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Research Article

Formulation and Evaluation of a Sunscreen Ingredient

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ABSTRACT

Sunscreen is essential to protect skin from harmful Ultraviolet (UV) radiation, which can cause oxidative stress, aging, and skin cancer. UV exposure generates Reactive Oxygen Species (ROS), increasing damage risk, especially in children. Ascorbic acid and Tocotrienols offer potent antioxidant protection by neutralizing ROS. Natural vegetable oils like Olive oil, Almond oil and Sesame oil contribute to SPF, nourish skin, and improve vitamin penetration. Using such oils reduces the need for organic UV filters, minimizing adverse effects and meeting consumer demand for safe and natural sunscreen. The aim of the present research work was to develop a "Sunscreen ingredient" that can be incorporated in any base to form a "Sunscreen" for kids (age 12 years and onwards). Oil- in- Water (O/ W) emulsion was prepared as Sunscreen Ingredient. Emulsion preparation was carried out in multiple steps like, selection of surfactant combination, determination of quantity of oil that can be loaded in emulsion, minimizing quantities of surfactant mixture in emulsion and optimization of oil ratio for maximizing SPF. The Optimized Sunscreen ingredient was formulated and incorporated in the Vanishing cream base, thus developing Prototype formulation. Both Optimized Sunscreen ingredient and Prototype formulation were subjected to various evaluation tests like in-vitro SPF, antioxidant activity, pH, accelerated stability studies, etc. Both demonstrated satisfactory results.

INTRODUCTION

The skin, referred to as the cutaneous membrane, is the body's outer layer.[1] While barrier integrity matures by six years of age, complete physiological and microbiological maturation extends into later childhood.[2, 3, 4, 5] Of the UV radiation reaching Earth, about 95% is UVA and 5% is UVB. UVA causes skin aging and tanning, while UVB leads to sunburn by penetrating deeper layers of skin. Both types generate ROS, initiating photochemical reactions and photosensitization.

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Prolonged UV exposure is linked to eye disorders, immune suppression, and skin cancers. UV radiation induces DNA mutations essential for carcinogenesis. Chronic exposure causes hyperpigmentation, collagen degradation, and mutation accumulation, with 86% of melanomas and 90% of non-melanoma skin cancers attributed to UV exposure. UVB (280–320 nm) causes DNA lesions like Cyclobutane pyrimidine dimers (CPDs) and Pyrimidine-pyrimidone (6-4) photoproducts, disrupting replication and transcription. UVA (320–400 nm) is needed in small amounts for circadian rhythm regulation but at high doses suppresses immunity and promotes ROS formation. ROS damages DNA, lipids, and proteins, contributing to photoaging and cancer. Melanin protects against UV but can act as a pro-oxidant under exposure, leading to CPD formation in melanocytes and facilitating melanoma.[6] Melanocytes and Interfollicular epidermal stem cells are present in the basal layer of skin. When these cells get high UV exposure in childhood, risk of developing skin cancer later in life increases. This is because epidermal stem cells have lifelong persistence, thus being an ideal site for carcinogenic effect. Also such UV exposure serves to be a starting point for melanocytic nevi development.[7] Skin sensitivity to UV varies by skin tone. Darker skin has greater resistance to sunburn and better UV tolerance but remains susceptible to damage. The World Health Organization (WHO) recommends using sunscreen along with protective clothing, hats, sunglasses, and shade. Daily sunscreen use helps prevent premature aging and skin cancer. Hence, it is recommended to use sunscreens daily, irrespective of skin type.[8]

All organic sunscreen agents can cause adverse effects like irritation, allergic contact reactions, photoallergy, and phototoxic effects.[9] Zinc oxide (ZnO) and Titanium dioxide (TiO_2) are widely used inorganic UV filters. However, ZnO's thicker consistency may cause clogged pores in certain people, particularly when used with other comedogenic substances. For those with acne-prone skin, this may result in outbreaks or skin irritation.[10] For TiO_2 the two main issues raised were the possibility that TiO_2 nanoparticles could produce ROS in response to UV light, which could harm skin cells' DNA, and the nanoparticles' ability to penetrate the skin.[11]

Vitamin C is a powerful antioxidant found in the aqueous compartment of the cells. It counteracts oxidative damage caused by a variety of causes, most prevalent of which is UV damage. Deep stratum corneum layers contain a large amount of Vitamin E, which serves as the skin's main defence against oxidative stress brought about by UV radiations. Topically Vitamin E shields the skin from UV- induced cutaneous damage as well as chemical agent's carcinogenic and mutagenic properties.[12] According to one study, tocotrienols have 1600 times the antioxidant capacity of alpha tocopherol. According to some reports, the unsaturation in the aliphatic tail of tocotrienol may be responsible for its superior antioxidant activity by facilitating easier tissue penetration.[13]

In the current research work attempt is being made to develop a sunscreen ingredient that can be incorporated in any base to develop Sunscreen formulation making use of antioxidants and vegetable oils. A blend of vegetable oils is expected to reflect the UV rays, thus contributing to the Sun Protection Factor (SPF). L- Ascorbic acid and Tocotrienol will contribute to antioxidant activity of "Sunscreen ingredient", this will take care of oxidative stress generated due to UV radiations exposure. Kaolin being an inorganic sunscreening agent, will help to improve the SPF. Also, it will aid in the stability of "Sunscreen

ingredient" which is Oil- in- Water (O/W) emulsion by acting as a suspending agent.

MATERIALS AND METHODS:

Materials:

Gift sample of Tocotrienol with brand name Orah Vit E (OE) was obtained from Orah NutriChem Pvt. Ltd., Pune. L- ascorbic acid (AA) was procured from Molychem, Mumbai. Olive oil, Almond oil, Sesame oil were purchased from Vishal Chem, Mumbai. Glycerin, Propylene glycol, Kaolin, Tween 80, Span 80, Stearic acid and Potassium hydroxide were procured from renounced sources.

Methods:

1. **Formulation of Sunscreen Ingredient (SI)**

Step 1: Determination of ratio of surfactant mixture (S_{mix})

Hydrophilic Lipophilic balance (HLB) value was determined assuming ratio of oils as 5: 3: 2 for Olive oil: Almond oil: Sesame oil and considering required HLB (RHLB) values of individual oils.

HLB of Oil blend = (Parts of Olive oil x RHLB of Olive oil) + (Parts of Almond oil x RHLB of Almond oil) + (Parts of Sesame oil x RHLB of Sesame oil)

HLB of Oil blend = 6.7

Thus, tentatively Oil blend was assumed to have RHLB of 6.7.

Now, by doing theoretical calculations the ratio of surfactant quantities was determined for getting HLB of 6.7.

Step 2: Procedure followed for developing emulsion

Aqueous phase consisted of AA, Kaolin, Tween, Glycerin, Propylene glycol and Water. Aqueous phase was stirred for 10 min. for uniformly dispersing Kaolin. Kaolin was weighed separately, then sieved from sieve no. 100 to break lumps, make it free flowing and then transferred to aqueous phase. The oil phase consisted of a blend of oils, OE and Span. Oil phase was stirred manually and added drop wise to aqueous phase. This was followed by stirring for 30 min. on a magnetic stirrer. Resultant course emulsion was subjected to probe sonication for 15 min., with 5 sec. pulse cycle.

Step 3: Determination of Concentration of the internal phase that can be loaded in emulsion

Various batches of emulsion were prepared having different concentrations of oil with 5% (w/w) S_{mix}. Emulsions were then exposed to stress conditions. Those stress conditions were Centrifugation (1000 RPM, 20 min.), Accelerated conditions (40°C, 75 % RH; 1 week) and Freeze Thaw cycles (48 hrs each, 3 cycles; freezing at 0°C and thawing at room temperature). The objective was to maximize the quantity of oils in emulsion.

Step 4: Minimizing the S_{mix} concentration

Emulsions were prepared of the selected S_{mix} by gradually reducing concentration of S_{mix} in them. Developed emulsions were exposed to above mentioned stress conditions to evaluate their stability. The objective was to minimize the quantity of S_{mix} in emulsion.

Step 5: Optimization of oil ratio

Using 2^3 Factorial design ratio of olive oil: almond oil: sesame oil was optimized, so that maximum SPF can be achieved. Output factor/ Response was *in- vitro* SPF. Input factors with their levels are summarized below,

Table no. 1: Coded and Uncoded levels of Factors for Factorial design

Name of oil	Low level (Coded)	Low level (Actual) Parts	High level (Coded)	High level (Actual) Parts
Olive oil	-1	3	+1	7
Almond oil	-1	1	+1	5
Sesame oil	-1	1	+1	3

Step 6: Development of Optimized emulsion, Sunscreen Ingredient

By executing experimental design and analyzing data using StatEase software, software recommended a 7: 1: 1 ratio of Olive oil: Almond oil: Sesame oil for achieving maximum SPF. Using oils in said ratio and executing procedure given in *Step: 2* optimized emulsion was developed in triplicates.

2. Formulation of Prototype formulation (PF)

Step 1: Formulation of Vanishing cream base

Vanishing cream base was used to develop PF. Formula of Vanishing cream given in literature (14) was modified to achieve desired organoleptic characteristics in final formulation. Stearic acid and Potassium hydroxide in formula will generate in- situ emulsifier, glycerin will act as humectant in the formulation. Modified formula for vanishing cream base is as follows:

Table no. 2: Formula of Vanishing Cream base

Name of Ingredient	Quantity (% w/ w)
Stearic acid	45
Potassium Hydroxide	0.8
Glycerin	8
Water	Q. S. to 100

Procedure: Aqueous phase and Oil phase were prepared separately. Aqueous phase consists of Potassium hydroxide, glycerin and water. The oil phase has Stearic acid. Required quantities of ingredients were weighed and transferred into respective beakers. Both beakers were heated in a water bath until stearic acid melts completely. Once both phases were liquid, the aqueous phase was transferred to the Oil phase. This was followed by vigorous stirring until formulation cooled. Thus, forming a vanishing cream base.

Step 2: Blending of Vanishing cream base and SI

It was predecided that base and ingredient will be mixed in 1: 1 ratio. For that purpose in one beaker vanishing cream base was weighed. Pre-formulated SI was transferred into the same beaker in small portions followed by mixing (Geometric dilution). This ensured uniform and thorough mixing. Once all the quantity of SI was added, the resultant mixture was stirred for 10 to 15 min. Thus, developing the Prototype formulation.

3. Evaluation of SI and PF

a. ***Organoleptic properties (Color, Odor):*** SI and PF were subjected to evaluation of color and odor. Visual observation was performed to record color. Identification of the odor was performed by manual assessment.

b. ***Texture:*** PF was evaluated for its texture by rubbing a small quantity of formulation over skin.

c. ***pH:*** 1 % w/v solution of SI and PF were made in distilled water. pH was determined using Digital pH meter. To prepare 1 % w/v solution 0.5 gm of each was weighed in a beaker and diluted with 50 ml of distilled water.

d. ***Dilution test[15]:*** A dilution test was performed to determine the type of emulsion. A small

amount of SI was mixed with water in one beaker and another small portion with oil in another beaker.

e. ***Homogeneity[15]:*** The PF was tested for Homogeneity by visual appearance and by touch.

f. ***Spreadability:*** To determine spreadability of PF two clean glass slides were used. On one of the glass slides 50 mg of formulation was placed. Over this slide another clean glass slide was placed. Now over these two slides weight of 100 gms was placed. Sample was allowed to spread for 5 min. Then by removing weight, spread of sample was recorded from three different sites and averaged to get spreadability of sample.

g. ***Washability:*** Glass slides used for spreadability testing of PF were used for accessing washability of formulation. A glass slide was held under running tap water to evaluate washability.

h. ***Viscosity[15]:*** PF's viscosity was measured using Brookfields viscometer spindle 7. Three replications of the measurements were made at 100 rpm. Viscosity was calculated in Centipoise (cP). Formula to calculate viscosity is given below,

Viscosity (cP) = Dial reading x Factor

i. ***In- vitro SPF determination[16, 17]:*** *In- vitro* SPF values were recorded for both PF and SI. 0.1 gm of sample was diluted to 10 ml with Ethanol: Water (40: 60) to get 1 % w/v solution. 1 ml of this 1 % w/v solution was diluted to 10 ml with the same solvent to get 0.1 % w/v solution. Absorbance of this 0.1 % w/v solution was recorded from 290 nm to 320 nm, at 5 nm intervals. Then using the Mansur mathematical equation and reported constants, SPF was calculated. Values of EE (**λ**) x I (**λ**) are constant and reported in literature.

$$SPF = CF \times \sum_{290}^{320} \times Abs \times EE \times I$$

Where,

- CF = Correction factor (10)
- EE (**λ**) = Erythmogenic effect of radiation with wavelength **λ**
- Abs (**λ**) = Spectrophotometric absorbance values at wavelength **λ**
- I (**λ**) = Intensity of solar light of wavelength **λ**

Table no. 3: Values of EE (λ) x I (λ) [16]

Wavelength (λ) (nm)	EE(λ) x I (λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
310	0.0837
320	0.0180

j. ***Antioxidant study, DPPH (2, 2- Diphenyl-1-picrylhydrazyl) method[12]:*** Antioxidant study was performed for both PF and SI. 1% w/v solution of sample was prepared by dissolving 0.1 gm in 10 ml Ethanol. 100 ppm concentration solution of DPPH was prepared in ethanol. In a test tube, 1 ml sample solution was mixed with 2 ml DPPH solution. The test tube was covered and subjected to sonication for 10 min. in a Bath sonicator. This was followed by incubation of samples at room temperature for 30 min. A Blank/ Control solution was also prepared. Post incubation absorbance of blank and sample were recorded at 517 nm, and anti- oxidant activity calculated using given formula,

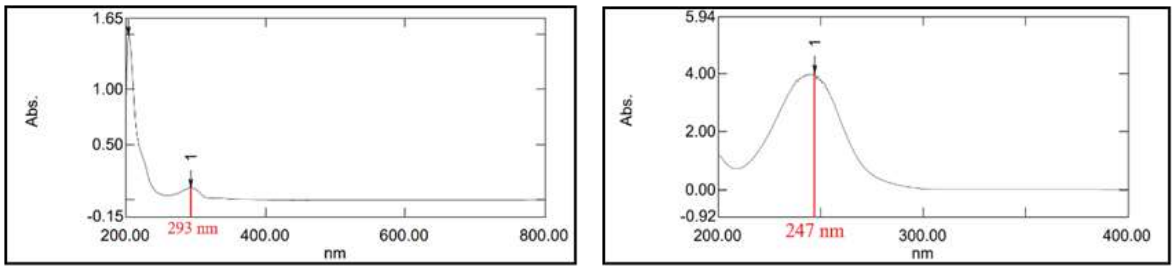
Antioxidant activity (%) =

$$\left(\frac{Absorbance\ of\ Blank - Absorbance\ of\ sample}{Absorbance\ of\ Blank}\right) \times 100$$

k. ***Particle Size and Polydispersity Index (PDI):*** SI was subjected to evaluation of particle size and PDI. 0.1 gm of emulsion was diluted with double distilled water to 10 ml, to get 1 % w/v solution. 1 ml of 1 % w/v solution was diluted to 10 ml to get 0.1 % w/v solution. This 0.1 % w/v solution was used for analysis. Before analyzing, samples were filtered from a 0.45 µm pore filter.

l. ***Zeta potential determination:*** SI was subjected to evaluation of zeta potential. Sample prepared for Particle Size and PDI determination was used for determination of Zeta potential.

m. ***Assay (for AA and OE content):*** 10 mg of PF was weighed and diluted to 10 ml with methanol to get stock solution. 4 ml of stock solution was diluted to 10 ml, this solution was used to record absorbance at 293 nm to quantitate OE in sample solution. 0.1 ml of stock solution was diluted to 10 ml, this solution was used to record absorbance at 247 nm in-order to quantitate AA. OE has maximum absorbance at 293 nm and AA at 247 nm in methanol. UV Visible spectrum revealed wavelength of maximum absorption of AA and OE.



{a} {b}

Fig. no. 1: UV Visible spectrum {a}: OE, {b}: AA

n. **In- vitro SPF determination according to COLIPA- 2011 guidelines:** A sample of 5 gms of PF was given for *in- vitro* SPF determination at KET's Scientific research center, Mulund.

4. Accelerated Stability Studies of SI and PF:

SI and PF were kept for Stability studies under Accelerated conditions, 40°C and 75 % RH for a duration of 45 days. Samples of both were collected periodically and analyzed. The FTIR spectrum of samples taken on day 1 and day 45 were compared, to comment on content of AA and OE in formulation. For that purpose, a small quantity of sample was solubilized in Chloroform and spread over a blank KBr pellet, then FTIR spectrum was recorded.

RESULTS:

1. Formulation of SI

Determination of ratio of surfactant mixture (S_{mix})

Following were the three ratios that were screened for emulsion development,

Span 80: Tween 20= 81: 19	Span 80: Tween 60= 77: 23	Span 80: Tween 80= 78: 22

Determination of Concentration of the internal phase that can be loaded in emulsion.

Results of stability on exposure to stress conditions of various batches of emulsion are

summarized below. Based on the results it was observed that, S_{mix} of Tween 80 and Span 80 gave stable emulsion with 40 % w/ w oil. However this emulsion showed separation of one oil droplet over the surface. Thus, this surfactant combination was chosen for development of SI and the quantity of oil in emulsion was reduced to 36 % w/ w.

Table no. 4: Stability data of various batches of emulsion prepared with different concentrations of oil

Surfactant combination	S_{mix} (% w/w)	Oil (% w/w)	Internal Phase (% w/w)	Post Centrifugation (1000 RPM, 20 min.)	Post Accelerated conditions exposure (40◦ C, 75 % RH, 1 week)	Post Freeze Thaw cycles (3, each of 48 hrs.)
Tween 20: Span 80	5	20	24	Stable	Stable	Stable
Tween 60: Span 80	5	20	24	Stable	Phase separation	Signs of Phase separation
Tween 80: Span 80	5	20	24	Stable	Stable	Stable
Tween 20: Span 80	5	40	44	Stable	Phase separation	Stable
Tween 80: Span 80	5	40	44	Stable	Stable	Stable (an oil droplet over surface)
Tween 80: Span 80	5	45	49	Phase separation	-	-

Minimizing the S_{mix} concentration

Attempt was made to reduce surfactant concentration ensuring that maximum quantity of oil is being loaded in emulsion with minimum S_{mix} concentration, such that resulting emulsions are stable enough. For that purpose emulsions having 40 % oil phase were prepared by gradually reducing the concentration of S_{mix}. Stability data for this study is summarized in table no. 5.

Table no. 5: Stability data of various batches of emulsion prepared with different concentrations of Smix

Surfactant combination	S_{mix} (% w/w)	Oil (% w/ w)	Internal Phase (% w/ w)	Post Centrifugation (1000 RPM, 20 min.)	Post Accelerated conditions exposure (40◦ C,75 %RH, 1 week)	Post Freeze Thaw cycles (3, each of 48 hrs.)
Tween 80: Span 80	5.0	36	40	Stable	Stable	Stable
Tween 80: Span 80	4.5	36	40	Stable	Stable	Stable
Tween 80: Span 80	4.0	36	40	Phase separation	-	-

From this study it was observed that 5.0 % w/ w and 4.5 % w/ w S_{mix} gave emulsions which were stable under multiple stress conditions. Emulsion with 4.0 % w/ w S_{mix} showed phase separation on centrifugation. Thus, the S_{mix} concentration was decided to be kept 4.5 % w/ w.

Optimization of oil ratio

StatEase software was used for optimization. Experimental design with response values was as follows,

Table no. 6: Experimental design with response values

Std	Run	Factor A: Olive oil (Parts)	Factor B: Almond oil (Parts)	Factor C: Sesame oil (Parts)	Response 1 *In- vitro* SPF
3	1	3	5	1	19.6042
6	2	7	1	3	18.2439

2	3	7	1	1	20.9450
7	4	3	5	3	20.6036
5	5	3	1	3	20.0470
4	6	7	5	1	19.0454
1	7	3	1	1	20.6826
8	8	7	5	3	19.4700

Software provided a Pareto chart. In that Pareto chart bars of A, B, C and AC were of blue color suggesting that the effect of these factors is Negative on the response. Bars of BC and ABC were of orange color suggesting that these factors have Positive effect on response.

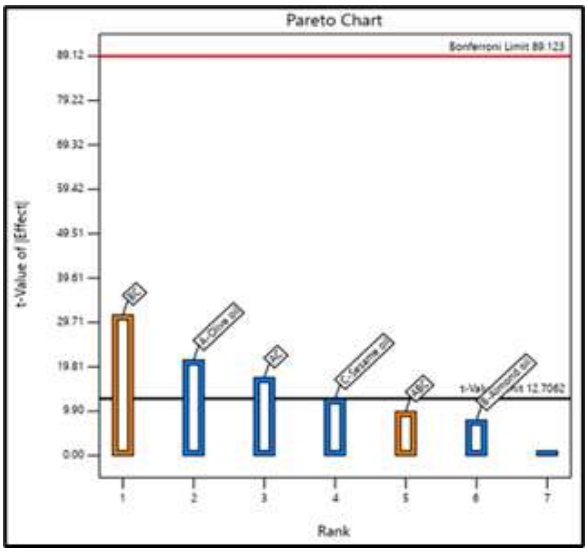


Fig. no. 2: Pareto chart

Software generated ANOVA table for the data which is given below (Table no. 7). Model F- value of 343.27 implies that the model is significant. Model terms A, AC and BC have their P- value less than 0.0500, indicating that those model terms are significantly affecting the response i.e. *in- vitro* SPF.

Table no. 7: ANOVA table

Source	Sum of Square	df	Mean Square	F value	P value	
Model	5.9200	6	0.9875	343.27	0.0413	Significant
A- Olive oil	1.3100	1	1.3100	454.22	0.0298	
B- Almond oil	0.1786	1	0.1786	62.08	0.0804	
C- sesame oil	0.4573	1	0.4573	158.97	0.0504	
AC	0.8714	1	0.8714	302.93	0.0365	
BC	2.8300	1	2.8300	984.85	0.0203	
ABC	0.2778	1	0.2778	96.56	0.0646	
Residual	0.0029	1	0.0029			
Cor Total	5.9300	7				

Software also provides equation in terms of Coded factors which helps to predict the relative effect of each factor, interaction factor and higher order interaction factor on the response by comparing the factor coefficients. Coded equation is,

Y = 19.83 - 0.4041A - 0.1494B - 0.2391C - 0.3300 AC + 0.5951BC + 0.1863ABC

2D Contour plots and 3D Surface response plots were generated by the software giving graphical representation of the relationship between factors and response variable.

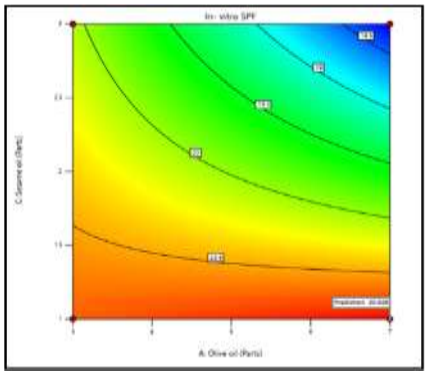


Fig. no. 3: 2D Contour Plot for factor A and factor C

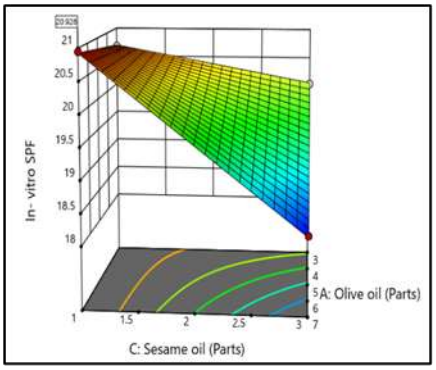


Fig. no. 4: 3D Surface Response plot for factor A and factor C

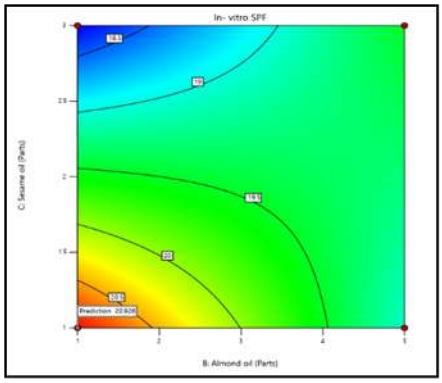


Fig. no. 5: 2D Contour Plot for factor B and factor C

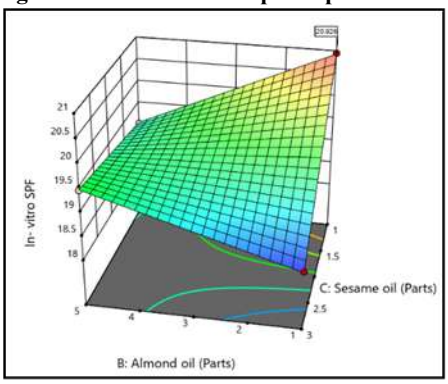


Fig. no. 6: 3D Surface Response plot for factor B and factor C

Overlay plots were obtained which depicted the design space according to the desired criteria (maximum *in- vitro* SPF). Yellow region is the design space. 64 solutions were provided by the software, from which the most suitable solution was adopted as the optimized batch. It was ensured that the chosen solution has desirability close to 1.

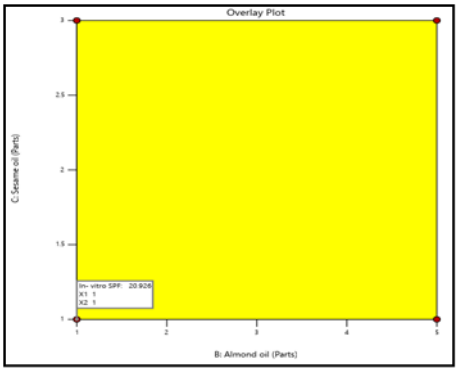


Fig. no. 7: Overlay plot for factor B and factor C

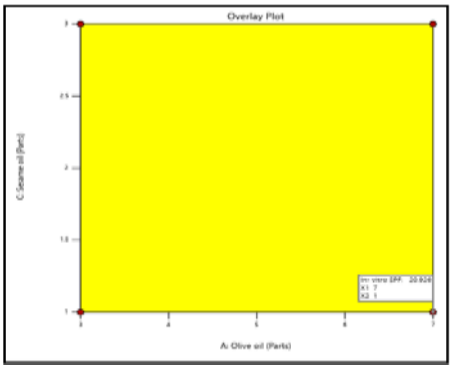


Fig. no. 8: Overlay plot for factor A and factor C

Table no. 8 illustrates the Fit Statistics data. The adjusted R^2 (0.9966) is in reasonable agreement with the predicted R^2 (0.9689), i.e., the difference is less than 0.2. Thus, it can be inferred that the model is significant. Adeq Precision measures signal to noise ratio. The Adeq precision value is 53.8389 which is greater than the threshold value of 4 and thus it indicates the model can be used to

navigate the design space. The C.V. % is 0.2705 which is less than 10%. This indicates that the model is reproducible. Table no. 9 gives the Optimized oil ratio, the predicted response values, and the experimental response values.

Table no. 8: Fit Statistics

Std. Dev.	0.0536	**R^2**	0.9995
Mean	19.83	**Adjusted R^2**	0.9966
C. V. %	0.2705	**Predicted R^2**	0.9689
		Adeq Precision	53.8389

Table no. 9: Optimized oil ratio

Independent variables		**Dependent variables**		
Factor	**Quantity (Parts)**	**Response**	**Predicted value**	**Experimental value**
Olive oil	7	*In- vitro* SPF	20.926	19.923 ± 0.790 (N=3)
Almond oil	1			
Sesame oil	1			

The results depict that the response values predicted by the software have been reproduced and hence the oil ratio 7: 1: 1 was concluded to be the Optimized oil ratio.

Through laboratory work and by analysing statistical data given by the StatEase software, a formula of the SI was developed which is given in table no. 10. Orah Vit E and L- ascorbic acid work as antioxidant, oil blend as Sunscreen agent, Glycerin and Propylene glycol as humectant, Span and Tween 80 as emulsifier, Kaolin plays dual role, it acts as Sunscreen agent and emulsion stabilizer. Optimized SI was formulated in triplicates and evaluated.

Table no. 10: Formula of SI

Ingredients	**Quantity (% w/ w)**
Orah Vit E	4.00
Olive oil	28.00
Almond oil	4.00
Sesame oil	4.00
L- ascorbic acid	2.00
Kaolin	1.00
Glycerin	10.00
Propylene glycol	10.00
Span 80	3.51
Tween 80	0.99
Water	Q. S. to 100

Fig. no. 9: Optimized SI formulated in triplicates

2. Evaluation of SI

Table no. 11: Results of evaluation of SI

Parameter	**Result**
Organoleptic Properties: Color	White
Organoleptic Properties: Odor	Slight and unpleasant
pH	3.69 ± 0.044
Dilution Test: With water	No phase separation
Dilution Test: With oil	Phase separation observed
In-vitro SPF Determination	19.923 ± 0.790
Antioxidant Study (DPPH method)	90.767 ± 0.549 %
Particle Size	179.867 ± 32.690 nm
PDI	0.118 ± 0.082
Zeta Potential	-77.733 ± 1.514 mV

3. Formulation and evaluation of PF

Formulation:

Vanishing cream was decided to be used as a base for developing sunscreen cream. For that purpose a vanishing cream base was prepared.

Fig. no. 10: Vanishing cream base

Fig. no. 11: Prototype formulation

Evaluation:

Table no. 12: Results of evaluation of PF

Parameter	Result
Organoleptic Evaluation	White color, odorless, smooth texture
Homogeneity	Achieved
pH	6.023 ± 0.015 (N=3)
Spreadability	2.113 ± 0.196 cm (N=3)
Washability	Easy with slight rubbing
In-vitro SPF Determination	12.725
Antioxidant Study (DPPH Method)	88.941 %
Viscosity	2466.667 cP (N=3)
Assay (AA and OE content)	Unable to quantitate
In-vitro SPF (COLIPA 2011 Guidelines)	1.08 ± 0.01

4. Accelerated stability studies of SI and PF

SI was subjected to stability studies for a duration of 45 days. Periodically samples were withdrawn and analyzed to characterize stability of the SI over a period. To the results obtained, a statistical test Single sample t- test was applied to evaluate if results vary significantly or not.

Table no. 13: Accelerated stability study data for SI

Evaluation test	Day 1	Day 7	Day 15	Day 30	Day 45
Color	White	White	Off White	Off White	Off White
Odor	Slight but unpleasant	Slight but unpleasant	Slight but unpleasant	Slight but unpleasant	Slight but unpleasant
pH	3.770	3.723	3.283	3.037	3.333
In- vitro SPF	21.1577	21.3068	19.2821	20.5989	19.7664
Anti-oxidation activity	88.538 %	85.950 %	85.923 %	78.149 %	58.283 %
Particle size	155.1 nm	209.9 nm	212.5 nm	257.2 nm	279.6 nm
Zeta potential	- 79.5 mV	- 71.3 mV	- 49.9 mV	- 44.9 mV	-48.6 mV
PDI	0.097	0.188	0.217	0.096	0.108

FTIR spectra of SI samples kept for accelerated stability studies were recorded on Day 1 and Day 45. FTIR spectra of SI were studied to ensure that AA and OE are present in the sample, sufficient to develop characteristic peaks. Given below are the FTIR spectra of samples,

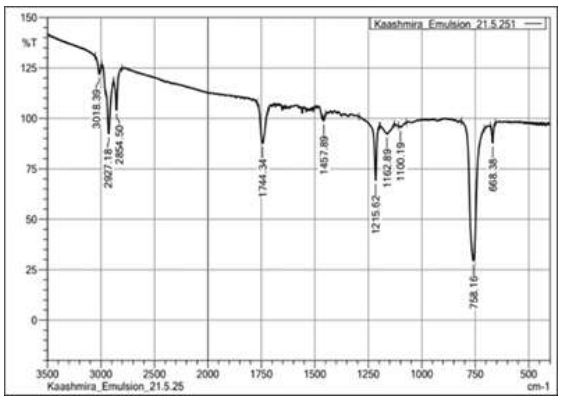


Fig. no. 12: FTIR spectra of SI Day 1 sample

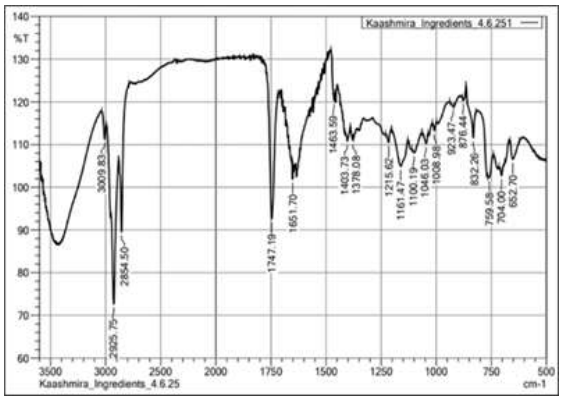


Fig. no. 13: FTIR spectra of SI Day 45 sample

Peaks specifically shown by AA and OE were retained in the FTIR spectrum after 45 days of accelerated stability studies. The following table gives the peaks specifically shown by OE and AA.

Table no. 14: FTIR peaks specifically shown by AA and OE in sample of SI

AA				OE			
Reference peaks[21] (cm^{-1})	Day 1 sample peaks (cm^{-1})	Day 45 sample peaks (cm^{-1})	Significance[21]	Reference peaks [18, 19] (cm^{-1})	Day 1 sample peaks (cm^{-1})	Day 45 sample peaks (cm^{-1})	Significance[18,19]
3040	3004.13	3009.83	C-H bond	2929.00	2927.18	2925.75	O-H bond
1750	1744.34	1747.19	C=O bond	2857.52	2854.50	2854.80	C-H bond
1450	1457.89	1463.59	C-H bond (bending)	1678- 1668	1744.34	1747.19	C=C bond
1125	1162.89	1161.47	C-O-C bond	1375- 1465	1457.89	1463.59	C-H bond
1030	1100.19	1100.19	C-O-H bond				
790	758.16	759.58	O-H bond				

PF was subjected to stability studies for a duration of 45 days. Periodically samples were withdrawn and analyzed to characterize stability of the PF over a period. To the results obtained, a statistical test Single sample t- test was applied to evaluate if results vary significantly or not.

Table no. 15: Accelerated stability study data for PF

Evaluation test	Day 1	Day 7	Day 15	Day 30	Day 45
Color	White	White	Off White	Off White	Off White
Odor	Absent	Slight but unpleasant	Slight but unpleasant	Slight but unpleasant	Slight but unpleasant
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
pH	6.023	5.747	5.867	5.380	4.373
Homogeneity	Achieved	Achieved	Achieved	Achieved	Achieved
Spreadability	2.113 cm	2.043 cm	1.690 cm	2.111 cm	1.800 cm
Washability	Easy with slight rubbing	Easy with slight rubbing	Easy with slight rubbing	Easy with slight rubbing	Easy with slight rubbing

In- vitro SPF	12.7250	10.0863	9.0990	9.8509	6.6376
Anti-oxidation activity	88.941 %	65.640 %	86.213 %	79.238 %	66.168 %

FTIR spectra of PF samples kept for accelerated stability studies were recorded on Day 1 and Day 45. FTIR spectra of PF were studied to ensure that AA and OE are present in the sample, sufficient to develop characteristic peaks. Given below are the FTIR spectra of samples,

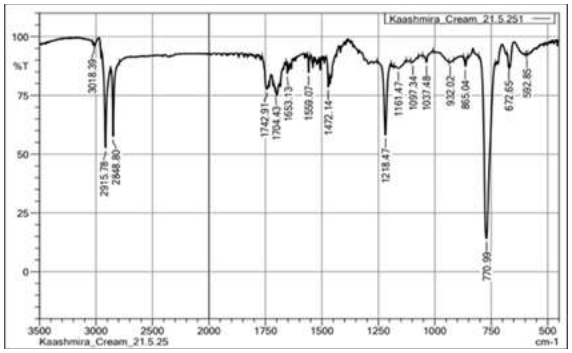


Fig. no. 14: FTIR spectra of PF Day 1 sample

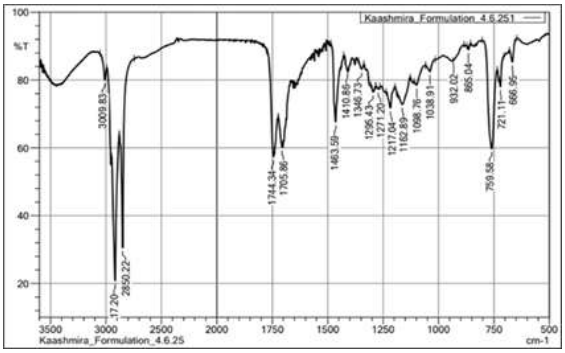


Fig. no. 15: FTIR spectra of PF Day 45 sample

Peaks specifically shown by AA and OE were retained in the FTIR spectrum after 45 days of accelerated stability studies. The following table gives the peaks specifically shown by OE and AA.

Table no. 16: FTIR peaks specifically shown by AA and OE in sample of PF

AA				OE			
Reference peaks[21] (cm^{-1})	Day 1 sample peaks (cm^{-1})	Day 45 sample peaks (cm^{-1})	Significance[21]	Reference peaks[18, 19] (cm^{-1})	Day 1 sample peaks (cm^{-1})	Day 45 sample peaks (cm^{-1})	Significance[18, 19]
3040	3004.13	3009.83	C-H bond	2929.00	2915.78	2917.20	O-H bond
1750	1744.34	1744.34	C=O bond	2857.52	2848.80	2850.22	C-H bond
1450	1457.89	1463.59	C-H bond (bending)	1678- 1668	1742.91	1744.34	C=C bond
1125	1162.89	1162.89	C-O-C bond	1375- 1465	1472.14	1463.59	C-H bond
1030	1100.19	1098.76	C-O-H bond				
790	758.16	759.58	O-H bond				

DISCUSSION:

1. Formulation of SI

Determination of Concentration of the internal phase that can be loaded in emulsion: Initially emulsions with 20 % w/ w oil were developed and exposed to stress conditions. However, emulsion having Tween 60 and Span 80 showed phase separation. Thus this combination was ruled out. With the remaining two S_{mix} using 40 % oil phase, emulsions were developed. From those emulsions, Tween 20 and Span 80 containing emulsion showed phase separation on exposure to stress conditions. The only left combination was of Tween 80 and Span 80, forming a stable emulsion containing 40 % oil. However post freeze thaw cycles, this emulsion showed separation of tiny droplet of oil. Further Tween 80 and Span 80 with 45 % oil showed phase separation on

centrifugation. Thus, the selected surfactant combination was Span 80 and Tween 80 and Oil phase concentration was decided to be kept 40 % w/w.

Minimizing the S_{mix} concentration: Exposure to excessive surfactants can lead to drying, irritation, itching of skin due to dehydration. So surfactants should be used in minimum concentration. Emulsion with 4.0 % w/ w S_{mix} showed phase separation on centrifugation. However, emulsion with 4.5 % w/ w S_{mix} was stable under all three stress conditions. Thus, S_{mix} quantity was reduced to 4.5 % w/ w.

Optimization of oil ratio: Considering the data given by Pareto chart, ANOVA table, Coded equation, 2D Contour plots and 3D Surface response plots we observe that factor A, interaction factor AC and interaction factor BC significantly affect the *In- vitro* SPF value. Effect of factor A i.e. Olive oil and interaction factor AC (Olive oil and Sesame oil) is Negative. This means that with an increase in the amount of Olive oil in the formulation, *in- vitro* SPF will reduce. However, the coefficient of factor A in the coded equation is small. This signifies that though an increase in the quantity of Olive oil will reduce SPF, this reduction will not be drastic. Interaction factor AC will also reduce SPF but as indicated by its small coefficient, reduction will not be drastic. When we observe the 3D Surface Response plot for AC factors, we can clearly observe that, as the quantity of Olive oil increases (with constant quantity of sesame oil), significant reduction in SPF is not observed. However, with increasing quantities of Sesame oil, SPF is drastically reducing. Thus, highlighting the need to minimize the quantity of Sesame oil to achieve maximum SPF. This also becomes evident through the negative coefficient associated with factor C, Sesame oil. The effect of interaction factor BC is Positive and largest amongst other factors. This means that interaction between Almond oil and Sesame oil is improving SPF. However, this positive trend is not observed throughout the concentration range of Almond oil and Sesame oil, after a certain concentration trend is getting reversed. Further increase in quantity of both oils is reducing SPF. The optimized oil ratio recommended by StatEase software is 7: 1: 1 for olive oil: almond oil: sesame oil to achieve maximum *in- vitro* SPF. High amount of Olive oil is acceptable due to minimum SPF reduction. Low amount Sesame oil is essential to minimize its strong negative effect on SPF. A low amount of Almond oil will avoid mild negative effects and will also preserve the beneficial effect of interaction factor BC.

2. Evaluation of SI

Table no. 17: Significance of evaluation of SI

Parameter	Significance
Organoleptic Properties	Acceptable
pH	Acidic, due to L- ascorbic acid content.
Dilution Test	Dilution with water showed no phase separation, confirming the nature of emulsion as Oil- in- Water (O/ W).
In-vitro SPF Determination	Normal Indians have Skin type III and type IV (Classification of skin types given by Fitzpatrick). SPF needed to protect these skin types from harmful UV radiations is at the most 5. For skin type I which is most sensitive to UV radiations, SPF required is at least 8. (Sharma, 2014) Developed SI has SPF of 19.923 ± 0.790. Thus, confirming that developed SI will offer sufficient UV protection.
Antioxidant Study (DPPH method)	A stable DPPH radical in ethanol can absorb light of up to 517 nm. The reaction between the molecules of the antioxidants and the DPPH radical is what causes the decrease in

	absorbance of the radical. Therefore, DPPH is frequently employed as a material to assess antioxidant activity.[20] Antioxidant study helped to understand if developed SI will show resistance towards ROS generated by sun exposure or not. SI showed 90.767 ± 0.549 % antioxidant activity. Indicating that SI will show good ROS scavenging activity.
Particle Size	Particle size and PDI help to comment on stability of emulsion. Mean particle size of emulsion was 179.867 ± 32.690 nm. The small value of particle size indicates that the oil phase is well dispersed in the aqueous phase, which is essential for stability of emulsion.
PDI	PDI was found to be 0.118 ± 0.082 confirming the Monodisperse nature of emulsion. Monodisperse emulsions are stable in nature.
Zeta Potential	For dispersion systems to remain stable, zeta potential should be ± 30 mV. (Malvern, 2015) Larger value of zeta potential indicates that there is sufficient charge available on the surface of dispersed phase droplets. This charge will prevent coalescence of dispersed droplets due to repulsive forces between the droplets. SI has a zeta potential of -77.733 ± 1.514 mV, indicating stability of SI.

3. Formulation and evaluation of PF

Formulation: Vanishing cream is designed to spread easily on skin and then rapidly vanish leaving behind a thin, invisible layer. (Rieger, 2009) They are light in feel being oil- in- water emulsion, formula is well established and formulation is simple. Thus, vanishing cream was decided to be used for developing PF.

Evaluation:

Table no. 18: Significance of evaluation of PF

Parameter	Significance
Organoleptic Properties	Acceptable
Homogeneity	Homogeneous mixing of SI with base was achieved as indicated by absence of clumps and grittiness on touch.
pH	PF had a pH of 6.023 ± 0.015 (N=3). pH of skin ranges from 4 to 6. (Bikiaris, 2023) PF has pH very close to that of pH of skin thus ensuring that formulation will not cause any irritation to skin.
Spreadability	Spreadability is essential for topical formulations like sunscreens because more the spreadability of formulation, more skin surface area will be covered by formulation thus better sun protection will be achieved. PF was observed to spread easily.
Washability	Sunscreen formulations should stay over the surface of skin even on exposure to water, to ensure that sun protection is achieved even while engaging in water sports. However, over a period formulation should get washed- off ensuring that it does not turn comedogenic. PF required rubbing of the glass slide to wash it off. This indicates that formulation will stay over the surface of skin and not get easily washed- off, which is desirable.
In-vitro SPF Determination	PF had SPF of 12.7253, which is more than that of SPF required to protect sensitive skin against sun damage. This indicates the capability of PF to protect skin against sun damage.
Antioxidant Study (DPPH method)	Antioxidant activity of PF was found to be 88.941 %. This indicates that PF was showing good enough antioxidant activity, having been formulated using well established anti-oxidants AA and OE.
Viscosity	Mean viscosity was found to be 2466.667 cP, meeting user expectations for texture and usability.
Assay (for AA and OE content)	When absorbance of sample solutions were recorded it was observed that recorded absorbance's were very high. Investigation revealed that oils absorbed heavily at around wavelength of maximum absorption of AA and OE. Several solvents were used like Acetonitrile, Ethanol, Methanol and their aqueous mixtures, blend of

	methanol and ethanol with 20 % and 40 % ammonia were also used. However, no solvent system was able to reduce interference from oils. AA having good absorptivity was showing significant absorbance at 293 nm, wavelength of maximum absorbance of OE. This further complicated the situation. Thin layer Chromatography (TLC) was performed to separate oils from AA and OE, however due to lipophilic nature, OE did not separate from oils. Instead, both oils and OE showed similar travel. As a result, quantitation of AA and OE in PF was not possible.
In- vitro SPF determination according to COLIPA- 2011 guidelines	As determined by this method *in- vitro* SPF of PF was found to be 1.08 ± 0.01. Significant difference is observed in the SPF determined using Mansur's equation and according to the method given by COLIPA guidelines which involves use of PMMA plates. PMMA plates mimic human skin, thus giving results that are more representative of *in- vivo* results. This SPF comparison highlights the need of developing a suitable *in- vitro* method of determining SPF, which can be performed easily on laboratory scale giving results like those obtained by the methods given by regulatory guidelines.

4. Accelerated stability studies of SI and PF

Organoleptic properties of SI were unaffected by exposure to accelerated conditions. Statistical analysis of data revealed that pH, *in- vitro* SPF, Antioxidant activity and PDI of SI are not significantly different from the day 1 results. This indicates that even on exposure to stress conditions *in- vitro* SPF and Antioxidant activity of SI are remaining unaffected. Statistical analysis reveals that particle size and zeta potential for SI has changed significantly in 45 days. Though there is significant decline in zeta potential over a period, zeta potential is more than ± 30 mV. Particle size though increasing significantly, values do not suggest large instability of emulsion. Thus, the conclusion is that although particle size and zeta potential are varying significantly, this variability is not capable of bringing about immediate instability in the developed SI.

Peaks specifically shown by AA and OE were retained in the FTIR spectrum after 45 days of accelerated stability studies, suggesting that AA and OE are present in considerable quantities in the formulation even after exposure to stress conditions.

Thus, based on accelerated stability studies conducted, we conclude that developed SI is remaining stable.

Organoleptic properties, Homogeneity and Washability of PF were unaffected by exposure to accelerated conditions. Statistical analysis of data revealed that pH, Spreadability and Antioxidant activity of PF are not significantly different from the day 1 results whereas, *in- vitro* SPF has declined significantly. Reasons for decline in SPF can be attributed to pH changes in formulation affecting stability of oils, degradation of oils and antioxidants. Reduction in pH of PF can be due to degradation of AA. Though not significant, reduction in antioxidant activity is observed, indicating loss of some amount of antioxidant.

An interesting observation is noted in antioxidant activity during accelerated stability studies of PF. After 7 days of study, the antioxidant activity of PF has dropped drastically to around 65 % however, on 15th day the activity has improved again to around 85 %. This can be attributed to the fact that, due to stress conditions one or both antioxidants in small quantities must have got oxidized, as indicated by reduced antioxidant activity. However, as reported in literature

Vitamin C and Vitamin E work synergistically. When one of the vitamins is oxidized the other one revives the oxidized vitamin. This chain continues, and thus stability of both improves. (Bikiaris et al.,2023, Packer et al.,2001). Similar behavior is observed here, a desired synergy between AA and OE is developed.

Peaks specifically shown by AA and OE were retained in the FTIR spectrum after 45 days of accelerated stability studies, suggesting that AA and OE are present in considerable quantities in the formulation even after exposure to stress conditions.

Overall based on accelerated stability studies conducted, we can conclude that developed PF is remaining stable, however reduction in SPF needs to be investigated.

CONCLUSION:

The present study successfully formulated and evaluated a novel sunscreen ingredient as an Oil-in-Water emulsion incorporating natural Vegetable oils, L-ascorbic acid, Tocotrienol, and Kaolin. The optimized emulsion demonstrated satisfactory SPF, antioxidant activity, stability, and appropriate physicochemical characteristics. Incorporation into a vanishing cream base produced a prototype formulation with acceptable SPF, antioxidant activity, spreadability, pH, and homogeneity. The approach highlights the potential of combining natural oils and antioxidants to minimize use of organic UV filters, addressing safety and consumer preferences for natural sun protection while ensuring effective skin care. Study was limited by the inability to quantify antioxidants and lacks *in-vivo* validation.

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REFERENCES

1. Tortora GJ, Derrickson B. Principles of Anatomy & Physiology. 15th ed. Hoboken, New Jersey: John Wiley & Sons, Inc; 2017.
2. Stamatas GN, Roux PF, Boireau-Adamezyk E, Imane Lboukili, Thierry Oddos. Skin maturation from birth to 10 years of age: Structure, function, composition and microbiome. Experimental Dermatology 2023;32:1420–9.
3. Telofski LS, Morello AP, Mack Correa MC, Stamatas GN. The Infant Skin Barrier: Can We Preserve, Protect, and Enhance the Barrier? Dermatology Research and Practice 2012;2012:1–18.
4. Visscher MO, Burkes SA, Adams DM, Hammill AM, Wickett RR. Infant skin maturation: Preliminary outcomes for color and biomechanical properties. Skin Research and Technology 2017;23:545–51.
5. Rahma A, Lane ME. Skin barrier function in infants: Update and outlook. Pharmaceutics 2022;14.
6. Vechtomova Y. UV Radiation in DNA Damage and Repair [Internet]. encyclopedia.pub [cited 10AD May];Available from: https://encyclopedia.pub/entry/17565
7. Volkmer B, Greinert R. UV and Children's skin. Progress in Biophysics and Molecular Biology 2011;107:386–8.
8. Chavda AsstProfV, Acharya D, Hala V, Vora L, Dawre S. Sunscreens: A comprehensive

review with the application of nanotechnology. Journal of Drug Delivery Science and Technology 2023;104720.
9. Kullavanijaya P, Lim HW. Photoprotection. Journal of the American Academy of Dermatology 2005;52:937–58.
10. de G, Helena C, José M, Berardo M. Development of Topical Formulations Containing 20% of Coated and Uncoated Zinc Oxide Nanoparticles: Stability Assessment and Penetration Evaluation by Reflectance Confocal Laser Microscopy. Cosmetics 2023;11:6–6.
11. Trivedi M, Murase J. Titanium Dioxide in Sunscreen. Application of Titanium Dioxide 2017;
12. Bikiaris ND, Ioanna Koumentakou, Katerina Hatzistamatiou, Smaro Lykidou, Panagiotis Barmpalexis, Nikolaidis N. Preparation and Investigation of the SPF and Antioxidant Properties of O/W and W/O Emulsions Containing Vitamins A, C and E for Cosmetic Applications. Cosmetics 2023;10:76–6.
13. Singh VK, Beattie LA, Seed TM. Vitamin E: tocopherols and tocotrienols as potential radiation countermeasures. Journal of Radiation Research 2013;54:973–88.
14. RIEGER MM. Harry's Cosmeticology. Boston, Mass: Chemical Publishing; 2009.
15. Manisha Sutar, Chaudhari SR, Chavan MJ. Formulation and In Vitro Evaluation of Sun Protection Factor of Herbal Sunscreen Cream Containing Butea monosperma, Neolamarckia cadama and Punica granatum Extracts. Journal of Drug Delivery and Therapeutics 2019;9:328–34.
16. Saraf S, Kaur C. In vitrosun protection factor determination of herbal oils used in cosmetics. Pharmacognosy Research 2010;2:22.
17. Ni Nyoman Yuliani, Siswandono Siswandono, Tristiana Erawati, Jefrin Sambara, Yulius Korassa, Poddar S. Bougenville Flower (Bougainvillae spectabilis Willd) Extract (In Vitro) Activity Test as Sunscreen. Research journal of pharmacy and technology 2024;849–54.
18. Infrared Spectroscopy Absorption TableAvailable from: https://chem.libretexts.org/Ancillary_Materials/Reference/Reference_Tables/Spectroscopic_Reference_Tables/Infrared_Spectroscopy_Absorption_Table
19. Büsing A, Ternes W. Separation of α-tocotrienol oxidation products and eight tocochromanols by HPLC with DAD and fluorescence detection and identification of unknown peaks by DAD, PBI-EIMS, FTIR, and NMR. Analytical and Bioanalytical Chemistry 2011;401:2843–54.
20. Sharma GK, Sharma S, Chasta P, Joshi RK, Tiwari A, Chandrul KK. Assessment of pharmacognostic parameters and antioxidant potential of bitter melon or karela (Momordica charantia L.) fruits by DPPH method. RESEARCH JOURNAL OF PHARMACY AND TECHNOLOGY 2021;14:437–41.
21. Sreeja V, Jayaprabha KN, Joy PA. Water-dispersible ascorbic-acid-coated magnetite nanoparticles for contrast enhancement in MRI. Applied Nanoscience 2014;5:435–41.
22. Sharma PP. COSMETICS-FORMULATION, MANUFACTURING & QUALITY CONTROL. 5th ed. Delhi-110034: Vandana Publications; 2014.
23. Zeta potential - An introduction in 30 minutes [Internet]. 2015. Available from: https://www.research.colostate.edu/wp-content/uploads/2018/11/ZetaPotential-Introduction-in-30min-Malvern.pdf
24. Packer L, Weber SU, Rimbach G. Molecular Aspects of α-Tocotrienol Antioxidant Action and Cell Signalling. The Journal of Nutrition. 2001 Feb 1;131(2):369S373S.

25. Firi Oktavia Hariani, Mohammad Adam Jerusalem, Tahir I, Maisari Utami, Oh WC, Wijaya K. Component, Formulation and Regulatory of Sunscreen Materials: A Brief Review. Korean J. Mater. Res 2023;33:87–94.
26. Shebis Y, Iluz D, Kinel-Tahan Y, Dubinsky Z, Yehoshua Y. Natural Antioxidants: Function and Sources. Food and Nutrition Sciences 2013;04:643–9.
27. Montenegro L, Santagati L. Use of Vegetable Oils to Improve the Sun Protection Factor of Sunscreen Formulations. Cosmetics 2019;6:25.
28. Required HLB for Oils and Lipids [cited 2025 Jan];Available from: https://www.researchgate.net/profile/Jean-Canselier/post/How-can-I-determine-the-Hydrophilic-Lipophilic-Balance-of-an-oil/attachment/59d6235879197b8077981ae7/AS:306873738170368@1450175808132/download/Required_HLB_for_Oils_and_Lipids.pdf

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