Plan of work

The aim of the present research was to design, develop, optimize, characterize and evaluate novel polymer stabilized chitosan capped gold nanoparticles loaded with dasatinib (BCS class II) anticancer drug as an active pharmaceutical ingredient for the effective and improved treatment of chronic myeloid leukaemia (CML). To achieve the aim, all the developed formulations were optimized, characterized and evaluated for *in-vitro* and *in-vivo* performance. In addition, their anticancer potential against associated cell lines (K562) and pharmacokinetics in Sprague Dawley rats were also evaluated. Thus, the objective of the work was to:

1. Design, develop, optimize, characterize and evaluate dasatinib loaded polymeric gold nanoparticulate based drug delivery system, and
2. Improve its stability, bioavailability, pharmacokinetic profile and therapeutic efficacy.

The study was planned as outlined below:

A. Preformulation studies

- ✓ Physical observation
- ✓ FTIR interpretation
- ✓ Analytical method development of DSB by UV-visible spectroscopy in *in vitro* samples and by HPLC for estimating drug in biological samples.

B. Formulation development

- ✓ Selection of manufacturing method for the synthesis of GNPs
- ✓ Preparation of chitosan capped GNPs (Ch-GNPs) by green reduction method
- ✓ Preparation of dasatinib loaded polymer stabilized Ch-GNPs

- DSB loaded PVP stabilized Ch-GNPs (DSB-PVP-Ch-GNPs)
- DSB loaded PEG stabilized Ch-GNPs (DSB-PEG-Ch-GNPs)
- DSB loaded PLGA stabilized Ch-GNPs (DSB-PLGA-Ch-GNPs)

✓ Formulation optimization

- Risk assessment studies
- Identification and selection of critical quality attributes (CQAs)
- Risk assessment screening by Placket-Burman design (PBD)
- Optimization of the formulation by QbD

C. Characterization studies

✓ Particle size (dynamic light scattering)

✓ Zeta potential (light diffraction velocimetry)

✓ Entrapment efficiency (% EE)

✓ Solid state characterization (FTIR and XRD)

✓ Physicochemical and morphological characterization (HR-SEM, EDXS, TEM, SAED, and AFM)

D. Assessment of stability of the optimized nanoformulation at three different storage conditions over a period of 6 months.

E. Assessment of i*n vitro* DSB release from the optimized nanoformulation in pH 7.4 phosphate buffer.

F. Assessment of i*n vitro* hemocompatibility of the optimized nanoformulation by using optical microscopy.

G. Assessment of cellular uptake (K562 - human myeloid leukaemia cell lines) of the optimized nanoformulation by using confocal fluorescence microscopy.

H. Assessment of i*n vitro* cytotoxicity (K562 - human myeloid leukaemia cell lines) of the optimized nanoformulation by SRB assay.

I. Assessment of apoptosis (K562 - human myeloid leukaemia cell lines) of the optimized nanoformulation by flow cytometry.

J. *In vivo* pharmacokinetic studies of the optimized nanoformulation following single i.v. administration in healthy Sprague Dawley rats.