Chapter 9
Publications

List of Publications

Research Publications

1. **Chintan Vora**, Riddhish Patadia, Karan Mittal and Rajashree Mashru. Risk Based Approach for Design and Optimization of Stomach Specific Delivery of Rifampicin. ***International Journal of Pharmaceutics,*** 2013, 455, 169-181 (Copy Attached).

2. **Chintan Vora**, Riddhish Patadia, Karan Mittal and Rajashree Mashru. Risk Based Approach for Design and Optimization of Site Specific Delivery of Isoniazid. ***Journal of Pharmaceutical Investigation***, Accepted (Copy Attached).

3. **Chintan Vora**, Riddhish Patadia, Karan Mittal and Rajashree Mashru. Formulation Development, Process Optimization and *In Vitro* Characterization of Spray Dried Lansoprazole Enteric Microparticles. ***Scientia Pharmaceutica*.** **(Manuscript Submitted)**

Review Publications

1. **Chintan Vora**, Riddhish Patadia, Karan Mittal and Rajashree Mashru. Recent Patents and Advances on Anti - Tuberculosis Drug Delivery and Formulations. ***Recent Patents on Drug Delivery & Formulation,*** 2013, 7, 138-149 (Copy Attached).

Conferences/Workshops Attended

1. Dissolution Studies, Workshop organized by LABINDIA, ELECTROLAB and Parul Institute of Pharmacy, Vadodara, September 2012.
2. Approaches in New Drug Discovery, AICTE sponsored National Seminar, organized by Pharmacy Department, The M.S. University of Baroda, Vadodara, February 2013.
3. QbD : An Essential Requirement in Pharmacy, National conference organized by Gujarat technological university, Ahmedabad and supported by De Montfort University ,& Parul Pharmacy held at Parul Institute of Pharmacy, Vadodara, March 2013.
4. Protection of Intellectual Property Rights: Patent or Perish, UGC sponsored national seminar, Pharmacy Department, The M.S. University of Baroda, Vadodara, January 2014.

International Journal of Pharmaceutics 455 (2013) 169–181

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Risk based approach for design and optimization of stomach specific delivery of rifampicin

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ARTICLE INFO

Article history:
Received 26 April 2013
Received in revised form 12 July 2013
Accepted 15 July 2013
Available online 31 July 2013

Keywords:
Risk management
Gastroretention
Failure mode and effects analysis
Box–Behnken design
Desirability function
Overlay contour plots

ABSTRACT

The research envisaged focuses on risk management approach for better recognizing the risks, ways to mitigate them and propose a control strategy for the development of rifampicin gastroretentive tablets. Risk assessment using failure mode and effects analysis (FMEA) was done to depict the effects of specific failure modes related to respective formulation/process variable. A Box–Behnken design was used to investigate the effect of amount of sodium bicarbonate (X1), pore former HPMC (X2) and glyceryl behenate (X3) on percent drug release at 1st hour (Q1), 4th hour (Q4), 8th hour (Q8) and floating lag time (min). 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 Q1 of 20.9%, Q4 of 59.1%, Q8 of 94.8% and floating lag time of 4.0 min. Akaike information criteria and Model selection criteria revealed that the model was best described by Korsmeyer–Peppas power law. The residual plots demonstrated no existence of non-normality, skewness or outliers. The composite desirability for optimized formulation computed using equations and software were 0.84 and 0.86 respectively. FTIR, DSC and PXRD studies ruled out drug polymer interaction due to thermal treatment.

1. Introduction

Though the realm of drug delivery system (DDS) has metamorphosed from conventional oral solid dosage form to intricate DDS, oral route has explicitly been the most sought by pharmaceutical industries and patients. Amongst the myriad of oral DDS controlled release (CR) formulations endows to be of greater interest to formulation scientists and pharmaceutical industry (du Toit et al., 2006; Singh et al., 2005). Though CR drug delivery offers many benefits, for certain drug substances, prolonging the gastric retention is desirable for achieving greater therapeutic benefit. The gastroretentive DDS can improve the controlled delivery of the drugs by continuously releasing the drug for a prolonged period at the absorption site thus ensuring its optimal bioavailability (Rouge et al., 1996).

Tuberculosis (TB) is one of the major infectious killers since decades throughout the world. TB occurs in every part of the world and is considered as worldwide crisis from many years. With respect to case notifications, 5.8 million newly diagnosed cases were notified to national TB control programmes conducted by World Health Organization (WHO) in 2011(Vora et al., 2013). Rifampicin (RIF) is the vital component in the current therapeutic treatment for TB having excellent effect even on dormant TB bacilli and is currently one of the frontline drugs recommended by WHO. Though it has excellent anti TB activity, it has many pitfalls like short half-life (1.5 to 4.5 h), adverse effects, pH-dependent degradation, bioavailability problems and concentration dependent autoinduction of its own metabolism which ends up in decrease in its bioavailability after repeated oral administrations (Hiremath and Saha, 2004). Concerning its solubility it is reported that RIF is more soluble at low pH (pH 1.5, 1 in 5 of 0.1 M HCl, at 37 °C), while it is less soluble at higher pH (pH 7.4, 1 in 100 of phosphate buffer, at 37 °C) (Gallo and Radeilli, 1976). Prankerd et al. (1992) reported solubility of RIF at 25 °C is 1 in ~10, 250, and 360 parts of water at pH 2, 5.3, and 7.5, respectively. Concerning its permeability, Mariappan and Singh (2003) reported that RIF exhibits permeability in stomach. It is also stated that site specific sustained drug delivery in stomach of RIF could be beneficial in improving its bioavailability (du Toit et al., 2006; Shishoo et al., 2001). This all reveal that

Abbreviations: FMEA, Failure Mode and Effects Analysis; DDS, Drug Delivery System; CR, Controlled Release; TB, Tuberculosis; WHO, World Health Organization; RIF, Rifampicin; MIC, Minimum Inhibitory Concentration; QbD, Quality by Design; BBD, Box–Behnken designs; HPMC, Hydroxy Propyl Methyl Cellulose; QTPP, Quality Target Product Profile; ICH, International Conference on Harmonization; CQA, Critical Quality Attributes; RPN, Risk Priority Number; DOE, Design of Experiments; USP, United States Pharmacopoeia; AIC, Akaike Information Criterion; MSC, Model Selection Criteria; SSR, Sum of the Squared Residuals; FT-IR, Fourier Transform Infra Red; DSC, Differential Scanning Calorimetry; PXRD, Powder X-Ray Diffraction; SEM, Scanning Electron Microscopy; HDPE, High Density Polyethylene; ES, Experimental Sequence; SD, Standard Deviation.

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http://dx.doi.org/10.1016/j.ijpharm.2013.07.043

RIF has high solubility in acidic pH and is better absorbed from stomach which demanded and necessitated the development of RIF gastroretentive CR formulation to improve its biopharmaceutical properties.

Several studies have been reported in the literature addressing the importance of RIF gastroretentive formulations. Shishoo et al. reported oral gastroretentive RIF formulation consisted of RIF pellets for immediate release as the loading dose and a bio/mucoadhesive RIF tablet for extended release (Pund et al., 2011). Gohel et al. developed floating tablets of RIF using hydrophilic HPMC polymer and calcium carbonate as gas generating agent which were subsequently filled together with delayed release INH capsule in one large capsule (Gohel and Sarvaiya, 2007). Floating microspheres were developed by Goyal et al. using solvent evaporation technique (Goyal et al., 2011).

RIF is an anti-microbial agent and its sufficient amount should be released initially as loading dose to achieve its minimum inhibitory concentration (MIC) to elicit required therapeutic effect in the body. On basis of its MIC, volume of distribution and fraction bioavailable, a minimum of 17.11% should be released as initial loading dose theoretically (data not shown). On succinct amount of literature search of gastroretentive drug delivery of RIF, no report has been found in the area of wax based oral DDS of RIF for biphasic release from single monolithic system using systemic quality by design (QbD) approach. The purpose of this work was to demonstrate suitability of a wax matrix system to achieve a bi-phasic release profile: a fast release within 1 h followed by extended drug release. Waxes give several benefits that embrace good stability at varying pH and moisture levels with well established safe applications (Sudha et al., 2010; Zhang and Schwartz, 2003). This type of systems would especially epitomize the objectives like cost-effective, feasible and save valuable life and resources in the management of TB which will be of prodigious concern for under-developed or developing countries with minimum industrial facilities.

The major aims of this study were: (i) step wise systemic formulation development and optimization using QbD approach (ii) applying principles of risk assessment and FMEA for formulation development and achieving biphasic release (iii) to implement Box–Behnken design (BBD) as optimization technique for establishment of mathematical equations and graphical results, thus portraying a complete picture of variation of the product/process response(s) as a function of the input variables and (iv) to better understand the underlying mechanisms.

2. Materials and methods

2.1. Materials

Rifampicin was purchased from Acme Pharmaceuticals Pvt. Ltd., Ahmedabad, India. Microcrystalline cellulose (Avicel® PH 102, Signet Chemical Corporation, India), Glyceryl Behenate (Compritol 888 ATO Gattefose Ltd., India), Hydroxypropylmethylcellulose (HPMC, Methocel E5, Colorcon Asia Pvt. Ltd., India) and colloidal silicon dioxide (Aerosil® 200 Evonik Ind., India) were generously gifted from the indicated sources. Sodium bicarbonate (Rankem Ltd., New Delhi, India), and magnesium stearate (S. D. Fine Chem Pvt. Ltd., India) were purchased from the indicated sources. All other ingredients, chemicals and reagents were of analytical grade and were used as received.

2.2. Quality Target Product Profile (QTPP) of RIF oral gastroretentive dosage form

The target product quality profile is enlisted as the quality properties that a drug product ought to possess so as to fulfill the objectives set in target product profile as quantitative attributes (Lionberger et al., 2008). The International conference of harmonization (ICH) Q8 (R2) (ICH harmonised tripartite guideline, 2009) recapitulates them as QTPP. The QTPP should furnish a quantitative surrogate to ascertain the aspects of clinical safety and efficacy. Thus it ought to form the basis for determining the critical quality attributes (CQAs), critical process parameters and control strategy (Fahmy et al., 2012, ICH harmonised tripartite guideline, 2009). QTPP for RIF gastroretentive CR formulation is depicted in Table 1.

2.3. Risk assessment by Failure Mode and Effects Analysis (FMEA)

ICH Q9, 2005 guidance document introduced the concept of quality risk management for evaluating, communicating, controlling and reviewing risks to the quality of drugs across product life cycle. The CQAs relies on the type of formulation, dosage form designed, manufacturing or production methodology, etc. employed and selected amongst many possible options. Thus based upon feasibility studies, we defined the formulation and manufacturing method as described in Section 2.4. 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. Using FMEA, the failure modes can be prioritized for a product or process for risk management purposes according to the seriousness of their consequences (effects), how frequently they occur and how easily they can be detected (Fahmy et al., 2012). The relative risk that each drug substance attributes presents was ranked according to risk priority number (RPN) (Hiyama, 2009). Those attributes that could have a high impact on the drug product attributes needed to be studied in detail whereas those attributes that had low impact on the drug product attributes required no further investigation. Table 2 depicts the FMEA for RIF gastroretentive tablet with their respective RPN for each failure mode. The RPN was calculated with the Eq. (1) mentioned as below:

$$\mathrm{RPN} = \begin{bmatrix}5\\4\\3\\2\\1\end{bmatrix} \mathrm{Ox} \begin{bmatrix}5\\4\\3\\2\\1\end{bmatrix} \mathrm{Sx} \begin{bmatrix}1\\2\\3\\4\\5\end{bmatrix} \mathrm{D} \quad (1)$$

where O is the occurrence probability or the likelihood of an event to occur; we ranked these as 5, frequent; 4, probable; 3, occasional; 2, remote and 1, improbable to occur. The next parameter S is the severity, which is a measure of how severe of an effect a given failure mode would cause; we ranked these as 5, catastrophic; 4, critical; 3, serious; 2, minor and 1, negligible or no effect. The final parameter D is the detectability which means the ease that a failure mode can be detected. Thus the more detectable a failure mode is, the less risk it presents to product quality. For D, we ranked 1, absolute certain or easily detectable; 2, high detectable; 3 moderately detectable; 4, low or remote detectable and 5 as hard to detect or absolute uncertain.

2.4. Preparation of RIF floating tablet

The tablets were prepared by direct compression method using glyceryl behenate as wax. Some preliminary studies were carried out before choosing suitable glyceryl behenate concentration, amount of HPMC as pore former and sodium bicarbonate as gas generating agent. Only the formulations and studies relevant for the present investigation are presented here. Briefly, accurately weighed quantity of RIF (150 mg/tab), MCC PH 102 (16.4–36.4 mg/tab), glyceryl behenate (20–30 mg/tab), HPMC E5

Table 1
QTPP of RIF gastroretentive tablet.

QTPP element	Target	Justification
Dosage form	Sustained release floating tablet	Tablet because commonly accepted unit solid oral dosage form. Floating because RIF is more absorbed from stomach due to its greater solubility and preferable site of absorption.
Route of administration	Oral	Dosage form designed to administer orally.
Dosage strength	150 mg	Commonly accepted strength
Stability	Short term stability of 3 months on accelerated condition 40 °C/75% RH and long term conditions 25 °C/60% RH.	Minimum time period (at least 3 months initially) decided to study stability of final formulation.
Drug Product Quality Attributes	Physical attributes	No physical defects like chipping, lamination, capping, etc.
	Assay	Meeting the compendial or other applicable quality standards (90 to 110% of label claims).
	Floating lag time	Keeping as minimum as possible.
	Dissolution	Initial burst release sufficient to achieve MIC followed by sustained release up to 8 h.
Container closure system	Suitable for storage of dosage form	To maintain product integrity and quality up to target shelf life.

Table 2
Risk assessment by FMEA analysis to identify criticality of failure modes.

Formulation/process parameter component	Failure mode	Failure effects	*S*	Potential causes or root of failure	*O*	Detectability method or control	*D*	RPN
Hardness	Inadequate hardness and its range	Drug release and friability	5	Machine failure, operator's error, excipient selection	3	Hardness tester, friability testing, dissolution	2	30
Amount of glyceryl behenate	Improper concentration	Drug release	5	Improper concentration	5	Dissolution	2	50
Amount of pore former (HPMC)	Improper concentration	Initial burst release	5	Improper concentration	5	Dissolution	2	50
Amount of gas generating agent (sodium bicarbonate)	Improper concentration	Floating lag time and floating duration	5	Improper concentration	5	Floating lag time and floating duration	2	50
Thermal sintering	Inadequate time	Drug release	5	Inadequate time	2	Dissolution, hardness	3	30
Packaging	Insufficient to protect drug from temperature, humidity and shipping.	Stability	5	Packaging material	3	Assay, dissolution, hardness	2	30

cps (5–15 mg/tab) and sodium bicarbonate (15–25 mg/tab) were sifted through sieve ASTM # 30 (Jayant Scientific Industries, Mumbai, India) and physically mixed for 10 min. Then silicon dioxide (as glidant) was sifted through ASTM sieve # 60 and blended for 5 min. Finally magnesium stearate (as lubricant) was sifted through ASTM sieve 60 # and blended for 3 min. The homogeneous blend was compressed on an eight station automatic rotary tablet machine (Modern Engineering Works, New Delhi, India) equipped with flat faced beveled edges punches of 7.0 mm diameter to a target weight of 240 mg/tab. Thermal sintering of tablets were done by individually placing it on aluminum foil on a stainless steel tray which was further put into an oven (Shree Kailash Industries, India) maintained at 80 °C for 20 min. From the preliminary experiments conducted, it was found that 20 min was sufficient for melting and redistribution of wax. Beyond this time tablet aspect ratio and dimensions were distorted (data not shown). Dissolution and physical tests were performed at least 24 h after the thermal treatment. The sintering time was fixed for all of the trials in order to exclude its effects on formulations.

2.5. Box–Behnken experimental design

A BBD with 3 factors, 3 levels, and 15 runs was selected for the optimization study (Box and Behnken, 1960; Lewis et al., 1999). Independent and dependent variables with their constraints are listed in Table 3. Q1, Q4, Q8 and floating lag time were selected as dependant variables while floating duration will be observed for each Design of Experiments (DOE) batch and will be correlated with

Table 3
Formulation variables and their levels for Box–Behnken design.

Factors	Coded levels	Actual Levels
X1: Amount of sodium bicarbonate (mg/tab)	−1	15
	0	20
	1	25
X2: Amount of HPMC (mg/tab)	−1	5
	0	10
	1	15
X3: Amount of glyceryl behenate (mg/tab)	−1	25
	0	30
	1	35

Responses	Constraints
Q1: Percent drug released in 1 h	$18\% \leq Q1 \leq 25\%$
Q2: Percent drug released in 4 h	$50\% \leq Q4 \leq 65\%$
Q8: Percent drug released in 8 h	$85\% \leq Q8 \leq 100\%$
Floating lag time (min)	As minimum as possible
Floating duration (h)	≥ 8 h

other dependant variables. For predicting the optimal region, the quadratic equation generated for the variables was explained as follows (Eq. (2)):

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} {x_i}^2 \quad (2)$$

where Y is the predicted response, β_0 is model constant/coefficient, β_i is the linear regression coefficient, β_{ii} is the squared regression coefficient, β_{ij} is the interaction effect regression coefficient and X_i is the dimensionless coded value of the independent variables (X_i). All statistical treatments of DOE were performed using Design Expert software (ver. 8.0.7.1, Stat- Ease Inc., USA) Main effect plots, interaction plots, residual plots and overlaid contour plots were generated using Minitab software (ver. 16.2.1., Minitab Inc., USA). All experimental trials were randomized to exclude any bias. Further the model was evaluated for best fit using parameters, coefficient of determination (r^2), adjusted r^2 (Adj r^2), predicted r^2 (Pred r^2), adequate precision (Shah et al., 2008) and Q^2 (Singh et al., 2005).

2.6. Physical characterization of the tablets

The compressed tablets were subjected to variegated physical investigations like appearance, weight variation, hardness and drug content. The weight variation was carried out on 20 tablets using electronic balance (Shimadzu AX 120, Japan). Tablet hardness was determined prior to thermal treatment and at least after 24 h using minimum 6 tablets for each batch with dial type tablet hardness tester (Scientific Engineering Corporation, Delhi, India) respectively. Friability was determined by Roche Friabilator for 4 min at 25 rpm. A validated RP-HPLC (Shimadzu, Kyoto, Japan) method was used for drug content measurement in triplicate. The method was modified as described by Shah et al. (Shah et al., 1992) and validated for drug content determination. Analysis was performed on Phenomenex C18 column (250 mm x 4.6 i.d., 5 micron) at 1.0 ml/min flow rate with 75:25% v/v mixture of methanol: 0.02 M disodium hydrogen phosphate pH 4.5 buffer as mobile phase at 254 nm. Linearity was demonstrated in range of 10–100 μg/ml. The recovery data was in the range of 98.6% –101.3%. The RSD for the precision was below 2%.

2.7. In vitro buoyancy study and in vitro drug release study

The studies were performed using United States Pharmacopeia (USP) 34 type II apparatus (VDA 6-DR,Veego Instruments Corporation, Mumbai, India) using 900 ml of 0.1 N hydrochloric acid (HCl) at 50 rpm rotation speed and 37 °C ± 0.5 °C temperature. Floating lag time was noted as the time interval between introduction of the tablet in the dissolution vessel and the time for tablet to the rise to surface of medium (Badhan et al., 2009). The time for which tablet constantly floated on the medium surface was considered as total floating time. Both the determinations were made in triplicate to ensure data reliability. Six dosage units were analyzed for dissolution profiling and data were recorded at 1, 2, 4, 6, and 8 h. Samples withdrawn were filtered through a 0.45 μm membrane filter and then analyzed for drug release by RP-HPLC method as described in Section 2.6.

2.8. Desirability function

The desirability function was applied to merge multicriteria responses in one single criterion measurement and reveal the possibility of predicting optimum levels for the independent variables. If the value of the response is on target or is at optimum, its desirability value was assigned as 1 and for totally unacceptable value its desirability was given as 0. The individual desirability for each response was calculated (Shah et al., 2008) using the approaches discussed below.

Q1 was desired to be the maximum so as to achieve initial burst release of RIF. Desirability *d1* for response Q1 was calculated by Eq. (3):

$$dl = \left[\frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}}\right] \quad (3)$$

Y_i is the experimental result, and Y_{min} and Y_{max} represent the minimum and maximum possible values. Y_{max} and Y_{min} for this response were 28.5 and 10.8 percent drug release respectively.

For Q4 there were no specific requirements for either obtaining maximum or minimum value. Q4 response justifies that the drug releases in sustain manner from the dosage form. The formulations having percentage release within 50% to 65% were considered as optimum having desirability of 1, while formulations having values out of this range have a desirability of 0. This can be explained by below Eq. (4):

$$d2 = 0 \text{ for } Y_i < Y_{\min}, \quad d2 = 1 \text{ for } Y_{\min} < Yi < Y_{\max}, \quad d2 = 0 \text{ for } Y_i > Y_{\max} \quad (4)$$

However for Q8 more than 85% drug should be release to ascertain complete release from the dosage forms. Y_{max} and Y_{min} for this response were 94.8 and 62.0 percent drug release respectively. Thus *d3* it was calculated with same formula as Eq. (3).

Floating lag time was desired as minimum as possible. Y_{max} and Y_{min} for this response were 7.3 and 3.5 min respectively. Thus desirability function for *d4* was calculated using the following Eq. (5).

$$d4 = \left[\frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}}\right] \quad (5)$$

The overall desirability was calculated from the individual values by using the following Eq. (6).

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} \quad (6)$$

2.9. Curve fitting and release mechanism

In order to study the drug transport mechanism from the formulations used, various models were considered to fit the experimental data using Microsoft Excel based DD solver software (Microsoft, USA) to perform and evaluate dissolution data modeling. The *in vitro* release pattern was evaluated to check the goodness of fit to the zero order (Costa and Sousa Lobo, 2001), first order (Wagner, 1969), Higuchi (Higuchi, 1963), Baker (Baker and Lonsdale, 1974), Hopfenberg (Costa and Sousa Lobo, 2001; Katzhendler et al., 1997), Hixson–Crowell's (Hixson and Crowell, 1931), Weibull (Sathe et al., 1996) and Korsmeyer–Peppas power law equation (Korsmeyer et al., 1983; Ritger and Peppas, 1987). For Korsmeyer–Peppas model, data were analyzed for first 60% of the drug release. Importantly, the goodness of fit was evaluated using adjusted r^2 (correlation coefficient) values. This is for the reason that r^2 will always increase as more parameters are included, whereas r^2 adjusted may decrease when over fitting has occurred (Zhang et al., 2010).

2.9.1. Akaike information criterion (AIC)

Many times, the sum of the squared residuals (SSR) can be used to distinguish the models that described the best fit. But sometimes greater number of model parameters could lead to a higher probability of obtaining a smaller SSR value, thus AIC was applied here to render the analysis independent of the number of parameters between studied models (Akaike, 1974; Zhang et al., 2010). Prominently, the AIC is dependent on the magnitude of the data and on the number of data points. The AIC is defined as

$$AIC = n\ln(WSS) + 2p \quad (7)$$

where n is the number of data points, *WSS* is the weighted sum of squares and p is the number of parameters in the model (Zhang et al., 2010).

2.9.2. Model selection criteria (MSC)

The MSC is another fascinating statistical criterion for model selection and evaluating goodness of fit. By and large, it is a modified reciprocal form of the AIC and has been normalized so as to make it independent of the scaling of the data points (Zhang et al., 2010); it is defined as:

$$MSC = \ln\left[\frac{\sum_{i=1}^{n} w_i(y_i\_obs - \bar{y}\_obs)^2}{\sum_{i=1}^{n} w_i(y_i\_obs - y_i\_pre)^2} - \frac{2p}{n}\right] \quad (8)$$

where w_i is the weighting factor, which is generally equal to 1 for fitting dissolution data, y_i_obs is the ith observed y value, y_i_pre is the ith predicted y value, $\bar{y}\_obs$ is the mean of all observed y data points, n is the number of data points and p is number of parameters.

2.10. Erosion studies

Tablet erosion test was performed under the same conditions described in the dissolution studies (Hiremath and Saha, 2004). At predetermined time intervals, individual tablets were removed during the dissolution studies and placed on aluminum foil and dried at 50 °C until a constant weight was achieved. The percentage of matrix eroded was calculated from the weight loss of the tablets (mean of three) at each time interval using the below Eq. (9).

$$\text{Percent tablet eroded} = \frac{w_i - w_t}{w_i} \times 100 \quad (9)$$

where w_i is the initial tablet weight and w_t is the weight of the dried tablets.

2.11. Characterization

Fourier transform infra-red spectroscopy (FT-IR) (Bruker, USA), Differential scanning calorimetry (DSC) (DSC-60, Shimadzu, Japan) and Powder X-ray diffraction (PXRD), (Panalytical, XPERTPRO software, Netherlands) (Shah et al., 2008) were carried out for examining any chemical interaction between the formulation ingredients before and after sintering. The Scanning electron microscopy (SEM) (JSM-5610LV, Jeol Ltd., Tokyo, Japan) (Shah et al., 2008) was used for topographical characterization of tablets before and after sintering as well to study effects of pore formers on tablet surface during dissolution respectively.

2.12. Packaging and stability study

The optimized batch was subjected to short term stability testing according to the ICH guidelines for zones III and IV (ICH Q1A (R2), 2003). Tablets were packed in count of 30 into high density polyethylene (HDPE) bottle with child resistant cap and were further induction sealed. Before induction sealed one silica bag was kept in bottle as desiccant. The sealed bottles were exposed to accelerated (40 ± 2 °C/75 ± 5% relative humidity) and long term (25 ± 2 °C/60 ± 5% relative humidity) stability for three months. The samples were withdrawn periodically (0, 15, 30, 60 and 90 days) and evaluated for different physicochemical parameters like visual inspection, drug content, hardness, floating lag time and *in vitro* drug release.

3. Results and discussion

3.1. QTPP of RIF oral gastroretentive dosage form

As discussed, defining QTPP varies upon the type of formulation and process chosen (Yu, 2008; Lionberger et al., 2008). Based on the preliminary studies conducted, the parameters that will be focused in our study were selected and enlisted as QTPP for RIF gastroretentive tablet (Table 1). Thus, other than describing our QTPP, the steps to define the QTPP are not discussed. The depicted QTPP will lay down the basis for determining CQA.

3.2. Risk assessment by FMEA

Table 2 enlists the factors that were considered in development of RIF gastroretentive tablet while performing FMEA. 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 (Hiyama, 2009). From the Table 2, it is clear that amount of glyceryl behenate, amount of HPMC and amount of sodium bicarbonate have RPN greater than 40 and needed thorough investigation. Hence, their optimization was done using response surface design for establishing design space which is discussed in detail in Section 3.4. Hardness and thermal sintering parameters were posted moderate risk and are discussed in Section 3.3. Packaging RPN value also falls under moderate risk category and is discussed in Section 3.9.

3.3. Physical evaluation of tablets

Physical appearance, hardness, friability, weight variation and assay of all the formulations were found to be satisfactory. Hardness was found to be 2–3.5 kg/cm^2 for unsintered tablets which increased after thermal treatment to 4.5–6.8 kg/cm^2. The hardness of all the batches was in comparable range and statistically no major difference in its range was found amongst different batches. Here, the increase in temperature boosted up the hardness which seems to be due to the fusion of the wax particles or the formation of welded bonds among the matrix particles after cooling (Singh et al., 2007). Secondly as discussed in Section 2.4, thermal sintering time was kept constant for all the batches. Hence, risk and criticality of this failure mode is low. Friability was found to be less than 0.5% (w/w). The formulated tablets comply with the weight variation and assay tests according to USP pharmacopoeial limits demonstrating that the direct compression method was suitable for preparing matrix tablets of RIF.

3.4. Effect of factors on the responses

3.4.1. Q 1

Results of the measured response for BBD are depicted in Table 4. ANOVA results and regression coefficients of response variables are shown in Table 5. From the results, it can be concluded that amount of HPMC was the most influencing factor affecting positively (Table 5) in initial burst release to achieve desired MIC. The same can be inferred from the contour plots and main effect plots for Q1 (Fig. 1 and Fig. 2A) respectively. However, amount of glyceryl behenate was affecting negatively (Table 6 and Fig. 2A) in significant amount. Looking inside deeply, it can be anticipated that HPMC will be dissolved faster and so the pores induced within short time will result in initial higher release. The dissolved HPMC generate higher porosity and produce even more channels in the matrix, facilitating the penetration of the dissolution medium into the matrix and/or more matrix erosion and dissolve the drug more rapidly. Similar mechanisms of influence of hyodrophillic materials in waxy carrier on drug release rate

Table 4
Matrix of the experiments for Box–Behnken design and results for the measured responses.

ES[a]	Amount of sodium bicarbonate (mg/tab)	Amount of HPMC (mg/tab)	Amount of glyceryl behenate (mg/tab)	Q1 (h)[b]	Q4 (h)[b]	Q8 (h)[b]	Floating lag time (min)[c]	Floating time (h)[c]
6	−1	−1	0	15.5 ± 1.1	46.8 ± 2.3	85.9 ± 3.9	7.0 ± 0.9	>8
9	1	−1	0	16.6 ± 0.9	50.6 ± 3.1	86.5 ± 2.1	3.9 ± 0.5	>8
4	−1	1	0	23.6 ± 1.6	68.8 ± 3.9	94.8 ± 2.1	6.9 ± 0.3	>7
8	1	1	0	24.1 ± 1.3	74.6 ± 2.2	93.9 ± 2.0	3.8 ± 0.3	>7
12	−1	0	−1	23.6 ± 0.8	79.8 ± 3.2	92.3 ± 3.0	7.3 ± 0.8	>6
1	1	0	−1	25.3 ± 1.5	82.6 ± 2.0	94.6 ± 2.2	4.6 ± 0.2	>6
14	−1	0	1	11.1 ± 0.9	40.8 ± 2.5	64.0 ± 2.0	6.7 ± 0.3	>8
7	1	0	1	12.9 ± 1.6	42.8 ± 2.0	68.0 ± 2.5	3.5 ± 0.5	>8
2	0	−1	−1	20.3 ± 1.2	83.6 ± 3.5	94.3 ± 1.8	5.5 ± 0.8	>6
13	0	1	−1	28.5 ± 1.5	90.8 ± 1.5	91.5 ± 1.5	5.5 ± 0.6	>4
10	0	−1	1	10.8 ± 0.8	35.6 ± 2.3	62.0 ± 2.1	4.1 ± 0.3	>8
15	0	1	1	14.2 ± 1.5	48.6 ± 1.5	89.5 ± 1.2	4.2 ± 0.2	>7
5	0	0	0	20.3 ± 1.1	58.5 ± 1.5	93.8 ± 1.1	4.1 ± 0.6	>7
11	0	0	0	19.8 ± 0.9	57.9 ± 1.9	92.9 ± 1.0	4.0 ± 0.5	>7
3	0	0	0	20.9 ± 1.2	59.1 ± 2.2	94.8 ± 1.1	4.0 ± 0.6	>7

[a] Experimental sequence.
[b] Mean ± SD (n = 6).
[c] Mean ± SD (n = 3).

Table 5
Regression analysis results.

Factors	Q1		Q4		Q8		Floating lag time	
	Coefficient	p value (Prob > F)	Coefficient	p value (Prob > F)	Coefficient	p value (Prob > F)	Coefficient	p value (Prob > F)
Intercept	20.33	<0.0001[a]	58.50	0.0015[a]	93.83	0.0041[a]	4.03	<0.0001[a]
X1	0.64	0.0742	1.80	0.3130	0.75	0.5930	−1.51	<0.0001[a]
X2	3.40	<0.0001[a]	8.27	0.0036[a]	5.12	0.0114[a]	−0.013	0.8484
X3	−6.09	<0.0001[a]	−21.13	<0.0001[a]	−11.15	0.0004[a]	−0.55	0.0003[a]
X1X2	−0.15	0.7234	0.50	0.8344	−0.37	0.8481	0.000	1.0000
X1X3	0.025	0.9527	−0.20	0.9332	0.43	0.8282	−0.13	0.2138
X2X3	−1.20	0.0302[a]	1.45	0.5511	7.57	0.0096[a]	0.025	0.7873
$X1^2$	−0.30	0.4984	−0.73	0.7713	−4.08	0.0888	1.03	<0.0001[a]
$X2^2$	−0.079	0.8569	2.43	0.3518	0.52	0.7985	0.33	0.0148[a]
$X3^2$	−1.80	0.0075[a]	3.72	0.1757	−10.03	0.0035[a]	0.46	0.0041[a]

Regression coefficients are in coded value.
[a] Statistically significant (p < 0.05).

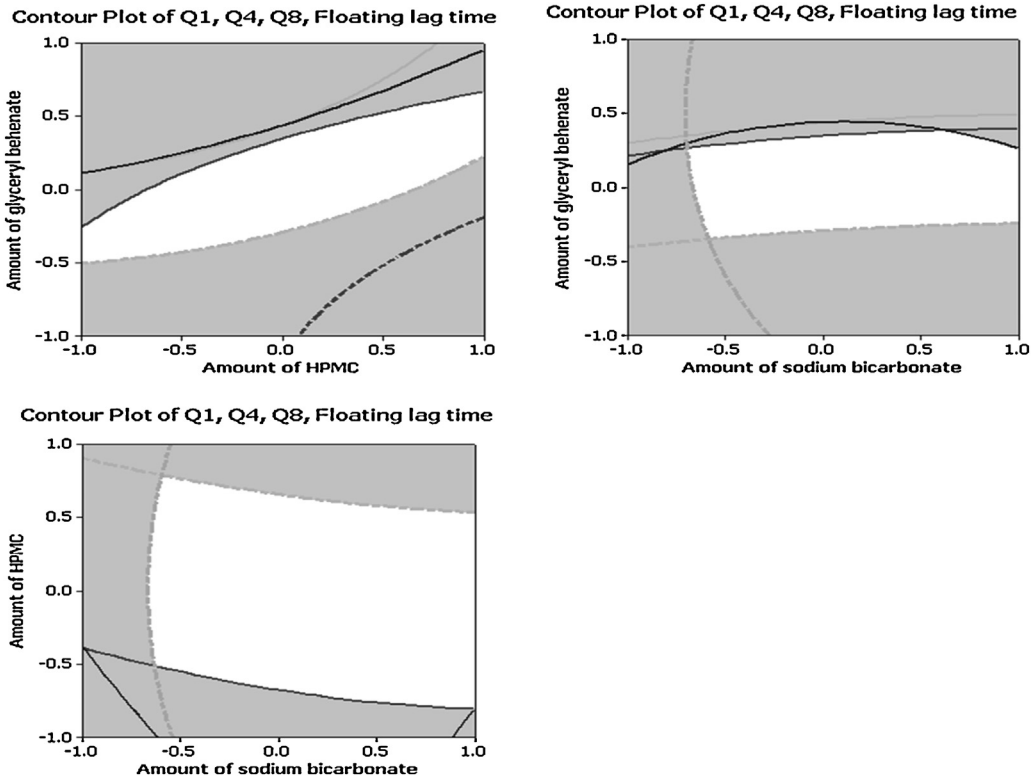

Fig. 1. Overlaid contour plots of Q1, Q4, Q8 and floating lag time as a function of amount of sodium bicarbonate, amount of HPMC and amount of glyceryl behenate.

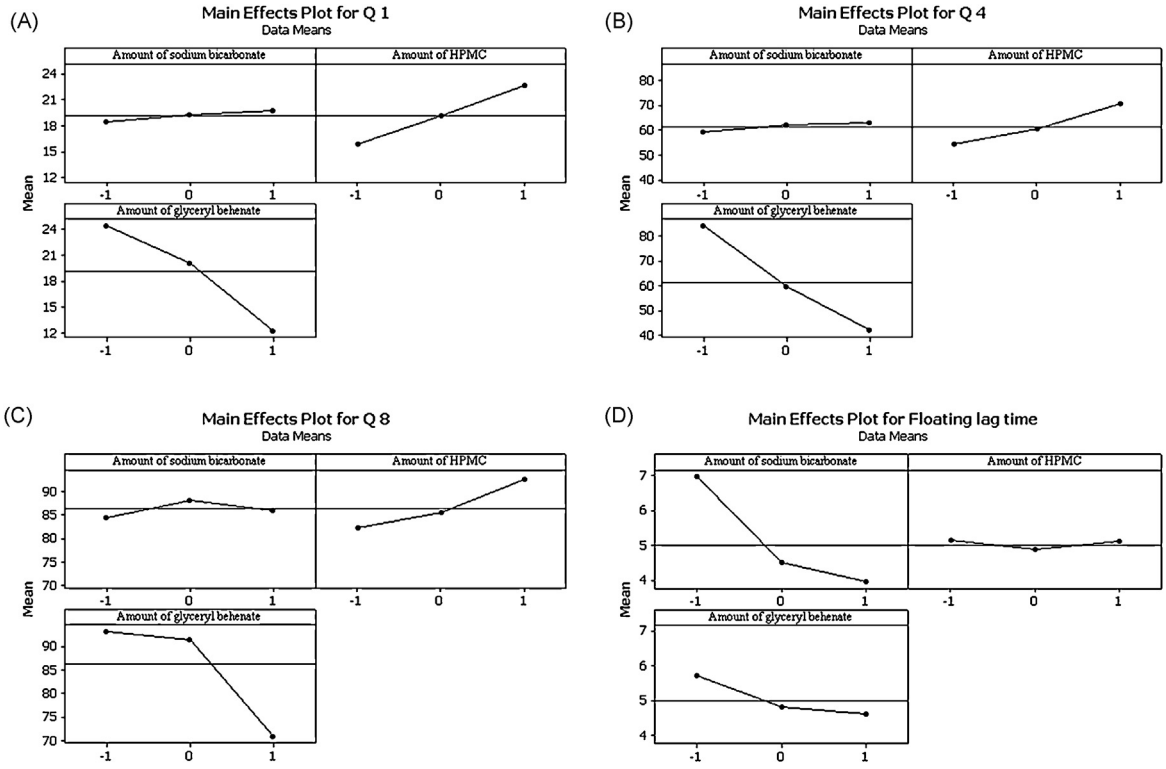

Fig. 2. Main effects plot for (A) Q1, (B) Q4, (C) Q8 and (D) floating lag time as a function of amount of sodium bicarbonate, amount of HPMC and amount of glyceryl behenate.

have been reported by Li et al. (2006). SEM was used to image the tablet surfaces before and after sintering and after 1 h of the drug released. SEM micrographs (Fig. 3B) of the surface of the tablets after sintering depicted a smooth structure covering the entire surface, promoting a better distribution of glyceryl behenate throughout the matrix and increasing the bonding strength. This is due to possible change in nature of the pores within the matrix with decreasing the porosity and increasing the tortuosity factor of the matrix which ultimately, is responsible for the retardation of drug release from matrix tablets with increasing glyceryl behenate amount in tablet. Similar mechanisms for thermal sintering of wax and its effects on drug release have been proposed by some researchers (Singh et al., 2007; Sudha et al., 2010; Zhang and Schwartz, 2003). The SEM image of tablet surface after 1 h (Fig. 3C) describes the porous surface which is due to HPMC dissolved as explained earlier.

The quadratic equation for reduced model in coded units is as below:

$$Q1 = 20.33 + 3.4X2 - 6.09X3 - 1.2X2X3 - 1.8X32 \quad (10)$$

3.4.2. Q 4

From Table 5 and Fig. 2B, it can be concluded that amount of glyceryl behenate was the most influencing factor affecting Q4 negatively (negative co-efficient; Table 5) to maintain the sustain release. On the contrary, amount of HPMC was affecting positively; (Table 5, Fig. 2B) in noteworthy amounts. In batches (Table 4) where wax amount was at lower level (−1); experimental sequence (ES) 12, 1 and HPMC amount at moderate level (0) or wax amount at lower level and HPMC at lower or higher level (−1) or (1) in ES 2 and 13 respectively, more than 75% drug released in 4 h suggesting incapable for sustain release up to 8 h. This can be prudently correlated with floating duration as seen from Table 4. This may be due to low concentration of wax and/or high amount HPMC which will create weak matrix and low hydrophobicity penultimately allowing higher ingress of water which further reduces matrix strength and ultimately releasing higher drug due to higher matrix erosion. Even in the batches ES 8 where HPMC level was at high level (1) and glyceryl behenate at moderate level (0) more than 70% drug has been released in 4 h. Contrary in batch ES 10 where glyceryl behenate concentration is high and HPMC at lower level, drug release

Table 6
ANOVA results showing the effect of independent variables on the measured responses.

Measured response	Model	Sum of squares (SS)	DF	Mean square (MS)	*F*-value	(Prob > *F*) 100	PRESS	r^2	Adj-r^2	Pred-r^2	Adeq Precision	Q^2
Q 1	FM	410.18	9	45.58	71.01	<0.0001	43.01	0.9922	0.9783	0.8960	29.008	0.89
	RM	406.49	4	101.62	147.15	<0.0001	18.13	0.9833	0.9766	0.9561	39.548	0.95
Q4	FM	4226.70	9	469.63	22.79	0.0015	1638.90	0.9762	0.9334	0.6215	15.863	0.61
	RM	4117.93	2	2058.96	116.64	<0.0001	375.75	0.9511	0.9429	0.9132	31.295	0.90
Q8	FM	1860.76	9	206.75	14.96	0.0041	1081.03	0.9642	0.8997	0.4398	12.336	0.42
	RM	1790.95	4	447.74	32.23	<0.0001	520.24	0.9280	0.8992	0.7305	18.007	0.71
Floating lag time	FM	25.46	9	2.83	91.73	<0.0001	2.38	0.9940	0.9831	0.9073	28.771	0.90
	RM	25.39	5	5.08	207.33	<0.0001	0.75	0.9914	0.9866	0.9707	41.677	0.97

FM – Full model.
RM – Reduced model.

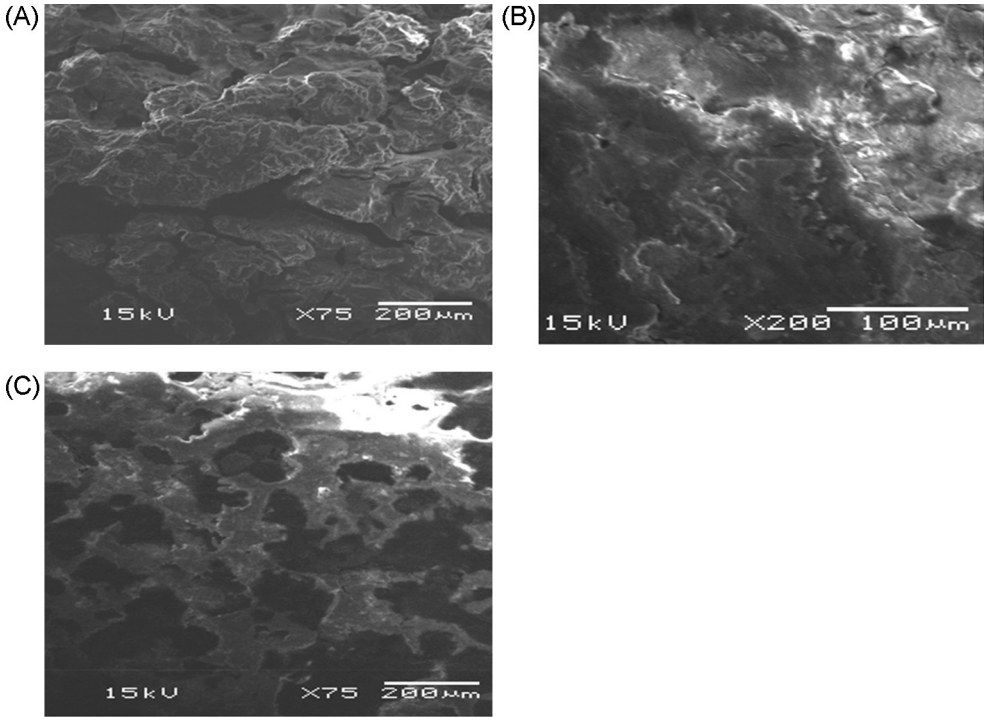

Fig. 3. SEM images of tablet surface (A) without heat treatment, (B) with heat treatment and (C) after dissolution.

was up to 35% only in 4 h which may be due to more hydrophobicity and less porous surface. The same can be correlated with floating duration (Table 4). Hence, there is an optimum range of glyceryl behenate and HPMC where you can get desired percentage release in the constraint range which is represented in contour plots (Fig. 1).

The quadratic equation for reduced model in coded units is as below:

$$Q4 = 58.50 + 8.27X2 - 21.13X3 \quad (11)$$

3.4.3. Q 8

As shown in Fig. 2C and Table 5 it can be concluded that amount of glyceryl behenate was the most influencing factor affecting Q 8 negatively and amount of HPMC was affecting positively in noteworthy amount. The batches (e.g. ES 5, 11 and 3) in which matrix was strong enough after pores created by HPMC could sustain drug release up to 8 h and simultaneously have released drug more than 85%. On the contrary, batches; ES 14 and 10 in which glyceryl behenate effects was prominent could sustain for 8 h but could not release drug more than 85%

The quadratic equation for reduced model in coded units is as below:

$$Q8 = 93.83 + 5.12X2 - 11.15X3 + 7.57X2X3 - 10.03X3^2 \quad (12)$$

3.4.4. Floating lag time

As highlighted in Fig. 2D and Table 5; amount of sodium bicarbonate was the most influencing factor affecting negatively (negative co-efficient; Table 5) floating lag time. However, surprisingly, amount of glyceryl behenate was also affecting negatively in noteworthy amount but not as significant as amount of sodium bicarbonate. This may be due to glyceryl behenate acting as floating enhancer which may be hypothesized due to hydrophobicity provided by it.

The quadratic equation for reduced model in coded units is as below:

$$\text{Floating lag time} = 4.03 - 1.51X1 - 0.55X3 + 1.03X12 + 0.33X22 + 0.46X3^2 \quad (13)$$

3.4.5. Model fitting and statistics of the measured responses

Statistically, high values of the r^2 for all dependent variables indicate a good fit. Adj-r^2 and Pred-r^2 values were also in reasonable agreement, particularly for reduced models signifying good model fit (Table 6). Better Pred r^2 obtained for reduced model might be due to elimination of insignificant terms. Further both full and reduced model, showed the adequate precision value greater than 4, indicating adequate model discrimination (Shah et al., 2008). A model fit value of $Q^2 > 0.5$ is considered as fairly good and value of $Q^2 > 0.9$ is generally taken as excellent (Singh et al., 2005). Q^2 were in good reasonable agreement, particularly for reduced models signifying good model fit.

3.4.6. Interaction between the factors and residual plots

An interaction is the failure of a particular factor to produce the same effect on the response at the different levels of the other factor. The ANOVA results (Table 5) and Fig. 4 depicts the interaction effects amongst the factors. Based on the p value X2X3 showed significant influence on percent drug release at Q1 and Q8 time point. The residual plots viz., normal probability plot of residuals, residual vs fit, residual vs order and histogram of residuals for Q1, Q4, Q8 and floating lag time are depicted in Fig. 5. The normal probability plot of residuals for responses reveals that the residuals appear to follow straight line and thus existence of non-normality, outliers, skewness or unidentified variables can be ruled out. From the plot of residual vs fit of all responses, it can be stated that residuals appear to be randomly scattered about zero and existence of missing terms, non-constant variance, outliers or influential points can be ruled out. Similar conclusions can be drawn out from histograms of residuals of all responses that skewness or outlier does not exist. Residual vs

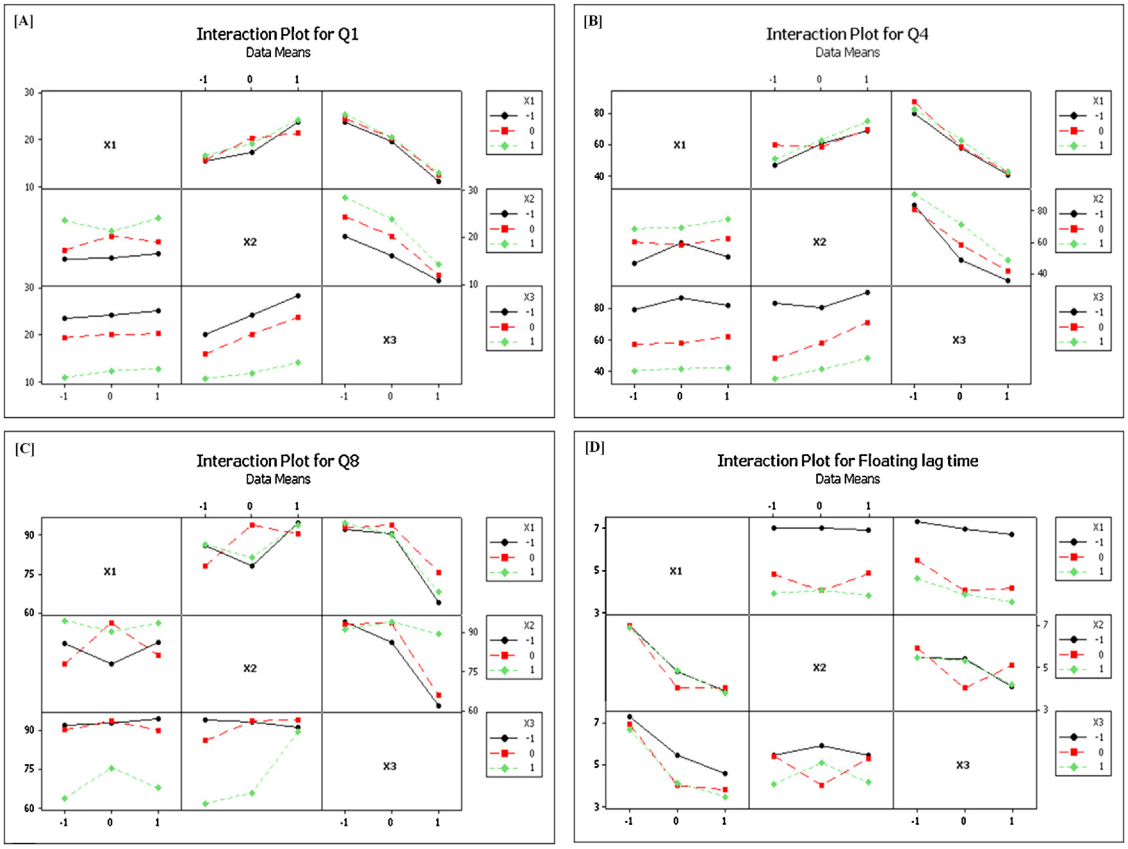

Fig. 4. Interaction profile of amount of sodium bicarbonate, amount of HPMC and amount of glyceryl behenate on (A) Q1, (B) Q4, (C) Q8 and (D) Floating lag time.

order is specifically helful to determine whether the order of the observations influence the results or not. From the Fig. 5, there exists no evidence of the error terms to be correlated with each other.

3.4.7. Evaluation of model using cross-validation

In order to assess reliability of the model, five experiments were conducted by varying the formulation variables at values other than that of the model. The predicted and experimental values for each response are shown in Table 7. Bias or percent relative error between predicted and experimental values for each response was calculated by the following Eq. (14):

$$\text{Bias} = \left[\frac{\text{Predicted value} - \text{experimental value}}{\text{predicted value}}\right] \quad (14)$$

There was a reasonable agreement between the predicted and the experimental value in all the five batches, due to low value of the bias was found. Thus it can be concluded that the equations express adequately the influence of the selected formulation variables on the responses under study.

3.5. Optimization using desirability function

Desirability function was calculated for floating lag time and percent drug release at Q1, Q4 and Q8 time. Based on the composite desirability data and overlay contour plots, ES 3 was identified as the optimum batch having desirability of 0.84. Composite desirability found out for optimized batch with the help of Minitab software was 0.86. The weight and importance was allotted 1 for each response respectively.

3.6. Curve fitting and release mechanism

Values of adjusted r^2, AIC and MSC value are presented in Table 8. The drug release data of the optimized batch ES 3 show a good fit to the Korsmeyer–Peppas' power law release kinetics which can be confirmed by comparing the values of adjusted r^2 with that of the other models. The values of release exponent (n) determined for the optimized formulation batch ES 3 was found to be 0.706 suggesting the probable release by anomalous transport (Ritger and Peppas, 1987). If one considers the adjusted r^2 values, Hixson–Crowell, Hopfenberg and Korsemeyer–Peppas; all the three models describe the dissolution data reasonably well. Thus AIC was applied as substitute which renders the analysis independent of the number of parameters between models. The lowest AIC value; 8.29, of optimized batch ES 3 indicates that Korsemeyer–Peppas power low is the best fit model in describing the dissolution behavior. Similarly, the highest MSC, 6.54, of the optimized batch indicates the same.

3.7. Erosion study

The results revealed that the matrix erosion followed a linear profile with the time (Fig. 6). There was linear matrix erosion corresponding with the linear release profile of drug. Hence, study supported the fact that the release was majorly dependent upon the erosion mechanism.

3.8. Characterization

3.8.1. FT-IR Study

During sintering there are possibilities of interaction between glyceryl behenate and RIF in which the drug release prolongation can be related to this interaction. To investigate the lack of

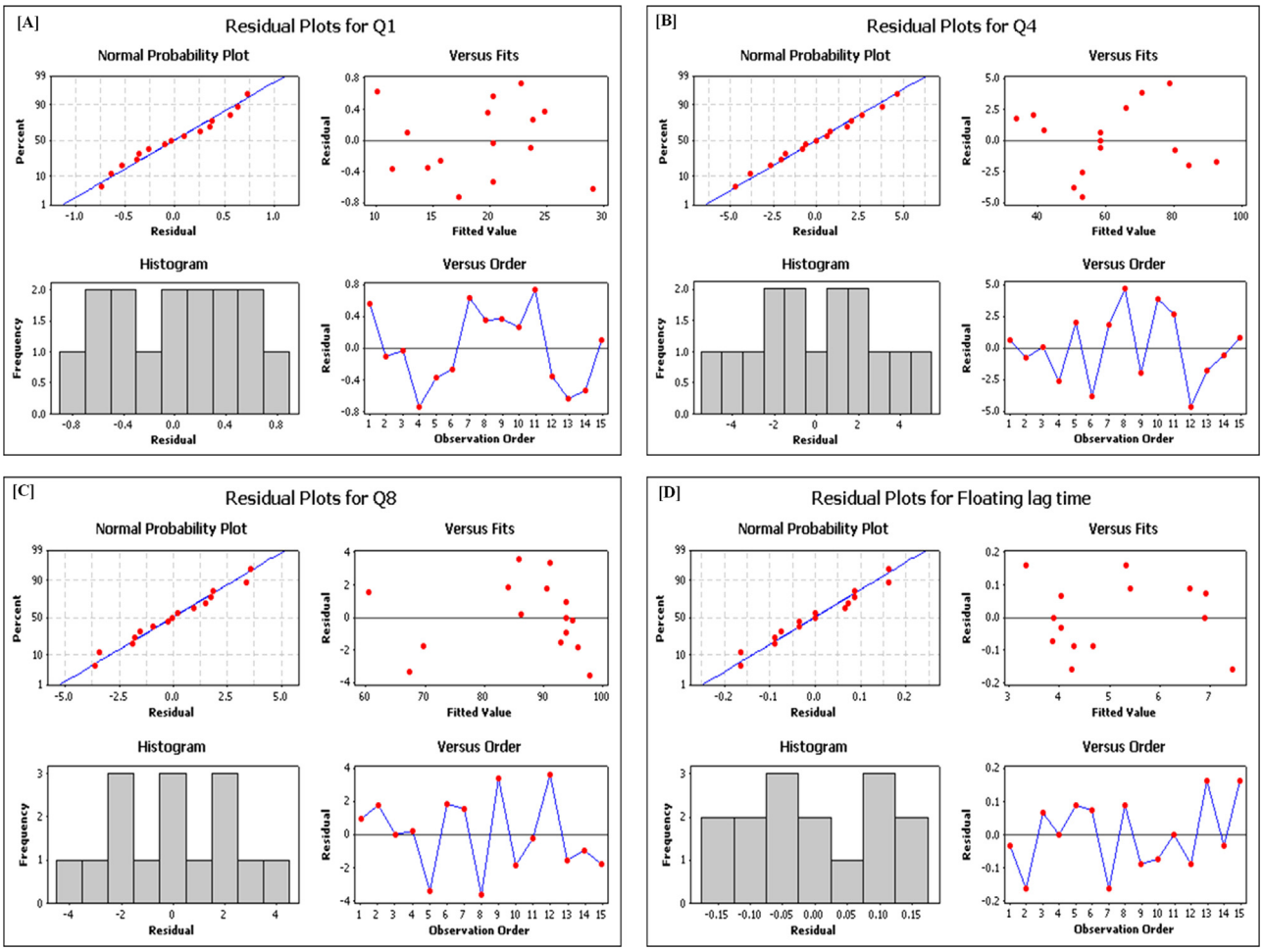

Fig. 5. Residual plots for (A) Q1, (B) Q4, (C) Q8 and (D) Floating lag time.

any drug polymer interaction during sintering FT-IR spectroscopy was used. The FT-IR spectra of RIF, physical mixture of RIF and excipients and thermally treated samples were recorded and are depicted in Fig. 7. The FT-IR spectra of RIF shows characteristics of form-II signifying double peaks at 1713 and 1733 cm^{-1} due to acetyl and furanone C=O, respectively, broad band over 3565–3150 cm^{-1} due to absorption of ansa OH, 1566 cm^{-1} due to amide C=O and 2883 cm^{-1} due to N-CH3 (Henwood et al., 2000; Agrawal et al., 2004). The spectra were compared with physical mixture and heat treated sample. The spectra revealed no

Table 7
Comparison of responses between predicted and experimental values for the cross validation set.

Responses	Test	Factors/levels			Experimental values	Predicted values	Bias (%)
		X1	X2	X3			
Q1	1	−1	−0.6	−0.6	19.10	19.82	3.63
	2	−0.6	0	0.4	17.92	17.11	−4.73
	3	−0.4	0.6	0	21.65	22.07	1.90
	4	0	−0.4	0.6	15.23	14.94	−1.94
	5	0.5	0.5	−0.5	25.90	25.11	−3.15
Q4	1	−1	−0.6	−0.6	64.85	65.56	1.08
	2	−0.6	0	0.4	50.10	49.35	−1.52
	3	−0.4	0.6	0	64.26	63.38	−1.39
	4	0	−0.4	0.6	42.96	43.89	2.12
	5	0.5	0.5	−0.5	72.30	75.27	3.95
Q8	1	−1	−0.6	−0.6	95.50	96.96	1.51
	2	−0.6	0	0.4	92.60	90.74	−2.05
	3	−0.4	0.6	0	96.12	101.23	5.05
	4	0	−0.4	0.6	86.98	84.75	−2.63
	5	0.5	0.5	−0.5	95.80	101.85	5.94
Floating lag time	1	−1	−0.6	−0.6	6.90	7.12	3.09
	2	−0.6	0	0.4	5.30	5.19	−2.12
	3	−0.4	0.6	0	5.18	4.91	−5.50
	4	0	−0.4	0.6	4.10	3.92	−4.59
	5	0.5	0.5	−0.5	4.15	4.02	−3.23

Table 8
Comparative characteristics of different drug release kinetic models for DOE batches.

		Batch number														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Zero Order	r^2	0.9837	0.9586	0.7565	0.6455	0.6584	0.5989	0.9441	0.9447	0.7106	0.2543	0.9739	0.9870	0.9210	0.9312	0.9179
	AIC	22.20	26.73	35.57	37.53	37.89	38.61	25.19	25.54	37.84	41.10	20.87	21.24	30.26	29.56	30.49
	MSC	3.72	2.79	1.01	0.64	0.67	0.51	2.48	2.49	0.84	−0.11	3.24	3.95	2.14	2.28	2.10
First order	r^2	0.9589	0.9738	0.9933	0.9926	0.9702	0.9740	0.9987	0.9981	0.9429	0.9567	0.9895	0.9514	0.9748	0.9746	0.9728
	AIC	26.82	24.44	17.58	18.21	25.69	24.93	6.34	8.67	29.72	26.86	16.29	27.85	24.55	24.58	24.97
	MSC	2.79	3.24	4.61	4.50	3.11	3.25	6.25	5.87	2.46	2.74	4.16	2.62	3.28	3.27	3.20
Higuchi	r^2	0.8725	0.9023	0.9667	0.9499	0.9117	0.9181	0.9099	0.9152	0.8805	0.8481	0.8849	0.8691	0.9349	0.9288	0.9373
	AIC	32.48	31.03	25.62	27.75	31.12	30.67	27.57	27.68	33.41	33.14	28.28	32.80	29.29	29.74	29.14
	MSC	1.66	1.93	3.00	2.59	2.03	2.10	2.01	2.07	1.72	1.48	1.76	1.63	2.33	2.24	2.37
Hixson–Crowell	r^2	0.9844	0.9934	0.9942	0.9910	0.9816	0.9869	0.9931	0.9945	0.9673	0.9595	0.9919	0.9782	0.9939	0.9945	0.9924
	AIC	21.98	17.57	16.89	19.16	23.27	21.50	14.73	13.98	26.93	26.53	15.00	23.83	17.44	16.94	18.56
	MSC	3.76	4.62	4.75	4.31	3.60	3.94	4.58	4.81	3.02	2.81	4.42	3.43	4.70	4.80	4.49
Baker–Lonsdale	r^2	0.7947	0.8237	0.8920	0.8901	0.8505	0.8617	0.8641	0.8649	0.8088	0.8555	0.8403	0.7890	0.8466	0.8415	0.8474
	AIC	34.86	33.98	31.51	31.67	33.75	33.29	29.63	30.00	35.77	32.89	29.92	35.19	33.58	33.73	33.59
	MSC	1.18	1.34	1.83	1.81	1.50	1.58	1.60	1.60	1.25	1.53	1.43	1.16	1.47	1.44	1.48
Weibull	r^2	0.9864	0.9904	0.9971	0.9985	0.9896	0.9934	0.9987	0.9982	0.9860	0.9719	0.9904	0.9789	0.9868	0.9881	0.9793
	AIC	23.29	21.44	15.38	12.17	22.45	20.07	8.33	10.52	24.69	26.71	17.87	25.68	23.33	22.78	24.16
	MSC	3.50	3.84	5.05	5.71	3.76	4.22	5.85	5.50	3.47	2.77	3.84	3.06	3.52	3.63	3.37
Hopfenberg	r^2	0.9957	0.9959	0.9975	0.9956	0.9762	0.9828	0.9983	0.9977	0.9602	0.9484	0.9893	0.9929	0.9938	0.9953	0.9920
	AIC	16.06	15.79	13.16	16.16	25.13	23.41	8.36	10.24	28.48	28.31	16.96	18.83	18.08	16.69	19.38
	MSC	4.94	4.97	5.50	4.91	3.23	3.55	5.85	5.55	2.71	2.45	4.02	4.43	4.57	4.85	4.32
K-Peppas	r^2	0.9973	0.9961	0.9834	0.9555	0.9213	0.9220	0.9934	0.9965	0.9007	0.8546	0.9955	0.9981	0.9989	0.9989	0.9991
	AIC	15.15	16.95	24.14	29.15	32.55	32.42	16.50	13.76	34.49	34.92	14.01	13.59	10.68	10.73	8.29
	MSC	5.12	4.74	3.30	2.31	1.74	1.75	4.22	4.85	1.51	1.13	4.61	5.48	6.05	6.04	6.54
	K	14.689	17.407	28.746	31.852	31.018	33.345	13.500	14.359	29.401	39.621	11.017	14.572	21.592	20.759	21.974
	n	0.855	0.781	0.590	0.549	0.567	0.541	0.756	0.752	0.605	0.454	0.823	0.867	0.711	0.725	0.706

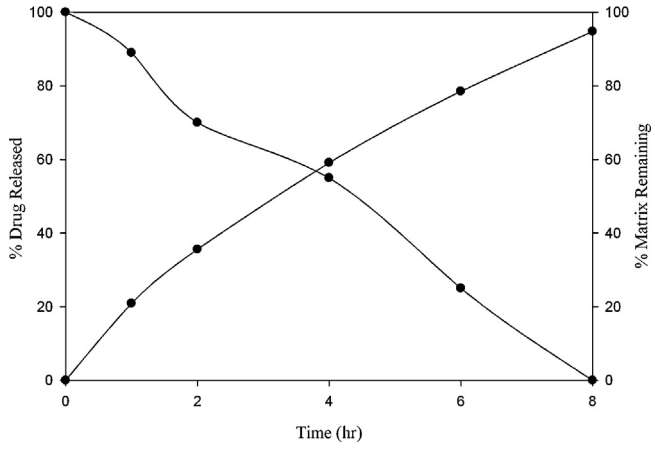

Fig. 6. Comparative release and erosion profile of optimized batch.

difference in the position of the absorption bands of RIF in physical mixture and heat treated samples. The spectra can be considered as the superposition of those of physical mixture and heat treated. This observation ruled out the possibility of chemical interaction and complex formation between these components by thermal treatment.

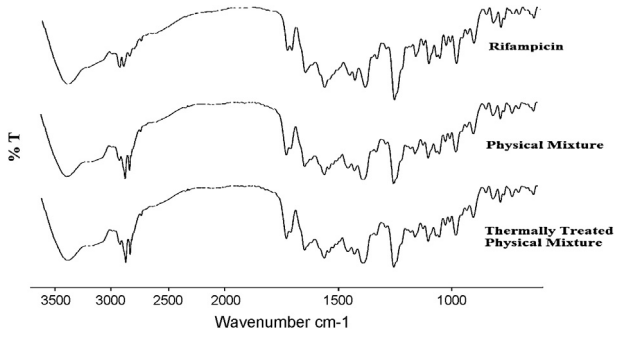

Fig. 7. FT-IR spectra of plain drug, physical mixture and heat treated physical mixture.

3.8.2. DSC study

DSC study also revealed form-II of RIF showing melting endotherm at 186 °C immediately followed by recrystallization to form I (exotherm at 196 °C) which is a characteristic of solid–liquid–solid transition and finally decomposes at 248 °C. The physical mixture, showed the melting endotherm at 70 °C corresponding to glyceryl behenate. Comparing the spectra of physical mixture and heat treated sample shows that no change has been occurred after heat treatment, therefore there is no evidence for the formation of solid solution or polymorphic changes during the heat-treatment.

3.8.3. Powder XRD study

The XRD study of RIF showed peaks at $2\theta = 9.91$ and 11.10 which are characteristic peaks of form-II of RIF. The other characteristics were observed at 15.74 and 19.92 (Henwood et al., 2000; Agrawal et al., 2004). The XRD peaks for RIF were found in the same position for both physical mixture and heat-treated sample. The results were in line with the DSC and FT-IR data conforming the absence of drug polymer interaction.

3.9. Packaging and stability study

The optimized formulations showed negligible change under the conditions of storage for parameters like appearance, drug content, hardness, floating lag time and *in vitro* drug release. The similarity factor (f2) (Costa and Sousa Lobo, 2001) was employed for comparison of dissolution profiles on each time point. It ranged from 84 to 96. Thus the data suggested that the formulation was stable for under the packaging material selected revealing that it risks it under control and low.

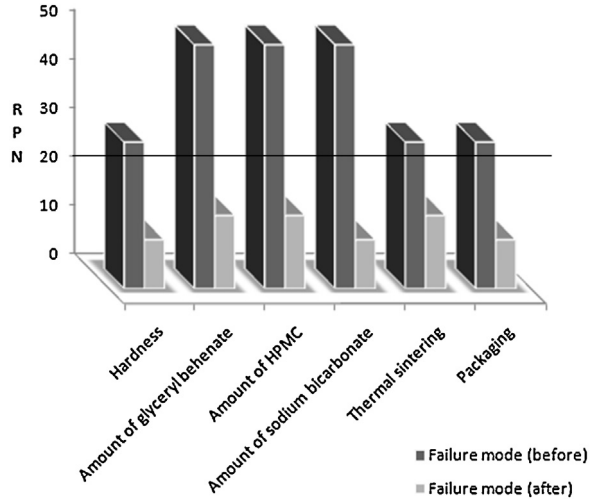

Fig. 8. FMEA analysis of RIF gastroretentive tablet depicting RPN number of failure mode before and after implementation of control strategy.

3.10. Risk mitigation and control strategy

BBD was employed to investigate the multidimensional interaction of input variables which were ranked as high risk in the initial risk assessment for establishment of a design space. The design space is the acceptable region within which the quality of the product can be built (Lionberger et al., 2008; Yu, 2008). The risk mitigation and control strategy is an amalgamated outline of how quality is ascertained based on product knowledge and current process.

For X1, it can be inferred from main effect plots (Fig. 2A–D), overlay contour plots (Fig. 1) and p value from ANOVA (Table 5) that it significantly affected floating lag time. Thus it has minor impact on the other dependant variables (p value > 0.05). Main effect plots and multiple linear regression analysis results depict that the floating lag time significantly decreases from low (−1) to moderate (0) level while from moderate (0) to high (1) level it does not have major impact on decreasing floating lag time. Thus we decided to use sodium bicarbonate between 0 to 1 level which also increases its operability range. The risk with operating in this range is low. The risk mitigation strategy is to monitor the floating lag time.

From the ANOVA table and p value (Table 5), overlay contour plots (Fig. 1), interaction (Fig. 4) and main effect plots (Fig. 2A–D) it is clearly observed that both factors X2 and X3 have major impact on percent drug release at Q1, Q4 and Q8. As discussed earlier there is an optimum range and ratio of glyceryl behenate and HPMC where you can get desired percentage release in the constraint range which is represented in overlay contour plot (Fig. 1) of X2 and X3 vs. all four responses. Working in this range, risk is low as all the responses are in the desired constraints. The risk mitigation strategy for the same is that the all the responses Q1, Q4 and Q8 are in the constraints range.

Regarding the moderate RPN failure modes, hardness and packaging were discussed in their respective Sections 3.3 and 3.9 respectively. Fig. 8 describes the FMEA analysis before and after the implementation of control strategy. RPN for all the possible failure modes were below 20 and which make them fall in the low range. The scalability of the design space can be evaluated in the transfer from lab to pilot and subsequent scale up batch manufacturing. Thus it may be further refined based on additional experience gained during the commercial lifecycle of the production.

4. Conclusion

With the rising awareness of QbD tools and risk management approaches, the utility of it has now permeated tangibly into research and industry for understanding of process or formulation variable rationally. The manuscript describes the overall QbD approach along with risk assessment using FMEA method, risk analysis and control strategy to mitigate the risk for development of RIF gastroretentive dosage form. In an endeavor to accomplish the objectives of QbD, response surface methodology using BBD was applied for evaluating the failure modes with high RPN number and defining the relationships between input variables and quality traits desired. 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. Finally, the design space was established and control strategy was developed to mitigate the risk in future. The RPN of updated risk assessment depict that all the failure modes were in low risk category (Fig. 8). Thus the shift in paradigm from traditional approach to QbD approach can provide astute insight for building quality within the product.

Hence, the developed CR formulation may provide prudently a better substitute for immediate release tablet in circumventing its hiccups; simultaneously providing biphasic release and may anticipate a better bioavailability. The developed formulation has shown promising results *in vitro* and is potential for assessing *in vivo* bioavailability. The further *in vivo* investigations in suitable animal models and human clinical trials are required to prove the clinical usability of the experimental extended release formulation. The manufacturing method employed is relatively simple and can easily be adopted in industries.

Conflict of interest

The authors declare that this article content has no conflicts of interest.

Acknowledgements

Mr. Chintan Vora is highly grateful to the University Grants Commission, New Delhi, Government of India, for availing Junior Research Fellowship. We greatly appreciate the Metallurgical Department, Faculty of Technology and Engineering, The M.S. University of Baroda, Vadodara, Gujarat, India and SAIF, Punjab University, Punjab, India for skillful assistance and providing SEM and PXRD testing facility in this study respectively. The authors are grateful to the industries that provided respective gift samples.

References

Agrawal, S., Ashokraj, Y., Bharatam, P.V., Pillai, O., Panchagnula, R., 2004. Solid state characterization of rifampicin samples and its biopharmaceutic relevance. Eur. J. Pharm. Sci. 22, 127–144.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 9, 716–723.

Badhan, A.C., Mashru, R.C., Shah, P.P., Thakkar, A.R., Dobaria, N.B., 2009. Development and evaluation of sustained release gastroretentive minimatrices for effective treatment of *H. pylori* infection. AAPS PharmSciTech 10, 459–467.

Baker, R.W., Lonsdale, H.S., 1974. Controlled release: mechanisms and rates. In: Taquary, A.C., Lacey, R.E. (Eds.), Controlled Release of Biologically Active Agents. Plenum Press, New York, pp. 15–71.

Box, G.E.P., Behnken, D.W., 1960. Some new three level designs for the study of quantitative variables. Technometrics 2, 455–475.

Costa, P., Sousa Lobo, J.M., 2001. Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci. 13, 123–133.

du Toit, L.C., Pillay, V., Danckwerts, M.P., 2006. Tuberculosis chemotherapy: current drug delivery approaches. Resp. Res. 7, 118.

Fahmy, R., Kona, R., Dandu, R., Xie, W., Claycamp, G., Hoag, S.W., 2012. Quality by design I: application of failure mode effect analysis (FMEA) and Plackett–Burman design of experiments in the identification of "main factors" in the formulation and process design space for roller-compacted ciprofloxacin hydrochloride immediate-release tablets. AAPS PharmSciTech 13, 1243–1254.

Gallo, G.G., Radeilli, P., 1976. Rifampicin. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances. Academic Press, New York, pp. 467–575.
Gohel, M.C., Sarvaiya, K.G., 2007. A novel solid dosage form of rifampicin and isoniazid with improved functionality. AAPS PharmSciTech 8, E1–E7.
Goyal, P., Gill, S., Gupta, U.D., Rath, G., Narang, R.K., Goyal, A.K., 2011. Development and characterization of rifampicin loaded floating microspheres. Artif. Cells Blood Substit. Immobol. Biotechnol. 39, 330–334.
Henwood, S.Q., de Villiers, M.M., Liebenberg, W., Lotter, A.P., 2000. Solubility and dissolution properties of generic rifampicin raw materials. Drug Dev. Ind. Pharm. 24, 403–408.
Higuchi, T., 1963. Mechanism of sustained action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci 52, 1145–1149.
Hiremath, S.P., Saha, R.N., 2004. Design and study of rifampicin oral controlled release formulations. Drug Deliv. 11, 311–317.
Hixson, A.W., Crowell, J.H., 1931. Dependence of reaction velocity upon surface and agitation. Ind. Eng. Chem. 23, 923–931.
Hiyama, Y., 2009. Quality overall summary mock P2 March 2009. <http://www.nihs.go.jp/drug/section3/English%20Mock%20QOS%20P2%20R.pdf/> (accessed 15.05.11).
ICH harmonised tripartite guideline, February 2003. Stability testing of new drug substances and products Q1A(R2).
ICH harmonised tripartite guideline, August 2009. Pharmaceutical development Q8 (R2).
ICH harmonised tripartite guideline, November 2005. Quality risk management Q9.
Katzhendler, I., Hoffman, A., Goldberger, A., Friedman, M., 1997. Modeling of drug release from erodible tablets. J. Pharm. Sci. 86, 110–115.
Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P., Peppas, N.A., 1983. Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm. 15, 25–35.
Lewis, G.A., Mathieu, D., Phan-Tan-Luu, R., 1999. Response surface methodology, in: Pharmaceutical Experimental Design. Marcel Dekker Inc., New York, pp. 185–246.
Li, F.Q., Hu, J.H., Deng, J.X., Su, H., Xu, S., Liu, J.Y., 2006. *In vitro* controlled release of sodium ferulate from Compritol 888 ATO based matrix tablets. Int. J. Pharm. 324, 152–157.
Lionberger, R.A., Lee, S.L., Lee, L., Raw, A., Yu, L.X., 2008. Quality by design: concepts for ANDAs. AAPS J. 10, 268–276.
Mariappan, T.T., Singh, S., 2003. Regional gastrointestinal permeability of rifampicin and isoniazid (alone and their combination) in the rat. Int. J. Tuberc. Lung Dis. 7, 797–803.
Prankerd, R.J., Walters, J.M., Parnes, J.H., 1992. Kinetics for degradation of rifampicin, an azomethine containing drug which exhibits reversible hydrolysis in acidic solutions. Int. J. Pharm. 78, 59–67.
Pund, S., Joshi, A., Vasu, K., Nivsarkar, M., Shishoo, C., 2011. Gastroretentive delivery of rifampicin: *in vitro* mucoadhesion and *in vivo* gamma scintigraphy. Int. J. Pharm. 411, 106–112.
Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J. Control. Release 5, 37–42.
Rouge, N., Buri, P., Doelker, E., 1996. Drug absorption sites in the gastrointestinal tract and dosage forms for site-specific delivery. Int. J. Pharm. 136, 117–139.
Sathe, P.M., Tsong, Y., Shah, V.P., 1996. *In vitro* dissolution profile comparison: statistics and analysis, model dependent approach. Pharm. Res. 13, 1799–1803.
Shah, P.P., Mashru, R.C., Rane, Y.M., Thakkar, A., 2008. Design and optimization of mefloquine hydrochloride microparticles for bitter taste masking. AAPS PharmSciTech 9, 377–389.
Shah, Y., Khanna, S., Jindal, K.C., Dighe, V.S., 1992. Determination of rifampicin and isoniazid in pharmaceutical formulations by HPLC. Drug Dev. Ind. Pharm. 18, 1589–1596.
Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., Vora, M.J., 2001. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. Int. J. Pharm. 228, 53–67.
Singh, B., Kumar, R., Ahuja, N., 2005. Optimizing drug delivery systems using systematic "design of experiments." Part I: fundamental aspects. Crit. Rev. Ther. Drug Carrier Syst. 22, 27–105.
Singh, R., Poddar, S.S., Chivate, A., 2007. Sintering of wax for controlling release from pellets. AAPS PharmSciTech 8, E1–E9.
Sudha, B.S., Sridhar, B.K., Srinatha, A., 2010. Modulation of tramadol release from a hydrophobic matrix: implications of formulations and processing variables. AAPS PharmSciTech 11, 433–440.
Vora, C., Patadia, R., Miattal, K., Mashru, R., 2013. Recent patents and advances on anti- tuberculosis drug delivery and formulations. Rec. Pat. Drug Deliv. Formul. 7, 138–149.
Wagner, J.G., 1969. Interpretation of percent dissolved time plots derived from *in vitro* testing of conventional tablets and capsules. J. Pharm. Sci. 58, 1253–1257.
Yu, L.X., 2008. Pharmaceutical quality by design: product and process development, understanding, and control. Pharm. Res. 25, 781–791.
Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, C., Xie, S., 2010. DDSolver: an add in program for modeling and comparison of drug dissolution profiles. AAPS J. 12, 263–271.
Zhang, Y.E., Schwartz, J.B., 2003. Melt granulation and heat treatment for wax matrix controlled drug release. Drug Dev. Ind. Pharm. 29, 131–138.

Journal of Pharmaceutical Investigation
DOI 10.1007/s40005-014-0170-z

RESEARCH ARTICLE

Risk based approach for design and optimization of site specific delivery of isoniazid

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Received: 27 September 2014 / Accepted: 16 December 2014

Abstract The research envisaged focuses on risk management approach for development and optimization of enteric coated tablet of isoniazid giving extended release in pH 6.8 phosphate buffer. Risk assessment using failure mode and effects analysis was done to depict the effects of unambiguous failure modes related to particular formulation/process variable. A 2^3 full factorial design was employed for optimization of core tablet to investigate effect of amount of Polyox WSR 303 (A), hardness (B) and amount of ethyl cellulose (C) on percent drug release in pH 6.8 phosphate buffer. Main effects and interaction plots were generated to study effects of variables. The selection of optimized formulation was done on overlay contour plots and desirability function. The optimized formulation exhibited percent drug release at first hour of 26.97 %, second hour of 44.20 %, fourth hour of 66.15 % and eighth hour of 97.9 % in phosphate buffer pH 6.8. Akaike information criteria and Model selection criteria revealed that the model was best described by Korsmeyer–Peppas power law. The Kopcha and Peppas–Sahlin model revealed diffusion as predominant mechanism of release which may be due to high solubility of drug and drug loading. Enteric coating optimization revealed weight gain of 10 % w/w as optimum; giving nil release of isoniazid in 0.1 N hydrochloric acid. The composite desirability for optimized formulation computed using equations and software were 0.91 and 0.90 respectively. Capability analysis on reproducibility batches revealed all indices above 1.33 signifying process was within control of producing batches as per desired specifications.

Keywords Quality by design · Site specific drug delivery · Failure modes and effects analysis · Full factorial design · Desirability function · Capability analysis

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Introduction

The drug delivery systems (DDS), which contemplates the carrier, the route and the target, has evolved into processes designed to enhance the efficacy of therapeutic agents through site-specific, modified or controlled release. This may involve augmented bioavailability, improved therapeutic index, improved biopharmaceutical properties, enhanced patient compliance, reduced side effects, etc. (du Toit et al. 2006; Batyrbekov et al. 1997). Amongst realm of DDS, oral route unambiguously, has been most sought especially in under developed or developing countries to epitomize the objectives like cost-effectiveness, feasibility and save resources (Singh et al. 2005). Of various oral DDS, site specific prolonged release formulations endow to be of greater interest to formulation scientists for ensuring optimal bioavailability or improve biopharmaceutical properties (du Toit et al. 2006).

Tuberculosis (TB) has remained, a significant health care problem since long times, particularly in developing countries. With respect to case notifications, 5.8 million newly diagnosed cases were notified to national TB control programmes conducted by World Health Organization in 2011 (Vora et al. 2013a). Isoniazid (INH) is the vital component in the current therapeutic treatment for TB. INH and rifampicin (RIF), the two most potent anti-TB

Published online: 27 December 2014

drugs, kill more than 99 % of tubercular bacilli within 2 months of initiation of therapy (du Toit et al. 2006; Mitchison 1985; Iseman and Madsen 1989). But, one of the major drawbacks in the use of INH for the treatment of TB is the severe toxic/adverse effects associated with it; primarily hepatotoxicity because of metabolism of INH, especially acetylation, by *N*-acetyltransferase (Schaberg et al. 1996; Burman et al. 2001; Katzung 2001). The effect is genetically prominent in rapid acetylators leading to plasma concentrations approximately one third to half of that in slow acetylators and average half-lives are less than 1–3 h, respectively. Moreover these toxic effects lead to discontinuation of the therapy because of the lack of patient compliance which results in subtherapeutic concentrations of the drug in the blood, leading to treatment failure and also encourages the INH resistant strains of *Mycobacterium tuberculosis* (Ellard et al. 1972; Hiremath and Saha 2008). Secondly, as addressed in earlier studies that in the acidic pH of stomach, RIF reacts with INH to form an insoluble compound 3-formylrifamycin resulting in reduction of bioavailability of RIF to the extent of 30 % (Shishoo et al. 2001; Singh et al. 2001). Moreover, permeability studies have demonstrated INH is less permeated through the stomach due to its protonated form at acidic pH (pKa = 2) and is well absorbed through all the three segments of intestine (Mariappan and Singh 2003). Hence, this all urgently demanded and necessitated development of site specific extended release formulations of INH in order to improve biopharmaceutical properties, minimize interaction with RIF and providing sustained release especially in rapid acetylators.

Several studies have been reported in the literature addressing the importance of INH site specific formulations. Pund et al. reported delayed release INH formulation consisted of INH pellets (Pund et al. 2010). Gohel et al. developed floating tablets of RIF using hydrophilic hydroxypropylmethyl cellulose (HPMC) polymer and calcium carbonate which were consequently filled together with delayed release INH capsule in one large capsule (Gohel and Sarvaiya 2007). Patent WO2011/012987A1 disclosed composition of RIF, INH and piperine wherein the bioavailability of RIF by piperine is maintained in presence of INH. The invention claims delayed release form of INH preferably tablet for minimizing physical interaction of both the drugs in gastrointestinal tract by segregating drug delivery (Bhatt et al. 2011).

INH is an anti-microbial agent and its satisfactory amount should be released initially as loading dose to achieve its Minimum Inhibitory Concentration (MIC) to elicit required therapeutic effect in the body. On basis of its MIC, volume of distribution and fraction bioavailable, a minimum of approximately 16 % should be released as initial loading dose theoretically (data not shown). On succinct amount of literature search of site specific drug delivery of INH, no report has been found in the area of site specific extended release formulation using stepwise systemic quality by design (QbD) approach.

The major aims of this study were: (i) step wise systemic formulation development and optimization using QbD approach (ii) applying principles of risk assessment and Failure Mode and Effects Analysis (FMEA) for formulation development (iii) to implement 2^3 full factorial design as optimization technique for establishment of mathematical equations and graphical results, thus depicting a complete picture of variation of the product/process response(s) as a function of the input variables and (iv) to perform capability analysis to investigate spread and control of process on reproducibility.

Materials and methods

Materials

Isoniazid was purchased from S. D. Fine Chem Pvt. Ltd., India. Microcrystalline cellulose (MCC) (Avicel® PH 101 and Avicel® PH 102) and Talc (Signet Chemical Corporation, India), Polyethyleneoxide (Polyox WSR 303), Hydroxypropylmethyl cellulose (HPMC E5) and Ethyl Cellulose (Ethocel N 10) (Colorcon Asia Pvt. Ltd., India), colloidal silicon dioxide (Aerosil® 200) and Eudragit L-100 55 (Evonik Ind., Mumbai, India) were generously gifted from the indicated sources. Magnesium stearate, Butylated Hydroxy Toluene (BHT), Polyethylene Glycol (PEG 400) and Triethyl citrate (S. D. Fine Chem Pvt. Ltd., India) were purchased from the indicated source. All other ingredients, chemicals and reagents were of analytical grade and were used as received.

Quality Target Product Profile (QTPP) of INH site specific extended drug delivery

The quality traits that a formulation must possess so as to accomplish the objectives set in target product profile as quantitative attributes are enlisted as target product quality profile (Lionberger et al. 2008). The International conference of harmonization (ICH) Q8 (R2) recapitulates them as QTPP (ICH Q8 (R2) 2009). The QTPP should endow a quantitative surrogate to ascertain the features of clinical safety and efficacy. Thus the pillar for identifying critical quality attributes (CQAs), critical process parameters and control strategy is ought to be QTPP. QTPP for INH site specific extended drug delivery is depicted in Table 1.

Table 1 QTPP of INH enteric coated sustained release tablet

QTPP element	Target	Justification
Dosage form	Enteric coated tablet which gives sustain action in pH 6.8	Tablet because commonly accepted unit solid oral dosage form. Enteric coated because INH is more absorbed from all segments of intestine due to its unionized form at intestinal pH and preferable site of absorption. To minimize interaction with rifampicin
Route of administration	Oral	Dosage form designed to administer orally
Dosage strength	75 mg	Generally accepted strength for combination with rifampicin (fixed dose combination)
Stability	Short term stability of 3 months on accelerated condition 40 °C/ 75 % RH and 3 months long term conditions 25 °C/60 % RH	Minimum time period (at least 3 months initially) decided to study stability of final formulation
Drug product quality attributes	Physical attributes	No physical defects in core tablet and no coating defects in coated tablet
	Assay	Meeting the compendial or other applicable quality standards. (90–110 % of label claims)
	Drug release in 0.1 N HCl	Less than 10 %
	Dissolution in pH 6.8 buffer	Initial burst release sufficient to achieve MIC followed by sustained release up to 8 h
Container closure system	Suitable for storage of dosage form	To maintain product integrity and quality up to target shelf life

Risk assessment by FMEA

The concept and spurts of quality risk management was introduced in ICH Q9, 2005 guidance (ICH Q9, 2005). The CQAs depend on dosage form designed; type of formulation, manufacturing method, etc. employed and is selected amongst many possible options. Thus based upon feasibility studies, we defined the formulation and manufacturing method as described in following below sections. An overall risk assessment of the formulation/ process variables was executed using FMEA method. By this method, the failure modes were identified that could have highest impact on product performance and greatest chance of eliciting product failure. FMEA method facilitates in prioritizing failure modes for risk management purposes according to the seriousness of their consequences (effects), how frequently they occur and how easily they can be detected. The relative risk that each formulation/process variable presents was ranked according to risk priority number (RPN) (Vora et al. 2013b).

Those attributes that could have a major impact on the drug product attributes needed to be studied in detail whereas those attributes that had minor impact on the drug product attributes required no further investigation. Table 2 portrays the FMEA for INH site specific extended release tablet with their respective RPN for each failure mode. The RPN was calculated with Eq. 1 mentioned as below:

$$\mathrm{RPN} = \begin{bmatrix} 5 \\ 4 \\ 3 \\ 2 \\ 1 \end{bmatrix} \mathrm{O} \times \begin{bmatrix} 5 \\ 4 \\ 3 \\ 2 \\ 1 \end{bmatrix} \mathrm{S} \times \begin{bmatrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{bmatrix} \mathrm{D} \quad (1)$$

where O is the occurrence probability or the likelihood of an event to occur; we ranked these as 5, frequent; 4, probable; 3, occasional; 2, remote and 1, improbable to occur. The next parameter S is the severity, which is a measure of how severe of an effect a given failure mode would cause; we ranked these as 5, catastrophic; 4, critical; 3, serious; 2, minor and 1, negligible or no effect. The final parameter D is the detectability which means the ease that a failure mode can be detected. Thus the more detectable a failure mode is, the less risk it presents to product quality. For D, we ranked 1, absolute certain or easily detectable; 2, high detectable; 3, moderately detectable; 4, low or remote detectable and 5 as hard to detect or absolute uncertain.

Preparation of INH sustained release core tablets

Some preliminary studies were carried out before selecting method of granulation, appropriate binder concentrations and polymer proportions. Only the formulations and studies pertinent for the present investigation are presented here. The tablets were prepared by wet granulation method. Briefly, accurately weighed quantity of INH (75 mg/tab), MCC PH 101, talc (as an anti-static agent), colloidal silicon

Table 2 Risk assessment by FMEA analysis to identify criticality of failure modes

Formulation/process parameter component	Failure mode	Failure effects	S	Potential causes or root of failure	O	Detectability method or control	D	RPN
Hardness	Inadequate hardness and its range	Drug release and friability	5	Machine failure, operator's error, excipient selection	4	Hardness tester, friability testing, dissolution	2	40
Amount of Polyox WSR 303	Improper concentration	Drug release	5	Improper concentration	5	Dissolution	2	50
Amount of binder (ethyl cellulose)	Improper concentration	Drug release	5	Improper concentration	5	Dissolution	2	50
Enteric coating	Improper coating	Gastric resistance	5	Improper weight gain, coating uniformity	5	Gastric resistance, dissolution	2	50
Granule sizes	Improper size	Drug release	5	Improper size	2	Dissolution	3	30
Packaging	Insufficient to protect drug from temperature, humidity and shipping	Stability	5	Packaging material	3	Assay, dissolution, hardness	2	30

dioxide and binder, ethyl cellulose (EC) were sifted through sieve ASTM # 30 (Wire metal GMP products, Mumbai, India) and physically mixed for 10 min. The premix was wet granulated using isopropyl alcohol (IPA) and dried in oven at 55 °C (Shree Kailash Industries, India). To the dried granules; MCC PH 102, Polyox WSR 303 (previously sifted through sieve ASTM # 30) and BHT (previously sifted through ASTM sieve 60 #) were added and blended for 10 min. Then colloidal silicon dioxide (as glidant, previously sifted through ASTM sieve 60 #) was added and blended for 10 min. Finally, magnesium stearate (as lubricant, previously sifted through ASTM sieve 60 #) was added and blended for 3 min. The homogeneous blend was compressed on an eight station automatic rotary tablet machine (Modern Engineering Works, New Delhi, India) equipped with shallow concave punches of 7.0 mm diameter to a target weight of 150 mg/tab. The dried granules of the optimized batch was subjected to downsizing in two different sizes range viz. through ASTM 18/24#, and 24/30# respectively and investigated for their effects on drug release. The granules to fine ratio were kept constant (70:30) in all the batches.

Seal coating on optimized tablet

Seal coat on core tablets of optimized batch was carried out with 8 % (w/w) aqueous dispersion of HPMC E5 and PEG 400 (82:18) using perforated coating pan apparatus (Solace Engineers (Mktg) Pvt. Ltd., Vadodara, Gujarat) to achieve 3 % weight gain. The process conditions were pre-warming of the tablets at 40 °C for 10 min; spray nozzle diameter, 1 mm; atomizing air pressure, 1–1.2 bar; inlet air temperature, 50–55 °C; product temperature 38–42 °C; spray rate, 5–8 g/min; pan rpm 6–8, post-drying at 40 °C for 30 min. The coating was performed to achieve weight gain of 3 % w/w.

Enteric coating on seal coated tablet

Enteric coating of tablets was done using perforated coating pan apparatus [Solace Engineers (Mktg) Pvt. Ltd., Vadodara, Gujarat] machine using aqueous dispersion of Eudragit L-100-55. To the aqueous dispersion, triethyl citrate as plasticizer (15 % w/w of dry polymer) and talc as anti-tacking agent (25 % w/w of dry polymer) previously homogenized were added and mixed for about 30 min. Final suspension was diluted with purified water under stirring to obtain 15 % w/v aqueous dispersion. Finally, the suspension was passed through ASTM 60# sieve and further used. The process conditions were pre warming of the tablets at 40° C temperature for 10 min, inlet air temperature (40–50 °C), bed temperature (29–31 °C) atomizing air pressure (1–1.2 bar), rotating speed of pan (7–10 rpm) and spray rate (4–6 g/min). After finishing of the coating, tablets were kept in the pan at 40° C for curing up to 30 min. The coating was performed to achieve three different weight gains viz. 8, 10 and 12 % w/w of average tablet weight.

2^3 Full factorial design for optimization of core tablet

A 2^3 factorial design with three factors, two levels, and eight runs was selected for the optimization study independent and dependent variables with their constraints are listed in Table 3. Percent drug release in phosphate buffer

Table 3 Formulation variables and their levels for 2^3 full factorial design

Factors	Coded levels	Actual levels
A: amount of Polyox WSR 303 (mg/tab)	−1	30
	1	45
B: Hardness (Kp)	−1	3–5
	1	6–9
C: amount of ethyl cellulose (mg/tab)	−1	7.5
	1	11.25
Responses		**Constraints**
Q3: Percent drug released in 3 h		20 % ≤ Q3 ≤ 30 %
Q4: Percent drug released in 4 h		30 % ≤ Q4 ≤ 40 %
Q6: Percent drug released in 6 h		60 % ≤ Q6 ≤ 75 %
Q10: Percent drug released in 10 h		90 % ≤ Q10 ≤ 100 %

pH 6.8 at first hour (Q3), second hour (Q4), fourth hour (Q6) and eighth hour (Q10) were selected as dependant variables. For predicting the optimal region, the linear polynomial equation generated for the variables was explained as follows (Eq. 2):

$$Y = \beta_0 + \sum \beta ixi + \sum \beta ijxixj \quad (2)$$

where Y is the predicted response, β_0 is model constant/coefficient, βi is the linear regression coefficient, βij is the interaction effect regression coefficient and Xi is the dimensionless coded value of the independent variables (Xi). All statistical treatments of design of experiment were performed using Design Expert software (ver. 8.0.7.1., Stat- Ease Inc., USA) Main effect plots, interaction plots and overlaid contour plots were generated using Minitab software (ver. 16.2.1., Minitab Inc., USA). All experimental runs were randomized to exclude any bias. Further the model was evaluated for best fit using parameters, coefficient of determination (r^2), adjusted r^2 (Adj-r^2), predicted r^2 (Pred-r^2), adequate precision (Shah et al. 2008) and Q^2 (Singh et al. 2005).

Physical characterization of the tablets

The compressed core tablets were subjected to various physical investigations like appearance, weight variation, hardness, friability and drug content. The weight variation was carried out on 20 tablets using electronic balance (Shimadzu AX 120, Japan). Tablet hardness was determined using minimum six tablets for each batch with dial type tablet hardness tester (Scientific Engineering Corporation, Delhi, India) respectively. Friability was determined by Roche Friabilator for 4 min at 25 rpm. Coated tablet was subjected to investigations like appearance, percentage weight gain, loss on drying and drug content. A validated RP-HPLC (Shimadzu, Kyoto, Japan) method was used for drug content measurement in triplicate. The method was modified as described by Shah et al. (1992) and validated for drug content determination. Analysis was performed on Phenomenex C18 column (250 mm × 4.6 i.d., 5 micron) at 1.0 ml/min flow rate with 75:25 % v/v mixture of methanol: 0.02 M disodium hydrogen phosphate pH 4.5 buffer as mobile phase at 254 nm. Linearity was demonstrated in range of 5–50 µg/ml. The recovery data was in the range of 98–102 %. The RSD for the precision was below 2 %. Percentage weight gain was computed using following equation.

$$\%\text{Weight gain} = (\text{Wt}_a - \text{Wt}_b)/\text{Wt}_b \times 100 \quad (1)$$

where Wt_a is the weight of tablet after coating and Wt_b is the weight of tablet before coating (Patel et al. 2010).

In vitro drug release

The study was performed using United States Pharmacopeia (USP) 30 type II apparatus (VDA 6-DR, Veego Instruments Corporation, Mumbai, India) using Method B for delayed release products as specified in USP for enteric coated tablets (USP 30/NF 25 2007a). Six dosage units were analyzed for dissolution profiling and data were recorded at 1, 2, 3, 4, 6, 8, and 10 h. For optimization of core tablets dissolution was performed in pH 6.8 phosphate buffer. Samples withdrawn were filtered through a 0.45 µm membrane filter and then analyzed immediately for drug release. The drug released in acidic medium was analyzed as per method specified in USP (USP30/NF25 2007b) and released in pH 6.8 phosphate buffer was measured by HPLC method as described in above section.

Desirability function

The desirability function is an excellent tool to merge multicriteria responses in one single criterion measurement. The

information obtained from it can be useful for predicting optimum levels of individual variables. If the value of the response is on target or is at optimum, its desirability value was allocated as 1 and for totally unacceptable value its desirability was given as 0. The individual desirability for each response was calculated (Shah et al. 2008) using the approaches discussed below.

Q3 was desired to be the maximum so as to achieve initial burst release of INH. But here target was specified for Q3 as it was anticipated that more of the initial release might not prolong drug release up to 8 h. Desirability $d1$ for response Q3 was calculated by Eq. 3:

$$d1 = \left[\frac{Yi - Ymin}{Ytarget - Ymin}\right] \tag{3}$$

Yi is the experimental result, and *Ymin* and *Ymax* represent the minimum and maximum possible values. *Ymax*, *Ymin* and *Ytarget* for this response were 33.16, 24.48 and 26.0 % drug release respectively.

For Q4 ($d2$) and Q6 ($d3$) there were no specific requirements for either obtaining maximum or minimum value. Q4 and Q6 response justifies that the drug releases in sustain manner from the dosage form. For Q4 and Q6; the formulations having percentage release within the constraint range selected (Table 3) was considered as optimum having desirability of 1, while formulations having values out of this range have a desirability of 0. This can be explained by below Eq. 4:

$$\begin{aligned} &d2, d3 = 0 \quad \text{for } Yi < Y\,min \\ &d2, d3 = 1 \quad \text{for } Ymin < Yi < Ymax \\ &d2, d3 = 0 \quad \text{for } Yi > Ymax \end{aligned} \tag{4}$$

However for Q10 more than 90 % drug should be release to ascertain complete release from the dosage forms. *Ymax* and *Ymin* for this response were 98.9 and 95.8 % drug release respectively. Thus $d4$ for Q10 was calculated by formula shown in Eq. 5.

$$d4 = \left[\frac{Yi - Ymin}{Ymax - Ymin}\right] \tag{5}$$

The overall desirability was calculated from the individual values by using the following Eq. 6.

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} \tag{6}$$

Curve fitting and release mechanism

In order to study the drug release mechanism from the formulations, various models were considered to fit the experimental data using Microsoft Excel based DD solver software (Microsoft, USA) to execute and evaluate dissolution data modeling. The in vitro release pattern was evaluated to check the goodness of fit to the zero order (Costa and Sousa Lobo 2001), first order (Wagner 1969), Higuchi (Higuchi 1963), Baker (Baker and Lonsdale 1974), Hopfenberg (Costa and Sousa Lobo 2001; Katzhendler et al. 1997), Hixson–Crowell's (Hixson and Crowell 1931), Weibull (Sathe et al. 1996) and Korsmeyer–Peppas power law equation (Korsmeyer et al. 1983; Ritger and Peppas 1987). For Korsmeyer–Peppas model, data were analyzed for first 60 % of the drug release. Importantly, the goodness of fit was evaluated using adjusted r^2 (correlation coefficient) values. This is for the reason that r^2 will always increase as more parameters are included, whereas r^2 adjusted may decrease when over fitting has occurred (Zhang et al. 2010). The Akaike information criterion (AIC) and Model selection criteria (MSC) criteria were also applied as described elsewhere (Akaike 1974; Zhang et al. 2010; Vora et al. 2013b).

In order to understand the drug release mechanism, the release data of the optimized batch (ES 5) was fitted to empirical equations proposed by Kopcha (Kopcha et al. 1991).

$$M = At^{1/2} + Bt \tag{7}$$

In the above equations, M ($\leq$70 %) is the percentage of drug released at time t, while A and B are, respectively, diffusion and erosion terms. According to this equation, if diffusion and erosion ratio, A/B = 1, then the release mechanism includes both diffusion and erosion equally. If A/B > 1, then diffusion prevails, while for A/B < 1, erosion predominates (Ratsimbazafy et al. 1996). Additionally, the data were also fitted into Peppas–Sahlin model to understand drug release mechanism (Peppas and Sahlin 1989; Grassi and Grassi 2005).

Capability analysis

Capability analysis is used to assess whether a process is capable of producing output that meets your desired quality traits. A capable process is able to produce products that meet desired specifications. The process here was assumed to be in statistical control. The normal probability plot was used to examine normal distribution of data (Bissell 1994). Additionally, Anderson–Darling, Ryan–Joiner and Kolmogorov–Smirnov test statistic at 5 % significance level were applied to assess whether data follows a normal distribution or not. Capability analysis was performed on five reproducibility batches (n = 30) using Minitab software (ver. 16.2.1., Minitab Inc., USA).Cp, CPU, CPL and Cpk were computed for potential within capability and Pp, PPU, PPL and Ppk for overall capability respectively (Rudisill and Litteral 2008; Shinde and Katikar 2012). The 3–σ standard deviation variation was everywhere considered for relating process spread to specification spread.

Packaging and stability study

The optimized batch was subjected to short term stability testing according to the ICH guidelines for zones III and IV (ICH Q1A(R2) 2003). Tablets were packed in count of 30 into high density polyethylene bottle with child resistant cap and were further induction sealed. Before induction sealed one silica bag was kept in bottle as desiccant. The sealed bottles were exposed to accelerated (40 ± 2 °C/75 ± 5 % relative humidity) and long term (25 ± 2 °C/60 ± 5 % relative humidity) stability for three months. The samples were withdrawn periodically (0, 15, 30, 60 and 90 days) and evaluated for different physicochemical parameters like visual inspection, drug content, gastric resistance and in vitro drug release.

Results and discussion

QTPP of INH site specific delivery

Laying down QTPP depends upon formulation type and process chosen (Yu 2008; Lionberger et al. 2008). Based on preliminary trials undertaken, the parameters that will be focused in our study were selected and enlisted as QTPP for INH site specific delivery (Table 1). Thus, except recitation of our QTPP, the further steps to describe the QTPP are not discussed. The said QTPP will lay down the foundation for determining CQA.

Risk assessment by FMEA

The factors that were embarked and assessed by FMEA in development of INH site specific delivery are highlighted in Table 3. In the current approach for development, the factors that exhibited RPN $\geq$ 40 was considered as high risk, $\geq$20 to <40 was considered as medium risk and <20 was considered as low risk (Vora et al. 2013b). From Table 2, it is clearly stipulated that amount of Polyox WSR 303, hardness, amount of EC and enteric coating have RPN $\geq$ 40 and require through investigation and optimization. Thus, the optimization of three main factors that affect the core tablet formulation i.e. amount of Polyox WSR 303, hardness and amount of EC was done statistically using 2^3 full factorial design for establishing design space. The enteric coating RPN also falls under high risk category and its optimization is discussed in its respective section. Granule sizes and packaging RPN fall under moderate risk category and are also discussed in their respective sections.

Physical evaluation of tablets

Physical appearance, friability, weight variation and assay of all the formulations of core tablets were found to be satisfactory. Hardness was studied in two ranges 3–5 Kp and 6–9 Kp. Friability of the core tablets was found to be less than 0.5 % (w/w). The coated tablet appearance was found to be satisfactory. Weight gain of seal coated tablets was checked by weighing different sets of 20 tablets three times and results were found to be comparable with low standard deviation (SD < 5 % of weight gain) while loss on drying was found to be 0.9 % considering initial pre-warmed uncoated tablet weight and loss on drying. Similar calculations were carried out for enteric coated tablet and results were found with low standard deviation (SD < 5 % of weight gain) and loss on drying of 1.1 %.

Effect of factors on the responses

Q3

Results of the measured response for 2^3 full factorial design are displayed in Table 4. Regression coefficients and ANOVA results of response variables are shown in Table 5. From the results, it can be concluded that hardness was the most influencing factor affecting negatively (Table 5) in initial burst release to achieve desired MIC. The same can be inferred from the Pareto chart, half normal plot and main effect plots for Q1 (Figs. 1a, 2a) respectively. The reason may be the higher hardness increased the bonding strength of the tablet which would have decreased the porosity and increased the tortuosity factor of the matrix which is responsible for its negative impact on drug release.

Moreover, the initial higher release was observed in all batches (Table 4). Looking deeply inside, it can be anticipated that in the beginning, drug close to surface of matrix might be released before the surrounding polymer reached the polymer disentanglement concentration. In addition, for water soluble drugs diffusional driving force would be highest and mean dissolution rates close to the mean water infiltration rates (Tahara et al. 1996). Also for high viscosity polymer like Polyox WSR 303, it would take a longer time to form a gel layer which provides enough time for initial burst release. Similar mechanisms have been reported in literature for initial high release of water soluble drug from matrix monolithic system (Barakat et al. 2008). Now interestingly, interaction effect was also observed between amount of Polyox WSR 303 and amount of EC which affected positively (Table 5; Fig. 1a). The same can be inferred from interaction plot (Fig. 3a). The quadratic equation for reduced model in coded units is as below:

$$Q3 = 28.39 - 3.05B + 0.79AC \quad (8)$$

Q4

From Table 5; Figs. 1b and 2b, it can be concluded that amount of Polyox WSR 303 and hardness were the most

Table 4 Matrix of the experiments for 2^3 full factorial design and results for the measured responses

ES[a]	Amount of Polyox (mg/tab)	Hardness (Kp)	Amount of ethyl cellulose (mg/tab)	Q3 (h)[b]	Q4 (h)[b]	Q6 (h)[b]	Q10 (h)[b]
8	−1	−1	−1	33.16 ± 1.2	51.79 ± 2.2	82.42 ± 1.9	96.50 ± 1.5
3	1	−1	−1	30.50 ± 0.9	44.90 ± 3.0	78.90 ± 2.2	96.80 ± 0.9
5	−1	1	−1	26.97 ± 1.3	44.20 ± 2.9	66.15 ± 1.1	97.90 ± 0.8
4	1	1	−1	24.50 ± 1.6	33.95 ± 2.1	63.75 ± 1.6	98.90 ± 0.9
6	−1	−1	1	30.90 ± 0.8	49.50 ± 2.6	80.10 ± 1.5	98.20 ± 0.8
2	1	−1	1	31.20 ± 1.6	48.90 ± 2.3	82.20 ± 1.2	97.80 ± 1.2
1	−1	1	1	24.48 ± 0.9	41.06 ± 1.5	58.72 ± 2.0	96.20 ± 1.3
7	1	1	1	25.40 ± 1.3	35.90 ± 2.0	62.62 ± 2.5	95.80 ± 0.5

[a] Experimental sequence

[b] Mean ± SD (n = 6)

Table 5 ANOVA results (p values): effect of the variables on Q3, Q4, Q6 and Q10

Factors	Q3		Q4		Q6		Q10	
	Coefficient	p value (Prob > F)	Coefficient	p value (Prob > F)	Coefficient	p value (Prob > F)	Coefficient	p value (Prob > F)
Intercept	28.39	0.0313*	43.78	0.0466*	+71.86	0.0171*	+97.26	0.1601
A	−0.49	0.0697	−2.86	0.0333*	0.010	0.9254	+0.062	0.6051
B	−3.05	0.0112*	−5.00	0.0191*	−9.05	0.0060*	−0.063	0.6051
C	−0.39	0.0864	0.065	0.7397	−0.95	0.0570	−0.26	0.2048
AB	+0.10	0.3107	−0.99	0.0957	+0.37	0.1457	+0.088	0.5000
AC	+0.79	0.0430*	1.42	0.0669	+1.49	0.0363*	−0.26	0.2048
BC	−0.004	0.9557	−0.36	0.2498	−1.19	0.0453*	−0.94	0.0592

Regression coefficients are in coded value

* Statistically significant ($p < 0.05$)

influencing factor affecting Q4 negatively (negative coefficient; Table 5) to maintain the sustain release. The reason for factor A to retard drug release might be due to greater chain entanglement produced by high viscosity polymers, ultimately resulting in thicker gel layer after hydration of it. Furthermore, on formation of gel by high viscosity polymers, it is difficult for longer chains to dissolve since high energy is required for pulling them off the matrix. Similar mechanism have been proposed for high viscosity polymers for sustained drug release by some researchers (Hiremath and Saha 2008; Colombo et al. 2000). Thus, the longer diffusional path length created by gel layer resulted in the decreased effective diffusion of the drug and therefore a reduction in the drug release rate. The mechanism of hardness for decreasing drug release has been already discussed. The quadratic equation for reduced model in coded units is as below:

$$Q4 = 43.78 - 2.86A - 5.00B \quad (9)$$

Q6

As shown in Fig. 1c, 2c and Table 5, it can be concluded that hardness was the most influencing factor affecting Q6 negatively. The batches (e.g. ES 5, 4 and 1) in which hardness was at higher level could sustain drug release in desired constraints. On the contrary, batches (e.g. ES 8, 3 and 6) in which hardness was at low level released more than 75 % drug. Interestingly, interaction effect was observed between AC and BC (Table 5) on percent drug release at Q6 time point. The same can be inferred from Pareto chart, half normal probability ploy (Fig. 1c) and p value of ANOVA table (Table 5). The results obtained surprisingly depict that there is no pronounced decrease in drug release on increasing binder EC concentration (Table 5) similar to results of Q4. The reason might be EC was added as dry binder in the premix. Then IPA was added to granulate the mass. In case of EC it is hypothesized that it dissolved on addition on IPA and it exhibits coating like effect. Here, addition of IPA was stopped on achieving end point in granulation. Thus it is hypothesized that quantity of IPA was not sufficient to dissolve dry binder in the premix to exhibit more hydrophobic coating like effect and as a result much binder remained in the dry state. As a result, there might not be significant difference in providing hydrophobic interaction as compared to lower binder concentrations. Secondary reason assumed may be

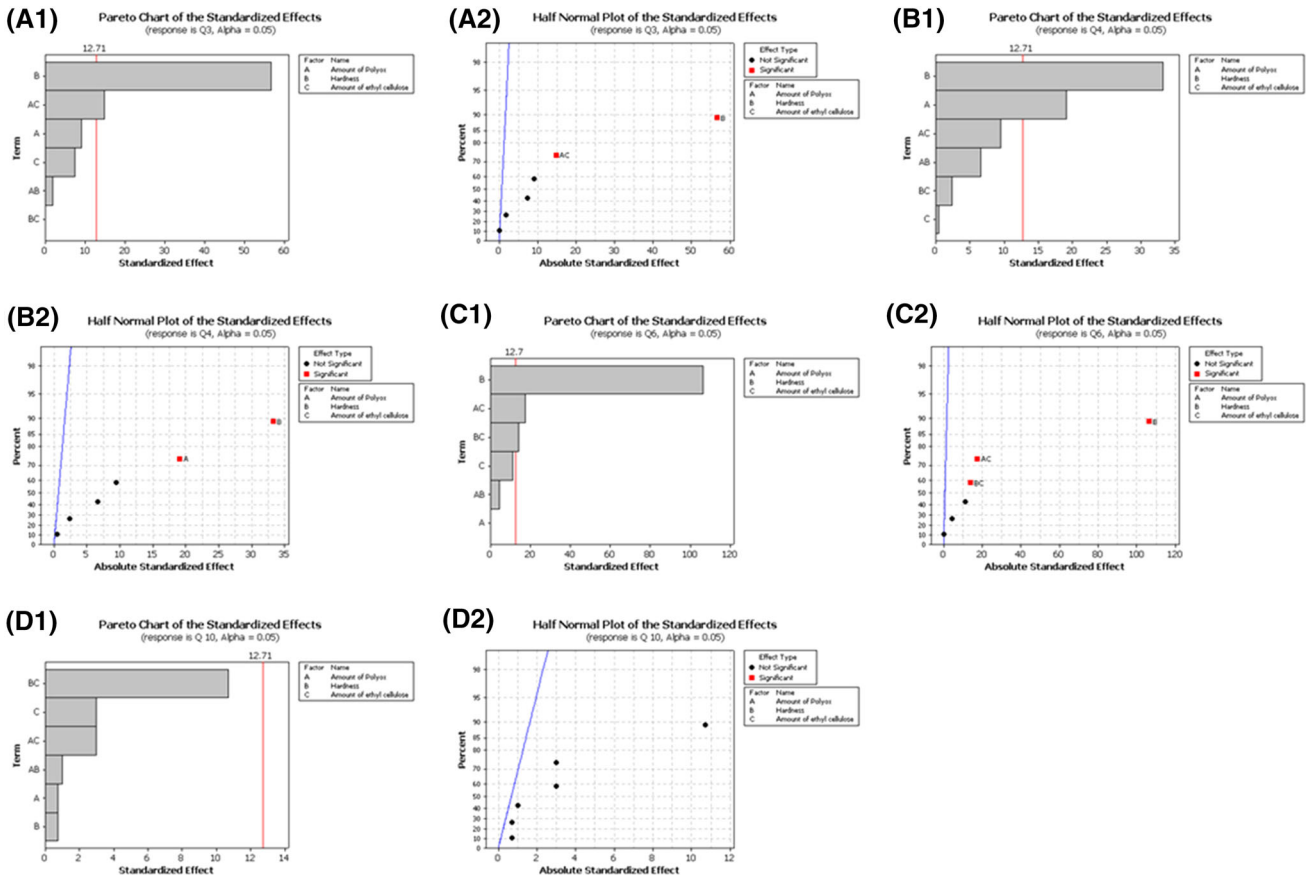

Fig. 1 Pareto chart and half normal plot of the standardized effects for responses **a** Q3, **b** Q4, **c** Q6 and **d** Q10

high solubility of the drug and drug loading. Hence, due to early achieving end point in granulation with less consumption of IPA, together with high solubility of drug there is not much significant decrease in drug release on increasing binder concentration. The exact mechanism yet needs to be investigated.

The quadratic equation for reduced model in coded units is as below:

$$Q6 = 71.86 - 9.05B + 1.49AC - 1.19BC \quad (10)$$

Q10

For Q10, more than 90 % drug release is desired to ensure complete drug release from developed formulation. As highlighted in Fig. 1d and p value from ANOVA table (Table 5), it is clearly stipulated that none factor neither interaction had influenced on percent drug release at Q10. All the batches showed more than 90 % drug release at Q10. The reason may be due to high solubility of drug and drug loading. Another factor is threshold level of retardation of drug release rate by polymer as drug release does not result solely from polymer erosion, but also on drug diffusion through the hydrated polymer layers.

The quadratic equation for model in coded units is as below:

$$Q10 = 97.26 + 0.062A - 0.063B - 0.26C + 0.088AB - 0.26AC - 0.94BC \quad (11)$$

Model fitting and statistics of the measured responses

Here, higher values of r^2 for all dependent variables were found which statistically signify a good fit. Additionally, Adj-r^2 and Pred-r^2 values were also in reasonable agreement signifying good model fit (Table 6). Further model showed the adequate precision value greater than 4, indicating adequate model discrimination (Shah et al. 2008). A model fit value of $Q^2 > 0.5$ is considered as fairly good and value of $Q^2 > 0.9$ is generally taken as excellent (Singh et al. 2005). Q^2 values for all the measured responses were good signifying good model fit.

Evaluation of model using cross-validation

The reliability of the model was assessed by conducting five experiments by varying the formulation variables at values other than that of the model. The experimental and

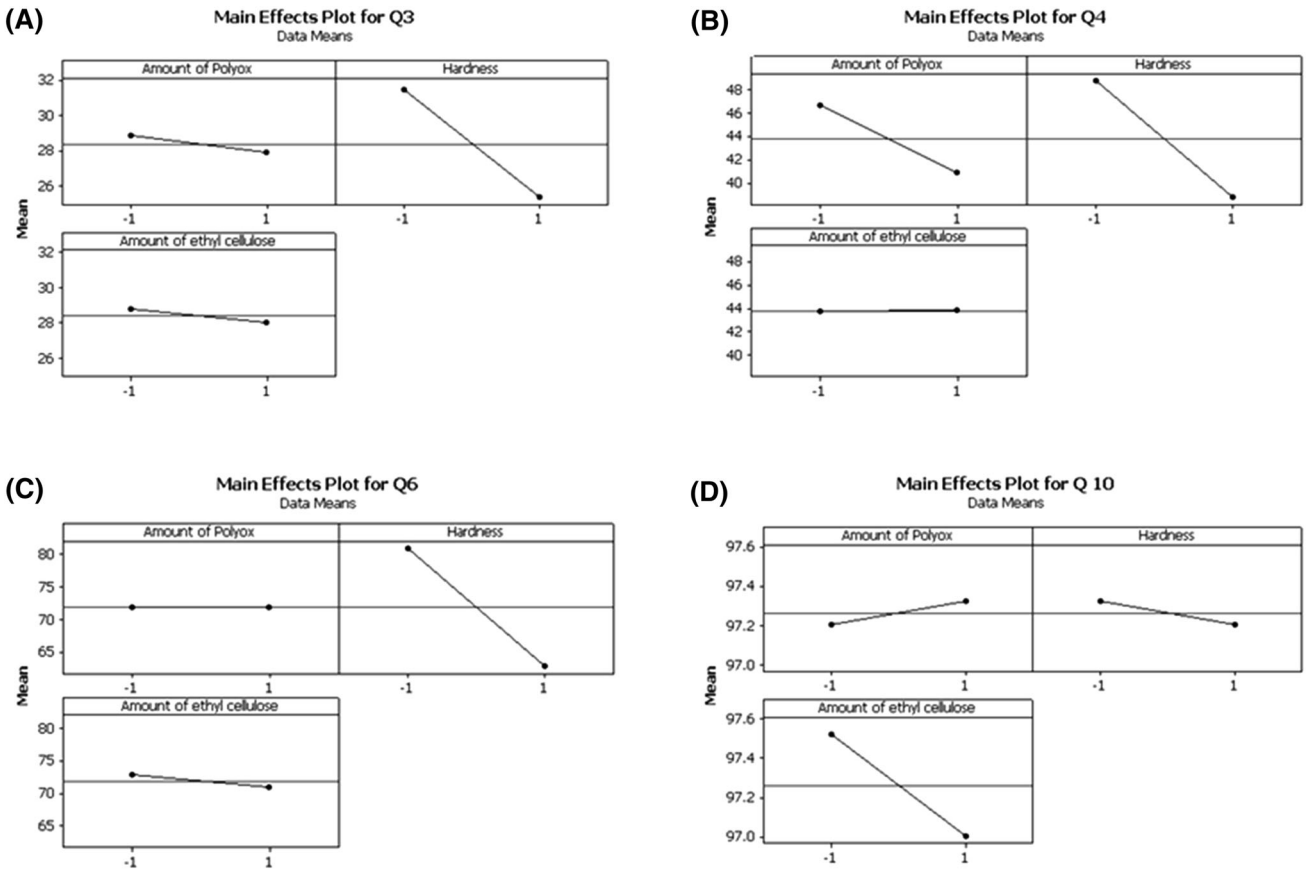

Fig. 2 Main effects plot for **a** Q3, **b** Q4, **c** Q6 and **d** Q10 as a function of amount of Polyox WSR 303, hardness and amount of ethyl cellulose

predicted values for each response are shown in Table 7. Bias or percent relative error between predicted and experimental values for each response was calculated by the following Eq. (12).

$$\text{Bias} = \left[\frac{\text{Predicted value} - \text{Experimental value}}{\text{Predicted value}}\right] \quad (12)$$

Results from Table 7 reveal reasonable agreement between the predicted and the experimental value in all the five batches, due to low value of the bias was found. Thus it can be concluded that the equations express satisfactorily the influence of the chosen formulation variables on the responses under study.

Optimization using desirability function

Desirability function was calculated for percent drug release at Q3, Q4, Q6 and Q10 time. Based on the composite desirability data and overlay contour plots (Fig. 4), ES 5 was identified as the optimum batch having desirability of 0.91. Composite desirability found out for optimized batch with the help of Minitab software was 0.90. The weight and importance was allotted 1 for each response respectively.

Curve fitting and release mechanism

Values of adjusted r^2, AIC and MSC value are presented in Table 8. The drug release data of the optimized batch ES 5 shows a good fit to the Korsmeyer–Peppas power law equation which can be confirmed by comparing the values of adjusted r^2 with that of the other models. The values of release exponent (n) determined for the optimized formulation batch ES 5 was found to be 0.592 suggesting the probable release by anomalous transport (Ritger and Peppas 1987). The lowest AIC value; 13.3878, of optimized batch ES 5 indicates that Korsemeyer–Peppas power law was the best fit model in describing the dissolution behavior. Similarly, the highest MSC, 5.4208, of the optimized batch ES 5 indicates the same.

To investigate the underlying release mechanism, Kopcha model was applied. The A/B ratio for drug release was found to be 4.46 which is greater than 1, showing predominance of diffusion relative to erosion. The value of constants k1 (26.575) and k2 (2.966) of Peppas–Sahlin model are displayed in Table 8. k1 denotes relative contribution of drug diffusion to drug release and k2 denotes relative contribution of polymer relaxation to drug release. From Table 8, it is clearly stipulated that diffusion is the

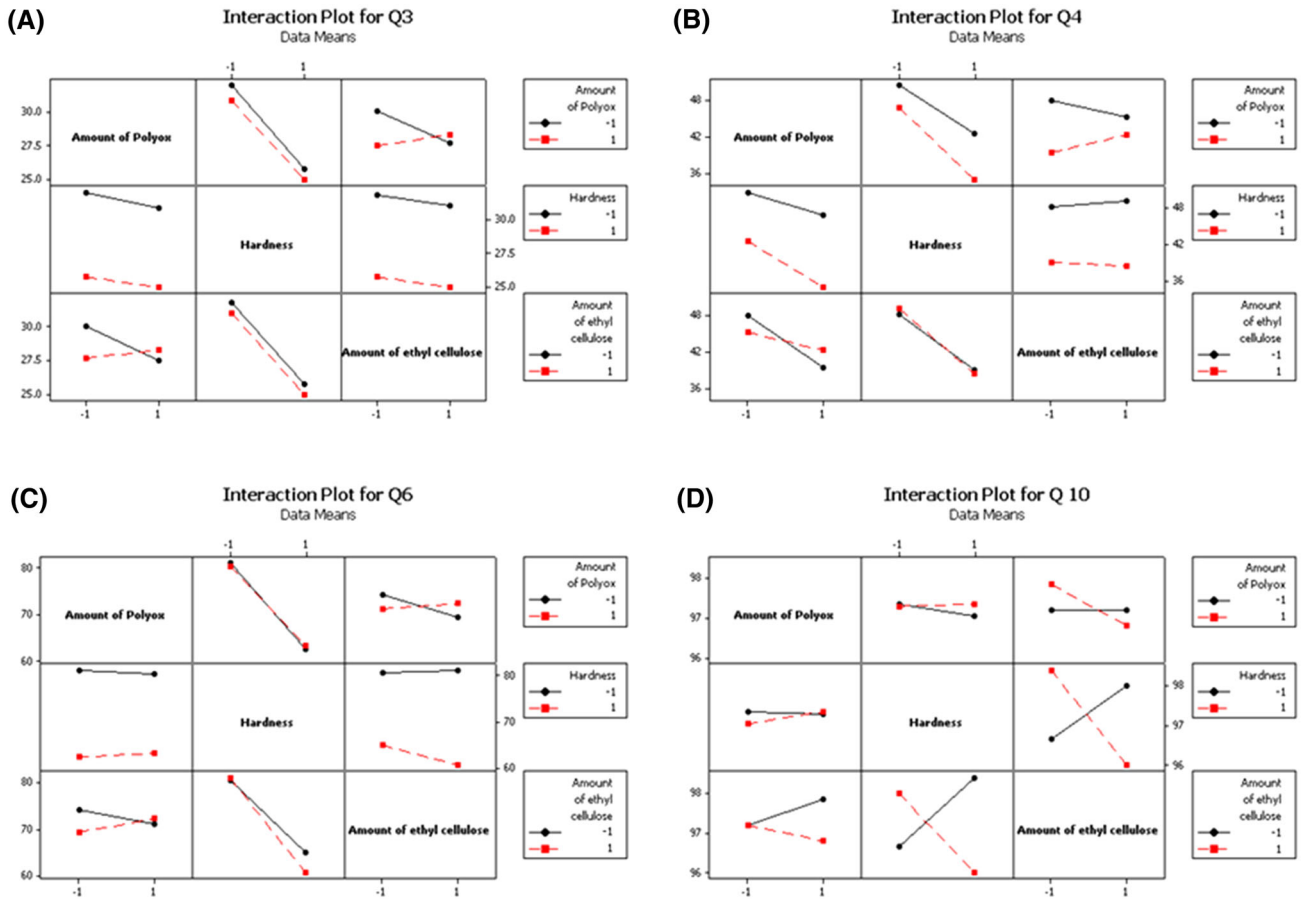

Fig. 3 Interaction profile of amount of Polyox WSR 303, hardness and amount of ethyl cellulose on **a** Q3, **b** Q4, **c** Q6 and **d** Q10

Table 6 ANOVA results showing the effect of independent variables on the measured responses

Measured response	Sum of squares (SS)	DF	Mean square (MS)	*F* value	(Prob > F) 100	PRESS	r^2	Adj-r^2	Pred-r^2	Adeq. precision	Q^2
Q3	82.75	6	13.79	596.75	0.0313	1.48	0.9997	0.9980	0.9821	61.037	0.9821
Q4	290.47	6	48.41	268.95	0.0466	11.52	0.9994	0.9957	0.9604	44.953	0.9603
Q6	629.24	6	115.37	1996.09	0.0171	3.70	0.9999	0.9994	0.9947	105.385	0.9946
Q10	8.26	6	1.38	22.47	0.1601	3.92	0.9926	0.9485	0.5288	12.635	0.5254

predominant mechanism for drug release. Thus data are in agreement with Kopcha model revealing diffusion as predominant mechanism which may be due to high solubility of the drug.

Effects of granule size

The granules of optimized batch ES 5 were subjected to downsizing in two particle sizes range viz. ASTM 18/24#, and 24/30# to investigate its effects on percent drug release. Results revealed that there is no statistically significant difference in drug release for two different sizes of granules ($p > 0.05$). The reason may be high water solubility of the drug together with its high percent drug loading. Probably, the high diffusion co-efficient of highly water soluble drug may have quash the effect of granule size. Hence, risk and criticality of this failure mode is low.

Effect of enteric coating

Here target was set to be zero percentage of drug release in acidic medium as it was anticipated that even small amount of INH release in acidic medium will augment the degradation of RIF.

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 (Fig. 5). Weight gain of

Table 7 Comparison of responses between predicted and values for the cross validation set

Responses	Test	Factors/levels			Experimental values	Predicted values	Bias%
		X1	X2	X3			
Q3	1	−1	−0.6	−0.6	28.55	30.69	6.99
	2	−0.6	0	0.4	30.55	28.20	−8.33
	3	−0.4	0.6	0	27.12	26.56	−2.11
	4	0	−0.4	0.6	31.35	29.61	−5.88
	5	0.5	0.5	−0.5	27.50	26.67	−3.12
Q4	1	−1	−0.6	−0.6	50.80	49.64	−2.34
	2	−0.6	0	0.4	46.90	45.5	−3.08
	3	−0.4	0.6	0	40.10	41.92	4.34
	4	0	−0.4	0.6	44.26	45.78	3.32
	5	0.5	0.5	−0.5	41.55	39.85	−4.27
Q6	1	−1	−0.6	−0.6	75.10	77.76	3.42
	2	−0.6	0	0.4	74.10	71.50	−3.64
	3	−0.4	0.6	0	68.23	66.43	−2.71
	4	0	−0.4	0.6	71.90	75.77	5.11
	5	0.5	0.5	−0.5	65.20	67.26	3.06
Q10	1	−1	−0.6	−0.6	95.50	96.95	1.50
	2	−0.6	0	0.4	98.66	97.18	−1.52
	3	−0.4	0.6	0	96.20	97.17	1.00
	4	0	−0.4	0.6	97.55	97.35	−0.21
	5	0.5	0.5	−0.5	95.66	97.71	2.10

8 % w/w provided similar dissolution profile as that of core tablet in phosphate buffer pH 6.8 but simultaneously drug release of 2.3 % (mean value) was obtained in acidic medium. On the contrary, 12 % w/w provided zero percentage drug release in acidic medium but slow down dissolution profile of the core tablet during initial hour in pH 6.8 phosphate buffer (Fig. 5).

Capability analysis

The probability plots result for detecting normality of distribution are depicted in Table 9 for Q3, Q4, Q6 and Q10 respectively together with p values of Anderson–Darling test, Ryan–Joiner (similar to Shapiro–Wilk test) and Kolmogorov–Smirnov test. The p values of all the three tests were greater than 0.5 indicating normal distribution of the data at 5 % significance level. Hence, capability analysis with normal distribution was undertaken.

Results of the various indices of capability analysis for Q3, Q4, Q6 and Q10 are displayed in Table 9. For a process to be capable to produce batches within specifications, all the indices value should be above 1.33 (Rudisill and Litteral 2008; Kane 1986). From the results of Table 9, it can be inferred that all the indices value were above 1.33 which indicates that the process passes the capability analysis at 3–σ standard deviation process spread and the process is capable of producing batches that conform to specifications. Therefore, the measurements are located within specification limits for Q3, Q4, Q6 and Q10.

For Q4, Cp is 2.10, which indicates that the specification spread is 2.10 times greater than the 3–σ spread in the process. Moreover Cp (2.10) and Cpk (2.05) are very close to one another, revealing that the process is centered. Regarding overall capability, Pp value is 2.13 indicating 2.13 times greater than the 3–σ spread in the process. Also Pp (2.13), Ppk (2.07) and Cpm (2.12) are very close to another, indicating that the process is centered on the target. Furthermore, the within and overall capability indices are very close to each other indicating process is within the control. For Q3 and Q6, Cp and Cpk are not as close to each other as compare to Q3 signifying the process is slight deviating from the center (Table 9). Nevertheless, all the indices of within and overall were above 1.33 (Table 9) and within desired constraints (Table 3) signifying process passes capability analysis at 3–σ standard deviation process spread. Regarding Q10, there is disparity between Cp (3.25) and Cpk (1.41), which is due to more than 95 % drug release in all the batches which was desired. The same can be inferred from CPL (5.10) value. Similar conclusions can be drawn for overall indices. Furthermore, all the indices were above 1.33 (Table 9) revealing process passes capability indices.

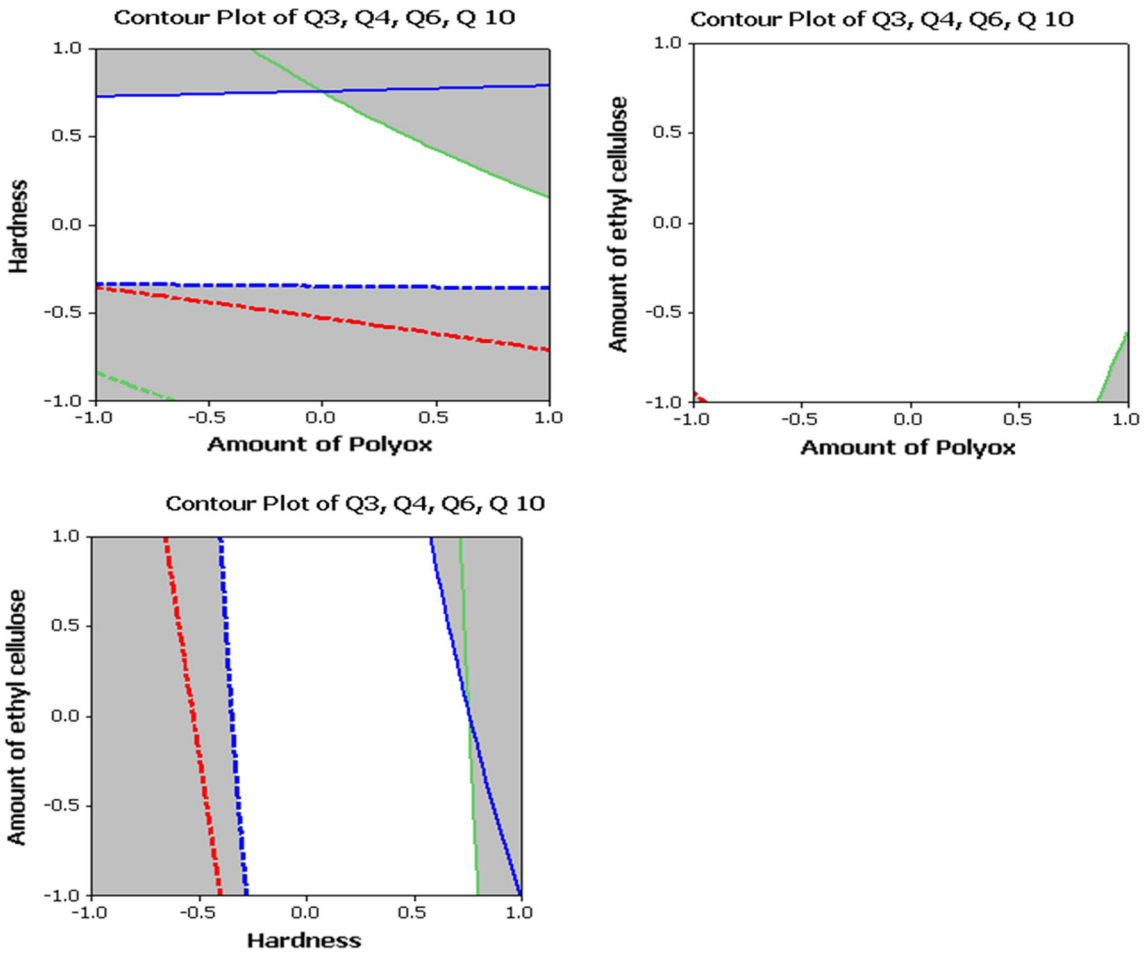

Fig. 4 Overlaid contour plots of Q3, Q4, Q6 and Q10 as a function of amount of Polyox WSR 303, hardness and amount of ethyl cellulose

Table 8 Comparative characteristics of different drug release kinetic models for optimized batch

Batch no.	Zero-order	First order	Higuchi	Hixon–Crowell	Hopfenberg	Baker Lonsdale	Weibull	Korsmeyer–Peppas	Peppas–Shalin
ES 5									
r^2	0.7746	0.9819	0.9806	0.9845	0.9833	0.8969	0.9779	0.9973	0.9953
AIC	35.0412	22.4381	22.7874	21.6436	22.5971	31.1288	24.0023	13.3878	16.2368
MSC	1.0899	3.6105	3.5407	3.7694	3.5787	1.8724	3.2977	5.4206	4.8508
								28.813 (k)	26.575 (k1)
								0.592 (n)	2.966 (k2)

Packaging and stability studies

The optimized formulation ES 5 showed negligible change under the conditions of storage for parameters like appearance, drug content, gastric resistance and in vitro drug release. The similarity factor (f2) (Costa and Sousa Lobo 2001) was employed for comparison of dissolution profiles on each time point. It ranged from 83 to 92. Thus the data suggested that the formulation was stable for under the packaging material selected revealing that it risks it under control and low.

Risk mitigation and control strategy

2^3 full factorial design was employed to examine the multidimensional interaction of input variables of the core tablet which were ranked as high risk in the initial risk assessment for establishment of a design space. The acceptable region within which a quality of the product can be constructed is called as design space (Lionberger et al. 2008; Yu 2008). The risk mitigation and control strategy is fused outline of how quality is established based on current process and existing product knowledge.

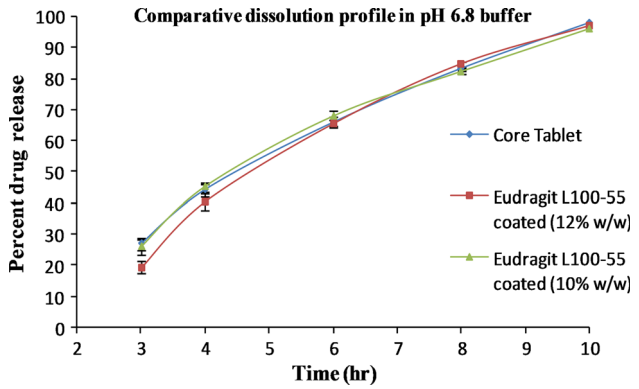

Fig. 5 Comparative dissolution profile of core tablet, coated tablet with Eudragit L100-55 (12 % w/w) and coated tablet with Eudragit L-100-55 (10 % w/w)

For factor C, it can be inferred from main effect plots (Fig. 2a–d), overlay contour plots (Fig. 4) and p value from ANOVA (Table 5) that it did not significantly affected any of the dependant variables (p value >0.05) as main effect but showed interaction effects with other factors on some of the responses (Fig. 3a–d). Using software, we modulated the range of C by changing its setting level and observed the change in overlay contour plot of A versus B.

The range of C where region of A versus B in overlay contour plot was found to be maximum was selected as range of C. We found range of C of −0.3 to 0.6 in coded units as optimum range and thus we decided to use EC in that range. The risk with operating in this range is low. The risk mitigation strategy is to monitor the dissolution within desired constraints range.

From the ANOVA table and p value (Table 5), overlay contour plots (Fig. 1) and main effect plots (Fig. 2a–d) it is clearly observed that factor A have major impact on percent drug release at Q3 and Q4 and B on Q3, Q4 and Q6.

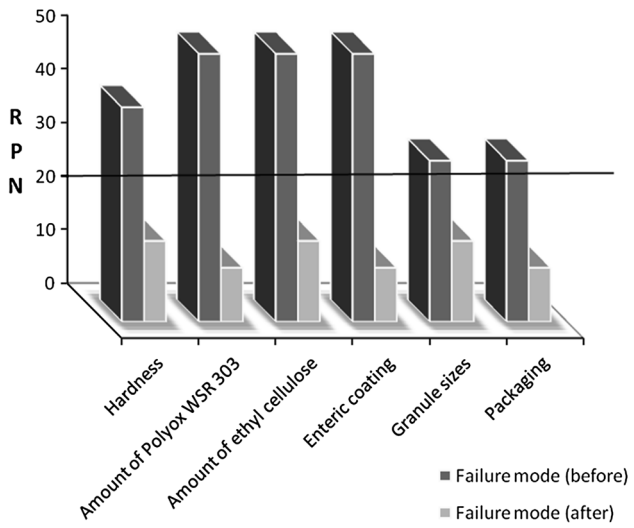

Fig. 6 FMEA analysis of INH site specific tablet depicting RPN number of failure mode before and after implementation of control strategy

Thus there is an optimum range for A and B where you can get the desired drug release in the set constraint range which is specified in overlay contour plot (Fig. 4) of B and C versus all four responses. Working in this zone, risk is low as all the responses will ascertain to be in the constraint range. The risk mitigation strategy for the same is to monitor percent drug release at Q3, Q4, Q6 and Q10 are in the constraint range.

The enteric coating was also in the high risk category which was optimized as discussed. Regarding moderate RPN failure modes, granule size and packaging were discussed in their respective sections. Figure 6 expresses the FMEA analysis before and after the execution of the control strategy. It was found that RPN of all the possible failure modes were below 20; making them to fall under

Table 9 Summary of the various capability indices

Variable	Potential within capability				Overall capability				
	Cp	Cpk	CPL	CPU	Pp	Ppk	PPL	PPU	Cpm
Q3	1.99	1.43	2.55	1.43	2.13	1.53	2.74	1.53	1.65
Q4	2.10	2.05	2.05	2.16	2.13	2.07	2.07	2.18	2.12
Q6	2.92	2.41	2.41	3.43	3.17	2.61	2.61	3.72	2.51
Q10	3.25	1.41	5.10	1.41	3.42	1.48	5.36	1.48	1.35

Normal probability test results at 5 % significance level

	AD value	p value	RJ value	p value	KS value	p value
Q3	0.381	0.379	0.988	>0.100	0.123	>0.150
Q4	0.324	0.510	0.990	>0.100	0.112	>0.150
Q6	0.435	0.280	0.985	>0.100	0.137	>0.150
Q10	0.541	0.152	0.986	>0.100	0.131	>0.150

AD Anderson–Darling, *RJ* Ryan–Joiner, *KS* Kolmogorov–Smirnov

the low risk category. The scalability can be further evaluated from subsequent transfer from lab to pilot and then scale up batch manufacturing. Thus it may be further cultured based on supplementary experience gained during the commercial lifecycle of the production.

Conclusion

There is no ambiguity that several initiatives are undertaken worldwide to circumvent development hiccups of anti-TB formulations. The formulation technology used here is simple, easily scalable and adopted in industries. Hence, it endows to be of greater interest especially in under developed or developing countries to epitomize the objectives like cost-effectiveness, feasibility and save resources. The manuscript describes the overall QbD approach along with risk assessment, risk analysis and control strategy to mitigate the risk for development of INH site specific sustained drug delivery. In an endeavor to accomplish the objectives of QbD, 2^3 full factorial design was employed for evaluating the failure modes with high RPN number of core tablet and defining the relationships between input variables and quality traits desired. The optimized formulation exhibited percent release at Q3 of 26.97 %, Q4 of 44.20 %, Q6 of 66.15 %, Q10 of 97.9 % and gastric resistance less than 10 %. Finally, the design space was established and control strategy was developed to mitigate the risk in future. The RPN of updated risk assessment represents that all the failure modes of FMEA analysis were in low risk category (Fig. 6). Finally, capability indices were performed on five reproducibility batches and results revealed that all indices were above 1.33 indicating process was significantly under control.

Thus the shift in exemplar from traditional approach to QbD approach can provide incisive insight for building quality within the product. Hence, the developed formulation may provide prudently a better substitute for conventional tablet in circumventing its hiccups; improve biopharmaceutical properties, providing biphasic release and may anticipate a better bioavailability. The developed formulation has shown promising results in vitro and is potential for assessing in vivo bioavailability. The further in vivo investigations in suitable animal models and human clinical trials are required to prove the clinical usability of the experimental tailored release formulation.

Acknowledgments This article does not contain any studies with human and animal subjects performed by any of the authors. All authors (C. Vora, R. Patatia, K. Mittal, R. Mashru) declare that they have no conflict of interest. Mr. Chintan Vora is highly grateful to the University Grants Commission, New Delhi, Government of India, for availing Junior Research Fellowship. The authors are grateful to the industries that provided respective gift samples.

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 9:716–723

Baker RW, Lonsdale HS (1974) Controlled release: mechanisms and rates. In: Taquary AC, Lacey RE (eds) Controlled release of biologically active agents. Plenum Press, New York, pp 15–71

Barakat NS, Elbagory IM, Almurshedi AS (2008) Controlled release carbamazepine granules and tablets comprising lipophilic and hydrophilic matrix components. AAPS PharmSciTech 9:1054–1062

Batyrbekov EO, Rukhina LB, Zhubanov BA, Bekmukhamedova NF, Smailova GA (1997) Drug delivery systems for tuberculosis treatment. Polym Int 43:317–320

Bhatt DD, Trivedi MR, Modi IA, Khamar BF (2011) Pharmaceutical composition of INH. WO2011012987

Bissell D (1994) Statistical methods for SPC and TQM. Chapman and Hall, London

Burman WJ, Gallicano K, Peloquin C (2001) Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin Pharmacokinet 40:327–341

Colombo P, Bettini R, Santi P, Peppas NA (2000) Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance. Pharm Sci Technol Today 3:198–204

Costa P, Sousa Lobo JM (2001) Modeling and comparison of dissolution profiles. Eur J Pharm Sci 13:123–133

du Toit LC, Pillay V, Danckwerts MP (2006) Tuberculosis chemotherapy: current drug delivery approaches. Resp Res 7:118

Ellard GA, Gammon PT, Laksminarayan S, Fox W, Aber VR, Mitchison DA, Citron KM, Tall R (1972) Pharmacology of some slow release preparations of isoniazid of potential use in intermittent treatment of tuberculosis. Lancet 1:340–343

Gohel MC, Sarvaiya KG (2007) A novel solid dosage form of rifampicin and isoniazid with improved functionality. AAPS PharmSciTech 8:E1–E7

Grassi M, Grassi G (2005) Mathematical modelling and controlled drug delivery: matrix systems. Curr Drug Deliv 2:97–116

Higuchi T (1963) Mechanism of sustained action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 52:1145–1149

Hiremath PS, Saha RN (2008) Controlled release hydrophilic matrix tablet formulations of isoniazid: design and in vitro studies. AAPS PharmSciTech 9:1171–1178

Hixson AW, Crowell JH (1931) Dependence of reaction velocity upon surface and agitation. Ind Eng Chem 23:923–931

ICH Harmonized Tripartite Guideline (2003) Stability testing of new drug substances and products Q1A(R2), February 2003

ICH Harmonized Tripartite Guideline (2009) Pharmaceutical development Q8 (R2), August 2009

ICH Harmonized Tripartite Guideline. Quality risk management Q9, November 2005

Iseman MD, Madsen LA (1989) Drug-resistant tuberculosis. Clin Chest Med 10:341–353

Kane VE (1986) Process capability indices. J Qual Technol 18:41–52

Katzhendler I, Hoffman A, Goldberger A, Friedman M (1997) Modeling of drug release from erodible tablets. J Pharm Sci 86:110–115

Katzung BG (2001) Basic and clinical pharmacology, 8th edn. McGraw-Hill, New York

Kopcha M, Lordi N, Tojo KJ (1991) Evaluation of release from selected thermosoftening vehicles. J Pharm Pharmacol 43:382–387

Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA (1983) Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm 15:25–35

Lionberger RA, Lee SL, Lee L, Raw A, Yu LX (2008) Quality by design: concepts for ANDAs. AAPS J 10:268–276

Mariappan TT, Singh S (2003) Regional gastrointestinal permeability of rifampicin and isoniazid (alone and their combination) in the rat. Int J Tuberc Lung Dis 7:797–803

Mitchison DA (1985) Mechanism of drug action in short-course chemotherapy. Bull Int Union Tuberc 65:30–37

Patel SR, Patel PR, Vora CN, Patel ND, Patel JK (2010) Formulation, process parameter optimization and evaluation of delayed release tablets of rabeprazole sodium. Int J Pharm Pharm Sci 2:144–156

Peppas NA, Sahlin JJ (1989) A simple equation for the description of solute release III. Coupling of diffusion and relaxation. Int J Pharm 57:169–172

Pund S, Joshi A, Vasu K, Nivsarkar M, Shishoo C (2010) Multivariate optimization of formulation and process variables influencing physico-mechanical characteristics of site-specific release isoniazid pellets. Int J Pharm 388:64–72

Ratsimbazafy V, Bourret E, Brossard C (1996) Drug release from matrix tablets and minitablets containing glycerides. Pharm Ind 58:442–446

Ritger PL, Peppas NA (1987) A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J Control Release 5:37–42

Rudisill F, Litteral LA (2008) Capability ratios: Comparison and interpretation of short-term and overall indices. Int J Qual Stand 2(1), Paper 3, 67–86. http://www.bsieducation.org/Education/downloads/ijqs/paper19.pdf. Accessed 18 Nov 2013

Sathe PM, Tsong Y, Shah VP (1996) In vitro dissolution profile comparison: statistics and analysis, model dependent approach. Pharm Res 13:1799–1803

Schaberg T, Rebhan K, Lode H (1996) Risk factors for side effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. Eur Respir J 9:2026–2030

Shah Y, Khanna S, Jindal KC, Dighe VS (1992) Determination of rifampicin and isoniazid in pharmaceutical formulations by HPLC. Drug Dev Ind Pharm 18:1589–1596

Shah PP, Mashru RC, Rane YM, Thakkar A (2008) Design and optimization of mefloquine hydrochloride microparticles for bitter taste masking. AAPS PharmSciTech 9:377–389

Shinde JH, Katikar RS (2012) Importance of process capability and process performance indices in machine tool. Int J Res Eng Appl Sci 2:1211–1217

Shishoo CJ, Shah SA, Rathod IS, Savale SS, Vora MJ (2001) Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. Int J Pharm 228:53–67

Singh S, Mariappan TT, Sankar R, Sarda N, Singh B (2001) A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int J Pharm 228:5–17

Singh B, Kumar R, Ahuja N (2005) Optimizing drug delivery systems using systematic "design of experiments". Part I: fundamental aspects. Crit Rev Ther Drug Carrier Syst 22:27–105

Tahara K, Yamamoto K, Nishihata T (1996) Application of model-independent and model analysis for the investigation of effect of drug solubility on its release rate from hydroxypropyl methylcellulose sustained release tablets. Int J Pharm 133:17–27

US Pharmacopoeia 30/NF25 (2007a) Chapter 711: dissolution. US Pharmacopoeial Convention, Rockville

US Pharmacopoeia 30/NF25 (2007b) Isoniazid tablets monograph. US Pharmacopoeial Convention, Rockville

Vora C, Patadia R, Miattal K, Mashru R (2013a) Recent patents and advances on anti- tuberculosis drug delivery and formulations. Recent Pat Drug Deliv Formul 7:138–149

Vora C, Patadia R, Mittal K, Mashru R (2013b) Risk based approach for design and optimization of stomach specific delivery of rifampicin. Int J Pharm 455:169–181

Wagner JG (1969) Interpretation of percent dissolved time plots derived from in vitro testing of conventional tablets and capsules. J Pharm Sci 58:1253–1257

Yu LX (2008) Pharmaceutical quality by design: product and process development, understanding, and control. Pharm Res 25:781–791

Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S (2010) DDSolver: an add in program for modeling and comparison of drug dissolution profiles. AAPS J 12:263–271

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Recent Patents and Advances on Anti - Tuberculosis Drug Delivery and Formulations

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Received: September 13, 2012; Revised: November 30, 2012; Accepted: December 4, 2012

Abstract: Tuberculosis has remained, unambiguously, a significant health care problem since long times, particularly in developing countries. The endeavoring battle against multi drug resistant TB, multiple dosing, their prominent side effects and bioavailability hiccups related to fixed dose combinations has undeniably become a Herculean task indicating rigorous research requirement in anti TB drug therapy. In view of the fact that patenting a drug molecule, a drug delivery system or a formulation has been very fruitful for the growth and sustainment of pharmaceutical industry, a meticulous review of recent developments, providing a balanced view on merits/demerits, will facilitate researchers to update themselves, thereby focusing their research in more relevant areas to furnish desired quality traits. This article reviews the present scenario in terms of drug delivery approaches for TB chemotherapy. The review encompasses and summarizes recent patents and advances on variegated facets of dosage forms, together with from conventional solid oral to novel controlled release oral formulations and additionally alternative weapons for anti TB drug delivery. A critical review of multidisciplinary approaches to boost anti TB therapy may facilitate the scientists to resolve existing technological gaps.

Keywords: Bioavailability, fixed dose combination, novel drug delivery, patents, stability, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is one of the topmost infectious killers since decades throughout the world. TB occurs in every part of the world and is considered as worldwide crisis from many years. Pragmatically no country has ever eliminated this disease till now. TB is an infectious bacterial disease, caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), which most commonly affects lungs. It can be transmitted from an infected individual to an uninfected individual via droplets or nuclei that are inhaled and lodge within the alveoli in the distal airways [1, 2]. Subsequently, *M. tuberculosis* is taken up by alveolar macrophages, thereby initiating a cascade of events which ultimately ends in either successful containment of infection or progression to active disease. In healthy people, infection with *M. tuberculosis* often causes no symptoms, since the person's immune system acts to "wall off" the bacteria. The common symptoms of active TB of the lung are coughing, sometimes with sputum or blood, chest pains, weakness, weight loss, fever and night sweats [3-5].

According to global TB report (2012) of World Health Organization (WHO), as of now the burden of TB is highest in Asia and Africa. India and China together solely account for almost 40% of the world's TB cases. About 60% of cases are in the south East Asia and western pacific regions. With respect to case notifications, 5.8 million newly diagnosed cases were notified to national TB control programs conducted by WHO in 2011. The combat against TB since years have appear to be winning from the same report stating that new cases fell at a rate of 2.2% between 2010 and 2011. The report also mentioned that the TB mortality rate decreased 41% since 1990 and at the moment the world is on a track to achieve the global target of 50% reduction by 2015. While foreseeing most difficult Multi Drug Resistant-TB (MDR-TB) cases amongst worldwide, 3.7% of new cases and 20% of antecedently treated cases were projected. India, China, the Russian Federation and South Africa have captured almost 60% of the total cases of MDR-TB throughout the world [6].

Notably, childhood TB has always been remained as focal point with regard to establishing an accurate diagnosis and dose adjustment [6, 7]. The foremost common clinical manifestations of pediatric TB are pulmonary parenchymal disease and intrathoracic adenopathy, accounting for 60%-80% of child cases. Many a times, in children less than 12 years of age, the diagnostic smear appears to be false negative, thus underestimating as well as permitting the disease to cross above danger line and eventually making it uncontrollable. With respect to TB diagnosis in children, there is merely little prospect of achieving a gold standard from widely available means i.e. culture, microscopy, polymerase chain reactions based tests or serological testing. Hence, clinicians have to altogether rely on clinical criteria, chest radiography, tuberculin testing and concomitantly attempts must be made to enhance the prognostic power of available diagnostic tools [7]. Recently WHO has published a docu-

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Table 1. Comparison of previous and current WHO recommendations on dose by weight of first line anti-TB drugs for children.

	Previous recommendation (mg/kg/day)	Current recommendation (mg/kg/day)
Rifampicin	8 to 12	10 to 20
Isoniazid	4 to 6	10 to 15
Pyrazinamide	20 to 30	30 to 40
Ethambutol	15 to 20	15 to 25

Table 2. Current FDCs strengths approved by the WHO-UN prequalification program for child treatment.

Drug combination	Strength
Rifampicin + Isoniazid	RIF 60 mg + INH 30 mg dispersible
Rifampicin + Isoniazid + Pyrazinamide	RIF 60 mg +INH 30 mg + PZ 150 mg dispersible
Rifampicin + Isoniazid	RIF 150 mg +INH 75 mg tablet
Rifampicin + Isoniazid + Ethambutol	RIF 150 mg + INH 75 mg +EMB 275 mg tablet
Rifampicin + Isoniazid + Pyrazinamide + Ethambutol	RIF 150 mg + INH 75 mg + PZ 400 mg + EMB 275 mg

ment regarding dosage regimen for the use of currently available fixed-dose combinations (FDCs) meant for children. Comparison of preceding and current WHO recommendations for dosage regimen in children on dose by weight basis is depicted in Table **1** [8, 9] and for FDCs is depicted in Table **2** [8, 9]. Global TB report also reported that estimating the burden of childhood TB (aged less than 15) was tough. According to the report, there were an estimated of 0.5 million cases and approximate 64000 deaths amongst children in 2011 [6].

One of the major constraints in the conventional treatment of TB is that patients have to take an enormous number of tablets, usually 9-16 per day for 2 months (initial phase of treatment), followed by 3-9 tablets daily for 4-6 months (continuation phase). Current TB treatment usually recommends FDCs therapy to avert the emergence of multiple drug resistant organisms. Additionally using FDCs, the number of tablets to be taken can be reduced to as few as three or four per day for the entire course of the treatment. Effective treatment of TB patients with short course multidrug chemotherapy is the keystone of the modern approach to the control of the disease. Use of FDCs tablets against TB is moreover recommended by WHO and the International Union Against Tuberculosis and Lung Disease as an additional step in ensuring proper treatment [10,11]. The present day short course chemotherapy (SCC) regimens consist of four first-line anti TB drugs namely rifampicin (RIF), isoniazid (INH), pyrazinamide (PZ) and ethambutol hydrochloride (EMB) used in an initial intensive treatment phase of two months and a continuation phase [10, 11]. The recommended daily doses and range of first-line anti-TB drugs are: INH 5 mg/kg/day (4 to 6 mg/kg/day), RIF 10 mg/kg/day (8 to 12 mg/kg/day), PZ 25 mg/kg/day (20 to 30 mg/kg/day), and EMB 15 mg/kg/day (15 to 20 mg/kg/day) [10, 12, 13] Table **3** depicts the FDCs strengths recommended by WHO [10, 12, 13].

DRAWBACKS OF ANTI TB FORMULATIONS

Since decades FDCs finds two major drawbacks; fall in bioavailability of RIF when combined with other anti TB drugs and instability of the formulations resulting in variant pharmacokinetic profiles [14-16, 18]. Singh *et al.* reported mechanism demonstrating that RIF is first hydrolyzed under acid conditions to 3-formylrifamycin, which reacts further with INH to form isonicotinylhydrazone (HYD). The HYD converts back to INH and 3-formylrifamycin, resulting in recovery of INH, but eventually inflicting the loss of RIF. Thus enhanced degradation of RIF in acidic medium in the presence of INH is one of the explanations for the poor bioavailability of the FDCs [15, 16]. The same phenomenon of INH triggering the degradation of RIF in acidic medium was reported by Shishoo *et al.* [17]. In an another study carried out by Singh and Mariappan signified the involvement of efflux-mediated and saturable absorption mechanisms of RIF in rat intestine, which act as barriers to the absorption of the drug. Now as P-glycoprotein (P-gp) varies from person to person to an extent of 2-8 folds, it can be one of the reasons for the interindividual variable pharmacokinetic profiles shown by RIF [18]. Secondly, complications of TB chemotherapy like multiple dosing over an extensive period of time, side effects, etc. have explicitly leaded to extensive progressive development of sustained release dosage forms and other carrier system to target the site of *M. tuberculosis* infection, scale back the dosing frequency and side effects.

Recent years have witnessed a spurt in the development of TB chemotherapy to surmount the pitfalls of traditional approaches. The article is an earnest attempt to acquaint the reader on variegated facets of novel drug delivery modules and recent patents on novel techniques patented for frontline anti-TB drugs. The article encompasses recent developments in the field of anti-TB drug delivery and briefly summarizes

Table 3. WHO recommended FDCs strengths.

Drug	Strengths (in mg)
Daily Use	
Rifampicin +Isoniazid+ Pyrazinamide+ Ethambutol	RIF 150 +INH 75 +PZ 400+EMB 275
Rifampicin+Isoniazid+Pyrazinamide	RIF 150+INH 75+PZ 400
Rifampicin +Isoniazid	RIF 300+ INH 150 RIF 150+ INH 75
Isoniazid + Ethambutol	INH 150+ EMB 400
Intermittent use (3 times Weekly)	
Rifampicin +Isoniazid+ Pyrazinamide	RIF 150 +INH 150 +PZ 500
Rifampicin +Isoniazid	RIF 150 +INH 150

the inventions of recently introduced patents particularly highlighting on United States (US) and WIPO (WO) patent.

NANOPARTICULATE DRUG DELIVERY APPROACH

Recent research on idealizing drug delivery systems which is progressing at a prodigious rate, aims at the development of drug delivery systems with maximum therapeutic benefits leading in safe and effective management of disease [19]. Amongst the myriad of drug delivery systems, nanoparticles (NP) are one of the prominents in circumventing the hiccups. NP appeal to scientists across many disciplines due the opportunity to engineer many properties that might otherwise be irreconcilable on a single device. Products of nanotechnology are expected to revolutionize modern medicine, as demonstrated by recent scientific advances and global initiatives to support nanotechnology and nanomedicine research [19, 20]. Such types of carrier or delivery systems are also developed in anti-TB therapy for sustained drug delivery, target the site of *M. tuberculosis* infection, reduce dosing frequency, minimize toxicity and improve patient compliance. Most of the anti-TB drugs are entrapped and delivered using biodegradable polymers which are biocompatible and release the drug in a controlled manner at therapeutic levels. The systems below discussion employ NP as means of delivering anti-TB drugs.

It is reported that recent TB therapy can also be treated with combination of moxifloxacin (MOX), azoles and RIF [21]. However rapid clearance of MOX and poor oral bioavailability of azole demarcate its applications and makes a requirement for formulation that overcomes its shortcomings. To address the above issues inventors of the US patent 20100310662A1 [21] developed an oral nanoparticulate drug delivery system for TB comprising poly DL - poly lactide-co-glycolide (PLG) NP encapsulating at least one azole, moxifloxacin and RIF. They prepared separate NP of drugs encapsulating it for increasing bioavailability and retention period of the drugs. The invention disclosed the multiple emulsion and solvent evaporation technique for preparation of NP. Concisely the procedure involved dissolving the active drug in the distilled water which was then added to dichloromethane (DCM) containing PLG. The mixture was sonicated to form the primary emulsion which was poured into aqueous polyvinyl alcohol (PVA) and resonicated. The secondary emulsion so formed was stirred for the removal of DCM, centrifuged to harvest the NP which were washed with distilled water and vaccum dried. The patent further discloses particle size of the NP having average particle size between 217 nm to 250 nm. *In vivo* drug disposition studies carried out by inventors revealed that single oral drug administration of PLG NP maintained therapeutic drug concentration in plasma for several days in comparison to oral free drug administration. The nicety of the invention is that though NP, it is to be administered orally cicrcumventing the pain and drawbacks caused by subcutaneously or intravenously. Hence, this type of formulation is not only convinient to patient but also can be a fruitful substitue of conventional long term multi dosing anti-TB therapy in future.

In an antibiotic treatment it is desirable to introduce the antibiotic *in vivo* in such a way that its effectiveness against the pathogen is increased and maximized. Hence, there has been continuing search and research to provide new and improved delivery vehicles and systems wherein the efficacy of an antibiotic with respect to a given pathogen is maximized. In this regard the US patent 6054133 [22] discloses methods and composition for targeting intracellular pathogens like M. tuberculosis. The invention is grounded on discovery that phagosomes which contains pathogens selectively take up transferrin. Therefore the antibiotics were conjugated with transferrin or other ligands to form conjugates that are selectively taken up by infected phagosomes. The invention states that similar to transferrin low density lipoprotein are also suitable vehicles for antibiotic drug delivery which like transferrin traffic to early endosomes. They prepared three types of formulations; PLG NP encapsulating RIF+INH+ PZ, PLG NP encapsulating RIF+ INH and PLG NP encapsulating EMB. The findings of this invention reveals that Minimum Inhibitory Concentration (MIC) is not achieved when EMB is part of four active substances encapsulation however separate encapsulation of EMB provides the required MIC. Thus the inventors have taken a special care in the formulation to insure that drug interactions are kept to

the minimum to attain the desired MIC. Hence, this novel invention has critical advantage of delivering antibiotics directly and selectively to the pathogens maximizing the efficacy of the antibiotics.

Pandey *et al*. describes oral drug loaded NP of RIF, INH and PZ encapsulated in PLG. NP were prepared by multiple emulsion technique and drug loading was found 570 to 680 mg drug per gram of polymer. They carried out *in vivo* studies in infected mice upon oral administration of drug loaded NP formulation. The findings of the *in vivo* studies revealed that administration on every 10^{th} day; no tubercle bacilli could be detected in the tissues after 5 oral doses of treatment. Thus the invention is advantageous in reducing the dosage frequency for better management of TB however sufficient clinical and toxicity data including in humans in future will suffice the purpose of research [23].

Mycobacterium tuberculosis is known to infect alveolar macrophages and affect the pathogenesis of TB. Thus administering the therapeutics through pulmonary route may prudently suffice the purpose of delivering drugs to alveolar macrophages [24]. Moreover to combat pulmonary TB, it requires sustained appreciable drug concentration for prolonged period which sometimes demarcate usefullness of oral drug administration therapy. Thus this all has leaded renewed interests in targeting of anti-TB drugs to these cells and development of formulations which delivers the therapeutic agent at high concentration into the alveolar macrophage [24-26]. In addition, delivering the drug directly to the lungs can increase absorption and bioavailability and decrease the dose and hence reduce side effects of therapeutics. In this concern Abdulla *et al.* (2010) developed lyophilized RIF loaded NP using two different molecular weights of poly-(ethylene oxide)-block- distearoylphosphatidylethanolamine (mPEG2000-DSPE and mPEG5000-DSPE) polymers for nebulization. The aerosolized particles will deposit on the lung periphery where they will be ingested by the alveolar macrophages and exert their actions. They found particle size of the NP in range of 162-395 nm. The entrapment efficiency (EE) results depicted that the EE was not pretentious by the copolymer's molecular weight, but was pretentious by drug to polymer ratio and the highest EE (100%) was obtained with drug to copolymer ratio of 1:5. The findings suggested that aerodynamic characteristic of the preparations was not influenced by the molecular weight of the copolymers. Thus this type of the rehydrated lyophilized mPEG–DSPE formulations given by the nebulizer may be promising for targeting pulmonary related diseases [24].

Zahoor *et al.* undertook pharmacokinetic and chemotherapeutic studies with aerosolized alginate (ALG) NP encapsulating INH, RIF and PZ and RIF, INH, PZ and EMB. NP were prepared by cation induced gelification of ALG for inhalation route of administration. They reported that majority of particles (80.5%) were in the respirable range, with a MMAD of 1.1 ± 0.4 μm and geometric standard deviation of 1.71 ± 0.1 μm. *In vivo* lung disposition and chemotherapeutic efficacy of drug loaded ALG NP nebulized was compared against free drug and findings reported that the drug loaded NP maintained drug level at or above MIC90 for 15 days. Hence, the research envisaged by aforesaid researchers can be potential controlled drug delivery for anti TB drugs [25, 26].

Further in the ambit of nanotechnology and nanoscience, RIF loaded PLG NP were prepared and characterized by Ghambhire *et al*. NP were prepared by multiple emulsion solvent evaporation methodology. The *in vitro* release studies indicated sustained drug release over period of 12 hours and the optimum batch showed particle size of 326 nm and EE of 61.70% [27].

MICROPARTICULATE DRUG DELIVERY SYSTEM

Recent developments in drug delivery have become increasingly important mainly due to the awareness of the difficulties associated with a variety of old and new drugs. Various approaches in delivering therapeutic substance to the target site in a sustained controlled release manner are employed of which microparticles holds a sturdy position. It is one of the protuberant approaches to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest with reduce untoward effects [28]. Secondly biodegradable polymers have been used widely as drug delivery systems in the form of microparticles as not only they are biocompatible and biodegradable but also they can be engineered to get desired product attributes [29, 30].

Treating intracellular infections may sometimes do not respond sufficiently with conventional regimen leading to the relapses and patient noncompliance. Secondly treatment regimen may involve multiple doses which may increase the risk of treatment failure and side effects. To circumvent the above issue researchers of the patent US 6264991B1invented formulation to treat or prevent intracellular infection particularly TB in animals [31] wherein it discloses two sets of biocompatible microspheres. First set having diameter less than or equal to 10 μm which releases drug at first effective rate. A second set having diameter greater than 10 μm may also be administered to provide continuing systemic drug release. The patent discloses microsphere comprising of polylactide (PLA) or PLG matrix, poly (anhydride) or poly (caprolactone) matrix as biocompatible materials. They compared effectiveness of drug delivery by microsphere versus free drug using J774 cells infected with *M. tuberculosis* H37Rv. Results of the *in vivo* mice study unconcealed substantial reductions in viable *M. tuberculosis* H37Rv at 26 days post infection. Further the invention states that it is not restricted to RIF solely however could be expanded to many anti-TB drugs. The findings of the invention edifies us that the small microsphere formulation alone did not result in a significant reduction in viable mycobacteria by 26 days. However treatment with either the large formulation or a combination of small and large formulations did result in a significant reduction in viable *M. tuberculosis* H37Rv by 26 days post infection. Post therapy plasma levels of RIF in microsphere treated mice were determined and the results disclose that the group of mice that received a combination of small and large RIF loaded microsphere had detectable levels of RIF in their plasma. Thus the regimen of this invention reduces intracellular counts better than equivalent doses administered in conventional treatment regimens.

There has been a continous need for developing a formulation to target the microorganisms present in the macrophages. One way to beat this problem is using carriers

for transporting the drugs such as liposomes and microparticles. Targetting macrophages with drug containing microparticles leads to transportation of drugs to all the sites where migrating macrophages go thereby mimicking the course spread of bacteria. As a result ample drug concentration may be achieved in the several tissues where bacteria tend to migrate. To explore this inventors of the patent US20050084455 A1 [32] have disclosed inhalable biodegradable microparticles for target specific drug delivery to manage pulomonary TB comprising two anti TB drug and one biodegradable polymer wherin drug combination preferred was Rifabutin and INH. The patent claims two anti TB drugs are used in ratio 1:2 or 2:1. The invention discloses that microparticles were prepared by spray drying. In brief procedure involved dissolving drug (s) in an aqueous solvent preferably, alcohol or any other suitable solution. In the next step dissolving the polymer in dichloromethane or any suitable solvent to obtin another solution. In the next step mixing the aforementioned solutions to obtain the final solution and spray drying the final solution to obtain the microparticles. The particle size of microparticles disclosed in the patent is between 1 to 15 μm wherein 90% particles are less than 10 μm. Hence, this kind of inhalation drug delivery can deliver drug directly to macrophages leading to higher concentration than when given orally.

Controlled release of drug can be obtained by the use of numerous polymers for prolonging the drug action. To explore identical, Bhise *et al.* developed RIF's loaded porous microspheres employing eudragit for sustained release action. They reported that microspheres of RIF were prepared by emulsion solvent diffusion method. The critical findings of the investigation suggest that ratio of drug to eudragit was critical for EE. SEM studies conducted by researchers confirmed the porous and spherical structure of microspheres. *In vitro* drug release studies reported that drug to polymer ratio of 2:1 showed more than 85% drug release over the period of 3 hr [33]. Hence, this type of RIF's loaded porous microspheres can be a favorable approach for improving biopharmaceutical properties of drug.

Polymeric carriers have been widely studied for controlled drug delivery of antimicrobial agents of which natural polymers have been widely explored. In this esteems, Pandey *et al.* studied effects of natural polymer ALG and chitosan (CHT) on developing their microspheres as drug carriers for prolonged drug therapy. Microsphere encapsulating three frontline drugs RIF, INH and PZ were formulated and evaluated. They administered orally therapeutic dose and a half therapeutic dose of the microsphere encapsulated drugs to guinea pigs for pharmacokinetic/chemotherapeutic evaluations, respectively. Results discovered that the half life and mean residence time of the drugs were increased 13 to 15 fold by microsphere encapsulation, along with an enhanced relative/absolute bioavailability. Moreover, results also reported that a half therapeutic dose of anti TB drugs loaded microspheres was also able to produce a satisfactory drug release profile as it was observed that sustained drug levels could still be maintained for 5 days in plasma and 7 days in organs. Thus ALG CHT based drug delivery systems can offer means for a much needed reduction in dose/dosing frequency and offer hope for a better tomorrow in the management of TB [34].

Zhou *et al.* prepared spherical microparticles of ionizable prodrug of INH, INH methanesulfonate, by precipitation with a compressed antisolvent process for sustained drug delivery to alveolar macrophages. The aerodynamic diameter of particle was found in the range of 1 to 3 μm. The charged prodrug was ionpaired with two different hydrophobic cations: tetrapentylammonium and tetraheptylammonium bromide. The research reports that loading of the prodrug into microparticles was necessary for realization of the target sustained effect. *In vitro* released studies reported initial burst effect with slower release afterwards. *In vivo* drug accumulation studies in cultured rat alveolar macrophages showed reduction in the blood levels of acetylisoniazid, a major and potential toxic metabolite of INH [35]. This novel prodrug approach by researchers teaches us preparation of formulation that reduces side effects which is ultimately very beneficial in long term TB therapy. Thus this type of approach opens the new prospects of research within the field of anti TB therapy.

Dutt and Khuller have prepared INH loaded PLG microparticles. The microparticles were prepared by double emulsification solvent evaporation technique for subcutaneous route of administration. The mean volume diameters found by researches were 62.11 μm, 71.95 μm and 11.75 μm for porous, non-porous and hardened microparticles, respectively. Researchers found that sustained drug delivery upto 7 weeks was observed from hardened PLGA microparticles and concentrations of INH obtained were higher than the MIC of INH. Further extending their work researchers prepared hardened PLG microparticles for combination of RIF and INH. The microparticles were prepared by same aforementioned technique. The volume mean diameters found by them were 11.75 μm for INH and 11.64 μm for RIF. The combination of *in vivo* and drug deposition studies reported the sustained release of INH and RIF for up to 7 and 6 weeks, respectively in comparison with free drugs which was detectable for upto 24 hours only [36, 37].

There have been persistent efforts for preparing palatable dosage forms for pediatric drug delivery of anti-TB drugs. In this respect Samad *et al.* developed reconstituted powder for suspension of RIF and INH, formulated as microspheres. The results stated that RIF slowly diffuses out through the hydrogel matrix formed due to swelling of gelatin in acidic environment thereby sustaining the drug release in an acidic environment, while release of INH was very low in simulated gastric fluid pH 1.2 but its release was sustained in simulated intestinal fluid pH 7.4. The drug delivery system was efficacious in preventing interaction due to reduced release of INH from ALG microspheres in the gastric medium concomitantly RIF releasing maximum in the gastric medium. Hence, researchers have taken special care in preparing formulation to minimize interaction between RIF and INH. They reported *in vitro* drug release for 36 hr for INH ALG microspheres and for 4 hr for RIF's gelatin microspheres. Gamma scintigraphy analysis carried out by researchers showed the presence of ALG microspheres in the intestine for a period of more than 24 hr. Hence, the developed formulation modulated the release of RIF and INH to minimize their interaction simultaneously providing pediatric compliance. This type of formulation can be of significant clinical usefulness for delivering above two said frontline anti TB drugs successfully [38].

CYCLODEXTRIN BASED DRUG DELIVERY

High-throughput screening approaches to drug development have led to an increasing number of lipophilic water insoluble drugs whose clinical usefulness is hampered by their insolubility in water. Poorly solubility remains one of the major hurdles in ascertaining desired bioavailability of many drugs. To be pharmacologically active, all drugs must possess some degree of aqueous solubility and should be lipophilic to permeate the biological membranes via passive diffusion. Cyclodextrin (CD) are multifunctional drug carriers that form inclusion complex or CD/drug conjugate and helps in improving solubility, stability, safety and bioavailability of drug molecules [39-41].

In this respects to improve solubility and bioavailability of RIF inventors of patent US7001893B2 [42] discloses RIF inclusion complex with beta cyclodextrin (CD) and hydroxy propyl beta CD (HP beta CD). The invention describes process of synthesizing inclusion complex of RIF with beta CD and HP beta CD. The process comprises of adding RIF to CD and grinding in an agate mortar to form a uniform powdery material of RIF CD inclusion complex.The findings of the invention reported that aforementioned complex has enhanced solubility and bioavailability of the drug and also improves stability of RIF in fixed dose combination.Various possible mechanisms for enhancing bioavailability of drugs by CD have been proposed in literature like hydrophilic CDs increase the solubility, dissolution rate and wettability of poorly water soluble drugs, CDs prevent the degradation or disposition of chemically unstable drugs in gastrointestinal tracts as well as during storage, CDs perturb the membrane fluidity to lower the barrier function, which consequently enhances the absorption of drugs and competitive inclusion complexation with third components (bile acid, cholesterol, lipids, etc.) to release the included drug [39-41]. Hence, the said formulation can be advantageous for treatment of childhood TB if it can be employed in palatable dispersible form.

Extensive efforts are made for preparation of stable dosage forms of FDCs. The RIF variable bioavailability and its interaction with INH are amongst frontiers topic of discussion and research in TB chemotherapy. There has been continous need of improvised formulation which can overcome the above problem together with reduced side effects. To address the above topic, WO2011126352A2 [43] patent discloses preparation based on CD inclusion complex of RIF and granulated INH with pH sensitive polymer and other excipients. The preferred molar ratio in which the complexation was carried out of CD with RIF was 1:1. The findings of the invention states that bioavailability of the RIF is maintained along with low toxicity which is an additional advantage. Another major advantage of the invention is that its application can be extended to children also thus enhancing its utility.

Besides bioavailability problems of RIF, FDCs tablet is of giant size and sometimes patient compliance to adhere to treatment is difficult. Additionally FDCs are only effective if the individual components are available in the tissue at correct concentration. Hence, if FDC is of substandard quality it may result in treatment failure and emergence of drug resistance. It is moreover been found that partial adherence to therapy is a crucial menace to community because the patient who does not take any therapy at all, transmits nonresistant tubercle bacilli to others whereas the patient who takes partial therapy develops multi drug resistance and transmits drug resistant tubercle bacilli. To deal with such problems inventors of patent WO2005074937 A1 [44] invented oral palatable mixture of at least two different anti-TB drugs out of four frontline anti-TB drugs RIF, INH, PZ and EMB for SCC. The palatable powder or granule mixture is filled in the sachet and can be disbursed by mixing the powder in a glass of water or juice with meal. The invention describes complexation method with schardinger sugar for preparation of palatable dosage form. The invention eliminates all the problems and process requirements of wet granulation, drying, mixing and lubrication, compression and coating. Further the nicety of the invention is that the process of complexation not solely helps in dissolution of not easily soluble drugs like RIF but concomitantly reduces the absorption of moisture for hygroscopic drugs like EMB. Such sort of palatable dosage form can be a favorable approach in pediatric and geriatric anti-TB therapy for enhancing patient compliance and adherence to long term TB therapy.

ENHANCEMENT OF TUBERCULAR EFFICACY USING NATURAL COMPOUNDS

Owing to numerous benefits of natural bioavailability enhancers, the recent years have witnessed a spurt in the development of dosage forms in combination with natural compounds. Natural bioavailability enhancers has been speedily permeating into the mindset and practice in the industrial environs [45, 46]. This popularity of using natural compounds as bioavailability enhancer in pharma circles is largely attributable to the recent impetus provided by their potential applications discovered in the modern era. Its domain of application is currently extended to anti TB drugs also.

As discussed, natural herbs are powdered and mixed with the formulations to increase their therapeutic efficacy and potency. With respect to this inventors of patent US5439891 [47] discloses pharmaceutical composition for treatment of TB and Leprosy with augmented therapeutic activity and bioavailability by use of piperine with anti-TB and anti leprotic drugs. They found noteworthy improvement in bioavailability of the said drugs on using piperine in combination with them. Further patent discloses concentration range of piperine and it should be preferably used in the range of 0.4-0.9% by weight of anti-TB or anti leprotic drugs. The finding also suggests that synergistic of piperine is not uniform but is selective. Different mechanisms for the bio enhancing activity of piperine have been proposed in literature including Deoxyribo Nucleic Acid (DNA) receptor binding, modulation of cell signal transduction, inhibits drug metabolizing enzymes, stimulates absorption by stimulating gut amino acids transporters, inhibits the cell pump responsible for drug elimination from cells, etc [48-50]. It is reported that RIF is P-gp inducer and piperine inhibits human P-gp and cytochrome P3A4 (CYP3A4) [51-53]. So it may be hypothesized that inhibition of P-gp may be one of the mechanisms for bioavailability enhancement of RIF by piperine. Studies conducted on *Mycobacterium smegmatis* by Balakrishnan *et al*. report that piperine alone shows no inhibitory effect for the growth of *M. smegmatis* even at

higher concentration but increases inhibitory potential of RIF and this inhibition is higher than RIF alone. RIF inhibits transcription of DNA template exclusively by binding to the s-subunit of Ribo Nucleic Acid (RNA) polymerase and piperine enhances the binding activity of RIF to RNA polymerase which may also be one of the mechanisms for inhibiting TB infections [54].

Similar to above patent, to escalate bioavailability of RIF inventors of the patent WO2011010214A1 [55] disclose formulation of RIF and piperine and enhanced bioavailability of RIF in presence of piperine. As per the invention, the enhanced bioavailability of RIF by piperine is observed with powder form of RIF with bulk density greater than 0.4 gm/cc and particle size d90 < 250 μm. They also found that bioenhancing property of piperine depends on physical property of RIF, selection of excipients and manufacturing process. They additionally found that powdered form of RIF shows greater bioavailability than compacted form of RIF. The patent also describes process and composition for preparing RIF blend and preferably piperine tablet in a specific manner and composition for the same. Thus making use of the prevailing traditional natural herbs for enhancing therapeutic efficacy of existing anti-TB drugs can be a promising advantageous approach for reducing the therapeutic dose, side effects and enhancing the patient compliance. It may be also a better alternative to searching newer drugs for reducing dose and side effects.

As mentioned above piperine enhances bioavailability of RIF. Once INH is separately administered to composition containing RIF and piperine, the bioavailability of RIF was found to be significantly reduced compared to composition containing RIF and piperine alone. The same effect was observed when INH was mixed with composition of RIF and piperine as long as all ingredients are in immediate release (IR) forms. To circumvent the above drawback, inventors of the patent WO2011012987A1 [56] disclosed composition of RIF, INH and piperine wherein the bioavailability of RIF by piperine is maintained in presence of INH. The said invention claims delayed release form of INH preferably tablet and discloses method for the preparation of the same. It discloses that RIF and piperine are release as IR form and INH is released in intestine thereby minimizing physical interaction of both the drugs in gastrointestinal tract by segregating drug delivery. The findings of the invention also describes that required amount of RIF in said invention is lower than conventional available formulation. Thus the invention is very fruitful in minimizing side effects of RIF by reducing the dose needed to provide same therapeutic effects which may be advantageous in terms of patient compliance.

Invention of the patent US 20050171116 A 1 [57] disclosed the enhancement of efficacy of the tubercular drugs by conjoint administration of α- tocopherol (α- TOCO) along with frontline anti TB drugs for treatment of pulmonary TB. The composition claims using α- TOCO in the concentration range of 0.5 to about 1gm. They conducted clinical studies using human volunteers having pulmonary TB and the results reported that the group which received α- TOCO concurrent with routine anti TB drugs showed synergistic effect in augmenting sputum negativity. The exact mechanism by which α- TOCO exerts beneficial effect is unknown. But the inventor gives postulation that α- TOCO quenches free radicals liberated and thereby causes reduction in cell membrane damage of macrophages and other cells involved in cell mediated immunity. This improved immune cell function results in exaggerated bactericidal activity by host defense cells. Hence there may be correct oxidant balance which caused the less lung damage. The results of the *in vitro* cell culture study revealed an outstanding outcome showing that α- TOCO is not only effective against *M. tuberculosis* strains but also effective against RIF resistant, INH resistant and multi drug resistant strains. Hence, this type of novel and unpretentious approach of exploiting naturally occurring substances with anti-TB drug can open new scope in TB therapy as these naturally occurring compounds can also show tremendous effect in multi-drug resistant TB. Similar to above approach of using natural compounds, use of vitamins with anti-TB drugs have been explored in anti-TB therapy. To explore the same inventors of patent RU2195937 [58] discloses combined anti-TB formulation of RIF, INH, PZ, EMB and pyridoxine in specific amounts. Invention provides the enhancement of effectiveness of therapy due to simplification of curative process and also found resistance overcoming in many patients and improvement of the preparation properties.

CONVENTIONAL SOLID ORAL DOSAGE FORMS AND THEIR MODIFICATIONS

Compare to the advanced and intricate drug delivery systems, oral intake has unambiguously been the most pursued by the patients and manufacturers. Development of an effective oral drug delivery system involves rational amalgamation of diverse functional and non-functional polymers and excipients. The intricate drug delivery systems provides substantial merits but still there is requisite to formulate oral system which addresses the issues related to FDCs. In this regards there have been tenacious efforts in improvising oral drug delivery products to furnish the desired quality traits.

FDCs stability and variable pharmacokinetic profiles are a major concern for successful TB therapy and research to resolve the said barriers and challenges have been amongst the keystone of the modern era. In order to surmount the said pitfalls, inventors of the patent US20120027853A1 [59] discloses the process and compositions for preparation of film coated, multilayered FDC or their acceptable salts thereof of four frontline anti TB drugs. The process comprises mixing individually of PZ, EMB and INH with excipients followed by wet granulation to obtain granules of said each mixture and mixing RIF with excipients to form powder. Thereafter preparing two to four pharmaceutical layers wherein RIF and INH are in different layers thereby segregating them. The invention further discloses application of subcoating to multilayered preparation. The invention reported excellent stability of the formulation, conducted for 6 months on conditions long term 25°C/60% relative humidity (RH) and accelerated 40°C/75%RH. The inventors compared *in vivo* pharmacokinetics to marketed preparation and the invention provided equivalent or higher Cmax and AUC for all four anti-TB drugs. Hence, the invention provides knowledge to us for preparation of solid oral dosage form for improvising both stability and bioavailability of FDCs.

One of the reasons of poor patient compliance on long anti TB therapy as discussed is side effects of anti-TB drugs. The major side effect of INH reported is hepatotoxicity and studies indicated that hydrazine is most likely responsible for INH induced hepatotoxicity. Researchers of the US 2011/0207684 A1 [60] patent describes preparation of low side effects composition of INH. The invention discloses that composition comprises of INH and a cytochrome P450 2E1 (CYP2E1) inhibitor. The CYP2E1 inhibitor can be disulfiram (DSF) and/or bis-p-nitophenyl phosphate (BNPP). Such complex can reduce INH induce hepatotoxicity. Inventors determined plasma 8-iso-PGF2 alpha by validated LCMS/MS as an indicator of oxidative stress. They found that its level increased in INH group than in control. BNPP-INH, DSF-INH and BNPP-DSF-INH groups also showed considerable reduction in 8-iso-PGF2alpha concentration. Further from Galactose Single Point (GSP) test, GSP level from liver function tests were in correlation with 8-iso-PGF2alpha levels. Thus this type of innovative methodology gives us a new path for preparation of low side effects formulations.

Inventors of the patent WO2012/013756A2 [61] developed a process for preparing FDCs of RIF, INH, PZ and optionally EMB. The process comprises of non aqueous wet granulation of INH, PZ and optionally EMB. To this RIF was added and compressed into tablet with other excipients. The patent discloses that resulting composition is advantageous compared to conventional anti-TB FDC and shows good anti TB activity when administered orally. To overcome incompatibility between RIF and INH, one of the approaches as discussed is the segregated drug delivery of both drugs in different locations of gastrointestinal tract (GIT). With respect to these inventors of patent CN 1437946 [62] discloses preparation method for oral drug delivery of RIF. The invention discloses that INH and other anti-TB drugs are gastric soluble and RIF is enteric soluble or INH is enteric soluble and others gastric soluble. By any of the above methods RIF and INH are released in segregated parts of GIT to reduce incompatibility. FDCs are now used as frontline treatment of TB and also recommended by WHO to improve patient compliance and to reduce drug resistant. Researchers of patent CN1611218 [63] discloses method of preparation of FDCs of frontline anti-TB drugs RIF, INH, PZ and EMB. The invention discloses dry granulation and subsequent compression into tablet for preparing FDCs. As stated earlier RIF has bioavailability and stability concerns reported with other anti-TB drugs. To address this issue CN1864681 [64] discloses preparation process of compound RIF micro tablet capsule. The invention discloses that out of four frontline anti TB drugs RIF, INH, PZ and EMB, at least one is formulated into micro tablet before encapsulation into capsule. Further the micro tablet is also coated to avoid excess contact between RIF and other anti-TB drugs inside the capsule. This improves stability of RIF by minimizing its contact.

In 2012, Zhonggui *et al.* developed enteric coated tablets of RIF and INH for improving bioavailability of theirs FDC. They studied RIF stability in presence and in absence of INH at various pH buffers from 1.0-7.4. The results reported that RIF degraded in the presence of INH to a higher extent at pH 1.0-4.5 and degradation of RIF is suppressed by the existence of INH in pH 6.8. Hence, enteric coated tablet of combination was developed by them and compared *in vivo* with reference product. Moreover they grinded RIF to improve its solubility and results revealed better dissolution from core tablets of RIF compared to reference. They found increase in AUC_{0-48} of RIF 353.79 ± 31.63 $\mu g \cdot hr \cdot ml^{-1}$ against reference 304.77 ± 42.27 $\mu g \cdot hr \cdot ml^{-1}$ which justified their rationale. The AUC_{0-48} of INH in both reference and test tablets they found were 17.14 ± 8.59 and 19.62 ± 10.57 $\mu g \cdot hr \cdot ml^{-1}$, respectively, which were also comparable [65]. Hence, this type of innovative approach can minimize interaction of RIF and INH in their FDCs and facilitate to resolve their problems.

In 2011, Avachat and Bhise developed FDCs of RIF and INH based on degradation studies of RIF in presence of INH in different ratios. They studied degradation in ratios RIF :INH (i) 1:0.1, (ii) 1:0.25, (iii) 1:0.5, and (iv) 1:0.75 in 250 ml solution having pH of 1.2, 2.0 and 3.0 medias in USP apparatus II at 25 rpm. Their studies revealed that degradation of RIF is reduced to less than 10% when the ratio of RIF: INH is below 1:0.25. Based on the above findings they prepared bilayered tablets with this concept and checked human bioavailability of formulation. Bioavailability studies revealed that Cmax and AUC for RIF increased by 18 and 20%, respectively. The finding also suggests the pH of microenvironment surrounding of RIF, specifically in the presence of INH affects its degradation significantly [66].

Shishoo *et al.* developed oral gastroretentive RIF's formulation which consisted of RIF's pellets for IR, as the loading dose and a bio/mucoadhesive RIF tablet for extended release. IR pellets were prepared by extrusion spheronization. They found porosity of the IR pellets to be 47.39% and dissolution greater than 75% at 45 min. They reported optimal calculated parameters for mucoadhesive extended release tablet were 19% (w/w) of carbopol and 2.5% (w/w) of microcrystalline cellulose having work of adhesion 327.8-344 g s and T50% 240-245 min. Gamma scintigraphy studies conducted by researchers showed that formulation is retained in the stomach for more than 6 hours [67].

Pillay *et al.* developed dry reconstitutable multiparticulate of anti-TB drugs RIF and INH. In that ionotropically crosslinked polymeric enterospheres for delivery of INH to the small intestine were developed through response surface methodology for the design and optimization of the formulation and processing variables. They varied concentration of zinc sulfate salting out and crosslinking electrolyte, the crosslinking reaction time, the drying temperature and the concentration of triethyl citrate plasticizer for determination of their effect on the molar amount of zinc (nZn) incorporated in the crosslinked enterosphere, drug entrapment efficiency (DEE), and mean dissolution time (MDT) at t_{2hr} in acidic media (0.1 M HCl). The results reported that the salting out and crosslinking agent and the plasticizer significantly affected nZn and the DEE. The temperature at which the enterospheres were annealed also significantly affected the DEE. For RIF they prepared reconstitutable granules for suspension. The results reported by investigators stated that for optimal formulation degradation did not exceed 3.06± 0.097% after 3 hr incubation in acidic media [68].

Hiremath and Saha developed once a day controlled release (CR) formulation which provides both initial release (loading dose) and controlled release (maintenance dose) of

RIF and INH from a single CR matrix tablet required for better therapeutic efficacy. They developed formulations using hydrophilic polymers hydroxypropyl methylcellulose (HPMC) and hydroxypropyl cellulose (HPC). They incorporated Eudragit L100-55 in the matrix tablets to compensate for the pH dependent release of RIF. The studies disclosed that the CR formulations containing 80% HPC and 60% eudragit was found to be of good quality and provided required release profile for both RIF and INH. These formulations gave good initial release for RIF (about 100mg in 2 hr) and INH (about 34% of the total dose in 2 hr) and also the release of both RIF and INH were extended up to 18–24 hr [69].

Hiremath and Saha developed and evaluated controlled release RIF formulation using HPMC as controlled release polymer. In one study they used HPMC 15 cps and found that release was controlled upto 16 hours using 40% w/w HPMC of the drug content. Further increase in concentration did not result in significant change in release rates. They also found variation in release rates and release character on varying in compression force. In another study they evaluated three different grades of HPMC low, medium and high viz. HPMC 100 cps, HPMC 4000 cps and HPMC 15000 cps respectively and studied the influence of drug: HPMC ratio, viscosity grade of HPMC, drug particle size and compression force on the formulation characters and drug release. Results reported that in general, decrease in the drug particle size decreased the drug release and lower viscosity HPMC polymer was found to be more sensitive to the effect of compression force than the higher viscosity. They developed series of formulations varied extending release from 12 hours to 24 hours with various release mechanism. Thus the research guides us that exploiting various HPMC grades and combination can be utilized to design target release profile of controlled release oral formulation of RIF [70, 71].

Gohel *et al.* developed a novel dosage form RIF and INH comprising two floating tablets of RIF 150 mg and enteric coated capsule containing INH 150 mg blend together in one size 00 hard gelatin capsules. They found that RIF was released over 4 hours following zero order kinetics and INH was released more than 90% in phosphate buffer pH 7.4. They reported that only 3.6% to 4.8% of RIF was degraded when the dosage form was tested in the modified dissolution apparatus [72]. This reduced degradation of RIF could be due to the separation of RIF and INH and/or the CR of RIF in acidic medium.

Sustained release osmotically asymmetric membrane capsular systems and dense semipermeable membrane coating capsules were developed for simultaneous oral delivery of RIF and INH in order to reduce the problems associated with the multi drug therapy of TB by Prabhakaran *et al. In vitro* release studies disclosed that asymmetric capsular systems are suitable for RIF with sufficient initial burst release but not for INH due to higher aqueous solubility. Moreover dense semipermeable membrane systems provided controlled release of both drugs but were devoid of initial burst release of INH. To overcome these shortcomings, they developed a modified asymmetric system by adding appropriate amount of hydrophilic polymer mixture with INH. This system provided satisfactory sustained release of RIF and INH with initial burst release which may be sufficient to achieve MIC in blood. They found drug release from the systems followed first order kinetics and statistical analysis of release rate data ascertained that modified asymmetric capsular system provided required controlled release rates of both drugs with initial burst release [73].

MISCELLANEOUS

TB infection also grows in alveoli during exacerbations and as a result it progressively causes inflammation and destruction of lung tissue. Aminoglycosides antibiotics are one of the classes used for treatment. One of the key concerns in aminoglycoside antibiotic therapy is that sputum which contains mucin, DNA and glycoproteins binds to aminoglycosides making it less effective. This inhibitory action can be overcome by increasing concentration in sputum by administering ten times larger dose of its MIC. Because of the above reasons larger doses are administered parenterally to achieve its desired concentration into sputum. The high doses of these drugs parenterally administered increases the risk of toxicity including ototoxicity and nephrotoxicity. The invention of patent US 6083922 [74] educates us the preparation of tobramycin (TBA) aerosol formulation for treatment, prevention and containment of acute and chronic pulmonary TB. The invention discloses formulation comprises of 40 to about 800 mg of TBA dissolved in full strength or diluted saline adjusted to pH between 5.5 to 7 and is administered using nebulizer. The investigators found MMAD between 1 to 5 μm for efficacious delivery of concentrated TBA in the endobronchial space.

With similar application of aminoglycodise antibiotics through pulmonary route, invention of the patent US2007 0128124 A1 [75] enlighten us with the preparation of capreomycin (CAP) aerosolized dosage form. CAP is poorly absorbed orally and must be administered parenterally and have similar toxicities and problems like that of TBA. When used as an inhalation therapy CAP can be introduced into gases form as a solution, suspension, a powder or a spray. Since particle size is very important for pulmonary route the invention discloses particle size of the formulation between 1 to 10 μm. They carried out *in vitro* studies comparing CAP with two frontline anti TB drugs, RIF and INH using Relative Light Units (RLU) as a parameter to determine anti microbial viability. The results disclosed that on comparing with RIF or INH at 10 times the MIC, the efficacy of CAP is superior to that of INH and is as efficacious as RIF in log phase growth conditions. Hence, this type of method for delivering aminoglycosides by pulmonary route can improve risk benefit ratio of the drug by permitting effective therapeutic levels to be reached in the lungs thereby increasing the patient population that can benefit from its use.

To overcome RIF bioavailability problems and degradation in presence of INH, inventors of patent US2003 0072800 A1 [76] discloses composition of RIF and INH wherein bioavailability of RIF is increased by preventing its degradation due to INH. Inventors have found during their experimentation that degradation of RIF is pH dependant in presence of INH. At pH 1 the degradation is minimum and increases abruptly between pH 2 and 3. Thereafter above pH 3 the degradation is minimal. Patent discloses interaction is

minimized by preventing contact of RIF and INH in solution state between pH 1-4 thereby preventing degradation of RIF in presence of INH. For achieving this, RIF and INH may be present in delayed release and/ or extended release forms. Delayed release forms are achieved by using pH dependant polymers and extended release by various pH independent polymers.

US patent 20090192173A1 [77] discloses methods and compositions for treating mycobacterial infections comprising ethylene diamines having improved mycobacterial activity. To assess the antimicrobial efficacy of substituted ethylene diamines compounds, one of the studies they carried out was lung study in which the compound 37, compound 59 and compound 109 each at 1 mg/kg and 10 mg/kg tested were directly compared to INH and EMB at 25 mg/kg and 100 mg/kg respectively. Simultaneously they also carried out lesion study for compound 59 and 109 to determine their ability to prevent gross pathology of diseases. The results from both the study demonstrated the ability of the drug to prevent development of disease pathology. Hence, this type of new molecular entities can start a new era of novel molecules which may show better activity than traditional molecules, may have lower side effects and also show promising activity against MDR-TB.

For enhancing product activity RIF, it was encapsulated in liposomes. RU2223764 [78] patent discloses method for preparing liposomes of RIF by thin film hydration technique to prepare multi lamellar vesicles followed by subsequent homogenization or passing through extruder to keep particle size of liposome between 0.1-0.5 μm. In brief procedure reported preparing RIF solution along with phospholipids in organic solvent followed by removal of solvent for preparing thin film which is dispersed in an aqueous medium to prepare suspension of multilamellar vesicles containing RIF. Prepared suspension was homogenized under high pressure or by extruding through filter under pressure for getting desired particle size of prepared liposomes of 0.1-0.5 μm.

CURRENT & FUTURE DEVELOPMENTS

A plenty of examples and strategies for anti-TB therapy were discussed in this article. Several intricate, novel and conventional drug delivery systems have proved their immense worth in sustained drug release behavior, targeting drug molecules to macrophages, augmenting rate and/or extent of bioavailability, reducing side effects, better stability and improving patient compliance, however still sufficient clinical and toxicological data are required in some studies for commercialization. It is expected that ongoing research in this area will lead to more breakthroughs and expand therapeutic options till new drugs are available in market. Current knowledge on anti-TB drug delivery, dosage forms available and existing clinical studies data with TB therapy should serve as lessons for designing appropriate dosage forms in future. Thorough envisioning of the formulation development exercise, understanding of the pharmacokinetics and bio pharmaceutics of anti-TB drugs together with coherent scientific methodology will resolve its drawbacks resulting in formulation more compliant to patient. The issues like patient compliance, risk benefit ratio and the cost should be considered while designing future dosage forms for effective anti-TB therapy.

Though the practice of systematic development of drug delivery systems has undoubtedly spiced up advancements several more initiatives need to be undertaken in future to circumvent development hiccups. The quest for more efficient and novel drug delivery system capable of delivering therapeutic agent with blend of systematic, wise and rational approaches will be of substantial research interest among pharmaceutical industries and academic research institutes. In future, the human prowess amalgamated with newer technologies will tend to divulge the degree of improvement in the product characteristics leading to the better products in terms of money, human resources, better safety and efficacy of anti-TB drugs. There are greater prospects for identification of new targets for drug development, successful commercialization of developed formulations and technologies and for the development of an effective vaccine in future.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

One of the authors, Mr. Chintan Vora is highly grateful to the University Grants Commission, New Delhi, Government of India for providing financial assistance in the form of research fellowship.

ABBREVIATIONS

TB	=	Tuberculosis
M. tuberculosis	=	*Mycobacterium tuberculosis*
WHO	=	World Health Organization
MDR	=	Multi Drug Resistant
FDC	=	Fixed Dose Combination
SCC	=	Short Course Chemotherapy
RIF	=	Rifampicin
INH	=	Isoniazid
PZ	=	Pyrazinamide
ETH	=	Ethambutol Hydrochloride
HYD	=	Isonicotinylhydrazone
P-gp	=	P-glycoprotein
US	=	United States
WIPO	=	WIPO
NP	=	Nanoparticles
MOX	=	Moxifloxacin
PLG	=	Poly lactide-co-glycolide
DCM	=	Dichloromethane
PVA	=	Polyvinyl Alcohol
MIC	=	Minimum Inhibitory Concentration
DSPE	=	Distearoylphosphatidylethanolamine
EE	=	Entrapment Efficiency

MMAD	=	Mass Median Aerodynamic Diameter
ALG	=	Alginate
PLA	=	Polylactide
CHT	=	Chitosan
CD	=	Cyclodextrin
HP beta CD	=	Hydroxy propyl beta CD
DNA	=	Deoxyribo Nucleic Acid
CYP3A4	=	Cytochrome P3A4
RNA	=	Ribo Nucleic Acid
M. smegmatis	=	Mycobacterium smegmatis
IR	=	Immediate Release
α- TOCO	=	α - tocopherol
RH	=	Relative Humidity
CYP2E1	=	Cytochrome P450 2E1
DSF	=	Disulfiram
BNPP	=	Bis-p-Nitophenyl Phosphate
GSP	=	Galactose Single Point
PLGA	=	Poly DL –lactide-co-glycolide
GIT	=	Gastrointestinal Tract
DEE	=	Drug Entrapment Efficiency
MDT	=	Mean Dissolution Time
CR	=	Controlled Release
HPMC	=	Hydroxyl Propyl Methyl Cellulose
HPC	=	Hydroxyl Propyl Cellulose
TBA	=	Tobramycin
CAP	=	Capreomycin
RLU	=	Relative Light Units
CFU	=	Colony Forming Units

REFERENCES

[1] World Health Organization: Tuberculosis. Available at http://www.who.int/tb/en.

[2] Williams DA, Lemke TL. Foye's Principles of Medicinal Chemistry. 5th ed. Lippincott, Williams and Wilkins: Philadelphia, USA 2002.

[3] Toit LCD, Pillay V, Dankwerts MP. Tuberculosis chemotherapy: Current drug delivery approaches. Resp Resear 2006; 7: 118-36.

[4] Ahmad S, Mokaddas E. Recent advances in the diagnosis and treatment of multidrug resistant Tuberculosis. Resp Med 2009; 103:1777-90.

[5] Ahmad S. New approaches in the diagnosis and treatment of latent tuberculosis infection. Resp Resear 2010; 11:169-86.

[6] World Health Organization: Global tuberculosis report 2012. Available at http://www.who.int /tb/publications /global_report/en.

[7] Swaminathan S, Rekha B. Pediatric tuberculosis: global overview and challenges. Clin Infect Disease 2010; 50: S 184-94.

[8] World Health Organization: Dosing instructions for the use of currently available fixed-dose combination TB medicines for children. Available at http://www.stoptb.org/assets/documents/gdf/whatis /Interim%20Paediatric%20FDCs%20dosing%20instructions%20for%20prescribers_Sept09.pdf.

[9] World Health Organization: Dosing instructions for the use of currently available fixed-dose combination TB medicines for children. Available at http://www.stoptb.org/assets/documents/gdf/whatis/ Interim%20Paediatric%20FDCs%20detailed%20dosing%20instructions_Sept09.pdf.

[10] Blomberg B, Spinaci S, Fourie, B. Laing R. The rationale for recommending fixed dose combination tablets for treatment of tuberculosis. Bulletin of the World Health Organization 2001; 79 (1).

[11] Nora de Souza MN. Promising drugs against tuberculosis. Rec Patents on Anti Infect Drug Discov 2006; 1: 33-44.

[12] World Health Organization: Guidelines for treatment of tuberculosis. Fourth Edition. 2010. Available at http://www.who.int/tb/publications/2010/9789241547833/en/index.html.

[13] World Health Organization: The use of essential drugs: ninth report of the WHO expert committee. Available at http://apps.who.int/medicinedocs/en.

[14] Somoskovi A, Parsons LM, Salfinger M. The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in *Mycobacterium tuberculosis*. Resp Resear 2001; 2:164-8.

[15] Singh S, Bhutani H, Mariappan TT. Quality problems of anti tuberculosis fixed-dose combinations (FDCs): A way forward. Indian J Tuberc 2006; 53:201-5.

[16] Bhutani H, Mariappan TT, Singh S. The physical and chemical stability of anti tuberculosis fixed dose combination products under accelerated climatic conditions. Int J Tuberc Lung Dis 2004; 8(9):1073-80.

[17] Shishoo CJ, Shah SA, Rathod IS, Savale SS, Kotecha JS, Shah PB. Stability of rifampicin in dissolution medium in presence of isoniazid. Int J Pharm 1999; 190: 109-23.

[18] Mariappan TT, Singh S. Evidence of efflux mediated and saturable absorption of rifampicin in rat intestine using the ligated loop and everted gut sac techniques. Mol Pharm 2004; 1(5): 363-7.

[19] Faraji AH, Wipf P. Nanoparticles in cellular drug delivery. Bioorg and Med Chem 2009; 17: 2950-62.

[20] Ramesh B, Ganesh NS, Kotha NS, Raju L, Kavitha K. Nanoparticulate drug delivery systems: An overview. J Pharm Res 2011; 4(6): 1688- 90.

[21] Khuller G.K., Verma J.N. Oral drug delivery system for azole, moxifloxacin and rifampicin. US20100310662 (**2010**).

[22] Horwitz M.A., Clemens D.L. Antimicrobial for targeting intracellular pathogens. US6054133 (**2000**)

[23] Pandey R, Sharma A, Zahoor A, Sharma S, Khuller GK, Prasad B. Poly (DL-lactide-co-glycolide) nanoparticles based inhalable sustained drug delivery system for experimental tuberculosis. J Antimicrob Chemother 2003; 52: 981-986.

[24] Abdulla JMA, Tan YTF, Darwis Y. Rehydrated lyophilized rifampicin loaded mPEG-DSPE formulations for nebulization. AAPS Pharm Sci Tech 2010; 11(2): 663-71.

[25] Zahoor A, Sharma S, Khuller GK. Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. Int J Antimicrob Agents 2005; 26(4): 298-303.

[26] Zahoor A, Pandey R, Sharma S, Khuller GK. Pharmacokinetic and pharmacodynamic behavior of antitubercular drugs encapsulated in alginate nanoparticles at two doses. Int J Antimicrob Agents 2006; 27(5): 409-16.

[27] Gambhire V, Bhalekar M, Gambhire M. Development of rifampicin nanoparticles by 3^2 factorial design. Int J Pharm Sci Nanotech 2010; 3 (3): 1085-91.

[28] Lassalle V, Ferreira ML. PLA Nano and microparticles for drug delivery: An overview of the methods of preparation. Macromol Biosci 2007; 7: 767-83.

[29] Park JH, Ye M, Park K. Biodegradable polymers for microencapsulation of drugs. Molecules 2005; 10: 146- 61.

[30] Singh P, Prakash D, Ramesh B, Singh N, Mani TT. Biodegradable polymeric microspheres as drug carriers; a review. Ind Jour of Nov Drug Del 2011; 3(2): 70-82.

[31] Barrow W.W., Barrow E.L., Quenelle D.C., Winchester G.A., Staas J.K. Compositions and methods for treating intracellular infections. US6264991 (**2001**).

[32] Sen H., Jayanthi S., Siha R., Sharma R., Muttil P. Inhalable biodegradable microparticles for target specific drug delivery in tuberculosis and process thereof. US20050084455 (**2005**).

[33] Bhise SB, More AB, Malayandi R. Formulation and *in vitro* evaluation of rifampicin loaded porous microspheres. Sci Pharm 2010; 78: 291-2.

[34] Pandey R, Khuller GK. Chemotherapeutic potential of alginate-chitosan microspheres as anti-tubercular drug carriers. J Antimicrob Chemother 2004; 53: 635-40.

[35] Zhou H, Zhang Y, Biggs DL, Manning MC, Randolph TW, Christians U, *et al*. Microparticle based lung delivery of isoniazid decreases isoniazid metabolism and targets alveolar macrophages. J Control Release 2005; 107(2): 288-99.

[36] Dutt M, Khuller GK. Sustained release of isoniazid from a single injectable dose of poly (DL-lactide-co-glycolide) microparticles as a therapeutic approach towards tuberculosis. Int J Antimicrob Agents 2001; 17:115-22.

[37] Dutt M, Khuller GK. Chemotherapy of *Mycobacterium tuberculosis* infections in mice with a combination of isoniazid and rifampicin entrapped in Poly (DL-lactide-co-glycolide) microparticles. J Antimicrob Chemother 2001; 47(6): 829-35.

[38] Samad A, Sultana Y, Khar RK, Aqil M, Chuttani K, Mishra AK. Reconstituted powder for suspension of antitubercular drugs formulated as microspheres for pediatric use. Drug Discov Ther 2008; 2(2):108-14.

[39] Davis ME, Brewster ME. Cyclodextrin based pharmaceutics: Past, present and future. Nature Rev Drug Discov 2004; 3: 1023-35.

[40] Rasheed A, Ashokkumar CK, Sravanthi VVNSS. Cyclodextrins as drug carrier molecule: A review. Sci Pharm 2008; 76: 567-98.

[41] Uekama K, Hirayama F, Arima H. Recent aspect of cyclodextrin-based drug delivery system. J Inclu Phenom Macrocyc Chem 2006; 56: 3-8.

[42] Rao K.R., Bhanumathi N., Yadav J.S., Krishnaveni S. Inclusion complex of rifampicin, an anti tubercular drug, beta cyclodextrins and hydroxyl propyl beta cyclodextrins and a process thereof. US7001893 (**2006**).

[43] Kunnaz M., Turarbek R., Marina K., Kaldybay P., Kairat B., Zhanbolat R., Temirkhanovna R. Anti tuberculosis preparation based on an inclusion complex of cyclodextrin with rifampicin and a method for the preparation thereof. WO2011126352 (**2011**).

[44] Sapte V.M. Oral cyclodextrin complexes of anti tuberculosis drug. WO2005074937 (**2005**).

[45] Atal N, Bedi KL. Bioenhancers: Revolutionary concept to market. J Ayurved Integr Med 2010; 1(2): 96-9.

[46] Singh A, Deep A. Piperine: a bioenhancer. Int J Pharm Res and Tech 2011; 1(1): 1-5.

[47] Kapil R.S., Zutshi U., Bedi K.L., Singh G., Johri R.K., Dhar S.K., Kaul J.L., Sharma S.C., Pahwa G.S., Kapoor N., Tickoo A.K., Tickoo M.K., Kaul U., Singh S., Zutshi R.K., Singh R. Process for preparation of pharmaceutical composition with enhanced activity for treatment of tuberculosis and leprosy. US5439891 (**1995**).

[48] Patil UK, Singh A, Chakraborty AK. Role of piperine as a bioavailability enhancer. Int J Rec Advan in Pharma Res 2011; 4: 16-23.

[49] Zutshi U, Bano G, Raina RK, Bedi KL, Influence of piperine on rifampicin blood labels in patients with pulmonary tuberculosis. J Associat Physici India 1984; 33: 223-24.

[50] Dahanuker SA, Kapadia AB, Karandikar SM. Influence of trikatu on rifampicin bioavailability. Ind Drug 1982; 19: 271-73.

[51] Schuetz EG, Schinkel AH, Relling MV, Schuetz JD. Pglycoprotein: A major determinant of rifampicin inducible expression of cytochrome P4503A in mice and humans. Proc Natl Acad Sci 1996; 4001-05.

[52] Kim KA, Park PW, Liu KH, Kim KB, Lee HJ, Shin JG, Park JY. Effect of rifampin, an inducer of CYP3A and P glycoprotein, on the pharmacokinetics of risperidone. J Clin Pharmacol 2008; 48(1):66-72.

[53] Westphal K, Weinbrenner A, Zschiesche M, Franke G, Knoke M, Oertel R, *et al*. Induction of P glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug/drug interaction. Clin Pharmacol Ther 2000; 68(4): 345-55.

[54] Balakrishnan V, Varma S, Chatterji D. Piperine auguments transcription inhibitory activity of rifampicin by several fold in mycobacterium smegmatis. Curr Sci 2001; 80: 1302-05.

[55] Bhatt D.D., Trivedi M.R., Modi I.A., Khamar B.F. Pharmaceutical compositions of rifampicin. WO2011010214 (**2011**).

[56] Bhatt D.D., Trivedi M.R., Modi I.A., Khamar B.F. Pharmaceutical composition of INH. WO2011012987 (**2011**).

[57] Sapte V.R. Enhancing the efficacy of tubercular drugs. US20050171116 (**2005**).

[58] Golyshevskaja V.I., Demikhova O.V., Erokhin V.V., Kiselev V.I., Perel Man M.I., Severin E. S. Combined antituberculosis preparation (rizobutol). RU2195937 (**2003**).

[59] Pao, L.H., Shiao, N.H., Yang, K.H., Chou, J.M. Process for preparation of anti tubercular combination and pharmaceutical composition prepared therefrom. US20120027853A1 (**2012**).

[60] Hu, Y.O., Young T.H. New low side effect pharmaceutical composition containing isoniazid. US20110207684 (**2011**).

[61] Yasmine, L.F., Myriam L.F. Method for preparing tablets combining rifampicin, isoniazid, pyrazinamide and optionally ethambutol. WO2012013756 (**2012**).

[62] Gao, H., Tonghong L. Medicine release system of compound rifampicin. CN1437946 (**2003**).

[63] Zhonggui, H., Sun, J. Tianhong Z. Fixed dose compound preparation of anti-tubercular drug and its preparing method. CN1611218 (**2005**).

[64] Yang, L.S. Preparation method of composite rifampicin micro tablet capsule. CN1864681 (**2006**).

[65] Wang Y, Liu H, Liu K, Sun J, He Z. Design and evaluation of enteric coated tablets for rifampicin and isoniazid combinations. Pharm Dev Tech 2012; 1-6.

[66] Avachat AM, Bhise SB. Tailored release drug delivery system for rifampicin and isoniazid for enhanced bioavailability of rifampicin. Pharm Dev Tech 2011; 16(2): 127- 36.

[67] Pund S, Joshi A, Vasu K, Nivsarkar M, Shishoo C. Gastroretentive delivery of rifampicin: *in vitro* mucoadhesion and *in vivo* gamma scintigraphy. Int J Pharm 2011; 411(1-2): 106-12.

[68] Toit LCD, Pillay V, Danckwerts MP, Penny C. Formulation and statistical optimization of a novel crosslinked polymeric anti tuberculosis drug delivery system. J Pharm Sci 2008; 97(6): 2176-07.

[69] Hiremath PS, Saha RN. Oral matrix tablet formulations for concomitant controlled release of anti-tubercular drugs: Design and *in vitro* evaluations. Int J Pharm 2008; 362: 118-25.

[70] Hiremath PS, Saha RN. Design and study of rifampicin oral controlled release formulations. Drug Deliv 2004; 11: 311-7.

[71] Hiremath PS, Saha RN. Oral controlled release formulations of rifampicin. Part II: Effect of formulation variables and process parameters on *in vitro* release. Drug Deliv 2008; 15: 159-68.

[72] Gohel MC Sarvaiya KG. A novel solid dosage form of rifampicin and isoniazid with improved functionality. AAPS Pharm Sci Tech 2007; 8 (3): E1-7.

[73] Prabakaran D, Singh P, Jaganathan KS, Vyas SP. Osmotically regulated asymmetric capsular systems for simultaneous sustained delivery of anti-tubercular drugs. J Contr Rel 2004; 95(2): 239-8.

[74] Montgomery A.B. Method and a tobramycin aerosol formulation for treatment prevention and containment of tuberculosis. US6083922 (**2000**).

[75] Kraus C.N., Barry, III C.E., Doan B.T. Aerosolized capreomycin for inhibition of pulmonary tuberculosis. US20070128124 (**2007**).

[76] Singh A., Jain R. Novel pharmaceutical compositions of anti-tubercular drugs and process for their preparation. US20030072800 (**2003**).

[77] Protopova M.N., Einck L., Nikonenko B., Chen P. Anti-tubercular drugs: Compositions and methods. US20090192173 (**2009**).

[78] Selishcheva A. A., Sorokoumova G. M., Kornilova Z.K.H. Method for preparing rifampicin liposomal form. RU2223764 (**2004**).