3.1 Aim of work

The aim of this research was to develop a novel PLGA based polymeric nanoparticles containing Hepatitis B Virus for the effective vaccination. To achieve the aim, developed formulations were characterized and evaluated for *in-vitro* and *in-vivo* performance. In addition, their immunogenic potential against virus was evaluated in developed Xenograft Humanized Mice Model.

3.1 Objectives

- To formulate and characterize Hepatitis B surface antigen loaded polymeric nanoparticles.
- To perform the biological evaluation of prepared Hepatitis B surface antigen loaded nanoparticles.
- To select the route of administration and immunological estimation of prepared Hepatitis B surface antigen loaded nanoparticles in BALB/c mice.
- To assess the immunological parameters in developed Humanized Xenograft Model.

3.3 Experimental plan

3.3.1 Preformulation studies

- Analytical method development by HPLC
- Standard calibration curve

3.3.2 Formulation of HBsAg loaded polymeric nanoparticles

- Selection of manufacturing method
- Formulation optimization

3.3.3 *In-vitro* characterization of prepared HBsAg loaded polymeric nanoparticles

- Particle size, polydispersity index and Zeta potential
- Entrapment efficiency

3.3.4 Surface characterization

- SEM (Scanning Electron Microscopy)
- TEM (Transmission Electron Microscopy)
- AFM (Atomic Force Microscopy)

3.3.5 *In-vitro* antigen release from nanoparticles

3.3.6 Structural integrity determination of HBsAg loaded nanoparticles

3.3.7 *In-vitro* cellular uptake study of nanoparticles

3.3.8 Haemocompatibility studies

- Evaluation of haemolysis
- Quantitative platelet aggregation evaluation
- Qualitative platelet aggregation study

3.3.9 Stability study

3.3.10 Selection of route of administration in BALB/c mice

3.3.11 *In-vivo* cellular internalization study

3.3.12 Immunological characterization and measurement of antibody levels

3.3.13 *In-vivo* Lymphocyte and T cells proliferation study

3.3.14 Assessment of immunological parameter in Humanized Xenograft model

- Development of Humanized Xenograft model
- Study design and vaccination
- Sample collection and antibody response measurement
