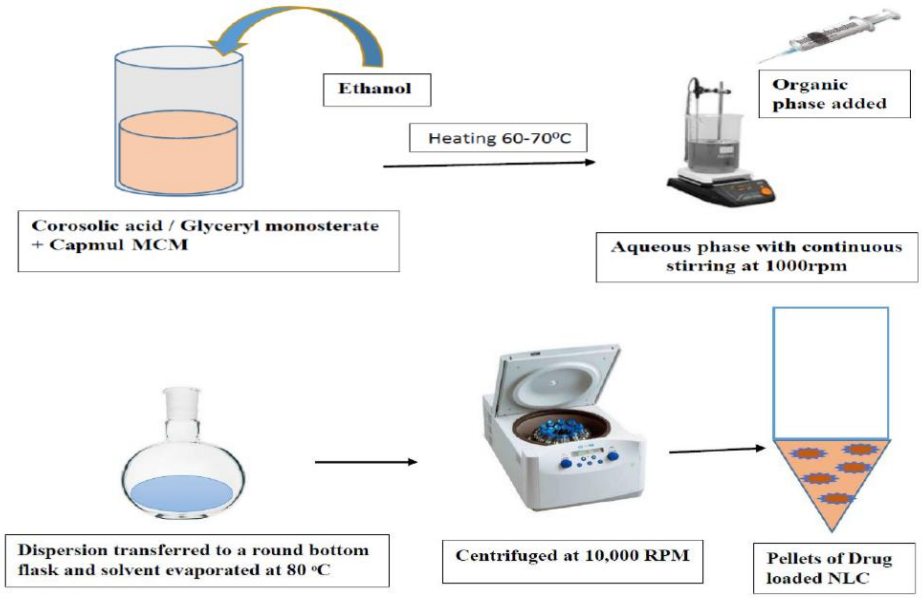

Fig. 1: Drug Loaded NLC

Determination of particle size and polydispersity index of the NLC

The MPS and PDI were determined by PCS with a Malvern Zeta sizer. The measurement using PCS is based on the light scattering phenomena in which the statistical intensity fluctuations of the scattered light from the particles in the measuring cell are measured. Prior to the measurements, all samples were diluted with double distilled water to produce a suitable scattering intensity. The z-average and PDI values were obtained at an angle of 90° using disposable polystyrene cells having 10 mm diameter cells at 25°C, which were equilibrating for 120 seconds. All measurements were performed in triplicate at 25°C (Araújo, *et al*., 2010; Liu, *et al.*, 2011).

Determination of Zeta potential (ZP) of the NLC

The measurements were performed with diluting in double-distilled water. It was measured using Dip cell with applying field strength 20 V/cm and the average of the zeta potential was given from 20 runs (Chalikwar, *et.al*., 2013)

Determination of *in - vitro* drug release study of the NLC

The dialysis bag diffusion technique was used to investigate the in vitro release of Corosolic acid-NLCs under sink condition. The amount of Corosolic acid released from the NLCs was determined spectrophotometrically at 210 nm from pre-constructed calibration curve. All experiments were performed in triplicate.

Determination of transmission electron microscopy (TEM) of the NLC

Morphological study of optimum formulation was done by taking TEM pictures of prepared NLCs.

Results and Discussion

Mean particle size and polydispersity index

The particle size of the NLCs suspension is a crucial factor because it determines the rate and extent of drug release as well as drug absorption. The particle size and Poly dispersity index (PDI) of the fabricated batches were in the range of 104.6 to 441 nm, and 0.501 to 0.764 respectively. The particle size and Polydispersity index (PDI) of the optimized Corosolic-NLC was found to be 205.8 nm and 0.271nmrespectively.

Zeta potential (ZP)

The measurement of ZP is the key to understanding the dispersion and aggregation processes. The measurements were performed with diluting in double-distilled water. It was measured using Dip cell with applying field strength 20 V/cm and the average of the zeta potential was given from 20 runs. ZP value ranges from-18to-43mW.

In-vitro drug release

The cumulative percentage drug release of Corosolic acid from Corosolic acid suspension and optimized Corosolic acid -loaded NLCs were observed in vitro by dialysis bag over a time period of 1h and5h, respectively. It was observed that, from Corosolic acid NLCs, drug was released slowly up to 70% at the end of 6h.

Transmission electron microscopy (TEM)

Morphological study of optimum formulation was done by taking TEM pictures of prepared NLCs. It was revealed that they were spherical in shape and uniformly distributed and considerably the size was found to be equivalent or nearby 200nm.

Conclusion

In this drug delivery system, Corosolic acid was successfully incorporated into NLCs by Solvent diffusion method with high entrapment efficiency due to its high lipophilicity. The prepared NLCs were found to have an amorphous structure and spherical shape morphology. The *in-vitro* release pattern showed a sustained and continuous release of Corosolic acid-NLCs. Thus, NLCs can be demonstrated as a potential carrier to improve oral bioavailability of Corosolic acid.

Conflict of Interest

The authors declare no conflicts of interest.

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