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OPTIMIZATION OF *WITHANIA SOMNIFERA* AND HYDROCORTISONE EMULGEL FOR MELASMA TREATMENT USING BOX-BEHNKEN DESIGN: FORMULATION AND EVALUATION

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ABSTRACT: The expanding consumer interest in natural products has recently increased demand for herbal mixtures. Nowadays, herbal cosmeceuticals are more common because they don't cause harmful side effects. A common hyperpigmentation condition called melasma is characterized by sporadic brown patches on skin exposed to the sun. While hydrocortisone is a corticosteroid with anti-inflammatory characteristics, *Withania somnifera*, often known as ashwagandha, is a well-known herb having antioxidant and anti-inflammatory activities. This study intended to formulate and evaluate an emulgel that would treat melasma by combining hydrocortisone with *Withania somnifera* extract.

KEYWORDS: Box Behnken Design; Emulgel; Hydrocortisone; *Withania somnifera*.

1. INTRODUCTION

Melasma is a facial pigmentary disorder that often affects the cheeks, forehead, and upper lips. One million individuals worldwide are affected by this acquired hyperpigmentation disorder (1). Photoprotection is required since hormonal variables and sun exposure worsen the condition.It is appearing as light brown to dark, muddy brown patches and macules on the face, particularly the chin, forehead, and malar regions Melasma causes facial disfigurement, it is often linked to a significant emotional stress (2). Due of its resistance and recurrence, melasma is challenging to treat. Therapies to treat this disorder may include topical corticosteroids, retinoids, kojic acid. Even with a wide range of choices, treating melasma can be highly challenging and stressful for the patient as well as the dermatologist (3).

In recent years, the usage of herbal medicines has grown in popularity all over the world. Ashwagandha is an herb commonly used in Ayurvedic medicine for various health benefits, may help cure hyperpigmentation by reducing the overproduction of melanin, according to research showed an increase in skin hydration and a decrease in transepidermal water loss (4).

For many years, Tyrosinase inhibitors have been the main treatment for melasma because they prevent melanocytes from producing melanin and there are various topical agents are marketed as pharmaceuticals (5), most widely used combinations are Hydroquinone, retinoic acid and corticosteroids such as hydrocortisone have been used in combination with other medications to treat melasma (6).

Melasma management can be challenging and requires topical medication over an extended period. Topical medications can sometimes result in serious adverse reactions, and the outcomes are often unsatisfactory for this reason we can use a combination of hydrocortisone with plant extracts like *Withania somnifera* (ashwagandha) (7).

Topical delivery systems provide localized effects at the application site as a result of medication penetration into the underlying skin layers. For dermatological use, Novel emulgels exhibit a variety of features including less greasy, ease of spreadability and removal, long shelf life, and bio-compatible.

2. MATERİALS AND METHODS

2.1. Materials

Ashwagandha Extract, Hydrocortisone, Tween 80, Span 20, Methyl Paraben, Propylene glycol, Paraffin oil, Propyl Paraben, Ethanol, Carbopol 934, Triethanolamine, Distilled water. Few ingredients were obtained from laboratory, few were purchased and Ashwagandha extract was done in the laboratory.

2.2. Preparation of *Withania somnifera* & Hydrocortisone loaded Emulgel

Table 1. Optimized formulation (F5)

S.No.	Ingredient	Quantity	Uses
1	Ashwagandha Extract	1.5	Antioxidant, Anti-inflammatory
2	Hydrocortisone	7.5	Anti-inflammatory
3	Tween 80	1.1	Surfactant
4	Span 20	1.4	Surfactant
5	Paraffin Oil	10	Emulsifying agent
6	Ethanol	5	Solvent
7	Propylene Glycol	2.5	Permeation enhancer
8	Methyl Paraben	0.18	Preservatives
9	Carbopol 934	1.5	Gelling agent
10	Triethanolamine	q.s	Neutralizer
11	Propyl Paraben	0.02	Preservatives
12	Distilled Water	100	Vehicle

2.3. Method of Preparation

2.3.1. Extraction of Withania Somnifera

The air-dried and finely powdered leaves of 50g were thoroughly extracted in ethanol using the Maceration process for approximately 72 hours. The extract was then filtered and evaporated to dryness using a water bath (8).

2.3.2. Preparation of Emulsion

A particular weighed amount of Span 20 was dissolved in Paraffin oil to create an oil phase, and the desired quantity of Tween 80 was dissolved in distilled water to form the aqueous phase. Herbal extract (Ashwagandha) dissolved in ethanol: water combination and both 0.18 gram and 0.02 gram of propyl and methylparaben were dissolved in 2.5 ml of propylene glycol before adding it to the aqueous phase. The aqueous and oily phases were heated to 70–80˚C individually. A small amount of ethanol was mixed with hydrocortisone while the mixture was heated to a temperature b/w 40 and 45˚C. Afterward, the aqueous and oil phases were mixed & stirred at 100 rpm until the mixture formed an emulsion after cooling to room temp (10).

2.4. Preparation of Emulgel

Using a magnetic stirrer set to 1500 rpm, Carbopol was dissolved in sufficient deionized water. The pH was then brought to 6.5 to 6 (9).

Every experimental batch was made by mixing the formed emulsions in a 1:1 ratio with the gel until a uniform emulgel was achieved (11).

3. Design model

Table 2. Box Behnken Design Model

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Run	Ashwagandha	Hydrocortisone	Carbopol	pH	Viscosity
1	1.5	5	1	6.9	7519
2	1.5	10	1	7.1	7969
3	1	10	0.5	7	6513
4	1	5	1.5	6.8	8212
5	1.5	7.5.	1.5	6.5	8520
6	0.5	10	1	6.4	7698
7	1.5	7.5	0.5	6.7	6432
8	1	10	1.5	6.9	8012
9	0.5	5	1	6.4	7648
10	0.5	7.5	0.5	6.5	5413
11	0.5	7.5	1.5	6.6	8103
12	1	5	0.5	6.5	6498

Effect of independent variables on pH

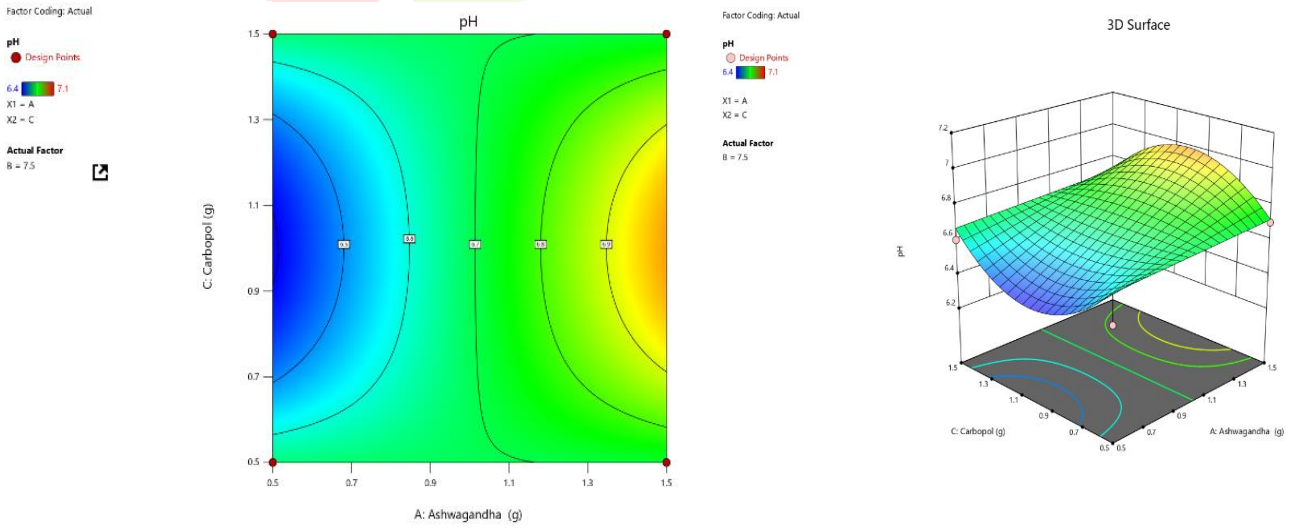

Figure 1. Contour and 3D plot for pH

The coded equations for the dependent variables demonstrated varying levels of significance and predictability.

For pH, the coded equation (+6.58+0.0250A+0.1750B^2+0.2750AB^2+0.1500BC^2) had an R^2 value of 0.8228, P-value = 0.0091< 0.0500 indicates model terms are significant, and an F-value of 8.13 implies the model is significant and indicating a moderately strong model.

Effect of independent variables on Viscosity

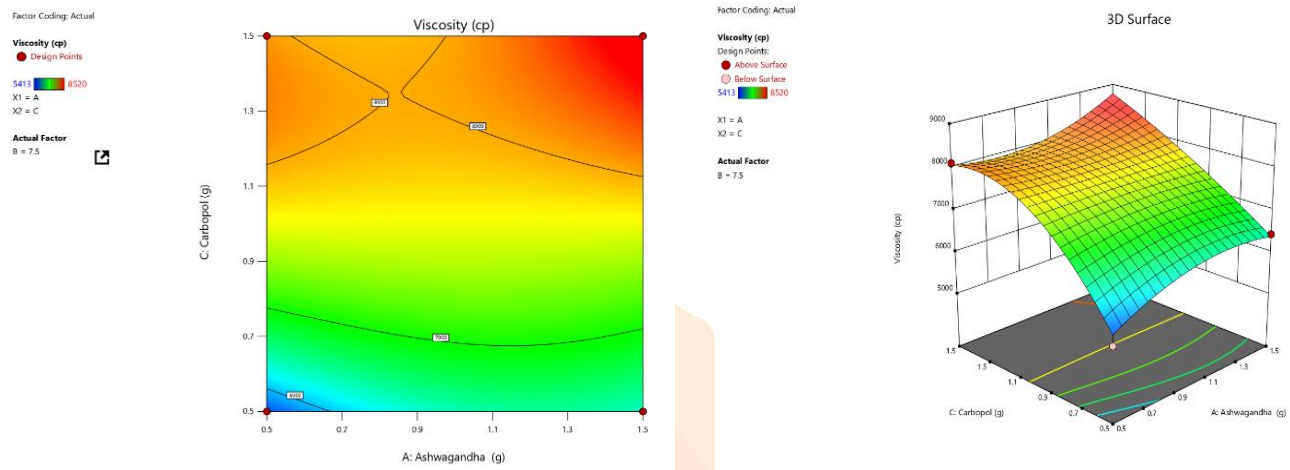

Figure 2. Contour and 3D plot for Viscosity

The coded equations for the dependent variables demonstrated varying levels of significance and predictability.

For Viscosity, the coded equation (+7708.50+803.25C-495.63C^2+391.25A^2C+359.00AC^2) had an R^2 value of 0.9701, P-value = 0.0001< 0.0500 indicates model terms are significant, and an F-value of 8.13 implies the model is significant and indicating a moderately strong model.

4. EVALUATİON TESTS FOR EMULGEL

4.1. Physical parameters

Visual assessment was done on the prepared emulgel formulations to check for color, phase separation, grittiness , homogeneity and consistency.

4.2. pH

Using a pH meter, the pH values of the prepared 1% aqueous solutions were determined. Three measurements of the pH of the emulgel formulations were made in triplicate, and the average value was determined.

4.3. Spreadability

The spreadability of the emulgel formulations was determined 24 hours after permeation, by gauging, after a minute, the spreading diameter of one gram of emulgel between two horizontal plates (20 cm x 20 cm).

Organoleptic properties

4.4. Color

The color is an important pharmacological characteristic of any product because it is an indication of the quality and the validity of the product such as gel, cream, and ointments.

4.5. Odor

The smell is an important pharmacological characteristic of any product because it is an indication of the quality and the validity of the product such as gel, cream, and ointments.

4.6. Viscosity

The viscosity of the emulgel formulations was measured at 10 rpm with a Brookfield viscometer using spindle no 4.

4.7. Drug content

With a UV spectrophotometer, the drug content in the emulgel was measured. *Withania somnifera* content in emulgels were studied by Using sonication for 20 minutes to dissolve a specified amount (5 ml) of gellified emulsion in 50 ml of ethanol. Then, using the same solvent in a volumetric flask, the volume was increased to 50 ml by filtering the remaining mix. For every dilution, the concentration was determined using the molarity formula. The amount of stock solution was taken with the help of a micropipette and mixed with dilution for UV-visible analysis. Dilutions were made 1-14 µg/ml. Using a UV/visible spectrophotometer, absorbance was measured at 237 nm.

4.8. *In-Vitro* drug release studies

The drug release analysis of emulgel was done using Franz diffusion. 1 ml of emulgel was applied onto the cellophane membrane. This membrane clamped carefully between the compartments of the donor and receptor. To solubilize the drug, The receptor compartment was filled with phosphate buffer (pH 7.4) and stirred using a magnetic stirrer. Each sample was taken out in 1 ml intervals at 0, 15, 30, 60, 90, 120, 180, 240, 360, and 480 minutes and the drug content of the samples was determined at 237 nm using a UV-visible spectrophotometer following the appropriate dilutions.

5. RESULTS & DİSCUSSİON

5.1. pH

Based on Optimization and evaluation **F5** formulation shows a **pH 6.5** and is considered as the optimized result than other formulations.

Table 7. Results of pH for F_1 to F_{12}

Formulation	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**	**F_8**	**F_9**	**F_{10}**	**F_{11}**	**F_{12}**
pH	6.9	7.1	7	6.8	6.5	6.4	6.7	6.9	6.4	6.5	6.6	6.5

5.2. Viscosity

The viscosity of the emulgel formulations were better in viscosity because of Carbopol and viscosity variables due to differences in surfactant concentration.

Table 8. Results of Viscosity for F_1 to F_{12}

Formulation	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**	**F_8**	**F_9**	**F_{10}**	**F_{11}**	**F_{12}**
Viscosity	7519	7969	6513	8212	8520	7698	6432	8012	7648	5413	8103	6498

The viscosity of the optimized formulation **F**5 was found to be **8520 centipoises**

5.3. Physical parameters

The physical parameters are observed visually.

Table 9. Results of Withania somnifera & Hydrocortisone loaded Emulgel (F5)

SI.No.	Parameters	Observation
1.	Color	Mustard Yellow
2.	Odor	Pleasant
3.	pH	6.5
4.	Viscosity (cps)	8520
5.	Phase separation	No
6.	Greasiness	Less greasy
7.	Spreadability	22.96 gm.cm/sec
8.	Washability	Easily Washable
9.	Patch test	There were no observed allergic responses
10.	Irritation	No irritation
11.	Grittiness	No gritty particles
12.	Consistency	Semi-solid
13.	Extrudability	Easily extruded

5.4. *In-vitro* drug release

Table 10. Results of *In-vitro* Drug Release (F5)

Time	Drug release (%) (F5)
0	0
15	4.58
30	6.02
60	12.01
90	17.14
120	28.9
180	45.25
240	60.21
360	75.21
480	89.02

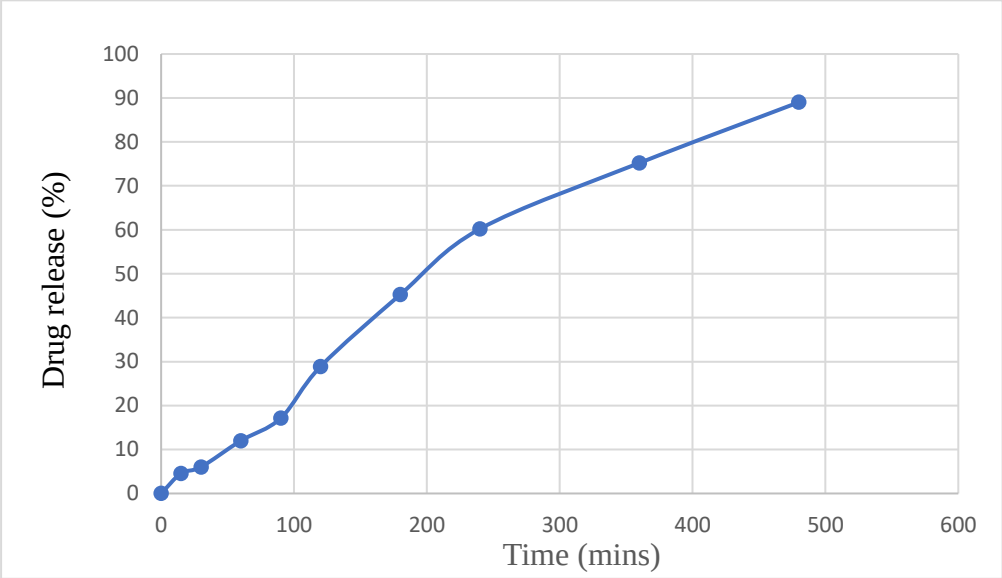

Figure 3. Results of *In-vitro* Drug Release (F5)

5.5. Stability test

Table 11. Result of Stability test (F5)

Parameters	**Temperature conditions**	
	Room temperature	**5^0 C**
Odor	Pleasant	Pleasant
Color	Yellow	Faded mustard yellow
Spreadability	No changes were observed and uniform	No changes were observed and uniform
Viscosity	8500 cps	8490
pH	6.4	6.3

6. CONCLUSİON

The emulgel produced good results and was stable at room temperature. Based on the results of the emulgel, it is determined to be suitable for use on the skin to treat Melasma and does not cause any harmful side effects. Out of many Trials performed, the F5 batch produced good results in terms of viscosity and pH. Furthermore, it improves the spreadability and smoothing action of the skin. So, it can be concluded that formulated *Withania somnifera* & Hydrocortisone-loaded Emulgel are useful for the treatment of Melasma-hyperpigmentation conditions on skin exposed to sunlight.

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