6 Summary and Conclusions

TNZ loaded nanofiber membrane as local drug delivery may be an advantageous form of treatment as it would probably eliminate side effects, which occur with systemic dosing. So, in the present research work, an attempt has been made to formulate three different types of nanofiber systems namely PCL nanofiber (TNZ-PCLNF), GE-PCL hybrid nanofiber (TNZ-PGHNF) and CH-PCL hybrid nanofiber (TNZ-PCHNF) of TNZ for the effective treatment of periodontal disease with local delivery into the periodontal pockets.

TNZ-PCLNF membrane were successfully prepared by electrospinning method and optimized by "Quality by Design" approach using Box-Behnken experimental design. The optimized TNZ-PCLNF membrane showed the diameter of 147.6±7.6 nm and EE of 84.36±1.5%. The solid-state characterizations of the optimized TNZ-PCLNF membrane using FTIR, DSC, and PXRD pointed towards the encapsulation of TNZ inside the nanofiber membrane without any physical as well as chemical interactions. Surface morphological study using HR-SEM and AFM showed the existence of cylindrical shaped, smooth surfaced nanofiber membrane with negligible bead defects. *In vitro* drug release and antibacterial study demonstrated capability of the developed nanofiber membranes for efficiently delivering TNZ in a sustained manner up to 20 days and its ability to inhibit bacterial growth, respectively. Higher contact angle (123.6±2.8º) revealed that PCLNF membrane was of hydrophobic nature which would prevent its adhesion to the site of action. Further, haemocompatibility results of formulations exhibited that the haemolysis percentage was under 3% for 0%, 10% and 20% TNZ loaded nanofiber membrane, demonstrating the good and acceptable character of anti-haemolytic property of all the nanofiber membrane. However, nanofiber membrane loaded with 30% TNZ showed maximum HP viz. 3.75±0.50%.

MTT assay and CLSM study indicated that optimized nanofiber membrane showed insignificant ($p > 0.05$) cytotoxicity on mouse fibroblasts (L-929 cell lines). The TNZ-PCLNF membrane, was found physically and chemically stable over the storage time period without any significant change ($p > 0.05$) in their physicochemical attributes, stored at room temperature (30±2 °C/65±5% RH), refrigerated condition (4±1 °C) and significant change ($p < 0.05$) at accelerated condition (40±2 °C/75±5% RH), suggesting instability due to biodegradation of polymer which would have expelled the drug molecule from nanofiber hence, it was strongly recommended that the nanofiber membrane should be stored at room temperature (30±2 °C) or at refrigerated condition (4±1 °C), to hold the pharmaceutical properties for safe and effective long-term use. Further, *in vivo* study by ligature-induced periodontitis in rats confirmed that TNZ loaded nanofiber membrane can significantly ($p < 0.05$) improve continuity of epithelium and transseptal fiber of Interdental papilla in comparison to control group.

TNZ-PGHNF were also prepared by electrospining technique and optimized with BBD experimental design. Box–Behnken design was employed for evaluating the influence of formulation and processing variables on entrapment efficiency (EE) and diameter of the nanofiber. The optimized batch selected by desirability approach was subjected to physicochemical characterization such as FTIR, DSC and PXRD which revealed entrapment of drug in a molecular dispersion form and devoid of any chemical interaction with the excipients. Electron microscopy showed the smooth structure in nanometre range, without any visible sign of fiber break-up or disruption within the nanofiber membrane. Optimized TNZ-PGHNF nanofiber membrane exhibited a diameter of 160.54±11.8 nm and EE 82.75±1.6%. *In vitro* release of TNZ from TNZ-PGHNF membrane in McIlvaine buffer pH, 6.6 showed sustained release up to 15 days by diffusion controlled process. Further, reduction of contact angle (from 123.6±2.8º to

55.2±1.6º) and increase in mucoadhesive force 120 gm/cm^2 (optimized batch) revealed that incorporation of GE enhanced the hydrophilicity as well as mucoadhesivity of the nanofiber membrane which would facilitate its adhesion to the site of action and instigate proliferation of cells. *In vitro* antibacterial study showed significant antimicrobial activity against *S. aureus*. Cytocompatibility with L-929 (mouse fibroblast) cell lines was also observed, and percentage haemolysis was found to be less than 4.54±0.43% for all batches. Further, TNZ-PGHNF were found physically and chemically stable over the storage period without any significant change ($p > 0.05$) in their physicochemical attributes when stored at room temperature (30±2 °C) and refrigerated condition (4±1 °C) whereas significant change ($p < 0.05$) was found at accelerated condition (40±2 °C/75±5% RH) of storage. Hence it is recommended that the nanofiber membrane should be stored at room temperature (30±2 °C) or at the refrigerated condition (4±1 °C). Moreover, *in vivo* study by ligature-induced periodontitis in rats confirmed that TNZ loaded nanofiber membrane can significantly ($p < 0.05$) improve continuity of epithelium and transseptal fiber of Interdental papilla in comparison to control group.

TNZ-PCHNF membrane was prepared by electrospinning method. A 3-level, 3-factor Box-Behnken design was employed for evaluating the influence of formulation and processing variables on quality of final formulation. Optimized nanofiber membrane was subjected to solid-state and surface characterization studies using FTIR, DSC, PXRD, SEM and AFM, which revealed that TNZ was entrapped in an amorphous form inside smooth and uniform cylindrical nanofibers without any physicochemical interaction with excipients. The optimized TNZ-PCHNF membrane had a diameter of 143.55±8.5 nm, entrapment efficiency of 83.25±1.8% and porosity of the nanofiber membranes were found in the range of 68% to 85%. Elemental analysis of TNZ-

PCHNF membrane indicated the presence of all the elements belonging to drugs and polymers and assured their homogeneous distribution. *In vitro* drug release and antibacterial study demonstrated capability of the developed nanofiber membranes for efficiently delivering TNZ in a sustained manner up to 18 days, and its ability to inhibit bacterial growth, respectively. Further, reduction of contact angle (from 123.4±2.5º to 27.4±2.3º) revealed that blending of CH with PCL increases hydrophilicity as well as mucoadhesivity of the nanofiber membrane. The mucoadhesive study demonstrated higher mucoadhesive detachment force (175 gm/cm^2) for optimized CH-PCL nanofiber membrane, as compared to GE-PCL nanofiber membrane (120 gm/cm^2). MTT assay and CLSM study suggested that optimized nanofiber membrane was devoid of cytotoxicity on mouse fibroblasts. The optimized TNZ-PCHNF were found physically and chemically stable over the storage time period without any significant change ($p > 0.05$) in their physicochemical attributes, stored at room temperature (30±2 °C / 65±5% RH) and the refrigerated condition (4±1 °C). On the other hand a significant change ($p < 0.05$) in the properties observed when the product was stored at accelerated condition (40±2 °C / 75±5% RH), suggesting instability due to biodegradation of polymer which would have expelled the drug molecule from nanofiber. Taking these results into consideration, it was strongly recommended that these nanofiber membrane should be stored at room temperature (30±2 °C) as well as refrigerated condition (4±1 °C), so as to hold the pharmaceutical properties intact for safe and effective long-term use. *In vivo* study by ligature-induced periodontitis in rat confirmed that TNZ loaded nanofiber membrane can significantly ($p < 0.05$) improve continuity of epithelium and transseptal fiber of Interdental papilla in comparison to control group. Furthermore, after developing and comparing all three types of nanofiber-based drug delivery systems, our results suggest that TNZ-PCHNF has a

better potential to deliver TNZ locally into the periodontal pockets, based on the homogenous distribution of diameter, porosity, hydrophilic and mucoadhesive nature of nanofiber, entrapment efficiency and drug release performance. Therefore, TNZ-PCHNF was further subjected to clinical evaluation in patients suffering from periodontitis to establish therapeutic potential of developed nanofiber membrane. Results of clinical studies on patients proved the therapeutic efficacy of the nanofiber membrane by eliciting a significant ($p < 0.05$) decrease in clinical markers of periodontitis like gingival index, clinical attachment level, bleeding on probing and probing pocket depth.

Thus, on the basis of our research findings, it could be concluded that the performance of the developed TNZ loaded CH-PCL hybrid nanofiber membrane with the hydrophilic and mucoadhesive property was found to be promising for the treatment of periodontal infections by prolonging the periodontal residence time and thereby better therapeutic effects. In addition, they provide intimate contact between dosage form and periodontal pocket which may result in high drug concentration in the local area. Hence, the developed TNZ loaded nanofiber drug delivery system is proved to be a novel approach for the better treatment of periodontal disease as it reduces the dose size, dosage frequency, dose-related side effects, bypasses the usual surgical procedures and improves patient compliance.