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Research Article

Formulation of grape seed oil and hibiscus extract for treatment of acne: optimization using D-optimal design and in vitro characterization

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Abstract

Background: Teenagers are the main demographic affected by acne vulgaris, also referred to as acne. In the pursuit of effective therapeutic ingredients for managing acne, grape seed oil, hibiscus extract, Borax, Beeswax, soft paraffin, and vitamin E are used. Given the popularity of cream formulations among teenagers, we have chosen this formulation to incorporate active ingredients because they have potential benefits, including antioxidants, antibacterial, and anti-inflammatory effects. Our cream falls into the category of water-in-oil (W/O) emulsions, which provide distinct advantages such as enhanced moisturization and the establishment of a long-lasting protective barrier on the skin.

Methods: To establish correlations between the independent variables (surfactant type and surfactant concentration) and dependent variables (pH, spreadability, and viscosity), we employed the D-optimal design using Design-Expert® software. In-vitro characterization tests were conducted on the topical cream to ensure homogeneity and absence of phase separation. The cream's appearance, like color, pearlescence, roughness, and washability, was evaluated.

Results: We assessed the pH, viscosity, and spreadability across 2^2 factorial designs, and four formulations (F1, F2, F3, and F4) were obtained. Based on the results, we selected the optimal formulation, which was F1. It exhibited a pH of 5.5, a spreadability value of 66 g·cm/s, and a viscosity of 520 cps.

Conclusions: These parameters were deemed favorable for achieving the desired properties, such as moisturizing ability, acceptable consistency, good spreadability, and a balanced pH, with the absence of irritants, and ensuring an efficacious cream formulation.

1. Introduction

Acne, also known as acne vulgaris, is a common skin condition that mainly affects teenagers. Comedones, papules, and pustules characterize it, and in severe cases, nodules and cysts. The development of acne involves four key factors: firstly, an androgen-induced increase in sebum production; secondly, abnormal keratinization leading to clogged follicles; thirdly, the growth of Cutibacterium acnes bacteria; and finally, inflammation of the skin around the affected area (1).

Acne can have a significant impact on patients' psychosocial health. It can severely affect a person's psychological and social well-being. Moreover, acne not only causes physical blemishes but can also result in emotional struggles such as low self-confidence, isolation, anxiety, and depression. Additionally, teenagers with acne may be seen by their friends as quiet, less outgoing, and at a higher risk of being bullied, which can hinder their professional opportunities (2). Furthermore, the trivialization of acne by others, including healthcare professionals, can exacerbate the sense of isolation and misunderstanding experienced by those affected. This lack of recognition and subsequent trivialization can lead to a diminished quality of life and may affect long-term treatment adherence and overall management of the condition (3). Among the ingredients investigated for their therapeutic properties in acne management, grape seed oil and hibiscus extract have gained attention due to their benefits.

Grape seed oil is renowned for its potent antioxidant properties. Specifically, it helps protect the skin from oxidative stress caused by environmental factors such as UV radiation and pollution. Additionally, grape seed oil exhibits antimicrobial properties, making it effective in combating bacteria that can contribute to various skin concerns, including acne. Grape seed oil has a high content of gallic acid, catechin, epicatechin, procyanidins, and proanthocyanidins. These compounds contribute to the oil's ability to combat oxidative stress and protect the skin (4). Notably, hibiscus extract demonstrates significant antibacterial effects against Propionibacterium acnes and Staphylococcus epidermidis, which are associated with inflammatory acne. Furthermore, it exhibits protective effects against oxidative stress caused by UVB rays, thereby reducing damage to collagen and elastin fibers in the skin (5).

Considering the convenience and popularity of cream formulations among teenagers, we have chosen water in oil (W/O) emulsion form to incorporate the active ingredient, which offers distinct advantages in terms of moisturization and the formation of a long-lasting protective barrier on the skin (6).

Moreover, the cream formulation includes other ingredients with specific functions. For instance, borax is used in cosmetic products as an emulsifier, buffering agent, or preservative. Similarly, beeswax acts as an occlusive, helping to create a semi-occlusive skin barrier and lock in hydration. Additionally, soft paraffin contains occlusive agents, humectants, and lubricants, making it an effective emollient (7) . Lastly, vitamin E for its antioxidant properties and skin-firming effects (8).

To obtain a statistically optimized formulation, we used Design Expert software to ensure that our cream is of the utmost quality and efficacy for its intended purposes.

In this study, we aimed to utilize Hibiscus sabdariffa extract and grape seed oil for their antioxidant, antibacterial, and anti-inflammatory properties to treat acne.

2. Materials and methods

2.1 Materials:

Grape seed oil and vitamin E were supplied by Raw African for Cosme pharmaceutical (Nasr City, Cairo, Egypt). Beeswax, soft paraffin, Borax, Tween, and Sodium salicylate were supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hibiscus Sabdariffa was supplied by El Shams Apothecary (Mohandessin, Giza, Egypt).

2.2 Methods

2.2.1 Experimental Design

The D-optimal design was used to find the correlation between independent and dependent variables by using Design-Expert® software, version 13. An initial screening of the independent factors, such as surfactant type (A) and surfactant concentration (B), was conducted to select the

Table 1. D-optimal design with the independent variables and the examined responses.

Factors	Low limit (-1)	High limit (1)
A: surfactant type	Borax	Tween 80
B: surfactant concentration (%)	1	2
Responses	Goals	
Y_1: PH	4-6	
Y_2: Spreadability	Maximize	
Y_3: Viscosity	Maximize	

most effective of them based on the chosen dependent factors. The formulations were optimized by 2^2 factorial designs. The factors were calculated by low and high, at 2 levels indicating (-1, +1) respectively, the dependent responses were PH (Y_1), spreadability (Y_2), and viscosity (Y_3) (9). Table 1 illustrates the independent factors with targeted responses. Furthermore, Table 2 lists the design's suggested four formulas and the resulting responses.

2.2.2 Preparation of Hibiscus sabdariffa L. Extract

For the preparation of water extraction, 25 g of roselle was added to 250 mL of distilled water, and the mixture was boiled for 10 min while stirring. Then, the extract was filtered through filter paper (10).

2.2.3 Formulation of Hibiscus sabdariffa L. cold cream

In the formulation of the cold cream water-in-oil (W/O) emulsion, the aqueous phase is formed by blending 4.8 ml of the Hibiscus extract with 0.22 g of either borax or tween and 0.25 g of sodium salicylate in a 50 ml beaker (11). This mixture is then heated to 75 °C using a water bath (Lab-Line Instruments, Melrose Park, IL, USA). Concurrently, the oil phase is prepared by melting 2.5 g of beeswax, 2.5 g of soft paraffin, 10 ml of grape seed oil, and vitamin E in a separate porcelain dish. This combination is also heated to 75 °C in a water bath. Finally, to create the homogenous cream, the aqueous phase is gradually added to the oil phase with constant stirring. This process continues until a uniform cream is achieved, ensuring the proper integration of both phases (12).

Table 2. Experimental formulas suggested by a D-optimal design

Ingredients	F1	F2	F3	F4
	Borax		Tween 80	
Surfactant (g)	0.2	0.4	0.2	0.4
Beeswax (g)	2.5	2.5	2.5	2.5
Soft paraffin (g)	2.5	2.5	2.5	2.5
Grape seed oil and Vitamin E (g)	10	10	10	10
Hibiscus extract (g)	4.8	4.8	4.8	4.8
Sodium salicylate (gm)	0.25	0.25	0.25	0.25

Figure 1. The prepared cold cream.

2.2.4 In-vitro characterization

2.2.4.1 Macroscopical examination

Topical cream was tested for homogeneity, phase separation, and evaluated for its appearance in terms of color, pearlescent, and roughness, and graded accordingly. The washability of the cream was assessed and observed in running water.

2.2.4.2 pH

Weigh 0.5g of grape seed oil and Hibiscus sabdariffa extract cream, and dissolve it in 50 mL of distilled water, and measure with the help of a digital pH meter (13).

2.2.4.3 Viscosity

The viscosity of the grape seed oil and Hibiscus sabdariffa extract W/O emulsion Cream was determined with the help of a Brookfield viscometer at 100 rpm with a spindle number 40 (14). Viscosity not only affects features such as spreadability, and skin feel but may also affect the skin penetration of incorporated actives and lead to higher physicochemical stability of the formulation

Viscosity is calculated using this equation, Eq. 1

$$\mathbf{cP = TK \times SMC \times \frac{10000}{RPM}}$$

The resistance to flow is measured by viscosity (Cp), while the rotational force is represented by torque (TK). Additionally, the spindle speed multiplier factor is indicated by the Spindle multiplier constant (SMC).

2.2.4.4 Spreadability test

Spreadability of cream formulations, that is, the ability of cream to evenly spread on the skin, plays an important role in the administration of a standard dose of a medicated formulation to the skin and the efficacy of topical therapy. The grape seed oil and Hibiscus sabdariffa extract W/O emulsion Cream was applied between the two glass slides and compressed to a uniform thickness by placing a 100g weight for 5 minutes, then the weight was added to the weighing pan. The time during which the upper glass slide moved over the lower slide was taken as a measure of spreadability (15).

Spreadability is calculated using this equation, Eq. 2

$$\mathbf{Spreadability} = \frac{M \times L}{T}$$

These include the weight of the upper slide (M), the length moved on the glass slide (L), and the time taken to separate the slide (T).

2.2.4.5. Accelerated stability studies

A centrifugation test (mechanical stress) is used for pre-screening regarding physical stability. Prognoses. Samples (6 ml) of each formulation were submitted to three cycles in a centrifuge (Sigma 3 K 30, Rodemark, Germany) at 3000 rpm for a duration of 30 minutes. At the end of each cycle, the samples were checked for the presence of any changes (16).

Table 3. Measurements of the three responses in the prepared formulations.

Formulations	PH	Viscosity	Spreadability
F1	5.5	520	66
F2	6.07	282	50
F3	5.3	412	56
F4	5.3	435	60

3. Results and discussion

3.1 Macroscopical examination

Different batches of cream were prepared and subjected to macroscopical examination to assess the cream homogeneity, appearance, and uniformity. The prepared formula had an acceptable and pleasant aroma. It was found that the cream was homogeneous, smooth, consistent, and uniform in appearance, with a complete absence of any grittiness particles in the texture analysis (17). The prepared cold cream is shown in Figure 1.

3.2 Evaluation of Herbal cold cream

The prepared herbal cold cream was evaluated for pH, spreadability, and viscosity. The results were observed as follows:

3.2.1 Variables influence on the pH

The cream's pH was found to be in the range of 5.3 to 6.07 (Table 3). This range aligns well with the naturally acidic pH of human skin, which typically falls between 4.0 and 6.0. Maintaining this range is crucial because preparations with a pH more acidic than skin can irritate during application, while those more alkaline can cause dryness (13,18). The impact of surfactant selection and its concentration on the final cream's pH was investigated as shown in Figure 2. It was found that the cream's pH increased in direct proportion to the concentration of borax, also referred to as sodium tetraborate decahydrate. When borax is added to water, it dissociates, producing sodium ions and borate ions. The hydroxide ion concentration rises as a result of this dissociation process, which raises the pH (19). Higher borax concentrations in the cream formulation will therefore result in a rise in the pH. However, the cream formulation's pH remained unchanged when the concentration of the non-ionic surfactant, Tween 80, increased due to its neutral properties (Figure 3) (20,21). This difference highlights the influence of surfactant choice on overall product acidity. Significantly, formulations fell within the acceptable pH range.

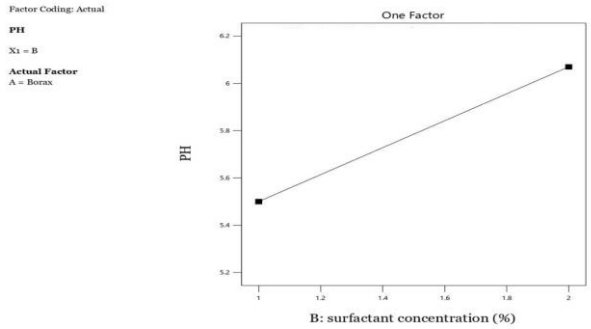

Figure 2. The response surface curve shows the interaction between PH and Borax.

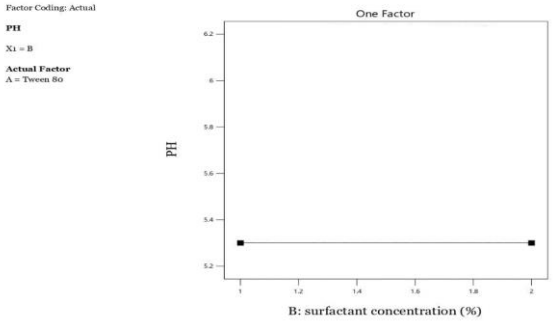

Figure 3. The response surface curve shows the interaction between PH and Tween 80 concentrations.

3.2.2. Variables influence on Spreadability

Spreadability is the ability of a cream to spread on the skin. It plays an essential role in the administration of a standard dose of medicated formulation to the skin and the efficacy of topical therapy. The type of surfactant and its interaction with the cream base also significantly influence spreadability. The spreadability of the prepared formulas was found to be in the range of 50 g·cm/s to 66 g·cm/s (Table 3). Changing the surfactant type and concentration significantly affects the spreadability. In formulas F1 and F2, which both contain Borax as the surfactant, a higher Borax concentration in F2 led to a

lower viscosity (282 cP) compared to F1 (520 cP). However, F2 exhibited reduced spreadability (50 g·cm/s) compared to F1 (66 g·cm/s) (Figure 4). This apparent inconsistency suggests that spreadability in Borax-based systems may not be solely governed by viscosity, but also by the interaction of Borax with the cream matrix, possibly increasing internal structuring and reducing the ease of spreading despite a lower viscosity reading (22). In contrast, formulas F3 and F4, which contain Tween 80, showed a more expected trend: F4 had a slightly higher viscosity (435 cP) than F3 (412 cP) but also demonstrated higher spreadability (60 g·cm/s vs 56 g·cm/s) (Figure 5). This supports the notion that Tween 80, as a nonionic surfactant, enhances the emulsion's internal consistency and spreadability through improved droplet dispersion and lower surface tension, outweighing the modest viscosity increase (23). Therefore, F1 is the optimum formula, containing the lowest concentration of Borax, showing the highest level of spreadability.

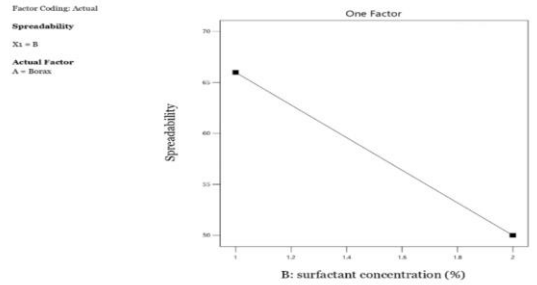

Figure 4. The response surface curve shows the interaction between spreadability and borax concentration.

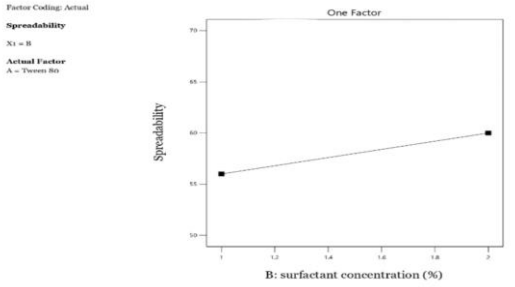

Figure 5. The response surface curve shows the interaction between spreadability and Tween 80 concentrations.

3.2.3. Variables influence on Viscosity

The viscosity of the cream formulations was significantly influenced by the type and concentration of the surfactant used. In formulations containing Borax (F1 and F2), an inverse relationship was observed between Borax concentration and viscosity (Figure 6). Specifically, F1, which had a lower concentration of Borax, exhibited higher viscosity (520 cP) compared to F2 (282 cP). This can be attributed to the unique behavior of borate ions, which form transient hydrogen bonds and dynamic crosslinked networks with the cream's aqueous phase (24). As the Borax concentration increases, these reversible gel-like networks become more fluid due to weakened or restructured hydrogen bonding under stress, resulting in decreased viscosity despite increased surfactant presence (24). In contrast, formulations containing Tween 80 (F3 and F4) exhibited a direct relationship between surfactant concentration and viscosity (Figure 7). As the Tween 80 concentration increased from F3 to F4, viscosity increased from 412 cP to 435 cP. This is consistent with the known emulsifying properties of Tween 80, a nonionic surfactant, which enhances interfacial film formation and molecular packing at the oil–water interface. These interactions increase internal friction within the system, thereby elevating the viscosity (25). Tween 80's ability to stabilize emulsions by reducing droplet size and increasing dispersion uniformity also contributes to this behavior (25).

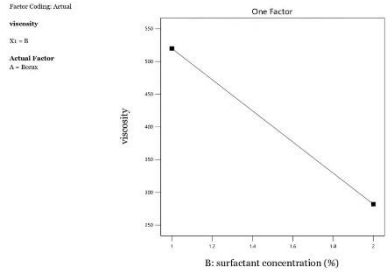

Figure 6. The response surface curve shows the interaction between viscosity and borax concentration.

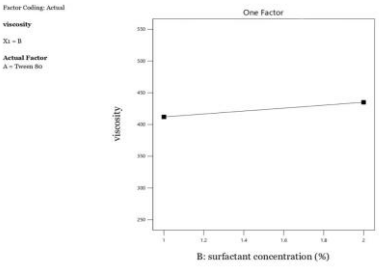

Figure 7. The response surface curve shows the interaction between viscosity and Tween 80 concentrations.

3.3 Optimization of formulation components

The process was optimized for all three responses. Optimum formulation was selected based on the constraints set on independent variables. The final optimal experimental parameters were calculated using the extensive grid search and feasibility search provided in the Design Expert software. After the generation of the polynomial equations to correlate the dependent and independent variables, the process was optimized for responses (26)The optimum formulation was selected based on the evaluation test results. Thus, the optimized formulation was F1, which had a pH of 5.5, spreadability of 66, and viscosity of 520 cps.

3.4 Accelerated stability studies

No phase separation was observed in formulation (F1) after the first cycle, and no other changes were observed after the three centrifugation cycles. As expected, preparations that withstand a high mechanical load generally showed more stability than those that do not show this property (16).

4. Conclusion

This study successfully formulated and optimized a novel topical cold cream using a D-optimal design to manage acne vulgaris, primarily affecting adolescents. The cream incorporated grape seed oil and Hibiscus sabdariffa extract, both known for their antioxidant, antibacterial, and anti-inflammatory properties. These bioactives were delivered through a water-in-oil (W/O) emulsion system, which enhanced skin moisturization and formed a long-lasting protective barrier.

Through factorial experimental design, we evaluated the effects of surfactant type (Borax vs. Tween 80) and surfactant concentration on key formulation parameters—pH, viscosity, and spreadability. Among the four formulations, F1 emerged as the optimal cream, exhibiting a pH of 5.5, viscosity of 520 cP, and spreadability of 66 g·cm/s. These characteristics align with dermatological standards for topical use, ensuring skin compatibility, ease of application, and therapeutic effectiveness. The findings demonstrate that the rational combination of natural ingredients and statistical formulation design can yield a stable, cosmetically elegant, and efficacious herbal cream. The optimized formula holds significant promise as a safe, natural, and effective treatment option for individuals suffering from acne vulgaris.

Conflict of Interest

The author declares no conflict of interest.

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