CHAPTER-6
SUMMARY AND CONCLUSION

Malaria has had a greater impact on world history than any other infectious disease. With the emergence of widespread chloroquine resistance and worldwide scarcity of quininine, a search for newer antimalarial drugs has become imperative. For effective treatment of malaria, WHO recommends the use of artemisinin, an active principle of the Chinese medicinal plant, *Artemisia annua* (Quinghaosu), and its semisynthetic derivatives viz. arteether, artemether, artesunate, and artemotil. Treatments containing an artemisinin derivative (artemisinin-combination therapies, ACTs) are now standard therapeutic approach worldwide for *P. falciparum* malaria.

Currently, only intramuscular injections of this drug are available in the market, which has lower patient acceptance and compliance due to being involved pricking, pain at the site of injection, numbness, redness, and even leading to muscle fibrosis, granuloma, tissue necrosis, and injuries. This formulation also suffers from various issues like erratic absorption patterns and increased cost of production. No oral dosage form of arteether is yet developed due to its low solubility (~17 μg/ml), high degradation in the stomach (~40%), and poor oral bioavailability.

The present work was based on initial positive findings at Mentor's lab regarding success in the enhancement of aqueous solubility of arteether by > 70 times using cyclodextrin encapsulation with micronization approach using spray drying method. Furthermore, various formulations for oral delivery like spheroids, extruders, matrix tablets for colon targeting, solid lipid nanoparticles, and enteric-coated tablets were pre-conceptualized for effective oral delivery of arteether, Preliminary studies showed promising results (data not published due to intellectual property and patent-related issues) regarding the successful development of an oral formulation of arteether for the very first time.

In this study, the preformulation studies revealed that the drug was in pure form and can be used for further studies. UV spectroscopy and HPLC based analytical methods were developed using QbD approach to further check the purity of drug. The analytical methods thus developed using QbD were validated as per ICH Q2 R1 guidelines. Further, to optimize HPLC based analytical method, the Placket Burman

method was used, and the influence of each parameter was analysed using an overlay plot in Design Expert version 13 software. Mobile phase (acetonitrile:water), column C_{18} 250 nmX4.6 mm, column temperature 30 ºC, wavelength: 254 nm, injection volume 20 µl/min, run time 10 mins, detector waters 2489 UV/Visible detector, retention time 4.149 min were among the critical parameters estimated for the development of an HPLC analytical method for estimation of ART.

In order to address the problems associated with lower aqueous solubility of ART, various solubility enhancement approaches alone or in combination, viz. solid dispersions and hydrotropy using hydrotropic agents and cyclodextrin complexation (binary and ternary) were explored. The prepared molecular inclusions having significant increment in water solubility of ART were characterized using various analytical approaches like FTIR, DSC, SEM, TEM, etc.

Various hydrotropic agents like sodium benzoate, nicotinamide, sachharine, PEG-6000, Poloxomer-407, cyclodextrin were tested to see whether these agents might improve arteether's water solubility. The aqueous solubility of arteether was increased by 50 times using a hydrotropic mix of 40 percent nicotinamide solution.

After preliminary studies, solid dispersions with the drug:carrier ratios of 1:3:2.5:5 (ART:PEG-6000, Poloxamer-407, HPMC K 100M), 1:3:2.5 (ART:PEG-6000, Poloxamer-407) (39.6±0.4), ART: CD:PEG600:Pol -407 (1:1:3.7:4.3), and arteether: saccharin sodium (1:5) were prepared. Maximum solubility enhancement was observed in the ratio ART: β-CD:PEG600:Pol-407 (67±1) and arteether: saccharin sodium(1:5) (61.06±0.6). PEG-6000 and Poloxamer-407 in combination resulted in a greater drug amorphization and hence enhanced the dissolution rate of the produced solid dispersion. As a result, the solid dispersion prepared with (ART:β CD:PEG600:Poloxamer-407) by melting meethod showed maximum solubility enhancement of arteether with a weight ratio of 1:1:3.7:4.3 (67%). This may be due to the drug being protected from acid degradation as it may be encapsulated in the cyclodextrin cavity. As the solid dispersion of arteether with saccharin sodium showed burst release in non uniform manner, it was not used for further studies.

Further, binary and ternary inclusion complex of ART-CD were prepared. The ratio was selected by implementing the QbD approach to get the best host-guest complex ratio. The enhancement in dissolution profile, as well as solubility, was compared

with the ART. By QbD approach as well as phase solubility studies, it was confirmed that molar ratio of 1:1 fits best for the ART-CD inclusion complex formation. This was substantiated by a saturation solubility investigation, which established that in the presence of PVP K30 (ternary inclusion complex), the solubility of ART was enhanced maximaly by 77.05 times. The results of scanning electron microscopy, X-ray diffraction, and differential scanning calorimetry indicated that the crystalline nature of drug was lost in the inclusion complex, confirmed that the drug was present in a solubilized form in the formulation. The ternary lyophilized ART-CD inclusion complex with maximum enhanced solubility and best dissolution profile was further used for permeability studies, and *in-vivo* and *ex-vivo* studies. Further, the complexed powder was filled in empty enteric-coated capsule shells. *In vitro* release studies for ART-CD filled enteric capsules was observed for 6 h by progressive dissolution method From the research, it was concluded that inclusion complexation is a suitable approach to improvise the solubility as well as bioavailability of hydrophobic drugs with limited aquous solubility like ART.

Various novel nanoformulations (SLNs, NLCs, SMEDDS) and solid dosage forms (spheroids, and enteric-coated tablets) were also formulated to enhance the oral bioavailability of drug.

ART loaded SLNs were prepared by solvent emulsification/evaporation method using Quality by Design approach. Surfactant concentration and acetone to ethanol volume ratio were selected as independent variable while particle size and entrapment efficiency was selected as responses using central composite design. The produced SLN were lyophilized and the powdered SLNs was encapsulated in an enteric coated capsule shell. The particle diameters of all the formulations were between 109 and 250 nm, and the entrapment effectiveness was 93.7 %. The XRD spectrum revealed that the ART was in amorphous form. The ART-SLNs release pattern revealed that ART was released in a slow yet time-dependent manner, which seems beneficial to prevent it from acid degradation.

The diffusion of solvent was used to prepare ART loaded NLCs. NLCs with a milky white hue were produced. The developed NLCs were then lyophilized. The NLCs were then placed inside enteric-coated capsule shells to prevent the medicine from being dissolved by acid. Using Design Expert version 13 software, a central

composite design was applied to optimize two chosen independent variables, the concentration of surfactant and lipids. Particle size and entrapment effectiveness were selected as the dependent response variables.

Optimized ART-NLCs dispersion exhibit an average particle size of 150.3 nm and PDI of 0.016. The low PDI value indicated homogenous NLC dispersion. The colloidal dispersion of the generated NLCs was stable and free of aggregation and settling, as shown by the zeta potential of the ART-NLC dispersion, which was -26.1 mV. Drug entrapment ratio was found to be 68.2%, showing th capability of formulation to deliver drug in required dosage. The optimized ART-NLCs had 88.6 % drug content. The pH of NLCs was discovered to be 6.9, which was a neutral value. Bulk density of solid NLCs was found to be 0.79. The tapped density was found to be 0.87. Compressibility index was found to be 7.2, which showed excellent flow. From the result, Hausners ratio was found to be 1.02. Angle of repose of solid NLC was found to be 22^{o}.

Self micro emulsified drug delivery system (SNEDDS) of ART was also developed using a QbD technique to increase the solubility and bioavailability. The mixture design was used to select the oil and the co- surfactant-surfactant ratio was employed as parameters in DoE for optimization of drug-loaded SMEDDS. The globule size and zeta potential of SMEDDS was found to be 120 nm and -19.8 mV respectively. The studies showed that 68 ±0.2% of the drug was entrapped in SMEDDS. The prepared SMEDDS were then adsorbed on surface of colloidal silica to encapsulate in enteric coated capsule shells. The solid SMEDDS were analysed by microscopic image, particle size, flow properties, *in -vito* drug release studies. The *in- vitro* drug release studies showed that in 12 h around 60 % of the drug was released .

The enteric encapsulated spheroids of ART-CD inclusion complex were prepared by extrusion spheronization using QbD approach. A Box-Behnken design was consequently employed for investigation of impact on the response on selected variables. The preparation of spheroids was confirmed by X-ray diffraction, solubility studies, scanning electron microscopy, differential scanning calorimetry and flow properties. *In vitro* release studies confirmed that around 80% drug was released in 4.5 h which validated that the drug was released in intestine, the preferred absorption site. Flow property characterization confirmed free flowing nature of prepared spheroids.

Encapsulated solidified nano-formulations (SLNs, NLCs, SMEDDS) and spheroids in enteric coated capsule shells were subjected to various quality control tests (weight variation, content uniformity, weight loss, size, moisture) and found acceptable within specified range as described in pharmacopoeia.

The enteric-coated tablets were prepared using a QbD approach. Box Behnken design was used for the optimization of tablets. Microcrystalline cellulose, magnesium stearate, lactose, and PVPK30 were taken as critical factors, and drug release was taken as a response. Response surface methodology was used to generate a highly significant mathematical model, which can adequately describe or predict the optimization of enteric-coated tablet formulation. The hardness of tablets was found to be in the ranges of 5.9±0.87 to 7.1±0.82 kg/cm^2 .Three batches of optimized formulations were evaluated for friability and the % weight loss in the friability test was found to be less than 1% for all batches indicating that prepared tablets can withstand mechanical shock or during handling.The average weight of tablets was found to be 215.42 mg for all batches and % deviation was also observed within specified limits.

The permeability of ART containing formulations was investigated using the Franz diffusion cell technique. The concentration of ART employing ART-SLN, ART-NLCs and ART-SMEDDS to pure ART in the pig's intestine was compared.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.

For establishing plasma drug concentration–time profile of pure drug suspension, enteric-coated tablets and spheroids and various nanoformulations (SLNs, NLCs, SMEDDS) in enteric coated capsule shells were administered orally in rabbits. The pharmacokinetic parameters of the prepared formulations revealed that the absolute bioavailabilities of the formulations SLNs (27.6%), NLC (18.4%), SMEDDS (8.5%), ART-CD spheroids (51.8%) and enteric coated tablets (48.2%) were comparable with marketed formulation i.e. *i.m.* injection (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 arteether.

In order to compare the antimalarial efficacy of ART and ART-CD complx, IC50 values against P. falciparum stain Pf3. The IC_{50} value of ART and ART-CD complex were estimated to be 0.76 ng/mL and 0.733 ng/ mL in dimethylsulfoxide as solvent. The IC_{50} value of ART-CD complex was almost equivalent to ART reflecting the acceptability of antimalarial activity of ART-CD complex drug. Therefore, it is concluded that present study have explored the feasibility to prepare oral formulation of ART with acceptable bioavailability.

With benefits including higher acceptance, particularly among adolescents and female patients, enhanced compliance, lesser production cost, and many dose-related difficulties, the suggested formulation may offer a new vista in the treatment of malaria. Application of various prevalent industrial approaches involving total quality management based on intergrative strategies like critically analysis, quality risk management, analytical method validation, formulation-by-design and ultimately quality-by-design in present study also provide a comprehensive solution to develop oral formulation of arteether with desired bioavailability at industrial scale.