CHAPTER-7

Summary and conclusions

CHAPTER 7

Summary and Conclusions

7.1. SUMMARY

Periodontal diseases are already a serious public health concern all over the world due to its high prevalence and serious symptoms. The identification and treatment of patients at high risk for rapid progression of periodontitis is a major challenge for future research and lack of proper treatment will lead to permanent tooth loss. Teeth are the most important part of human body and have invaluable superficial value. Considering the limitations of systemic therapy, the current treatment strategies have moved to localized therapeutic modalities.

The work was performed with the hypothesis that crosslinked CS microspheres could be formulated by optimizing with scientific QbD approach which could help in screening of most desirable independent formulation variables and response variables, and minimize the traditional hectic experimental procedure involved in the formulation of crosslinked CS based multiparticulate drug delivery systems of OZ and DX. No any intrapocket formulations containing OZ and DX are available in the market worldwide. CS is non-toxic, biodegradable, biocompatible polymer but alone it could not control or prolong the drug release due to its hydrophilic properties. Thus, statistical QbD approach have been explored to develop and optimize CS crosslinked microspheres using different crosslinkers which could provide prolonged and sustained release of drugs within the periodontal pockets.

The experimental work is embodied in Chapter 4, 5, and 6. **Chapter 4** dealt with the preformulation studies assessing the feasibility of combination of OZ and DX in a single dosage form. FTIR studies revealed compatibility between two drugs for administration in a combined dosage form. Further, two UV method (Verodt's method (Method I) and Q-analysis method (Method II)) was developed and validated

for simultaneous analysis of both drugs when present in combined dosage form. Method I, involved solving of simultaneous equations and Method II, was based on iso-absorptive point of both the drugs *viz.* 292 nm. The λ_{max} of OZ was obtained at 319 nm and of DX at 274 nm in PBS pH 6.8. The linearity was observed in the concentration range of 5-25µg/ml (R^2>0.999) for OZ and 5-30µg/ml (R^2>0.999) for DX at all the selected wavelengths. The linearity, accuracy, precision, LOD and LOQ were found to comply ICH guidelines and both methods can be used for simultaneous estimation of both drugs in a formulation during routine analysis. Solubility studies revealed both drugs has sufficient solubility to get released in periodontal pocket fluids as represented by PBS pH 6.8.

Chapter 5, provided an account of design, optimization and characterization of crosslinked CS microspheres. CS microspheres were formulated using three types of crosslinkers such as SA (Part A) which forms polyelectrolyte complexes with CS; TPP (Part B) as ionic crosslinker; and GLU and VAN as covalent crosslinkers (Part C). *Part A*, provided deep insight into the captivating features of CS-Ca-SA over Ca-SA microspheres for the simultaneous controlled delivery of OZ and DX for the treatment of periodontitis. Microspheres were formulated by application of PBFD by employing collective effect of seven critical formulation variables *i.e.* SA, CS, CaC, SC, CT, AS and aqueous/oil PR on minimizing PS and burst release as well as for maximizing EE and $T_{80\%}$. Regression analysis (ANOVA) results approved the validity of the generated mathematical model. The significantly influencing variables included SA, AS, SC affecting PS; SA, CaC, PR affecting EE; SA, AS affecting burst release; and SA, CS affecting $T_{80\%}$ were screened by Pareto analysis. The optimized microspheres exhibited average PS of 257 µm, EE of 79.89 % with burst release of 14.36 % and $T_{80\%}$ of 72.43 h. Microspheres provided controlled and prolonged delivery of drugs for 120 h with desirable burst effect and followed non-Fickian (n>0.43) based diffusion and swelling controlled drug release. SEM images showed spherical to pear-shaped microspheres. EDXA plots evidenced the complexation of Ca^{2+} ions in the microstructure of CS-Ca-SA microspheres. FTIR spectra supported

ionic complexation between SA and CS. DSC and XRD studies ascertained the conversion of crystalline drugs into amorphous form after encapsulation into microspheres. CS-Ca-SA microspheres exhibited moderate swelling, less erosion and improved mucoadhesion properties as compared to Ca-SA microspheres. The optimized microspheres had acceptable neutral surface pH of 6.5±0.5. Antimicrobial activity ensured zones of inhibitions above the required standard values indicating activeness of optimized CS-Ca-SA microspheres against *E. coli* and *S. aureus*. SRB assay established cytocompatibility of optimized CS-Ca-SA microspheres on L929 mouse fibroblast cells suggesting their safety for intrapocket delivery. Results of stability studies advocated the prolonged stability and shelf-life under refrigeration (19.87 months) and room temperature (12.35 months) conditions as compared to high temperature conditions. The outcomes of study supported the success of dual polymer and dual drug based microspheres in periodontal drug delivery with the usefulness of the experimental design approaches for optimization of biodegradable, stable, biocompatible, mucoadhesive, controlled and prolonged drug release microspheres. However, due to use of dual polymers like CS and alginate large microspheres were obtained. Also, the drug release was prolonged only for 4 days with $T_{80\%}$ of 72 h.

Part B, dealt with the application of PBFD to investigate the effect of processing factors on the fabrication of ionically crosslinked CSTPP microspheres. Microspheres were screened for fabrication variables *viz.* MOP, CS, TPP, CT, AS and DT with the constraints of maximizing process yield (PY), %EE, and T_{80}% and minimizing burst and PS, for successful application in periodontitis. The optimum formulation variables were obtained as method B (o/w emulsion crosslinking), CS (2.5%w/w), TPP (5%w/w), CT (120 min), AS (2000 rpm), DT (freeze-dried) and provided PY- 95.67% w/w, PS- 168.45 µm, EEOZ- 85.56%, EEDX- 91.34%, BOZ- 15.26%, BDX- 12.91%, TOZ- 47.09 h and TDX- 67.95 h. Biphasic drug release was observed for four days with non-Fickian kinetics. FTIR illustrated compatibility between excipients and ionic complexation of CS and TPP. XRD and DSC showed loss of crystallinity of entrapped drugs in microspheres. Further, microspheres

exhibited good mucoadhesivity (82.51 %), antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, cytocompatibility for L929 cells and long-term stability particularly at refrigerated conditions. Although CSTPP microspheres showed great potential in the treatment of periodontal disease and were smaller in size than CS-Ca-SA microspheres they were weak as about 25 % microspheres got eroded during drug release period of 120 h.

In *Part C*, dual design approach based on screening by PBFD and final optimization using BBED was applied. QbD based covalently crosslinked CS microspheres loaded with DX had been developed using w/o emulsion crosslinking method and freeze-drying technique as optimized formulation variable in Part B. PBFD investigated and screened out the most significant variables influencing the fabrication of microspheres. The eight formulation variables chosen were CS, TCl (GLU or VAN), CLC, CT, AS SC, PR and OP (soyabean oil or liquid paraffin) optimized for PS, EE and $T_{80\%}$. The results concluded that a lower PS could be obtained at a high AS and SC and lower TCL and CS. Also, higher drug %EE could be achieved using CS, CLC, and CT. The prolonged drug release potential (as measured by $T_{80\%}$) was improved by increasing CS and CLC. No statistically significant effect of paraffin oil or soyabean oil had been observed. The optimized CSV microspheres were produced by the combined effect of all the significant factors with of PS of 75.45 µm, %EE of 88.87 % and $T_{80\%}$ of 10.41 days at CS (3.5%), TCL (VAN), CLC (6%), CT, 120 min, AS 2000 rpm, PR (1:4), SC (2.5%) and OP (soyabean oil). Both optimized CSV and corresponding CSG microspheres exhibited prolonged release for about 12 days. NMR and FTIR reaffirmed Schiff base formation (covalent bond) in VAN crosslinked microspheres. XRD plots indicated changes in the crystallinity of drug within microspheres. SEM images evidenced irregular CSG microspheres and spherical CSV microspheres with smooth surfaces. EDXA revealed the uniform elemental composition of microspheres. Moreover, CSV microspheres exhibited slow swelling with high mucoadhesion attributed to its high hydrophobicity and low degree of crosslinking than CSG microspheres. SRB assay demonstrated high

cytocompatibility of CSV than CSG microspheres against L929 cell lines based on calculated IC_{50} values. The optimized CSV microspheres maintained a significant zone of inhibition against selected microbes and prolonged stability at refrigerated condition.

After preliminary experiments and screening process, three critically selected independent variables were: CS, VS, and SC were evaluated at three levels (-, 0, +) using a response surface methodology BBED. The CSV microspheres were optimized for constraints of PS < 50 µm, EE > 80 %, *in-vitro* drug release ($T_{80\%}$) > 7 days and acceptable mucoadhesion (%M). The final optimized batch generated at CS-2.62 % w/w, VAN-5.52 % w/w, and SC-2.22 % w/v with PS of 49.95 µm, EEOZ-80.20 %, EEDX-82.73 %, TDX-8.29 days, TOZ-7.73 days, and %M-75.45 %.

In **Chapter 6,** systematic development of optimized CS-VAN microspheres loaded pluronic based *in-situ* gel forming implants (MLIG), containing OZ and DX. The optimization of both microspheres and MLIG was successfully carried out for G_{temp} and viscosity with target of attaining 34-37 °C and < 1000 cps respectively. The optimized MLIG implants exhibited G_{temp} of 35.23 °C and viscosity of 663.27 cps with formulation variables of P127 (15.93 % w/v), P68 (3.25 % w/v) and COM (35.38 % w/v) at desirability of 1. The *in-vitro* drug release study presented extended release of drugs for twelve days following non-Fickian type of drug release mechanism. The optimized MLIG implants exhibited acceptable pH, syringeability, and mucoadhesion. FTIR disclosed compatibility among drugs and polymers. DSC and XRD graphs observed alterations in crystallinity of drugs within microspheres however crystallinity of pluronics remained unaffected after formulation. SEM images disclosed crystalline surface of Pluronic® gels. Stability studies ascertained MLIG implants were sterilizable and stable for about 11.29 months on refrigeration. The formulations exhibited significant ($p<0.001$) antimicrobial activity against *Staphylococcus aureus, Escherichia coli*, and *Enterococcus faecalis*, and were found biocompatible and biodegradable during preclinical studies. Ligature-induced periodontal rat model,

corroborated significant growth ($p<0.05$) of gingival tissue after two weeks. Clinical trials revealed, intra-pocket administration of MLIG along with SRP provided significant reduction in clinical parameters as compared to SRP alone.

7.2. **CONCLUSION**

In conclusion, a successful attempt has been made to perform a complete study on CS based multiparticulate drug delivery systems of OZ and DX using systematic QbD approach. Both drugs cab be analyzed accurately and precisely by using developed UV methods which can also be a better substitute for chromatographic techniques for routine analysis. The PBFD emerged as the best screening design, rapid and efficient technique which produces minimum experimental runs, and is devoted to the evaluation of existing relationship between a cluster of controlled experimental variables and measured responses according to the selected criteria. Microspheres were easy to formulate, appeared cost-effective and required minimal sophisticated instruments for fabrication and characterization. Further the CS microspheres were injectable, mucoadhesive, biodegradable, and non-toxic due to use of all FDA approved polymers (chitosan, sodium alginate, tripolyphosphate, vanillin and pluronics).

Out of various all successful attempts of microspheres formulation, VAN crosslinked CS showed prolonged drug release for more than a week and can be used as a substitute as a crosslinker for toxic glutaraldehyde based on results of cytocompatibility studies, degree of crosslinking, swelling and mucoadhesion. These properties confirmed its high potential and applicability in chronic periodontitis.

Loading of CS-VAN crosslinked microspheres into in-situ gelling systems (MLIG implants) have provided benefits of small size, hydrogel properties and improved patient compliance together. The extensive research work concluded that formulated optimized MLIG implants are beneficial, in terms of biodegradability, biocompatibility, and safety as confirmed by *in-vivo* preclinical studies. The studies

also proved that multiparticulate systems are better alternatives to existing treatment methods including mechanical therapy (SRP) based on preclinical and clinical studies performed. Inclusion of dual antimicrobials in single drug delivery systems increased the spectrum of the antimicrobial activity against the microorganisms was confirmed by disc diffusion method.

Conclusively, all the desirable properties including stability, biocompatibility, biodegradability, mucoadhesivity, syringeability, broad antimicrobial spectrum, prolonged and controlled release as compared to existing or prior systems make MLIG implants more patients compliant, beneficial and cost-effective for the treatment of periodontal pocket infections. Such MLIG formulations has potential of reaching market after their scale-up and clinical testing on large scale of patients.