3 Plan of work

3.1 Objective and plan of work

The objective of the present research work was:

- To develop, optimize and evaluate TNZ loaded electrospun nanofiber membrane for the effective treatment of periodontal disease.
- To deliver TNZ at a controlled rate locally in the periodontal pocket and maintain required therapeutic drug concentration for the treatment duration with the ultimate aim to reduce dose size and dosing frequency while improving the patient compliance and also maximizing therapeutic outcome.

3.2 Study Design

To achieve the objectives mentioned above, the present study was planned carefully and divided into the following parts:

Part I: ***Analytical method development and validation for estimation of TNZ***

- Development and Validation of simple, rapid, reliable and reproducible analytical methods using UV-Visible spectrophotometer for quantitative estimation of TNZ during *in vitro* studies.

Part II: ***Development, optimization and evaluation of TNZ loaded homogeneous electrospun poly (ε-caprolactone) nanofiber membrane.***

- Formulation of TNZ encapsulated PCL nanofiber membrane (TNZ-PCLNF) using electrospinning technique.
- Optimization of formulation and process parameters affecting the diameter and entrapment efficiency of TNZ-PCLNF by using Box-Behnken experimental design followed by desirability approach.
- Solid state characterization of the optimized TNZ-PCLNF membrane using Fourier Transformed Infra-Red spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction (PXRD).

- Morphological characterization of the optimized TNZ-PCLNF membrane using High-Resolution Scanning Electron Microscopy (HR-SEM) and Atomic Force Microscopy (AFM).
- Determination of surface wettability of the nanofiber membranes by measurement of contact angle.
- Determination of *in vitro* release characteristics of TNZ from optimized TNZ-PCLNF membrane in McIlvaine buffer pH 6.6 and mathematical modeling of release kinetics.
- Determination of *in vitro* antibacterial study as well as cytocompatibility study of different formulations.
- Determination of the Biocompatibility of developed TNZ-PCLNF membrane by assessing haemocompatibility with red blood cell through haemolysis method.
- Assessment of real-time and accelerated stability of optimized TNZ-PCLNF membrane by storing at different environmental conditions.
- Assessment of *in vivo* performance of the developed TNZ-PCL nanofiber membrane using Ligature-induced rat model for periodontitis.

Part III: ***Development, optimization and evaluation of TNZ loaded homogeneous electrospun GE/PCL hybrid nanofiber membrane***

- Formulation of TNZ encapsulated GE/PCL hybrid nanofiber membrane (TNZ-PGHNF) using electrospinning technique.
- Optimization of formulation and process parameters affecting the diameter and entrapment efficiency of TNZ-PGHNF using Box-Behnken experimental design followed by desirability approach.
- Solid state characterization of the optimized TNZ-PGHNF membrane using FTIR, DSC, and PXRD.

- Morphological characterization of the optimized TNZ-PGHNF membrane using HR-SEM and AFM.
- Determination of surface wettability of the nanofiber membranes by measuring contact angle using sessile drop method.
- Assessment of mucoadhesion of nanofiber formulation by measuring adhesion force using texture analyzer.
- Determination of *in vitro* release characteristics of TNZ from optimized TNZ-PGHNF membrane in McIlvaine buffer pH 6.6 and mathematical modeling of release kinetics.
- Determination of *in vitro* antibacterial study as well as cytocompatibility study of different formulation.
- Determination of Biocompatibility of developed TNZ-PGHNF membrane by evaluating haemocompatibility with red blood cell through haemolysis method.
- Assessment of real-time and accelerated stability of optimized TNZ-PGHNF membrane by storing at different environmental conditions.
- Assessment of *in vivo* performance of the developed TNZ-PGH nanofiber membrane using Ligature-induced rat model for periodontitis.

Part IV: ***Development, optimization and evaluation of TNZ loaded homogeneous electrospun CH/PCL hybrid nanofiber membrane***

- Formulation of TNZ encapsulated CH/PCL hybrid nanofiber membrane (TNZ-PCHNF) using electrospinning technique.
- Optimization of formulation and process parameters affecting the diameter and entrapment efficiency of TNZ-PCHNF using Box-Behnken experimental design followed by desirability approach.

- Solid state characterization of the optimized TNZ-PCHNF membrane using FTIR, DSC, and PXRD.
- Morphological characterization of the optimized TNZ-PCHNF membrane using HR-SEM and AFM.
- Determination of surface wettability of the nanofiber membranes through contact angle measurement.
- Assessment of mucoadhesion of nanofiber formulation by measuring adhesion force using texture analyzer.
- Determination of *in vitro* release characteristics of TNZ from optimized TNZ-PCHNF membrane in McIlvaine buffer pH 6.6 and mathematical modeling of release kinetics.
- Determination of *in vitro* antibacterial study as well as cytocompatibility study of different formulation.
- Determination of Biocompatibility of developed TNZ-PCHNF membrane by evaluating haemocompatibility with red blood cell through haemolysis method.
- Assessment of real-time and accelerated stability of optimized TNZ-PCHNF membrane by storing at different environmental conditions.
- Assessment of *in vivo* performance of the developed TNZ-PCH nanofiber membrane using Ligature-induced rat model for periodontitis.
- Performing Clinical study in patients suffering from periodontitis to assess the efficacy and therapeutic potential of the optimized TNZ-PCHNF formulation.