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Development and Rheological Properties Evaluation of a Burn Healing Ointment Composed of Pomegranate Peels and Beta-Sitosterol Using Factorial Design

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ABSTRACT

The present study was aimed to develop and optimize the ointment based formulation for treating skin burns and wounds using factorial design. The composition includes beeswax, Vaseline, seams seed oil, Beta-sitosterol, and pomegranate peels extract. A 2^3 randomized full factorial design was utilized in the present study. The responses observed in this study were Hardness, Viscosity, and Spreadability (rheological properties). Pareto charts and main effects plots of each response were done to obtain an optimum proportion of ingredients. The Ointment formulation of pomegranate peel extract significantly shows a good result in all the evaluation test parameters such as Hardness, viscosity, and Spreadability. A multi-response optimization was done to obtain a combined optimum that gave us 5% Beeswax (BW), 1.5% Vaseline(VAS) and 60% Seams oil(SO) . It can be concluded that the ointment base obtained with the optimal rheological properties. The optimum formula for the ointment base properties was estimated using the statistical a multi-response prediction analysis, Pareto charts, and main effects plots of responses. This ointment base with desired rheological properties can be used for cosmetic and pharmaceutical usages. It can be concluded that the ointment base obtained with optimal rheological properties can be used for healing burns, scars, and wounds.

1. INTRODUCTION

In the last few years, there has been rapid growth in the field of herbal medicine and these drugs gaining popularity both in developing & developed countries due to their natural origin and fewer side effects. Appropriate treatment of burns is necessary to minimize pain, avoid infection, and promote healing. Minor burns are typically treated with conventional drugs, including antiphlogistics, antiseptics, or analgesics, to mitigate inflammation, prevent infection or relieve pain or discomfort of patients. More severe burns, however, cannot adequately be treated with conventional drugs alone. Severe burns, such as deep secondary and third degree burns, invade dermal and subcutaneous tissues, resulting in the destruction of most of the follicles and sebaceous glands. Obvious scars appear after healing and usually affect the function and outward appearance.

Many efforts have been made to explore new agents that can enhance healing and allow for the speedy recovery of injuries while saving patients from amputation, similar complications, and additional problems. Medicinal plants were shown to play an important role in curing skin disorders like cuts and burns.[1] At present, the genus *Punica* consists of two species, the *Punica protopunica* and one under consideration. The pomegranate is one of the oldest known edible fruits.

Punica granatum has been used for a long time as a therapeutic agent for the treatment of inflammatory diseases. The aqueous ethanolic extracts of fruit rind, flower, and leaves of *Punica granatum* have shown anti-inflammatory activity. The pomegranate peels considered as a waste material contained active chemical constituents such as tannins, flavonoids β-Sitosterol responsible for anti-inflammatory activity. Herbs such as pomegranate have been widely used in traditional systems of medicines due to their antiseptic and anti-inflammatory properties[2] Pomegranate is one of the important fruits stated in the Holy Qur’an. *Punica granatum* belongs to the family of *Punicaceae* and is more commonly known as pomegranate, granats, grenade, and punica apple.[3] Also,The peel, pulp, and seed, of pomegranate fruit had the highest antioxidant activity.[4] The ointment includes only natural ingredients that are unadulterated or minimally processed. The topical use of the ointment does not result in harmful side effects. Therefore, this study focused on the objective to formulate an ointment base with optimal rheological properties by the valorization of local resources as ingredients (Pomegranate peels extract, Beeswax, Beta-sitosterol, soft paraffin, and sesame oil). The ointments and their rheological properties were studied using a full factorial design.

2. MATERIALS AND METHODS

Materials: Dried peels of *Punica granatum* were collected from the local market (Sana'a, Yemen), Beta-sitosterol, AEROSIL® 200 (Silicon dioxide), the hard thickener Beeswax, the soft thickener white soft paraffin (soft paraffin), the lubricant, sesame oil were obtained from Research and Development Center at Modern Pharmaceutical Company (Sana'a, Yemen).

Plant extraction: The peels were washed with purified water, rinsed well, and dried at room temperature for about 30 min in the open air. The dried material was properly ground into powder. This powder material was then extracted by maceration with ethanol with shaking using a shaker for three days at room temperature. The extract was concentrated at 40˚C of temperature to obtain semisolid extract (crude extract). The crude extract was dried by using drying agent AEROSIL® 200 (Silicon dioxide) in (3:1).

Factorial experimental design of herbal ointment: It is desirable to develop an acceptable pharmaceutical formulation in the shortest possible time using the minimum number of hours and raw materials. Designing drug delivery formulations with the minimum number of trials is very crucial for pharmaceutical scientists [5]. Moreover, it may be difficult to develop an ideal formulation using this classical technique (One factor at a time) since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of many variables, and their interactions [6].

The selection of variables (independent, dependent), The different variables used in this study are shown in Table No.1.

Factorial experimental design for formulation ointment base: The three ingredients for ointment base formulation, which are Hard thickener (BW), Soft thickener (VAS), and Lubricant(SO) are shown in the Implicit Domain for optimization plan Table No. 2 was used. Based on the above implicit plan proposed by the Minitab (18) program, the following design of experiments (Full Factorial design) were made according to a full 2^3 factorial design for optimization with two replicates, without center points and blocks. The factors studied were the Beeswax – (BW) as a hard thickener (5 and 10%), the percentage of Soft paraffin (VAS) in the ointment base – (1.5 and 3%), and the sesame oil for lubricant – (60 and 75%). All the factors studied were independent of each other. The design layout and coded value of inputs (independent) factor are shown in Table No. 3.

Preparation of herbal ointment: Ointments were prepared by the fusion method as described by Alalor et al.[7]. The beeswax was melted at 70ºC then soft paraffin was added at 70ºC. After melting the ingredients were stirred gently. β -sitosterol powder (0.25% W/W) was dispersed in half part of sesame oil then the mixture was added to previous ingredients. Pomegranate peels extract was dispersed in the remaining quantity of sesame oil, then was added to ingredients, the ingredients were stirred gently at 70ºC for 10 minutes and then cooled in the open air.

Hardness evaluation of ointment base: The hardness of formulations was determined by using the Brookfield Texture analyzer. It is based on the speed of displacement of the probe into the sample (ointment) at a given distance. The probe was moving down at a speed of 1mm/s till a 1 g surface trigger was attained. At this point, the probe was in full contact with the sample surface. Then the probe continued to penetrate to a depth of 5 mm at a speed of 1 mm/s. At this point, the probe returned to its starting position. The peak load (maximum force) was registered, and is considered as a measure of firmness of the product – the bigger the force the thicker/harder is the sample. The values of peak force were expressed in N (Newton).

Spreadability evaluation of ointment base: Spreadability is a term expressed to denote the extent of the area to which the ointment and gel readily spread on application to the skin or affected part. A special apparatus has been designed by Multimer [9] to study the spreadability of formulations. The spreadability is expressed in terms of times in seconds taken by two slides to slip off from ointment and placed in between the slides under the direction of a certain load. The time is taken for the separation of two slides, the resultant the better spreadability [10]. Spreadability was calculated by using the formula. (S = M.L/T). Where S = Spreadability, M = Weight tied to upper slide, L = Length of glass slides, and T = Time taken to separate the slides from each other. In this study, M = 45 gm, L = 7.5 cm and. Spreadability was calculated and expressed in g.cm/s. was recorded in Table[4].

Viscosity evaluation of ointment base: The rheological behaviour of different formulations was done by measuring the viscosity. This viscosity expressed in centipoise (cP) was determined by Brookfield viscometer by a modified method of Akanksha et al.[8]. The test sample was taken in a clean and dry 250 ml beaker, and the viscosity of the test sample was determined by the standard operating procedure of Viscometer using spindle No. 5. This spindle was used for finding the viscosity of the sample at speeds of 25, 50, and 80 rpm. The reading near to 100% torque was noted. Samples were measured at 25 ± 1°C.

Statistical analysis: Eight experiments were carried out at each experimental design point with two replicate and the mean values were stated as observed responses. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. Statistical analyses ($p < 0.05$) were performed using Minitab software (Version 18). The optimal formulation for ointment base formulation was estimated through Pareto Charts and Main Effects plots for responses of the independent variables and each dependent variable (Response).

3. RESULTS

Optimization of formulation of ointment base: The results for the analysis of different formulation bases aimed at choosing the best proportion of ingredients as Hard thickener (beeswax), Soft thickener (soft paraffin) and lubricant (sesame oil) are shown in the Table No. 4. The responses observed in the course of this analysis are Hardness, Viscosity, and Spreadability. The experimental results obtained are between, 249.5-1831 N, 1013.5-12805 cP, and 13.2 - 23.3 g.cm/s.min, for Hardness, Viscosity, and Spreadability respectively. Concerning the viscosity, the range obtained 2027 and 12800 cP is slightly comparable to the result of the values obtained by Akanksha et al. [8] During their formulation, the emulsifying wax, white soft paraffin, and liquid paraffin were used as a hard thickener, soft thickener, and lubricant respectively. From this, our formulation ointment base from natural ingredients could be used for burn ointment. Because the viscosity governs many properties of the ointment formulation such as spreadability, pourability of the product from the container, etc. [16]. To get a stable and optimal formulation, it is necessary to understand the proportion and nature of each component. Besides, each formulation must be specifically designed according to the desired purpose of its use, site of application, and patient acceptability. From the above observations, the BW (beeswax) must not be high in the formulation to facilitate spreadability, the lubricant must not equally be too much to avoid the formula from flowing off its target. Thus a multi-response optimization analysis was done to obtain the combined optimum that was used to formulate the final product with BW:VAS:SO to be 5:1.5:60 is given in the following Figure No. 1 and 2. it can be concluded that the formula with BW:VAS:SO to be 5:1.5:60 considers an optimized formula with the appropriate rheological characteristics.

Effect of formulation ingredients on Hardness: The Pareto Charts [Figure No. 3. (a)] significantly shows that the hard thickener (beeswax) has the maximum effects on the hardness of the ointment base while the lubricant (sesame oil) has the minimum effect. Also, the binary effects of hard thickener (beeswax) and soft thickener (soft paraffin) together exert a synergistic effect on the hardness. However, the weakest effect could be of two groups of ingredient soft thickener (soft paraffin), lubricant (sesame oil). The main effects Plot for hardness [Figure No. 3. (b)] shows positive effect in case of the increase of the percentage of hard thickener (beeswax) and soft thickener (soft paraffin) lead to very high hardness. However, it shows negative effect instead of the increase lubricant (sesame oil) leads to reduce the hardness.

Effect of formulation ingredients on Viscosity: The Pareto Charts [Figure No. 4. (a)] significantly shows that hard thickener (beeswax) has the maximum effects on the hardness of ointment while the binary effects of soft thickener (soft paraffin) and lubricant (sesame oil) have the minimum effect.

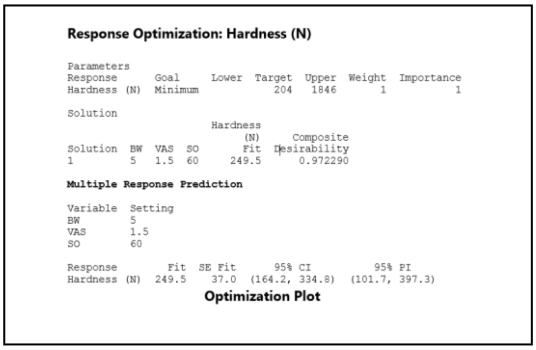
The main effects Plot for viscosity [Figure No. 4. (b)] shows positive effect in case of the increase of the percentage of hard thickener (beeswax) and soft thickener (soft paraffin) lead to high viscosity. However, it shows negative effect instead of the increase lubricant (sesame oil) leads to reduce the viscosity.

Effect of formulation ingredients on spreadability: The Pareto Charts [Figure No. 5. (a)] significantly shows that hard thickener (beeswax) has the maximum effects on spreadability of ointment while the binary effects of soft thickener hard thickener (beeswax) and soft thickener (soft paraffin) together have the minimum effect.

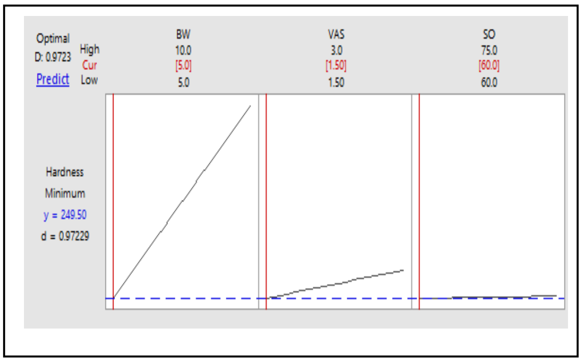
The main effects Plot for spreadability [Figure No. 5. (b)] shows negative effect in case of the increase of the percentage of hard thickener (beeswax) and soft thickener (soft paraffin) lead to low spreadability. However, it shows positive effect instead of the increase lubricant (sesame oil) leads to increase the spreadability.

3.1. Figures and Tables

All figures and tables are cited in the main text as Figure No. 1, Table No, 1, etc.

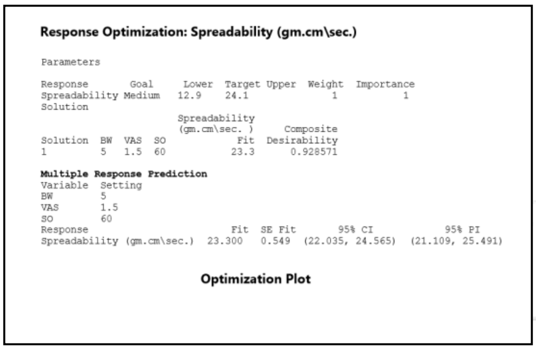


(a)

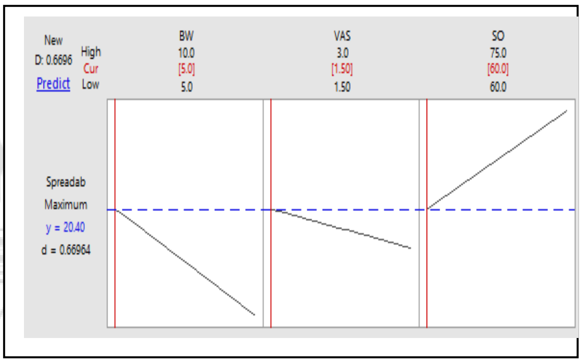


(b)

Figure No.1: Optimization Plot For Minimum Hardness. (a) Multiple Response Prediction For Minimum Hardness; (b) Optimization Plot For Minimum Hardness.

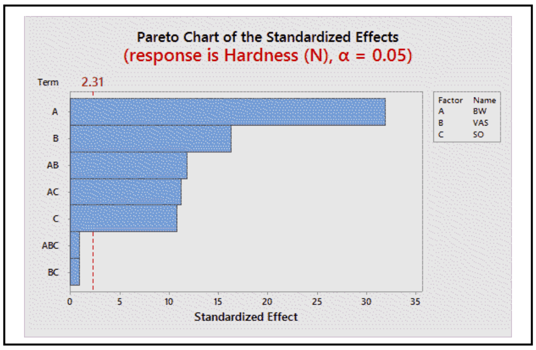


(a)

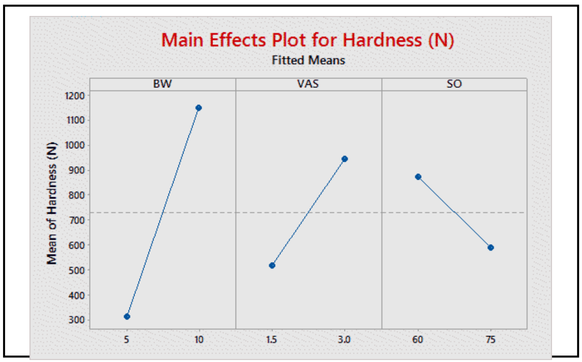


(b)

Figure No. 2: Optimization Plot Medium Spreadability (grm.cm). (a) Multiple Response Prediction For Medium Spreadability; (b) Optimization Plot For Medium Spreadability.

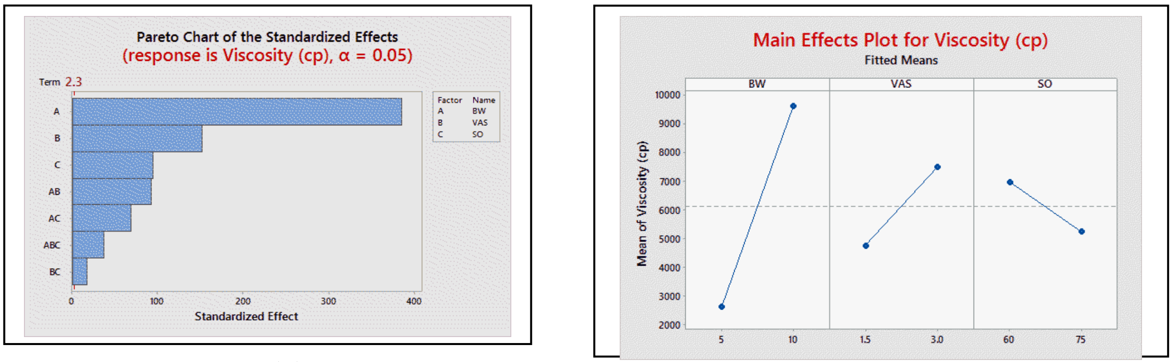


(a)



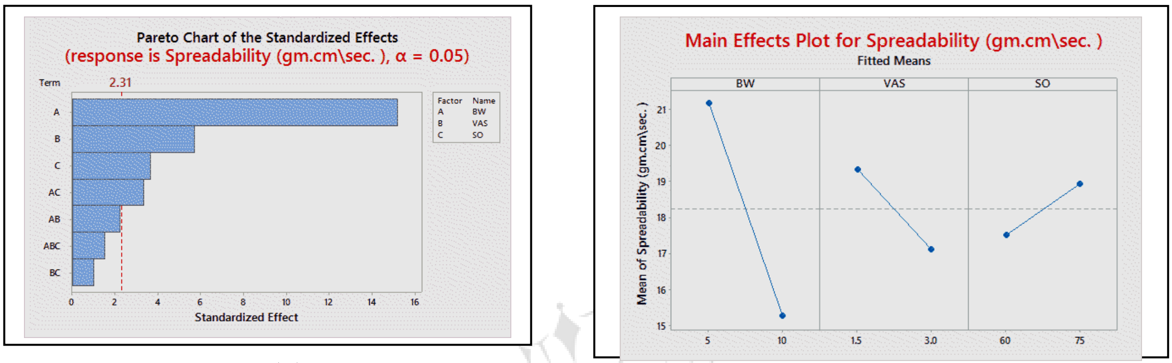
(b)

Figure No. 3: Effects charts for Hardness. (a) Pareto Charts for Hardness; (b) Main Effects plots for Hardness.



(a) (b)

Figure No. 4: Effects charts for Viscosity. (a) Pareto Charts for Viscosity; (b) Main Effects plots for Hardness.



(a) (b)

Figure No. 5: Effects charts for Spreadability. (a) Pareto Charts for Spreadability; (b) Main Effects plots for Hardness.

Table No.1: Selection Variables.

Independent (Ingredient)	**Dependent (Response)**
Hard thickener (Beeswax)	Hardness
Soft thickener (Soft paraffin)	Viscosity
Lubricant (Sesame oil)	Spreadability

Table No. 2: Implicit Domain for optimization plan.

Independent (Ingredient)	**Abbreviation**	**Lower limit (%)**	**Upper limit (%)**
Hard thickener (Beeswax)	BW	5	10
Soft thickener (Soft paraffin)	VAS	1.5	3
Lubricant (Sesame oil)	SO	60	75

Table No. 3: Full 2^3factorial design layout

Run Order	Inputs (%)			Outputs (Responses)		
	BW	VAS	SO	Hardness (N)	Viscosity (cp)	Spreadability (gm.cm\sec.)
1	5	3	60	-	-	-
2	5	1.5	75	-	-	-
3	10	3	60	-	-	-
4	5	3	75	-	-	-
5	10	3	75	-	-	-
6	5	1.5	60	-	-	-
7	10	1.5	60	-	-	-
8	10	1.5	75	-	-	-

Table No. 4: Full 2^3 factorial design for optimization with responses results

Run Order	Inputs (%)			Outputs (Responses)		
	BW	VAS	SO	Hardness (N)	Viscosity (cp)	Spreadability (gm.cm\sec.)
1	5	3	60	352	3575	19.25
2	5	1.5	75	259.5	1013.5	23.3
3	10	3	60	1831	12805	13.2
4	5	3	75	379.5	2746.5	21.75
5	10	3	75	1203.5	10815	14.3
6	5	1.5	60	249.5	2149.5	20.4
7	10	1.5	60	1045.5	9348	17.25
8	10	1.5	75	516	5440	16.4

[4] Full 2^3 factorial design for optimization with responses results.

4. DISCUSSION

Several studies showed that burn infection is the main cause of mortality in patients with extensive burns. Therefore, many researchers tried to achieve appropriate treatment methods to reduce the risk of wound infections and to shorten the period of treatment of patients with burn wounds. [11] Some of these treatments involve using topical antimicrobial agents which effectively reduce the mortality rate of burns.[12, 13] Healing of burn is still a challenge in modern medicine and there are a few drugs capable of accelerating wound healing and as an alternative plants were rich sources to survey.[12-13,15] For many years, the effect of herbal medicine on burn wound has been noted. Herbal products seem to possess moderate efficacy with no or less toxicity and are less expensive as compared with synthetic drugs. Many plants and plant-derived products have been shown to possess potent wound-healing activity.[12-14] According to these facts, burn wound, which undergoes high inflammation process, should be treated with anti-inflammatory and antioxidant substances to prevent the probability of delayed wound healing. Generally, pomegranate has various active substances that individually can act as an antibacterial, anti-inflammatory, and strong antioxidant. Some of the potential constituents of the pomegranate are tannin, flavonoid, punicic acid, and phytoestrogen. In addition, tannins can specifically act as astringent and natural wound protector to let the skin heal naturally.[16]

Ellagic acid is a simplified form of ETs (Ellagitannins), which genuinely include in tannins. Ellagic acid is an active substance that acts dominantly inside the pomegranate. Ellagic acid individually also has antibacterial, antiinflammatory, and strong antioxidant effects.[17] The addition of ellagic acid in pomegranate standardization can strengthen the function of pomegranate as a topical drug, as explained by Patwardhan et al.[18]. In previous research, pomegranate can mimic the role of ampicillin [17]. Although the antibacterial mechanism of pomegranate is not fully understood, the active substance inside pomegranate can create toxicity inside the body of bacteria through the change and interaction with enzymes and substrates [19] In previous research, the result showed of good collagen formation can be linked with an optimal healing process of the burn wound. The ointments can create an optimal process of burn wound healing. Also, it is known that the addition of 40% ellagic acid in the standardization of pomegranate extract presumably creates a synergic mechanism to support an optimal process of burn wound healing. In fact, beeswax is a large and complex group of lipophilic compounds that generally consist of mixtures of esters of fatty acids with long-chain monohydric alcohols [20]. The lubricant here which is sesame oil is made up of unsaturated fatty acid (90%) and this will tend to reduce the hardness of the ointment base. However, the binary effects of soft thickener (soft paraffin) and lubricant (sesame oil) together significantly dose show any effects.

Hard thickener contributes to increasing viscosity of the ointment base. This is due to the lipophilic compounds that generally consist of mixtures of esters of fatty acids with long-chain monohydric alcohols which constitute the wax constituting the hard thickener (beeswax). Waxes are recognized as a potential excipient for enhancing the aesthetic and maximizing the therapeutic benefits of topical formulations by increasing the viscosity[20]. Pattanayak et al. [21] had shown by a comparative study that when the viscosity of formulation increases, the spreadability decreases, and vice versa.

5. CONCLUSION

This study aimed to formulate an ointment base with optimal rheological properties by the available ingredients from local market resources as ingredients (Pomegranate peels extract, Beeswax, Beta-sitosterol, soft paraffin, and sesame oil). Using the full factorial design experiment, it was found that the mixtures of Beeswax, soft paraffin, and sesame oil not only improve the spreadability of the ointment base but also modify its viscosity and hardness. The optimum formula for the ointment base properties were done, using the statistical Pareto charts and main effects plots of responses. This ointment base with desired rheological properties can be used for further work as a matrix for the incorporation of extracts for cosmetic and pharmaceutical usages. It can be concluded that the ointment base obtained with optimal rheological properties can be used for cosmetic and pharmacological applications.

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Conflicts of Interest: The authors declare no conflicts of interest regarding the publication of this paper.

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