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Dissolution test for site-specific release isoniazid pellets in USP apparatus 3 (reciprocating cylinder):
Optimization using response surface methodology

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Abstract

The present work aims to predict drug release from novel site-specific release isoniazid pellets, in USP dissolution test apparatus 3, using the response surface methodology (RSM). Site-specific release isoniazid pellets were prepared by extrusion-spheronization followed by aqueous coating of Acryl-EZE®. RSM was employed for designing of the experiment, generation of mathematical models and optimization study. A 3^2 full factorial design was used to study the effect of two factors (at three levels), namely volume of dissolution medium (150, 200, 250 ml) and reciprocation rate (5, 15, 25 dips per min). Amount of drug released in 0.1 N hydrochloric acid at 2 h and in pH 6.8 phosphate buffer at 45 min were selected as responses. Results revealed that both, the volume of medium and reciprocation rate, are significant factors affecting isoniazid release. A second order polynomial equation fitted to the data was used to predict the responses in the optimal region. The optimized conditions resulted in dissolution data that were close to the predicted values. The proposed mathematical model is found to be robust and accurate for optimization of dissolution test conditions for site-specific release isoniazid pellets. © 2007 Elsevier B.V. All rights reserved.

Keywords: Isoniazid; Site-specific release; USP apparatus 3; Response surface methodology; Optimization; Full factorial design

1. Introduction

Isoniazid, an isonicotinic acid hydrazide, a first-line antitubercular agent, is an integral part of intensive as well as continuation phase of six months treatment schedule against tuberculosis [1]. Isoniazid has an aqueous solubility of approximately 125 mg ml^{-1} [2]. In order to minimize its interaction with rifampicin in acidic environment of stomach, Shishoo et al. [3] emphasized the need to develop a site-specific release formulation of isoniazid. Isoniazid is less permeated through the stomach and is mainly absorbed through the intestine because it occurs in the protonated form at acidic pH (pK_a = 2) [4]. Therefore, it can be considered as a good candidate for the development of a site-specific release formulation. Enteric coating is a popular and a widely accepted technique for achieving the site-specific drug release in the intestine. Considering the popularity and the robustness of the multiparticulate system (e.g., pellets, granules, etc.) as a means of tailoring the release profile of a drug [5], this approach has been adopted in the formulation of isoniazid pellets. Pellets offer various advantages over single unit dosage form including minimal risk of dose dumping, flexibility of blending units with different release patterns, as well as short and reproducible gastric residence time [6].

Dissolution test has proved to be an essential in vitro test to characterize the performance of an oral drug delivery system [7]. The significance of a dissolution test is that, for a drug to be absorbed from gastrointestinal tract and to be available to the systemic circulation, it must be previously solubilized [8]. Therefore, dissolution test is used not
only for the quality control of a finished product to assess batch-to-batch consistency of drug release from solid dosage forms, but is also essential in the development of a formulation for screening and proper assessment of different formulations. In fact, the creative use of dissolution technique can speed up the formulation development, particularly in the case of modified-release products, enabling prompt identification of potential problems in drug release rate. Essentially, dissolution test makes it possible to assess the dissolution properties of the drug itself and thereby to select the most appropriate excipients and appropriate proportions among them for obtaining the desired drug release behavior. Among the several dissolution methods specified in United States Pharmacopoeia (USP), apparatus 1 (basket) has been extensively employed to evaluate the dissolution of site-specific release formulations. However, USP apparatus 3 (reciprocating cylinder) provides sound hydrodynamic conditions for the evaluation of pellets. In contrast to the movement of media in USP apparatus 1, the dosage form moves freely through the dissolution medium in case of reciprocating cylinder. USP apparatus 3 is considered as the first line apparatus in product development of controlled release products and especially the pellets, because of its usefulness and convenience in exposing products to mechanical as well as variety of physicochemical conditions which eventually influence the release of a product in the gastrointestinal tract. USP apparatus 3 has a relatively short history and was incorporated into USP in 1991 as apparatus 3 [9]. There exist only a few reports in the literature on the use of USP apparatus 3 for testing drug release rate and for comparing it to those obtained from other methods. Most of these reports, however, focus on extended release dosage forms [10–13].

Response surface methodology (RSM) is a widely practiced approach in the development and optimization of drug delivery systems [14,15]. Based on the principle of design of experiments, the methodology encompasses use of various types of experimental designs, generation of polynomial equations and mapping of the response over the experimental domain to optimize formulation as well as processing conditions. The advantage of such methodology is in providing a rationale for simultaneous evaluation of several variables. The technique requires minimum experimentation and time, thus proving to be far more efficient and cost effective than conventional methods of product development. For implementation of RSM, factorial designs (FDs; full or fractional) are the most popular statistical designs. Full factorial design (FFD) involves the study of the effect of all factors at various levels and is considered as an efficient approach to estimate the influence of individual variables (main effect) and their interactions. Until date, application of RSM has not been reported in the development and optimization of the dissolution test method for USP apparatus 3. Most of the publications however focus on the optimization of dissolution test conditions for USP apparatus 1 and 2 [14,16].

The current study illustrates the evaluation of in vitro release characteristics of site-specific release isoniazid pellets, under defined hydrodynamic conditions in USP apparatus 3. Computer-aided optimization techniques using $3^2$ FFD were employed to investigate the effect of two factors viz., volume of the dissolution medium and reciprocation rate, on release of isoniazid from the site-specific release pellets. A FD for 2 factors at 3 levels each ($3^2$) is considered identical to a two-factor composite design and has an added advantage of determining a quadratic response surface [14,15,17].

2. Materials and methods

2.1. Materials

Isoniazid I. P. was kindly supplied by Cadila Pharmaceuticals Ltd., Ahmedabad, India. Microcrystalline cellulose (Avicel® PH 101, Signet Chemical Corporation, Mumbai, India), Polyvinylpyrrolidone (PVP K-90, Kolli- don® 90, BASF, Germany) and Acryl-EZE® (Colorcon Asia Pvt. Ltd., Mumbai, India) were used as excipients and were obtained from the indicated sources. All other ingredients and reagents were of analytical grade and were used as received.

2.2. Preparation of site-specific release isoniazid pellets

2.2.1. Preparation of isoniazid loaded pellets

Powder components of the formulation (Isoniazid – 55% w/w, Avicel® PH 101 – 42% w/w and Kollidon® 90 – 3% w/w; Batch size – 500 g) were mixed in a small scale planetary mixer (Kalweka, Karnavati Eng. Ltd., India) for 10 min. Purified water (40% w/w of total solids) was added to get a wet mass. Extrudates were obtained by feeding the wet mass in gravity fed cylinder extruder (R. R. Enterprises, India). Extrudates were spheronized in a spheronizer (R. R. Enterprises, India) to obtain spherical pellets. The pellets were dried in fluid-bed dryer (Nero-Aeromatic, Switzerland) at 50 °C for 20 min. Fraction of pellets, 16/25#, was subjected to coating process.

2.2.2. Enteric coating of isoniazid pellets for site-specific release of isoniazid

Isoniazid pellets were coated with 10% w/w aqueous suspension of Acryl-EZE® using fluid-bed coater (Nero-Aeromatic, Switzerland) to achieve 35% weight gain. The process conditions were ‘pre-warming of the cores at 40 °C for 10 min; spray nozzle diameter, 1 mm; atomizing air pressure, 1 bar; air flow rate, 80 m³ h⁻¹; inlet air temperature, 40 °C; product temperature 32–35 °C; spray rate, 1.5 ml min⁻¹; post drying at 40 °C for 10 min.”

2.3. Dissolution methodology

Dissolution studies were carried out in USP dissolution apparatus 3 (Hanson Research B-3 release rate tester;
Hanson Research Corporation, Chatsworth, CA). For carrying out release rate study, USP method B, for delayed release formulations, was followed [18]. Test was carried out in 0.1 N hydrochloric acid (HCl) for 2 h followed by pH 6.8 phosphate buffer USP for 45 min. Dissolution medium, pH 6.8 phosphate buffer were prepared by combining appropriate amounts of HCl and tri-basic sodium phosphate. Table 1 summarizes the general conditions followed in this study. During the study, the reciprocating cylinder containing pellets moved between the rows successively and switched from one medium to another.

All the dissolution samples were filtered through 0.22 μm Millipore® (Polyvinylidene difluoride, PVDF) filter and analyzed immediately after the completion of dissolution test by UV-Visible spectrophotometer (Shimadzu UV-2450, UV-vis scanning spectrophotometer, Japan). Isoniazid released in 0.1 N HCl was estimated as per method specified in USP [19] and isoniazid released in pH 6.8 phosphate buffer was measured at λmax 263 nm by a validated spectrophotometric method [20]. The analytical method was found to be specific, linear in the concentration range of 5–30 μg/mL, precise (%CV: 1.05–3.16) and accurate (98.5–102.0%). For each dissolution run, a mean of six determinations was recorded.

2.4. Experimental design

A 3² FFD was used for the dissolution testing optimization procedure. Volume of dissolution medium (X₁, ml) and reciprocation rate (X₂, dips per minute, dpm) were the two factors (independent variables) studied. The levels for X₁ and X₂ were chosen in accordance with the preliminary data and were representative of the entire range of operating conditions of the USP apparatus 3. The responses (dependent variables) studied were amount of isoniazid released in 0.1 N HCl at 2 h (Y₁, %) and amount of isoniazid released in pH 6.8 phosphate buffer at 45 min (Y₂, %). Table 2 summarizes independent and dependent variables along with their levels. Experimental dissolution testing runs are listed in Table 3.

2.5. Statistical analysis of the data and validation of the model

Various RSM computations for the current study were performed employing Design-Expert® software (Version 7.1.2, Stat-Ease Inc., Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. Statistical validity of the polynomials was established on the basis of ANOVA and the 3D response graphs were constructed using Design-Expert® software. To validate the chosen experimental design and polynomial equations, optimum test condition was selected. The tests corresponding to this optimum dissolution condition and three additional random dissolution test conditions were carried out in the experimental matrix to determine the validity of the model generated. Subsequently, the resultant experimental data of the response properties were quantitatively compared with those of the predicted values. Also, the linear regression plots between observed and predicted values of the response properties were drawn using MS-Excel.

3. Results and discussion

In developing a novel drug delivery system, particularly, in the case of site-specific release product, dissolution test is a helpful in vitro tool for the assessment and adjustment of the drug release profile from a candidate formulation, enabling easy and fast evaluation of the effects of formulation changes. However, this test is sensitive to many...
parameters such as temperature, agitation, dissolution medium, pH of the medium, volume of dissolution medium and shape of the vessel [21,22]. The precision of dissolution test is essential for the reliability of the results. Earlier experiments in our laboratory using USP dissolution apparatus 1 indicated a non discriminatory dissolution test (data not shown). Therefore, USP dissolution apparatus 3 was chosen for the current study. A multivariate optimization strategy was carried out with the aim of finding the optimum conditions for the testing of drug dissolution behavior from the site-specific release isoniazid pellets.

3.1. Experiments of 3² FFD

Response data for all the 9 experimental runs of 3² FFD, performed in accordance with Table 3, are presented in Table 4. In 0.1 N HCl only 11.40% to 15.90% of isoniazid was released in 2 h and pellets were found to be completely intact at the end of 2 h. Acid resistance test is a significant index of drug dissolution performance of an enteric coated formulation. Polymers used for formulating an enteric coated formulation should be able to withstand the enteric coated formulation. Acryl-EZE® effectively controls the release of isoniazid (a borderline BCS class-I and class-III drug, [23]) from site-specific release isoniazid pellets.

3.2. Mathematical modeling

Mathematical relationship was generated between the factors (dependent variables) and responses (independent variables) using the statistical package Design-Expert® for determining the levels of factors, which yield optimum dissolution responses. A second order polynomial regression equation that fitted to the data is as follows:

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2, \]

where \( \beta_0 \) is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs; \( \beta_1 \) to \( \beta_3 \) are the coefficients computed from the observed experiments values of \( Y \) and \( X_1 \) and \( X_2 \) are the coded levels of factors. The terms \( X_1X_2 \) and \( X^2_i \) (\( i = 1 \) and 2) represent the interaction and quadratic terms, respectively. The equations of the responses are given below:

\[ Y_1 = 11.52 - 0.58X_1 + 0.01X_2 + 0.58X_1X_2 + 2.82X_1^2 + 0.47X_2^2 \]
\[ Y_2 = 70.72 + 1.88X_1 + 3.38X_2 + 4.9X_1X_2 + 0.88X_1^2 - 1.32X_2^2 \]

The equation represents the quantitative effect of factors \( X_1 \) and \( X_2 \) upon the responses \( Y_1 \) and \( Y_2 \). Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors.

Analysis of variance (ANOVA) was applied for estimating the significance of the model, at 5% significance level. A model is considered significant if the \( p \)-value (significance probability value) is less than 0.05. From the \( p \)-values presented in Table 5, it can be stated that for both the responses the linear contribution of the model was not significant. However, for response \( Y_1 \), quadratic contribution of the response was significant, whereas, for response \( Y_2 \), the cross product contribution was significant.

In Table 6, factor effects of 3² FFD model and associated \( p \)-values for the responses \( Y_1 \) and \( Y_2 \), are presented. A factor is considered to influence the response if the effects significantly differ from zero and the \( p \)-value is less than 0.05.

Data in Table 6 show that the response \( Y_1 \) was significantly affected by the synergistic effect of quadratic term

Table 4
Results of dissolution studies carried out on site-specific release isoniazid pellets as per 3² full factorial experimental design: response \( Y_1 \) (amount of isoniazid released in 0.1 N HCl at 2 h, %) and response \( Y_2 \) (amount of isoniazid released in pH 6.8 phosphate buffer at 45 min, %)

<table>
<thead>
<tr>
<th>Dissolution test run</th>
<th>Response ( Y_1 (%) )</th>
<th>Response ( Y_2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.9 ± 0.7</td>
<td>68.9 ± 3.6</td>
</tr>
<tr>
<td>2</td>
<td>15.3 ± 1.5</td>
<td>70.6 ± 3.6</td>
</tr>
<tr>
<td>3</td>
<td>14.5 ± 1.2</td>
<td>67.9 ± 4.2</td>
</tr>
<tr>
<td>4</td>
<td>11.4 ± 0.9</td>
<td>69.8 ± 1.7</td>
</tr>
<tr>
<td>5</td>
<td>11.6 ± 0.7</td>
<td>68.1 ± 4.5</td>
</tr>
<tr>
<td>6</td>
<td>12.5 ± 1.2</td>
<td>72.5 ± 3.1</td>
</tr>
<tr>
<td>7</td>
<td>14.0 ± 0.5</td>
<td>62.0 ± 4.5</td>
</tr>
<tr>
<td>8</td>
<td>13.3 ± 0.8</td>
<td>76.1 ± 2.1</td>
</tr>
<tr>
<td>9</td>
<td>14.9 ± 0.5</td>
<td>80.6 ± 2.3</td>
</tr>
</tbody>
</table>

* Mean of 6 ± SD.

Table 5
Summary of analysis of variance (ANOVA) for the measured response \( Y_1 \) (amount of drug released in 0.1 N HCl at 2 h) and response \( Y_2 \) (amount of drug released in pH 6.8 phosphate buffer at 45 min)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>( Y_1 )</th>
<th>( Y_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( F )</td>
<td>( p )-value</td>
</tr>
<tr>
<td>Linear contribution</td>
<td>0.34</td>
<td>0.7273</td>
</tr>
<tr>
<td>Quadratic contribution</td>
<td>10.36</td>
<td>0.0413</td>
</tr>
<tr>
<td>Cross-product contribution (2FI)</td>
<td>0.33</td>
<td>0.8067</td>
</tr>
</tbody>
</table>

Table 6
A summary of each factor effect and its \( p \)-values for, response \( Y_1 \) (amount of isoniazid released in 0.1 N HCl at 2 h) and for response \( Y_2 \) (amount of isoniazid released in pH 6.8 phosphate buffer at 45 min)

<table>
<thead>
<tr>
<th>Factor</th>
<th>( Y_1 )</th>
<th>( Y_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Factor effect</td>
<td>( p )-value</td>
</tr>
<tr>
<td>( X_1 )</td>
<td>-0.580</td>
<td>0.1035</td>
</tr>
<tr>
<td>( X_2 )</td>
<td>+0.100</td>
<td>0.7179</td>
</tr>
<tr>
<td>( X_1X_2 )</td>
<td>+0.580</td>
<td>0.1592</td>
</tr>
<tr>
<td>( X_1^2 )</td>
<td>+2.820</td>
<td>\textbf{0.0075}</td>
</tr>
<tr>
<td>( X_2^2 )</td>
<td>+0.432</td>
<td>0.3632</td>
</tr>
</tbody>
</table>

Significant effects of factors on individual responses are shown in bold type.
of volume of dissolution medium ($X_1^2$) ($p$-value, 0.0075). Significant factors affecting the response $Y_2$ were reciprocation rate ($X_2$) with $p$-value, 0.0309 and interaction effects (cross-product terms) with $p$-value, 0.0169. Both the above-mentioned factors show the synergistic effect and increase the release of isoniazid from site-specific release isoniazid pellets.

3.3. Response surface analysis

The 3-dimensional response surface plots were drawn to estimate the effect of independent variables on each response. Figs. 1 and 2 show the effect of two hydrodynamic conditions in the dissolution test on the release of isoniazid in 0.1 N HCl and release of isoniazid in pH 6.8.

Fig. 1. Response surface plot showing the influence of volume of dissolution medium and reciprocation rate on response $Y_1$ (amount of isoniazid released in 0.1 N HCl at 2 h, %).

Fig. 2. Response surface plot showing the influence of volume of dissolution medium and reciprocation rate on response $Y_2$ (amount of isoniazid released in pH 6.8 phosphate buffer at 45 min, %).
respectively. The Fig. 1 shows ‘a region of minima’ lying between the intermediate to higher levels of both the factors. However, the effect of volume of dissolution medium seems to be more pronounced as compared with that of speed. This receives confirmation from the mathematical model generated for response (Eq. 2).

Fig. 2 depicts a nonlinear twisted relationship for \( Y_2 \) at intermediate and high levels of both the factors. This can be attributed to the potential occurrence of interaction between the two independent variables at the corresponding factor levels, construing that each independent variable is tending to modify the effect of another towards the release of isoniazid in pH 6.8. However, the effect of speed seems to be more pronounced as compared with that of volume of dissolution medium. This is in agreement with Eq. (3) as well as Fig. 2.

With the help of polynomial equation, the process was optimized for both the responses. The final optimal experimental parameters were calculated by satisfying the requirements for each response in the set. Thus, to obtain site-specific release of isoniazid, it is desirable to minimize \( Y_1 \), and maximize \( Y_2 \). In this study optimization was performed with constraints for \( Y_1 \) (\( \leq 15\% \)) and \( Y_2 \) (\( \geq 80\% \)).

The optimal calculated parameters were

- Volume of dissolution medium, \( X_1 = 225 \text{ ml} \)
- Reciprocation rate, \( X_2 = 25 \text{ dpm} \)

The test carried out with the above-mentioned dissolution test conditions showed \( Y_1\text{Experimental} \) as 12.00% \( (Y_1\text{Predicted}, 12.31\%; \text{percentage prediction error, } -2.58) \) and \( Y_2\text{Experimental} \) as 82.63% \( (Y_2\text{Predicted}, 80.6\%; \text{percentage prediction error, } 2.46) \) as shown in Table 7. Low values of prediction percentage error indicate that the predicted and observed values are in good agreement.

### 3.4. Validation of response surface model

In order to assess the reliability of the developed mathematical model, dissolution tests corresponding to the above-mentioned optimum dissolution conditions and three additional random dissolution tests with conditions covering the entire range of experimental domain were performed. For each of these test runs, responses were estimated by use of the generated mathematical model and by the experimental procedures. Table 7 lists the dissolution test conditions of the optimum and the random check points, their experimental and predicted values for both the response variables. The graphs demonstrate high values of correlation coefficient, \( r^2 (>0.9) \) indicating excellent goodness of fit. Therefore, it can be concluded that, model functions \( Y_1 \) and \( Y_2 \) are well represented by the fitted polynomial equations:

\[
y_1 = 1.1891x - 2.2932 \quad R^2 = 0.9663
\]

\[
y_2 = 0.7798x + 15.538 \quad R^2 = 0.9631
\]

Table 7

<table>
<thead>
<tr>
<th>Dissolution test</th>
<th>Test conditions ( X_1 ) (ml)/( X_2 ) (dpm)</th>
<th>Response</th>
<th>Experimental value</th>
<th>Predicted value</th>
<th>Percent prediction error ( b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>225/25</td>
<td>( Y_1 )</td>
<td>12.00</td>
<td>12.31</td>
<td>-2.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Y_2 )</td>
<td>82.63</td>
<td>80.6</td>
<td>2.46</td>
</tr>
<tr>
<td>B</td>
<td>200/10</td>
<td>( Y_1 )</td>
<td>11.91</td>
<td>11.83</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Y_2 )</td>
<td>67.15</td>
<td>68.9</td>
<td>-2.60</td>
</tr>
<tr>
<td>C</td>
<td>225/20</td>
<td>( Y_1 )</td>
<td>12.54</td>
<td>12.25</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Y_2 )</td>
<td>76.49</td>
<td>74.76</td>
<td>2.26</td>
</tr>
<tr>
<td>D</td>
<td>150/10</td>
<td>( Y_1 )</td>
<td>14.71</td>
<td>15.27</td>
<td>-3.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Y_2 )</td>
<td>71.96</td>
<td>70.44</td>
<td>2.11</td>
</tr>
</tbody>
</table>

\[ a \quad X_1, \text{volume of the dissolution medium (ml)} \text{ and } X_2, \text{reciprocation rate (dpm).} \]

\[ b \quad \text{Percent error was calculated using the formula (Experimental value – Predicted value)/Experimental value × 100.} \]
and $Y_2$ well interpreted, the variable data of isoniazid release in 0.1 N HCl at 2 h and isoniazid release in pH 6.8 phosphate buffer at 45 min. Thus, the lower magnitude of error (−3.8 to 2.31 for $Y_1$ and −2.6 to 2.46 for $Y_2$) as well as significant values of $R^2$ (>0.9) in the current study indicate the robustness of the mathematical model and high prognostic ability of RSM.

4. Conclusion

A statistical model has been established to predict the release properties of the isoniazid from the site-specific pellets, by simultaneously studying the effect of various hydrodynamic factors in USP dissolution test apparatus 3, using RSM. The $3^2$ FFD strategy was found to point out the significant factors affecting drug release from the site-specific release isoniazid pellets, in the considered experimental domain. A set of optimum conditions for dissolution test, with respect to the release of the isoniazid, were found to be 225 ml of dissolution medium with 25 dpm reciprocation rate. High degree of prognosis obtained for $3^2$ full factorial design corroborates that RSM is an efficient tool in optimization experiments.

This approach could be applied for other dissolution procedures as well as for other solid dosage forms. Examination of dissolution data discussed in this work will help research scientist in collection of scientifically sound data and its interpretation.

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References

Multivariate optimization of formulation and process variables influencing physico-mechanical characteristics of site-specific release isoniazid pellets

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A B S T R A C T

In the present study, isoniazid was formulated as site-specific release pellets with high drug loading (65%, w/w) using extrusion–spheronization followed by aqueous coating of Suretic® (35% weight gain). A statistical experimental strategy was developed to optimize simultaneously the effect of the two formulation variables and one process variable on the critical physico-mechanical properties of the core pellets of isoniazid. Amount of granulating fluid and amount of binder were selected as formulation variables and spheronization speed as a process variable. A $2^3$ full factorial experimental design was employed for the present study. Pellets were characterized for physico-mechanical properties viz. usable yield, pellet size, pellips, porosity, abrasion resistance, mechanical crushing force, residual moisture and dissolution efficiency. Graphical and mathematical analysis of the results allowed the identification and quantification of the formulation and process variables active on the selected responses. A polynomial equation fitted to the data was used to predict the responses in the optimal region. The optimum formulation and process parameters were found to be 44.24% (w/w) of granulating fluid, 2.13% (w/w) of binder and spheronization speed of 1000 rpm. Optimized formulation showed usable yield 84.95%, particle size 1021.32 μm, pellips 0.945, porosity 46.11%, and abrasion resistance 0.485%. However, mechanical crushing force, residual moisture and dissolution efficiency were not significantly affected by the selected independent variables. These results demonstrate the importance of, amount of water, binder and spheronization speed, on physico-mechanical characteristics of the isoniazid core pellets with high drug loading.

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1. Introduction

Isoniazid, in combination with rifampicin, is used as a first-line drug for the treatment of tuberculosis. However, poor and impaired bioavailability of rifampicin from a number of dosage forms of rifampicin and its combination with isoniazid continues to be a subject of much concern (Shishoo et al., 2001a,b). Earlier studies have established that in the acidic pH of stomach rifampicin reacts with isoniazid to form an inactive compound, isonicotinyl hydrazone resulting in reduction of bioavailability of rifampicin to the extent of 30%. Further, the solid–solid interaction between the two drugs in a fixed dose combinations degrades rifampicin to the extent of 10%. Hence, there is an urgent need to develop an oral system, which will directly address the issues of unacceptable rifampicin bioavailability. The fabrication of a multiparticulate formulation of principal anti-TB drugs which attains segregated delivery of rifampicin and isoniazid for improved rifampicin bioavailability could be a step in the right direction (Shishoo et al., 2001b; Singh et al., 2001; Toit et al., 2006). Since, isoniazid occurs in the protonated form at acidic pH ($pK_a = 2$), it is less permeated through the stomach and is mainly absorbed through the intestine (Mariappan and Singh, 2003). Therefore, isoniazid was formulated for site-specific release in intestine.

Considering the popularity and the robustness of the multiparticulate system (e.g., pellets, granules, etc.) as a means of tailoring the release profile of a drug, this approach has been adopted, in the formulation of isoniazid pellets. Pellets offer various advantages over single unit dosage form including minimal risk of dose dumping, flexibility of blending units with different release patterns, as well as short and reproducible gastric residence time (Kramer and Blume, 1994; Melia et al., 1994).

Extrusion–spheronization process is the most widely accepted method of pellet manufacturing (Ghebre-Sellassie and Knoch, 2007). However, it is likely to fail when slight changes in formulation and process are made. Nevertheless, pelletization is a rather complicated multivariable process (Hellén et al., 1993; Sousa et al., 1996; Neau et al., 2000). A large number of factors, including the physico-chemical properties of the raw materials, both drug and excipients, the composition and the component's relative amounts in the formulations, as well as the manufacturing process parameters, can influence various properties of the formulation (Sousa et al., 1996, 2002). Thus, identifying the influence of these vari-
Multivariate optimization methodologies are powerful, efficient and systematic tools in the design of pharmaceutical dosage forms, allowing a rational study of the influence of formulation and/or processing parameters on the selected responses with a shortening of the experimentation time and an improvement in the quality of research and development work (Furlanetto et al., 2003, 2006; Kramar et al., 2003; Singh et al., 2006; Kim et al., 2007; Joshi et al., 2008). Experimental design is thus the preferred strategy, especially when complex formulations, such as multiparticulate systems, are to be developed (Neau et al., 2000). Multivariate optimization methodologies has been successfully applied in developing multiple-unit delivery systems, allowing a rapid and efficient quantification and prediction of the effects of formulation changes on the considered responses (Neau et al., 2000; Gupta et al., 2001; Paterakis et al., 2002; Akhgari et al., 2005; Howard et al., 2006).

Considerable amount of work has been reported which identifies the factors involved in pelletization, still there are areas of uncertainty remaining; especially for pellets with high drug loading. The ability to produce pellets with high drug loading is one of the claimed advantages of the process of extrusion-spheronization, but it is not possible to achieve this with all the drugs, especially those with very high aqueous solubility. There are very few reports which provide evidence for the ability to prepare pellets with high drug loading using Avicel PH 101, although various other grades such as Avicel RC 591, Avicel 955 have been suggested as alternatives for successful pelletization (Jover et al., 1996; Podczeck et al., 2008).

The present study deals with the core pellet optimization with high drug loading for isoniazid The objective of this work was to establish the effect of formulation as well as process variables and their eventual interactions over various micromeritic, mechanical and release characteristics of the isoniazid pellets. In order to do so, the experimental plan chosen was the $2^3$ full factorial analysis along with graphical interpretation of the effects and mathematical modelling. Two formulation variables; amount of granulating fluid and the binder concentration and a process variable; spheronization speed were studied.

## 2. Materials and methods

### 2.1. Materials

Isoniazid was received as gift sample from Litaka Pharmaceuticals Pvt. Ltd., Pune, India. Microcrystalline cellulose (Avicel® PH 101, Signet Chemical Corporation, Mumbai, India), polyvinylpyrrolidone (Kollidon® 90, BASF, Germany) and Sureticon (Colorcon Asia Pvt. Ltd. Mumbai, India), were used as excipients and obtained from the indicated sources. All other ingredients and reagents were of analytical grade and were used as received.

### 2.2. Preparation of isoniazid core pellets

#### 2.2.1. Experimental design

Before application of the design, a number of preliminary trials were conducted to determine the conditions at which the process resulted to pellets. The levels of the factors were also determined by this procedure. A $2^3$ full factorial design was used for optimizing the formulation. The studied factors were: the amount of granulating fluid; purified water ($X_1$, % w/w of dry blend) and amount of binder; Kollidon® 90 ($X_2$, % w/w of dry blend) and the spheronization speed ($X_3$, revolutions per minute, rpm). The responses studied were usable yield ($Y_1$, %theoretical), pellet size ($Y_2$, μm), pellips ($Y_3$), porosity ($Y_4$, %), abrasion resistance ($Y_5$, %), mechanical crushing force ($Y_6$, N), residual moisture ($Y_7$, %) and dissolution efficiency (DE) at 15 min ($Y_8$, %). These studied factors along with their levels and the corresponding responses are summarized in Table 1 and experimental formulations are listed in Table 2.

#### 2.2.2. Preparation of isoniazid core pellets

Powder components of the formulation (Isoniazid, 65% (w/w); Avicel® PH 101, 32–35% (w/w) and Kollidon® 90.0–3% (w/w)) were mixed in a small-scale planetary mixer (Kalweca, Karnavati Eng. Ltd., India) for 10 min. The required quantity of water (35–55, w/w of dry powder blend) was added as per the factorial design. The wet mass was processed for further 10 min with occasional pauses to allow scraping of the bowl and blade. Extrudates were obtained by using gravity fed cylinder extruder (R.R. enterprises, India), extruding at a constant speed of 125 rpm, through a roller die having holes 1 mm in diameter and 4 mm in length. A sizeronizer (R.R. enterprises, India), equipped with a rotating plate of regular cross hatch geometry was used for the spheronization. The extrudates were spheronized for 10 min at speed (700–1000 rpm) as per the experimental design. The contents emptied from the sizeronizer were dried in the Fluidized Bed dryer (Niro-Aeromatic, Switzerland) at 50 °C for 20 min.

#### 2.2.3. Characterization of uncoated isoniazid pellets

#### 2.2.3.1. Usable yield (% theoretical)

The size distribution of uncoated pellets was determined by sieving using standard set of sieves (800–2360 μm) on a sieve shaker (Electromagnetic sieve shaker, EMS-8, Electrolab, India) for 5 min at a frequency of 50 Hz with amplitude of 1 mm. The fraction of pellets, 700–1190 μm, was considered as the usable yield (Howard et al., 2006).

#### 2.2.3.2. Pellet size

Particle size for each batch was determined using Laser Light Scattering system (Malvern Mastersizer 2000, Malvern Instruments, Malvern, UK). All the measurements were carried out in triplicate and 50th percentile diameter of the cumulative particle size distribution was considered as mean pellet size (Koo and Heng, 2001).

#### 2.2.3.3. Determination of the shape using image analysis

For shape analysis, the images were captured using a stereomicroscope Leica S4E (Leica, Germany). The captured images were analyzed using Image analysis software (AnalySIS® Soft Imaging system, v. 5.2, Münster, Germany). Analysis was carried out on 50 pellets from usable yield fraction. In this study, pellips was calculated for the characterization of the shape by using the following equation (Koo and Heng, 2001; Almeida-Prieto et al., 2007)

$$\text{pellips} = \frac{P}{\pi \times d_{\text{max}}}$$

where $P$ is the perimeter and $d_{\text{max}}$ is maximum diameter of the pellet, calculated directly by using Image analysis software.

#### 2.2.3.4. Mechanical crushing force

At least 20 pellets from the usable yield fraction of each formulation were evaluated for their diametral crushing force using a tablet strength tester (EH 01, Electrolab, India) (Sousa et al., 2002; Newton et al., 2007).

#### 2.2.3.5. Abrasion resistance

The resistance to abrasion was analyzed using Roche friabilator (Veego instruments corporation, India). A pre-weighed sample (approximately 6 g) taken from the usable yield fraction was placed in a friabilator along with 25 steel spheres, each 2 mm in diameter. After 100 revolutions at 25 rpm, the mass retained on the sieve (1190 μm) was weighed and the abrasion resistance was calculated as the percentage loss of mass...
between initial and final weights of each pellet batch (Howard et al., 2006). Each batch was analyzed in triplicate.

2.2.3.6. Porosity. Pellet porosity was determined using Helium pycnometry (SmartPycno 30, Smart Instruments, India). All the values are mean of three replicates (Chopra et al., 2001; Steckel and Mindermann-Nogly, 2004).

2.2.3.7. Residual moisture. The residual water content present in the pellets after drying was determined by USP Method A using Karl Fischer titrator (Systronics Universal titrator 353, India). The equipment was pre-calibrated and standardised with disodium tartrate dihydrate. Pellets, approximately 250 mg, were accurately weighed and immediately placed in the moisture analyser for titration with Karl Fischer reagent. Each batch was analyzed in triplicate (USP 30/NF25, 2007a).

2.2.3.8. Dissolution efficiency; DE. Dissolution study on uncoated pellets was carried out in pH 6.8 phosphate buffer in USP dissolution apparatus I (Hanson Research Corporation, Chatsworth, CA) and DE at 15 min was calculated. Isoniazid released in the dissolution media was measured at \( \lambda_{max} \) 263 nm by a validated spectrophotometric method (Joshi et al., 2008; Rastogi et al., 2007). For each dissolution run, a mean of six determinations was recorded.

2.3. Statistical analysis of the data and validation of the optimization model

The NEMRODW software (LPRAI SARL, Marseille, France) was used in the current study for the generation and evaluation of statistical experimental design. Polynomial models including interaction terms were generated for all the response variables using multiple linear regression analysis. The influence of factors and their interaction, on each of the response are represented graphically.

In order to validate the polynomial equations, one optimum checkpoint (formulation composition and process) and two random checkpoints were selected by intensive grid search, performed over the entire experimental domain. The criterion for selection of optimum check point was mainly based on the highest possible values of response parameters, i.e. usable yield, porosity, mechanical crushing force, DE and pellips; while lowest possible values of responses, namely, size, abrasion resistance and water content. Formulations corresponding to these three check points were prepared and evaluated for all the eight responses \((Y_1 - Y_8)\). The resultant experimental data of response properties were subsequently compared quantitatively with the predicted values.

2.4. Enteric coating of optimized isoniazid core pellets for site-specific release and evaluation of coated pellets

A coating of drug-loaded pellets with optimum composition was carried out with 10% (w/w) aqueous suspension of Sureteric \(^{\text{®}}\) using fluid-bed coater (Niro-Aeromatic, Switzerland) to achieve 35% weight gain. The process conditions were pre-warming of the cores at 40 °C for 10 min; spray nozzle diameter, 1 mm; atomizing air pressure, 1 bar; air flow rate, 80 m\(^3\)h\(^{-1}\); inlet air temperature, 40 °C; product temperature 32–35 °C; spray rate, 1.5 ml min\(^{-1}\); post-drying at 40 °C for 10 min.

2.4.1. Dissolution testing of site-specific release isoniazid pellets

The enteric-coated isoniazid pellets were characterized for the complete release profile. Method B for delayed release products, specified in USP, was followed (USP 30/NF 25, 2007b). All the dissolution samples were analyzed immediately after the completion of dissolution test by UV–vis spectrophotometer (Shimadzu UV-2450, UV–vis scanning spectrophotometer, Japan) (Joshi et al., 2008). For each dissolution run, a mean of six determinations was recorded.

2.4.2. Surface topography

Morphological examination of the surface of uncoated as well as coated pellets of optimized isoniazid formulation was carried out using a scanning electron microscope. Scanning electron microphotographs of pellets were obtained using JEOL (JEOL JSM-6100, Tokyo, Japan). The particles were vacuum dried, coated with thin gold–palladium layer by sputter coater unit (JEOL JFM-1100, Tokyo, Japan) and observed microscopically at an accelerating voltage of 5.0 kV.

Table 1

<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels of the factors used in the formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1</td>
</tr>
<tr>
<td></td>
<td>+1</td>
</tr>
<tr>
<td>(X_1) = amount of granulating fluid, water</td>
<td>35% (w/w) of dry mix</td>
</tr>
<tr>
<td>(X_2) = amount of binder, Kollidon (^{\text{®}}) 90</td>
<td>0% (w/w) of dry mix</td>
</tr>
<tr>
<td>(X_3) = Spheronization speed</td>
<td>700 rpm</td>
</tr>
<tr>
<td>Responses</td>
<td></td>
</tr>
<tr>
<td>(Y_1) = usable yield (% theoretical)</td>
<td>+1</td>
</tr>
<tr>
<td>(Y_2) = pellet size ((\mu)m)</td>
<td>3 = pellips</td>
</tr>
<tr>
<td>(Y_3) = porosity (%)</td>
<td>6 = mechanical crushing force (N)</td>
</tr>
<tr>
<td>(Y_4) = dissolution efficiency; DE (%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Formulation run</th>
<th>Formulation variable - (X_1) (amount of granulating fluid, % w/w of dry mix)</th>
<th>Formulation variable - (X_2) (amount of binder, % w/w of dry mix)</th>
<th>Process variable - (X_3) (spheronization speed, rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>+1</td>
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<td>7</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>8</td>
<td>+1</td>
<td>+1</td>
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</table>
3. Results and discussion

3.1. Preliminary experiments

The purpose of pelletization process is to produce spherical particles of acceptable size and size distribution along with good mechanical strength and desired release properties. The common way for the delivery of pellets is by filling them in hard gelatin capsules. Also, they may be coated to produce desired drug release profile. Therefore, it is important to determine the pellet size, size distribution, shape, abrasion resistance and mechanical strength as these parameters determine the quality of pellets produced. Also, filling in hard gelatin capsules is uniform, and their coating procedure becomes successful (Paterakis et al., 2002). Although, with Avicel PH 101 formulations, there did not appear to be an issue with distribution, shape, abrasion resistance and mechanical strength as capsules. Also, they may be coated to produce desired drug release properties. The common

3.2. Usable yield, pellet size and size distribution

The purpose of pelletization process is to produce spherical particles of acceptable size and size distribution along with good mechanical strength and desired release properties. The common way for the delivery of pellets is by filling them in hard gelatin capsules. Also, they may be coated to produce desired drug release profile. Therefore, it is important to determine the pellet size, size distribution, shape, abrasion resistance and mechanical strength as these parameters determine the quality of pellets produced. Also, filling in hard gelatin capsules is uniform, and their coating procedure becomes successful (Paterakis et al., 2002). Although, with Avicel PH 101 formulations, there did not appear to be an issue with distribution, shape, abrasion resistance and mechanical strength as capsules. Also, they may be coated to produce desired drug release properties. The common

<table>
<thead>
<tr>
<th>Formulation run</th>
<th>Y1</th>
<th>Y2</th>
<th>Y3</th>
<th>Y4</th>
<th>Y5</th>
<th>Y6</th>
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<tbody>
<tr>
<td>1</td>
<td>95.5</td>
<td>707.25</td>
<td>0.861</td>
<td>48.81</td>
<td>2.00</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>84.8</td>
<td>1015.06</td>
<td>0.888</td>
<td>46.32</td>
<td>1.50</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>85.2</td>
<td>1003.26</td>
<td>0.873</td>
<td>45.94</td>
<td>0.20</td>
<td>8.2</td>
</tr>
<tr>
<td>4</td>
<td>80.9</td>
<td>1108.43</td>
<td>0.892</td>
<td>43.71</td>
<td>0.10</td>
<td>8.9</td>
</tr>
<tr>
<td>5</td>
<td>94.7</td>
<td>750.23</td>
<td>0.933</td>
<td>43.99</td>
<td>1.60</td>
<td>4.7</td>
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<tr>
<td>6</td>
<td>87.3</td>
<td>979.12</td>
<td>0.952</td>
<td>42.86</td>
<td>1.30</td>
<td>5.3</td>
</tr>
<tr>
<td>7</td>
<td>83.5</td>
<td>1063.76</td>
<td>0.933</td>
<td>43.91</td>
<td>0.20</td>
<td>8.5</td>
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<tr>
<td>8</td>
<td>81.3</td>
<td>1107.5</td>
<td>0.941</td>
<td>41.45</td>
<td>0.00</td>
<td>8.3</td>
</tr>
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</table>

The purpose of pelletization process is to produce spherical particles of acceptable size and size distribution along with good mechanical strength and desired release properties. The common way for the delivery of pellets is by filling them in hard gelatin capsules. Also, they may be coated to produce desired drug release profile. Therefore, it is important to determine the pellet size, size distribution, shape, abrasion resistance and mechanical strength as these parameters determine the quality of pellets produced. Also, filling in hard gelatin capsules is uniform, and their coating procedure becomes successful (Paterakis et al., 2002). Although, with Avicel PH 101 formulations, there did not appear to be an issue with distribution, shape, abrasion resistance and mechanical strength as capsules. Also, they may be coated to produce desired drug release properties. The common
Table 4
A summary of p-values for coefficients of factors for response: $Y_1$ (usable yield), $Y_2$ (pellet size), $Y_3$ (pellips), $Y_4$ (porosity), $Y_5$ (abrasion resistance), $Y_6$ (mechanical crushing force), $Y_7$ (residual moisture) and $Y_8$ (dissolution efficiency at 15 min).

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>$Y_1$</th>
<th>$Y_2$</th>
<th>$Y_3$</th>
<th>$Y_4$</th>
<th>$Y_5$</th>
<th>$Y_6$</th>
<th>$Y_7$</th>
<th>$Y_8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_1$</td>
<td>0.0310</td>
<td>0.0171</td>
<td>0.0261</td>
<td>0.0416</td>
<td>0.1695</td>
<td>0.4511</td>
<td>0.1037</td>
<td>0.1625</td>
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<tr>
<td>$b_2$</td>
<td>0.0243</td>
<td>0.0142</td>
<td>0.3440</td>
<td>0.0530</td>
<td>0.0323</td>
<td>0.0604</td>
<td>0.1450</td>
<td>0.1145</td>
</tr>
<tr>
<td>$b_3$</td>
<td>0.7952</td>
<td>0.1749</td>
<td>0.0078</td>
<td>0.0249</td>
<td>0.2578</td>
<td>0.4097</td>
<td>0.0655</td>
<td>0.4097</td>
</tr>
<tr>
<td>$b_{12}$</td>
<td>0.0656</td>
<td>0.0303</td>
<td>0.1000</td>
<td>0.2650</td>
<td>0.3440</td>
<td>0.7952</td>
<td>0.1331</td>
<td>0.1344</td>
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<tr>
<td>$b_{13}$</td>
<td>0.3932</td>
<td>0.0828</td>
<td>0.1000</td>
<td>0.0830</td>
<td>0.7952</td>
<td>0.7048</td>
<td>0.4823</td>
<td>0.0604</td>
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<tr>
<td>$b_{23}$</td>
<td>0.2422</td>
<td>0.2117</td>
<td>0.0700</td>
<td>0.0438</td>
<td>0.3440</td>
<td>0.5577</td>
<td>0.5817</td>
<td>0.0692</td>
</tr>
</tbody>
</table>

Significant effects of factors ($p < 0.05$) on individual responses are shown in bold type.

Fig. 1. Graphical representation of effect of factors on various responses ($Y_j$). (a) Usable yield ($Y_1$); (b) pellet size ($Y_2$); (c) pellips ($Y_3$); (d) porosity ($Y_4$); (e) abrasion resistance ($Y_5$); (f) mechanical crushing force ($Y_6$); (g) residual moisture ($Y_7$) and (h) DE ($Y_8$).
rial that is critical for a number of processing advantages (e.g., a free flowing and uniformly coated product). It is eventually the work of spheronization process, to fragment the extrudate through interactions with the frictional plate, and subsequently smoothen the fragments into the spherical pellets. A variety of parameters have been used to express the shape of the pellets like aspect ratio, roundness score, circularity, pellets, elongation, projection sphericity, etc. (Podczeck et al., 1999; Steckel and Mindermann-Nogly, 2004; Howard et al., 2006; Almeida-Prieto et al., 2007). For the current study, pellets was used for the characterization of shape of pellets. A pellets of 1 represents a perfect sphere. In the present study, pellets ranged from 0.861 to 0.952 (Table 3). The pellets produced with low amount of granulating fluid and low spheronization speed were comparatively non-spherical.

The regression equation for the pellets is

\[ Y_2 = 0.909 + 0.009X_1 + 0.0006X_2 + 0.031X_3 
- 0.002X_1X_2 - 0.0002X_1X_3 - 0.003X_2X_3 \]  
(5)

where \( F = 1237.59, p = 0.0218 \) and \( r^2 = 0.999 \).

Our graphical analysis (Fig. 1c) shows that pellets is significantly affected by the amount of granulating fluid; water and the spheronization speed (p-value < 0.05, Table 4). This is ascribed to the fact that rounding of pellets in the spheronizer is a function of plasticity of the extrudates, where water acts as a plasticizer (Lustig-Gustafsson et al., 1999). During spheronization agglomeration undergo densification resulting in increased availability of surface water and in increased surface plasticity. This will allow faster rounding of extrudates but excessive surface water will result in further pellet growth (Heng and Koo, 2001).

### 3.4. Pellet porosity

Pellet porosity, a vital characteristic, strongly depends on composition of pellet, volume of the wetting liquid, spheronization and drying conditions. This will critically determine the relevant properties such as friability, flowability, wettability, adhesion to various substrates and drug release profile in different ways. This also has the potential to change the ability of a film to adhere to the surface of the pellets (Gómez-Carracedo et al., 2009). Values of porosity for all the eight batches range from 42.86% to 48.81% (Table 3)

\[ Y_4 = 44.874 - 0.7894X_1 - 0.621X_2 - 1.321X_3 
+ 0.116X_1X_2 + 0.391X_1X_3 + 0.749X_2X_3 \]  
(6)

where \( F = 217.61, p = 0.0251 \) and \( r^2 = 0.995 \).

There appears to be significant negative influence of individual components, i.e. amount of granulating fluid and spheronization speed on the porosity of the isoniazid pellets (p-value <0.05, Table 4); as depicted in the graph (Fig. 1d and Eq. (6)). This can be explained by the fact that during spheronization of extrudates, water migrates to the surface resulting in reduction of voids, which in turn, leads to further densification and reduced porosity.

### 3.5. Abrasion resistance and mechanical crushing force

Abrasion resistance is designed to assess the resistance of the pellet surface to abrasion, which pellets will encounter during further processing and shipping, whereas, mechanical crushing force gives indication of its mechanical robustness. The values for both the parameters are shown in Table 3. Pellets with high resistance to abrasion are desirable, as they are likely to retain their integrity on handling and during further processing, such as coating. The regression equations for abrasion resistance and mechanical crushing force are shown as Eqs. (7) and (8), respectively

\[ Y_5 = 0.862 - 0.137X_1 - 0.737X_2 - 0.088X_3 + 0.062X_4X_5 
+ 0.013X_3X_2 - 0.062X_5X_3 \]  
(7)

\[ Y_6 = 6.9 + 0.175X_1 + 1.575X_2 - 0.200X_3 - 0.450X_1X_2 
- 0.075X_3X_1 + 0.125X_2X_3 \]  
(8)

where \( F = 68.56, p = 0.0092 \) and \( r^2 = 0.983 \).

The amount of binder was found to have significant influence on abrasion resistance (p<0.05) as shown in Table 4. Graphical analysis (Fig. 1e) and Eq. (7) reveals that amount of binder is inversely affecting the abrasion resistance. This implies that in order to minimize the abrasion resistance, amount of binder needs to be maximized. Pellets lacking sufficient binding property at the surface will experience greater damage during attrition and make them more vulnerable to wear and tear.

### 3.6. Residual moisture

Uncoated pellets of all the eight runs were found to have had lower moisture content (1.7–1.9%; Table 3). This is in agreement with the graphical representation (Fig. 1g), in which none of the factor or their cross-product term were found to be significant (Eq.
(9) and Table 4)

\[ Y_7 = 1.815 - 0.027X_1 + 0.019X_2 - 0.042X_3 + 0.021X_1X_2 - 0.005X_1X_3 + 0.003X_2X_3 \]

where \( F = 28.89, p = 0.014 \) and \( r^2 = 0.959 \).

For the standard drying conditions, it was found that the differences in the residual moisture of the pellets was very small (statistically non-significant), indicating that in spite of the different initial water contents of the pellets, the drying process efficiently removed the free water added during the initial wet massing stage.

### 3.7. Dissolution efficiency (DE)

DE is a model-independent parameter widely employed as a significant index of drug dissolution performance (Costa and Lobo, 2001; Menegola et al., 2007). In the present study, all the formulation batches showed statistically non-significant and comparable DE. \( p \)-Values of each coefficient indicate non-significant effect of the individual factors or their interactions on the response (Table 4). This behaviour can be attributed to the highly soluble nature of isoniazid (\( \sim 125 \text{ mg ml}^{-1} \)), which is a borderline of Class I and Class III of BCS (Becker et al., 2007)

\[ Y_8 = 67.65 - 0.575X_1 + 0.825X_2 - 0.200X_3 + 0.700X_1X_2 - 1.575X_1X_3 - 1.3753X_2X_3 \]

where \( F = 43.79, p = 0.0152 \) and \( r^2 = 0.973 \).

### 3.8. Validation of multiple response optimization model

In order to assess the reliability of the developed mathematical model, formulations corresponding to optimum composition and two additional random compositions covering the entire range of experimental domain were performed. For each of these formulations, the responses were estimated by the use of generated mathematical models and by the experimental procedures. The formulation parameters of the optimum and the random check points, their experimental and predicted values for all the eight response variables are listed in Table 5. The lower magnitudes of the error in current study indicate the robustness of the model and high prognostic ability of multiple response optimization technique.

### 3.9. Analysis of site-specific release coated isoniazid pellets

The isoniazid core pellets formulated using with optimum formulation composition and process condition were evaluated for the above-mentioned physico-chemical properties. The optimum formulation batch composition and the values for its responses results are enlisted in Table 4. The usable yield of core pellets based on the sieve analysis was found to be 84.95%, whereas the abrasion resistance and mechanical crushing force were found to be 0.485% and 7.32 N, respectively. This indicted that the core pellets are quite hard and are able to withstand the mechanical stresses of subsequent coating process. Coated pellets had residual moisture 2.59% which is significantly higher than the core pellets. It is possible that the additional moisture content was in the coat. In general, moisture would plasticize the dry film coat making it softer and more flexible.

The complete release profile of site-specific release isoniazid pellets is shown in Fig. 2. Only 10.0% of the isoniazid was released at 120 min in 0.1 N hydrochloric acid which indicates the significant gastric acid resistance of the coated pellets. While isoniazid released in intestinal pH 6.8 buffer was found to be within the acceptance criteria (\( >85\% \) of the loaded amount). The external morphology of the core and coated pellets, under scanning electron microscope, is shown in Fig. 3a and b, respectively. The coated pellet was spherical with a smoother surface in comparison to core pellet.
4. Conclusion

In pellets prepared with high drug loading and low level of Avicel PH101, identification of correct level of formulation variables and process variable is essential for desired physico-mechanical properties. Qualitative relationship between the formulation variables, amount of granulating fluid and binder and a process variable, speed of spheroidization for the formulation have been identified. In particular, graphical analysis of the effects enabled identification for each examined variable which are active on the selected responses. The mathematical model for each of the response developed using multiple regression analysis quantitatively describes the influence of the selected variables on the responses under study. From the significance of main effects and their interactions found in this work, it was possible to predict the influence of the factors within the defined experimental domain.

A set of optimum parameters for preparing the cores of site-specific release isoniazid pellets with respect to its desired range of physico-mechanical properties were found to be 44.24% (w/w) of granulating fluid, 2.13% w/w of binder and spheroidization speed, 1000 rpm. Additional experiments performed at optimal and random variables settings confirmed the validity of the proposed model.

It is evident that, the identification of critical levels of granulating fluid, binder and speed spheroidization could be of potential benefit in preparing pellets with high loading of water soluble drug. This approach will help to retain the ability of Avicel PH 101 to prepare satisfactory pellets, even at its low level.

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