International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Formulation Development and Stability Study of Sunscreen

S.Santhosh[1], Dr. P. Satheesh Kumar[2], Dr. E. Thilagam[3], Dr. N. Senthil Kumar[4]

[1]Student, JKKMMRF's Annai JKK Sampoorani Ammal College of pharmacy, B. Komarapalayam - 638183, Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai - 600032
[2]Professor, JKKMMRF's Annai JKK Sampoorani Ammal College of pharmacy, B. Komarapalayam - 638183, Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai - 600032
[3]Professor & HOD, JKKMMRF's Annai JKK Sampoorani Ammal College of pharmacy, B. Komarapalayam -638183, Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai - 600032
[4]Principal JKKMMRF's Annai JKK Sampoorani Ammal College of pharmacy, B. Komarapalayam - 638183, Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai - 600032

ABSTRACT

The paper explores the realm of herbal cosmetics, blending conventional cosmetic ingredients with herbal components to deliver specific beauty benefits. It delves into the history of cosmeceuticals, a term coined in the 1990s for skincare products claiming therapeutic advantages from plant-based actives like alpha-hydroxy acid and retinoic acid. These natural ingredients offer diverse benefits such as skin elasticity enhancement, wrinkle reduction, UV radiation protection, and collagen degradation prevention. Skin and hair health are influenced by various factors, including health, habits, climate, and maintenance. Excessive heat exposure in summer can lead to skin dehydration, while extreme winter conditions may cause skin cracks and infections. Skin diseases can result from exposure to microbes, chemicals, and environmental toxins, as well as malnutrition. Ayurveda, an ancient Indian science, has long utilized herbs and botanicals in cosmetics, believed to enrich the body without side effects. The Drugs and Cosmetics Act defines cosmetics as products intended for body application for cleansing, beautifying, or altering appearance, not requiring a drug license. Herbal cosmetics incorporate phytochemicals from various botanical sources, influencing skin functions and providing nutrients for healthy skin or hair. The use of natural herbs and products for aromatic value in cosmetic preparation is also termed as herbal cosmetics. There is a growing consumer preference for herbal and natural products in cosmetics, driven by a belief that chemical-based cosmetics can be harmful. The regulatory framework for herbal cosmetics is similar to that for other chemical ingredients in cosmetic formulations. The two approaches to ensuring pharmaceutical product quality are Quality by Testing (QbT) and Quality by Design (QbD). QbT relies on end-product testing with limited process understanding, while QbD is a systematic approach emphasizing predetermined objectives and process understanding, aiming to enhance formulation design and development.

Additionally, the study details the development of a sunscreen gel using herbal components in a nanoemulsion. Various tests and assays were conducted on the nanoemulgel and its raw ingredients, including standardization tests, antioxidant activity tests, and microbial tests. The nanoemulsion preparation and evaluation involved optimization using a central composite design and assessment of particle size, zeta potential, and nanoemulgel properties. The findings showed higher antioxidant activity

and peroxide scavenging capacity in thymol compared to cinnamon oil, with the nanoemulsion demonstrating suitable properties for a sunscreen gel, and the gel was prepared with both thymol and cinnamon oil, and stability was initiated.

Keywords: Sunscreen, nanoemulgel, essential oil, Cosmetics, Stability.

INTRODUCTION

Herbal Cosmetics

Herbal cosmetics are formulated using permissible cosmetic ingredients as a base, with added herbal components providing specific benefits. Since the 1990s, the term 'cosmeceutical' has been used for OTC skincare products that claim therapeutic benefits, incorporating plant-based ingredients like alpha-hydroxy acids, retinoic acid, and ascorbic acid. These ingredients enhance skin elasticity, reduce wrinkles, protect against UV radiation, and prevent collagen degradation. Skin and hair health are influenced by factors such as habits, climate, and maintenance. Excessive heat can dehydrate the skin, leading to wrinkles, freckles, blemishes, and sunburns, while extreme cold causes cracks and infections. Skin diseases arise from exposure to microbes, chemicals, toxins, and malnutrition. Ayurveda utilizes herbs and floras in cosmetics, providing nutrients without side effects. The Drugs and Cosmetics Act defines cosmetics as products applied to the body for beautification or altering appearance and does not require a drug license. Herbal cosmetics, containing phytochemicals from botanicals, offer nutrients for healthy skin or hair. Increased consumer awareness has driven demand for natural products, although regulatory requirements for herbal cosmetics are the same as for chemical ingredients..

Quality by testing

In the Quality by Testing (QbT) approach, product quality and performance are ensured by end-product testing, with a limited understanding of the process and critical process parameters (1). The quality is assessed after the final product is designed and manufactured. Root causes for failure are usually poorly understood due to poor process understanding and uncertainty about how the characteristics of substances impact the target product profile (2). Quality by Testing (QbT) was the only way to guarantee the quality of drug products before the FDA launched the Current Good Manufacturing Practice (CGMP). In QbT, product quality is ensured by following a sequence of steps, including raw material testing, the fixed drug product manufacturing process, and end-product testing. Only when all the specifications of the FDA or other standards comply can the materials be used for manufacturing or come into the market. If not, they need to be reprocessed. Root causes for failure are usually poorly understood due to a flawed understanding of the process and uncertainty about how substances impact the target product profile. This method also creates problems moving from laboratory piloting to full-scale production. Production specifications are typically derived from test data from small batches in the laboratory environment. Scaling up to commercial production can reveal new complexities that were not evident during development (3). As a result, the manufacturers must restart the procedure until the root causes of failure are understood and addressed. With the development of the concept of Quality by Design (QbD), there will be a significant transformation in pharmaceutical quality regulation from an empirical process to a more scientific and risk-based approach. Quality by Design takes a different approach to ensuring consistent levels of quality by allowing for flexibility during the manufacturing process (3).

Quality by Design (QbD)

Pharmaceutical Quality by Design (QbD) is a systematic approach to dev proves manufacturing quality performance for the consumer's safe and effective drug supply. QbD uses a systematic approach to

understand a process before starting product design and development to enhance the formulation design's elopement that begins with predetermined objectives, product and process understanding, process control based on sound science, and quality risk management (4-6). QbD in development capability and speed (6). Usually, several factors are involved in the formulation. Design of Experiments (DoE) is a statistical method of optimization to obtain design space, a component of QbD. DoE helps to understand various interactions among the factors while establishing a relationship between the factors and process output (5). Although QbD positively impacts the pharmaceutical industry, it has not yet been fully explored. The main challenge is the lack of understanding. For implementing QbD, tools like Risk Assessment (RA), Design of Experiments (DoE), and Multi-Variety Data Analysis (MVDA) are required. Among these, DoE is the most popular and widely used. However, the pharmaceutical industry and scientific domain are still not familiar with the various models of DoE and their merits and limitations (7, 8).

Components of QbD

Quality Target Product Profile (QTPP)is summarizes the drug product characteristics that should be achieved to ensure quality, safety, and efficacy" (9). QTPP are clinical and patient-related aspects that determine a dosage form's quality, safety, and efficacy.

Critical quality attributes (CQA) is defined as a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit or range to ensure the desired product quality" (9). Critical Quality Attributes are dosage form-related aspects identified based on the severity of harm to the patient (safety and efficacy) resulting from a failure to meet the quality attributes.

Quality risk management (QRM) is a systematic process for the assessment, control, communication, and review of risks to the quality of the drug product across the product life cycle"(10).

Design space

It is a multidimensional combination and interaction of input variables (material attributes and process parameters) that have been demonstrated to assure quality" (9). Design space is one of the essential components in QbD that can be established using DoE, which investigates the interactions between the input variables, such as Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs), which determine the product quality. The control strategy is a planned set of controls derived from the current product, and process understanding that guarantee process performance and product quality" (9).

Effect of Controlled Factors

The effect of the controlled factors on the final product quality characteristics differs according to the pattern of the Pareto principle, which states that a relatively small number of factors are responsible for the substantial effect. This is popularly known as the 20:80 rule, which asserts that 20% of the factors (causes) are responsible for 80% of the responses (results) (11, 12). DoE is an approach where the controlled input factors of the process are systematically and deliberately varied to identify and analyze their effects on the responses.

Full Factorial Design

A complete factorial design is primarily used to understand whether the variables are essential to the process. This is handy for preliminary studies or initial optimization steps (13). This design can screen significant factors from unimportant factors and study individual factors and their interactions with the product and process performance. This is also called a 2-level Full Factorial Design because each factor has two levels (+ and -). 2-Level Full Factorial Design is widely used because it is easy to design, efficient

to run, and straightforward to analyze (14). Complete Factorial Design supports both numeric and categorical factors (14). The primary and interaction effects can be examined, and significant factors can be isolated. A single factor causes the main effect, and the interaction effect is caused by two or more factors that affect the outcome of the process. The number of runs in a factorial design is always $\mathbf{2K}$, where K is the number of factors to be studied (15). Let us assume that the effect of lipid and surfactant concentration on nanoparticle entrapment efficiency is being investigated. As we have two factors, the number of runs will be 22i.e., 4.

Factors and their levels

Run/Trail	Factor Levels (e.g.)	
	Lipid Conc.	Surfactant Conc.
1	+	+
2	+	-
3	-	+
4	-	-

Design is more efficient when compared to OFAT (One Factor at a Time) experiments. OFAT requires many resources and time, contrary to the factorial design. In a full factororial, as the number of factors increases, the number of experimental runs also increases, which is the main disadvantage of this design.

Box-Behnken Design

Box-Behnken Design is a quadratic design that allows the study of 3 or more factors, all at 3 levels, i.e., (-1, 0, +1). This design can be obtained by combining a 2-level full factorial design with an incomplete block design (19). In BBD with 3 factors, two factors are combined with a 22-factor design, and the third factor is maintained at a zero level or centre point. The number of experimental runs obtained in a BBD is $\mathbf{N} = (\mathbf{K} - \mathbf{1}) + \mathbf{CP}$, where K is the number of factors in the experiment, and CP is the number of centre points (20).

BBD is more cost-effective and efficient when compared to 2-level and 3-level full-factorial designs. The design points are located at the centre of all the edges of the cube. This design is mostly used for a safe operation zone process because, when a circle is drawn, touching all the points in the design, the circle does not go out of the cube. Whereas in a CCD, where axial points are located outside the cube, the circle goes out of the cube, which indicates an increased number of experiments that are out of the safe operating zone. The design layout of BBD and CCD is shown in Figure 4.

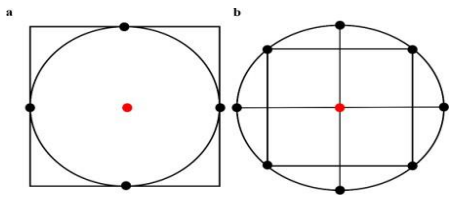


Figure 4. Design layout of BBD and CCD (a) Layout of BBD (all the factor combinations lie inside the working space) (b) Layout of CCD (some of the factor combinations lie outside the working space)

Central Composite Design

CCD is a mixture of Full or Fractional Factorial Design, centre points, and axial points (represented by α (alpha)). CCD has 5 levels of each factor i.e., extreme low (-α), low (-1), centre point (0), high (+1), and extreme high (+α). The extremely low and extremely high are the low and high axial points respectively. These axial points are located at a specific distance from the centre of the cube. The number of experiments in a CCD is $\mathbf{N = 2K + 2K + CP}$, where Cp is the number of centre points, and K is the number of factors in the experiment. This Design's versatility lies in selecting axial point/distance (α) and the number of centre points in the Design. Depending on the axial distance (α), there are two types of CCD. When the α value is equal to 1, the axial point lies on the cube's face, and this is called Face-centred Central Composite Design (FCCD) (15). A Circumscribed Central Composite Design (CCCD) has $\alpha > 1$, i.e., the axial point lies outside the cube. In FCCD, factors have 3 levels, and in CCCD, the factors have 5 levels (15).

Skin

The skin, the body's largest organ, consists of three layers: the epidermis, dermis, and hypodermis. The epidermis, the outermost layer, provides a waterproof barrier and contributes to skin tone. Beneath it, the dermis contains connective tissue, hair follicles, blood vessels, lymphatic vessels, and sweat glands. The deepest layer, the hypodermis, consists of fat and connective tissue. Skin functions include protecting against pathogens, UV light, chemicals, and mechanical injury. It regulates temperature and water loss through mechanisms like the epidermal water barrier, formed by cross-linked proteins and lipids. Keratinocytes in the epidermis produce substances that strengthen this barrier. Langerhans cells in the epidermis provide immune defense. The skin also aids in vitamin D production, converting 7-dehydrocholesterol into vitamin D with UV light, essential for keratinocyte function and skin health. Sweat and sebaceous glands perform exocrine functions, while nociceptors in the skin detect touch, heat, cold, and pain. The skin's appearance and turgor can reflect overall health.\

UV Radiation:

UV radiation from the sun, categorized into UVA (320-400 nm), UVB (280-320 nm), and UVC (100-280 nm), affects the skin, with only UVA and UVB reaching Earth due to atmospheric filtering. UV radiation's intensity varies with factors such as atmospheric conditions, latitude, season, time of day, altitude, and ozone layer thickness. UV rays can penetrate glass and light clouds, causing sunburns even in less direct sunlight. Reflective surfaces like snow and water increase UV exposure, and UV radiation is more intense near the equator, in summer, and at high altitudes. Depletion of the ozone layer, due to chemicals like chlorofluorocarbons, allows more UV radiation to reach Earth. Artificial UV sources, like tanning beds, emit more UVA, falsely marketed as "safer" but still pose significant risks, including skin cancer. UV exposure causes acute effects like sunburn and chronic damage such as skin thickening, wrinkling, actinic keratosis, and cancer. It also reduces epidermal Langerhans cells, impairing immune function. Skin types vary in UV sensitivity, with darker skin providing more natural protection but not immunity. Fair-skinned individuals, especially those with albinism or vitiligo, are particularly vulnerable to UV damage.

Stability Testing and Shelf-Life Determination Ayurvedic Drugs:

Shelf life, a mandatory requirement for licensed Ayurvedic medicines, is influenced by factors such as product nature, ingredients, and packaging. Stability studies ensure a product remains suitable for use during its shelf life under specified storage conditions. If no specific storage condition is listed, the product is assumed to be stable at room temperature (below 30°C). Stability testing assesses how environmental factors like temperature, humidity, and light affect product quality over time. Two methods for determining shelf life include storing samples under standard and accelerated conditions. (31)

Testing frequency:

For long-term stability studies of drugs with a shelf life of 12 months or more, testing should occur every 6 months during the first year, annually thereafter. At accelerated conditions, test at least three time points (e.g., 0, 3, and 6 months). Reduced testing designs are acceptable if justified.

Storage condition:

The world can be divided into four climatic zones: I–IV. This guideline addresses zone IV. The choice of test conditions defined in this guideline is based on an analysis of the effects of climatic conditions in the zone. Recommended storage conditions are the Accelerated Stability Study at 40°C±2°C/75% RH±5% for 6 months and the Long-Term Stability Study at 30°C±2°C/60% RH±5% for a minimum 12 months

MATERIALS AND METHODS

Active Raw Materials:

Thymol (*Trachyspermum ammi***)** as per National Formulary & Ayurvedic Pharmacopeia of India, The Ayurvedic Pharmacopeia Of Indian ., Part I, Vol –VI, pg. no-226**, Darusita taila** (*Cinnamomum zeylanicum*) as per Food Chemicals Codex & Ayurvedic Pharmacopeia of India, The Ayurvedic Pharmacopeia Of Indian., Part I, Vol –VI, pg. no-200

Excipients:

DM- Water, Carbomer - USP-NF, Glycerin - USP-NF, Coconut oil – FSSAI, Tocopherol – FSSAI, Tween 80 – IP, Erithrosin – FDC, Orange oil – FCC, Sodium hydroxide - IP

Methods

DPPH Assay: The antioxidant activity of ethanol extracts of thymol and cinnamon oils was evaluated using the DPPH assay. DPPH, a stable nitrogen-centered free radical, can accept electrons or hydrogen radicals. When reduced by antioxidants, DPPH loses its color, which can be measured spectrophotometrically at 517 nm. The scavenging activity is quantified by plotting a graph of extract concentration versus percent inhibition, allowing the determination of the IC50 value—the concentration needed to reduce DPPH radicals by 50%. A lower IC50 indicates higher antioxidant capacity.

Peroxide Scavenging Assay: This assay uses nitro blue tetrazolium (NBT) reduction to assess superoxide radical scavenging. Superoxide radicals, generated from riboflavin autooxidation under light, are highly reactive and damaging. They reduce NBT to a blue formazan, measurable at 560 nm. The degree of blue color reduction indicates the amount of superoxide scavenged by the extracts.

Reducing Power Assay: This test measures an antioxidant's ability to donate electrons, assessed by the reduction of Fe^{3+} to Fe^{2+}. The change in color from green to blue, observed at 700 nm, reflects the reducing power of the extracts. Strong reducing agents cause a pronounced shift to a blue hue due to the formation of a Prussian blue complex.

Preparation and Optimization of Nano Emulsion: Nano emulsions were prepared by dispersing an aqueous phase (DM water and Tween 80) into an oil phase containing thymol or cinnamon oil using a high-speed homogenizer. Optimization was carried out using a Design-Expert software (Version 7.1.6) with a 13-run, 2-factor, 3-level central composite design. This model allows for the construction of polynomial models and evaluation of quadratic response surfaces. Key factors included surfactant concentration (X1) and homogenization time (X2), with particle size (Y1) and zeta potential (Y2) as response variables. The design matrix involved 13 trial runs to determine optimal conditions, with the software generating nonlinear quadratic model equations:

$$Y=A0 + A1\ X1 + A2\ X2 + A3\ X1\ X2 + A4\ X12 + A5\ X2\ 2$$

Where, A0 - the intercept representing the arithmetic average of all 13 runs, A1, A2, A3, A4, A5 – regression coefficient of the observed experimental values of response variable Y, X1, X2 – independent variable with coded levels, X1 X2 – linear interaction terms, X12, X2 2 – quadratic terms The concentration of surfactant between 80 (X1) and the homogenization duration (X2), which were coded with +1 and -1 to denote the low and high values, respectively, were the independent variables in this design matrix. Particle size (Y1) and zeta potential (Y2) are the dependent variables. The response obtained following the production of these 13 formulations was fitted into the design.

Evaluation of Nano emulsion

Particle Size and PDI: Particle size of the nanoemulsion was measured using a Malvern Zetasizer Nano ZS 90 via photon correlation spectroscopy based on Mie theory. Measurements were taken at 25°C with appropriately diluted samples in disposable polystyrene cells.

Zeta Potential: The Malvern Zetasizer Nano ZS 90 also assessed zeta potential using photon correlation spectroscopy. The procedure was similar to particle size analysis, with diluted samples measured at 25°C in disposable polystyrene cells.

Preparation of Nanoemulgel: Carbopol was dissolved in water, and the nanoemulsion was added gradually while stirring. Tocopherol was included as a preservative, coconut oil as a stabilizer, orange oil for fragrance, glycerin as a humectant, erythrosin for color, and sodium hydroxide as a neutralizer.

Evaluation of sunscreen nanoemulgel

Description

Take 5 to 10 gram of gel in a clear petri dish and view it under white light. Check for appearance and aroma

Identification.by GC

Instrument: Agilent technologies – 7890A, *Cinnamomum zeylanicum* (Cinnamon oil /Darusita taila) and *Trachyspermum ammi* (Thymol / Yavani satva). The retention time of the peaks in the chromatogram of the sample preparation corresponds to that in the chromatogram of the standard preparation.

Viscosity:

At room temperature, the viscosity of the formulation was measured using a Brookfield viscometer. The sample size was in the ULA cylinder, and the ULA S00 spindle was adjusted at 4 rpm. The viscosity was measured with the torque level set to 10.

Spreadablity:

The Shimaszu texture analyzer was used to determine the Spreadability of the Nanoemulgel.

pH:

The formulation's pH is assessed to prevent skin irritation. There will be less likelihood of skin irritation with a formulation that has a pH close to that of the skin. A digital CyberScan pH Tutor Meter (Eutech instrument s Pvt Ltd India) that has been calibrated is used to measure the pH of the optimized formulation. The pH of the gel being tested is dipped into the rod of the pH meter, and the reading is noted.

Assay by GC

- *Trachyspermum ammi* (Thymol / Yavani satva):Between 90% and 110%
- *Cinnamomum zeylanicum* (Cinnamon oil /Darusita taila): Between 90%and 110%

Standard Preparation:

For cinnamon sun screen gel: Weigh accurately about 0.20g of Dimethyl phthalate (Internal standard), 0.025g of Cinnamon oil W.S. (Darusita taila) into a 50-ml. volumetric flask. Dissolve it in isopropyl alcohol and make up to the volume with isopropyl alcohol.

For Thymol sun screen gel: Weigh accurately about 0.20g of Dimethyl phthalate (Internal standard), 0.025g of Thymol W.S. (Yavani satva) into a 50-ml. volumetric flask. Dissolve it in isopropyl alcohol and make up to the volume with isopropyl alcohol.

Sample Preparation: Weigh accurately about 2.5 g of sample in a squad weighing bottle or beakers and add 10 ml of Iso Propyl Alcohol. Mix well with triturating of solid mass with the help of a glass rod having flat end at the bottom, to extract the actives in the Iso propyl alcohol and carefully transfer the extracted solution into a 50-ml volumetric flask. Repeat the extraction process for 3 times. Add 0.20 g of Dimethyl Phthalate (Internal standard) into the above 50-ml volumetric flask and make up to the volume with Iso propyl alcohol. Inject in replicate.

Chromatographic system: Column: BP-5 or Equivalent (30mx0.32mm ID x1.0µm film thickness), Oven Temperature: 120°C– 6 min.-5°C/min.-160°C-0 min.-25°C/min.-280°C-5 min. Injection Temperature: 260°C, Detection Temperature: 280°C, Carrier gas: Nitrogen, Flow rate: 1.0 ml/min, Injection volume: 2.0 µl, System Suitability: The relative standard deviation for replicate injections of standard preparation is not more than 2.0%.

Procedure:

Separately inject equal volumes of the standard preparation and sample preparation. Measure the peak response factor of standard preparation. *Trachyspermum ammi* (Thymol / Yavani satva) and *Cinnamomum zeylanicum* (Cinnamon oil /Darusita taila)

Microbiology:

As per Indian Pharmacopeia – Volume – I 2022, Total Plate Count: NMT1000 Cfu/gm, Total Yeast Mould: NMT100 Cfu/gm, E. coli, Salmonella Spp, S. aureus, P. aeruginosa

Stability study:

Stability plan as per ICH guidelines. Sampling intervals Long Term Stability Study month– 0^{th} Initial, 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 18^{th}, 24^{th} & 36^{th}. Accelerated Stability Study – Month 0^{th} Initial, 3^{rd}, 6^{th}.

RESULTS AND DISCUSSION

Standardization of Active raw materials

Thymol: The sample Complies the NF monograph

Cinnamon leaf oil: The sample Complies the FCC monograph

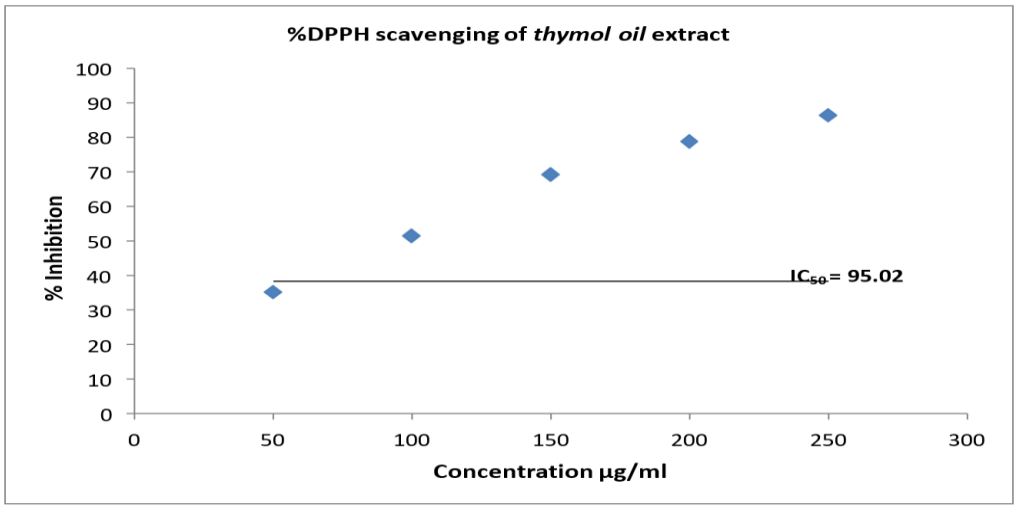
DPPH Assay: The IC50 values of the ethanol extracts of thymol and cinnamon oil were 95.02 and 126.64, respectively, as indicated in the table and figure. Comparatively, the cinnamon oil extract scavenged DPPH radical with an IC50 of 126.64, whereas the thymol extracts strongly scavenged DPPH radical with an IC50 of 95.02. It was discovered that the scavenging was dosage dependent. Ascorbic acid, a common medication, scavenged the DPPH radical with an IC50 of 95.28.

Free radical scavenging capacity of ethanol extracts of thymol

Concentration (µg/ml)	**DPPH Scavenging %**	
	Ethanol Extract	**Ascorbic Acid**

50	35.23±1.28	95.28±1.53
100	51.57±0.64	-
150	69.34±1.03	-
200	78.82±1.57	-
250	86.39±0.95	-
IC_{50}	**95.02**	-

Values are mean ± SEM of six determinations

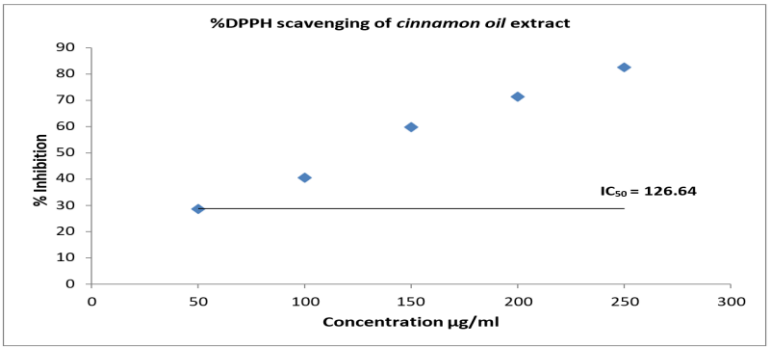


IC_{50} values of ethanol extract of *thymol*

Free radical scavenging capacity of ethanol extracts of cinnamon oil

Concentration (µg/ml)	**DPPH Scavenging %**	
	Ethanol Extract	**Ascorbic Acid**
50	28.61±2.05	95.28±1.53
100	40.43±0.52	-
150	59.78±0.91	-
200	71.29±1.43	-
250	82.51±1.12	-
IC_{50}	**126.64**	-

Values are mean ± SEM of six determinations



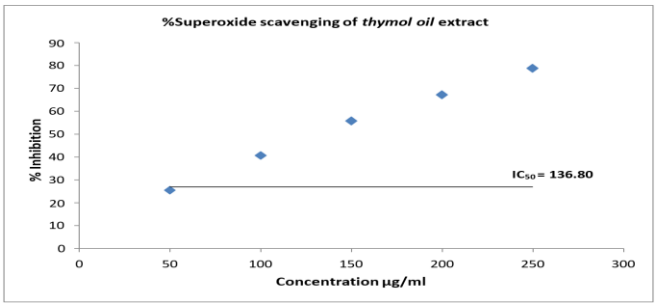
IC50 values of ethanol extract of cinnamon oil

Peroxide scavenging assay: The IC50 values of the ethanol extracts of thymol and cinnamon oil were 136.80 and 154.86, respectively, as indicated in the table and figure. Comparatively, the cinnamon oil extract scavenged peroxide radical with an IC50 of 154.86, whereas the thymol oil extracts strongly scavenged peroxide radical with an IC50 of 136.80. It was discovered that the scavenging was dosage dependent. Ascorbic acid, a common medication, scavenged the DPPH radical with an IC50 of 95.28.

Superoxide scavenging capacity of ethanol extracts of thymol

Concentration (µg/ml)	**Superoxide Scavenging %**	
	Ethanol Extract	**Ascorbic Acid**
50	25.48±1.43	88.23±1.78
100	40.72±1.72	-
150	55.81±2.16	-
200	67.23±1.34	-
250	78.91±1.65	-
IC_{50}	**136.80**	-

Values are mean ± SEM of six determinations

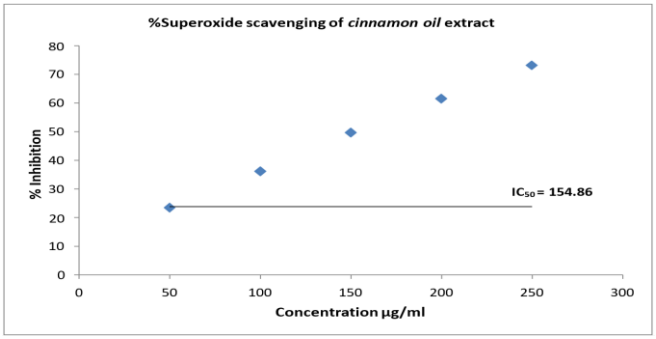


IC50 values of ethanol extract of thymol oil

Superoxide scavenging capacity of ethanol extracts of cinnamon oil

Concentration (µg/ml)	**Superoxide Scavenging %**	
	Ethanol Extract	**Ascorbic Acid**
50	23.54±1.27	88.23±1.78
100	36.21±1.53	-
150	49.67±0.98	-
200	61.58±2.31	-
250	73.18±1.15	-
IC_{50}	**154.86**	-

Values are mean ± SEM of six determinations



IC50 values of ethanol extract of cinnamon oil

Reducing Power Assay

Ascorbic acid was set at 100% antioxidant activity, showing greater reducing power than all tested extracts. Thymol oil extract demonstrated higher reducing potential. Combining extracts with ascorbic acid enhanced antioxidant activity, with thymol oil and ascorbic acid yielding the highest reducing power (137.21%), indicating a synergistic effect..

Antioxidant activity determination of various extracts

Extract	Absorbance at 700 nm	Antioxidant activity (%)
Ascorbic acid	0.825±0.04	100.00
Thymol	0.349±0.01	42.30
Cinnamon oil	0.324±0.05	39.27
Ascorbic acid and Thymol	1.132±0.05	137.21
Ascorbic acid and Cinnamon oil	1.045±0.07	126.67

Values are mean ± SEM of triplicate determinations

Optimization of nano emulsion: By running the required factors predetermined by the software we obtained the responses and were fitted into the model.

Factors and responses in the DoE run

Standard	Run	Factor 1: Tween 80	Factor 2: Homogenization time	Response 1: Particle Size	Response 2: Zeta Potential
1	1	2	10	240.7	-12.8
11	2	3.5	20	162.5	-25.3
2	3	5	10	196.6	-21.4
7	4	3.5	5.86	234.6	-15.4
5	5	1.38	20	212.5	-15.9
10	6	3.5	20	145.8	-26.4
12	7	3.5	20	158.5	-25.9
13	8	3.5	20	170.7	-24.2
4	9	5	30	100.7	-29.8

9	10	3.5	20	157.3	-25.8
8	11	3.5	34.14	112.5	-28.9
3	12	2	30	184.9	-23.8
6	13	5.62	20	141.8	-28.1

Variables and their levels in central composite design

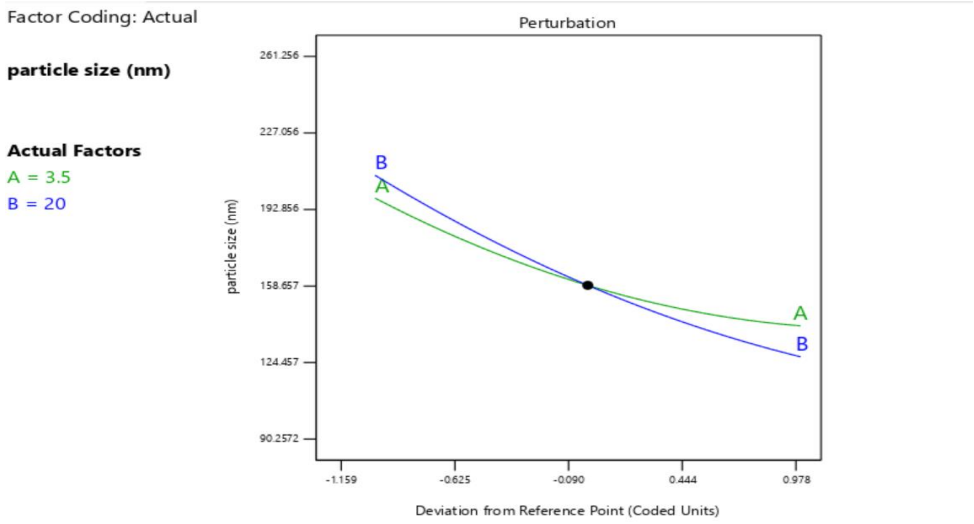
Factor	**Levels**	
	Low (-)	High (+)
Independent variables Amount of surfactant(%)X1 Homogenization time(min) X2	 2 10	 5 30
Dependent variables Particle size Y1 Zeta potential	 Minimize In range	

Analysis of the response:

Response Y1 (Particle size): The quadratic model for the Y2 response is suggested by the sum of squares, mean squares, F-value, P-value, residuals, adjusted R2, projected R2, adeq precision, and predicted residual error sum of square (PRESS) values. The answer Y1's model F-value of 55.02 indicates that the model is significant. This kind of huge F-value has a 0.01% probability of being caused by noise. Model terms are considered significant when P-values are less than 0.0500. A, B, AB, A2, and B2 are important model terms in this instance. This ANOVA revealed that the surfactant concentration and homogenization time are the independent variables influencing the Y1 response. The Y1 Lack of Fit F-value of 0.8698 suggests that the Lack of Fit is not statistically significant in comparison to the pure error. By coded factors the quadratic equation for the response Y2 was given in the equation

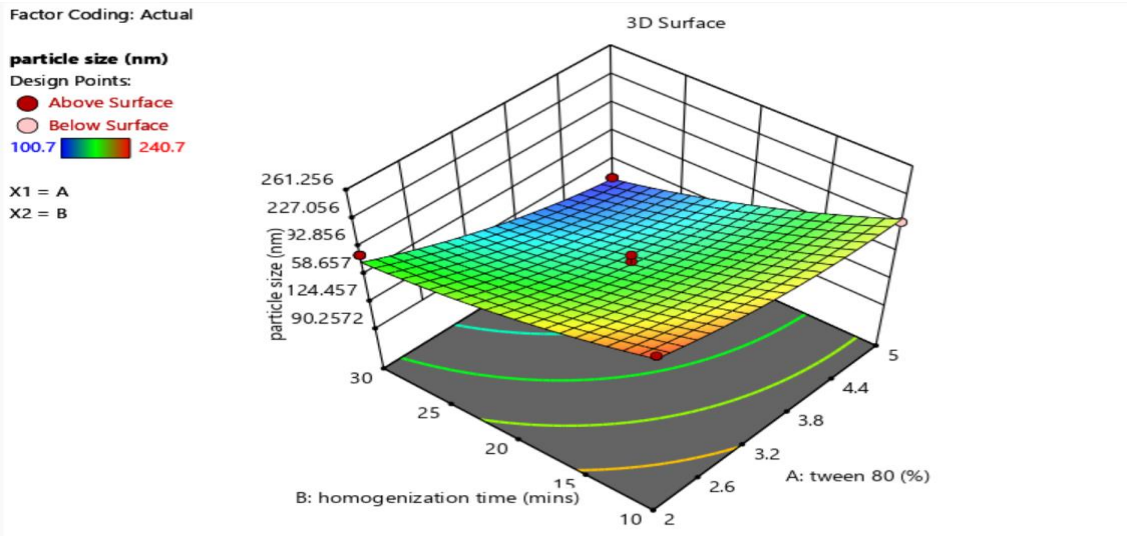
Y1= 158.96-28.54 X1-40.55X2 -10.02X1 X2+ 10.44 X12 + 8.64 X22

The two independent variables, X1 and X2, are the primary factors influencing the response, Y2, as indicated by this equation. The interaction terms X1, X2, X1 X2, X12, and X22, assuming they were altered concurrently, demonstrate a non-linear connection between the variable and answer Y1.



Perturbation plot of Response Y1

Two or more factors that have the most impact on the response can be identified via the perturbation plot. Both component A and factor B for response Y1 exhibit a modest slope with sight curvature. It suggests that the two factors that affected the nanoemulsion the most were the surfactant content and homogenization duration. A decrease in the nanoparticle's particle size is observed when the factor's concentration rises.



Response Surface area of Response Y1

The connection between the independent and dependent variables is expressed by the response surface graphic. An analysis was conducted on the interaction between the X1 and X2 factors in relation to the particle size response. The response plot made it clear that the surfactant concentration and homogenization duration interact with the nanoemulsion's particle size. The formulation's particle size will decrease when the concentration of both factors rises.

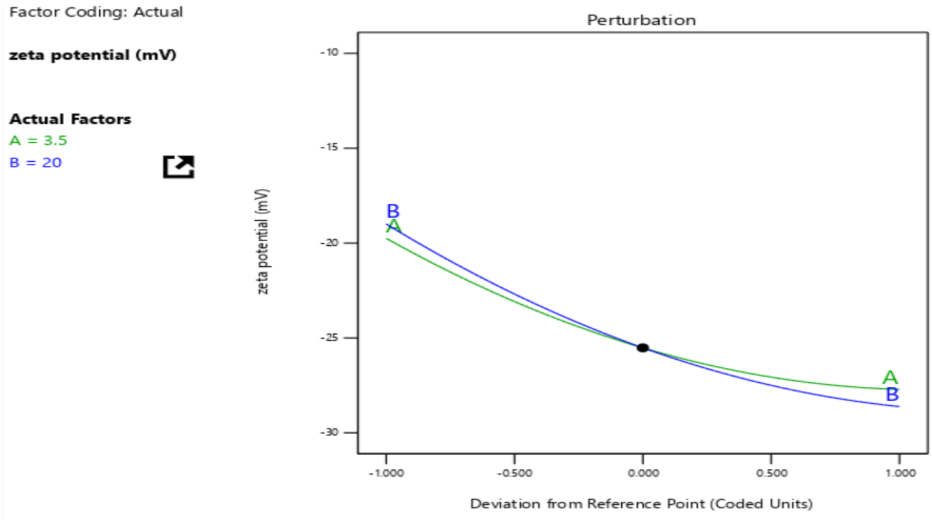
Response Y2 (Zetapotential):

The quadratic model for the Y2 response is suggested by the sum of squares, mean squares, F-value, P-value, residuals, adjusted R2, projected R2, adeq precision, and predicted residual error sum of square

(PRESS) values. The answer Y2's model F-value of 132.64 indicates that the model is significant. This kind of huge F-value has a 0.01% probability of being caused by noise. Model terms are considered significant when P-values are less than 0.0500. A, B, AB, and B2 are important model terms in this instance. This ANOVA revealed that the surfactant concentration and homogenization time are the independent variables influencing the Y2 response. The Y2 Lack of Fit F-value of 0.4415 indicates that the Lack of Fit is not statistically significant in comparison to the pure error. By coded factors the quadratic equation for the response Y2 was given in the equation

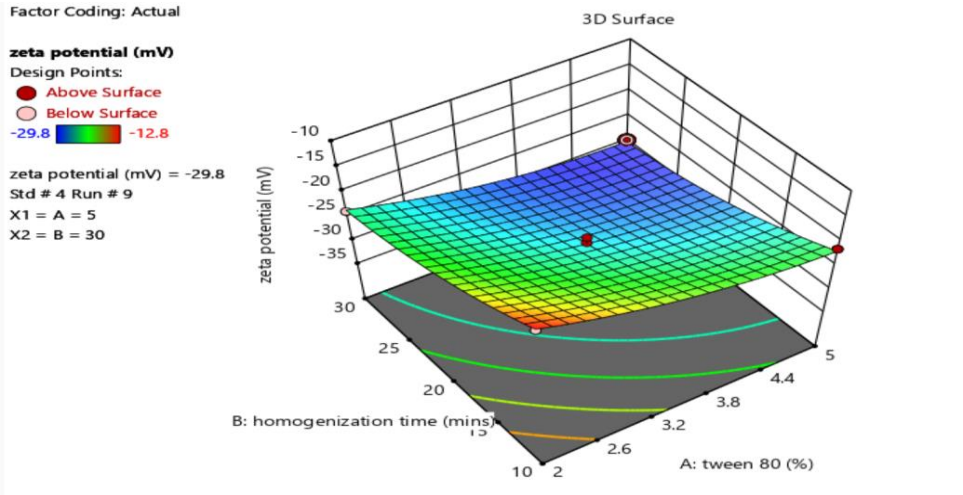
Y2=-25.52 -3.98 X1 -4.81X2 +0.6500 X1 X2 +1.79 X12 +1.72 X22

The two independent variables, X1 and X2, are the primary factors influencing the response, Y2, as indicated by this equation. The interaction terms X1, X2, X1 X2, X12, and X22, assuming they were altered concurrently, demonstrate a non-linear connection between the variable and answer Y2. X1, X2, X12, and X22's negative signs exhibit an antagonistic influence on the reaction Y2.



Perturbation plot of Response Y2

Both Factor A and Factor B have positive slopes for reaction Y2. It suggests that there is interaction over the zeta potential between the surfactant concentration and homogenization time. It is evident from the positive slope that there is an interaction in the zeta potential when the concentrations of both factors are increased.



Response Surface area of Response Y2

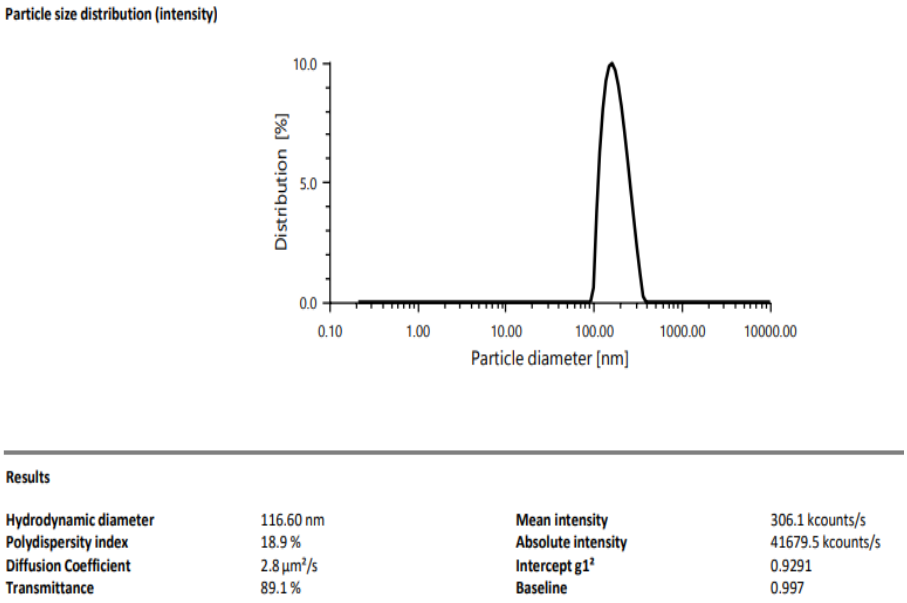
The connection between factors X1 and X2 over the zeta potential is displayed in the response surface

graphic. The figure made it clear that the compound's zeta potential would drastically shift as the concentration of both factors increased.

Evaluation of optimized Nanoemulsion

Particle size and PDI

A particle analyzer was used to carry out the quantitative measurement of particle size and PDI. According to the data, the PDI was 18.9% and the particle size was 116.60 nm.

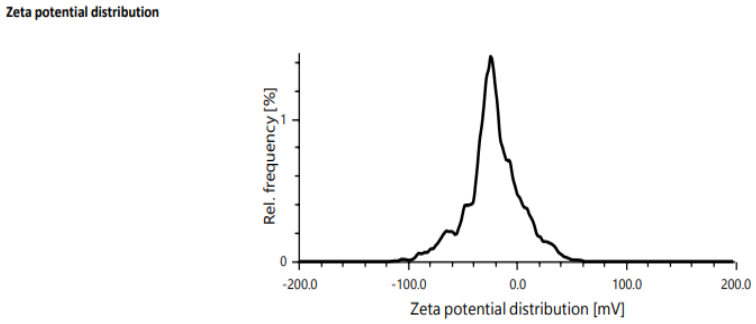


Results			
Hydrodynamic diameter	116.60 nm	Mean intensity	306.1 kcounts/s
Polydispersity index	18.9 %	Absolute intensity	41679.5 kcounts/s
Diffusion Coefficient	2.8 µm²/s	Intercept g1²	0.9291
Transmittance	89.1 %	Baseline	0.997

Particle size and PDI

Zeta Potential

The Zeta Potential was found to be -26.9 mV



Results			
Mean zeta potential	-26.9 mV	Mean intensity	534.1 kcounts/s
+/- Standard deviation	1.4 mV	Filter optical density	3.3029
Distribution peak	-24.3 mV	Conductivity	0.023 mS/cm
Electrophoretic mobility	-2.0972 µm*cm/Vs	Transmittance	85.0 %

Zeta Potential

Evaluation of Sunscreen Nanoemulgel

Description:

Of Thymol sunscreen gel: Pinkish gel with characteristic aromatic odour.

Of cinnamon sunscreen gel: Pinkish gel with characteristic aromatic odour.

Identification: (GC):

Cinnamomum zeylanicum (Cinnamon oil /Darusita taila) and Trachyspermum ammi (Thymol / Yavani satva). The retention time of the peaks in the chromatogram of the sample preparation corresponds to that in the chromatogram of the standard preparation.

Viscosity:

Of Thymol sunscreen gel was found to be 3912.80 cps.

Of Cinnamon sunscreen gel was found to be 3848.02 cps.

Spreadablity:

Of Thymol sunscreen gel

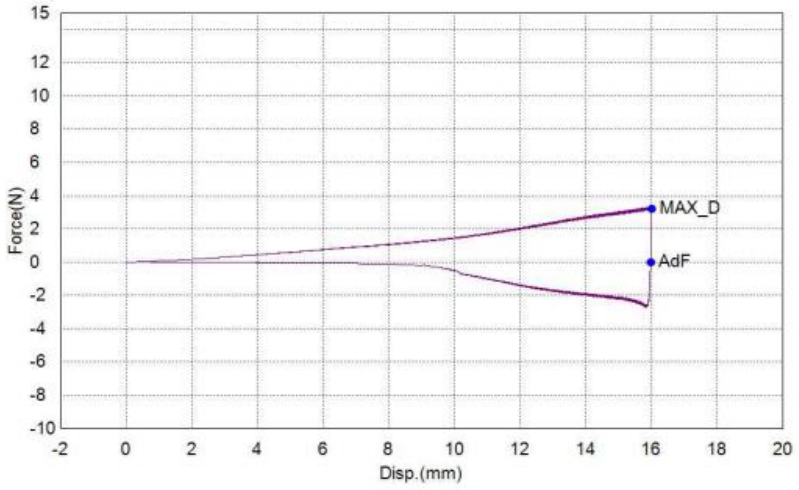
Name	GetData1_Force_Lp1	GetData1_Stress_Lp1	GetData1_Stroke_Lp1	GetData1_Disp._Lp1
Parameters				
Unit	N	N/mm2	mm	mm
1 _ 1	3.83584	0.19541	16.0004	16.0004
1 _ 2	3.41495	0.17397	16.0005	16.0005
1 _ 3	3.28814	0.16751	16.0005	16.0005
Average	3.51298	0.17896	16.0005	16.0005
Standard Deviation	0.28671	0.01460	0.00006	0.00006
Range	0.54770	0.02790	0.00010	0.00010

Name	GetData1_Strain_Lp1	Max_Disp._Force	Max_Disp._Stress	Max_Disp._Stroke
Parameters		Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	%	N	N/mm2	mm
1 _ 1	800.019	3.74985	0.19103	16.0033
1 _ 2	800.025	3.33363	0.16982	16.0034
1 _ 3	800.025	3.20953	0.16350	16.0034
Average	800.023	3.43100	0.17478	16.0034
Standard Deviation	0.00349	0.28302	0.01442	0.00006
Range	0.00604	0.54032	0.02753	0.00010

Name	Max_Disp._Strain	Adhesive_Force_Force	Adhesive_Force_Stroke
Parameters	Calc. at Entire Areas	2th Time	2th Time
Unit	%	N	mm
1 _ 1	800.163	−0.0986	15.9769
1 _ 2	800.169	−0.0146	15.9794
1 _ 3	800.169	−0.0324	15.9795
Average	800.167	−0.0485	15.9786
Standard Deviation	0.00345	0.04426	0.00147
Range	0.00598	0.08400	0.00260

Figure 15: Spreadablity Data Thymol sunscreen gel

Figure 16: Spreadablity Chart of Thymol sunscreen gel



Spreadablity:

Of Cinnamon sunscreen gel

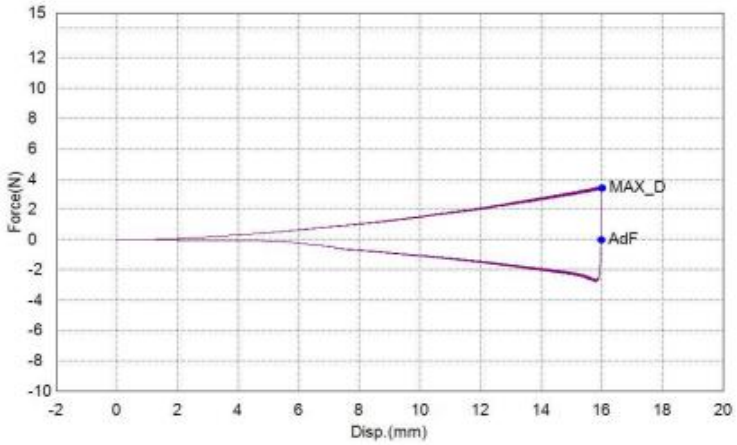
Figure 17: Spreadablity Data of cinnamon oil sunscreen gel

Name	GetData1_Force_Lp1	GetData1_Stress_Lp1	GetData1_Stroke_Lp1	GetData1_Disp._Lp1
Parameters				
Unit	N	N/mm2	mm	mm
1 _ 1	3.91153	0.19926	16.0005	16.0005
1 _ 2	3.55136	0.18091	16.0005	16.0005
1 _ 3	3.47799	0.17718	16.0005	16.0005
Average	3.64696	0.18578	16.0005	16.0005
Standard Deviation	0.23204	0.01182	0.00000	0.00000
Range	0.43354	0.02208	0.00000	0.00000

Name	GetData1_Strain_Lp1	Max_Disp._Force	Max_Disp._Stress	Max_Disp._Stroke
Parameters		Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	%	N	N/mm2	mm
1 _ 1	800.025	3.82710	0.19496	16.0034
1 _ 2	800.025	3.45586	0.17605	16.0023
1 _ 3	800.025	3.38240	0.17231	16.0031
Average	800.025	3.55512	0.18111	16.0029
Standard Deviation	0.00007	0.23839	0.01214	0.00057
Range	0.00000	0.44470	0.02265	0.00110

Name	Max_Disp._Strain	Adhesive_Force_Force	Adhesive_Force_Stroke
Parameters	Calc. at Entire Areas	2th Time	2th Time
Unit	%	N	mm
1 _ 1	800.169	−0.0128	15.9768
1 _ 2	800.113	−2.9515	15.8154
1 _ 3	800.156	−0.0047	15.9793
Average	800.146	−0.9897	15.9238
Standard Deviation	0.02933	1.69900	0.09391
Range	0.05603	2.94680	0.16390

Figure 18: Spreadablity Chart of cinnamon oil sunscreen gel



pH@25°C: Thymol sunscreen gel formulation was found to be 6.48, Cinnamon sunscreen gel formulation was found to be 6.52

Assay by GC: Thymol sunscreen gel *Trachyspermum ammi* (Thymol / Yavani satva): 99.85%, **Cinnamon sunscreen gel-** *Cinnamomum zeylanicum* (Cinnamon oil /Darusita taila): 99.71%

Microbiology

Study	**For thymol sun screen gel**	**For cinnamon sunscreen gel**
Total Plate Count	10 Cfu/gm	10 Cfu/gm
Total Yeast Mould	10 Cfu/gm	10 Cfu/gm
E. coli, Salmonella Spp, S. aureus, P. aeruginosa	Absent	Absent

Summary

This thesis explores the formulation and evaluation of a novel sunscreen gel incorporating Ayurvedic-approved essential oils—thymol and cinnamon oil—within a nanoemulsion. Rigorous standardization tests, including titration assays, infrared spectroscopy, and residue analysis, confirmed the quality of the essential oils. Antioxidant activities were evaluated using DPPH, peroxide scavenging, and reducing power assays, demonstrating thymol's superior scavenging and reducing abilities compared to cinnamon oil. The nanoemulsion was prepared by dispersing the aqueous phase into the oil phase using a high-speed homogenizer. Optimization was achieved through central composite design, focusing on particle size, zeta potential, and nanoemulgel preparation. Characterization involved tests on description, GC identification, viscosity, spreadability, and pH, all confirming that the nanoemulgel met desirable properties for topical use. Microbial tests ensured product safety in compliance with ICH guidelines, while stability studies under accelerated and long-term conditions assessed shelf-life. The developed nanoemulgel sunscreen showed excellent antioxidant activity, optimal particle size, and favorable physical characteristics.

Conclusion

This study represents a significant advancement in pharmacognosy by developing a herbal-based sunscreen gel using advanced nanoemulsion technology. We successfully formulated a gel with thymol and cinnamon oil, showcasing thymol's superior antioxidant activity. The nanoemulsion's high zeta potential and small particle size ensure stability and enhanced skin penetration. Comprehensive

evaluations confirmed the nanoemulgel's suitability for topical use, with all physical properties within acceptable ranges. Our research not only contributes a novel herbal sunscreen but also highlights the potential of pharmacognosy in skincare. Future work may optimize formulations for different skin types and explore additional herbal oils.

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