ABSTRACT

Artemisnin, a sesquiterene latone obtained from *Artemisia annua* (quinghaosu), and its semisynthetic derivatives are major therapeutic armamentium to treat chloroquinine resistant and cerebral malaria. One of the most widely used derivative, arteether, is having low aqueous solubility (~17 µg/mL) and about 40% degradation in acidic environment of the stomach. Due to which, it suffers from various issues like need of large dose, limited bioavailability following oral administration etc. Presently available intramuscular injection also suffers from disadvantages like pain at injection site, low patient compliance, erratic absorption, numbness, redness, and even muscle fibrosis, granuloma, tissue necrosis, and injuries, etc.

Present study was aimed to improve aqueous solubility of arteether and develop suitable dosage form(s) to achieve comparable oral bioavailability.

In order to achieve the same, various solubility enhancement approaches viz. solid dispersion, hydrotropy and inclusion complexation were explored. The developed molecular inclusions were also tested by various spectroscopic, thermal and microscopic techniques to validate the formation and understand the molecular changes associated with resultant enhancement of aquous solubility of arteether, a BCS class II drug.

Maximum solubility increment (77.05 times) was obtained with ternary complexation of arteether with cyclodextrin in presence of PVP K30. The cyclodextrin complexed arteether also exhibited comparable *ex-vivo* antimalarial activity when tested on Pf3D7 strain of *P. falciparum.*

Analytical methodologies by ultraviolet spectroscopy (UV) and chromatography (HPLC) were developed and validated on the basis of ICH Q2 R1 guidelines for industrial acceptance.

For formulation development and optimization studies, various arteether loaded nanoformulations (SLNs, NLCs, SMEDDS) were prepared and solidified. In order to develop an oral formulation with prevention of drug degradation in acidic environment and to reach at preferred absorption site i.e. intestine, enteric delivery approaches through filling in specific capsule shells were explored in view of their easy scale up and industrial acceptability. Solid dosage form (enteric coated tablets and spheroids in enteric capsules) containing cyclodextrin complexed arteether were also prepared to explore the feasibility to develop oral formulations with desired

bioavailability. Total quality management practices to meet current industrial approach and FDA requirments were applied for development of all formulations, as mentioned above. In order to understand the product development process with deeper insight on critical variables, process parameters and their impact on target quality attributes was studied by applying 'Quality by Design' methodology. For all formulations, quality target product profile(s) was identified and Ishikawa-fishbone correlation diagrams were designed to study the correlation between all possible variables for formulation the correlation between all possible variable for formulation designing. 'Quality Risk Assessment Metrices' were then established to identify critical variables affecting the quality of each formulation. Based on this, critical variables were identified and their concentrations were optimized using suitable Formulation by Design strategies. Best fit statistical design were employed to generate experimental recipe and ANOVA was used to define the relationship between selected variables and responses. Response surface methodology involving contour plots, overlay plots and other statistical techniques were employed to study the interactive effect of selected critical parameters on response. Point prediction features was used to find out the optimum concentration was validated experimentally.

Solid lipid nanoparticles (SLNs) containing arteether were developed by optimizing surfactant concentration and organic phase on the basis of entrapment efficiency and particle size responses using central composite design. The developed formulation was prepared by spontaneous precipitation method with some modifications. The developed formulation showed particle size (109 ±15 nm), entrapment efficiency was (93.7±6.2%) and drug loading of (78.54±0.62%). The developed SLNs were then lyophilized to convert it into feasible to develop enteric coated capsule based formulation.

Nano lipid carrier (NLC) containing arteether were prepared by applying central composite design and reciepe suggested by Design Expert software by combining high and low levels of critical variables (lipid and surfactant/ co-surfactant ratio) was experimentally explored using solvent diffusion method on the basis of entrapment efficiency and particle size as responses. Polynomial (quadratic) model, as suggested by ANOVA, defined the interrelation and 2 dimentional and 3 dimentional contour plots were plotted. For optimization, point prediction features was applied. The suggested formulation was then validated experimentally.

The developed NLCs were characterized on the basis of particle size 150.3 nm, polydispersity index 0.016 and SEM/TEM images confirmed the shape and size of nanoformulations. Entrapment efficiency of 68.2% and drug loading 88.6% also assured the feasibility to load required dose of arteether. NLCs so prepared were also lyophilized and converted into free flowing powder for further development of enteric coated formulations.

Self micro emulsified drug delivery systems (SMEDDS) were also prepared to explore lipophilic nature of arteether using self emulsification method. Statistical design based studies to find out optimum concentration of selected oil phase (arachis oil), surfactant (Tween 80), co-surfactant (Span 80) on the basis of drug release (%) and % RSD drug release as critical quality attributes. The optimized composition was experimentally verified and developed SMEDDS were solidified by absorbing on colloidal silica. The powdered SMEDDS were then filled in enteric coated capsule shells for further studies. Globule size (120 nm), zeta potential (-19.8 mv), entrapment efficiency (68 ± 0.2%) were characterised to confirm the acceptability of formulation.

All powdered nanoformulations were also studies to ensure their acceptable flow properties on the basis of shape, size and other parameters viz. bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose.

Cyclodextrin complexed arteether loaded (ART-CD) spheroids were also prepared by extrusion spheronization method to enhance the solubility as well oral biovailability of drug. The Box Behnken Design applied for the optimization of selected critical factors such as stirring time, stirring speed, disintegration concentration and binder concentration on the basis of selected response i.e., % dissolution. The developed spheroids were characterized on the basis of particle size of spheroids (0.560-0.780 mm), friability (0.54 %), tapped density (0.84), compressibility index (7.1) drug content (97.37%), Hausner's ratio (1.14), angle of repose of (20), reflecting the excellent flow. Further, spheroids were then filled into enteric-coated capsules for *in-vitro* drug release studies and pharmacokinetic studies.

All capsule based formulations were tested for finished product quality control tests viz. weight variation, content uniformity, weight loss, size, moisture and the formulations separately containing various noanoformulations as well as spheroids were found acceptable within pharmacopoeial units.

ART-CD containing enteric coated tablets were also prepared by direct compression method. The Box-Behnken design was used to optimise tablet composition taking microcrystalline cellulose, magnesium stearate, lactose, and PVPK30 as critical factors and drug release (%) as response. The optimized enteric coated tablets was experimentally verified and developed ART-CD complexed enteric coated tablets were characterized on the basis of in- process quality control tests like tapped density (0.88), compressibility index (6.8), Hausners ratio (1.14), and angle of repose (26). Further, the hardness of tablets varied from 5.9 kg/cm^2 to 7.1 kg/cm^2, friability (1±0.2%), average weight (215.42±2.4 mg) also confirmed pharmacopoeial compliance of developed tablets.

All the prepared nanoformulations viz. SLNs, NLCs, SMEDDS and ART-CD spheroids were encapsulated in enteric coated capsule shells and their dissolution parameters of these formulations and enteric coated tablets were analysed using progressive dissolution technique. The results of dissolution studies suggested that SLNs (40% in 10 h), NLCs (46.8% in 12 h), SMEDDS (42% in 10 h), ART-CD spheroids (96% in 7 h), enteric coated tablets (86.23% in 8 h) shown desired release profiles. The results confirmed that in lipid nanoformulations as the lipid matrix that encapsulates the drug can act as a barrier, slowed down the release of the drug. The release profile was improved when arteether was complexed with cyclodextrin. Cyclodextrins are cyclic oligosaccharides that can form inclusion complexes with various drugs. When arteether was complexed with cyclodextrin, it become more soluble and stable, which lead to improved drug release. Cyclodextrins can also protect drugs from degradation and metabolism, which can increase their bioavailability and therapeutic efficacy.

The Franz diffusion cell approach was used to investigate the permeability of prepared formulations of ART, which correlates with enhanced bioavailability as a component of improved dissolvability. A nearly 7.1 fold, 6.8 fold, 7 fold, 4.6 fold increase in permeability compared to ART in its pure form was observed with SLNs, NLCs, SMEDDS, ART-CD inclusion complex respectively showing positive effect on permeability of drug across membrane. The findings supported the feasibility of various formulations approaches for enhancing the bioavailability of ART.

Plasma drug concentration–time profile of pure drug suspension, enteric-coated tablets, spheroids and various nanoformulations (SLN, NLCs, SMEDDS) containing

ART were established following oral administered in rabbits. The pharmacokinetic parameters of the prepared formulations revealed that the absolute bioavailabilities of the formulations (SLN=27.6%, NLC=18.4%, SMEDDS= 8.5%, ART-CD spheroids=51.8%, enteric coated tablets=48.2%) was comparable with marketed *i.m.* injaection (43.73%). Further, the *in vivo* studies also confirmed the enhancement in absolute bioavailability by 48.29%, when compared with *i.v.* data of pure drug.

The IC_{50} value of ART and ART-CD complex were estimated to be 0.76 ng/mL and 0.733 ng/mL [equivalent to 3.4 ng/mL (calculated by molecular ratio)] in dimethylsulfoxide as solvent. This suggested that the antimalarial activity of the ART-CD complex was almost equivalent to ART.

Hence from the research, we can conclude that use of nanoformulation and cyclodextrin complexation have shown great promise in enhancing the solubility and bioavailability of poorly soluble drugs like ART. These techniques have the potential to revolutionize drug delivery systems, allowing for more effective treatments and improved patient outcomes. Additionally, the use of cyclodextrins as complexing agents offers a safe and cost-effective solution for improving drug solubility. Hence, present study may offer as suitable base study to develop oral formulation of arteether with comparable bioavailability and industrially acceptable production methodologies.