CHAPTER-3

Objective and Plan of Work

The present study was aimed with an objective to develop, optimize and characterize four different types of nanophytoformulations: A) Curcumin (CUR) encapsulated lipopolysaccharides based nanocarriers (C-LPNCs), B) CUR encapsulated Eudragit E 100 based nanoparticles (CENPs), C) Naringenin (NAR) encapsulated lipopolysaccharides based nanocarriers (N-LPNCs) and D) NAR encapsulated Eudragit E 100 based nanoparticles (NENPs).

With the above objective in mind the present research work was focused to:

- Develop, optimize, characterize and investigate the potential of C-LPNCs, CENPs, N-LPNCs and NENPs for passive targeting to the colon-26 cancer cells in the treatment of colorectal cancer
- Avoid gastric as well as systemic degradation of drugs
- Reduce the total required dose in the formulation
- Bring about improvement in bioavailability and pharmacokinetic profile of drugs.
- Target (passively) the drugs to colon-26 cancer cells and thus, to provide an intense, safe and efficacious treatment option for colorectal cancer

As a goal to achieve highlighting bioavailability enhancement more efficacy with lower dose and increase compliance, the study was planned in following steps:

Step 1: Pre-formulation studies of CUR and NAR

- Organoleptic characteristics of the drugs
- Melting point of the drugs

- Solubility studies of the drugs
- Analytical and bioanalytical method development, validation and preparation of calibration curve of CUR and NAR by UV-Visible Spectrophotometer for *in-vitro* samples and High performance liquid chromatography (HPLC) method for plasma samples, respectively

Step 2: Preparation of physical mixture and formulation of CUR encapsulated lipopolysaccharide based nanocarriers (C-LPNCs)

- Preparation of CUR-lipopolysaccharide physical mixture
- Formulation of lipopolysaccharide based CUR nanocarriers using high speed homogenization technique
- Optimization of formulation using L9 Taguchi orthogonal experimental design as design of experiment for the dependent variables, such as mean particle size and percent entrapment efficiency.
- Solid state characterization of optimized formulation using Fourier transform infrared spectroscopy, Differential scanning calorimetry and Powder X-Ray diffraction
- Microscopic characterization of the prepared optimized formulation by using Transmission electron microscopy and Atomic force microscopy
- Qualitative drug distribution study of optimized formulation by using confocal laser scanning microscopy
- Assessment of *in-vitro* drug release characteristics and kinetic studies of CUR from the optimized formulation in media of phosphate buffer solution, pH 7.4
- Assessment of stability of the optimized formulation at room temperature (25±2°C)
- *In-vivo* Pharmacokinetic studies of optimized formulation following oral administration in Wister rats
- Quantitative *in-vitro* cytotoxicity study (Sulphorhodamine B assay) of optimized formulation in colon-26 cancer cell line

- Qualitative *in-vivo* anticancer efficacy studies of optimized formulation following oral administration in murine colon-26 tumor-bearing BALB/c mice by measuring:
  - ✓ Tumor volumes, Tumor weights and Body weights
  - ✓ Percent Survival using Kaplan-Meier survival plot

Step 3: Preparation of physical mixture and formulation of CUR encapsulated Eudragit E 100 nanoparticles (CENPs)

- Preparation of CUR-Eudragit E 100 physical mixture
- Formulation of CUR-Eudragit E 100 nanoparticles using emulsification-diffusion-evaporation technique
- Optimization of formulation using L9 Taguchi orthogonal experimental design as design of experiment for the dependent variables, such as mean particle size and percent entrapment efficiency.
- Solid state characterization of optimized formulation using Fourier transform infrared spectroscopy, Differential scanning calorimetry and Powder X-Ray diffraction
- Microscopic characterization of the prepared optimized formulation by using Transmission electron microscopy and Atomic force microscopy
- Qualitative drug distribution study of optimized formulation by using confocal laser scanning microscopy
- Assessment of *in-vitro* drug release characteristics and kinetic studies of CUR from the optimized formulation in media of phosphate buffer solution, pH 7.4
- Assessment of stability of the optimized formulation at room temperature (25±2°C)
- *In-vivo* Pharmacokinetic studies of optimized formulation following oral administration in Wister rats
- Quantitative *in-vitro* cytotoxicity study (Sulphorhodamine B assay) of optimized formulation in colon-26 cancer cell line

- Qualitative *in-vivo* anticancer efficacy studies of optimized formulation following oral administration in murine colon-26 tumor-bearing BALB/c mice by measuring:
  - ✓ Tumor volumes, Tumor weights and Body weights
  - ✓ Percent Survival using Kaplan-Meier survival plot

Step 4: Preparation of physical mixture and formulation of NAR encapsulated lipopolysaccharide based nanocarriers (N-LPNCs)

- Preparation of NAR-lipopolysaccharide physical mixture
- Formulation of lipopolysaccharide based NAR nanocarriers using high speed homogenization technique
- Optimization of formulation using L9 Taguchi orthogonal experimental design as design of experiment for the dependent variables, such as mean particle size and percent entrapment efficiency.
- Solid state characterization of optimized formulation using Fourier transform infrared spectroscopy, Differential scanning calorimetry and Powder X-Ray diffraction
- Microscopic characterization of the prepared optimized formulation by using Transmission electron microscopy and Atomic force microscopy
- Assessment of *in-vitro* drug release characteristics and kinetic studies of CUR from the optimized formulation in media of phosphate buffer solution, pH 7.4
- Assessment of stability of the optimized formulation at room temperature (25±2°C)
- *In-vivo* Pharmacokinetic studies of optimized formulation following oral administration in Wister rats
- Quantitative *in-vitro* cytotoxicity study (Sulphorhodamine B assay) of optimized formulation in colon-26 cancer cell line
- Qualitative *in-vivo* anticancer efficacy studies of optimized formulation following oral administration in murine colon-26 tumor-bearing BALB/c mice by measuring:

- ✓ Tumor volumes, Tumor weights and Body weights
- ✓ Percent Survival using Kaplan-Meier survival plot

Step 5: Preparation of physical mixture and formulation of NAR encapsulated Eudragit E 100 nanoparticles (NENPs)

- ❖ Preparation of NAR-Eudragit E 100 physical mixture
- ❖ Formulation of NAR-Eudragit E 100 nanoparticles using emulsification-diffusion-evaporation technique
- ❖ Optimization of formulation using L9 Taguchi orthogonal experimental design as design of experiment for the dependent variables, such as mean particle size and percent entrapment efficiency.
- ❖ Solid state characterization of optimized formulation using Fourier transform infrared spectroscopy, Differential scanning calorimetry and Powder X-Ray diffraction
- ❖ Microscopic characterization of the prepared optimized formulation by using Transmission electron microscopy and Atomic force microscopy
- ❖ Assessment of *in-vitro* drug release characteristics and kinetic studies of CUR from the optimized formulation in media of phosphate buffer solution, pH 7.4
- ❖ Assessment of stability of the optimized formulation at room temperature (25±2°C)
- ❖ *In-vivo* Pharmacokinetic studies of optimized formulation following oral administration in Wister rats
- ❖ Quantitative *in-vitro* cytotoxicity study (Sulphorhodamine B assay) of optimized formulation in colon-26 cancer cell line
- ❖ Qualitative *in-vivo* anticancer efficacy studies of optimized formulation following oral administration in murine colon-26 tumor-bearing BALB/c mice by measuring:
  - ✓ Tumor volumes, Tumor weights and Body weights
  - ✓ Percent Survival using Kaplan-Meier survival plot