RESEARCH ARTICLE

Formulation and Evaluation of Antiaging Ointment Containing Microencapsulated Turmeric and Jojoba Oil

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ABSTRACT

Introduction: Skin health and beauty is essential for maintaining the overall well-being of human life. In the current fast-paced world, factors like busy schedules, unhealthy diets, and harsh environmental conditions can lead to noticeable signs of aging. Therefore, it is crucial to find effective solutions to address these concerns and maintain healthy, youthful-looking skin. To address this problem, we prepaid anti-aging ointment. And the beauty of the formulation is it contains microencapsulated turmeric and jojoba oil.

Material and methods: Microcapsules were synthesized through the utilization of the ionic gelation method. In this method, sodium alginate is used as polymer and calcium chloride as cross-linker. These microcapsules were then incorporated into the ointment base to formulate the anti-aging ointment. Turmeric and jojoba oil were incorporated into the ointment to provide anti-aging benefits.

Results: After the assessment of the microcapsules, it was determined that the prepared microcapsules fall within the range of 400 to 1000 µm. From FTIR spectra revealed successful microencapsulation of oil by sodium alginate. TGA analysis indicates that the prepared microcapsules exhibited thermal stability. The physical properties, spreadability, viscosity, and pH of the ointment were observed within the specified range. Formulation shows sustained release of turmeric oil 68.81% and jojoba oil 74.81% over 6 hours and DPPH assay shows with increase in concentration of oil the free radical scavenging activity also increases.

Conclusion: The oils incorporation of microcapsules has significantly improved the antiaging properties of the ointment. The microencapsulation of these oils enables a sustained release of the active ingredients within the ointment formulation. The DPPH assay results confirm that the formulated ointment possesses robust antioxidant properties.

Keywords: Turmeric oil, Jojoba oil, Ionic gelation method, Microcapsules, Antiaging, Ointment.

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INTRODUCTION

The process of skin aging occurs as a result of ongoing deterioration caused by cellular DNA and protein damage.[1] Aging is marked by a decline in both the structure and function of the body, accompanied by a decrease in adaptability and resistance.[2] Skin aging represents a multifaceted process triggered by continuous exposure to ultraviolet light/rays' exposure. UV rays instigate the generation of reactive O_2 species, which in turn leads to a depletion of collagen, ultimately culminating in the formation of skin wrinkles. Other environmental factors such as air pollution and smoking are responsible for skin aging.[3] The growing aspiration to maintain a youthful appearance has generated a substantial market demand for anti-aging products, specifically focusing on anti-aging ointments and creams. These products assert their ability to mitigate the visible signs of aging and rejuvenate the complexion, aiming to restore a more youthful look.[4]

As the largest organ of the body, the skin prominently displays the visible effects of aging as the individual grows

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older. Consequently, a significant portion of daily expenses, particularly for women, is allocated towards cosmetics and medications aimed to preventing skin aging. This is widespread demand for a cosmetic solution continues to drive research into skin aging and the development of various formulations that purpose to possess anti-aging properties.[5,6]

Recently, there has been notable increase in research and development endeavors within the cosmetic and pharmaceutical industries, all aimed at formulating effective anti-aging ointments.[7,8] Based on the process of skin aging, three methods can be identified to resolve it: safeguarding the skin against external environmental factors, eliminating free radicals within cells, and providing reparative nourishment to skin cells. Therefore, utilizing specific types of herbal essential oil keeps skin moisturized and reduces aging. [9]

Essential oils (EOs) are fluid substances found in plants, which can be described as complex, natural combinations of volatile secondary lipophilic compounds.[10] These compounds contribute to the distinct aroma and color found in various plants and spices.[11,12] EOs exhibit antioxidant properties, and research has indicated that EOs such as turmeric oil[13] and jojoba oil[14] demonstrated the ability of the percent radical scavenging effect.[15] Turmeric oil may potentially help to reduce the appearance of fine lines and wrinkles by minimizing reactive oxygen species and inflammation.[13] Jojoba oil, is derived from the seeds of the jojoba plant. It is abundant in essential nutrients such as vitamin E and B-complex, zinc, and copper. These nutrients play a vital role in promoting skin health by helping to maintain the skin's natural elasticity and moisture level.[15] Mechanism behind the antiaging effect of turmeric and jojoba oil is as shown in Figure 1.

EO is prone to instability and it is easily influenced by external factors like light, temperature, oxygen, and humidity.[16] Their high volatility and reactivity pose significant challenges for their utilization across various industries. To overcome these problems, microencapsulation is frequently employed to preserve the functional and biological properties of these compounds, also controlling their release.[17]

Microcapsules are small particles or tiny spheres with a size typically ranging from 1 to 1000 µm. It consists of an active constituent enclosed within a natural or synthetic polymeric membrane. These capsules are composed of two main parts: the core, which is the covering of active ingredients such as essential oil, vitamins, peptides or antioxidants, and the shell, responsible for shielding the core from the external environment.[18] Microencapsulation finds application in safeguarding fragrances and other active agents against oxidation due to heat, light, and moisture during extended storage. It also serves to protect active substances from contact with other materials, prevent evaporation of volatile compounds, and control the rate of their release.[19]

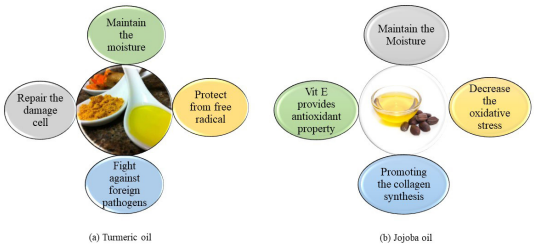

Figure 1: Mechanism behind the antiaging effect of turmeric and jojoba oil

The anti-aging effect of turmeric and jojoba oil is achieved by incorporating microcapsules into the ointment base. Preparing a formulation that can adhere to the skin for an extended period is essential and this is achieved through the use of semisolid preparations like ointments.[6] Ointment is a semi-solid formulation characterized by soft, easily applicable and simple-to-clean texture. It is commonly utilized as an external medication on both the skin and mucous membranes.[20] The prepared microcapsules were added into the ointment base and they are stable into it. The antioxidant activity of ointment was evaluated by performing DPPH assay.

The objective of this research paper is to offer a thorough evaluation of the existing scientific literature concerning the effectiveness of anti-aging ointments. Through a meticulous analysis of relevant studies, we aim to assess the performance of key ingredients and formulations commonly utilized in these products and ascertain their potential advantages.

MATERIALS AND METHODS

Materials

Yarrow Chem Products Pvt. Ltd Mumbai, supplied the sodium alginate. The turmeric and jojoba oil were acquired from Nature's Natural India Oils Pvt. Ltd in Ghaziabad. Forbes Pharmaceutical Mumbai provided the calcium chloride. White soft paraffin, tween 80, and glycerol monostearate were purchased from Research-lab Fine Chem. Ind., Mumbai. Cetostearyl alcohol was procured from West Coast laboratories, in Mumbai. While propylene glycol was purchased from Ozone International, Mumbai. Preservative paraben was obtained from Loba Chemie Pvt. Ltd, Palghar.

Synthesis of Microcapsules

Microcapsules of turmeric and jojoba oil were prepared by using the ionic gelation method. In this method, turmeric and jojoba oil are used as active ingredients. Sodium alginate as a shell-forming agent and calcium chloride as a cross-linker, as shown in Table 1.

The complete procedure for the synthesis of microcapsule is shown in Figure 2.

Table 1: Formulation table for microcapsules

S. No.	*Ingredient*	*Quantity*	*Role*
1	Turmeric oil/Jojoba oil	4 mL	Active ingredient
2	Sodium alginate	1 g	Shell forming agent
3	Calcium chloride	15 g	Cross-linker
4	Deionized water	150 mL	Aqueous solvent

Oil phase

In 1-g of sodium alginate was dissolved in 50 mL of water, with continuous magnetic stirring at 1700 rpm for 20 minutes. Then 4 mL of essential oil (Turmeric/Jojoba oil) was added in the solution with continuous stirring for 10 minutes. The oil phase for turmeric and jojoba oil were prepared separately.

Aqueous phase

In 15 g of calcium chloride was dissolved with 50 mL of water, then it was filled into the flask with a volume of 100 mL and made up the final volume with deionized water.

Formation of microcapsule

The prepared oil phase was added dropwise by the syringe into the aqueous phase with continuous stirring. The microcapsules were formed. Further, it was stirred continuously at 1700 rpm for 15 minutes. After that, the prepared microcapsules were filtered by using Whatman filter paper 1 (125 mm) washed 2 to 3 times with deionized water and dried at RT.

Formulation of ointment

The fusion method was used to prepare ointment. Firstly, white soft paraffin, cetostearyl alcohol, Tween 80 and glycerol monostearate melted together by heating on a water bath, stirring and keep the temperature of the mixture at about 75°C. For preparation of the aqueous phase methyl and propylparaben were added to propylene glycol and melted by warming. This aqueous phase was added to the above solution with continuous stirring to form an emulsion, cool and stir thoroughly until it congeals. The synthesized microcapsules of turmeric and jojoba oil were added to the ointment base to form an antiaging ointment. Figure 3 indicates the procedure for the formulation of antiaging ointment.

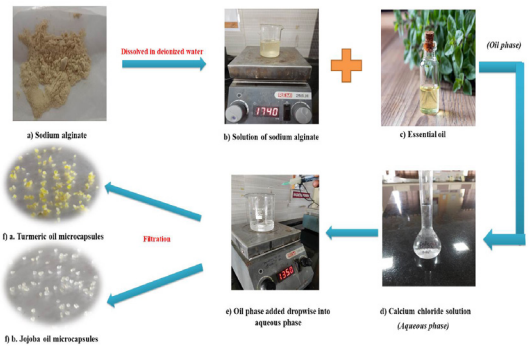

Figure 2: Synthesis of turmeric and jojoba oil microcapsule by ionic gelation method.

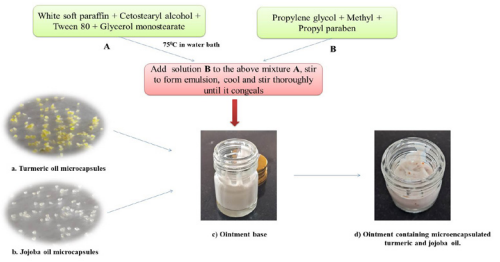

Figure 3: Formulation of antiaging ointment

Formulation table

By varying the concentrations of different excipients 5 formulations were prepared and the formula for all ointments is given in Table 2.

Characterization of Microcapsules

Scanning electron microscopy

The morphological study was performed by scanning electron microscopy (SEM) for turmeric and jojoba oil microcapsules. The microcapsules were coated with gold on a Nova NanoSEM (NPEP303) with an applied voltage 5.00 kV. The photomicrographs were obtained from Image J Software.

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) were used to characterize turmeric and jojoba oil, also microcapsules containing these oils. FTIR spectroscopy was carried out in the range of 4000 to 400cm^{-1}.

Thermogravimetric analysis

The thermogravimetric analysis (TGA) was conducted using the simultaneous thermal analyzer-SDT 650 Instruments. A 10 mg sample (microcapsules) was placed in a cup (alumina) and subjected to heating from RT to 600°C at a rate of 10°C min^{-1}, with a nitrogen flow of 50 cm^3 min^{-1}. The T_{onset}, and

Table 2: Formulation table of antiaging ointment

S. No	*Ingredients*	*Category*	*F_1*	*F_2*	*F_3*	*F_4*	*F_5*
1	Turmeric oil microcapsule	A. P. I	1	1	1	1	1
2	Jojoba oil microcapsule	A. P. I	1	1	1	1	1
3	White soft paraffin	Moisturizer	6.25	6.25	6.25	5	6
4	Cetostearyl alcohol	Emulsifier	5	5	4	3	3
5	Propylene glycol	Humectant	3	3	3	3	3
6	Tween 80	Non-Ionic Surfactant	1	1.5	1	1	1
7	Glycerol monostearate	Emulsifier	0.25	0.25	0.25	0.25	0.25
8	Methyl paraben	Preservative	0.025	0.025	0.025	0.025	0.025
9	Propyl paraben	Preservative	0.025	0.025	0.025	0.025	0.025
10	Purified water	Vehicle	QS	QS	QS	QS	QS

* All quantities in g

Table 3: Thermogravimetric analysis

Sample	*T_{onset} (°C)*	*T_{max} (°C)*
Turmeric oil microcapsule	173.96	285.08
Jojoba oil microcapsule	65.38	378.31

T_{max} values were determined to gain insights into the thermal properties of samples.[21]

Loading efficiency

The TG analysis technique was used to calculate the loading efficiency of turmeric and jojoba oil in microcapsules. The loading efficiency (LE) is calculated through equation I.

$$LE\ (\%) = W_{EO} / W_{Capsule} \times 100\% \quad \ldots (I)$$

Where, W_{EO} and $W_{capsule}$ refer to essential oil weight loss and the initial microcapsule weight, determined by TGA, respectively.

Evaluation of Formulation

The formulation was evaluated for organoleptic characteristics, pH, spreadability, viscosity, microbial study, *in-vitro* drug release study, skin irritation study and free radical scavenging activity.

Organoleptic characteristics

All formulations undergo for the assessment of their physical attributes mentioned in Table 4. Visual observation was used to evaluate these characteristics. Homogeneity and texture were evaluated by squeezing a small quantity of ointment between the thumb and index fingers. The uniformity of the formulations and the detection of coarse particles were considered as criteria for evaluating texture and homogeneity, respectively. The washability of ointment was checked by washing the applied area with tap water.

Determination of pH

pH of the ointment formulations was determined at room temperature by using a calibrated glass electrode pH meter (CONTECH pH- 102). The readings were recorded in triplicate for each of the formulations and the averages of the readings were considered.

Spreadability study

The spreadability of ointments was assessed by evaluating their slip and drag characteristics. Approximately 1-g of the ointment was applied onto a ground slide, forming a layer between this slide and a glass slide. To eliminate air, an 80 g weight was placed on the top of the slides for 5 minutes, resulting in a consistent film of the ointment. Subsequently, the top slide was pulled with a force of 60 g, and the time (in seconds) taken for it to travel a distance of 5 cm was recorded using a string attached to a hook. [1]

Spreadability was calculated by using the following formula

$$S = M \times L / T \quad (II)$$

Where,

S = Formulation Spreadability

M = weight in the pan

L = Length moved

T = Time taken to separate the slide

Viscosity

Rheological characterizations of prepared ointments were performed using Brookfield Viscometer RV DV II+ PRO. The measurements were performed using small volume adapter. Viscosity in centipoise was calculated by multiplying display reading by a factor provided in the Brookfield viscometer user manual.

Table 4: Physicochemical evaluation of ointments

Formulation	*Physical appearance*	*Texture*	*Phase separation*	*Homogeneity*	*Wash ability*
F1	Opaque	Rough	No	Homogenous	Poor
F2	Opaque	Smooth	No	Homogenous	Poor
F3	Opaque	Smooth	No	Homogenous	Good
F4	Opaque	Smooth		Homogenous	Good
F5	Opaque	Smooth	No	Homogenous	Good

Microbial study

Using the streak plate method, the formulated ointment was applied onto plates containing Muller Hinton agar media. As a control, a plate without the ointment was also prepared. Subsequently, the plates were placed in an incubator and kept at a temperature of 37°C for 24 hours. After the incubation period, the plates were removed and the microbial growth was assessed by comparing it with the control plate.

In-vitro drug release profile

Franz diffusion cell apparatus was used for the drug release study. The study was performed as per the method mentioned in Maru *et al*., (2019).[22]

Skin irritation study

In-vivo study was conducted on healthy wistar albino rats weighing 200 to 300 g. The animals were divided into three group each containing six animals. Group I is considered as control and treated with placebo formulation, group II is considered as standard and treated with marketed anti-aging ointment 2% w/w, group III, is treated with formulation (ointment) 2% w/w.

The back and flanks of each rat were completely shaved 24 hours before to the test. The optimized (F4) formulation was evenly applied on skin and the reaction at site of application will be observed.

Erythema and edema will be assessed at the first hour of dosing and first day it checked every hr for the first 12 hours. and then every 12 hours for the next 7 days.

%Free radical scavenging activity

The %free radical scavenging activity was carried as per the procedure mentioned in Harmita *et al*., (2020)[23] and it was get calculated by using the following formula:

$$\%\ RSA = Abs.\ of\ ctr. - Abs.\ of\ sample\ /Abs.\ of\ ctr. \times 100$$

Accelerated stability studies

The stability investigation was conducted in accordance with ICH guidelines focusing on Q1 A (R2), which addresses the stability testing of both new drug substances and products. The ointment was stored in air-tight glass containers. Samples were maintained in a stability chamber under accelerated conditions with humidity and temperature control.

RESULTS

Characterization of Microcapsules

SEM study

SEM was done to determine the shape and surface morphology of microcapsules. As shown in Figure 4, the microcapsules of turmeric (a), (b) and jojoba oil (c), (d) shows nearly spherical shape with smooth and porous surface. The size obtained from SEM was 400 µm to 1000 µm. The size of the microcapsule is an important parameter because it affects the stability and release of active ingredients from microcapsules.

Therefore, the smooth surface morphology of microcapsules has proven to be advantageous for protecting and sustaining the release of the inside core material.

FTIR study

The FTIR spectra of turmeric oil, jojoba oil and their prepared microcapsules are shown in Figures 5 and 6.

As observed in Figure 5 (a) and (b), the broad band around 3019 and 2920 cm^{-1} indicates C-H (aromatic) and C-H stretching (amide) is evident at 1684 cm^{-1}. The stretching vibration associated with C-O bond (esters) in the materials is evident at 1113 cm^{-1}, and the band at 1036 cm^{-1} aligns with the vibration of C-O groups. The bending vibration observed at 3420 cm^{-1} is corresponds to sodium alginate which is evident for the successful encapsulation of turmeric oil.

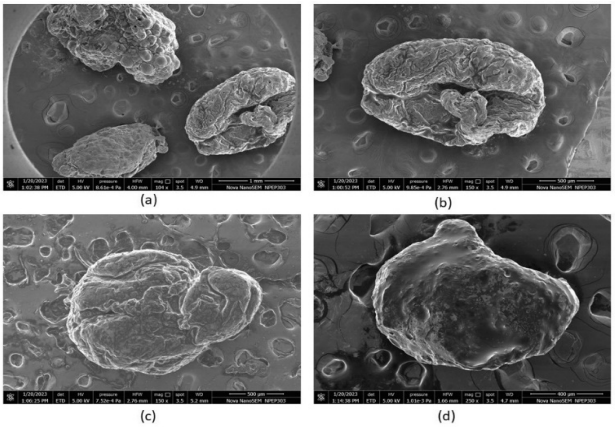

Figure 4: SEM images of turmeric oil microcapsules (a), (b) and jojoba oil microcapsules (c), (d).

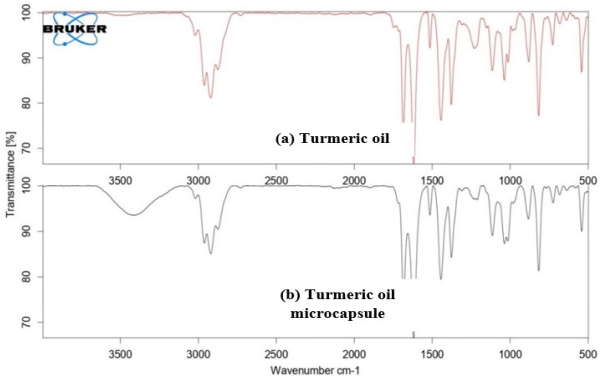

Figure 5: FTIR spectra of (a) Turmeric oil and (b) Turmeric oil microcapsule

For jojoba oil microcapsule functional groups observed are as follows. As shown in Figure 6 (a) and (b) the bands at 2922 and 2852 cm^{-1} are relative to the stretching of CH bonds. The presence ester (carbonyl) and alkene are evident at 1738 and 1654 cm^{-1}, respectively. The stretching vibration associated with the C-O bond of esters within the core materials is observable at 1170 cm^{-1}, and the band at 1017.96 cm^{-1} aligns with the vibration of the C-O group in the oil. The bending vibration observed at 3420 cm^{-1} is corresponds to sodium alginate which is evident for the successful encapsulation of jojoba oil.

TGA analysis

TGA was used to verify the stability of microcapsule and given oils. The curve is presented in Figure 7 and T_{onset} and T_{max} are presented in Table 3. Two thermal events were seen in the microcapsules containing EO, one linked to the sodium alginate polymer degradation and the other caused by the presence of the EO. These events are depicted at lower temperatures and are highlighted in Figure 7. It is possible to observe that turmeric and jojoba oil microcapsules presented T_{onset} of 173.97 and 65.38°C, respectively. It is possible to see a greater temperature

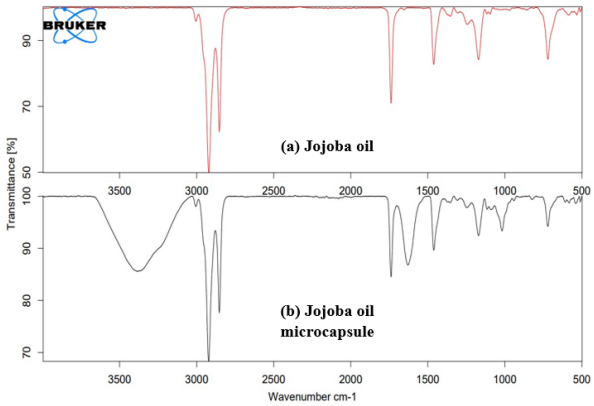

Figure 6: FTIR spectra of (a) Jojoba oil and (b) Jojoba oil microcapsule.

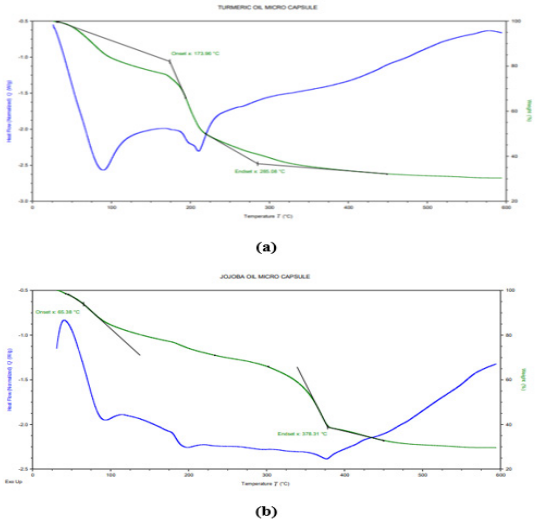

Figure 7: Thermal curves, for (a) Turmeric and (b) Jojoba oil microcapsules

(about 20°C) for the start of the oil degradation process in comparison to the pure EO. It implies that the microcapsules preserve the EO against thermal deterioration. Compared to pure EO, the T_{max} value for microcapsules is lower. It is critical to keep in mind that EO, sodium alginate, and water may be responsible for these outcomes.

Loading efficiency

The essential oil loading capacity was evaluated by the temperature curves seen on TGA curves of microcapsules using the following equation I

$$LE\ (\%) = W_{EO} / W_{Capsule} \times 100\% \quad \ldots\ldots. (I)$$

The loading efficiency of turmeric oil and jojoba oil was found to be 71 and 76%, respectively.

Evaluation of Formulation (Ointment)

Organoleptic characteristics

The organoleptic characteristics of ointment are given in Table 4. The ointments were all homogenous, with no evidence of phase separation, and the results demonstrated that they had a good appealing appearance and smooth texture. They have poor to good washability.

Determination of pH

All formulations pH levels were found within the allowable range of 5.24 ± 0.01 to 5.76 ± 0.02, which is shown in Table 5. All formulation has pH levels that are within the usual range for skin.

Spreadability study

There are three types of ointment spreadability: low, medium, and high. The spreadability of each formulation was evaluated and it was found that formulation F4 spreads more easily than the others (Table 6).

Table 5: pH of antiaging ointment formulations

Formulation	pH			Mean SD
	T1	T2	T3	
F1	5.73	5.75	5.72	5.730.015
F2	5.24	5.23	5.25	5.240.01
F3	5.76	5.74	5.78	5.760.02
F4	5.74	5.75	5.77	5.750.015
F5	5.52	5.51	5.51	5.510.005

T: Trial, SD: Standard deviation.

Table 6: Spreadability of antiaging ointment Formulations

Formulation	*Spreadability*			*Mean SD*
	T1	*T2*	*T3*	
F1	37.5	36.58	37.97	37.350.70
F2	73.17	71.42	75	73.19
F3	85.71	88.23	83.33	85.752.45
F4	115.38	107.14	111.11	111.214.12
F5	96.77	90.90	90.90	92.853.38

T: Trial, SD: Standard deviation.

Viscosity

Table 7 displays the results of measuring the viscosity of each formulation, which was found to range from 2845.66 ± 11.01 to 4335 ± 37.74 cps at 100 rpm. The standard deviation and average of the three readings were calculated.

Microbial study

When optimized formulation (F4) was tested for growth of microbes, after incubation of 24 hours it was found that there is no growth of microbes. So, the ointment formulation is safe to use for skin (Figure 8 and Table 6).

In-vitro drug release study

As shown in Figure 9, the outcome of Franz diffusion cell experiment showing the *in-vitro* release pattern of ointment shows that, after 6 hours 68.81 and 74.81% of the drug was released from turmeric oil and jojoba oil, respectively. From the in vitro drug release, we can say that formulated ointment shows sustained release.

Skin irritancy test

The optimized formulation (F4) shows no redness, edema, inflammation, and no irritation during these studies compared with standard marketed formulation. These formulations are safe to use for the skin (Figure 10).

Table 7: Viscosity of antiaging ointment formulations

Formulation	*Viscosity*			*Mean± SD*
	T1	*T2*	*T3*	
F1	3540	3505	3520	3521.66
F2	3800	3780	3815	3798.33
F3	4375	4330	4300	4335
F4	3600	3595	3610	3601.66
F5	2835	2857	2845	2845.66

T: Trial, SD: Standard deviation.

Table 8: Stability study of optimized formulation (F4)

Condition	*pH*	*Spreadability*	*Viscosity*
25°C ± 2°C/ 60% ± 5% RH	5.770.02	111.680.60	360013.22
30°C ± 2°C/ 65% ± 5% RH	5.760.015	111.720.54	3602.336.42
40°C ± 2°C/ 70% ± 5% RH	5.780.005	111.740.31	3603.335.85

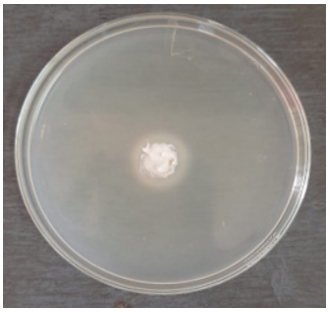

Figure 8: Microbial study

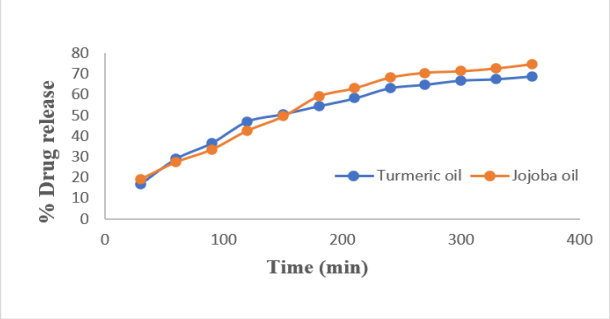

Figure 9: Percent amount of turmeric and jojoba oil diffused from ointment

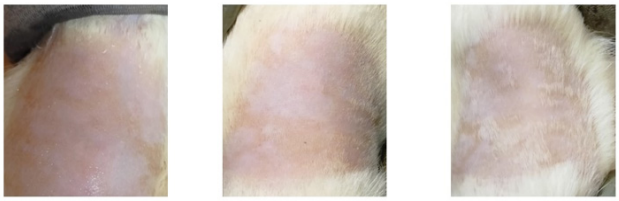

Figure 10: a) Before application; b) 1 hour After application c) After 7 days of continual application

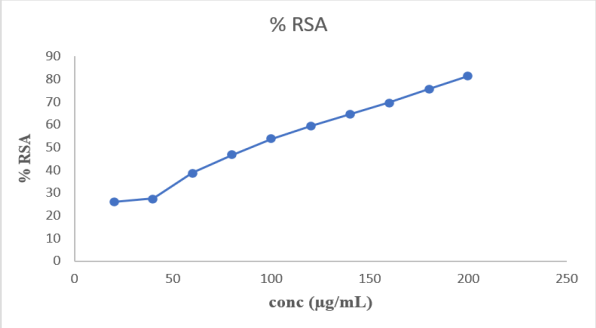

Figure 11: % Radical scavenging activity of ointment

DPPH assay

The ability of ointment to scavenge free radicals was estimated by adding DPPH to various concentrations of 20 to 200 µg/mL of ointments, which was examined using UV-visible spectroscopy at 517 nm. As seen in Figure 11, with the increase in concentration, rise in free radical scavenging capacity was seen, going from 25.92 to 81.25%. A progressive color change form purple to yellow was seen while treating DPPH with ointment.

Stability study

The optimized formulation (F4) was evaluated to stability study as per ICH guidelines and results are shown in Table 8. This study indicates that there was no significant fluctuation in pH, spreadability, and viscosity.

DISCUSSION

The developed ointment formulations were evaluated using different parameters and the results were within the permitted ranges, as shown in Tables 4 to 7. The pH of all formulations was found to be slightly acidic, which is equivalent to healthy skin. Based on viscosity, each formulation exhibits pseudoplastic flow. In comparison to other formulations, F4 has a higher spreadability. Based on the results of spreadability, viscosity and texture the formulation F4 was chosen as the optimized formulation. The ointment was made with turmeric oil and jojoba oil which is a model drug. It shows sustained drug release, after 6 hours 68.81% of turmeric oil and 74.81% of jojoba oil were released from ointment through the cellulose membrane. The optimized formulation (F4) showed no evidence of redness on the rat skin, as shown in Figure 10. The result of DPPH assay indicates that formulated ointment has strong antioxidant activity. Accelerated stability study indicates that optimized formulation is stable for period of 1 month.

CONCLUSION

In the modern world, the aging problem has become a serious issue for people. They consequently began to rely on anti-aging cosmetics. However, they had server-side effects, so they were forced to rely on cosmetics made with herbal substances because they had fewer side effects. The addition of microcapsules containing turmeric oil and jojoba oil has enhanced the anti-aging attributes of ointment. These microcapsules were formulated with the aim of improving stability, regulating the gradual release of active components, and prolonging the overall shelf life of ointment. Due to the microencapsulation of oils, it shows sustained drug release in ointment formulation. The different methods for characterization of microcapsules and ointment were performed and the results obtained from evaluation were good. The DPPH assay indicates that formulated ointment has strong antioxidant properties. The outcome of all evaluations demonstrated the successful formulation of anti-aging ointment which contains microencapsulated turmeric and jojoba oil.

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