Chapter 8
Summary and Conclusion

SUMMARY AND CONCLUSION

Without any hesitations, we are at a phase where we need improved formulations and treatments for combating life threatening diseases. The ongoing research against this endeavoring battle has lead to more breakthroughs in development of vital therapies but lack of efficient drug delivery systems many a times become a rate limiting step in efficient transfer of them in practice. Moreover, developing systems that can epitomize objectives like enhance patient compliance, minimize drug-drug interaction, reduce costs, improve biopharmaceutical properties, etc. would be of prodigious concern in improving therapeutic treatment.

The objective of the research work envisaged was to develop and evaluate various enterosoluble formulations of drugs for the treatment of Tuberculosis, Ulcers and Heart disease. The rationales for developing such formulations are summarized as below:

1. To minimize drug-drug interaction of two drugs by segregated drug delivery.
2. To protect the acid labile drug from acidic stomach environment and specifically targeting its release in intestine.
3. To improve solubility and prevent precipitation of weakly basic drug at intestinal pH.

In view of accomplishing above rationales, research work was bifurcated into three parts. The first part comprises of Quality by Design development of various enteric coated sustained release formulations of Isoniazid (INH) and study its interaction with developed rifampicin (RIF) formulation from its fixed dose combination. The RIF extended release floating dosage form was also developed as an add on formulation to minimize its individual drawbacks. The foundation was to segregate their zones of delivery with simultaneously providing extended release at their preferable site of absorption. The outcome of the novel fabricated dosage form revealed that it not only averted drawbacks related to individual drugs but also significantly reduced formation of 3-Formyl rifamycin; major degradant of RIF.

The goal of the second part was to formulate gastroresistant microparticles of lansoprazole (LSP) in single step employing spray drying technique to minimize its processing steps than existing commercial processes for preparation of tablets and pellets dosage forms. Additional goal was also to address solubility issues related to it. The outcome of the work reveals good resistance from developed novel

formulation together with significant enhancement in solubility as compared to plain drug.

The motive of the third part was to prevent precipitation of weakly basic drug dipyridamole (DPL) at intestinal pH by preparation of its solid dispersion with diverse polymers and complex formation with pH modifier. The outcome of the work revealed significant improvement in dissolution as compared to plain drug from solid dispersion prepared HPMC E50 and complex prepared from fumaric acid. Moreover, the said formulations significantly prevented precipitation and extended supersaturation of dipyridamole upon acid to neutral pH transition.

The key highlights of the research work are enlisted below:

- **Part A: Segregated drug delivery of two incompatible drugs: RIF and INH**

1. Quality by design development and optimization of floating sustained release RIF tablet for site specific delivery to stomach.
2. Quality by design development and optimization of enteric coated sustained release INH tablet for site specific delivery to intestine.
3. Quality by design development and optimization of enteric coated sustained release INH multiparticulate system i.e. pellets for site specific delivery to intestine.

- **Part B: Acid labile drug that require to be release in intestine: LSP**

1. Preparation and optimization of spray dried enteric microparticles of LSP

- **Part C: Prevent precipitation of weakly basic drug at intestinal pH: DPL**

1. Preparation of solid dispersion of weakly basic drug DPL with diverse polymers.
2. Preparation of DPL complex with pH modifier.

The brief details of each of the three parts are summarized below:

PART A: SEGREGATED DRUG DELIVERY OF TWO INCOMPATIBLE DRUGS

Drugs: Isoniazid (INH) and Rifampicin (RIF)

The objective of the study was to prepare two enteric coated sustained release formulations of INH; tablet and pellets and study its interaction with RIF from its fixed dose combination.

1. Quality by design (QbD) development and optimization of enteric coated sustained release tablet of INH

Quality Target Product Profile (QTPP) was selected as it ought to form the basis for determining the critical quality attributes (CQAs), critical process parameters and control strategy. Next CQAs were identified. Then an overall risk assessment of the drug product formulation components was performed using the Failure Modes and Effects Analysis (FMEA) method, which could identify the failure modes that have the greatest chance of causing product failure, i.e., not meeting the QTPP. The relative risk that each drug substance attributes presents was ranked according to risk priority number (RPN). Those attributes that could have a high impact on the drug product attributes were studied in detail whereas those attributes that had low impact on the drug product attributes required no further investigation. In the present study, the RPN ≥40 was considered as high risk, ≥20 to <40 was considered as medium risk and < 20 was considered as low risk. In an endeavor to accomplish the objectives of QbD, response surface methodology using 2^3 full factorial experimental design was applied for optimizing the core tablet formula. Amount of Polyox WSR 303 (A), hardness (B) and amount of ethyl cellulose (C) were taken as independent variables and percent drug release at 1^{st} hour (Q3), 2^{nd} hour (Q4), 4^{th} hour (Q6) and 8^{th} hour (Q10) in phosphate buffer pH 6.8 as dependent variables. The tablets were prepared by wet granulation technique. Before performing experimental design, preliminary trials were undertaken for selection of manufacturing method, type of binder and type of Polyox.

The preliminary trials revealed that wet granulation approach was more advantageous than direct compression method in controlling the drug release of highly water soluble INH. Moreover, hydrophobic binder ethyl cellulose (EC) was more efficient in retarding drug release than hydrophilic binders (PVP K30 and HPC). Notably, no impact of granule size was observed on percent drug release which might be due to high water solubility of the drug together with its high percent loading. Probably, the

high diffusion co-efficient of highly water soluble drug may have quash the effect of granule size.

The results of full factorial design revealed hardness as the major factor affecting percent drug release and was inversely proportional to it at Q3, Q4 and Q6. The results obtained surprisingly depict that there was no pronounced decrease in drug release on increasing binder concentration. Interaction effects were seen between A and C for percent drug release at Q3 and Q6. The same was inferred from p value of ANOVA, pareto chart, half normal probability plot and interaction plots. The increase in Polyox WSR 303 concentration affected the release pattern but did not prolong the release. Akaike information criteria and Model selection criteria revealed that the model was best described by Korsmeyer-Peppas power law. To prevent INH release in gastric conditions, the core tablets were seal coated with HPMC followed by enteric coating with Eudragit L-100-55 and examined for gastric resistance. Enteric coating of 10% w/w was found to be optimum to achieve zero percentage of drug release in 0.1 N hydrochloric acid and similar dissolution profile as of core tablet in pH 6.8 phosphate buffer. Lastly, capability analysis was performed on reproducibility batches of optimized formulation to investigate spread of process. Anderson-Darling test, Ryan-Joiner and Kolmogorov-Smirnov test revealed normal distribution of the data. All capability indices were above 1.33 signifying process was within control of producing batches as per desired specifications. The accelerated (40±2 ◦C/75±5% relative humidity) and long term stability studies (25±2 ◦C/60 ± 5% relative humidity) for 3 months suggested negligible changes in the formulation packed in the selected packaging material.

Finally, the design space was established and control strategy was developed to mitigate the risk in future. The RPN of updated risk assessment depicted that all the failure modes were in low risk category.

2. Quality by design (QbD) development and optimization of enteric coated sustained release pellets of INH:

Initially, QTPP was selected and CQAs were identified as discussed earlier. Then an overall risk assessment of the drug product formulation components was performed using the FMEA method, which could identify the failure modes that have the greatest chance of causing product failure, i.e., not meeting the QTPP. The relative risk that

each drug substance attributes presents was ranked according to RPN as discussed earlier.

Preliminary trials were undertaken to screen polymer not only feasible for extrusion but also provide sustain action from matrix pellets in pH 6.8 phosphate buffer. Out of the six polymers screened, glyceryl behenate showed the slowest release which was taken up in the subsequent trials for further improvement. In the next set of trials, type of binder was evaluated and results showed that HPMC K4M depicted the maximum slow down in the dissolution in comparison to other binders. Lastly, combinations of various polymers were explored to further retard the drug release and results revealed that batch containing glyceryl behenate and carbopol 934P was found to be optimum. The optimized batch showed good usable yield and aspect ratio together with low abrasion resistance. Akaike information criteria and Model selection criteria revealed that the model was best described by first order kinetics. Enteric coating optimization disclosed that 20% w/w was found to be optimum to achieve zero percentage of drug release in 0.1 N hydrochloric acid and similar dissolution profile as of core pellets in pH 6.8 phosphate buffer. The accelerated (40±2 ◦C/75±5% relative humidity) and long term stability studies (25±2 ◦C/60 ± 5% relative humidity) for 3 months suggested negligible changes in the formulation packed in the selected packaging material.

Finally, the design space was established and control strategy was developed to mitigate the risk in future. The RPN of updated risk assessment depicted that all the failure modes were in low risk category.

3. Quality by design (QbD) development and optimization of RIF gastroretentive tablet:

Initially, QTPP was selected and CQAs were identified as discussed earlier. Then an overall risk assessment of the drug product formulation components was performed using the FMEA method, which could identify the failure modes that have the greatest chance of causing product failure, i.e., not meeting the QTPP. The relative risk that each drug substance attributes presents was ranked according to RPN as discussed earlier. Preliminary trials were undertaken to screen type of wax and concentration, amount of gas generating agent and combinations of various release enhancers with wax. From the preliminary trials, results revealed glyceryl behenate as suitable wax

for matrix forming and HPMC E5 as suitable pore former. The amount of gas generating agent in reducing floating lag time was also evaluated. Further response surface methodology using BBD was applied for evaluating the failure modes with high RPN and defining the relationships between input variables and quality traits desired. Amount of sodium bicarbonate (X1), pore former HPMC (X2) and glyceryl behenate (X3) were selected as independent variables and percent drug release at 1st hour (Q1), 4th hour (Q4), 8th hour (Q8) and floating lag time (min) as dependent variables. The tablets were prepared by direct compression technique and thermally sintered for 20 minutes at 80^{0}C for melting and redistribution of wax. Main effects and interaction plots were generated to study effects of variables. Selection of the optimized formulation was done using desirability function and overlay contour plots. The optimized formulation exhibited percent release at Q1 of 20.9%, Q4 of 59.1%, Q8 of 94.8% and floating lag time of 4.0 min. The optimization process suggests that floating lag time was majorly dependant on concentration of sodium bicarbonate and exhibited inverse correlation with it. The surprising findings were that it was also dependant on amount of glyceryl behenate. The percent release at Q1, Q4 and Q8 time points were directly proportional to HPMC amount and inversely proportional to amount of glyceryl behenate. There is an optimum range and ratio of glyceryl behenate and HPMC where you can get desired percentage release in the constraint range which was represented by overlay contour plots. The mathematical analysis of different drug release models indicated that the drug release follows Korsmeyer-peppas power law equation with anomalous transport mechanism. The Akaike information criteria and Model selection criteria were also in agreement stating drug release can be best described by Korsmeyer-peppas power law equation. Fourier transform infra-red (FT-IR) spectroscopy study, Differential scanning calorimetry (DSC) study and Powder X-Ray Diffraction (PXRD) study studies ruled out the possibilities of polymorphic transition and formation of solid solution due to thermal treatment. Finally, capability analysis was performed on reproducibility batches of optimized formulation to investigate spread of process. Anderson-Darling test, Ryan-Joiner and Kolmogorov-Smirnov test revealed normal distribution of the data. All capability indices were above 1.33 signifying process was within control of producing batches as per desired specifications. The accelerated (40±2 ◦C/75±5% relative humidity) and long term stability studies (25±2 ◦C/60 ± 5% relative humidity) as per ICH Q1 A (R2) conditions was conducted for 3 months. Results suggested negligible

changes in the formulation packed in the selected packaging material. Finally, the design space was established and control strategy was developed to mitigate the risk in future. The RPN of updated risk assessment depicted that all the failure modes were in low risk category.

***In vitro* drug degradation from novel dosage form**

Fig.1 portrays the RIF and INH fixed dose combination final formulation schematic diagram

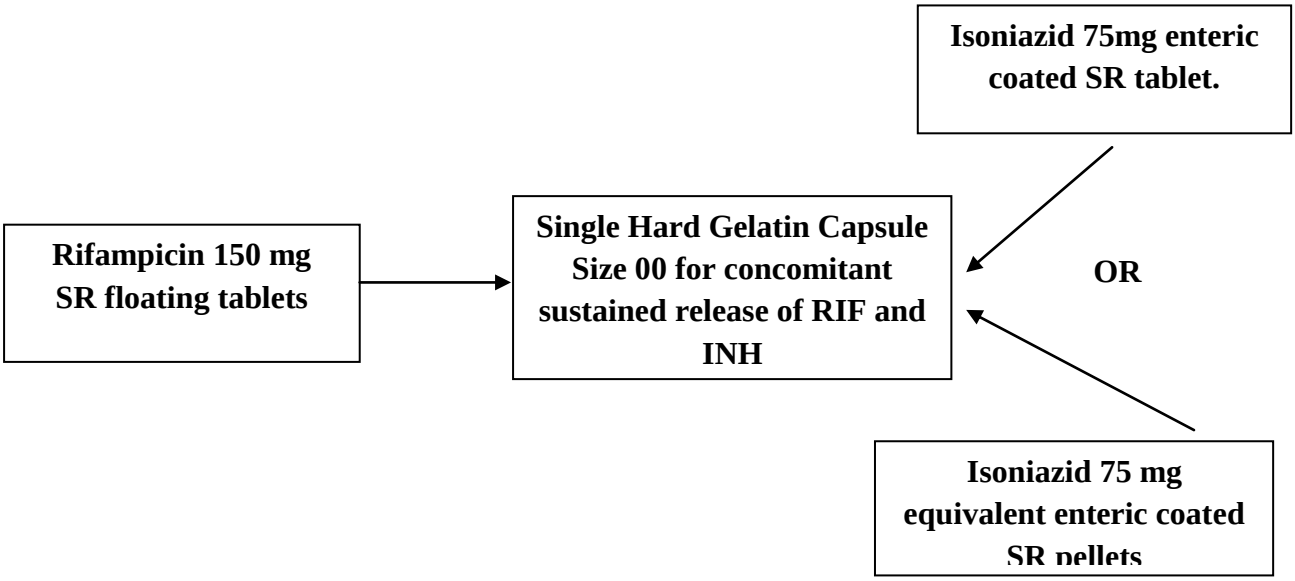

Fig.1: RIF and INH fixed dose combination final formulation schematic diagram

Shishoo et al reported that around 12% of RIF degraded to 3FRSV in acidic medium in 45 minutes from 150 mg dose of RIF [1] and Gohel and Sarvaiya reported 22% of 3FRSV formation in acidic medium from 300 mg dose of RIF in 75 minutes [2].

On the while Shishoo et al reported around 21% of RIF degradation in 45 minutes when a RIF release study was performed in the presence of INH. Furthermore, Singh et al reported that 17% to 24% of RIF degradation in 0.1 N HCl at 37^{o}C in 50 minutes when RIF was released with INH [3].

The results of *in vitro* degradation study of developed novel extended release dosage forms are represented in Table 1.

Table 1: *In vitro* degradation study of RIF

Formulation	Percentage of RIF degraded to 3-FRSV
RIF powder 150 mg	11.12%
RIF developed floating sustained release tablet 150 mg	6.53%
RIF developed floating sustained release tablet 150 mg+ INH enteric coated sustained release tablet 75mg	6.95%
RIF developed floating sustained release tablet 150 mg + INH enteric coated sustained release pellets 75 mg	6.65%

From Table 1 it can be concluded that degradation was minimized to great extent from developed novel extended release formulation. This diminish degradation could be due to sustained release of RIF in the acidic medium and segregating RIF and INH zones of drug release and delivery. The results of the research envisaged reveal the effectiveness of novel dosage form segregating drug delivery of RIF and INH and thereby reducing the degradation of RIF in presence of INH. Thus, delivering INH in the intestine and its enteric coating is justified.

PART B: ACID LABILE DRUG THAT REQUIRE TO BE RELEASE IN INTESTINE

Drug: Lansoprazole (LSP)

LSP, a BCS class II compound belongs to the category of compounds called proton pump inhibitors (PPIs). It is well recognized that the LSP and other PPIs are susceptible to degradation in acidic media. The research envisaged focuses on preparation of enterosoluble microparticles in single step by spray drying procedure that not only protects LSP from acidic pH but also addresses its solubility issues.

The spray drying operation was performed using a laboratory scale spray dryer. In general, polymer was dispersed in half quantity of required water which was subsequently neutralized by addition of sodium hydroxide. To this neutralized solution of enteric polymer, finely grounded LSP (sifted through ASTM # 150) was slowly added with continuous stirring and dispersed into it. Finally, the water was added to adjust the desired feed solution concentration.

Preliminary trails were under taken to optimize type of enteric polymers and its various levels. The results revealed Eudragit S 100 as the paramount polymer giving highest gastric resistance in comparison to other enteric polymers. Moreover, the findings also disclosed that entrapment efficiency was independent of Eudragit grades and thus not affected by type of Eudragit. Further trials included incorporation of plasticizer triethyl citrate and combinations of other polymers with Eudragit S100. Incorporation of plasticizer not only influenced entrapment efficiency but also diminished gastric resistance severely. The reason may be that incorporation of the plasticizer lowered the glass transition temperature (Tg) of the polymer and simultaneously increased the permeability of the microparticles membrane resulting in more ingress of the acid inside the microparticles and augmented drug release in acidic media. On the contrary, polymeric combinations reduced entrapment efficiency for both sodium alginate and glyceryl behenate but significantly influenced gastric resistance for only sodium alginate and not for glyceryl behenate.

Finally, various process parameters were varied to investigate its effect on microparticles properties. The optimized process parameters comprised of inlet temperature of 150°C, atomizing air pressure of 2 kg/cm^2, feed solution concentration of 6%w/w, feed solution spray rate of 3ml/min and aspirator volume of 90%. The microparticles resulted in significant enhancement of gastric resistance together with improved solubility in the dissolution medium pH 7.4 phosphate buffer. The scanning electron microscopy analysis revealed smooth and spherical shape morphologies. DSC study revealed absence of melting endotherm as well degradation exotherm of drug and PXRD study revealed halo diffractogram revealing amorphous nature of the drug. FT-IR study ruled out the possibility of interaction between drug and polymer indicating that the LSP was physically dispersed in the Eudragit S-100. Regarding stability, the product was found to be stable under 3 months accelerated (40±2 ◦C/75±5% relative humidity) and long term (25±2 ◦C/60 ± 5% relative humidity) stability conditions. Moreover, the manufacturing method employed can be easily adopted in industries.

PART C: PREVENT PRECIPITATION OF WEAKLY BASIC DRUG AT INTESTINAL pH

Drug: Dipyridamole (DPL)

Amorphous DPL, solid dispersions and complex formation was prepared by melting the drug or drug and excipients in a stainless steel beaker at about 168 °C and consequent quench cooling of the melt over crushed ice. The drug and excipients were physically mixed by triturating using mortar and pestle. The quench cooled product was ground and sieved (ASTM 45 #). HPMC and PVP were investigated to prepare solid dispersions and fumaric acid for complex formation. For HPMC, three viscosity grades were explored viz; HPMC E5, HPMC E15 and HPMC E 50 and for PVP two grades were investigated PVP K-30 and PVP K-90.

In this study, polymers having discrete hydrogen bond characteristics were investigated; eg., PVP which is proton acceptor and HPMC which is proton donor for evaluating intermolecular interactions. Complex was also prepared with fumaric acid which acts as a pH modifier and may increase the solubility in the neutral pH. Three different viscosity grades of HPMC and two grades of PVP were also investigated to study effect of higher molecular weight of homologous series on precipitation. The results revealed that glassy DPL sank in the phosphate buffer and precipitated in short time achieving equilibrium solubility to that of crystalline drug. Solid dispersions showed enhanced solubility with all the polymeric excipients. The DSC and PXRD study revealed that the drug was present as amorphous form inside polymeric matrix of PVP or HPMC. The acid to neutral pH transition dissolution study revealed the importance of intermolecular interactions between DPL and polymers. The HPMC E50 was found out to be the best polymer for inhibiting precipitation and extending the supersaturation. The effect of molecular weight was prominently seen in case of HPMC but not in case of PVP which may be due to interaction between drug and HPMC which was confirmed from FT-IR studies. Finally, the rapid disintegrating tablets were prepared with HPMC E50 and results revealed significant improvement in dissolution as compared to conventional DPL tablet which was clearly evident from mean dissolution time, cumulative percent drug released at 30 minutes and dissolution efficiency values of prepared formulations.

Preparation of complex with fumaric acid also increased solubility and prevented precipitation and extended supersaturation upon acid to neutral pH transition similar

to that of HPMC E50. Rapid disintegrating tablets prepared with fumaric acid also resulted in significant improvement in dissolution as compared to conventional DPL tablet.

Results of the *in vitro* Caco-2 cells transport studies showed that the enhancement of permeation for different DPL formulations as compared to plain drug. The P_{app} increased for different formulations as compare to plain drug. Moreover the cytotoxicity study using MTT assay revealed that the tested excipient concentrations were found to be non-toxic to the cell monolayer.

In nutshell, judicious selection of polymer is needed for preparing SD/complex which can stabilize and maintain supersaturation of DPL for longer period of time. The formulations develop with HPMC E50 and fumaric acid can be a promising alternative method for attaining greater supersaturation and prevent precipitation of weakly basic drug upon acid to neutral pH transition.