7. Summary

Different nanoformulations were developed to improve the antimicrobial activity of silver sulfadiazine and ciprofloxacin against the biofilm infection in diseases like burn wound and cystic fibrosis, respectively.

7.1. Silver sulfadiazine loaded solid lipid nanoparticles (SSD-SLNs)

SSD-SLNs were efficaciously prepared by double emulsification-sonication method. The process and formulation factors were optimized by Box-Behnken Design (BBD) using Design Expert. Further various techniques were utilized for physicochemical characterizations of SSD-SNLs. The optimized SSD-SLNs possessed 295.5±15.4 nm particles size, 0.261±0.023 PDI, -21.3±1.8 mV zeta potential and 75.9±3.4% entrapment efficiency. The SSD-SLNs had smooth and spherical shape and showed initial burst release up to 35.3±3.9% in 3 h followed by sustained release up to 83.1±2.6% of SSD in 24 h indicating sustained and diffusion controlled release of SSD from the SLNs. Further, findings of FT-IR revealed that, SSD and other components of the SSD-SLNs did not show any sign of potential physical or chemical interaction as most of the transmittance peaks specific to functional groups of SSD in FTIR spectra were also present in SSD-SLNs spectra. However, loss or broadening of sharp endothermic peak in DSC thermogram and diminished X ray diffraction intensity indicated either the conversion of crystalline SSD to amorphous or a uniform molecular distribution in a solid matrix of lipids characterization revealed that physicochemical interaction was absent amongst the component and the drug was amorphously dispersed in the nanoparticle matrix.

Furthermore, findings of antimicrobial evaluation demonstrated that SSD-SLNs maintained the minimum inhibitory concentration for prolonged period of time (48 h)

in comparison with pure drug. Amongst the different treatment groups, SSD-SLNs with DNase-I was more effective in treating the biofilm and had least minimum biofilm eradication concentration (0.5 µg/mL). Also, the efficacy of SSD-SLNs with DNase-I in reducing the established biofilm of the *P. aeruginosa* after three subsequent dosing (3 day) was highest (96.8%). Also, the biofilm thickness (24.6±3.4 µm) was minimum, when it was treated with SSD-SLNs with DNase-I. Besides, SSD-SLNs with DNae-I possessed the least fibroblast toxicity, indicating that the side effect of the SSD was reduced significantly when delivered in SLN with DNase-I enzyme.

The SSD-SLNS with DNase-I was further incorporated in the 1% chitosan gel for topical application on wounds, which exhibited the ideal mechanical property suitable for the topical application. Eventually, SSD-SLNs with DNase-I containing chitosan gel showed faster, complete and normal re-epithelialization of the wounds. The gel was also characterized for the rheological properties and found to have ideal mechanical properties suitable for topical application.

7.2. Alginate lyase conjugated chitosan nanoparticles of ciprofloxacin

The alginate lyase functionalized chitosan nanoparticles of ciprofloxacin were developed for the inhalational therapy of cystic fibrosis infections. The prepared functionalized nanoparticles were lyophilized and used after reconstitution. Initially, the chitosan nanoparticles were prepared by ionotropic gelation technique. Later, the alginate lyase was conjugated on nanoparticle's surface using the carbodiimide coupling. The developed nanoparticles exhibited desired quality attributes such as 205.5±9.0 nm particle size, 0.302±0.031 polydispersity index, 12.2±2.1 mV zeta potential, 51.8±2.1% entrapment efficiency and displayed spherical shape. Furthermore, the nanoparticles exhibited initial burst release up to 44.1±4.3% in first 3 h followed by sustained release up to 89.3±5.3% in 24 h and followed the Higuchi

release kinetics. Further, FTIR study suggested that the significant interaction which can cause loss of drug activity or toxicity was absent, as all the IR peaks signifying the CIPR functional group were intact in CIPR-CH-NPs spectra. Additionally, the loss of diffraction peaks in XRD spectra of CIPR-CH-NPs suggested that encapsulation of CIPR in chitosan nanoparticles resulted in amorphization or dispersion in molecular form in the chitosan matrix during the NPs formation.

Interestingly, nanoparticles demonstrated the prolonged microbial inhibitory action for 48 h (MIC 0.125 µg/mL) and were also efficient in preventing the bacterial surface adhesion and biofilm growth in planktonic bacterial suspension. Furthermore, the functionalized nanoparticles demonstrated the maximum anti-biofilm potential and completely eradicated the *P. aeruginosa* biofilm at least concentration of 0.5 µg/mL and also exhibited the complete biofilm elimination after 72 h on three successive in vitro dose at MIC (0.125 µg/mL). Moreover, the functionalized nanoparticles maximally reduced the biofilm thickness (14.2±2.03 µm) and biomass (9.1±1.3 $\mu m^3/\mu m^2$) to least value, consolidating the findings of above studies. Beside the improved antimicrobial efficacy, the optimized nanoparticles did not show any sign of in-vitro and in-vivo toxicity.