3. Objective and plan of work

3.1 Objective

The present research work was aimed at the development, optimization and evaluation of four different types of nano-carrier systems; (A) CS encapsulated polymeric nanoparticles (CS-PNs), (B) CS encapsulated solid lipid nanoparticles (CS-SLNs), (C) CS encapsulated core-shell polymer-lipid hybrid nanoparticles (CS-PLHNs), and (D) CS encapsulated poly-ε-caprolactone-chitosan based core-shell polymeric nanoparticles (CS-PCCSNs). The specific objectives of the present research work are summarized as follow:

- To formulate stable nano-carrier systems carrying poorly permeable hydrophilic drug, i.e., CS.
- To improve the encapsulation efficiency of CS.
- To screen and optimize the various formulation and process parameters by using "Quality by Design" approach.
- To formulate and characterize the optimized nano-carrier systems.
- To improve the oral permeability of CS via nano-carrier system.
- To improve the pharmacokinetic profile of CS upon oral administration.
- To deliver CS at a sustained rate in systemic circulation and thus, to reduce dosing frequency.
- To reduce the total dose delivered.

3.2 Study Design

To achieve above mentioned specific objectives, the present study was planned carefully as mentioned below:

(A) Analytical method development and validation

- UV-visible Spectrophotometric method development for estimation of CS during *in-vitro* studies.
- Reverse phase high performance liquid chromatographic (RP-HPLC) method development for estimation of CS in blood plasma.

(B) Formulation and development of CS encapsulated polymeric nanoparticles (CS-PNs)

- Formulation of CS encapsulated polymeric nanoparticles (CS-PNs) by using modified double emulsification ($W_1/O/W_2$) solvent evaporation method.
- Preliminary screening of formulation and process parameters affecting the physicochemical properties (i.e., particle size, encapsulation efficiency (EE), polydispersity index (PDI)) of CS-PNs by using Plackett-Burman screening design.
- Optimization of formulation and process parameters affecting the physicochemical properties (i.e., particle size, EE, PDI) of CS-PNs by using Box-Behnken experimental design followed by desirability approach.
- Physicochemical characterization of CS-PNs for particle size, EE, PDI, zeta potential and drug loading.
- Solid state characterization of optimized CS-PNs using Fourier Transformed Infra-Red spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction (PXRD).
- Morphological characterization of optimized CS-PNs using High-Resolution Transmission Electron Microscopy (HR-TEM) and Atomic Force Microscopy (AFM).

- Determination of *in-vitro* release characteristics of CS from optimized CS-PNs in phosphate buffer pH 7.4 and mathematical modeling of release kinetics.
- Assessment of real time and accelerated stability of optimized CS-PNs at different environmental conditions.
- Assessment of intestinal permeation of optimized CS-PNs via *ex-vivo* studies.
- Visualization of *in vivo* permeation behavior of optimized CS-PNs across rat intestine by using confocal laser scanning microscopy (CLSM)
- *In-vivo* pharmacokinetic and pharmacodynamic studies of optimized CS-PNs after oral administration in rats.

(C) Formulation and development of CS encapsulated solid lipid nanoparticles (CS-SLNs)

- Formulation of CS encapsulated solid lipid nanoparticles (CS-SLNs) by using modified double emulsification ($W_1/O/W_2$) solvent evaporation method.
- Preliminary screening of formulation and process parameters affecting the physicochemical properties (i.e., particle size, EE, PDI) of CS-SLNs by using Plackett-Burman screening design.
- Optimization of formulation and process parameters affecting the physicochemical properties (i.e., particle size, EE, PDI) of CS-SLNs by using Box-Behnken experimental design followed by desirability approach.
- Physicochemical characterization of CS-SLNs for particle size, EE, PDI, zeta potential and drug loading.
- Solid state characterization of optimized CS-SLNs using FTIR, DSC and PXRD.
- Morphological characterization of optimized CS-SLNs using HR-TEM and AFM.

- Determination of *in-vitro* release characteristics of CS from optimized CS-SLNs in phosphate buffer pH 7.4 and mathematical modeling of release kinetics.
- Assessment of real time and accelerated stability of optimized CS-SLNs at different environmental conditions.
- Assessment of intestinal permeation of optimized CS-SLNs via *ex-vivo* studies.
- Visualization of *in vivo* permeation behavior of optimized CS-SLNs across rat intestine by using CLSM.
- *In-vivo* pharmacokinetic and pharmacodynamic studies of optimized CS-SLNs after oral administration in rats.

(D) Formulation and development of CS encapsulated core-shell polymer-lipid hybrid nanoparticles (CS-PLHNs)

- Formulation of CS encapsulated core-shell polymer lipid hybrid nanoparticles (CS-PLHNs) by using modified double emulsification ($W_1/O/W_2$) solvent evaporation method.
- Preliminary screening of formulation and process parameters affecting the physicochemical properties (i.e., particle size, EE, PDI) of CS-PLHNs by using Plackett-Burman screening design.
- Optimization of formulation and process parameters affecting the physicochemical properties (i.e., particle size, EE, PDI) of CS-PLHNs by using Box-Behnken experimental design followed by desirability approach.
- Physicochemical characterization of CS-PLHNs for particle size, EE, PDI, zeta potential and drug loading.

- Solid state characterization of optimized CS-SLNs using FTIR, DSC and PXRD.
- Morphological characterization of optimized CS-SLNs using HR-TEM, AFM and CLSM.
- Determination of *in-vitro* release characteristics of CS from optimized CS-PLHNs in phosphate buffer pH 7.4 and mathematical modeling of release kinetics.
- Assessment of real time and accelerated stability of optimized CS-PLHNs at different environmental conditions.
- Assessment of intestinal permeation of optimized CS-PLHNs via *ex-vivo* studies.
- Visualization of *in vivo* permeation behavior of optimized CS-PLHNs across rat intestine by using CLSM.
- *In-vivo* pharmacokinetic and pharmacodynamic studies of optimized CS-PLHNs after oral administration in rats.

(E) Formulation and development of CS encapsulated poly-ε-caprolactone-chitosan based core-shell polymeric nanoparticles (CS-PCCSNs)

- Formulation of CS encapsulated poly-ε-caprolactone-chitosan based core-shell polymeric nanoparticles (CS-PCCSNs) by using modified nanoprecipitation method.
- Preliminary screening of formulation and process parameters affecting the physicochemical properties (i.e., particle size, EE, PDI) of CS-PCCSNs by using Plackett-Burman screening design.

- Optimization of formulation and process parameters affecting the physicochemical properties (i.e., particle size, EE, PDI) of CS-PCCSNs by using Box-Behnken experimental design followed by desirability approach.
- Physicochemical characterization of CS-PCCSNs for particle size, EE, PDI, zeta potential and drug loading.
- Solid state characterization of optimized CS-PCCSNs using FTIR, DSC and PXRD.
- Morphological characterization of optimized CS-PCCSNs using HR-TEM, AFM and CLSM.
- Determination of *in-vitro* release characteristics of CS from optimized CS-PCCSNs in phosphate buffer pH 7.4 and mathematical modeling of release kinetics.
- Assessment of real time and accelerated stability of optimized CS-PCCSNs at different environmental conditions.
- Assessment of intestinal permeation of optimized CS-PCCSNs via *ex-vivo* studies.
- Visualization of *in vivo* permeation behavior of optimized CS-PCCSNs across rat intestine by using CLSM.
- *In-vivo* pharmacokinetic and pharmacodynamic studies of optimized CS-PCCSNs after oral administration in rats.