JOMDI

Journal of Medical and Dental Investigations

J Med Dent Invest 2023; 4: e230345
https://doi.org/10.5577/jomdi.e230345

Original Article Open Access

Design and characterization of the cream formulation containing rosemary and green tea extract against *Propionibacterium acnes*

Mert Can Güzel[1], **Mahmut Ozan Toksoy**[2]

[1] Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Izmir, Türkiye
[2] Dicle University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Diyarbakır, Türkiye

Received: 15 October 2023
Accepted: 13 December 2023
Published: 30 December 2023

Correspondence:

Dr. Mert Can GÜZEL
Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Izmir, Türkiye.
E-mail: eczmertcan.guzel@gmail.com

How to cite this article:

Güzel MC, Toksoy MO. Design and characterization of the cream formulation containing rosemary and green tea extract against *Propionibacterium acnes*. J Med Dent Invest 2023; 4: e230345. https://doi.org/10.5577/jomdi.e230345

Abstract

Aim: Acne vulgaris is an inflammation of the pilosebaceous unit. Pharmaceutical and cosmetic companies continue to invest heavily in research and development for acne treatments. Medicinal plants are being studied as potential options to prevent antibiotic resistance and reduce the cost of treating diseases. This study aimed to prepare cream formulations containing various amounts of rosemary extract (RE) and green tea extract (GTE) with antibacterial properties, perform characterization studies, and determine formulation activity against *Propionibacterium acnes* (*P. acnes*).

Methods: The cream's basis was prepared by the fusion method. Different amounts of rosemary extract and green tea extract were added. The cream formulations were characterized by microscopy, pH, rheological properties, texture properties, droplet size measurement, and a stability test. The *P. acnes* antibacterial test was carried out using the agar diffusion method.

Results: The cream formulations F2 and F1 were effective against *P. acnes* and met all of the necessary criteria, including rheological, hardness, compressibility, stickiness, elasticity, physical stability, droplet size measurement, and pH. In the antibacterial test results for the F1 formulation, the zone diameter was 10 ± 0.15 mm, and for the F2 formulation, it was 12 ± 0.15 mm ($p < 0.05$).

Conclusion: In our study, we found that formulations F1 and F2 can be administered topically because they show pseudoplastic flow, an appropriate pH value, appropriate textural properties, and antibacterial activity against *P. acnes*-induced acne vulgaris.

Keywords: Cream formulation, green tea extract, rosemary extract, texture profile analysis, acne vulgaris, rheology

Introduction

Acne is a family of skin disorders whose pathogenesis and clinical characteristics vary widely [1]. On the surface of the skin, acne bacteria belong to the three main genera of *Corynebacteria*, *Propionibacteria*, and *Staphylococci* [2]. Hyperkeratinization, blockage of sebaceous glands, stimulation of sebum secretion by androgen hormones of sebaceous glands, genotypic factors, and bacterial colonization of sebaceous glands by *Propionibacterium acnes* (*P. acnes*) are the primary pathogenic factors [3]. The dominant pathogen in sebaceous glands, *Propionibacterium acnes*, regulates skin homeostasis and prevents colonization by other harmful pathogens, while also acting as an opportunistic pathogen in acne vulgaris [2]. The presence of excess sebum and a clogged pilosebaceous site provide an anaerobic environment for *P. acnes* growth [4]. Since it was first found, *Propionibacterium acnes* has been called *Bacillus acnes*, *Corynebacterium acnes* [5], *Corynebacterium parvum* [6], and *Cutibacterium acnes* [7].

Acne consists of comedones, papules, pustules, nodules, and cysts. Microcomedones develop roughly eight weeks before the acne lesion appears on the skin's surface [8]. As sebum and skin cells accumulate within micro comedones, larger, clinically prevalent closed or open comedones form [9]. The contents of closed comedones are not exposed to the skin's surface. Open comedones (blackheads) are small follicles with enlarged skin surface openings. The pigmentation is induced by the oxidation of residues within the follicle. Papules are small, typically red, elevated skin bumps. Pustules resemble papules but include pus-filled pockets. Nodules and cysts are larger, more unpleasant growths, typically exceeding 5 mm in diameter [10].

Propionibacterium acnes (*Cutibacterium acnes*) is a Gram-positive, anaerobic, rod-shaped, non-spore-forming, pleomorphic bacterium [11]. Since *P. acnes* modulates the differentiation of keratinocytes and increases local inflammation, it is regarded as the causative agent of both micro comedone (an imperceptible structure) and inflammatory acne lesions in the early stages of acne [12]. Recent research shows that *P. acnes* may activate parts of the innate and adaptive immune systems, and biofilms made by *P. acnes* may cause follicular hyperkeratosis [13].

For the treatment of acne, multiple topical and oral antibacterial drugs are commercially available, notably clindamycin, salicylic acid, isotretinoin, erythromycin, triclosan, tetracycline, minocycline, and metronidazole. However, prolonged use of these drugs can lead to an increase in bacterial resistance. These medications' toxicity and side effects include skin dryness, headaches, and nausea. To overcome these side effects, it is necessary to develop anti-acne formulations that are effective, safe, and economical. Herbal research may contribute to the advancement of anti-acne formulations currently under investigation [14]. Antibacterial, anti-inflammatory, anti-lipogenic, and antiandrogen activities of phytochemicals or extracts from various herbs, fruits, and vegetables have been investigated extensively in recent years as a screening strategy for anti-acne agents [15, 16].

Rosemary (Rosmarinus officinalis L.) is a confirmed anti-inflammatory, antioxidant, anticarcinogenic, antimicrobial, and another health-benefiting herb [17-19]. For the known pharmacological effects of this plant, the bioactive substances 1,8-cineole, rosmarinic acid, α-pinene, camphor, camphene, carnosic acid, and carnosol are important [20, 21].

Green tea's anti-inflammatory, antioxidant, antimicrobial, and antimutagenic properties are related to its high catechin and polyphenol content (flavan-3-ols). Epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC) are the major catechins found in green tea. Among these catechins, EGCG is the green tea polyphenol with the highest concentration [22, 23].

This study aimed to fabricate a cream formulation containing rosemary and green tea extracts for *P. acnes*.

Materials and Methods

Materials

Stearyl alcohol, cetyl alcohol, isopropyl myristate, green tea extract, rosemary extract, and propylene glycol were purchased by ScienceLab (Texas, USA). Tween 80 and Span 80 were purchased by Sigma-Aldrich (St. Louis, Missouri, USA). All chemicals were of analytical grade.

Methods

Preparation of cream formulations

The cream formulations were prepared by using the fusion method (Fig. 1). The oil phase was prepared with stearyl alcohol (SA), cetyl alcohol (CA), isopropyl myristate (IPM), and green tea extract (GTE). Pure water, Tween 80, propylene glycol (PG), and rosemary extract (RE) were mixed to prepare the water phase. Oil and water phases were warmed to 75 °C using a heater. Then the water phase was mixed with the oil phase using a mechanical mixer (Janke & Kunkel Ka-Work RW 20 DZM, Germany) at 800-1000 rpm for 10 min.

Hydrophilic-lipophilic balance (HLB) determination

HLB measures the hydrophilicity or lipophilicity of a surfactant. Griffin noted that HLB gives a practical method for selecting the optimal surfactant for a certain application [24, 25]. In our study, six different cream formulations were developed, and the ideal HLB values by the amount of surfactants were determined (Table 1). The ideal HLB value of an oil phase is for producing the most stable and dispersible emulsion possible [26]. The

following equation was used to determine the amount of each emulsifier to be added [27].

HLB: xA + (1-x)B (1)

Where x is the proportion of a surfactant having an HLB value of A, and the other surfactant has an HLB value of B [28].

Table 1. The concentration of emulsifiers in emulsions [HLB:4.3-15.0].

Batch code	HLB	Tween 80 (%)	Span 80 (%)	Tween 80 (g)	Span 80 (g)
E1	15.0	100	0	1	0
E2	14.27	95	5	0.95	5
E3	13.93	90	10	0.9	0.1
E4 (F0)	14.15	85	15	0.85	0.15
E5	12.86	80	20	0.8	0.2
E6	12.33	75	25	0.75	0.25

(E: Serial run abbreviation for preparing stable F0 formulation)

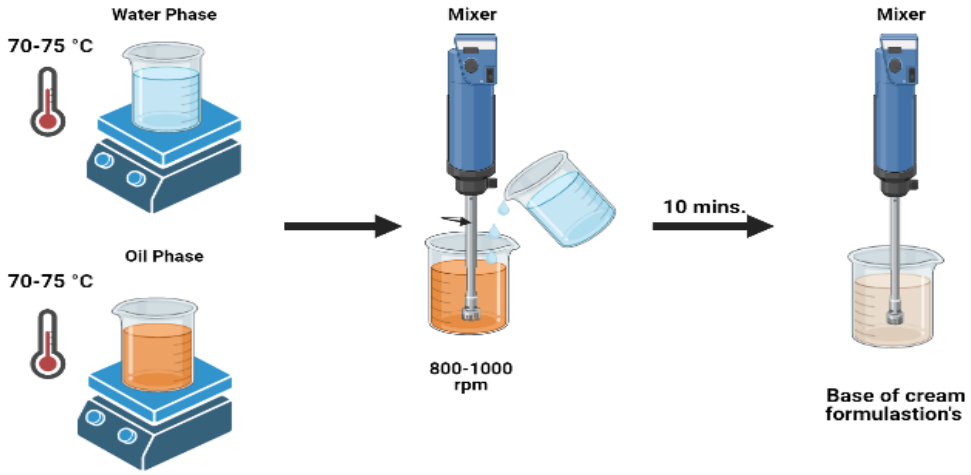

Figure 1. Preparation of formulation by fusion method (Created with BioRender.com).

Formulations were centrifuged at 4500 rpm / 30 minutes to evaluate their physical stability (SIGMA 2-16KL, Germany). The only batch without phase separation was E4 (Fig. 2). Since there is no phase separation in the E4 series, it was chosen as the cream base (F0).

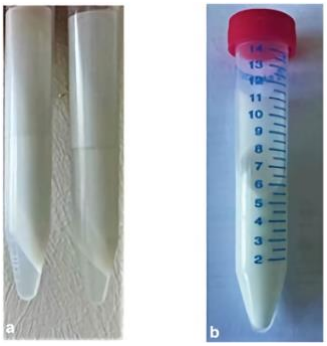

Figure 2. (a) Centrifuged other formulations, and (b) centrifuged E4 formulation.

Two different formulations (F1 and F2) were prepared (Table 2) by adding increasing amounts of rosemary and green tea extracts to the base cream formulation (F0).

Table 2. Extracts on cream formulations.

Formulation	Rosemary Extract (%)	Green Tea Extract (%)	Tween 80 (%)	Span 80 (%)
F0	-	-	85	15
F1	0.5	0.5	85	15
F2	1.5	1.5	85	15

Optical microscopic observation

F0, F1, and F2 formulations were analyzed using an optical microscope. (Zeiss Primo Star Transmission Microscopy, Germany).

pH measurement

The pH of F0, F1, and F2 formulations was measured using a pH meter (Thermo Scientific Orion 5 Star, Waltham, Massachusetts, USA), (n=3, mean ± SD).

Rheological properties

Rheological properties were characterized using a TA-TX Discovery HR1 (TA Instruments, New Castle, UK) rheometer at 25 ± 0.1 °C and 32 ± 0.1 °C. Samples were given at least 2 min to rest and acclimate to their surroundings before being measured. The studies were performed with a steel probe of 40 mm in diameter and a fixed pitch of 0.3 mm, and the shear rate varied between 10 and 2000 1/s. While the shear rate varied between these values, 40 measurements of shear stress were taken. Shear stress and shear rate graphs were formed, as well as flow curves. For each sample, the study was repeated three times (n=3).

Texture properties

Mechanical properties (hardness, compressibility, cohesiveness, elasticity, and adhesiveness) were measured using a software-controlled penetrometer (TA-XT Plus texture analyzer; Stable Micro Systems, Godalming, TA Instruments, New Castle, UK). The load cell was calibrated with a weight of 500 grams. Analysis: pre-test speed of 1.00 mm/sec, test speed of 5.00 mm/sec, post-test speed of 5.00 mm/sec, a distance of 10.00 mm, and time of 5.00 sec at 25 °C / %60 RH. Each experiment was repeated three times (n=3, mean ± SD).

Droplet size distribution

Using laser diffraction and a dry sample dispersion technique, the droplet size of the formulations was evaluated (Mastersizer 3000, Aero S, Malvern Instruments, Malvern, Worcestershire, UK). Before measuring, formulations were diluted with pure water (1:1000) and stirred slightly before analysis. For each sample, the study was repeated three times (n=3, mean ± SD).

Antibacterial activity

The agar diffusion method was used to determine the antibacterial activity of cream formulations against *Cutibacterium acnes* organism (Kirby-Bauer assay). *C. acnes* ATCC 11827 culture was grown on Modified Reinforced Clostridial agar medium at 37 °C under anaerobic conditions for 72 hours, and the suspension was prepared with PBS. 0.1 mL of bacterial culture adjusted to 0.5 McFarland (Grant Inst, Cambridge, UK) was inoculated onto Reinforced Clostridial agar plates and spread with sterile baguettes. A well of 6 mm in diameter was opened in the center of the medium with a sterile pipette tip, and cream samples were transferred into it. Petri dishes were incubated for 72 hours at 37 ± 1 °C, and the inhibition zone diameters were measured and recorded (n=3, mean ± SD).

Stability studies

Under extreme stress, F0, F1, and F2 formulations were monitored in an oven at 50 °C for three days. Long-term stability investigations of F0, F1, and F2 formulations were conducted in a stability chamber at 25 °C and 60% relative humidity (RH) for three months. The centrifuge test (SIGMA 2-16KL, Osterode am Harz, Germany) was performed at 4500 rpm for 30 minutes for physical stability.

Statistical analysis

Statistical analyzes were performed using IBM SPSS 22.0 software (IBM, Armonk, New York, USA).

All the experiments were performed in triplicate (n=3). The values were used for determining mean and standard deviation (SD), and one-way ANOVA was applied to confirm the value of $p < 0.05$.

Results

Appearance, content uniformity, and pH measurement

Formulations were smooth, uniform, and white and did not agglomerate. Table 3 shows the pH results for F0, F1, and F2 cream formulations.

Table 3. Appearance, content uniformity, and pH values of cream formulations.

Formulations	Appearance	Content Uniformity	pH Values
F0	Smooth, White	Uniform	5.11 ± 0.01
F1	Smooth, White	Uniform	5.24 ± 0.01
F2	Smooth, White	Uniform	5.66 ± 0.01

Optical microscopic observation

The microscope samples were examined at room temperature under x10 magnification. The formation of an emulsion structure is demonstrated in Figure 3.

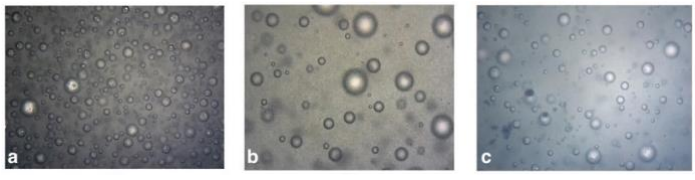

Figure 3. Microscopic view of F0 (a), F1 (b), and F2 (c) cream formulations

Rheological properties

The viscosity of F0, F1, and F2 formulations were determined. In each of the three formulations, the viscosity at 32 °C was lower than at 25 °C. Pseudoplastic flow with non-Newtonian rheological behavior was observed (Fig. 4).

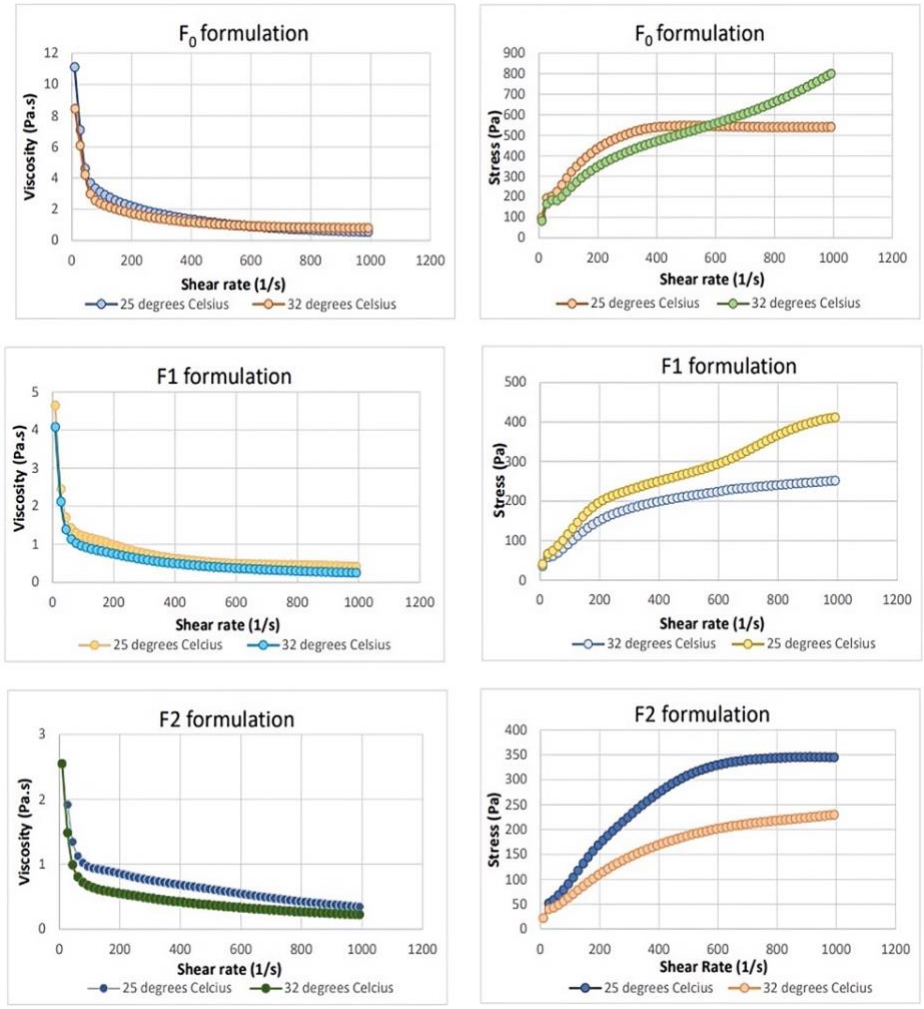

Figure 4. Rheological results of formulations (n=3)

Texture properties

TPA was used to examine the cream's hardness, adhesiveness, compressibility, elasticity, and cohesiveness. Results are shown in Table 4.

Droplet size distribution

The droplet size of emulsions is one of the main factors of their optical appearance, texture properties, physical stability, and finally, emulsion quality profile [29]. Dx 10 [µm] means that 10% of the particle dispersion is below this value, Dx 50 [µm] means that 50% of the dispersion is above this value and 50% is below this value, and Dx 90 [µm] means that 90% of the particle dispersion is below this value [30]. Table 5 and Figure 5 show droplet size distribution results of F0, F1, and F2 formulations.

Antibacterial activities of formulations

This study examined the antimicrobial activity of F1 and F2 formulations against *P. acnes*. The zone diameter for formulation F1 was 10 ± 0.15 mm and 12 ± 0.15 mm for formulation F2 (Fig. 6). According to these results, as the percentages of GTE and RE increase, the zone diameter also increases.

Table 4. TPA results of formulations (n=3, mean ± SD).

	F0	F1	F2
Hardness	14.727 ± 0.03	10.831 ± 0.04	10.554 ± 0.05
Compressibility	16.109 ± 0.07	19.441 ± 0.06	21.131 ± 0.07
Adhesiveness	18.855 ± 0.03	25.460 ± 0.05	30.129 ± 0.06
Cohesiveness	1.223 ± 0.05	1.186± 0.04	1.223 ± 0.04
Elasticity	1.0003 ± 0.01	1.0005± 0.01	0.985 ± 0.01

Table 5. Droplet size results of formulations (n=3, mean ± SD).

	F0	F1	F2
Dx 10 [µm]	3.89 ± 0.48	0.956 ± 0.14	1.17 ± 0.22
Dx 50 [µm]	15.0 ± 1.43	6.88 ± 0.94	8.45 ± 1.04
Dx 90 [µm]	32.3 ± 3.44	17.1 ± 2.46	22.5 ± 2.83

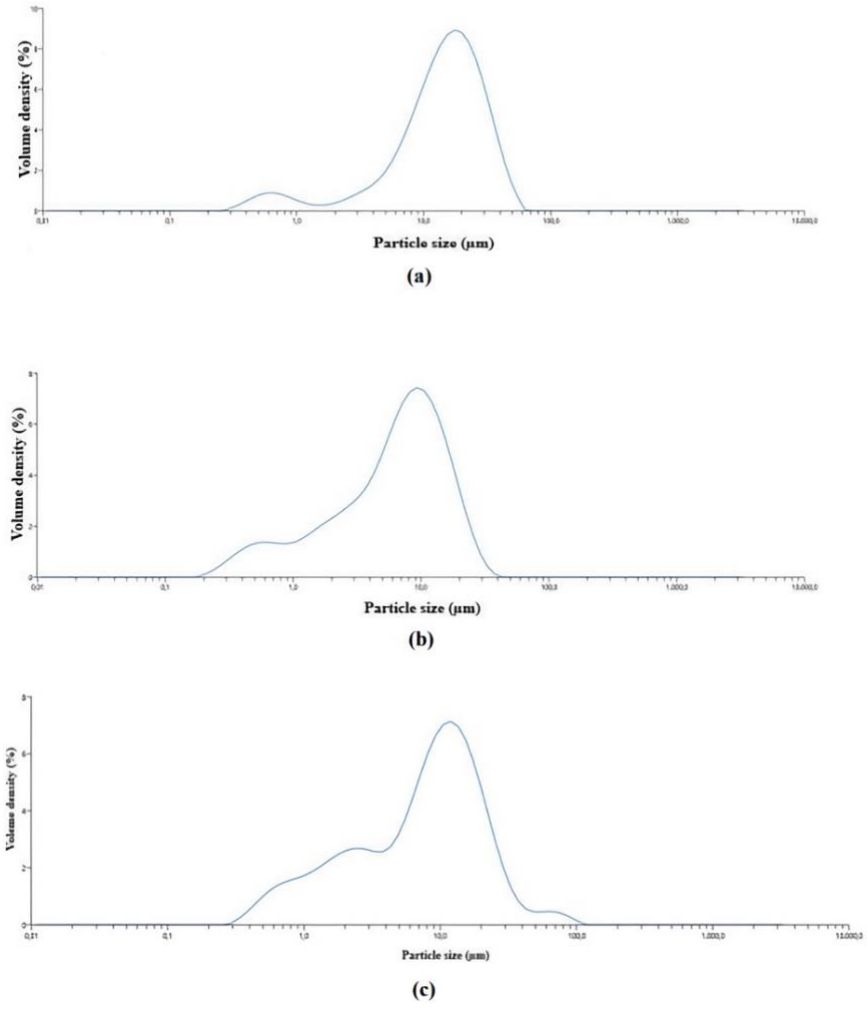

Figure 5. Graph of the droplet size distribution for formulations (a) F0, (b) F1, and (c) F2.

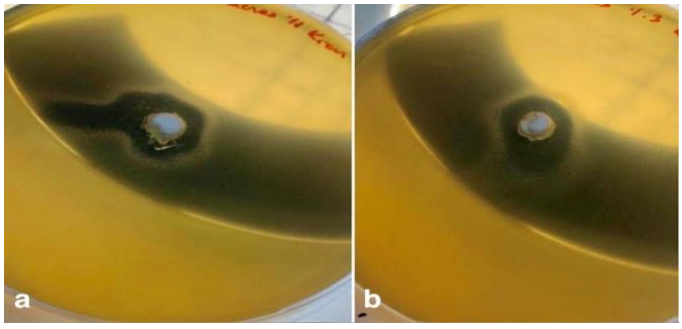

Figure 6. Representative images of F1 (a) and F2 (b) formulations of antibacterial test on Reinforced Clostridial agar plates

Stability studies

Under extreme stress, the formulations were monitored for three days in an oven at 50°C. By the conclusion of the third day, phase separation was observed. In studies of the long-term stability of cream formulations [at 25 °C and 60% relative humidity (RH)], no change was detected after the first month. Long-term stability studies are still ongoing.

Discussion

In our study, cream formulations containing rosemary and green tea extracts were prepared. Characterization studies were carried out. Effectiveness against *P. acnes* was investigated.

The centrifuge test accelerates particle motion as particles increase their kinetic energy and simulates an increase in gravitational force, thereby predicting the formulation's potential phase separation, precipitation, or coalescence. The emulsion is physically stable under normal gravity conditions if its physical stability does not change after centrifugation [28, 29].

Topical pharmaceuticals should be designed with a suitable pH range because pH impacts drug solubility, stability, and the risk of skin irritation [31]. Most of the time, the pH of the skin is between 4 and 6 [32, 33]. Making the surface of the skin more acidic can help acne. The inflammatory TH2 response decreases by lowering the pH, and the barrier function recovers faster [34, 35].

Understanding the behavior of creams during processing, packaging, storage stability, spreadability, drug release, and skin permeation can be enabled by their rheological characterization. Commonly, the shear rate and shear stress are indicative of the cream's viscosity and spreadability [36]. The flow properties of cream can tell us how easy it is to apply and spread the cream on the skin. It has been suggested that a skin cream should have a low viscosity at high shear for easy application and a high viscosity at low shear so that it does not spill out of the container [37]. Pseudoplastic liquids, such as formulations for cosmetics, are characterized by flow curves in which viscosity leads to a lower shear rate [38]. The purpose of analyzing the rheological properties at 25°C and 32 °C is to examine the flow properties of the formulations at room temperature and determine formulation spreadability and spreadability on the skin. Because of shear-thinning behavior, the viscosity of F0, F1, and F2 formulations was decreased. In each of the three formulations, the viscosity at 32 °C is lower than at 25 °C. Changes in shear stress with shear rate were used to determine if the formulation exhibited Newtonian or non-Newtonian rheological behavior. On the graph of shear stress versus shear rate, shear stress was increasing. In all formulations, pseudoplastic flow with non-Newtonian rheological behavior was observed.

Characterizing semisolid dosage forms has become a valuable technique for the analysis of pharmacy in recent years. Cream formulations need to have adequate mechanical properties [39]. TPA is a penetration test in which a solid probe is inserted to a specified level and then withdrawn. The clinical application of a product is directly impacted by its textural properties [40]. Ideal cream formulation generally requires low hardness compressibility and high adhesion. Owing to its low hardness and compressibility, the cream is simple to remove from its container and apply. Elasticity is the rate at which a sample returns to its original shape after being deformed [41-43]. The pH, color, odor, rheological, and texture features of F0, F1, and F2 cream formulations were unchanged considerably during a one-month long-term stability study. The formulations were an increase in droplet size. Ostwald ripening is the intrinsic thermodynamic instability of emulsions and involves droplet size polydistribution, which means that larger droplets grow by affecting smaller droplets. There is an obvious relationship between the increased emulsion droplet diameter, creaming index, and apparent viscosity of the emulsion [44]. Creaming is the process of separating larger lipid droplets from smaller droplets in an emulsion. Because of density differences, the larger lipid droplets float on the liquid's surface to form a layer of lipids-rich cream. There was no sign of phase separation or creaming in the formulations [45].

Conclusion

In this study, a cream basis (F0) was developed for F1 and F2 cream formulations. F1 and F2 cream formulations have different concentrations containing GTE and RE. The optimal HLB value for the F0 formulation's physical stability has been investigated. After obtaining a physically stable base formulation, characterization, stability, and efficiency tests were performed on F1 and F2 formulations containing various amounts of GTE and RE. Non-Newtonian flow, acceptable textural properties, an optimum pH, and antibacterial activity against *P. acnes* were observed in the F1 cream formulation in this study. Compared to the F1 formulation, the F2 formulation demonstrated high physical stability, rheological properties, textural properties, and activity against *P. acnes*. The formulations had good fragrance, spreadability, penetration, and lack of adhesion performance.

Disclosures

Acknowledgments: The authors thank Professor Özgen ÖZER for contributions.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.C.G., M.O.T.; Design – M.C.G.; Supervision – M.O.T.; Funding – M.C.G., M.O.T.; Materials – M.C.G., M.O.T.; Data Collection and/or Processing – M.C.G., M.O.T.; Analysis and/or Interpretation – M.C.G.; Literature Review – M.O.T.; Writer – M.C.G.; Critical Review – M.C.G.

Conflict of Interest: There is no any conflict of interest.

Funding: There is no any funding.

References

1. Muizzuddin N, Giacomoni P, Maes, D. Acne - a multifaceted problem. Drug Discovery Today: Disease Mechanisms, 2008;5(2):e183-e188. https://doi.org/10.1016/j.ddmec.2008.08.001
2. Dréno B, Pécastaings S, Corvec S, Veraldi S, Khammari A, Roques C. Cutibacterium acnes (Propionibacterium acnes) and acne vulgaris: a brief look at the latest updates. Journal of the European Academy of Dermatology and Venereology, 2018;32: 5-14. https://doi.org/10.1111/jdv.15043
3. Firlej E, Kowalska W, Szymaszek K, Roliński J, Bartosińska, J. The Role of Skin Immune System in Acne. Journal of Clinical Medicine, 2022;11(6): 1579. https://doi.org/10.3390/jcm11061579
4. Vary JC. Selected Disorders of Skin Appendages-Acne, Alopecia, Hyperhidrosis. Medical Clinics of North America, 2015;99(6):1195-1211. https://doi.org/10.1016/j.mcna.2015.07.003
5. Marples RR, McGinley KJ. Corynebacterium Acnes and Other Anaerobic Diphtheroids From Human Skin. Journal of Medical Microbiology, 1974;7(3): 349-358. https://doi.org/10.1099/00222615-7-3-349
6. Eady EA, Ingham E. Propionibacterium acnes-friend or foe? Reviews in Medical Microbiology, 1994;5(3):163-173. https://doi.org/10.1097/00013542-199407000-00003
7. Corvec S, Dagnelie MA, Khammari A, Dréno B. Taxonomy and phylogeny of Cutibacterium (formerly Propionibacterium) acnes in inflammatory skin diseases. Annales de Dermatologie et de Vénéréologie, 2019;146(1):26-30. https://doi.org/10.1016/j.annder.2018.11.002
8. Thiboutot DM. The role of follicular hyperkeratinization in acne. Journal of Dermatological Treatment, 2000;11(2), 5-8. https://doi.org/10.1080/095466300750163645
9. Gollnick HPM. From new findings in acne pathogenesis to new approaches in treatment. Journal of the European Academy of Dermatology and Venereology, 2015;29:1-7. https://doi.org/10.1111/jdv.13186
10. Mahto A. Acne vulgaris. Medicine, 2017;45(6):386-389. https://doi.org/10.1016/j.mpmed.2017.03.003
11. Perry AL, Lambert PA. Propionibacterium acnes. Letters in Applied Microbiology, 2006;42(3):185-188. https://doi.org/10.1111/j.1472-765X.2006.01866.x
12. Kistowska M, Gehrke S, Jankovic D, Kerl K, Fettelschoss A, Feldmeyer L, Fenini G, Kolios A, Navarini A, Ganceviciene R, Schauber J, Contassot E, French LE. IL-1β Drives Inflammatory Responses to Propionibacterium acnes In Vitro and In Vivo. Journal of Investigative Dermatology, 2014;134(3):677-685. https://doi.org/10.1038/jid.2013.438
13. Hazarika N. Acne vulgaris: new evidence in pathogenesis and future modalities of treatment. Journal of Dermatological Treatment, 2019;32(3): 277-285. https://doi.org/10.1080/09546634.2019.1654075
14. Kumar A, Baboota S, Agarwal S, Ali J, Ahuja A. Treatment of acne with special emphasis on herbal remedies. Expert Review of Dermatology, 2008;3(1):111-122. https://doi.org/10.1586/17469872.3.1.111
15. Nam C, Kim S, Sim Y, Chang I. Anti-Acne Effects of Oriental Herb Extracts: A Novel Screening Method to Select Anti-Acne Agents. Skin Pharmacology and Physiology, 2003;16(2):84-90. https://doi.org/10.1159/000069030
16. Tang Y, Liu L, Han J, Zhang Z, Yang S, Li S, Fan Z, Zhao H. (). Fabrication and Characterization of Multiple Herbal Extracts-loaded Nanofibrous Patches for Topical Treatment of Acne Vulgaris. Fibers and Polymers, 2021;22(2):323-333. https://doi.org/10.1007/s12221-021-0156-1
17. Baumann LS. Less-known botanical cosmeceuticals. Dermatologic Therapy, 2007;20(5):330-342. https://doi.org/10.1111/j.1529-8019.2007.00147.x
18. Peng C, Su J, Chyau C, Sung T, Ho S, et al. Supercritical fluid extracts of rosemary leaves exhibit potent anti-inflammation and anti-tumor effects. Bioscience, Biotechnology, and Biochemistry 2007;71(9):2223-2232. https://doi.org/10.1271/bbb.70199
19. Tai J, Cheung S, Cheah S, Chan E, Hasman D. In vitro anti-proliferative and antioxidant studies on Devil's Club Oplopanax horridus. Journal of ethnopharmacology, 2006;108(2):228-235. https://doi.org/10.1016/j.jep.2006.05.018
20. Mohamed WAM, Abd-Elhakim YM, Farouk SM. Protective effects of ethanolic extract of rosemary against lead-induced hepato-renal damage in rabbits. Experimental and Toxicologic Pathology, 2016;68(8):451-461. https://doi.org/10.1016/j.etp.2016.07.003
21. Sinha P, Srivastava S, Mishra N, Yadav NP. New Perspectives on Antiacne Plant Drugs: Contribution to Modern Therapeutics. BioMed Research International, 2014:1-19. https://doi.org/10.1155/2014/301304
22. Gaur S, Agnihotri R. Green tea: A novel functional food for the oral health of older adults. Geriatrics & Gerontology International, 2013;14(2):238-250. https://doi.org/10.1111/ggi.12194
23. Zaveri NT. Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications. Life Sciences, 2006;78(18), 2073-2080. https://doi.org/10.1016/j.lfs.2005.12.006
24. Griffin WC. Calculation of HLB values of non-ionic surfactants. J. Soc Cosmet Chem, 1954;5:249-256"
25. Griffin WC. Classification of Surface-Active Agents by “HLB”. The Journal of the Society of Cosmetic Chemists, 1949;1:311-326.
26. Schmidts T, Schlupp P, Gross A, Dobler D, Runkel F. Required HLB Determination of Some Pharmaceutical Oils in Submicron Emulsions. Journal of Dispersion Science and Technology, 2012;33(6):816-820. https://doi.org/10.1080/01932691.2011.584800
27. Rao MRP, Aghav S, Sukre G, Kumar M. Determination of Required HLB of Capryol 90. Journal of Dispersion Science and Technology, 2014;35(2): 161-167. https://doi.org/10.1080/01932691.2013.777824
28. Banker GS, Siepmann J, Rhodes C (Eds.) (2002). Preformulation. In: Tadros, T. F. (Ed.), Modern Pharmaceutics, (pp. 237-287). New York: Marcel Dekker. https://doi.org/10.1201/9780824744694-10
29. Simões A, Veiga F, Vitorino C. Developing Cream Formulations: Renewed Interest in an Old Problem. Journal of Pharmaceutical Sciences, 2019;108(10), 3240-3251. https://doi.org/10.1016/j.xphs.2019.06.006
30. Jones DS, Woolfson AD, Djokic J. (). Texture profile analysis of bioadhesive polymeric semisolids: Mechanical characterization and investigation of interactions between formulation components. Journal of Applied Polymer Science, 1996;61(12): 2229-2234. https://doi.org/10.1002/(SICI)1097-4628(19960919)61:12<2229::AID-APP24>3.0.CO;2-0
31. Tadros TF. (Ed.) Emulsion Formation, Stability, and Rheology. Tadros, T. F. (Ed.), Emulsion Formation and Stability, 2013, (pp. 1-75). Wokingham: Wiley-VCH. https://doi.org/10.1002/9783527647941.ch1
32. Demoliner LB, Agnol LD, Zanotti SB, Dias FTG, Angeli VW, Bianchi O. Correlation between rheological properties and capillary fixation for modelling creams. Brazilian Journal of Pharmaceutical Sciences, 2018;54(3). https://doi.org/10.1590/s2175-97902018000317642
33. Eren B, Tuncay Tanrıverdi S, Aydın Köse F, Özer Ö. Antioxidant properties evaluation of topical astaxanthin formulations as anti-aging products. Journal of Cosmetic Dermatology, 2018;18(1), 242-250. https://doi.org/10.1111/jocd.12665
34. Prakash C, Bhargava P, Tiwari S, Majumdar B, Bhargava RK. Skin surface pH in acne vulgaris: insights from an observational study and review of the literature. The Journal of clinical and aesthetic dermatology, 2017;10(7):33-39.
35. Kao JS, Garg A, Mao-Qiang M, Crumrine D, Ghadially R, Feingold KR, Elias PM. Testosterone Perturbs Epidermal Permeability Barrier Homeostasis. Journal of Investigative Dermatology, 2001;116(3):443-451. https://doi.org/10.1046/j.1523-1747.2001.01281.x
36. Pappas A, Johnsen S, Liu JC, Eisinger M. Sebum analysis of individuals with and without acne. Dermato-Endocrinology,

2009;1(3):157-161. https://doi.org/10.4161/derm.1.3.8473
37. Kamal NS, Krishnaiah YSR, Xu X, Zidan AS, Raney S, Cruz CN, Ashraf M. Identification of critical formulation parameters affecting the in vitro release, permeation, and rheological properties of the acyclovir topical cream. International Journal of Pharmaceutics, 2020;590:119914. https://doi.org/10.1016/j.ijpharm.2020.119914
38. Ali A, Skedung L, Burleigh S, Lavant E, Ringstad L, Anderson, C, Wahlgren M, Engblom J. Relationship between sensorial and physical characteristics of topical creams: A comparative study on effects of excipients. International Journal of Pharmaceutics, 2022;613:121370. https://doi.org/10.1016/j.ijpharm.2021.121370
39. Binks BP. Emulsions - Recent Advances in Understanding. In: Binks, B.P. (Eds.), Modern Aspects of Emulsion Science, 1998, (pp. 1-55). London: Royal Society of Chemistry. https://doi.org/10.1039/9781847551474-00001
40. Jones DS, Irwin CR, David Woolfson A, Djokic J, Adams V. (). Physicochemical characterization and preliminary in vivo efficacy of bioadhesive, semisolid formulations containing flurbiprofen for the treatment of gingivitis. Journal of Pharmaceutical Sciences, 1999;88(6):592-598. https://doi.org/10.1021/js9803095
41. Coviello T, Coluzzi G, Palleschi A, Grassi M, Santucci E, Alhaique F. Structural and rheological characterization of Scleroglucan/borax hydrogel for drug delivery. International Journal of Biological Macromolecules, 2003;32(3-5):83-92. https://doi.org/10.1016/S0141-8130(03)00041-2
42. Güneş M, Turan G, Dönmezer A, Aydın Köse F, Özer KÖ, Karavana SY. Mucoadhesıve Orodispersible Film Formulations of Rasagiline Mesylate For Parkinson Treatment. Ankara Universitesi Eczacilik Fakultesi Dergisi, 2022;47(1):2-2. https://doi.org/10.33483/jfpau.1150792
43. Olejnik A, Goscianska J, Zielinska A, Nowak I. (). Stability determination of the formulations containing hyaluronic acid. International Journal of Cosmetic Science, 2015;37(4):401-407. https://doi.org/10.1111/ics.12210
44. Gadkari PV, Shashidhar MG, Balaraman M. Delivery of green tea catechins through Oil-in-Water (O/W) nanoemulsion and assessment of storage stability. Journal of Food Engineering, 2017;199:65-66. https://doi.org/10.1016/j.jfoodeng.2016.12.009
45. Filho DCM, Ramalho JBVS, Spinelli LS, Lucas EF. Aging of water-in-crude oil emulsions: Effect on water content, droplet size distribution, dynamic viscosity and stability. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2012;396:208-212. https://doi.org/10.1016/j.colsurfa.2011.12.076