ABSTRACT

Objective: The aim of this study was to improve the aqueous solubility of a poorly water soluble drug, griseofulvin.

Methods: To accomplish this goal, a technique of solid dispersion with polyvinyl pyrrolidone (PVP) and poloxamer 407 was used. Solubility of griseofulvin alone and the solid dispersion were performed in 10 ml purified water and measured spectrophotometrically at λ max 295 nm to detect the coefficient of improvement.

Results: Solubility of griseofulvin alone was found to be 0.812 mg and the solubility of solid dispersion ranged from 1.64 to 9.56 mg. After generating polynomial models correlating the variables using a D-Optimal mixture design, an optimal formulation with desired response was proposed by the statistical package. For validation, a new solid dispersion formulation based on the optimized composition was prepared and tested for solubility of griseofulvin. The optimized solid dispersion formulation enhances the solubility of griseofulvin by about 12 folds. Increase the amount of poloxamer in the optimized formulation increases the griseofulvin solubility to about 6 folds more than the optimized form.

Conclusion: Solubility of poorly water soluble drugs is a great challenge facing the pharmaceutical researchers. Many techniques have been developed to enhance drug water solubility such as salt formation, simple grinding, grinding with additives, inclusion the drug with cyclodextrin, or solid dispersion. Therefore, increasing water solubility is an essential challenge that facing the pharmaceutical researchers. Many techniques have been developed to enhance drug water solubility such as salt formation, simple grinding, grinding with additives, inclusion the drug with cyclodextrin, or solid dispersion.

Keywords: Solid dispersion technique in conjunction with statistical design was shown to be very efficient for the optimization and improvement of aqueous solubility of poorly water soluble drugs.

INTRODUCTION

Oral dosage forms could be considered as the most convenient way to deliver the drug to patients. However, many problems must be solved before formulating the drug as an oral dosage form. One of these problems is the solubility of the drug. A lot of poorly water soluble drugs have been developed in the pharmaceutical field. Therefore, increasing water solubility to increase bioavailability of these drugs is a great challenge facing the pharmaceutical researchers. Many techniques have been developed to enhance drug water solubility such as salt formation, simple grinding, grinding with additives, inclusion the drug with cyclodextrin, or solid dispersion.

Solid dispersion could be defined as the dispersion of the active drug in an inert matrix and was first introduced by Sekiguchi and Ohi [5]. Many trials to prepare solid dispersion containing the desired drug as solvent dispersion [6-7], hot melting [8-9], solvent evaporation [10], spray drying [11, 12] and supercritical fluid [13] have been reported in the literature to improve aqueous solubility of poorly water soluble drugs and consequently their bioavailability. As more researchers are attracted to spend much effort concerning solid dispersion techniques, better understanding of solid dispersion molecular structure associated with its in vitro/in vivo performance [14-16] could be achieved and consequently more solid dispersion dosage forms will be introduced to pharmaceutical market.

Griseofulvin is white or yellowish-white, microfine powder, the particles of which generally have a maximum dimension of up to 5 μm, although larger particles that may exceed 30 μm may occasionally be present [17]. Griseofulvin is a broad spectrum antifungal agent used to treat mycotic disease of the skin, Hair, and Nails due to Microsporum, Trichophyton, or Epidermophyton respond to the drug therapy. It has a poor water solubility which limits its bioavailability and consequently medicinal uses. Many investigations were reported to increase its aqueous solubility and therefore bioavailability [16, 18, 19].

Polyvinylpyrrolidone (PVP), also commonly called Polyvidone or Povidone, is a water-soluble polymer made from the monomer N-vinylpyrrolidone. When dry, PVP is a light flaky powder, which readily absorbs up to 40% of its weight in atmospheric water. In solution, it has excellent wetting properties and readily forms films. PVP is a branched polymer, meaning its structure is more complicated than linear polymer, though it lies in a two-dimensional plane [20].

Poloxamers are nonionic polyoxyethylene–polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents [21]. The polyoxyethylene segment is hydrophilic while the polyoxypropylene segment is hydrophobic. All of the poloxamers are chemically similar in composition, differing only in the relative amounts of propylene and ethylene oxides added during manufacture. Their physical and surface-active properties vary over a wide range and a number of different types are commercially available. Poloxamers are used as emulsifying agents in intravenous fat emulsions, and as solubilizing and stabilizing agents to maintain the clarity of elixirs and syrups.

The traditional method of experimental design (one-factor-at-a-time) has many disadvantages as cost prohibitive, does not provide any information about the position of the optima and may call for an unnecessarily large number of runs. The application of statistical experimental design in pharmaceutical product development has been demonstrated to be an efficient and satisfactory method to acquire the necessary information that correlate the independent variables, or factors, with the dependent variables, or responses relevant to formulation composition, and/or manufacturing processing parameters [22-24]. One of the most popular experimental design methods is response surface methodology (RSM). RSM, such as the D-optimal design, is commonly used to reveal the main effects and interaction effects between the independent variables of the experiment [25, 26].

The objective of this work was to improve the aqueous solubility of a poorly water soluble drug (griseofulvin was selected as a model for this study).
To achieve this objective, i) solid dispersions made from different proportions of griseofulvin, polyvinyl pyrrolidone, and poloxamer as generated from D-optimal experimental design were prepared, ii) the dissolution of the different solid dispersions was investigated, and iii) optimized solid dispersion that generated from the experimental design was prepared and investigated in term of, solubility, thermal analysis, X-ray diffraction, and scanning electron microscopy (SEM).

**MATERIALS AND METHODS**

**Material**

Griseofulvin was obtained from Hawkins Inc., Pharmaceutical group (USA). Polyvinylpyrrolidone k30 and poloxamer 407 were provided by Sigma-aldrich (Germany). Absolute ethyl alcohol was purchased from HOLYLAND Inc., KSA. Water used in this study was purified by water purification system, Direct-Q5, millipore (USA). All chemicals were used as supplied without further modification.

**Methods**

**Experimental design**

A 14-run, three-factor, two-level D-Optimal mixture design was employed in this study to construct polynomial models in the form:

$$ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_12 X_1X_2 + \beta_13 X_1X_3 + \beta_23 X_2X_3 + \epsilon $$

where

- $A$- $\beta_0$ are the coefficients of the respective variables and their interaction terms, and $E$ is an error term. These models were used to describe the effect of three formulation ingredients: griseofulvin (X1), polyvinylpyrrolidone (X2), and poloxamer (X3) on the solubility of griseofulvin. A summary of the dependent and independent variables that were evaluated in this study and the constraints that were placed on the response are given in Table 1. The range of each factor was chosen based on preliminary studies.

To generate these empirical models, an experimental design was created using the Design-Expert software ($\times$5.07; State-Ease, Inc., Minneapolis, MN). In this design (Table 2), 14 different ternary-blends were suggested based on the constrains and limits given in Table 1. These design points represented factorial points (high and low level from the constrains on each factor), centers of edges (points midway between adjacent factorial points), constrains plane centroid points, axial check points, and an overall center point. A schematic representation of the design is given in fig. 1.

**Preparation of solid dispersions**

Solid dispersions were prepared from different griseofulvin, PVP and poloxamer ratios as illustrated in Table 1 using solvent method [27]. The accurate amount of the drug and poloxamer was dissolved in 100 ml ethanol and sonicated for 10 minutes (VWR,Model 75DOM, Bristol, CT, USA). The accurate amount of PVP was added to the solution and sonicated for another 10 minutes. The sonicated solution was evaporated under reduced pressure using Rotavapor RII (BUCHI, Germany). Then the drug solid dispersion was kept in drying oven (VWR, Model 1350GM, Bristol, CT, USA) at 45 °C for 48 hours. The dried material was ground in a mortar, sieved through a #80 sieve and subjected to further evaluation.

**Solubility study**

Saturation solubility measurements were assayed through ultraviolet absorbance determination at 295 nm using an UV spectrophotometer (6800 UV-VIS, JENWAY, UK). An excess amount of griseofulvin powder and solid precipitate samples was added to 10 ml of de ionized water contained in volumetric flasks. The flasks were stirred in the shaking water bath (Lab Tech, Korea) at 100 rpm for 72 hours at a temperature of 25 ± 0.5°C.

Visual inspection was carefully made to ensure that there was an excess sample in solid state indicating that saturation had been reached. Samples were centrifuged (NF 200, nive, Turkey) for 30 minutes at 5000 rpm and diluted to determine the solubility of griseofulvin from the different samples.

**Differential scanning calorimetry**

Differential scanning calorimetry equipped with a liquid nitrogen cooling system (DSC-60, SHIMADZU, Japan) was used to measure the thermal behavior of the pure griseofulvin powder, pure polymer and the optimized solid precipitates. Samples weighting 2-5 mg were heated in hermetically sealed aluminum pans over a temperature range of 25-300 °C at a constant rate of 30 °C / min.

**X-Ray Powder Diffraction (PXRD)**

Samples were analyzed using powder X-ray (XRD- XMD-300, Unisantis, Germany) diffractometer equipped with a Bragg-Brentano optical setup. Copper Ka radiation (wavelength 1.5406 A°) was used with a long fine focus X-ray tube. The tube voltage and amperage were set to 40 kV and 30 mA, respectively. The divergence and scattering slits was set at 0.50 and the receiving slit was set at 0.15 mm. DIFFRACT radiation was detected by a NaI scintillation detector. Samples were scanned at 0.02 2θ/s from 5 to 40°/2θ. Scans of each sample were run in triplicate. All powder samples were front filled using a circular area aluminum holder. A silicon standard was analyzed to check the instrument alignment each time before sample measurements.

**Scanning electron microscopy**

Powder samples were analyzed by scanning electron microscope (SEM). The particles were deposited on double-sided carbon tap
of griseofulvin, are given in table 2. Data generated for the response that was investigated in this study, aqueous solubility were analyzed using the Design-Expert software.

The independent and dependent variables that were examined in this study to correlate the effect of the level of griseofulvin, PVP, and poloxamer on the enhancing the aqueous solubility of griseofulvin. A 14-run, three-factor, two-level D-optimal design was utilized in this study to correlate the effect of the level of griseofulvin, PVP, and poloxamer (X3) on the solubility of griseofulvin was investigated by measuring the absorbance of the dissolved griseofulvin at λ max 295 nm. A contour plots showing the effect of X1, X2 and X3 on the solubility of griseofulvin is given in fig. 2. As seen from the figure, an increase in the amount of X1 and X2 led to an increase in the solubility of griseofulvin. On the other hand, increase the amount of X3 resulted in a decrease in griseofulvin solubility. These findings could be explained on the basis of the surface area and wetting of the drug. Increasing the amount of griseofulvin, increased the total surface area of the drug and consequently increased the amount of drug available for dissolution. PVP is a two-dimensional plane polymer [20], which is highly water soluble without exerting swelling properties.

Therefore increasing the amount of PVP in the solid dispersion increased the surface area of griseofulvin dispersed on the surface of PVP. On dissolution, PVP takes water and increased the wetting of

**RESULTS AND DISCUSSION**

**Experimental design**

A 14-run, three-factor, two-level D-optimal design was utilized in this study to correlate the effect of the level of griseofulvin, PVP, and poloxamer on the enhancing the aqueous solubility of griseofulvin. The independent and dependent variables that were examined in this study are listed in table 1. The composition of the 14 runs and the response that was investigated in this study, aqueous solubility of griseofulvin, are given in table 2. Data generated for the response were analyzed using the Design-Expert software.

The probability value (α) for determination of statistical significance was set at 0.05, which indicates that a "hypothesis" theory would be rejected if the calculated p-value was less than 0.05 in favor of an alternative theory. The first step toward an optimal statistical analysis was to select the model that best describes and fits the data. Therefore, results were analyzed by the sequential model analysis was to select the model that best (1) describes and (2) fits the data. As seen in table 4, large p values of the linear model (p > 0.0702) for griseofulvin solubility indicates that the linear model adequately fits the data. The linear model that describe griseofulvin solubility will be in the following form:

\[ Y = 4.576 X_1 + 0.1041 X_2 - 0.530 X_3 \]

Where Y represents griseofulvin solubility, X1, X2, and X3 represent the amount of griseofulvin, PVP and poloxamer respectively.

A more elaborate discussion of the effect of study factors is given in the following sections.

**Table 2: Composition of the three-component blend in each of the 14-runs of the D-Optimal mixture design**

<table>
<thead>
<tr>
<th>Run</th>
<th>A: Griseofulvin (gm)</th>
<th>B: PVP (gm)</th>
<th>C: Poloxamer (gm)</th>
<th>Solubility (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.25</td>
<td>1.25</td>
<td>0.00</td>
<td>9.36</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
<td>0.50</td>
<td>0.00</td>
<td>8.64</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>1.75</td>
<td>0.25</td>
<td>2.46</td>
</tr>
<tr>
<td>4</td>
<td>0.50</td>
<td>1.50</td>
<td>0.50</td>
<td>3.35</td>
</tr>
<tr>
<td>5</td>
<td>0.50</td>
<td>2.00</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>6</td>
<td>2.00</td>
<td>0.50</td>
<td>0.00</td>
<td>9.56</td>
</tr>
<tr>
<td>7</td>
<td>1.50</td>
<td>0.50</td>
<td>0.50</td>
<td>6.56</td>
</tr>
<tr>
<td>8</td>
<td>1.56</td>
<td>0.81</td>
<td>0.13</td>
<td>8.56</td>
</tr>
<tr>
<td>9</td>
<td>0.50</td>
<td>1.50</td>
<td>0.50</td>
<td>2.39</td>
</tr>
<tr>
<td>10</td>
<td>1.50</td>
<td>0.50</td>
<td>0.50</td>
<td>9.35</td>
</tr>
<tr>
<td>11</td>
<td>1.00</td>
<td>1.00</td>
<td>0.50</td>
<td>3.07</td>
</tr>
<tr>
<td>12</td>
<td>0.81</td>
<td>1.44</td>
<td>0.25</td>
<td>1.71</td>
</tr>
<tr>
<td>13</td>
<td>1.75</td>
<td>0.50</td>
<td>0.25</td>
<td>3.85</td>
</tr>
<tr>
<td>14</td>
<td>0.50</td>
<td>2.00</td>
<td>0.00</td>
<td>1.64</td>
</tr>
</tbody>
</table>

**Table 3: Sequential model comparison**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>DF</th>
<th>Mean square</th>
<th>F value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>375.45</td>
<td>1</td>
<td>375.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>91.84</td>
<td>2</td>
<td>45.92</td>
<td>10.76</td>
<td>0.0026</td>
</tr>
<tr>
<td>Quadratic</td>
<td>17.67</td>
<td>3</td>
<td>5.89</td>
<td>1.61</td>
<td>0.2620</td>
</tr>
<tr>
<td>Special Cubic</td>
<td>11.87</td>
<td>1</td>
<td>11.87</td>
<td>4.77</td>
<td>0.0651</td>
</tr>
<tr>
<td>Cubic</td>
<td>12.56</td>
<td>3</td>
<td>4.19</td>
<td>3.45</td>
<td>0.1309</td>
</tr>
<tr>
<td>Residual</td>
<td>4.84</td>
<td>4</td>
<td>1.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>514.23</td>
<td>14</td>
<td>36.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4: Lack of fit of different models**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>DF</th>
<th>Mean square</th>
<th>F value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>42.10</td>
<td>7</td>
<td>6.01</td>
<td>4.97</td>
<td>0.0702</td>
</tr>
<tr>
<td>Quadratic</td>
<td>24.42</td>
<td>4</td>
<td>6.11</td>
<td>5.05</td>
<td>0.0730</td>
</tr>
<tr>
<td>Special Cubic</td>
<td>12.56</td>
<td>3</td>
<td>4.19</td>
<td>3.46</td>
<td>0.1309</td>
</tr>
<tr>
<td>Cubic</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure error</td>
<td>4.84</td>
<td>4</td>
<td>1.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
griseofulvin and consequently increase solubility of griseofulvin. On the other hand, poloxamer had a negative impact on griseofulvin solubility as it appears from the negative sign of poloxamer coefficient (-0.530) in the linear model. Increasing the amount of poloxamer, decreased the solubility of griseofulvin. This finding could be explained depending on the amounts of poloxamer. The amount of poloxamer used (up to 0.5 gm) may be lower than the concentration required to produce micelles which could enhance the solubility of griseofulvin.

Fig. 2: Contour plot showing the effect of solid dispersion composition and the level of griseofulvin, PVP, and poloxamer on the solubility of riseofulvin

Numbers in the boxes are the estimated solubility in milligrams

Optimization of solid dispersion formulation

After generating the linear equation relating the dependent and independent variables, the amount of each ingredient ($X_1 - X_3$) was optimized to yield a high griseofulvin solubility. Based on the linear model and constraint, an optimal solid dispersion formulation was suggested by the statistical package. The suggested solid dispersion composition was a binary blend of griseofulvin (2 gm), PVP (0.5 gm), and no poloxamer with a maximum predicted solubility of 9.204 mg of griseofulvin. To validate the optimization process, a new solid dispersion was prepared based on the proposed composition and tested for dissolution. Solubility of the optimized formulation was found to be 9.870 mg compared to 0.812 mg of griseofulvin alone under the same condition.

Differential scanning calorimetry (DSC)

Studying the behavior of the substances during heating was performed using DSC to detect if there is any complex formation between griseofulvin and PVP or if there is any change in the crystal structure of griseofulvin in the optimized solid dispersion formulation.

Fig. 3: Thermogram of griseofulvin, PVP, and optimized formulation

Thermogram of griseofulvin, PVP, and optimized formulation is illustrated in fig. 3. A sharp peak at 223.7°C representing the melting point of griseofulvin, while PVP shows a broad peak starts at about 45 °C and ends at 120 °C representing the evaporation of the moisture and melting of PVP [27]. The presence of the peaks of griseofulvin and PVP in the thermogram of the optimized solid dispersion formulation means that there is no possibility of complex formation between griseofulvin and PVP.

X-ray powder diffraction (XRPD)

Presence of the substance as either crystalline or amorphous form could be detected by performing X-ray powder diffraction test. There is only one crystalline form for griseofulvin reported in the literature [28]. Fig. 4 shows the PXRD pattern of the griseofulvin, PVP and the optimized solid dispersion formulation.

Data were shown in the fig. indicate that the griseofulvin in the optimized formulation corresponds to the pure crystalline form and there is no change in the peaks intensities which means that griseofulvin present as a crystalline form and not changed to amorphous form. This finding was in close agreement with what was reported in the literature that drug in solid dispersion could be dispersed as crystalline particles [29].

Fig. 4: XRPD of griseofulvin, PVP, and optimized solid dispersion formulation

Scanning electron microscopy

Scanning electron microscope was used to detect the morphological difference between the optimized form and the pure griseofulvin powder. As showed in fig. 5, pure griseofulvin presents as a clumps because of the high lipid solubility of the drug. On the other hand, griseofulvin in the optimized form presents as a crystalline particles that are separated by the carrier PVP. Consequently, griseofulvin in the optimized form will have a high water solubility (9.870 mg) than griseofulvin alone (0.812 mg) because of the high surface area.

Fig. 5: SEM photos of (a) Pure griseofulvin and (b) optimized griseofulvin formulation

Effect of increasing amount of poloxamer on griseofulvin solubility

Effect of incorporating high amounts of poloxamer 409 on the aqueous solubility of griseofulvin was investigated. Poloxamer was added to the optimized form during preparation of the solid dispersion in a ratio ranged from 0.5: 1, 1: 1, and 2: 1 of poloxamer to optimized form respectively. Total amount of griseofulvin dissolved from the resulting solid dispersion was measured.
spectrophotometrically. Incorporating high amount of poloxamer resulted in increasing the aqueous solubility of griseofulvin than the optimized form. The amount of dissolved griseofulvin was 57.17, 50.38, and 36.15 mg for 0.5: 1; 1: 1, and 2: 1 respectively. High amount of griseofulvin dissolved was obtained from solid dispersion prepared from 0.5: 1 poloxamer to optimized form. This finding could be attributed to the ability of poloxamer to produce micelles which will enclose griseofulvin and consequently increasing its aqueous solubility. Increasing poloxamer amount to 1: 1 and 2: 1 of the optimized form resulting in decreasing the solubility of griseofulvin which could be explained on the basis of micelles aggregation. At high amount of poloxamer, produced micelles will aggregate to each other without enclosing more amounts of griseofulvin resulting in the observed decrease of griseofulvin amount dissolved.

CONCLUSION
Solid dispersion prepared from poorly aqueous soluble drug with a polymer that has high water solubility and surface area could be a useful technique to improve the aqueous solubility of the drug to about 12 folds. In this study for example, a D-optimal design was proven efficient in optimizing griseofulvin solid dispersion made of ternary blend of griseofulvin, PVP, and poloxamer. While high concentrations of griseofulvin in the solid dispersion were deemed essential for solubility improvement, addition of PVP was found to enhance griseofulvin aqueous solubility. Poloxamer amount was found to have a versatile effect on griseofulvin solubility. At small amounts, poloxamer was found to be inefficient in enhancing griseofulvin solubility. On the other hand, high amounts of poloxamer to optimized formulation (0.5: 1) increased griseofulvin solubility to about 6 folds than the optimized formulation, while further increase of poloxamer amounts decreased griseofulvin aqueous solubility.

CONFLICT OF INTERESTS
Declared None

REFERENCES