Screening of Sacrificial Excipients for Arresting Devitrification of Itraconazole from Solid Dispersion

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Abstract

Objective: Present investigation was aimed at developing the solid dispersion of itraconazole (ITR) using sacrificial excipients like pregelatinized starch, spray dried lactose alongside HPMC E5 and Poloxamer 188, thereby arresting the conversion of amorphous form of ITR to crystalline form and to assess the dissolution stability of an amorphous form of the drug on short-term storage.

Materials and Method: Itraconazole loaded solid dispersions were prepared by kneading method. Formulation optimization was achieved by using 2^4 full factorial design on the basis of cumulative percent drug released at t_{30}, t_{60} and t_{120} min. Artificial neural network (ANN) was also applied as a statistical tool for obtaining better predictive ability and the outcomes of ANN were compared with that of design expert software.

Results: The spectral data revealed no drug-carrier interactions. The P-XRD study of optimized batch showed decrease in crystallinity of drug as compared to the untreated drug. The *in vitro* dissolution studies of the optimized batch showed highest dissolution (92% at 120 min.) in comparison to the other formulations. Dissolution stability study of was performed at 40ºC and 75% RH for 90 days for the optimized formulation. Results of optimized batch showed insignificant changes in cumulative percentage drug release on storage.

Conclusion: Dissolution stability could be attributed to the presence of sacrificial excipients as they tend to absorb the moisture on storage and possibly itself get converted into crystalline form thereby minimizing the recrystallization of ITR.

Key words: *Solid dispersion, Itraconazole, ANN, Sacrificial Excipients, Devitrification*

1. INTRODUCTION

More than forty percentage of newly discovered active pharmaceutical ingredients (API) fail to enter the market due to low aqueous solubility.[1] Micronization, pH modification, hydrotropy and solid dispersion have been examined for improvement of apparent drug solubility in aqueous medium, API release rate and possibility of bioavailability. Solid dispersions can be incorporated in tablets, capsules, bioadhesive film, implants and dry powder inhalers.[2] The other merits of solid dispersion includes generation of fine particles of API without excessive use of energy and availability of variety of formulation options.

The main limitation of solid dispersion is, as reported in literature, its physical stability and recrystallization of API on standing, due to absorption of moisture by carrier and particle growth. The phenomena of reverse crystallization of API results in retarded API dissolution.[3] Maintenance of the amorphous state of the drug in a dosage form is always a challenge to the formulators. This is one of the reasons for availability of limited number of formulations in the market.

We hypothesized that if amorphous excipients are added in the solid dispersion containing amorphous API than the probability of recrystallization of API will be arrested to certain extent due to competition between the API and excipient. Such excipients are referred to as sacrificial excipient in the present investigation. The term “sacrificial excipient” is coined from the widely used term sacrificial antioxidants (ascorbic acid and others), which are added to formulation containing oxygen sensitive API.

The examples amorphous excipients are spray-dried lactose, pregelatinized starch, low-substituted hydroxypropyl ether of cellulose (L-HPC), and Neusilin. The sacrificial excipient will preferentially absorb moisture on standing and preferentially get converted in crystalline form and afford protection to amorphous physical state of the API. In the present investigation, the use of quality by design is also demonstrated to speed up the formulation development work at plant. Comparison is also done between the use of design of experiments (DoE) and artificial neural network (ANN).

The main objectives of the present study were to improve the apparent solubility of itraconazole and to test the proposed hypothesis of using sacrificial amorphous excipients for imparting dissolution stability of API on storage.

2. MATERIALS AND METHOD

2.1 Materials

Itraconazole (ITR) was received as a gratis sample from Alembic Pharmaceuticals (Baroda, India). The samples of hydroxypropyl methylcellulose (HPMC E5) and pregelatinized starch were procured from Colorcon Asia Pvt. Ltd. (Goa, India). Spray-dried lactose was procured from Signet Chemicals (Mumbai, India). Poloxamer 188 was purchased from BASF (Mumbai, India) and the solvents used were obtained from Astron Chemicals Pvt. Ltd., (Ahmedabad, India)

2.2 Preparation of solid dispersion by kneading method:

Solid dispersions of ITR were prepared by kneading method. Accurately weighed quantity of HPMC E5, Poloxamer 188 and sacrificial excipients (spray-dried lactose or pregelatinized starch) were mixed with sufficient quantity of water to obtain a smooth and homogeneous paste, after that weighed quantity of ITR was added to the paste and kneaded for 30 min. Finally the paste was dried in an oven at 45°C for 3h and then passed through sieve #100. The samples were stored in a screw-capped glass vial until use.[4]

2.3 Experimental Design:

The concentration of spray-dried lactose (X_1), pre-gelatinized starch (X_2), HPMC E5 (X_3) and Poloxamer 188 (X_4) were selected as independent variables in 2^4 full factorial design. All the other formulation factors were kept constant throughout the experiment. The percentage drug dissolved at 30 (Y_1), 60 (Y_2), and 120 (Y_3) min in 0.1N HCl were selected as dependent variables. Design-Expert software (version 9.0.0.7) was used for the evolving of the mathematical models.[5] The design layout with the results are shown in Table 1. Polynomial models including interaction terms were generated for all the response variables. The full polynomial equation is mentioned below (Eq 1).

$$Y= b_0+ b_1X_1+ b_2X_2+ b_3X_3+ b_4X_4+ b_{12}X_1X_2+ b_{13}X_1X_3+ b_{14}X_1X_4+ b_{23}X_2X_3+ b_{24}X_2X_4+ b_{34}X_3X_4+ b_{1234}X_1X_2X_3X_4 \quad \text{..... (Eq.1)}$$

Where, b_0 is the intercept representing the arithmetic average of all quantitative outcomes of factorial runs; b_1 to b_4 are the main effects. The terms b_{12}, b_{13}, b_{14}, b_{23}, b_{24} and b_{34}

represent the interaction terms. Statistical validity of the model was established on the basis of ANOVA (analysis of variance). Subsequently, the feasibility and grid searches were performed to locate the composition of optimum formulation. Contour plots were also constructed in MS-Excel environment using the output files generated by the Design Expert software.

Artificial Neuronal Network

Artificial neuronal network (ANN) are machine based computational techniques which attempt to simulate some of the neurological processing ability of the human brain. Fundamentally, ANN are interconnected networks of processing units termed as 'neurons', which are responsible for the completion of the decision-making process. They have the ability to discern complex and latent patterns in the information presented to them. This feature of ANN, to extract latent information from the data presented to them, proves them to be powerful tools for modeling and predictive purposes and offers great potential for application in a variety of disciplines. ANN's have attracted attention of many computer scientists and have been successfully applied to solve a multitude of problems in diverse areas of sciences, engineering and business.[6]

A three layer network with a different activation function was applied in this study. NeuroSolutions version 7.0.0 evaluation software was downloaded from NeuroDimension, Inc (www.nd.com). The independent variables X_1, X_2, X_3 and X_4 were used as inputs and the responses recorded were cumulative drug release at 30, 60 and 120 min. Generally, the neural network methodology has several empirically determined parameters. These include: the number of iterations or epochs, the processing element, learning rate and momentum terms. The optimum values for ANN parameters were evaluated by obtaining those values, which yielded the lowest prediction error. The multilinear perceptron (MLP) network model was selected from the customized new network. The different functions like TanhAxon, SigmoidAxon, LinearTanAxon, LinearSigmoidAxon, BiasAxon, LinearAxon, and Axon were used to predict the output response.[7]

2.4 Evaluation Parameters:

2.4.1 Drug Content

Solid dispersions equivalent to 10 mg of ITR were weighed accurately and dissolved in suitable quantity of methanol. The drug content was analyzed at 260 nm by using UV spectrophotometer (Shimadzu, Japan). Each sample was analyzed in triplicate.

2.4.2 In-vitro drug release study

Dissolution experiments were conduct on the untreated drug and solid dispersion. The in-vitro release test was performed using USP type-I (Basket type) dissolution apparatus (Electrolab TDT 08L, India). The dissolution medium was 0.1 N HCl (pH 1.2) maintained at a temperature of 37 ± 1°C with a paddle speed of 100 rpm. The powdered samples (sieved through a 100µm sieve) of pure drug and solid dispersion batches, equivalent to 100 mg of ITR were separately added to the dissolution vessels while stirring. Samples (5ml) were drawn at 30, 60 and 120 min and fresh dissolution medium (5 ml) was added after sampling to maintain sink condition. The samples were immediately filtered through 0.45 µm filters. The first 2 ml of the filtrate was discarded and the samples were assayed for drug content after appropriate dilution with the dissolution medium. The cumulative amounts of the drug dissolved (expressed as % of the total drug added) were plotted as a function of time to produce the dissolution profiles.[8]

2.4.3 Fourier Transform Infrared (FTIR) spectroscopy

Spectroscopy was conducted using an FTIR Spectrophotometer (Spectrum GX-FT-IR, Perkin Elmer,USA) for the untreated ITR and optimized batch of ITR solid Dispersion. The spectrum was recorded in the range of 4000–400 cm^{-1}. The procedure consisted of dispersing a sample in KBr followed by gentle mixing. The spectrum was scanned at a resolution of 0.15 cm^{-1} and scan speed was 20 scan/s.

2.4.4 Differential scanning calorimetry (DSC)

Differential scanning calorimeter (DSC-PYRIS-1, Phillips, Netherlands) was used to study the thermal behaviour of the untreated ITR and optimized batch of ITR solid Dispersion. The experiments were performed in a dry nitrogen atmosphere. The samples (2-4 mg) were heated in hermetically sealed flat-bottomed aluminium pans under nitrogen flow (20ml/min) at a scanning rate of 10°C/min from 25°C to 200°C. Empty aluminium pans were used as the reference standard.

2.4.5 X-ray diffraction (XRD)

The X-ray diffraction study was carried out to characterize the physical form of ITR in samples of untreated ITR and optimised batch of ITR solid dispersion. Vacuum grease was applied onto the glass slide to stick the sample. The sample was allowed to spread on the glass slide in approximately 0.5 mm thickness. The slide was then placed vertically at 0°angle in the X-ray diffractometer (X'Pert Model, Phillips, Netherlands) so that the X-ray beam fell on it properly. The results were recorded over a range of 0–90° (2θ) using the Cu-target X-ray tube and Xe-filled detector. The operating conditions were: voltage 40 kV; current 20 mA; scanning speed 1/min; temperature of acquisition: room temperature; detector: scintillation counter detector and sample holder: non-rotating holder.

2.4.6 Moisture uptake study

Accurately weighed amount of optimized solid dispersion (ITR + HPMC E5 + Poloxamer 188), solid dispersion with sacrificial excipient (ITR + HPMC E5 + Poloxamer 188 +PGS + spray-dried lactose) and also amorphous excipients PGS and spray dried lactose were exposed to 75% RH for a fixed period of time. The stated humidity was obtained by using saturated aqueous solution of sodium chloride in sealed desiccator at temperature of 40 ± 1°C. The samples were observed in two different conditions i.e. with packaging system (samples sealed with aluminium foil) and without packaging system.

2.4.7 Stability study

Short-term dissolution stability study was carried out under accelerated stability condition. The optimized batch with and without sacrificial excipient was stored at ambient conditions in capped amber vials (40˚C/75% RH). Samples were evaluated at an interval of 30, 60 and 90 days for drug content and *in-vitro* release characteristics study. In addition, further confirmation of stability was obtained by performing XRD study so as to confirm the amorphous property of drug on storage.[9]

3. RESULTS AND DISSCUSION

3.1 Drug Content

The drug content of the solid dispersions were found to be in the range of 98.7–101.3% (Table 1), which is acceptable according to the United States Pharmacopeia.[10]

3.2 In vitro release study

The cumulative drug release for the batches (SD1 to SD16) at t_{120} showed a wide variation of 33% to 98% (Table 1 and Fig. 1a). The fitted polynomial equations (full and reduced model) relating the response at t_{30}, t_{60} and t_{120} to the transformed factors are shown in Table 2. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e., positive or negative. The significance level of coefficients, which was found to be >0.05, were omitted from the full model equation to generate the reduced model equation for all the three responses. The coefficients which were found to be significant at *p value* less than 0.05 were retained in the reduced model. Table 2 shows the results of regression analysis. The high values of correlation coefficients of %CDR at t_{30}, t_{60} and t_{120} indicates a good fit. Table 3 shows the results of analysis of variance (ANOVA). The p value is less than 0.05 for all the three responses. It can, therefore be conclude that at least one of the independent variable influences the release of the drug from solid dispersion.

The change in %CDR at t_{30}, t_{60} and t_{120} as a function of X_3 and X_4 is depicted in the form of response surface plot (Fig. 1b) based on full factorial design. Low level of X_3 and high level of X_1, X_2 and X_4 were found to be favourable conditions for obtaining faster dissolution. Multiple linear regression analysis (Table 2) revealed that coefficient X_1, X_2, and X_4 are positive and X_3 is negative. This indicates that on increasing factor X_1, X_2 and X_4, the drug release rate at each time points increases. Higher amount of HPMC may lead to gelation.[11] This is due to the tendency of HPMC E5 to form a hydrogel which slowly erodes in water, which probably explains the delayed dissolution. On the contrary, Poloxamer is a water-soluble nonionic surface-active agent and has been used in solid dispersions to improve apparent solubility of API. It is proposed that the amorphous state of ITR in Poloxamer 188 solid dispersions and the solubilizing effect of Poloxamer are attributable to the high dissolution rate.[12] Pre-gelatinized starch and spray-dried lactose improved dissolution of ITR by virtue of its ability to arrest the devitrification process and

there by keeping the drug in amorphous state. Checkpoint batches C1, C2 and C3 were prepared as per the composition given in Table 4a. The theoretical % cumulative drug release at t_{120} of batches C1, C2 and C3, were 92.30, 92.17 and 92.38%, respectively. The experimental values were found to be 92.22, 92.00 and 92.16% respectively for the three batches (Table 4b), which are in good agreement with theoretical values. This confirms the validity of model. The optimized batch obtained from the solutions of DoE was 99.21 mg HPMC E5, 98.90 mg Poloxamer 188, 100 mg pre-gelatinized starch and 100 mg spray-dried lactose which met the set dissolution criteria i.e. more than 75% drug release at 120 min. Dissolution profile comparison of Pure ITR with the optimized formulation clearly indicated enhanced solubility of the drug in solid dispersion form rather than pure form as seen in Fig 1c.

Overlay Plot

US FDA insists that while submitting the ANDA application, the design space shall be submitted. Hence, design space was generated by overlapping the three contour plots (Fig. 2). The area in the right top corner indicate the design space. It is the space within which if variations occurs, than the FDA should not be approached for SUPAC, i.e. scale-up and post-approval changes.

Artificial neural network

In ANN, the training data set (a couple of data points are picked up from the experimental runs) were used to develop a mathematical model and thereafter test data (The data points not included in training) were uploaded for prediction. Then the observed value of response and computed values of the selected responses were compared. The difference between the two responses is expressed as root mean square of error (RMSE). If the model is perfect, the value of RMSE is zero. Low value of RMSE is an indication of better fit. The data collected were charged in the Neurosolutions software and for each response ANN was run to get the values of RMSE. [6,7] The number of epochs needed by the various options and the MSE values for all responses is summarized in the Table 5. The TanhAxon function showed the least RMSE value for all the responses. Eighteen, eighteen and fifteen epochs were required by the software to arrive at the minimum mean

square of error of 0.07, $1.972*10^{-25}$, 0.017 for all three responses respectively when TanhAxon option was selected in the software. The value of MSE is very close to zero. When the observed value of a response and a calculated value of response are exactly identical MSE is equal to zero. It means that the fit is perfect (predicted value is very close to the observed value). The software generally achieves this by an iteration technique. Comparison of RMSE in DoE and ANN, showed that ANN serves as a better predictive tool as shown in Table 6.

3.3 FTIR study

The FTIR spectrum of pure ITR and that of optimized solid dispersions are shown in Fig. 3a and 3b, respectively. The spectrum of ITR showed characteristic bands at 2935 and 2833 cm^{-1} (O-H stretching), 3320 cm^{-1} (N-H stretching), 1697cm^{-1}(C=O stretching), 1375 and 1465 cm^{-1} (O-H in plane bending), 1040cm^{-1}(O-H out plane bending) and 722 and 749 cm^{-1} (out plane bending for N-H). If we focus on spectra of solid dispersion, then prominent peaks of the drug are seen at 2935 cm^{-1} for O–H stretching, which is shifted to lower frequencies 2922 cm^{-1} in its kneaded particles with the same ratio. The reason for this observation might be interpreted as a consequence result of O-H stretching, which was found to be very week in its kneaded particles. These suggest that there must be strong hydrogen bounding of drug with HPMC E-5. It can be inferred that itraconazole molecules were entrapped in the matrix structure of HPMC E-5 and its physical movement in matrix was minimum and so re-aggregation and recrystallization chances were minimum with the HPMC E-5.[13]

3.4 DSC thermogram

The DSC curves obtained for untreated ITR and solid dispersions are shown in Fig. 4A and 4B. Pure ITR showed a sharp endotherm at 167.38 ºC corresponding to its melting point. DSC thermogram of ITR solid dispersion (Fig 4B) shows characteristic peaks at 50.17 ºC, and 216.42 ºC corresponding to melting point of Poloxamer 188 and HPMC E5 respectively. Absence of characteristic peak of the drug was noticed in solid dispersions. These suggest that the physical state of drug has been changed from crystalline to amorphous form. It is well-known that transforming the physical state of the drug to

amorphous or partially amorphous state leads to a high energy state and high disorder, resulting in enhanced solubility and faster dissolution.[14]

3.5 XRD

The XRD pattern of untreated ITR, solid dispersions and solid dispersion after stability study of 90 days are shown in Figs. 5a, 5b and 5c, respectively. The XRD scan of pure ITR showed intense peaks of crystallinity; whereas the XRD pattern of prepared solid dispersion and solid dispersion after stability exhibited a reduction in both number and intensity of peaks compared to the plain ITR indicating the decrease in crystallinity or partial amorphization of the drug in its kneaded form. Untreated ITR drug powder showed sharp intense peaks at diffraction angles of 2θ, 7.62, 10.33, 14.23, 15.41, 18.66, 19.73, 20.70, 21.86, 22.73, 23.61, 25.03, 27.61 and 28.62. These sharp peaks were present in the diffractograms of all the samples. Number of peaks and peak height in the diffractograms of solid dispersion decreased as compared to that of untreated ITR crystalline powder. Relative crystallinity at 2θ angle 20.70 was found to be 0.675. This indicate decrease in crystallinity or amorphization of the drug. After 90 days of stability study at 40ºC±75%RH, same XRD pattern was obtained indicating that the solid dispersion was stable and the drug was in amorphous state.

3.6 Moisture uptake study:

It is well understood that amorphous drugs formulated in a solid dispersion tend to undergo devitrification process upon storage at high temperature and humidity.[4] The optimized solid dispersion, without sacrificial excipients, was found to be highly hydroscopic (>30% water uptake) in nature at 75% RH. As the exposure time to the humidity was increased, the moisture content also increased. The plasticizing effect of absorbed moisture can reduce the T_g of amorphous substance and lead to further instability. Hence, decrease in dissolution rate is expected on long term storage. The samples of SD containing PGS and spray dried lactose picked up less amount of moisture. The results of the moisture uptake study of SD with and without sacrificial excipients and amorphous excipients (PGS and Spray dried lactose) are shown in Table 7. Table data reveals that, presence of sacrificial excipients absorbs the moisture in preference to the

amorphous drug, thereby stabilizing the solid dispersion on storage. The improved stability of amorphous ITR in presence of sacrificial excipient could be explained on the basis of combination of several effects: (a) elevation of T_g; (b) hydrogen bonding between the drug and the polymer; (c) antiplasticizing effect of the polymers.[15] Therefore, it can be concluded that the sacrificial excipients could be useful to prevent devitrification process of an amorphous drug by decreasing the plasticizing effect of adsorbed water.

3.7 Stability studies:

Effect of aging on performance of amorphous ITR was investigated by performing accelerated dissolution stability study of optimized formulation for a period of 3 months (40°C/75% RH). The result of evaluation are shown in Table 8.

Solid dispersion containing only plasticizer i.e HPMC E5 and Poloxamer 188 showed relatively less drug release (80.32% after 3 months). Whereas solid dispersion with sacrificial excipients i.e PGS and spray dried lactose, showed 91% drug release after 3 month which is comparable to its dissolution profile at the time of its manufacturing. From the study, it can inferred that, if formulation is exposed to a high humidity, the spray dried lactose and PGS may absorb moisture and therefore less amount of moisture (or no moisture) will be available to the drug. Therefore possibility of conversion of drug from amorphous form to crystalline form may be reduced. Thus, it can be concluded that sacrificial excipients plays a major in keeping the drug in amorphous form.[16,17]

4. Conclusion

The results of the study indicates that the dissolution rate of ITR can be significantly enhanced from its solid dispersion with HPMC E5 and pre-gelatinized starch. Itraconazole solid dispersion prepared by kneading method showed higher dissolution than the untreated drug. Moreover, the presence of sacrificial excipients like pre-gelatinized starch and spray-dried lactose in solid dispersion aided in maintaining the amorphous state of drug and preventing devitrification on storage, by self-absorption of moisture in place of the drug. As moisture is one of the main reasons for conversion of an amorphous form to crystalline form of drug on storage, smart choice of excipients can help in maintaining the drug in amorphous state on long term storage. The innovative use of pre-gelatinized starch and spray-dried lactose has not been reported in literature as sacrificial excipient.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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