INTRODUCTION

Celecoxib (CLX) CLX is a benzenesulfonamide, a lipophilic molecule and selective inhibitor of the enzyme cyclo-oxygenase-2, a non-steroidal anti-inflammatory drug. CLX is used to treat rheumatoid arthritis, acute pain, osteoarthritis, and rheumatoid arthritis. The poor aqueous solubility of certain active pharmaceutical ingredients poses a problem of the formulation; these molecules generally represent classes 2 and 4 of the biopharmaceutical classification system. Thus, CLX’s oral bioavailability is mainly controlled by its low aqueous solubility of 1.15 g/mL, as measured in the enhancement of aqueous solubility study, as well as a slow rate of dissolution. As a result, improving their solubility becomes a challenge through their formulation. Many technologies are being researched in various drug delivery systems to overcome poor aqueous solubility and bioavailability, such as microemulsions, nanoparticles, nanosuspensions, solid lipid nanoparticles, all these techniques are complicated and require facilities not available in all laboratories. However, emulsion’s economic and simple manufacturing facilities differentiate it from other solubilization techniques. Accordingly, we chose emulsion in this study as a method to improve the aqueous solubility of CLX. The International Union of Pure and Applied Chemistry (IUPAC) defines emulsions as “a colloidal fluid system in which liquid droplets and/or liquid crystals are dispersed in a liquid.” Suppose the emulsion’s continuous phase is an aqueous solution. In that case, the emulsion is oil-in-water and is denoted by the symbol O/W, whereas if the continuous phase is oil, the emulsion is W/O. An emulsifier is a surfactant or surface-active agent which reduces surface tension and/or interfacial tension. The three factors, including oil phases, surfactants, and co-surfactants, will be discovered based on the results of solubility screening. Regarding that, a pseudo-ternary phase diagram will be constructed in order to obtain the optimization emulsion. The final formulation's appearance, pH, homogeneity, stability, drug concentration, globule size, and zeta potential will be evaluated.

MATERIALS AND METHODS

Reagents

The sample of Celecoxib was obtained as a gift sample from the Pharmaceutical Institute (Pharmaceutical Company, Morocco, and Oleic acid was purchased from FlukaChemie AG (Switzerland). Tween 80, Labrafil, and Labrasol polyethylene glycol with an average molecular weight of 400 (PEG 400), were procured from Sigma-Aldrich GmbH (Germany). A certified high-purity Moroccan argan oil (Arganiaspinosa L.), olive oil, and soya oil were selected for the study and purchased...
from Somaprol Company (Morocco). Freshly distilled and filtered water was used throughout the study.

**Selection of Emulsion Components**

The solubility of CLX was tested in various oils: synthetic oils such as oleic acid and natural oils such as olive oil, argan oil, and soya oil. The surfactants were chosen according to their HLB value because those with high HLB have the ability to facilitate the formation of emulsions under a minimum quantity of co-surfactants. Several surfactants, including Tween® 80, Labrafil, and Labrasol, have been tested. Co-surfactants were selected depending on the maximum solubility of CLX, including polyethylene glycol 400 (PEG 400), alcohol (ethanol, methanol, octanol, and 1-butanol), ethylene glycol, and propylene glycol. For the determination of the solubility, sealed conical flasks were used in which an excess of CLX was deposited and 1 mL of the oil to be tested was added. The flasks were shaken in a vortex mixer for 10 minutes and then kept at a constant temperature of 25°C for 24 hours. The next day the saturated solution was centrifuged to remove the undissolved drug and the supernatant was filtered. The drug concentration in the saturated solution was measured by UV-vis absorption spectrophotometry.

**Construction of Pseudo-ternary Phase Iigrams**

Phase diagrams were constructed by the titration method to obtain appropriate components and their concentration ranges, allowing a large emulsion surface. The diagrams as well as the corresponding tests were carried out in two steps:

**Step 1:** Solvent-cosolvent mixture (So$\text{mix}$) was prepared in fixed weight ratios (3:1, 2:1, and 1:1), then each So$\text{mix}$ mixture was mixed with oil. For each phase diagram, the ratios of oil to So$\text{mix}$ were varied between 9:1, 7:3, 5:5, 3:7, and 1:9 (w/w). After mixing the oil and the So$\text{mix}$, 100 μL of distilled water was added to each mixture at room temperature (25°C). The amount of water added varied between 10%, 30%, 50%, 70%, and 90% w/w. The samples were vigorously mixed with a vortex mixer for 10 min and held at 25°C for 24 hours to reach equilibrium. On the next day, each mixture’s macroscopic aspect, fluidity, and homogeneity were noted to determine the emulsion’s domain at the level of the ternary phase diagram. After standing for 24 hours, depending on their macroscopic appearance, the mixtures were classified into thick emulsions, liquid emulsions, gels, or phase separation. Highly viscous mixtures that showed no change after being tilted at a 90° angle and were homogeneous were considered promising emulsions for further study. Based on this procedure, soya oil was selected for further study. Concerning surfactants and co-surfactants, the choice was based on subsequent published studies.

**Step 2:** The sample which gave the emulsion which best meets the established criteria was considered as a point around which a mini-diagram was established with more or less 10% oil, more or less 10% water and more or less 10% of the solvent-cosolvent mixture (So$\text{mix}$), to determine the optimal proportions for the preparation of Celecoxib loaded emulsions.

**Preparation of Celecoxib Emulsions**

Celecoxib was added to the So$\text{mix}$ in the chosen ratio and vortexed together until the drug was completely dissolved, next the oil was added with continuous mixing. Water was added lastly dropwise with continuous mixing. This mixture was kept for 24 hours at 25°C, in a shaking incubator, to attain equilibrium.

**Thermodynamic Stability Studies**

The pseudo-ternary diagram was used to pinpoint a constrained zone of emulsion production. As a result, nine of the formulations were regarded as emulsions. To conduct experiments for thermodynamic stability. The formulations were centrifuged at 4000 rpm for 30 minutes as part of these testing.

**Optimization of Celecoxib Emulsion by D-optimal Experimental Design**

The concentration range of the various components was established to produce stable emulsion formulations based on the findings of the thermodynamic stability experiments. In order to optimize the final formulation, an optimal mixture-design was created with 4 independent variables (water, oil, surfactant, and co-surfactant). Nine experimental assays totaling 8 pattern points, 5 repeats, 5 lack of fits, and a midpoint of analysis were used to build the experimental matrix. Each one of the formulations contained 5% (w/w) of CLX. The response that was studied was the homogeneity of the formulations.

**Characterization of the Drug-loaded Emulsion**

**Emulsions aspect**

Visual examination on a black backdrop at room temperature was used to assess the homogeneity, clarity, and optical shine of drug-loaded emulsions.

**Globule size and zeta potential determination**

Dynamic light scattering analyses utilizing Zetasizer 3000HS (Malvern Instruments, France) were used to quantify globule size, polydispersity index, and Zeta potential at 25°C. A glass electrode pH meter was used to measure the pH at room temperature (25°C) (Bante 920, Bante Instruments L. China). Before each usage, the pH meter was calibrated with buffer solutions of pH 4.0, 7.0, and 9.0.

**Drug Solubility Studies**

By dissolving the CLX in PEG 400, it was added in excess to the optimum emulsion formulation. Undissolved Celecoxib was removed by centrifugation at 4000 rpm for 30 minutes after 2 hours of continuous shaking at room temperature and a 24 hours standing time. The UV spectrophotometric technique was used to determine the concentration of CLX in the supernatant.
Stability Studies
For 90 days, the optimized emulsion containing Celecoxib was stored in three different conditions: (i) cold (4 ± 2°C), (ii) controlled room temperature (25 ± 2°C), and (iii) accelerated storage (40 ± 2°C). The phase separation, globule size, refractive index, and pH of the emulsion were then determined. The concentration of CLX was determined using UV spectrophotometry at 520 nm.\textsuperscript{16,17}

Statistical Analyzes
The experiments were carried out in triplicate and the results were expressed as the mean standard deviation. The statistical data was analyzed using the Student’s t-test at the $p < 0.05$.

RESULTS AND DISCUSSION

Solubility Study of Celecoxib
The results of the CLX solubility tests in the different oils tested are presented in Table 1. It’s reported that the highest solubility of CLX in cosolvant was obtained with Tween\textsuperscript{®} 80 (150.51 mg/mL),\textsuperscript{19} and the highest solubility of CLX in solvent was obtained with PEG400 (414.8 mg/mL).\textsuperscript{20} The three components for the emulsion formulation were chosen based on the CLX solubility data (PEG was selected as cosolvant and Tween\textsuperscript{®} 80 as was selected solvent and Soya oil was selected as oily phase). Polyethylene glycols have grown in popularity as alternative reaction media due to interesting qualities such as non-toxicity, biodegradability and complete miscibility with water and organic solvents.\textsuperscript{21} The extraordinarily high solubility of pharmaceuticals, particularly CLX (Figure 1), in PEG 400 (Figure 2) is most likely due to extensive hydrophobic interactions between the drug and PEG400, as PEG 400 has a long non-polar region compared to other solvents and full miscibility with water and organic solvents.\textsuperscript{22}

Pseudo-ternary Phase Diagrams
The required concentration ranges of the emulsion’s components might be found using the pseudo-ternary phase diagrams. Using Tween 80\textsuperscript{®} as the surfactant, soybean oil as the oil, and PEG400 as the co-surfactant, two phase diagrams with two distinct ratios of surfactant/co-surfactant mixture (3:1) and (1:1) were created (Figure 3). Four different appearances of results were obtained: translucent microemulsion, gel, liquid emulsion, and phase separation representing. Based on these findings, different sections of the pseudo-ternary diagram could be identified. The pseudo-ternary diagram region where the emulsions were obtained was selected for optimization. In fact, according to several studies, it was reported that the pseudoternary phase diagram makes it possible to establish the relationship between the composition of a mixture and its phase behavior.\textsuperscript{25-27} The pseudo-ternary diagram with the $S_{\text{mix}}$ ratio (1:1) did not produce stable emulsions, whereas the pseudo-ternary diagram with the $S_{\text{mix}}$ ratio (3:1) produced emulsions that were much more stable and had long-term stability when the $S_{\text{mix}}$ ratio was between 25% and 80% of the total composition of the emulsion (figures 3 and 4). Based on these findings, we have decided to use a $S_{\text{mix}}$ ratio of 3:1 for the formulation of the emulsion, which will contain Celecoxib and will be optimized. It is observed that the emulsion’s stability increases as the $S_{\text{mix}}$ ratio in the formulation increases. The phase diagram is generally used to arrive at this determination of the adequate region for the intended formulation.\textsuperscript{28-31} However, a high proportion of $S_{\text{mix}}$ in an emulsion could cause skin toxicity, thus a balance had to be established between emulsion stability and a percentage of $S_{\text{mix}}$ that was non-toxic to humans.\textsuperscript{16}

Stability of CLX Emulsions
Six of the 25 formulations of the pseudo-ternary diagram were selected as being more stable than the others based on emulsion parameters including appearance (cloudy/clear), viscosity (high/low), globule size,\textsuperscript{16,17} homogeneity, and stability after a 24 hour equilibrium time.\textsuperscript{32-35} We developed a second pseudo-ternary diagram based on these six formulations, varying the component proportions around the six formulations, and adding nine points to improve the accuracy of our research (Figure 3). As an outcome, a matrix comprising 15 test formulations, numbered F1 through F15 was provided.

**Figure 1:** Chemical structure of Celecoxib (CLX).\textsuperscript{23}

**Figure 2:** Chemical structure of PEG 400.\textsuperscript{24}

**Figure 3:** Diagram of the pseudo-ternary phase with the $S_{\text{mix}}$ (3:1) ratio
Experimental Design

According to the findings of the second pseudo-ternary diagram, the ratios of the mixture must be between 24% and 63% So_{mix} (3:1), between 15% and 63% oil, and between 10% and 50% water in order to produce an optimal emulsion. The experimental formulation optimization studies were carried out using this concentration range as the design space employing a statistical experimental design D-optimal with three levels and three factors. To determine the effects of composition variations on the response studied, 15 assays were tested (Table 2). Even though straight lines and outliers were not encountered, the experimental design included a normal probability plot, which indicates whether the residuals follow a normal distribution.

![Figure 4: Images of emulsions made using So_{mix} (3:1) ratio in (A) and So_{mix} (1:1) ratio in (B)](image)

Table 2: Experimental runs

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Independent variables</th>
<th>Response variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1=Water (%)</td>
<td>C2=Oil (%)</td>
</tr>
<tr>
<td>E1</td>
<td>0.2</td>
<td>0.32</td>
</tr>
<tr>
<td>E2</td>
<td>0.3</td>
<td>0.28</td>
</tr>
<tr>
<td>E3</td>
<td>0.4</td>
<td>0.24</td>
</tr>
<tr>
<td>E4</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>E5</td>
<td>0.3</td>
<td>0.35</td>
</tr>
<tr>
<td>E6</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>E7</td>
<td>0.2</td>
<td>0.48</td>
</tr>
<tr>
<td>E8</td>
<td>0.3</td>
<td>0.42</td>
</tr>
<tr>
<td>E9</td>
<td>0.4</td>
<td>0.36</td>
</tr>
<tr>
<td>E10</td>
<td>0.1</td>
<td>0.27</td>
</tr>
<tr>
<td>E11</td>
<td>0.3</td>
<td>0.21</td>
</tr>
<tr>
<td>E12</td>
<td>0.5</td>
<td>0.15</td>
</tr>
<tr>
<td>E13</td>
<td>0.1</td>
<td>0.45</td>
</tr>
<tr>
<td>E14</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>E15</td>
<td>0.1</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Prediction Point Analysis

The statistical model was validated by characterization of the formulations prepared according to the predicted formulations using three random points. The mean prediction point analysis with an alpha risk of 0.05 is compared to the two-sided prediction interval. There were no outliers discovered using Student’s residual analysis. Because the confirmation experiments were within the prediction interval of the confirmation node, the observed values for response variables were very close to the predicted values. As a result, the obtained mathematical model equations can be used to predict the response values.

Modeling of Response Variables

According to the ratios of the components employed, the quadratic model proved to be the most effective at capturing the evolution of our mixture. In order to predict the homogeneity, we therefore used a higher order polynomial. It is possible to compare the coefficients of the components to determine their relative impact by using the equation of the mathematical model expressed in terms of code factors, which is represented in Eq (1).

Homogeneity = -22.58 A – 5.95 B – 11.26 C + 53.7 AB + 83.8 AC + 24.4 BC (1)

A = water
B= oil
C= Solvent-cosolvant mixture

The F-value of 11.69 and the p-value < 0.001 indicate that the homogeneous model result is significant. The adjusted R^2 of 0.7924 is in reasonable agreement with the R^2 of 0.8665. The residuals plot depicts a normal probability plot.

To determine probability values for this design, statistical significance was set at 0.05. Contour plots and 3D surface response plots were used to depict the interaction between independent variables and experimental responses. Checkpoint analysis confirmed the model’s reliability (Figure 5).

As a result, this template can be used to navigate the design space. The influence of the independent variables on the experimental responses is schemed in the contour plots and surface response plots, as shown in Figures 6 and 7. The plots showed that the homogeneity varied significantly as a function of the variation in So_{mix} content and oil content. Indeed, the homogeneity increases by increasing the So_{mix} content and decreases by reducing the oil content.

Figure 5: The residual trace (Response variables=Homogeneity)
Celecoxib loaded emulsion system using optimal mixture design

Noted that by maintaining a So\textsubscript{mix} percentage greater than 40% and an oil percentage less than 20%, better homogeneity and a globule size less than 120 nm can be obtained. The coefficients of the factors also show that increasing the amount of oil and water at the same time causes an increase in the size of the globules.\textsuperscript{36} Indeed, these findings are consistent with previous research that used a surfactant to allow condensation and stability of the interfacial film between the oily and aqueous phases, as well as a co-surfactant to expand and strengthen the stability of this interfacial film.\textsuperscript{37,38}

**Optimized Formulation**

The optimal values of the mixture’s components were found by numerical optimization based on the criteria of minimizing globule size and maximizing homogeneity. The optimized formula was selected by Minitab\textsuperscript{®} software (4\textsuperscript{th} version) based on the desirability function.

The proportions of water and oil must be maximized and the proportion of the solvent/co-solvent mixture must be minimized in order to achieve the goal. By increasing the debit rate and permeability coefficient, the purpose is to promote good skin tolerance.\textsuperscript{39}

The optimal emulsion contains a proportion (w/w) of water of 36.74%, oil of 19.81%, and 43.44% of So\textsubscript{mix} (3: 1) based on the optimum desirability score of 1,000 obtained by the Minitab\textsuperscript{®} software.

**Optimized Formulation Characterization**

**Macroscopic Aspect**

The celecoxib-optimized emulsion has a homogeneous appearance, adequate viscosity for topical application, a brilliant white color, is easy to wash with water, and the two phases are completely mixable without phase separation or solid precipitation.

**Physicochemical Characteristics**

The optimized emulsion had a mean globule size of 116,6 nm (Figure 8), and the polydispersity index (PDI) was 0.231. It’s reported that when the homogeneity and stability of the preparation are ensured, the system’s polydispersion and distribution are low.\textsuperscript{40,41}

The zeta potential was -5.98 mV, it’s slightly negative, which is good for system stability.\textsuperscript{16} Figure 8 depicts the intensity and mass distribution of globule size. The optimized emulsion had a pH of 6, which was softly acidic. This pH is related to CLX’s acidity (pKa: 11.1).

This pH is acceptable for cutaneous application because it is compatible with the nature of the skin.\textsuperscript{17} A higher conductivity value than that of distilled water confirmed the sense of the oil-in-water emulsion. Because conductivity is close to zero in the absence of water (W/H).\textsuperscript{16}

**Celecoxib Solubility**

The optimal emulsion had an 83.52% soluble fraction of CLX according to UV-vis spectrophotometry (Figure 9). This finding supports the use of an emulsion to solubilize a drug that is insoluble in water. Emulsions have been reported to be...
Celecoxib loaded emulsion system using optimal mixture design

| Temperature test (°C) | Globule size (nm) | Percentage CLX | Phase separation | pH  
|------------------------|-------------------|----------------|-----------------|--------
| 4 ± 2°C                | 111, 6            | 84,01 ± 0.5    | No              | 5,9 ± 0.1|
| Room Temperature       | 116, 5            | 83,52 ± 0.5    | No              | 6 ± 0.1 |
| Accelerated (40 ± 2°C) | 105, 2            | 82,11 ± 0.5    | No              | 6,1 ± 0.1|

*(mean ± SD, n=3)

Figure 9: The calibration range of the UV-Vis spectrophotometric assay of Celecoxib in the optimized formulation.

used to improve the solubilization and thus bioavailability of lipophilic drugs due to their hydrophilic and lipophilic nature.\(^6\)

**Stability Studies**

According to the results of the stability studies (Table 3), the optimized CLX-based emulsion remained stable for three months. The clarity of the optimized emulsion was maintained throughout storage. No phase separation was observed, and no significant change in the CLX concentration was observed.

**Limitation of the Study**

However, in-vitro release and skin permeability studies would be required to validate this system as an effective method of delivering this drug.

**CONCLUSION**

The optimal concentration ranges for the emulsion formulation able of solubilizing Celecoxib might be found by using pseudo-ternary phase diagrams. By using experimental design software the concentrations of the components were fixed and optimized. The optimized emulsion included (w/w) 43.44% of So\(_{\text{mix}}\) (3:1) as a surfactant/co-surfactant, 19.81% of soya oil, and 36.74% of water. The modeling of the responses revealed that the emulsion’s homogeneity and globule size are directly influenced by the proportion of the surfactant/cosurfactant mixture. CLX formulation in emulsion it seems to significantly improve its solubility.

**CONFLICT OF INTERESTS**

Declared none

**REFERENCES**


Celecoxib loaded emulsion system using optimal mixture design