# CHAPTER-3
# RESEARCH ENVISAGED & PLAN OF WORK

## 3.1 Problem formulation

### 3.1.1 *Research problem*

Malaria has a greater impact on world history than other infectious diseases. With the emergence of widespread chloroquine resistance and worldwide scarcity of quinine, WHO recommends the use of artemisinin and its semisynthetic derivatives viz. arteether, artemether, artesunate, and artemotil for *P. falciparum* malaria. Among these, arteether (ART), being more lipophilic and having high solubility in oil, has the advantages of its accumulation in brain tissues and lesser toxicity as compared to artemether.

Currently, only intramuscular injections of this drug are available in the market which has an erratic absorption pattern, higher cost of production, lower patient acceptance and compliance due to pricking, pain at the site of injection, numbness, redness, even leading to muscle fibrosis, granuloma, tissue necrosis and injuries. No oral dosage form of ART is yet developed due to its low aqueous solubility (~17 μg/mL), high stomach degradation (~40%), and poor oral bioavailability.

Various solubility and bioavailability enhancement techniques of drugs with poor aqueous solubility include techniques like hydrotropy, cyclodextrins, solid dispersions, nanoformulations, etc.

In view of this, it was proposed to explore various strategies for solubility enhancement of arteether for comparative studies specially cyclodextrin complexed drug as well as conjugating it with bioavailability enhancement approaches. It was then explored for the development of suitable dosage forms for oral delivery of ART involving a Quality-by-Design approach based on Total Quality Management strategies for intense industrial viability with pharmaceutical acceptance.

## 3.2 Aim and objectives

### 3.2.1 *Aim*

Intensifying solubility and bioavailability of arteether through molecular encapsulation based oral drug delivery system

### 3.2.2 *Specific research objectives [As approved by Department Doctoral Research Committee (DDRC)]*

- To explore various techniques for solubility enhancement of arteether with special emphasis on cyclodextrin complexation technique, hydrotrophy, and solid dispersion techniques.
- To address issues related to the administration of ART viz. low aqueous solubility, gastric degradation, less absorption, instability, and hence poor oral bioavailability.
- To develop novel oral formulations for the very first time of ART with desired bioavailability and efficacy using the Formulation by Design approach.
- To perform antimalarial activity assay and bioavailability studies of developed formulations in animals.
- To apply Total Quality Management practices for industrial scale-up and feasibility.

## 3.3 Plan of work

### 3.3.1 *Methodology adopted*

The major steps considered are as follows:

**a. Preformulation studies of the drug**

- Physical appearance
- Melting point
- Solubility studies
- Fourier transform infrared (FT-IR) analysis
- UV spectroscopy and HPLC based analytical method development
- Differential scanning calorimetric (DSC) analysis
- X-Ray diffraction (XRD) studies

**b. Solubility enhancement techniques**

- **Solid dispersions**
  - Selection of hydrophilic carriers and their ratios
  - Optimization of their concentrations (alone or in combination)
  - Solvent evaporation method (as applicable)
  - Spray drying method

- Optimization studies

➢ **Preparation of CD complex**

- Physical mixture
- Kneading method
- Lyophilisation
- Optimization by using QbD approach

➢ **Characterization of solid dispersions and CD complexes**

- Solubility studies
- Equilibrium solubility studies
- Solubility enhancement analysis
- Fourier transform infrared (FT-IR) analysis
- Particle size measurement/ distribution
- Differential scanning calorimetric (DSC) analysis
- X-Ray diffraction (XRD) studies
- *In vitro* dissolution studies
- *Ex vivo* permeability studies

**c. Formulation design and characterization:**

Using statistical optimization methods, a variety of noval oral dosage forms, including spheroids, enteric-coated preparations, and nanoformulations like SLN, NLCs, and SMEDDS, were formulated and characterized.

➢ **Formulation development and optimization of SLNs.**

- Preparation of solid lipid nanoparticles.
- Optimization of SLNs by Design of Experiments.
- Physicochemical characterization of ART-loaded SLN.
- Particle size analysis and polydispersity index studies of ART-loaded SLNs.
- Morphology of ART-loaded SLNs.
- Entrapment Efficiency (%) of ART- loaded SLNs.
- Determination of total drug content.
- Determination of pH of ART-loaded SLNs.
- Particle size analysis of ART-loaded SLNs.
- Flow properties of lyophilized ART-loaded SLNs.
- *In-vitro* release studies of ART-loaded SLNs.

- **Formulation development and optimization of NLCs.**

- Risk assessment studies of NLCs.
- Outlining quality target product profile and CQAs of NLCs.
- Preparation of ART Loaded NLCs.
- Central composite design for systematic optimization of NLCs.
- Characterization of optimized NLCs.
- Particle size, potential, and PDI of NLCs.
- Evaluation of surface topography of NLCs.
- Flow properties of lyophilized NLCs.
- Drug entrapped and drug loading of NLCs.
- Drug release studies of NLCs.

- **Formulation development and optimization of SMEDDS.**

- Risk assessment studies of SMEDDS.
- Outlining Quality Target Product Profile (QTPP) and CQAs of SMEDDS.
- CMA of SMEDDS.
- CPP of SMEDDS.
- Risk assessment of SMEDDS.
- Formulation development, optimization, and characterization of SMEDDS.
- Equilibrium solubility studies of ART.
- Constituents of SMEDDS.
- Solubility studies for screening of oil.
- Surfactant screening through emulsification.
- Emulsification of the maximal oil quantity Possible.
- Co-surfactant screening through emulsification.
- Emulsification of the maximal oil quantity possible with both surfactant and cosurfactant.
- Construction of ternary phase diagram.
- Drug-excipients compatibility studies for preparation of SMEDDS.
- SMEDDS development.
- Selection of design for preparation of SMEDDS.
- Visual evaluation of SMEDDS.
- Size of globules and zeta potential of SMEDDS.

- Determination of percentage drug entrapped in SMEDDS.
- High-resolution Transmission Electron Microscopy of SMEDDS.
- Determination of total drug content of SMEDDS.
- Determination of pH of prepared SMEDDS.
- Determination of drug release (%) from SMEDDS.
- Thermodynamic stability studies/stability under centrifugation of SMEDDS.
- Encapsulation of lyophilized SMEDDS in enteric-coated capsule shells.

➢ **Formulation development and optimization of ART-CD complex spheroids.**

- Evaluation of spheroids preparation parameters of CD inclusion complexes.
- QbD-based analysis for the optimization development of spheroids.
- Physicochemical characterization of optimized spheroids.
- Flow properties of ART-CD spheroids.
- Disintegration time of ART-CD spheroids.
- *In - vitro* drug release from ART-CD spheroids.

➢ **Formulation development and optimization of enteric-coated tablets.**

➢ Quality profiling and identification of QTPP, CQAs, and CPPs of enteric coated tablets.

➢ Risk assessment of enteric coated tablets.

➢ Formulation by Design strategy for optimization of critical variables of enteric coated tablets.

➢ Defining of design space of enteric coated tablets.

➢ Validation of model of enteric coated tablets.

➢ In-process quality control evaluation of enteric coated tablets.

➢ Finished product quality control evaluation of enteric coated tablets.

o Weight variation test of enteric coated tablets.

o Hardness of enteric coated tablets.

o Content uniformity of enteric coated tablets.

o *In -vitro* release studies of the enteric-coated tablets.

➢ **Permeability studies of prepared formulations.**

- ***In- vitro* antimalarial activity of plain and CD-complexed drug**

The antimalarial activity of prepared ART-CD complex was checked on the Pf3D7 strain of *P. falciparum.*

- **Pharmacokinetic and bioavailability studies of prepared formulation**

The absolute bioavailability enhancement parameters were determined and pharmacokinetic investigations using the rabbit model were conducted.

### 3.4 Expected outcome

Various oral formulations are explored for better therapeutic index using the Quality by Design methodology having better therapeutic efficacy. Developed oral formulations may offer a better low-cost alternative to the existing intramuscular injections of ART with increased patient compliance and therapeutic effectiveness to treat malaria worldwide for the very first time.

The proposed developed formulations may open a new vista in the treatment of malaria worldwide using artemisinin derivatives with advantages like better acceptability, especially among children and female patients, improved compliance, low production cost, and various dose-related issues.