International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 3, 2016

Original Article

SOLUBILIZATION OF CELECOXIB USING ORGANIC COSOLVENT AND NONIONIC SURFACTANTS OPTIMIZED BY EXPERIMENTAL DESIGN

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Received: 04 Nov 2015 Revised and Accepted: 25 Jan 2016

ABSTRACT

Objective: The solubility of drug substances in water is one of the major factors taken into account in the formulation of oral solutions and parenteral forms. The present study aims to evaluate the utility of a mixture design in improving water solubility of celecoxib through a micellar system by the use of organic co solvent and nonionic surfactants that are well tolerated by the parenteral route.

Methods: In our study, a design of experiments approach was tested using a mixture design of nonionic surfactants (Tween® 80 and Solutol®HS 15), an organic cosolvent (ethanol) and celecoxib. Solubility determination was based on the analysis of samples absorbance at 215 nm. A particles size measurement was conducted using a Dynamic Light Scattering at the point showing the maximum of solubility.

Results: The results showed a significant solubility increase in most of tested mixtures. The analysis of the design space showed that the solubility of celecoxib varies very closely with the concentration of Tween® 80 associated with ethanol and Solutol®HS 15 in water. Run 19 containing 0.8% of celecoxib, 10% of ethanol, 2% of Tween® 80, 2% of Solutol®HS 15 and water q. s. for 100% w/w improved celecoxib solubility by about 90 %, and showed an average particles size of 9.67 nm.

Conclusion: Micellar solubilisation associating a cosolvent and nonionic surfactants seems to improve celecoxib solubility significantly. Mixture design provides maximum information about the effects and the proportions of each component from a limited number of experiments.

Keywords: Solubility, Celecoxib, Mixture design, Cosolvent, Surfactants

INTRODUCTION

Today about 40% of newly developed active pharmaceutical ingredients are discarded in early development phases because of their poor water solubility and bioavailability [1]. Also, up to 70% of synthesized drug molecules present solubility problems [2]. Solubilization of poorly water-soluble drugs is a crucial step in the preparation of many commercially available oral solutions, parenteral, soft gelatin, and topical pharmaceutical formulations [3].

Many efforts to improve drugs solubility using capable vehicles to enclose hydrophobic drugs, such as inclusion complexes with cyclodextrins, microemulsions, dendrimers or liposome formulations have been established to date [4-7]. However, all these systems reveal disadvantages. For example cyclodextrins need special guest molecule structures for complexation. Likewise, microemulsion systems are characterized by high surfactant concentrations which mostly are not well tolerable. Also, those systems are often stable at only an explicit composition of surfactants, co-surfactants, oil and water [8].

Adding miscible organic solvents (or cosolvents) is the most common and feasible method to increase drugs solubility. Generally, one organic solvent is able to solve the solubility problem. However, in some cases, the addition of a second and even a third cosolvent is necessary to reach the desired drug concentration. Ethanol is one of the most important and common cosolvents in the pharmaceutical industry. It is used in many commercially available oral, parenteral, and soft gelatin formulations [3]. In our context, it has been shown that the aqueous solubility of celecoxib, rofecoxib, and nimesulide could be significantly enhanced by using ethanol as a cosolvent [9].

Likewise, surfactants play an essential role in many processes related to fundamental and applied science. They form colloidal-sized clusters in solutions, known as micelles, which have particular significance in pharmacy since they are able to increase the solubility and bioavailability of poorly water soluble drugs [10, 11]. Beside these molecular conditions, the surfactant should also have an HLB-value above 10 (HLB = hydrophilic-lipophilic balance) to guarantee an adequate water solubility.

Among these surfactants, Macrogol 15 Hydroxy stearate: Solutol®HS 15 (which is a mixture of ~70% lipophilic molecules consisting of polyglycol mono-and diesters of 12-hydroxystearic acid and ~30% hydrophilic molecules consisting of polyethylene glycol) and polysorbate 80: Tween® 80 are two commonly used nonionic surfactants. They are widely used as formulation stabilizers and also as excellent solubilizers for parenteral use.

In this work, we tried to increase the solubility of celecoxib in water using surfactants and organic cosolvent. Celecoxib is the first specific inhibitor of cycloxygenase-2 (COX-2) [12]. It was given preference thanks to its popular use as an analgesic, antipyretic and anti-inflammatory agent. It is also used as an adjunct to standard therapy to reduce the number of colorectal adenomatous polyps in patients with familial adenomatous polyposis [13]. It's a class II drug of biopharmaceutical classification system, and its solubility is classified "low" according to this classification system [14]. However, in the formulation of liquid dosage forms, its solubility should be increased because of formulation volume limitation. According to the European pharmacopoeia 8.0, celecoxib is practically insoluble in water (one part in more than 10 000 parts of water), and its water solubility is 0.0033 mg/ml at 25 °C according to the literature [15, 16]. Knowing that the standard therapeutic oral dose is 200 mg with a bioavailability of about 40%, the therapeutic dose by parenteral route is expected to be 80 mg per a vial of a convenient volume of 10 ml [17]. However, an injection of 80 mg of celecoxib needs over 24 liters of water for injection.

For this reason, our study aims to evaluate the utility of a mixture design to determine the optimum composition of nonionic surfactants and co-solvent for obtaining a significant increase in

celecoxib water solubility, which would permit to reduce its volume of injection [18-28].

MATERIALS AND METHODS

Instruments and reagents

A sample of celecoxib ($C_{17}H_{14}F_3N_3O_2S$) (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide) was obtained as a donation from Promopharm Pharmaceutical Company (Morocco). Nonionic surfactants, Solutol® HS 15 and Tween® 80, were purchased from BASF (Ludwigshafen, Germany) and Merck (Germany), respectively. Ethanol was purchased from VWR BDH Prolabo® (France). Freshly distilled and filtered water was used for the preparation of all solutions.

In order to determine the maximum amount of celecoxib which can be solubilized by the mixtures of surfactants and cosolvent, absorbance measurements were carried out using UV/visible spectrophotometer (Shimadzu UV 2450, Japan). For size control in dispersion, a dynamic light scattering (DLS) by Zetasizer 3000HS (Malvern Instruments, France) was used.

Experimental design

To carry out this study, we were based on the protocol previously used by our team to study the solubilization of acetaminophen using phospholipids and nonionic surfactants optimized by experimental design [29]. Indeed, to define the formulation space for the celecoxib mixtures, we tested an experimental design by using Design-Expert® software, which is a statistical tool that enables calculation of factorial designs and drawing graphs for design evaluation.

The statistical study made by Design Expert consisted in the analysis of variance (ANOVA), the R-Squared and precision. The significance of the model was estimated by applying ANOVA at the 5% significance level. A model is considered significant if the p-value is less than 0.05. The signal-to-noise ratio is determined to evaluate measurements precision and should be greater than 4.

In this article, a D-optimal experimental design (mixture design) was selected to evaluate and model the effects of surfactants and co-solvent on enhancing the solubility of celecoxib in water. This has the advantage to provide maximum information from a limited number of experiments. The studied factors are: the amounts of ethanol (X_1 = D), Tween® 80 (X_2 = B) and Solutol® HS15 (X_3 = C). Output parameters included drug solubility and size measurement.

To make this experimental design, we used a constant concentration of celecoxib at 0.8% w/w in all experiences. This concentration is about 2400 times higher than the concentration usually soluble in water. Table 1 shows the ranges of these components for the determination of functional design space. The lower and upper limits of other components were determined to permit a solubilizing effect and a suitable concentration for parenteral administration [30-32].

Table 1: Lower and upper limits of surfactants and co-solvent used to make the experimental design

Components	Lower limit (%)	Upper limit (%)
X_1: ethanol	0	10
X_2: Tween® 80	0	2
X_3: Solutol® HS 15	0	2

With Design Expert® software, we experimented a matrix of 20 formulations at different ratios of all components (table 2).

Preparation of the samples

Mixed surfactant-co solvent system was prepared by a direct dispersion method according to methods previously described in the literature [33-35].

Pure surfactant stock solutions were prepared by accurately weighting the appropriate quantity of each component and diluting with distilled water to the final volume. Stocks solutions of water soluble surfactants were dispersed together in phosphate buffer 0.067 M at pH 7.4 in conical vials, by weighting the appropriate amounts of surfactants and then adding the desired amounts of ethanol [36, 37]. The respective amounts are defined according to the mixture design already realized.

The samples got equilibrated at 37 °C for at least 24 h in a thermostated water bath (GFL1083, Germany) [30, 38, 39]. The final concentration of surfactants (Tween® 80, Solutol® HS 15) and cosolvent (ethanol) in each vial is varying from 0 to 14% w/w according to our mixture design.

Drug solubilization study was conducted at room temperature following direct dispersion method where the model drug, at a fixed concentration of 0.8% w/w, was mixed with the surfactants-cosolvent dispersion previously prepared. Vials were then shaken in a thermostated water bath at 37 °C for at least 24 h. Then, samples were stored during 24 h at room temperature to reach equilibrium. Excess amounts of the drug were then separated by 12 min centrifugation at 12 000 rpm in a centrifuge (Industria EPF-12, Argentina).

A "blank" for each sample was simultaneously prepared following the same protocol and comprising the same proportions of different components, excluding the drug.

Solubility determination

A definite quantity of the clear supernatant solution properly collected from all conical vials was filtered and diluted with ethanol. The absorbance of each sample was determined against its blank at 215 nm [15]. The amount of solubilized drug was then obtained from the standard curve drawn with absorbance versus concentration.

All reported data are the average of three independent assays. For the calibration curve, five different concentrations in a range from 0% w/w to $0.02x10^{-3}$% w/w were prepared by dilution of drug stock solution in methanol. The concentration-absorbance relationship obeyed the Beers–Lambert law ($r^2 = 0.987$).

Size determination

Dynamic Light Scattering was used to determine particles size in a range between 0.3 nm and 10 µm [40, 41]. It was employed for the point having the maximum of solubility. The measurement was carried out at 25 °C after 5 min of equilibration. To avoid any losses (like larger vesicles), the produced sample was analyzed without any dilution or filtration step.

RESULTS AND DISCUSSION

Solubility of celecoxib

All mixture experiments were conducted in random order and calculations were performed by Design Expert® software. The solubility results of the 20 mixtures of celecoxib with various ratios of surfactants and cosolvent are shown in table 2.

Experimental design and mathematical modeling

Experiments were carried out to determine the mathematical relationship between the factors influencing the performance and the characteristics of the formulation. A first order polynomial regression model represented by a linear equation was selected as follows:

$$Y = a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4$$

Where Y is the solubility prediction of celecoxib, a_1, a_2, a_3 and a_4 are the estimated coefficients from the observed experimental values of solubility for X_1 (ethanol), X_2 (Tween® 80), X_3 (Solutol® HS 15) and

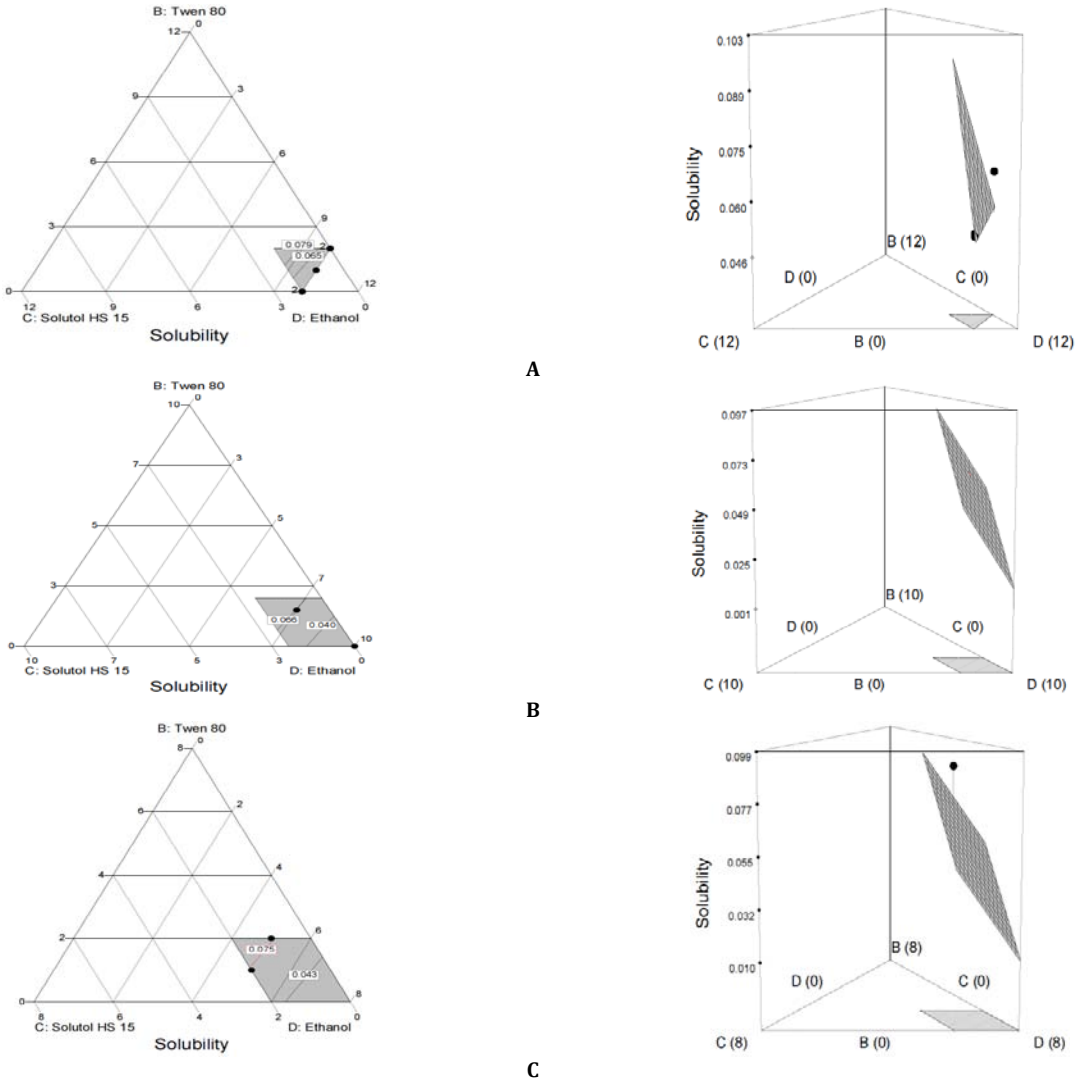
X_4 (water). The response of celecoxib solubility expressed by a linear equation was as follows:

$$\text{Solubility} = 0.02311X_1 + 0.01989X_2 + 0.00025X_3 + 0.00009X_4$$

With solubility measurements, mixtures were designed by Design Expert® to explore the feasibility zone presenting the maximum solubility for celecoxib. Fig. 1 represents the experimental domain inside the ternary diagram at different ratio of water.

Table 2: Mixture design of experiments and solubility results of the 20 celecoxib mixtures

Run	Celecoxib % w/w	X_1: Ethanol % w/w	X_2: Tween® 80 % w/w	X_3: Solutol® HS 15 % w/w	X_4: Water % w/w	Solubility % w/w
1	0.8	10	2	2	85.2	0.090
2	0.8	10	0	2	87.2	0.052
3	0.8	10	2	0	87.2	0.055
4	0.8	10	1	1	87.2	0.050
5	0.8	5	1	2	91.2	0.082
6	0.8	5	2	1	91.2	0.090
7	0.8	0	2	2	95.2	0.072
8	0.8	10	0	0	89.2	0.001
9	0.8	0	1	0	98.2	0.027
10	0.8	0	0	1	98.2	0.036
11	0.8	5	0	0	94.2	0.002
12	0.8	7.5	1.5	1	89.2	0.063
13	0.8	2.5	0.5	0.5	95.7	0.035
14	0.8	2.5	1.5	1.5	93.7	0.081
15	0.8	7.5	0.5	0.5	90.7	0.028
16	0.8	5	1	1	92.2	0.072
17	0.8	10	0	2	87.2	0.052
18	0.8	10	2	0	87.2	0.066
19	0.8	10	2	2	85.2	0.094
20	0.8	0	0	0	99.2	0.001



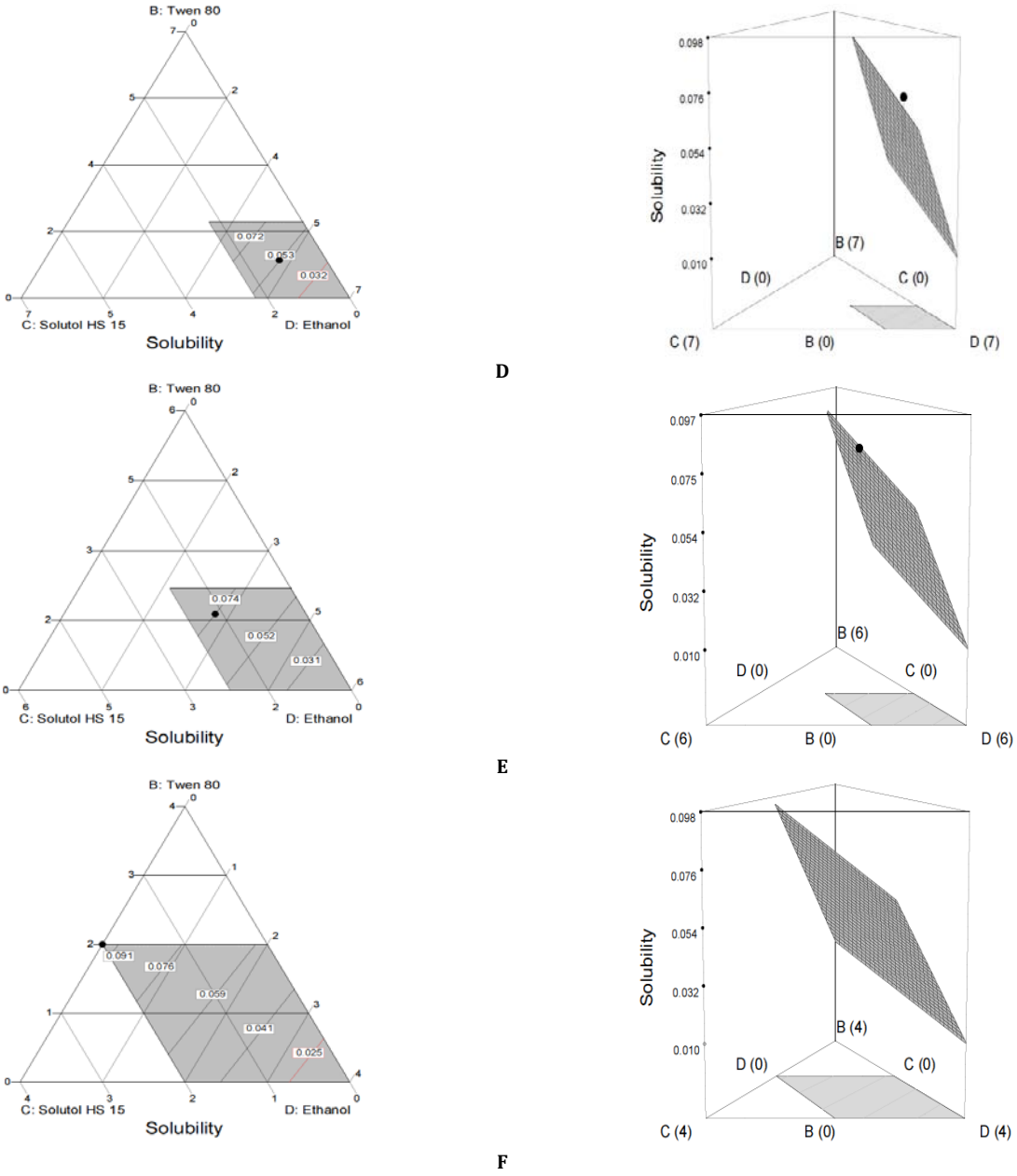


Fig. 1: Contours plots and surface plots of estimated solubility of celecoxib (% w/w) with a, b, c, d, e and f respectively at 87.2%, 89.2%, 91.2%, 92.2%, 93.7% and 95.2% of water

Size determination

Particles size measurement by DLS, of run 19 who had the maximum celecoxib solubility, shows a mean size of 9.67 nm with a narrow size distribution (fig. 2).

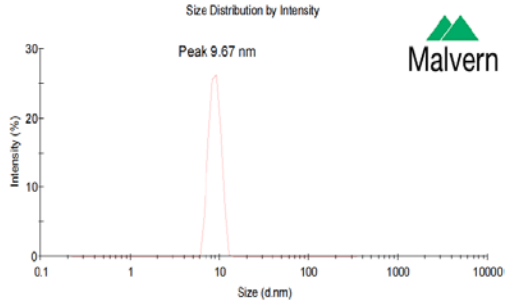


Fig. 2: Average particles size distribution of run 19

Statistical analysis

Our model's F-value is 37.82, which implies that the model is significant. At most there is only a 0.01% chance that this large could occur due to noise. Our signal-to-noise ratio of 17.9 indicates an adequate signal (greater than 4). Therefore, this model can be used to navigate the design space.

Model and results analysis

These experiments show an improvement of solubility, up to 90% with run 1 and run 19, compared to the solubility of celecoxib in water, without additives, examined by run 20. Indeed, exploring the proportion of surfactants and co-solvent shows that solubility is affected by their association.

Increasing the proportion of the three components together is in favor of higher solubility, as the optimum composition which permitted to solubilize the maximum of celecoxib contains the maximum amounts of surfactants and co-solvent in our matrix.

Also, it has already been reported that Solutol® HS 15 forms mixed micelles when mixed with Tween® 80. These mixed micelles are known to present a more excellent solubilizing capacity and stability than single Solutol® HS 15 or Tween® 80 micelles [42]. This indicates that dissolution is probably made through a micellar dispersion of celecoxib. In fact, water-miscible surfactant molecules contain both a hydrophobic and hydrophilic portion and can solubilize many poorly water-soluble drugs, especially in the presence of a cosolvent. Surfactants can also self-assemble to form micelles once the surfactant monomer concentration reaches the critical micelle concentration. In our model, the solubility obtained by maximum proportions of surfactants and cosolvent (run 1 and run 19) is better than that obtained without cosolvent with the same proportions of surfactants (run 7). This indicates that Tween® 80 and Solutol® HS 15 can probably solubilize celecoxib molecules by both a direct ethanol effect and by uptake into micelles [43].

The weakest solubility values of our matrix were observed with run 8 and run 11 that contain no surfactants. Then, with this model, the use of ethanol alone does not improve solubility. This result can be explained by the fact that cosolvent formulation has more tendency of precipitation on dilution comparing with micellar formulation [44].

Considering the coefficients a_1 (ethanol), a_2 (Tween® 80), a_3 (Solutol® HS 15) and a_4 (water) given by our model's solubility equation, we notice that a_1 and a_2 are the two coefficients that affect the most celecoxib solubility. However, as high values of solubility are observed with high proportions of cosolvent relative to surfactant; we can say that in our model ethanol is not a limiting factor, all the more, so ethanol alone does not improve solubility. Furthermore, response surface plots presented in fig. 1 and illustrating the effect of surfactants and cosolvent on solubility show that with this model, response surface is closely related to the concentration of Tween® 80 in the mixture.

The prediction by the model of a better solubility did not give additional points. This shows that the proportions of the various components bringing the maximum of solubility are already included in our experiment matrix.

CONCLUSION

Optimization by mixture design of the solubility of a hydrophobic molecule like celecoxib associating a cosolvent and nonionic surfactants seems to improve significantly the solubility and defines the effects and the proportions of each component from a limited number of experiments.

ACKNOWLEDGEMENT

The authors thank the technical research platform of Rabat Faculty of Medicine and Pharmacy (*Plateau Technique Central de Recherche*) for supporting this study.

CONFLICT OF INTERESTS

Declare none

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