International Journal of Pharmaceutics 388 (2010) 64–72

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

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

Swati Pund, Amita Joshi, Kamala Vasu, Manish Nivsarkar, Chamanlal Shishoo*

B.V. Patel PERD Centre, Department of Pharmaceutics, Sarkhej-Gandhinagar Highway, Thaltej, Ahmedabad 380054, Gujarat, India

ARTICLE INFO

Article history:
Received 24 April 2009
Received in revised form 9 December 2009
Accepted 16 December 2009
Available online 24 December 2009

Keywords:
Isoniazid
Multiple response optimization
Pellets
Physico-mechanical characteristics
Full factorial design

ABSTRACT

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 Sureteric® (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.

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-

* Corresponding author. Tel.: +91 79 27439375; fax: +91 79 27450449.
E-mail address: perd@perdcentre.com (C. Shishoo).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.12.034

ables is especially essential in achieving a controlled process and the product.

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 Sureteric® (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 (Kalweka, 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 spheronizer (R.R. enterprises, India), equipped with a rotating plate of regular crosshatch 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 spheronizer 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 (600–2360 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)

$$\text{pellips} = \frac{P}{\pi \times d_{\max}} \quad (1)$$

where P is the perimeter and $d_{\max}$ 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

Table 1
2^3 Full factorial experimental design: factors and responses.

Factors	Levels of the factors used in the formulation	
	−1	+1
X_1 = amount of granulating fluid, water	35% (w/w) of dry mix	55% (w/w) of dry mix
X_2 = amount of binder, Kollidon® 90	0% (w/w) of dry mix	3% (w/w) of dry mix
X_3 = Spheronization speed	700 rpm	1000 rpm
Responses		
Y_1 = usable yield (% theoretical)	Y_5 = abrasion resistance (%)	
Y_2 = pellet size (μm)	Y_6 = mechanical crushing force (N)	
Y_3 = pellips	Y_7 = residual moisture (%)	
Y_4 = porosity (%)	Y_8 = dissolution efficiency; DE (%)	

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 λ_{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® 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 2
Formulations as per the 2^3 Full Factorial experimental design.

Formulation run	Formulation variable - X_1 (amount of granulating fluid, % w/w of dry mix)	Formulation variable - X_2 (amount of binder, % w/w of dry mix)	Process variable - X_3 (spheronization speed, rpm)
1	−1	−1	−1
2	+1	−1	−1
3	−1	+1	−1
4	+1	+1	−1
5	−1	−1	+1
6	+1	−1	+1
7	−1	+1	+1
8	+1	+1	+1

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 the ability to make pellets, our preliminary work revealed that the amount of granulating fluid and the binder along with spheronization speed are significant variables influencing the formulation of isoniazid pellets. This may be attributed to high drug loading and low level of Avicel PH 101. Thus, optimization of core isoniazid pellets was further investigated in depth in order to draw maximum advantage from its potential effectiveness for site-specific release.

In the present study, experimental design methodology was exploited systematically for evaluating the effect of varying the amount of granulating fluid; water, binder; Kollidon® 90, and spheronization speed as well as to highlight any interaction among the components on the micromeritic, mechanical and release properties of isoniazid pellets. This will facilitate the identification of the most significant factors influencing these properties and establishing their best levels for optimizing the considered experimental responses.

Mathematical relationship was generated between the factors and responses for determining the levels of factors, which yield optimum responses. A first order polynomial regression equation that fitted to the data is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (2)$$

where b_0 is the intercept representing the arithmetic averages of all the quantitative outcomes of eight experimental runs; b_1–b_3 are the coefficients computed from the observed experimental values of Y; and X_1, X_2 and X_3 are the coded levels of factors. The terms X_iX_j (i and j = 1, 2 and 3) represent the interaction terms. The equation represents the quantitative effect of factors (X_1, X_2 and X_3) upon each of the responses; Y_1–Y_8. Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor represent the interaction between those factors. A 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 is less than 0.05. In addition, graphical analysis of responses was carried out. This analysis allowed the important factors for the considered responses to be pointed out and the optimum factor level to be selected. The bar graphs were constructed in which the bars that exceed the two reference lines, calculated according to the experimental variance, correspond to the factors that are active on the response. In particular, the active factors are those where a level change determines a response variation which is statistically different from the variation due to the experimental error (Furlanetto et al., 2003, 2006).

3.2. Usable yield, pellet size and size distribution

For a successful extrusion-spheronization process and the formulation, a high percentage of pellets should be produced within a desired size range. Pellets prepared by the process of extrusion and spheronization generally have a mean size between 0.5 and 1.5 mm depending on the diameter of the hole in the extruder die plate. Size polydispersity of pellets is an important factor to be considered if the pellets are to be coated for modifying the release of the drug. The uniformity of the coating requires narrow and homogeneous pellet size distribution that remains unchanged from batch to batch. The size and size distribution of pellets also affect the coating capture by the cores and thereby the release kinetics of the drugs (Mehta, 1989). In order to decrease the variation in fill weight, Rowe et al. (2005) have emphasized the importance of narrow pellet size distribution using computer simulation studies.

Table 3
Result data of mean values of various responses, i.e. usable yield (% theoretical, Y_1), pellet size (μm, Y_2), pellips (Y_3), porosity (%, Y_4), abrasion resistance (%, Y_5), mechanical crushing force (N, Y_6), residual moisture (%, Y_7) and dissolution efficiency at 15 min (%, Y_8).

Formulation run	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8
1	95.5	707.25	0.861	48.81	2.00	5.5	1.889	65.5
2	84.8	1016.06	0.888	46.32	1.50	5.8	1.795	65.8
3	85.2	1003.26	0.873	45.94	0.20	8.2	1.870	68.2
4	80.9	1108.43	0.892	43.71	0.10	8.9	1.876	71.9
5	94.7	750.23	0.933	43.99	1.60	4.7	1.798	70.7
6	87.3	979.12	0.952	42.86	1.30	5.3	1.703	65.3
7	83.5	1063.76	0.933	43.91	0.20	8.5	1.810	68.5
8	81.3	1107.5	0.941	43.45	0.00	8.3	1.780	65.3

Our results indicated that increasing the concentration of binder, increases the mean pellet size but decreases the yield in the desirable size range; 700–1190 μm (Table 3). Usable yield appears to be inversely influenced by the amount of binder as well as amount of granulating fluid (p-value <0.05, Table 4); however, the response is more dominated by the amount of binder as seen in Eq. (3) and Fig. 1a. This may be because of higher cohesiveness and inter-particulate adherence provided to the wet mass by binder and granulating fluid, resulting in pellet agglomeration. This agglomeration increases the quantity of abnormally large particles thereby, decreasing the usable yield.

The largest pellets are the ones made with high amount of binder along with higher amount of granulating fluid. This clearly implies that the solvent influences the packing of the particles during processing. This is evident from the highest positive value of coefficient of term X_2 (Eq. (4)) and highest bar length seen in graphical analysis (Fig. 1b). In addition to X_2, X_1 and the interaction term X_1X_2, significantly contribute to the pellet size as evident from the p-values of their coefficients (Table 4). However, negative influence of X_1X_2 can be attributed to the potential occurrence of interaction between the factors, construing that each factor is tending to modify the effect of another towards the pellet size. Nevertheless, all the batches demonstrated narrow size distribution and followed Gaussian curve

$$Y_1 = 86.65 - 3.075X_1 - 3.925X_2 + 0.050X_3 + 1.45X_1X_2 + 0.675X_1X_3 - 0.375X_2X_3 \quad (3)$$

where F = 204.17, p = 0.0435 and r^2 = 0.994

$$Y_2 = 966.951 + 85.826X_1 + 103.786X_2 + 8.201X_3 - 48.599X_1X_2 - 17.669X_1X_3 + 6.691X_2X_3 \quad (4)$$

where F = 652.82, p = 0.0300 and r^2 = 0.998.

3.3. Shape analysis

If the process of extrusion and spheronization is not optimized, pellet shapes can vary ranging from rounded cylinders to dumbbells and ellipsoids. It is desirable to obtain high usable yield of durable pellets, it is ultimately the shape of the collected mate-

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).

Coefficient	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8
b_1	**0.0310**	**0.0171**	**0.0261**	**0.0416**	0.1695	0.4511	0.1037	0.1625
b_2	**0.0243**	**0.0142**	0.3440	0.0530	**0.0323**	0.0604	0.1450	0.1145
b_3	0.7952	0.1749	**0.0078**	**0.0249**	0.2578	0.4097	0.0655	0.4097
b_{12}	0.0656	**0.0303**	0.1000	0.2650	0.3440	0.7952	0.1331	0.1344
b_{13}	0.1392	0.0828	0.1000	0.0830	0.7952	0.7048	0.4823	0.0604
b_{23}	0.2422	0.2117	0.0700	**0.0438**	0.3440	0.5577	0.5817	0.0692

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

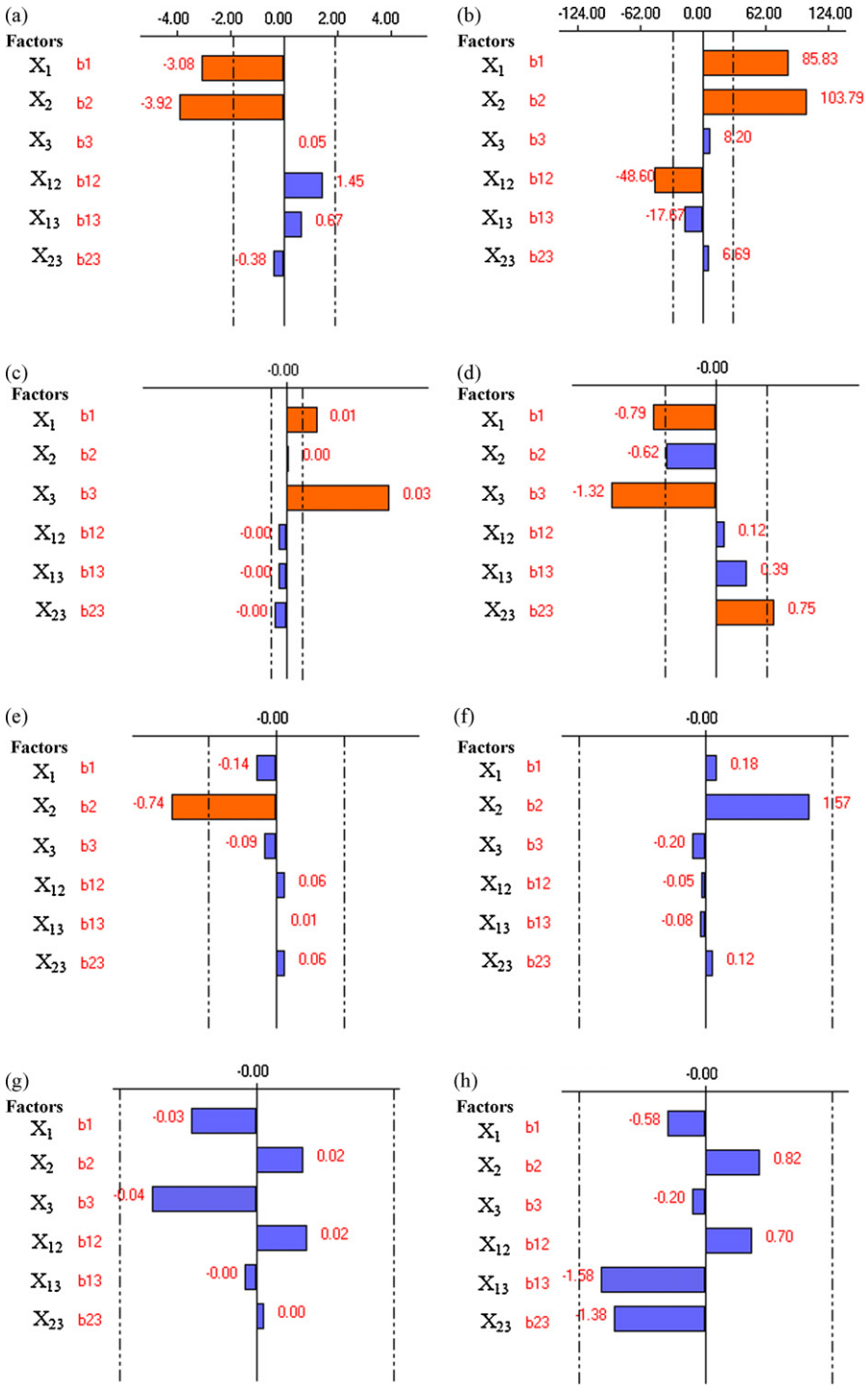

Fig. 1. Graphical representation of effect of factors on various responses (Y). (a) Usable yield (Y_1); (b) pellet size (Y_2); (c) pellips (Y_3); (d) porosity (Y_4); (e) abrasion resistance (Y_5); (f) mechanical crushing force (Y_6); (g) residual moisture (Y_7) and (h) DE (Y_8).

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, pellips, 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, pellips was used for the characterization of shape of pellets. A pellips of 1 represents a perfect sphere. In the present study, pellips 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 pellips is

$$Y_3 = 0.909 + 0.009X_1 + 0.0006X_2 + 0.031X_3 - 0.002X_1X_2 - 0.002X_1X_3 - 0.003X_2X_3 \quad (5)$$

where $F = 1237.59$, $p = 0.0218$ and $r^2 = 0.999$.

Our graphical analysis (Fig. 1c) shows that pellips 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 agglomerates 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)

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

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

$$Y_5 = 0.862 - 0.137X_1 - 0.737X_2 - 0.088X_3 + 0.062X_1X_2 + 0.013X_1X_3 - 0.062X_2X_3 \quad (7)$$

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 inversly 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

$$Y_6 = 6.9 + 0.175X_1 + 1.575X_2 - 0.200X_3 - 0.050X_1X_2 - 0.075X_1X_3 + 0.125X_2X_3 \quad (8)$$

where $F = 19.07$, $p = 0.0173$ and $r^2 = 0.939$.

As far as, mechanical strength of individual pellet is concerned, values for all the eight batches are comparable and contribution of each factor and their interaction was found to be statistically non-significant ($p > 0.05$, Table 4). This might be due to the fact that strength measurements were carried out on pellets of same size fraction, 0.8–1.0 mm.

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.

Table 5
The experimental and predicted values for all the eight responses (Y_1–Y_8) along with percentage prediction error[a] observed for optimum formulation (A) and random formulation (B and C).

Response	A (44.24, 2.13, 1000)[b]			B (46.76, 1.7, 1000)[b]			C (42.55, 1.59, 780)[b]		
	Experimental value	Predicted value	% Prediction error[a]	Experimental value	Predicted value	% Prediction error[a]	Experimental value	Predicted value	% Prediction error[a]
Y_1	84.95	85.01	−0.07	84.74	85.74	−1.18	87.68	87.21	0.54
Y_2	1021.32	1018.29	0.29	996.11	1000.66	−0.46	955.101	946.835	0.87
Y_3	0.945	0.938	0.74	0.944	0.940	0.42	0.889	0.892	−0.34
Y_4	46.11	45.02	2.36	44.21	45	−1.79	44.00	45.04	−2.36
Y_5	0.485	0.496	−2.26	0.671	0.66	1.64	0.922	0.891	3.36
Y_6	7.32	7.413	−1.27	7.01	6.94	0.99	6.91	7.03	−1.74
Y_7	1.729	1.783	−3.12	1.73	1.77	−2.31	1.86	1.841	1.02
Y_8	68.25	67.35	1.31	67.35	67.01	0.51	67.22	67.78	−0.83

[a] Percent prediction error was calculated using the formula (experimental value − predicted value)/experimental value × 100.
[b] The values represented in the brackets are the amount of granulating fluid in %w/w, amount of binder in %w/w and the speed of spheronization in rpm, respectively for A, B and C formulations.

(9) and Table 4)

$$Y_7 = 1.815 - 0.027X_1 + 0.019X_2 - 0.042X_3 + 0.021X_1X_2 - 0.005X_1X_3 + 0.003X_2X_3 \quad (9)$$

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 (~125 mg ml^{-1}), which is a borderline of Class I and Class III of BCS (Becker et al., 2007)

$$Y_8 = 67.65 - 0.575X_1 + 0.825X_2 - 0.200X_3 + 0.700X_1X_2 - 1.575X_1X_3 - 1.3753X_2X_3 \quad (10)$$

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.

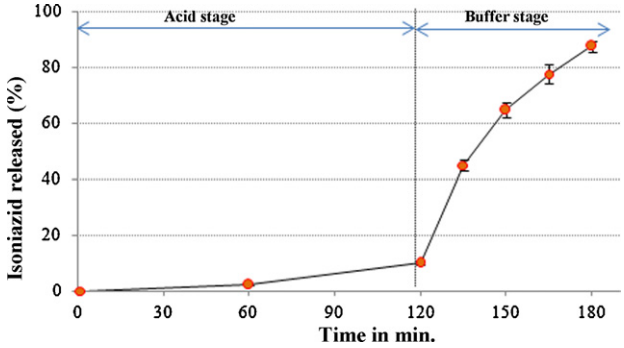

Fig. 2. Complete dissolution profile of site-specific release isoniazid pellets with optimized core formulation and coated with Sureteric® ($n = 6$).

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%, where as 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.

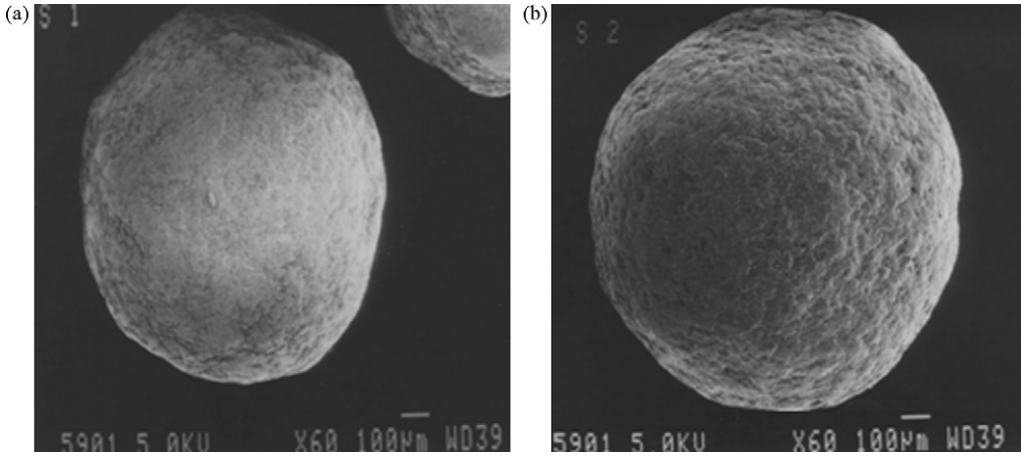

Fig. 3. Scanning electron microphotographs of isoniazid pellets: (a) core pellet; (b) coated pellet

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. Quantitative relationship between the formulation variables, amount of granulating fluid and binder and a process variable, speed of spheronization 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 spheronization 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 spheronization 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.

Acknowledgements

The authors are thankful to T. Nagai Foundation (Japan) for providing financial aid to carry out the research work. AJ is thankful to CSIR, New Delhi for the award of Senior Research Fellowship. The generosity of M/s. TwiLight Litaka Pharmaceuticals Pvt. Ltd. (Pune, India) and Colorcon Asia Pvt. Ltd. (Mumbai, India) is gratefully acknowledged for providing the gift samples of isoniazid and Sureteric®, respectively.

References

Akhgari, A., Garekani, H.A., Sadeghi, F., Azimaie, M., 2005. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int. J. Pharm. 305, 22–30.

Almeida-Prieto, S., Blanco-Méndez, J., Otero-Espinar, F.J., 2007. Microscopic image analysis techniques for the morophological characterization of pharmaceutical particles: influence of the software and the factor algorithms used in the shape factor estimation. Eur. J. Pharm. Biopharm. 67, 766–776.

Becker, C., Dressman, J.B., Amidon, G.L., Junginger, H.E., Kopp, S., Midha, K.K., Shah, V.P., Stavchansky, S., Barends, D.M., 2007. Biowaiver monographs for immediate release solid oral dosage forms: isoniazid. J. Pharm. Sci. 96, 522–531.

Chopra, R., Newton, J.M., Alderborn, G., Podczeck, F., 2001. Preparation of pellets of different shape and their characterization. Pharm. Dev. Technol. 6, 495–503.

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

Furlanetto, S., Maestrelli, F., Orlandini, S., Mura, P., 2003. Optimization of dissolution test precision for a ketoprofen oral extended-release product. J. Pharm. Biomed. Anal. 32, 159–165.

Furlanetto, S., Cirri, M., Maestrelli, F., Corti, G., Mura, P., 2006. Study of formulation variables influencing the drug release rate from matrix tablet by experimental design. Eur. J. Pharm. Biopharm. 62, 77–84.

Ghebre-Sellassie, I., Knoch, A., 2007. Pelletization techniques. In: Swarbrick, J.E. (Ed.), Encyclopedia of Pharmaceutical Technology, vol. 3. Informa Healthcare, New York, pp. 2651–2663.

Gómez-Carracedo, A., Alvarez-Lorenzo, C., Coca, R., Martínez-Pacheco, R., Concheiro, A., Gómez-Amoza, J.L., 2009. Fractal analysis of SEM images and mercury intrusion porosimetry data for the microstructural characterization of microcrystalline cellulose-based pellets. Acta Mater. 57, 295–303.

Gupta, V.K., Assmus, M.W., Beckert, T.E., Price, J.C., 2001. A novel pH- and time-based multi-unit potential colonic drug delivery system. II. Optimization of multiple response variables. Int. J. Pharm. 213, 93–102.

Hellén, L., Yliruusi, J., Kristoffersson, E., 1993. Process variables of instant granulator and spheronizer: II. Size and size distribution of pellets. Int. J. Pharm. 96, 205–216.

Heng, P.W.S., Koo, O.M.Y.K., 2001. A study of the effects of the physical characteristics of microcrystalline cellulose on performance in extrusion spheronization. Pharm. Res. 18, 480–487.

Howard, M.A., Neau, S.H., Marvin, J.S., 2006. PEO and MPEG in high drug load extruded and spheronized beads that are devoid of MCC. Int. J. Pharm. 307, 66–76.

Joshi, A., Pund, S., Nivsarkar, M., Vasu, K., Shishoo, C., 2008. Dissolution test for site-specific release isoniazid pellets in USP apparatus 3 (reciprocating cylinder): Optimization using response surface methodology. Eur. J. Pharm. Biopharm. 69, 769–775.

Jover, I., Podczeck, F., Newton, M., 1996. Evaluation, by a statistically designed experiment, of an experimental grade of microcrystalline cellulose, Avicel 955, as a technology to aid the production of pellets with high drug loading. J. Pharm. Sci. 85, 700–705.

Kim, M., Kim, J., You, Y., Park, H.J., Lee, S., Park, J., Woo, J., Hwang, S., 2007. Development and optimization of a novel oral controlled delivery system for tamsulosin hydrochloride using response surface methodology. Int. J. Pharm. 341, 97–104.

Koo, O.M.Y.K., Heng, P.W.S., 2001. The influence of microcrystalline cellulose grade on shape and shape distributions of pellets produced by extrusion-spheronization. Chem. Pharm. Bull. 49, 1383–1387.

Kramar, A., Turk, S., Vrečer, F., 2003. Statistical optimization of diclofenac sustained release pellets coated with polymethacrylic films. Int. J. Pharm. 256, 43–52.

Kramer, J., Blume, H., 1994. Biopharmaceutical aspects of multiparticulates. In: Sellassie, I.G. (Ed.), Multiparticulate Oral Drug Delivery, vol. 65. Marcel Dekker Inc., New York, pp. 307–355.

Lustig-Gustafsson, C., Johal, H.K., Podczeck, F., Newton, J.M., 1999. The influence of water content and drug solubility on the formulation of pellets by extrusion and spheronization. Eur. J. Pharm. Sci. 8, 147–152.

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.

Mehta, A.M., 1989. Evaluation and characterization of pellets. In: Ghebre-Sallassie, I. (Ed.), Pharmaceutical Pelletization Technology, vol. 37. Marcel Dekker Inc., New York, pp. 241–265.

Melia, C.D., Washington, N., Wilson, C.G., 1994. Advantages and disadvantages of multiparticulate delivery systems. In: Melia, C.D., Washington, N., Wilson, C.G. (Eds.), Multiparticulate Oral Dosage Forms: Technology and Biopharmaceutics. Scottish Academic Press, Edinburgh, pp. 135–140.

Menegola, J., Steppe, M., Schapoval, E.E.S., 2007. Dissolution test for citalopram in tablets and comparison of in vitro dissolution profiles. Eur. J. Pharm. Biopharm. 67, 524–530.

Neau, S.H., Chow, M.Y., Hileman, G.A., Durrani, M.J., Gheyas, F., Evans, B.A., 2000. Formulation and process considerations for beads containing Carbopol® 974P NF resin made by extrusion-spheronization. Int. J. Pharm. 199, 129–140.

Newton, J.M., Pinto, M.R., Podczeck, F., 2007. The preparation of pellets containing a surfactant or a mixture of mono- and di-glycerides by extrusion/spheronization. Eur. J. Pharm. Sci. 30, 333–342.

Paterakis, P.G., Korakianiti, E.S., Dallas, P.P., Rekkkas, D.M., 2002. Evaluation and simultaneous optimization of some pellets characteristics using 3^3 factorial design and the desirability function. Int. J. Pharm. 248, 51–60.

Podczeck, F., Rahman, S.R., Newton, J.M., 1999. Evaluation of standardised procedure to assess the shape of the pellets using image analysis. Int. J. Pharm. 192, 123–138.

Podczeck, F., Knight, P.E., Newton, J.M., 2008. The evaluation of modified microcrystalline cellulose for the preparation of pellets with high drug loading by extrusion/spheronization. Int. J. Pharm. 350, 145–154.

Rastogi, R., Sultana, Y., Aqil, M., Ali, A., Kumar, S., Chuttani, K., Mishra, A.K., 2007. Alginate microspheres of isoniazid for oral sustained drug delivery. Int. J. Pharm. 334, 71–77.

Rowe, R.C., York, P., Colbourn, E.A., Roskilly, S.J., 2005. The influence of pellet shape, size and distribution on capsule filling—a preliminary evaluation of three-dimensional computer simulation using a Monte-Carlo technique. Int. J. Pharm. 300, 32–37.

Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., Vora, M.J., 2001a. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. Int. J. Pharm. 228, 53–67.

Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., 2001b. Impaired bioavailability of rifampicin from fixed dose combination formulations with isoniazid. Indian J. Pharm. Sci. 63, 443–449.

Singh, B., Chakkal, S., Ahuja, N., 2006. Formulation and optimization of controlled release mucoadhesive tablets of Atenolol using response surface methodology. AAPS PharmSciTech. 7, E3.

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

Sousa, J.J., Sousa, A., Podczeck, F., Newton, J.M., Sousa, J.J., 1996. Influence of process conditions on drug release from pellets. Int. J. Pharm. 144, 159–169.
Sousa, A., Podczeck, F., Newton, J.M., 2002. Factors influencing the physical characteristics of pellets obtained by extrusion-spheronization. Int. J. Pharm. 232, 91–106.
Steckel, H., Mindermann-Nogly, F., 2004. Production of chitosan pellets by extrusion/spheronization. Eur. J. Pharm. Biopharm. 57, 107–114.
Toit, L.C., Pillay, V., Danckwerts, M.P., 2006. Tuberculosis chemotherapy: current drug delivery approaches. Respiratory Res. 7, 118.
US Pharmacopoeia 30/NF25, 2007a. US Pharmacopoeial Convention, Rockville, MD, pp. 385.
US Pharmacopoeia 30/NF25, 2007b. US Pharmacopoeial Convention, Rockville, MD, pp. 282.