Chapter - 9

9. Summary and Conclusion

The goal of education should be the physical, mental, ethical & spiritual development of the Individual." - Mahatma Gandhi
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In situ gel forming systems are described as low-viscosity solutions that undergo phase transition in the respective physiological condition to form viscoelastic gels due to conformational changes of polymers in response to physiological environment.

We have explored development and optimization of in situ gelling systems for ocular and periodontal application. We have employed simultaneously ion sensitive gellan gum and mucoadhesive/pH sensitive carbopol 934P to formulate ocular in situ gelling systems containing Olopatadine HCl. We have also included benzododecenium bromide as corneal permeability enhancer. We have also employed simultaneously temperature sensitive poloxamer-407 and mucoadhesive/pH sensitive chitosan to formulate periodontal in situ gelling systems containing Doxycycline hyclate. We have also included PEG 600 as gelation temperature modulator for thermo-sensitive in situ gelling system. These three excipients together were being used to formulate and optimize in situ gelling system by Box-behnken Design.

The drugs and polymers were identified and characterized in our work were found to be pure as per UV, FTIR and DSC techniques. The compatibility study done by DSC, FTIR technique along with storage at 25°C and 40°C concluded that there was no interaction found between drug — excipient and excipient — excipient and hence they were used for developing in situ gelling system.

There was no significant change in drug content, viscosity, appearance and pH of in situ gelling systems after autoclaving. Double distilled deionized water was selected as vehicle for Ocular and Periodontal in situ gelling system based on the results obtained of good solubility and stability of Olopatadine HCl and Doxycycline hyclate in it.

The results of preliminary batches of in situ gelling systems inferred that combination approach would be beneficial for an effective in situ gelling system. The results of preliminary batches also helped us to decide the levels/concentration of polymers to be used for formulating optimum ocular and periodontal in situ gelling systems by Box-behnken design.
In order to optimize the in situ gelling systems Box-Behnken statistical screening design was used to statistically optimise the formulation parameters and evaluate effects of the formulation ingredients (independent variable) on various evaluation parameters (dependent variable-response) of in situ gelling systems. According to this design a total of 17 batches including five centre points were successfully formulated for ocular and periodontal in situ gelling systems. In the study of ocular in situ gelling system three independent variables selected were Gellan gum (0.25-0.75%), Carbopol 934P (0.15-0.45%) and Benzododecenium bromide (0.006-0.018%). In the study of periodontal in situ gelling system three independent variables selected were Chitosan (0.5-1.5%), Poloxamer 407(10-20%) and PEG 600(1-5%).

These 17 batches each of ocular and periodontal in situ gelling systems were successfully characterized and evaluated as described in chapter 7. These formulations were characterised for clarity, pH, flowability, gel strength, drug content, gelling capacity, mucoadhesive strength, rheological study and in vitro drug release. These formulations were also evaluated for getting various responses like viscosity at physiological and non-physiological condition, gel strength, mucoadhesive strength, in vitro drug release at 1 hr and t90%. Ocular in situ gelling systems were also evaluated for its trans-corneal permeation. Periodontal in situ gelling systems were also evaluated for gelation temperature and syringeability. Statistical significance of results was obtained considering P<0.01 as significant. The pH of all in-situ gelling formulation was found in satisfactory range of 5.5 and 4.6. The drug content of all batches of in situ gelling systems was found to be between 96.5 % and 103.0% which could be considered as acceptable and indicated uniform distribution of drugs in in-situ gelling formulations. It was also observed that when formulations were brought to physiological condition (pH 7.4 and 37 °C) increase in viscosity and clear gel was formed; which preserved its integrity without dissolving or eroding for a prolonged period of time which would facilitate sustained release of drug to the tissue. The viscosity of in situ gelling system at non-physiological condition was low enough that they could be easily administered through dropper or syringe for drug delivery. Hence it was concluded that in situ gelling system had viscosity that allowed easy administration and would also undergo a rapid sol-gel transition at physiological condition.
All the data obtained from characterization and evaluations were subjected to multiple regression analysis to yield second order polynomial equations. These analyses confirmed that selected independent variables significantly affected independent variables studied in our work. It was also concluded that model of each response had good $R^2$ values (> 0.91), high F-value and P-Value<0.0001 which indicated good correlation and significance of model for our study. Two-dimensional contour plots and three-dimensional response surface plots helped us to create an overlay plot which was applied to reach an optimal batch of in situ gelling system. The entire experimental design and polynomial equations were validated by preparing five check-point batches of in situ gelling systems which proved the high prognostic ability of the applied Box-behnken experimental design and Response Surface Methodology used in our work. The various constraints of responses were set to come down to an optimal point of responses for respective in situ gelling system.

**Ocular In Situ Gelling Systems**
The observed value for viscosity at physiological condition for all 17 batches O1-O17 of ocular in situ gelling system varied from 471 to 6500 cps. The gels studied exhibited shear thinning behavior after sustained shearing, i.e. as the shear rate increased the measured viscosity decreased. This result clearly demonstrated that viscosity at physiological condition was strongly affected by the polymers used in the study. Thus without increasing the concentration of individual polymer solution, the combination system could be administrated into the eye as drops and to form a stronger gel following the phase transition in ocular cul-de-sac of eye. The gel strength for all 17 batches O1-O17 varied significantly (P<0.01) from 23 to 58 sec. The effect of gellan gum on gel strength was 4 fold as compared to variable carbopol 934P which was as per our hypothesis that gellan gum could be used to improve the gel strength and thereby decrease the drainage of formulation and can withstand blinking movement occurring in eye. The mucoadhesive strength for all 17 batches O1-O17 varied significantly (P<0.01) from 9.3 to 33.3 gm. The effect of carbopol 934P on mucoadhesive strength was 3.5 fold as compared to gellan gum. This was in agreement with our hypothesis that combination of gellan gum and carbopol 934P had synergistic effect on mucadhesive strength.
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The in-vitro drug release studies observed that increasing the concentration of gellan gum from 0.25 to 0.75% and the carbopol 934P from 0.15 to 0.45% has markedly increased t90%. The t90% for all 17 batches O1-O17 varied significantly (P<0.01) from 584 to 1388 min. It was concluded that this combination approach made possible to sustain the release up to 24 hrs. Drug was released by Fickian (n < 0.5) as well as non Fickian (n > 0.5) diffusion mechanism as interpreted from the value of release exponent of korsmeyer-peppas model obtained from release kinetic data.

The permeability coefficient/rate of permeation for all 17 batches O1-O17 varied significantly (P<0.01) from 0.56 to 1.46 (x 10^{-5}) cm/sec. The rate of permeation of Olopatadine HCl solution was found to be only 0.456 x 10^{-5} cm/sec. The permeability characteristics of Olopatadine HCl through the excised cornea significantly (P<0.05) increased with the increased Benzododecenium bromide (permeation enhancer) concentration. Histopathological evaluation of corneal tissue showed neither cell necrosis nor removal of the epithelium from the cornea after permeation of in situ gel containing Olopatadine HCl. Thus, gel formulations seemed to be safe with respect to ocular administration.

Upon trading of various response variables in overlaying the contour plots, comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with gellan gum (X1) concentration of 0.48%, carbopol 934P (X2) concentration of 0.37% and Benzododecenium bromide (BDB – X3) concentration of 0.012% was found to fulfil the maximum requisite of an optimum formulation considering the applied constraints on Y1 to Y7 for ocular drug delivery system. This optimum in situ gelling system was then characterized, evaluated and compared with marketed preparation of Olopatadine HCl. The contact angle of optimized ocular in situ gelling system, marketed gel forming system and water was found to be 24°, 34° and 33° respectively on hydrophilic surface. Therefore optimized ocular in situ gelling system exhibited better spreading ability over time with respect to marketed gel forming preparation. This better spreadability would improve patient compliance and also enhance the permeation of drug across cornea.

Ocular irritation studies according to the Draize technique revealed no ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were not visible. Therefore, optimized ocular in situ gelling system of Olopatadine HCl was perceived
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as being suitable and safe for in vivo use. Olopatadine HCl retained its anti-histaminic and mast cell stabilizing activity when formulated as in situ gelling system and was similar to marketed preparation of Olopatadine HCl. It was observed from the results of *in vivo* contact time that optimized ocular in situ gelling system of Olopatadine HCl maintained as gel and was retained in eye for about 10 to 19 hours and better than marketed gel forming system and conventional eye drops of Olopatadine HCl.

**Periodontal In Situ Gelling System**

The observed value for viscosity at physiological condition for all 17 batches P1-P17 varied significantly (P<0.01) from 750 to 10,150 cps. The gels studied exhibited pseudoplastic flow. It was observed that without increasing the concentration of individual polymer solution, the mixed vehicle using poloxamer-407 and chitosan could be administrated into the periodontal pocket using syringe. The gel strength for all 17 batches P1-P17 varied significantly (P<0.01) from 12 to 50 sec. On increasing the concentration of chitosan a firm gel was produced which resisted piston for about 50 seconds. The mucoadhesive strength for all 17 batches P1-P17 varied significantly (P<0.01) from 1960 to 13230 dyne/sq.cm. The effect of chitosan on mucoadhesive strength was 4 fold as compared to poloxamer-407. It was concluded that combination of chitosan and poloxamer-407 had synergistic effect on mucadhesive strength. The *in-vitro* drug release studies observed that increasing the concentration of poloxamer and chitosan has markedly increased t90%. The t90% for all 17 batches P1-P17 varied significantly (P<0.01) from 603 to 1423 min. It was concluded that this combination approach made possible to sustain the release up to 24 hrs. Drug was released by Fickian (n < 0.5) as well as non Fickian (n > 0.5) diffusion mechanism as interpreted from the value of release exponent of Korsemeyer-Peppas model. It was also observed that 10-15% of Doxycycline hyclate was released in one hour which was above MIC of Doxycycline hyclate. The gelation temperature for all 17 batches P1-P17 widely varied significantly (P<0.01) from 22 to 59 °C. In the gelation temperature study as the concentration of polymers increased, the gelation temperature of gel decreased. It was concluded that periodontal in situ gelling system having gelation temperature 35–37 °C and remaining in liquid form at room/storage temperature of 25°C could be developed using PEG 600 and chitosan even when low

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content of poloxamer-407 was used. It was also concluded that desired syringeability was achieved to administer in situ gel into periodontal pocket by manipulating the concentration of chitosan and PEG 600 as both these polymers did not much affect syringeability.

Upon trading of various response variables in overlaying the contour plots; the formulation composition with chitosan (X1) concentration of 1.1 %, carbopol 934P (X2) concentration of 14.8 % and PEG 600 (X3) concentration of 2.0 % was found to fulfil the maximum requisite of an optimum formulation considering the applied constraints on Y1 to Y8 for periodontal drug delivery system. This optimum in situ gelling system was then characterized, evaluated and compared with marketed preparation of Doxycycline hyclate. Antimicrobial activity of the optimized periodontal in-situ gelling formulation against *Staphylococcus aureus* and *Escherichia coli* showed the Zone of Inhibition values either similar or higher than the ZOI values of the marketed preparation of Doxycycline hyclate. The optimized periodontal in situ gelling system had sufficient viscosity that it could be delivered through syringe and formed a stiff gel (viscosity 4900 cps) at physiological condition (35 °C and pH 7.4) with sufficient gel and mucoadhesive strength. The optimized periodontal in situ gelling system could sustain the drug release up to 24 hours with $t_{90\%}$ of about 1200 min. The gelation temperature of optimized periodontal in situ gelling system was found to be $34 \pm 1{\degree}C$.

It was found that both optimized in situ gelling systems were isotonic and sterile hence would not cause irritation on administration. The results of Texture Profile Analysis (TPA) experiments concluded that optimized formulations had suitable mechanical properties for ocular administration that would prolong pre-corneal residence time and would not cause crusting of eye lids or blurring of vision. The results of TPA experiments concluded for optimized Doxycycline hyclate in situ gelling systems also had suitable mechanical properties for syringeable delivery that would prolong residence time in periodontal pocket.

Stability studies of optimized in-situ gelling systems were conducted as per ICH guidelines ($75\% \pm 5$ RH, $40\pm2{\degree}C$ for 180 days). It was concluded from the stability results that no significant change was observed in optimized in situ gelling system
after 6 months with respect to its drug content, viscosity pH and physical appearance. Since the overall degradation was < 5%, a tentative shelf life of 2 years was assigned to the optimized in situ gelling systems.

The developed optimized in situ gelling systems were easy to manufacture on large scale and were also cost effective as compared to other novel dosage forms. These developed and optimized in situ gelling systems could be easily administered through dropper and syringe. Both optimized in situ gelling systems were therapeutically efficacious, sustained drug release for 24 hours with sufficient gel and mucoadhesive strength to reside at respective physiological site. These optimized in situ gelling systems would be a once a day application dosage form, so it would also improve patient compliance in the treatment of all types of conjunctivitis and periodontitis. Hence we concluded that an optimum ocular in situ gelling system was formulated developed and optimized using gellan gum, carbopol 934P and corneal permeability enhancer benzododecinium bromide. The optimized ocular in situ gel also had sufficient permeability across cornea so it would prove beneficial in treatment for conjunctivitis of posterior segment of eye. The optimized ocular in situ gelling system was stable, safe and non-irritant hence it could be a viable alternative to conventional eye drops of Olopatadine HCl.

We finally also concluded that an optimum periodontal in situ gelling system was formulated developed and optimized using poloxamer-407, chitosan and PEG 600. The optimized periodontal in situ gel also had sufficient gelation temperature and syringeability. This optimized periodontal in situ gel maintained minimum inhibitory concentration of Doxycycline hyclate for 24 hours with 2.0% of Doxycycline hyclate in optimized formulation. The marketed preparation contained 4.0% of Doxycycline hyclate so it has also reduced the dose of Doxycycline hyclate which would be beneficial for patients from safety and economy point of view. The optimized periodontal in situ gelling system was stable, safe and non-irritant hence it could be a viable alternative to conventional gel or patch or film of Doxycycline hyclate.

We also concluded that concept of in situ gelling system has a strong potential and served as a very good platform for improvement of bioavailability of drugs to ocular and periodontal cavity.