Improvement of econazole nitrate solubility by using surfactants and phospholipids according to optimized experimental design

Ismail Bennani¹, Lamya Yachi¹, Yassir El Alaoui¹, Aicha Fahry¹, Nawal Cherkaoui¹, Abdelkader Latiris¹, Jaouad El Harti², Younes Rahali³

¹Laboratory of Pharmaceutics, Faculty of Medicine and Pharmacy of Rabat, University Mohamed V, 10000 Rabat, Morocco
²Laboratory of Medicinal Chemistry, Faculty of Medicine and Pharmacy of Rabat, University Mohamed V, 10000 Rabat, Morocco

ABSTRACT
The main purpose of this study is to improve and boost the solubility of Econazole Nitrate in water using phospholipids and surfactants, until it becomes possible to evaluate the utility of a mixture design in order to determine the optimal composition of non-ionic surfactants and phospholipids needed to obtain a significant improvement in the solubility of Econazole Nitrate in water. The design of experiments approach was tested using a mixture design of Lipoid 75® as phospholipid and Econazole Nitrate, Tween® 80 and Solutol® HS 15 as surfactants. Solubility was determined by the analysis of samples absorbance at 225 nm and the measurement size of particles conducted using a Dynamic Light Scattering at the maximum point of solubility. The final results displayed an improvement in solubility with a statistically significant increase in many tested mixtures. Analysis of the design space showed that, the solubility of Econazole Nitrate is importantly affected by the concentration of surfactants. The best obtained test encloses 1% Econazole Nitrate, 2% Tween® 80, 0.5% Lipoid 75®, 2% Solutol®HS 15 and water q. s. for 100% w / w. Our study has demonstrated that optimized experimental design determines the proportions and the effects of every component based on a limited number of experiments.

*Corresponding Author
Name: Ismail Bennani
Phone: +212667185145
Email: bennani.ismail@gmail.com

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v11i2.2036

INTRODUCTION
The solubilization of insoluble or poorly watersoluble drugs is an important step while preparing many oral, parenteral, soft gelatin topical solutions available in the market (Jouyban et al., 2010). Actually, almost 70% of the synthesized drug molecules trigger solubility problems (Keck et al., 2008). That’s why about 40% of recently developed pharmaceutical ingredients are abandoned at their very first stage of development and that’s due to their very low solubility in water and thus their bioavailability (Lukyanov and Torchilin, 2004).

It has been the main purpose of many studies to improve drugs’ solubility by using vehicles adept to contain hydrophobic drugs, such as inclusion complexes with dendrimers, cyclodextrins, microemulsions or liposome formulations have currently been used (Balte et al., 2012; Wafaa et al., 2016). Though, these systems may present some disadvantages. There is, for instance, the issue of the complexity of cyclodextrins which require distinct structures of...
molecules invited to complex. Similarly, microemulsion systems are characterized by important concentrations of surfactants, that are not so tolerated most of the time. In addition, these systems are often stable only by a specific composition of surfactants, co-surfactants, oil and water (Rupp et al., 2010b).

In practice, a phospholipid can solve the problem of solubility, hence the interest of adding miscible organic solvents or phospholipids is a familiar and efficient way to improve and increase the drugs’ solubility. Yet, in some cases, the addition of two or three phospholipids seem to be necessary in order to achieve the needed drug concentration. Lipoid remains one of the most important and universal phospholipids in the pharmaceutical industry, which is involved in the application of many formulations and it’s a fat free phospholipid used for parenteral preparation (Sefrioui et al., 2015; Alaoui et al., 2014). In the same context, it has been proven that the solubility in water of Econazole can be significantly increased by using Lipoid as a phospholipid (Ghareeb, 2019).

Surfactants are also of a particular importance in pharmacy because they boost the solubility and bioavailability of drugs that are poorly soluble in water and play a major role in many basic and applied scientific processes. They form clusters of colloidal size in solutions, which are the micelles and must have an HLB value greater than 10 (HLB = hydrophilic-lipophilic balance) so as to establish adequate water solubility (Seedher and Kanojia, 2008; Rahali et al., 2013).

Several surfactants are used, among which Solutol® HS 15 or MacrogolHydroxy Stearate: mixture of about 70% of lipophilic molecules composed of 12-hydroxy acid polyglycol mono- and diesters composed of Tween® 80: polysorbate 80 and polyethylene glycol.

The main purpose of this study was to improve and boost the solubility of Econazole Nitrate in water using phospholipid and surfactants.

Econazole Nitrate is a fungistatic antimycotic with a high broad-spectrum synthesized from imidazole, universally used to fight many fungi, especially Candida Albicans (Ogata et al., 1983). Econazole Nitrate is poorly soluble in water (1.48 μg / ml) yet very soluble in methanol (Suñer-Carbó et al., 2017). According to the biopharmaceutical classification system, Econazole Nitrate is classified in IV class: low solubility and permeability (Amidon et al., 1995). For this purpose, our study aims to evaluate the utility of a mixture design to determine the optimal composition of non-ionic surfactants and phospholipids to obtain a significant increase in the solubility of Econazole in the water.

**MATERIALS AND METHODS**

A sample of Econazole Nitrate was obtained from Maphar Eura Pharma (a pharmaceutical company in Morocco). The nonionic surfactants Tween® 80 and Solutol® HS 15, were bought from Merck and BASF, Lipoid® S75 from Lipoid GmbH. For the preparation of all solutions, freshly distilled and filtered water was utilized.

A Shimadzu UV 2450 UV / Vis Spectrophotometer is used for absorbing measurements that have been arrived at, to determine the maximum amount of Econazole solubilized by surfactant and phospholipid mixtures, a dynamic scattering of Zetasizer light 3000HS (Malvern Instruments, France) was used for size control in dispersion.

**Experimental design**

In order to study the solubility of Econazole and ibuprofen, we made use of the same protocol previously used by our team and that’s by using phospholipids and nonionic surfactants optimized by experimental design (Wafaa et al., 2016; Sefrioui et al., 2015).

For determining the formulation space of the nitrate Econazole mixtures, we tested an experimental model using the Design-Expert® software which is a statistical tool that calculates the factorial models, then elaborate graphs for the evaluation of the model.

The statistical study supervised by Design Expert consisted of an analysis of variance (ANOVA), R-squared and precision. The importance of the model was predicted by applying the ANOVA at the 5% significance level. A model is evaluated as significant if the p value is less than 0.05. The signal-to-noise ratio is determined to evaluate the accuracy of the measurements and must be greater than 4.

During this study, a D-optimal experimental model (mixing model) was elected to evaluate and model the effects of surfactants and phospholipid on developing the solubility of Econazole in water. This enabled providing as much information as possible based on a limited number of experiments. The main aspects studied are: the quantities Tween® 80 (X1 = D), Solutol® HS15 (X2 = B) and Lipoid® S75 (X3 = C). Outlet parameters included drug solubility and size measurement.

In the experimental part, we adopted a concentration of Econazole at 1% w / w constant in all the experiments. the ranges of the components chosen for the experiment to determine the design space
Table 1: Lower and upper limits of surfactants used to make the experimental design

<table>
<thead>
<tr>
<th>Components</th>
<th>Lower limit %</th>
<th>Upper limit %</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1:Tween %80</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>X2:Solutol® HS15</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>X1:Lipoid® S75</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>X4:Water</td>
<td>94.5</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Mixture design of experiments and solubility results of Econazole Nitrate mixtures

<table>
<thead>
<tr>
<th>Run</th>
<th>Econazole %</th>
<th>X1:Tween %80</th>
<th>X2:Solutol® HS15</th>
<th>X3:Lipoid® S75</th>
<th>X4:Water</th>
<th>R:solubility %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>2.00</td>
<td>2.00</td>
<td>0.50</td>
<td>94.50</td>
<td>0.99</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>0.00</td>
<td>2.00</td>
<td>0.00</td>
<td>97.00</td>
<td>0.32</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>1.50</td>
<td>1.50</td>
<td>0.38</td>
<td>95.62</td>
<td>0.61</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>2.00</td>
<td>0.00</td>
<td>0.25</td>
<td>96.75</td>
<td>0.90</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.50</td>
<td>97.00</td>
<td>0.30</td>
</tr>
<tr>
<td>6</td>
<td>1.00</td>
<td>0.00</td>
<td>2.00</td>
<td>0.50</td>
<td>96.50</td>
<td>1.08</td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>99.00</td>
<td>0.14</td>
</tr>
<tr>
<td>8</td>
<td>1.00</td>
<td>0.00</td>
<td>1.00</td>
<td>0.25</td>
<td>97.75</td>
<td>0.90</td>
</tr>
<tr>
<td>9</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.25</td>
<td>96.75</td>
<td>0.94</td>
</tr>
<tr>
<td>10</td>
<td>1.00</td>
<td>0.50</td>
<td>0.50</td>
<td>0.13</td>
<td>97.87</td>
<td>0.43</td>
</tr>
<tr>
<td>11</td>
<td>1.00</td>
<td>2.00</td>
<td>2.00</td>
<td>0.00</td>
<td>95.00</td>
<td>0.75</td>
</tr>
<tr>
<td>12</td>
<td>1.00</td>
<td>1.50</td>
<td>0.50</td>
<td>0.38</td>
<td>96.62</td>
<td>0.95</td>
</tr>
<tr>
<td>13</td>
<td>1.00</td>
<td>0.00</td>
<td>2.00</td>
<td>0.50</td>
<td>96.50</td>
<td>0.93</td>
</tr>
<tr>
<td>14</td>
<td>1.00</td>
<td>2.00</td>
<td>1.00</td>
<td>0.00</td>
<td>96.00</td>
<td>0.68</td>
</tr>
<tr>
<td>15</td>
<td>1.00</td>
<td>1.00</td>
<td>2.00</td>
<td>0.25</td>
<td>95.75</td>
<td>0.99</td>
</tr>
<tr>
<td>16</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.50</td>
<td>97.50</td>
<td>0.87</td>
</tr>
<tr>
<td>17</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>97.00</td>
<td>0.60</td>
</tr>
<tr>
<td>18</td>
<td>1.00</td>
<td>1.00</td>
<td>1.50</td>
<td>0.13</td>
<td>96.37</td>
<td>0.98</td>
</tr>
<tr>
<td>19</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.50</td>
<td>97.50</td>
<td>0.87</td>
</tr>
<tr>
<td>20</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>99.00</td>
<td>0.10</td>
</tr>
</tbody>
</table>

have been shown in Table 1. For a concentration suitable for parenteral administration and to obtain a solubilizing effect, the limits of the other components have been defined. (Rupp et al., 2010a; Rowe et al., 2009).

Samples preparation

According to the literature methods, surfactant-phospholipid system was mixed by dispersion method (Bakshi et al., 2005, 2006).

The surfactant solutions were prepared by weighing the appropriate amount of each component followed by dilution with distilled water to obtain the final volume. The prepared solutions of water-soluble surfactants were mixed together in 0.067 M phosphate buffer (pH 7.4), followed by addition of ethanol. (Serajuddin, 2007).

The prepared samples were kept for 24 hours at 37 °C in a thermostatically controlled water bath (Szni-
towska et al., 2008; Rupp et al., 2010b). The final concentration of surfactants and phospholipid in each test tube ranges from 0 to 14%.

The solubility operation was carried out at room temperature using the direct dispersion method, in which the econazole nitrate at a fixed concentration of 1%, is mixed with the surfactant-phospholipid mixture previously prepared. The samples were then stored for 24 hours at room temperature to reach equilibrium. 12 min centrifugation at 12,000 rpm was in a centrifuge to separate and remove excess amounts of insoluble drug. A blank for each sample was prepared simultaneously according to the same protocol and comprising the same proportions of different components.

Determination of Solubility

A well-defined amount of the clear supernatant solution of exact measure was taken from all vials,
was filtered and diluted with methanol. The absorbance of each sample was determined relative to its blank at 220 nm (EDQM, 2016). The amount of soluble drug was then obtained from the standard curve plotted with absorbance as a function of concentration.

All reported data is the average of three analyses. To establish the calibration curve, five different concentrations in a range from 0% w / w to 0.02 x 10-3% w / w were prepared by diluting the primary solution of the active ingredient in methanol. The concentration-absorbance relationship obeyed the Beers-Lambert law (r2 = 0.999).

**Determination of size**

The size measurement was carried out at 25 ° C (after 5 minutes of equilibration). The sample was analyzed without any additional steps (dilution or filtration). The determination of the particle size in the range from 0.3 nm to 10 µm is carried out by dynamic light scattering. (Kaushik et al., 2007; Wei et al., 2009).

**RESULTS AND DISCUSSION**

With the use of Design Expert® software all the mixing experiments were done randomly and the calculations were done. Table 2 summarizes the solubility scores of mixtures of Econazole with percentages variation of phospholipids and surfactants.

**Mathematical modeling of the experimental design**

The experiments were performed mathematically to define the relationship between the factors influencing the characteristics and performance of the formulation. The regression model (first order) represented by the following linear Equation (1),

\[
Y = a1X1 + a2X2 + a3X3 + a4X4
\]  

(1)

Y is the solubility prediction of Econazole, a1, a2, a3 and a4 are the coefficients determined from the observed solubility test values: X1 = Tween® 80, X2 = Solutol® HS 15, X3 = Lipoid® S75, X4 = water.

The solubility response of our study is expressed by the following Equation (2),

\[
Y = 0.1143X1 + 0.0826X2 + 0.8839X3 + 0.0035X4
\]  

(2)

For solubility measurements, Design Expert® has designed mixtures to explore the suitable zone which shows the maximum score solubility of Econazole nitrate. The ternary diagram of the experimental domain at different water ratios is illustrated in Figure 1.

**Determination of size**

The particle size measured by DLS, for the Run 1, which exhibited maximum solubility in Econazole Nitrate, showed a 8.25 nm size average with a narrow size distribution (Figure 2).

**Statistical analyzes**

Significant models indicate that P values less than 0.05. In our case, B, C, D, E are significant models. Tests 1,4,8,9,12,13,15 and 18 showed an improvement in solubility 90% higher than that of Econazole in water, examined by test (control). In fact, the optimization of the proportion of surfactants and phospholipids show that their association and proportion directly affect the solubility.

Our results indicate that the optimal composition for solubilizing the maximum of Econazole contains the maximum quantities of surfactants and phospholipids in our matrix, this means that a high solubility is obtained by raising the proportion of the three components together. In addition, we have indicated that mixed micelles are formed by mixing Solutol® HS 15 and Tween® 80. These mixed micelles are known to have better soluble power and stability (Su et al., 2015).

This confirms that the dissolution is probably carried out via a micellar dispersion of Econazole Nitrate, by the effect of water-miscible surfactant molecules which contain both a hydrophobic and a hydrophilic part and can solubilize several drugs poorly soluble in water, with the help of a phospholipid. Surfactants can also assemble synergistically to form micelles once the surfactant monomer concentration has reached the critical micelle concentration.

In our work, the solubility obtained by the high proportions of surfactants and phospholipids (tests 1 and 15) is better than that obtained without phospholipids with the same proportions of surfactants (tests 7). This indicates that Tween® 80 and Solutol® HS 15 can probably solubilize the Econazole molecules by both direct phospholipid and micelle absorption (Strickley, 2004; Zhang and Isaacs, 2014). In addition, our study showed more important results than those shown in other works realized in our laboratory with optimal values of surfactants (Wafaa et al., 2016; Alaoui et al., 2014), and this makes our study an ideal model.

In the other side, the lowest values of solubility in our matrix were obtained with tests 5 which prepared without surfactants; with this proposed model, the use of phospholipid alone proves that does not improve the solubility. This result can be explained by the fact that the phospholipid formula-
Figure 1: Contours and surface plots of obtained solubility of Econazole Nitrate (% w/w) with A, B, C, D, E respectively at 94.5%, 95.75%, 96.37%, 96.62%, and 96.75% of water

Figure 2: particles size Average distribution of run1

tion is more prone to dilution precipitation than the micellar formulation (Liu, 2008).

If we analyze the coefficients a1 (Tween® 80), a2 (Solutol® HS 15), a3 (Lipoid® S75) and a4 (water) obtained by the solubility equation of our model, we find that a1 and a2 and partially the a3 are the coefficients that affect the highest solubility in Econazole. In contrast, high values of solubility are observed with high proportions of surfactant relative to the phospholipid; therefore, phospholipid is not a limiting factor in our model, especially since lipoid alone does not improve solubility, which is shown in the response surface plots presented in Figure 1 illustrating the effect surfactants and phospholipids on solubility show that the response surface depends on the concentration of Tween® 80 in the studied mixture.

The prediction of better solubility by the proposed
model gave other additional points, which showed that the proportions of the different components offering better solubility are included in the matrix of experiments.

According to our results, this study has a potential to develop interest in new galenic forms of econazole nitrate, as well as to solubilize it in the aqueous phase.

**CONCLUSIONS**

Our study has shown that design optimization by mixing the solubility of a hydrophobic molecule such as Econazole by the addition of phospholipid and nonionic surfactants has improved solubility with a statistically significant result and determine the proportions of each component and their effects from a limited number of experiments. This will make it possible to developing other routes of administration of the molecule for a better effectiveness of the treatment.

**REFERENCES**


