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FORMULATION AND EVALUATION OF EFLORNITHINE GEL FOR HIRSUTISM BY QBD APPROACH

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ABSTRACT

Objective: The goal of this study was to formulate and optimize and evaluate a topical gel for Hirsutism containing Eflornithine hydrochloride and Allantoin using a Quality by Design approach (QbD).

Methods: To obtain a quality target product profile, the effects of critical parameters (Carbopol 934 and Triethanolamine concentrations) were investigated using a design of experimentation (DOE) with a Randomized Central Composite Design. Viscosity and pH were chosen as CQAs. Multiple regression analysis and ANOVA were used to assess and identify the effect of formulation variables and generate their relationship with CQA and optimized formulation was obtained. The optimized formulated gel was evaluated for their physical appearance, pH, viscosity, spreadability, drug content, in vitro drug release and ex vivo drug permeation and skin irritation.

Results: The pH of the optimized formulation was 5.68 ± 0.02 which was optimum and viscosity of 234.43±0.57 and spreadability of 4.33 ± 0.11 cm and drug content of 94.74±0.16.

Drug release across a cellulose membrane for the formulation was carried out and showed 81.23% and the ex-vivo permeation study carried out showed drug release to be 76.88% and showed no signs of irritation.

Conclusion: The topical gel containing Eflornithine hydrochloride along with Allantoin showed better therapeutic efficacy than conventional gel.

Keywords: Eflornithine hydrochloride, Allantoin, Hirsutism, Topical Gel, Quality by Design

INTRODUCTION

Hirsutism is defined as "excessive terminal hair growth in women in places usually associated with androgen dependent areas of the female body (i.e., face, chest, abdomen, lower back, upper arms, and thighs) **[1]**." 5% to 10% of reproductive-aged women are affected by this common endocrine disorder and is commonly associated with acne and oily skin **[2]**. There are several factors that can influence hair follicle growth and development **[3]**.

Hirsutism is caused by an extended anagen stage and abnormal enlargement of the hair follicles. The difference in hair growth on the body is caused by genes. Androgens are the most important hormones in hair growth modulation, as they are involved in keratinization, anagen phase prolongation, and stimulation of the transformation of vellus hairs into terminal hairs in androgen-dependent areas **[4]**.

Eflornithine is the only topical therapy available in market to treat Hirsutism. It was the first substance recognised to assist in slowing the rate of excessive hair growth and given topically as a cream. It is sold as a cream that contains 13.9% Eflornithine hydrochloride under the trade name Vaniqa®.The compound is an ornithine decarboxylase inhibitor that has been shown to reduce facial Hirsutism. The mechanism of action involves the inhibition of ornithine decarboxylase, which is an enzyme involved in keratin synthesis. The inhibition of this enzyme affects the hair follicle growth. One of the most commonly observed side effects of this medication is local irritation and itching of the skin **[5]**.

Allantoin is a compound that possess moisturizing effects on the skin and also possess the ability to soothe irritated skin. It can be found naturally in the roots & leaves of the comfrey plant, but can also be produced synthetically. It is regarded as a great anti- irritant **[6]**.

In the present study the aim was formulating a gel containing Eflornithine with Allantoin using suitable formulation method wherein the efficacy of Eflornithine can be improved by using Allantoin which reduces the itchiness considerably.

MATERIALS AND METHODS

Materials

Eflornithine hydrochloride was obtained as a gift sample from Rusan Pharma Ltd and Allantoin was purchased from Otto Chemie Pvt Ltd. The gelling agent that was used was Carbopol 934 procured from Hi Media Laboratories Pvt ltd. All other chemicals were of the analytical grade.

Method [7-10, 25]

Accurately weighed quantity of Carbopol 934 was dispersed in a small quantity of water to form a homogeneous dispersion. Then in another beaker accurately weighed quantity of Eflornithine hydrochloride and Allantoin was dissolved in water and propylene glycol was then added to the above solution. Methyl paraben and propyl paraben were dissolved in small quantity of water and added to above mixture with continuous stirring.

Quality by Design Approach [11-14]

Quality by Design is a systemic approach to developing a pharmaceutical product that ensures the intended performance of the final drug product. In the formulation in this study Quality by Design Approach was used for the formulation of the topical gel wherein the formulation F1-F4 were prepared based on analysis of a range of statistical parameters offered by DOE software, the best fit model was chosen.

Analysis of variance (ANOVA) was utilised to find significant influences on the coefficients of the response regression. Response surface plots were used to demonstrate how the dependent and independent variables were related (Contour and 3-D surface).

The Concentration of Carbopol 934 and Triethanolamine were selected as Quality Target Product Profile for the study. For the Critical Quality Attributes the selected parameters were viscosity and pH. For the development and optimization of topical gel, a 2^2-randomized factorial design was used. Two factors were studied at two levels in the design, and an experimental trial was carried out. To check the validation of the optimization process, Optimized Formulation of the topical gel having predicted values of the independent factors were prepared.

Evaluation [20-24]

Physical Appearance:

The gel was visually inspected for homogeneity, as well as for the appearance and presence of any aggregates, and for grittiness to check for the presence of any visible particulate matter.

pH

Using a digital pH meter, the pH of gel formulation was assessed. 1gram of gel was dissolved in distilled water in 100ml volumetric flask.

Viscosity

Viscosity of the gel was determined by using Brookfield digital DV-II+Pro viscometer. Equipped with a Spindle no. 51 at 25±0.3°C.

1gm of the gel was taken and the spindle was rotated at a speed of 50 RPM

Spreadability

The spreadability of the gel formulation was determined by measuring the diameter of 1 gm of gel and holding it in the centre of the one petri plate and carefully placing the second petri plate on top. A weight of 50 g was held on the plate and the gel allowed to spread for 2 minutes. The diameter of the gel was then measured with the help of measuring scale and the mean values were calculated.

Drug Content

Drug content was studied by accurately weighing a gel (about 100 mg) and was dissolved in 0.1 NaOH and then the solution was sonicated for 2 hours. After sonication and subsequent filtration, drug in solution was estimated spectrophotometrically by appropriate dilution and measured at 217nm for Eflornithine Hydrochloride.

In-vitro Drug Release

In-vitro drug release study of Eflornithine Hydrochloride through the optimized formulated gel was performed by using Franz diffusion cells with 1.6 cm^2 diffusion area and cellulose dialysis membrane-150 was used as a permeation barrier. The dialysis membrane was saturated in Phosphate buffer pH 5.4 for 24 hours prior to carrying out the study and then the membrane was clamped between the donor and the receptor compartment of Franz diffusion cell. 1 g of gel equivalent to 100 mg of Eflornithine was evenly applied on to the surface of cellulose dialysis membrane-150. Phosphate buffer pH 5.4 was used as dissolution medium and was filled in receptor compartment, stirring of solution was carried out using magnetic bead and the entire assembly was maintained at 37ºC ± 0.5 under constant magnetic stirring. The receptor chamber was covered with aluminium foil to prevent drying out. 0.5 ml of samples were withdrawn at predetermined time intervals over a time period by replacing it with 0.5 ml of fresh PBS pH 5.4 to maintain the sink condition. Dilutions were made and the sample was analysed by UV-VIS spectrophotometer at 217 nm for Eflornithine hydrochloride and % CDR was calculated.

Ex-vivo Drug Permeation

Ex-vivo drug release study of Eflornithine Hydrochloride through the formulated gel was performed by using Franz diffusion cells with 1.6 cm^2 diffusion area. The permeation barrier used was Wistar rat skin from the dorsal region. The hair, sub dermal fat and fascia were removed from the rat skin and the obtained skin was cleansed properly with a mild skin cleanser. This skin was clamped between the donor and the receptor compartment of Franz diffusion cell with the stratum corneum facing upwards. 1 g of gel equivalent to 100 mg of was evenly applied on to the surface of skin.

PBS pH 5.4 was used as dissolution medium and was filled in receptor compartment, stirring of solution was carried out using magnetic bead and the entire assembly was maintained at 37ºC ± 0.5 under constant magnetic stirring. The receptor chamber was covered with aluminium foil to prevent drying out. 0.5 ml of samples were withdrawn at predetermined time intervals over a time period of by replacing it with 0.5 ml of fresh PBS pH 5.4 to maintain the sink condition. Dilutions were made and the sample was analysed by UV-VIS spectrophotometer at 217 nm for Eflornithine hydrochloride and % CDR was calculated. The readings were taken in triplicates and mean values of % CDR were used for plotting graphs.

Drug Release Kinetics

To investigate the release kinetics of drug release from topical gel, the drug release and ex-vivo permeation data were subjected to fit various kinetic models using PCP Diss over 2 software Pune India. The r^2 and n values are determined.

Skin irritancy Studies

This study was carried out on healthy Wistar Albino rats, and were divided into two groups (n=6 in each group). Group I were treated with gel loaded with Eflornithine and Group II were treated with experimental gel (Gel loaded with drug Eflornithine and Allantoin) was applied on the skin and they were housed in cages in the animal house under controlled temperature and light conditions.

The dorsal surface of the rats was cleared and the hair was removed by shaving, 24 hrs. prior to the experiment. The formulations were topically applied to the hairless skin region and the animals were placed back to labelled respective cages and were inspected at time intervals of

1hr, 24hr., 48 h., 72h hr and 7^{th} day. Then, the applied sites were observed for dermal reactions such as erythema and edema.

The mean erythemal scores were recorded according to Draize scale relying upon level of erythema: no erythema=0, slight erythema (barely noticeable light pink) =1, moderate erythema (dark pink) =2, moderate to extreme erythema (light red) =3 and serious erythema (extreme redness) =4.

RESULTS AND DISCUSSION:

Quality by Design Approach

Response 1 – Viscosity

From graphical representation i.e., from the response surface plot and Contour Plot **(Figure 1 and 2)** observed that as the concentration of Carbopol 934 the viscosity increases and as the Concentration of triethanolamine decreases the viscosity decreases.

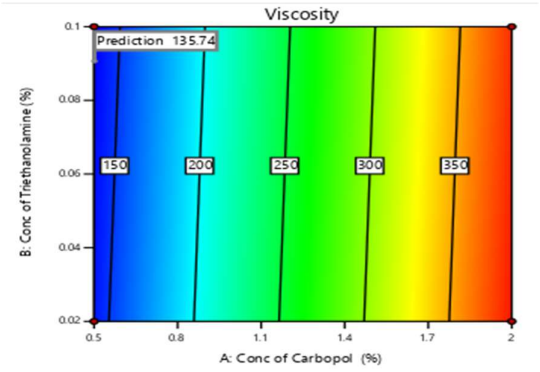

Figure 1: Contour Plot

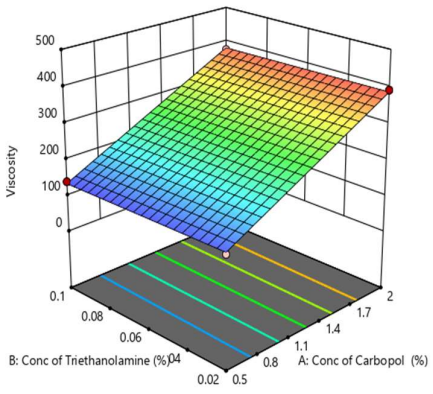

Figure 2: Response Surface plot

Response 2 -pH

From graphical representation i.e., from the response surface plot and Contour Plot **(Figure 3 and 4)** observed that as the level of Concentration of Carbopol 934 upsurges the value pH also increases and by increasing the level of Concentration of Triethanolamine the value of pH increases.

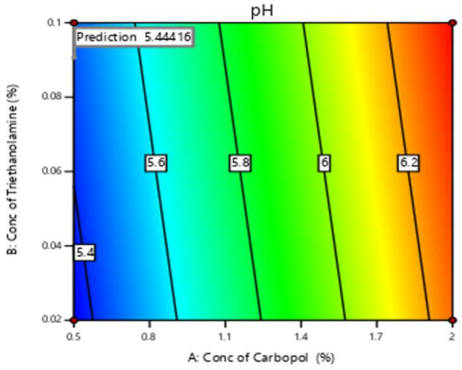

Figure 3: Contour Plot

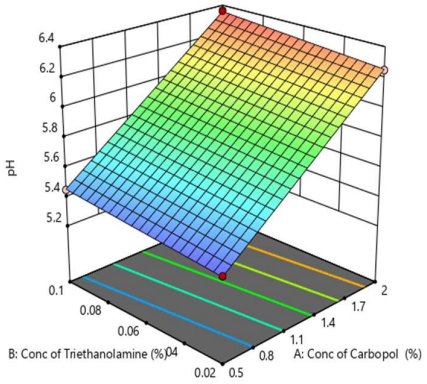

Figure 4: Response Surface plot

Establishing Design Space:

From the Design Space, the optimized formulation falls under the region of successful operating ranges. Therefore, Optimized Formulation (Concentration of Carbopol 934 – 1.18% and Triethanolamine –0.07%) completes the norms of QTPP and CQA for the formulation of topical gel.

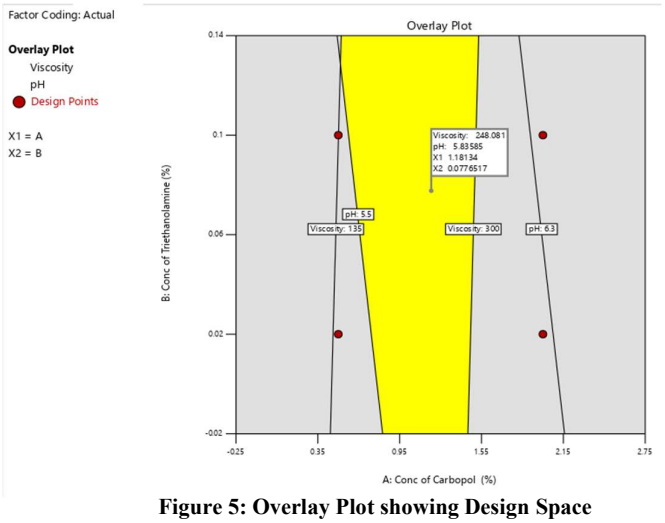

Figure 5: Overlay Plot showing Design Space

Validation of an Optimized Formulation

The predictable and experimental values of the optimized formulation are shown in **Table 1**. Statistical equivalence between predicted and experimental values can be seen there is a good accordance between the two values and emphasizes on the validity of selected variables.

Table 1: Comparison of Predicted and Experimental values for Optimized Formulation (OF)

Response	Predicted	Experimental
Viscosity (Cp)	248.08	234.43
pH	5.83	5.68

Evaluation

Physical appearance*:*

The prepared formulations and the optimized formulation were translucent in colour, having a smooth texture with no grittiness. According to the results, all of the formulations had good homogeneity and no grittiness. The results are given in **Table 3**.

Measurement of pH:

In order to prevent skin irritation, the pH of the formulated gel must be close to the skin pH as topical gels are directly applied on the skin. The pH of the formulations was found to be in the range of 5.78 -7 and that of the optimized formulation was found to be 5.68 which correlates with the skin pH. The result is given in **Table 3**.

Viscosity:

The viscosity of the formulated gels was determined using a Brookfield viscometer at of 25 ± 0.3°C. Viscosity of the gels was found to be in the range of 183 -417 cps and the optimized formulation showed viscosity of 234 cps. The results are in **Table 3**.

Spreadability study:

The spreadability of the formulated gels was found in the range of 3.36 – 4.3 and the optimized formulation showed spreadability of 4.33. The results are given in **Table 3**.

Drug content:

The percent drug content of formulated gel was found to be in the range of 90 -93% and that of the formulation was found to be 94.74%. The results are given in **Table 3**.

In vitro Drug Release

The in-vitro drug release of Eflornithine Hydrochloride from the formulated optimized topical gel was carried out using Franz diffusion cell. The optimized formulation (OF) showed maximum release of 81.23% ±0.26. A graph of %CDR v/s time is shown in **Figure 6**.

Ex- Vivo Drug Permeation

The ex vivo drug permeation of Eflornithine Hydrochloride from the formulated optimized topical gel was carried out using Franz diffusion cell. The optimized formulation (OF) showed maximum release of 76.88% ± 0.35. A graph of %CDR v/s time is shown in **Figure 7**.

Drug release Studies

To investigate the release kinetics of drug release of topical gel, the drug release and ex vivo permeation data are subjected to fit various kinetic models using PCP Diss ver 2 software Pune India. The r2 and n values are determined. The gel showed n value indicating that the drug release followed by and the best fit model observed to be as shown in **Table 4**.

Skin Irritancy Studies

The results of skin irritation studies were assessed on the basis of scoring system as per the by Draize scale- scoring system. In this study group I was treated with gel loaded only with Eflornithine and groupie were treated with optimized gel. Group I on treatment with the gel showed slight erythema at the end of 24 hours and moderate edema after 48 hours. Group II showed no signs of formation of erythema and edema formation during the duration of the study. Since there was no sign of edema, erythema and redness on the skin of Wistar rats with optimized gel, which was assigned with score of '0' therefore passed the skin irritation test. Scores obtained are displayed in **Table 5** and images which showcase the irritation are shown in **Figure 8 and 9**.

Table 2: Evaluation Data of Eflornithine Gel

Formulation Code	Physical appearance	Homogenity	Grittiness	Spreadability	pH	Viscosity	Drug Content
F1	Translucent	+++	-	3.56 ± 0.30	6.96 ±0.24	417 ± 0.37	93.29 ±0.20
F2	Translucent	++	-	3.65 ± 0.23	7± 0.16	208±0.47	92.39 ±0.15
F3	Translucent	+++	-	3.36 ± 0.13	5.86±0.34	183 ± 0.25	91.25 ±0.23
F4	Translucent	++	-	3.53 ±0.15	5.79 ± 0.29	389±0.32	90.96 ± 0.22
OF	Translucent	+++	-	4.33 ±0.11	5.68±0.02	234.43±0.5	94.74 ± 0.16

Key: +++Excellent; ++Very Good; ++Good

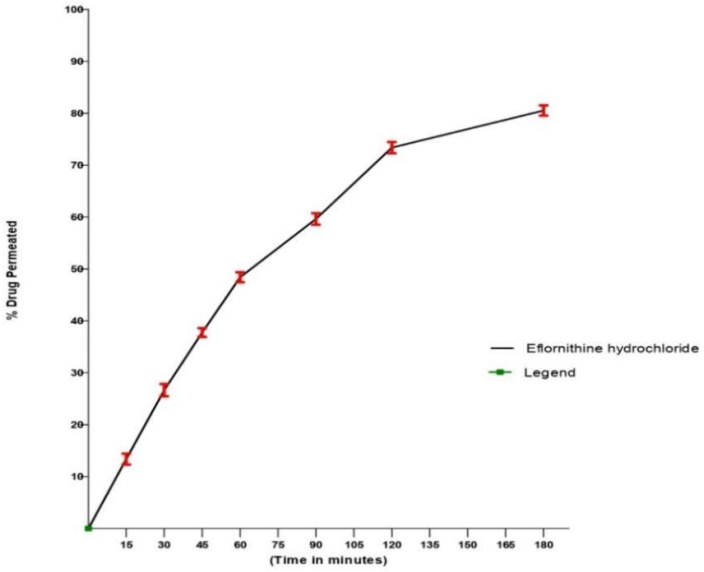

Figure 6: Graph of *In vitro* Drug Release of Eflornithine Hydrochloride

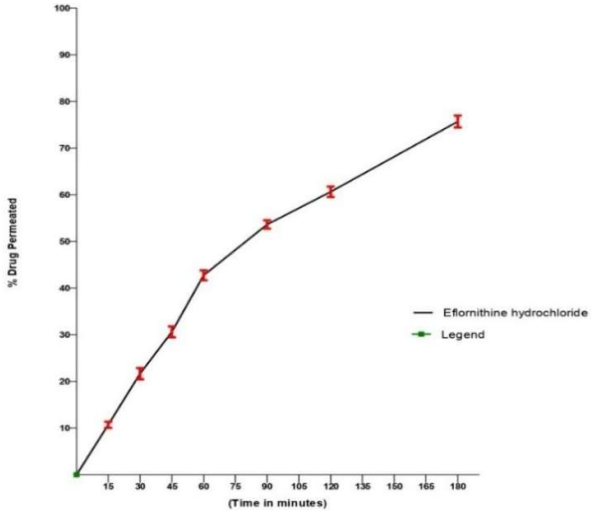

Figure 6: Graph of *Ex ivo* Drug Permeation of Eflornithine Hydrochloride

Table 3: Drug Release Study of Optimized Formulation (OF)

Formulation Code	n value	r^2	Mechanism	Best fit model
OF	0.755	0.946	Non fickian diffusion	Higuchi

Table 4: Erythema and Edema Formation

Skin Responses	Time (hrs)	SCORE Group 1	Group 2
Erythema Formation	1	0	0
	24	1	0
	48	0	0
	7^{th} day	0	0
Edema Formation	1	0	0
	24	0	0
	48	2	0
	7^{th} day	0	0
Primary Irritation Index (PII)		pII = ¾ = 0.75	pII = O/4 =00

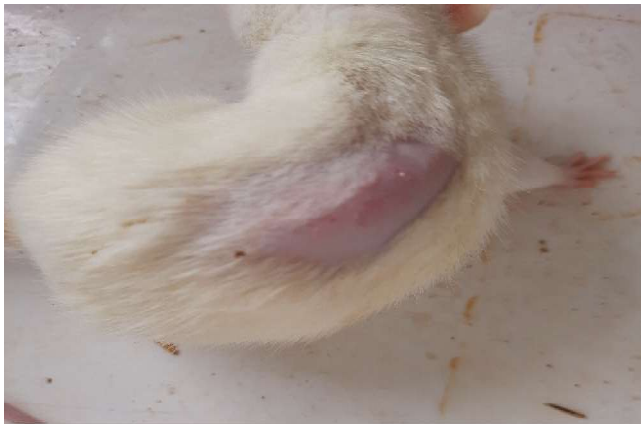

Figure 8: Group I – Gel loaded with Eflornithine

Figure 9: Group II – Gel loaded with Eflornithine and Allantoin

CONCLUSION

The current research showed the effective execution of QbD for formulation of a topical gel that was evaluated for physical appearance, pH, viscosity, spreadability, and drug content. To achieve the desired final product quality, the desired QTPP and CQAs were preset. The concentrations of Carbopol 934 and triethanolamine were chosen as the two major critical parameters for meeting QTPP goals. Based on the selection criteria, a randomly generated 2^2 Central Composite Design was utilized to conduct the design of experiment. To interpret the effect of formulation and achieve the desired QTPP in the design space, statistical data from ANOVA, polynomial equations, and response surface plots were used. It is possible to deduce that prepared formulation in the design space is capable of satisfying CQA, resulting in a product with the desired QTPP. Combining Eflornithine Hydrochloride and Allantoin to create a topical gel formulation for the treatment of Hirsutism was successful by using polymers Carbopol 934 as the gelling agent, the direct dispersion method was effectively used to formulate a topical gel. The prepared formulation was then evaluated for its physical appearance, pH determination, spreadability, viscosity, drug

content, in-vitro drug release, and skin irritancy.

Thus, we may reach the conclusion that the topical gel formulation for the treatment of hirsutism comprising eflornithine and allantoin can be successfully formed by direct dispersion method, producing the results for all the evaluation parameters within the acceptable range.

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