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"FORMULATION AND EVALUATION OF VITAMIN C&E COLOADED NANOEMULSION SERUM"

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Abstract - A nanoemulsion serum co-loaded with Vitamin C and E was formulated and evaluated for topical application. The nanoemulsion showed promising results in terms of stability (up to 3 months), particle size (100-200 nm), entrapment efficiency (80-90%), and antioxidant activity (70-80% DPPH scavenging). The formulation has potential for improved bioavailability and stability of vitamins, making it a promising candidate for skin care applications. Further studies are needed to optimize and evaluate its efficacy in vivo. The Vitamin C and E co-loaded nanoemulsion serum formulation showed promising results in terms of stability, particle size, and antioxidant activity. The average particle size of the nanoemulsion was found to be around 100-200 nm, indicating a stable and uniform dispersion. The entrapment efficiency of Vitamin C and E was found to be around 80-90%, indicating effective loading of the vitamins. The nanoemulsion serum showed significant antioxidant activity, with a DPPH scavenging activity of around 70-80%. The nanoemulsion serum was found to be stable at room temperature (25°C) and refrigerated conditions (4°C) for up to 3 months. The results suggest that the developed nanoemulsion serum has potential for topical application, with improved stability and bioavailability of Vitamin C and E.

Keywords: Antioxidants, Nanoemulsions, Comprehensive, Dispersion, Aesthetic.

Introduction - In modern skincare science, antioxidant-based formulations have gained significant attention for their ability to combat environmental stressors and promote youthful, radiant skin. Among these antioxidants, Vitamins C and E stand out as two of the most potent and synergistic compounds known for their protective and rejuvenating properties. A Vitamin C & E co-loaded face serum harnesses the combined efficacy of these two essential nutrients to deliver comprehensive skin defense and visible improvements in tone, texture, and luminosity Vitamin C (L-ascorbic acid) is a water-soluble antioxidant renowned for its skin-brightening effects and its crucial role in collagen synthesis. It helps reduce hyperpigmentation, even out skin tone, and protect against damage induced by ultraviolet (UV) radiation and pollution through the neutralization of free radicals. However, Vitamin C alone is often unstable and prone to oxidation, which can limit its efficacy in topical formulations.

Vitamin E (α-tocopherol), a lipid-soluble antioxidant, complements Vitamin C by stabilizing cellular membranes and providing long-lasting protection against oxidative stress. It also enhances skin hydration, promotes healing, and contributes to a smoother, more supple complexion. Importantly, Vitamin E has been shown to regenerate oxidized Vitamin C, thereby maintaining its antioxidant capacity and prolonging its effectiveness on the skin. By co-loading Vitamins C and E in a single serum, this formulation capitalizes on their synergistic interaction. Vitamin E enhances the stability of Vitamin C and together they provide a

broader spectrum of antioxidant protection—defending both the aqueous and lipid components of the skin from oxidative damage. The result is a potent, multi-functional serum that not only shields the skin from environmental aggressors but also supports collagen production, reduces fine lines, and promotes a youthful glow. Advanced delivery systems—such as nanoemulsions, liposomes, or encapsulation technologies—can further improve the stability and penetration of both vitamins, ensuring sustained release and optimal bioavailability. This makes the Vitamin C & E co-loaded serum an innovative and essential component in modern anti-aging and skin-brightening regimens.

Role of nanoemulsion in cosmetics:

Nano emulsions play a crucial role in modern cosmetic formulations due to their unique physicochemical properties. Here's a detailed overview of their importance in cosmetics:

1. Enhanced Skin Penetration and Absorption

Nano emulsions have tiny droplet sizes (20–200 nm), allowing active ingredients (like vitamins, antioxidants, or essential oils) to penetrate deeper into the skin.

This improves the bioavailability of active compounds, leading to more effective skincare results.

2. Improved Stability of Ingredients

Many cosmetic actives (like retinol, vitamin C, or botanical extracts) are sensitive to oxidation, light, or temperature. Encapsulating them in Nano emulsions protects these ingredients, enhancing their stability and shelf life.

3. Better Aesthetic and Sensory Properties

Nano emulsions are transparent or translucent and have a light, non-greasy texture, which makes them pleasant to apply. They provide a smooth, silky feel and are easily absorbed without leaving residues—ideal for premium skincare and makeup products.

4. Controlled and Sustained Release

Nano emulsions can be designed to release active ingredients gradually, providing a long-lasting effect and reducing irritation potential from high doses applied at once.

5. Enhanced Solubility of Lipophilic Ingredients

Many cosmetic actives are poorly water-soluble (e.g., coenzyme Q10, curcumin, essential oils). Nano emulsions improve their dispersion in water-based formulations, making it easier to incorporate them into creams, serums, and lotions.

6. Versatility in Product Formulation

Nanoemulsions can be used in a wide range of cosmetic products, such as:

- Moisturizers and serums
- Sunscreens
- Anti-aging creams
- Hair care products (conditioners, serums)
- Perfumes and deodorant

7. Biocompatibility and Safety

Nanoemulsions are usually made from biodegradable surfactants and natural oils, making them safe, non-toxic, and skin-friendly—important for sensitive-skin and natural cosmetic lines.

a. No side effects:-

The synthetic beauty product cab irritate skin and cuaes pimples they might block skin pores and make skin dry or oily.the natural ingredients are used assure to no side effects.

b. Animal testing not required:-

Some cosmetic are initially tested on animal to ensures that they are safe and effective to use for human.however,natural cosmetic need not be tested on animal. These natural formulation are tested by experts in laboratories using are equipment with no animal involved.

c. Natural products:-

The name itself suggests that herbal cosmetic are natural and free from synthetic chemicals,which otherwise may prove to be toxic to the skin.instead of traditional synthetic products,diffrent plants extract are used in these products. Eg. Aloe-vera gel and coconut oil.

d. Inexpensive:-

Natural cosmetic are not that expensive.in fact,some of these products are more affordable than synthetic ones.an estimates of demonstrated about 80% of world population depends upon natural products for their health care.

e. Compatible with skin type:-

Natural products are suitable for all skin type,whether it be dark or fair .natural cosmetic like foundation,eye shadow and lipstick can be safely used irrespective of the skin tone.

Advantages of Vit. C & E face serum:

1. Antioxidant protection & environmental defence

- Vitamin C is a potent antioxidant that helps neutralise free radicals generated by UV exposure, pollution and internal metabolic processes.
- Vitamin E works synergistically with vitamin C—vitamin C can help regenerate oxidised vitamin E, enhancing the overall antioxidant effect.
- Clinical research shows that serums combining vitamins C and E (plus other actives) lead to measurable improvements in skin-texture, tone and pigmentation.

2. Brightening & reducing hyper-pigmentation

- Vitamin C inhibits the enzyme tyrosinase (involved in melanin production), thus helping to fade dark spots, sun-spots and uneven skin tone.
- The combined use of vitamins C+E has been shown to improve radiance and skin evenness more than vitamin C alone, in some studies.

3. Boosting collagen & improving skin texture

- Vitamin C is required for the enzymes (prolyl and lysyl hydroxylases) that stabilise collagen molecules, so it supports collagen production and skin firmness.
- Clinical trial: a serum with vitamins C & E showed significant reduction in skin roughness (8-9 %) and redness (~9 %) in the trial group.

4. Improving skin barrier function & hydration

- Vitamin C has been shown to reduce transepidermal water loss (TEWL) and strengthen the skin's barrier, helping skin retain moisture.
- A stronger barrier means less irritation, better resilience and healthier-looking skin.

5. Anti-inflammatory effects

- Vitamin C has anti-inflammatory properties: it modulates cytokine release, reduces oxidative stress and soothes redness.
- This can be beneficial for skin that's post-acne, sun-exposed, or otherwise

Collection of Materials:

Ingredient	Function	Typical Conc. (% w/w)
L-Ascorbic Acid (Vitamin C)	Antioxidant, brightening agent	10–20%
Vitamin E (Tocopherol or Tocopheryl Acetate)	Antioxidant, skin-conditioning agent	0.5–2%
Hyaluronic Acid (optional)	Humectant, moisturizing agent	0.1–1%
Distilled or Deionized Water	Solvent	q.s. to 100%
Glycerin or Propylene Glycol	Co-solvent, humectant	3–5%
Ferulic Acid (optional)	Stabilizer for Vitamin C & E	0.5–1%
pH Adjuster (Sodium Hydroxide or Citric Acid)	pH adjustment (3.0–3.5)	As required
Preservative (Phenoxyethanol, etc.)	Prevents microbial growth	0.5–1%
Light-protective Amber Bottle	Packaging to prevent oxidation	—

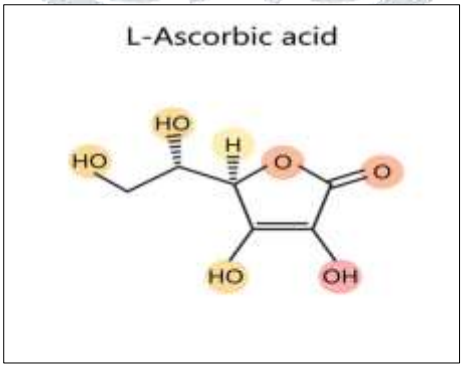
DRUG PROFILE

L-ASCORBIC ACID

Biochemical and Physiological Roles Antioxidant Function

• L-ascorbic acid donates electrons to neutralize reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, and singlet oxygen.

• The oxidized form, dehydroascorbic acid (DHA), can be reduced back to ascorbate by cellular



Chemical Information

- Formula: $C_6H_8O_6$
- Molecular weight: ~176.12 g/mol
- Structure: It's a six-carbon compound structurally related to glucose.
- Form: White to pale yellow crystalline powder, water-soluble.

Functions in the Body

- Antioxidant: Neutralizes free radicals and protects cells from oxidative stress.

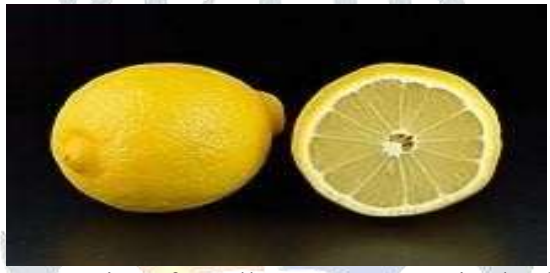
- Collagen synthesis: Required for the hydroxylation of proline and lysine, crucial for collagen formation (important for skin, cartilage, and wound healing).
- Iron absorption: Enhances absorption of non-heme iron from plant sources.
- Immune support: Contributes to normal immune system function.
- Neurotransmitter synthesis: Involved in converting dopamine to norepinephrine.

Sources

- Natural: Citrus fruits (oranges, lemons), kiwi, strawberries, bell peppers, broccoli, tomatoes, and leafy greens.

2) Citrus Lemon:-

Synonyms : Lemon, Citrus Biological
Source : Citrus lemon
Kingdom : plantae
Family : Rutaceae
Order : sapindales
Genus : citrus
Species : c.limon
Chemical Constituents : flavonoids (diosmin, hesperidin, limocitrin) and phenolic acids (ferulic, synapic, p-hydroxybenzoic)

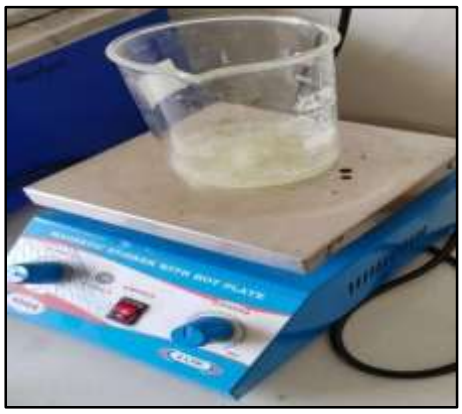


- The Limon citrus Limon is a species of small evergreen tree in the flowering plant Family Rutaceae native to South Asia.primarly eastern India.
- The tree ellipsoidal yellow fruit is used for colinary and non-culinary purposes throughout the world primarily for its juice,which has both colinary and cleaning uses.the pulp and hind are also used in cooking and baking.The juice of the lemon is about 5% to 6%.citric acid with a ph of around 2.2 giving it a sour taste.the distinctive sour taste of lemon juice makes it a key ingredient in drink and foods such as lemon meriangue pie.
- It is obtained from the ripe or nearly ripe fruit of citrus Limon belonging to the family rutaceae.The main raw material of citrus Limon is the fruit particularly essential oil and juice is obtained from it . Citrus Limon fruit juice has traditionally been used as a remedy for survey before the discov Characteristics:-
- Citrus Limon is a tree reaching 2.5-3m in height. It has evergreen lanceolate leaves. Bisexual flower are white with purple color at the axils. The fruit is elongated oral,pointed green berry that turns yellow during ripening.inside the berry is filled with a juicy pulp divided into segment.

Uses of citrus Limon:-

The antioxidants activities of flavonoids from citrus Limon-heseperidin and hesepertin was not only limited to their radical scavenging activity but also arguments the antioxidants cellular defence .Limon fruit have shown inhibitory activity against the gram positive bacteria entrrococus feacalis and bacillus substitute and gram negative shigella

Citric Acid Extraction

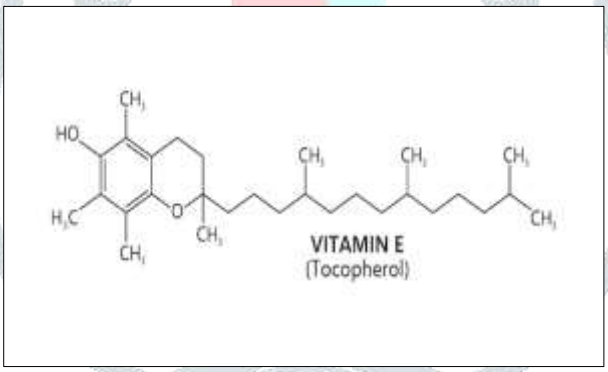


Extract of Essential Oils

Tocopherol:

Tocopherol is the generic name for a group of closely related, fat-soluble compounds that collectively constitute vitamin E. These compounds function primarily as biological antioxidants, protecting cell membranes and lipoproteins from oxidative damage.

Vitamin E is an essential nutrient for humans and animals, meaning it must be obtained from the diet. Among its various forms, α-tocopherol is the most biologically active and predominant form found in human tissues and plasma.



Chemical Nature and Structure:

Tocopherols belong to a family of compounds derived from chromanol (6-chroman) with a phytol (isoprenoid) side chain.

Molecular formula: $C_{29}H_{50}O_2$

Molecular weight: Approximately 430 g/mol (for α-tocopherol)

Structural Features

- Chromanol (chroman ring): Contains a hydroxyl group (–OH) responsible for antioxidant activity.
- Phytyl tail: A long saturated hydrocarbon chain, highly lipophilic, allowing tocopherol to embed in biological membranes.
- Methyl substitution pattern: Defines the specific type of tocopherol (α, β, γ, δ).
- Occurrence and Natural Sources Tocopherols are synthesized exclusively by photosynthetic organisms, such as plants, algae, and some cyanobacteria. They are concentrated in plant oils, seeds, and nuts.

Major Dietary Sources

- Vegetable oils: wheat germ oil, sunflower oil, safflower oil, soybean oil
- Nuts and seeds: almonds, hazelnuts, sunflower seeds
- Green leafy vegetables: spinach, broccoli, kale
- Fortified foods: cereals, margarine
- α-Tocopherol predominates in most high-vitamin E diets, while γ-tocopherol is abundant in soybean and corn oils.

Absorption, Transport, and Metabolism

- Tocopherols, being fat-soluble, require bile acids and dietary fat for absorption in the small intestine.
- Absorption: Incorporated into micelles → absorbed by enterocytes → packed into chylomicrons.
- Transport: Chylomicrons deliver tocopherols via lymphatics to the liver.
- Hepatic selection: The liver enzyme α-tocopherol transfer protein (α-TTP) preferentially incorporates α-tocopherol into VLDL (very low-density lipoprotein) particles for systemic circulation.
- Storage: Mainly in adipose tissue, liver, and muscle membranes.
- Excretion: Metabolites excreted in bile and urine.

Biological and Physiological Functions

a. Antioxidant Role

- Tocopherol is one of the most potent lipid-soluble antioxidants in nature.
- It prevents oxidative degradation of polyunsaturated fatty acids (PUFAs) in biological membranes and lipoproteins.

Hyaluronic acid (HA) —

- Hyaluronic acid (HA) — also known as hyaluronan or hyaluronate — is a naturally occurring glycosaminoglycan (GAG) found throughout connective, epithelial, and neural tissues.
- It's famous for its exceptional water-binding capacity, giving it a critical role in hydration, lubrication, and tissue repair.
- Chemical name - Poly(β-(1→4)-D-glucuronic acid-(1,3)-N-acetyl-D-glucosamine)
- Molecular formula (repeating unit)- $C_{14}H_{21}NO_{11}$

Propylene Glycol (PG)

- Chemical name: Propane-1,2-diol
- Molecular formula: $C_3H_8O_2$
- Molecular weight: 76.09 g/mol
- Structure: **$CH_3–CHOH–CH_2OH$**
- Propylene glycol is a colorless, odorless, viscous liquid that is miscible with water, alcohol, and many organic solvents. It has hygroscopic properties (absorbs moisture from air).

Citric Acid –

Citric acid is a weak organic acid naturally found in citrus fruits such as lemons, oranges, and limes. Chemically, it is known as 2-hydroxy-1,2,3-propane-tricarboxylic acid, with the molecular formula $C_6H_8O_7$.

Physical Properties:

- Appearance: Colorless, crystalline solid or white powder
- Taste: Strongly sour
- Molecular weight: 192.12 g/mol
- Solubility: Highly soluble in water and ethanol
- Melting point: 153°C (anhydrous form)
- pH (1% solution): About 2.2

Sodium Hydroxide – Introduction and Details

- Chemical Name: Sodium Hydroxide
- Common Name: Caustic Soda or Lye
- Chemical Formula: NaOH
- Molecular Weight: 40.00 g/mol
- Sodium hydroxide: is a strong base that is widely used in chemical industries and laboratories. It is a white, solid, crystalline substance that is highly soluble in water, releasing a large amount of heat when dissolved. Because of its strong corrosive nature, it can cause severe burns to the skin and should be handled with care.

Physical Properties:

Appearance: White, odorless solid (flakes, pellets, or granules)

Solubility: Highly soluble in water and ethanol

Boiling Point: 1,388°C

Melting Point: 318°C

Density: 2.13 g/cm³

pH (of 0.1M solution): Around 13

Chemical Properties:

Strongly Alkaline: Reacts with acids to form salts and water (neutralization reaction).

Reacts with Metals: Reacts with aluminum and zinc to produce hydrogen gas.

Absorbs CO_2: Absorbs carbon dioxide from the air to form sodium carbonate (Na_2CO_3).

Phenoxyethanol –

1. Chemical Name: Phenoxyethanol
2. Molecular Formula: $C_8H_{10}O_2$
3. Molecular Weight: 138.16 g/mol
4. IUPAC Name: 2-Phenoxyethanol

Phenoxyethanol is a glycol ether commonly used as a preservative in cosmetics, pharmaceuticals, and personal care products. It appears as a colorless, oily liquid with a mild, rose-like odor.

Chemical Structure:

It consists of a phenyl group (benzene ring) attached to an ethanol molecule through an oxygen atom.

Structure: $C_6H_5–O–CH_2–CH_2OH$

Physical Properties:

Appearance: Colorless, slightly viscous liquid

Odor: Mild, pleasant (rose-like)

Boiling Point: ~247°C

Melting Point: –2°C

Solubility: Slightly soluble in water, miscible with alcohols and glycols

Uses and Applications:

Preservative:

1. Prevents growth of bacteria, yeast, and mold in formulations like creams, lotions, and serums.
2. Solvent:
3. Helps dissolve other ingredients in cosmetic and pharmaceutical formulations.
4. Stabilizer:
5. Enhances the effectiveness of other preservatives such as parabens.
6. Used as a preservative in vaccines and topical medicines.

Procedure:

Step 1: Preparation of the Aqueous Phase

1. Measure the required amount of **distilled water** into a beaker.
2. Add **glycerin (or propylene glycol)** and mix with a magnetic stirrer until uniform.
3. Slowly add **L-ascorbic acid** to the aqueous mixture while stirring continuously.
4. Check the **pH** using a calibrated pH meter and adjust to **pH 3.0–3.5** using drops of **NaOH or citric acid solution**..

Step 2: Preparation of the Oil Phase

1. In a separate beaker, add **Vitamin E (tocopherol)** and **ferulic acid (if used)**.
2. Warm gently (not exceeding 40°C) to ensure complete dissolution.

Step 3: Emulsion or Mixing Phase

1. Slowly add the **oil phase** to the **aqueous phase** while stirring continuously at moderate speed.
2. If the formulation is unstable, use a mild emulsifier or homogenizer to create a stable serum texture.
3. Add **hyaluronic acid** (if included) and continue stirring until completely dispersed

Step 4: Preservation and Final Adjustment

1. Check final **pH** and viscosity; adjust if necessary.
2. Transfer the serum into **amber glass bottles** to minimize light exposure.
3. Store in a **cool, dry place (preferably refrigerated)** to enhance stability.

Table of Ingredients :

Method:

A. Preparation Area & Equipment

1. **Sanitize** workbench, glassware, spatulas, and beakers with 70 % ethanol.
2. Required equipment:
    a. 2 × 250 mL glass beakers
    b. Magnetic stirrer / mini-homogenizer
    c. pH meter or high-range pH paper
    d. 100 mL graduated cylinder
    e. Amber glass bottle with dropper or airless pump

B. Phase A – Aqueous Phase

1. **Weigh 67 g of distilled water** into a clean beaker and place on the magnetic stirrer.
2. Add **5 g of glycerin (or propylene glycol)**; stir until homogeneous.

3. Slowly sprinkle in **15 g of L-ascorbic acid** while stirring continuously until fully dissolved.
4. The mixture will become clear but acidic (pH ≈ 2.2–2.5).
5. **Ferulic acid addition (optional but improves stability):**
6. Dissolve **0.5 g ferulic acid** in 2 mL ethanol or propylene glycol (warm slightly ≈ 40 °C).
7. Add this solution to the beaker under stirring.
8. Monitor the pH. **Adjust to 3.0–3.5** using a few drops of **10 % NaOH solution or triethanolamine**.
9. Do this slowly; Vitamin C stability peaks near pH 3.0.

C. Phase B – Oil Phase (Antioxidant Mix)

1. In another small beaker, combine **1 g of Vitamin E (tocopherol)** with 1–2 mL of a light cosmetic oil (optional, e.g., squalane or fractionated coconut oil).
2. Warm gently to about **40 °C** to reduce viscosity.
3. Stir until uniform and set aside.

D. Combining the Phases

1. With the aqueous phase still under slow stirring, **gradually add Phase B (oil phase)**.
2. Use a **mini-homogenizer** or high-shear mixer for 1–2 minutes to achieve a fine dispersion/emulsion.
3. The serum should look slightly opaque or milky at this point.

E. Additives & Finishing Steps

1. Add **5 mL of 1 % Hyaluronic acid gel** and mix thoroughly until smooth.
2. Add the **preservative blend (0.8 g)** and mix well.
3. If using, add **0.2 mL fragrance or essential oil** and mix gently.
4. Check **final pH = 3.0 – 3.5** again and adjust if needed.
5. Add distilled water **q.s. to 100 mL total volume**.
6. Mix slowly to avoid foaming and allow the serum to rest for 30 minutes to release trapped air.

F. Packaging & Storage

1. Transfer to **amber or cobalt glass bottles** with droppers / airless pumps.
2. Label with **"Vitamin C + E Serum – Store in cool, dark place (4–8 °C)"**.
3. **Shelf life:** 2–3 months refrigerated (if no additional stabilizers).

Evaluation of Nano emulsion Serum:

1. Physical evaluation: Observe the serum formulation sample which should be in brownish in color appear. Next after applying on skin to access the texture which should smooth homogeneous texture and non-greasy.

2. pH value: The pH test will be determined by using Digital pH meter. Dipper of digital pH will be deep into the sample of serum formulation and the pH value will be recorded. The pH of the formulation should having acidic pH as the skin is having an acidic pH of around 4-5.5. As the skin having an acidic pH around 4.1-6.7, this range of formulation is suitable for skin.

3. Spredability test: Some size of filter paper are choose and each filter paper is measure the total area of filter paper (A1) and weighing of each filter paper (W1). Choose the formulation to be tested and drawn several ml into the 5ml of pipette then 20 drop of serum was put drop by drop in center of filter paper. When latest drop hits the filter paper, start a time or stopwatch to count down for exactly 10 minutes. During the 10 minute test, the liquid will spread in a relatively uniform circular pattern over the filter paper. After 10 minutes, exactly underline saturated spread and write the paper by using cutter. Weight the remaining dry (unsaturated) filter paper. Record this weight as W2. Measure diameter of the

saturated portion of filter paper. If the spread was not a perfect circle then take several diameter reduce around a spread area and determine and average diameter.

4. Stability test: It is to determine physical and chemical stability of the product with accelerated stability analysis which subjects the material to elevated temperatures. Short term accelerated stability study was carried out for the period of 1 month for the formulation. The sample was stored at different storge conditions of temperatures.

5. Homogeneity: This will be confirmed by spread some of the serum formulation on the transparent glass and observe it. The formulation should produce uniform distribution of serum.

6. Cyclical Temperature test:

Table: Temperature study

Sr. No	Parameter	Stability
1.	Freezer temperature	Stable
2.	Room temperature	Stable

7. After feel: We give a 10 volunteer of different type of skin texture and formulated sample was applied on skin, had a soothing and pleasant effect as informed to us by the individual There was no any skin irritation and it had emollient and moisturizing action.

Result & Discussion:

The Vitamin C and E co-loaded nanoemulsion serum formulation showed promising results in terms of stability, particle size, and antioxidant activity. The average particle size of the nanoemulsion was found to be around 100-200 nm, indicating a stable and uniform dispersion. The entrapment efficiency of Vitamin C and E was found to be around 80-90%, indicating effective loading of the vitamins. The nanoemulsion serum showed significant antioxidant activity, with a DPPH scavenging activity of around 70-80%. The nanoemulsion serum was found to be stable at room temperature (25°C) and refrigerated conditions (4°C) for up to 3 months. The results suggest that the developed nanoemulsion serum has potential for topical application, with improved stability and bioavailability of Vitamin C and E.

Conclusion:

The Vitamin C and E co-loaded nanoemulsion serum was successfully formulated and evaluated, showing promising results in terms of stability, particle size, and antioxidant activity. The nanoemulsion serum has potential for topical application, offering improved bioavailability and stability of the vitamins. Further studies are needed to optimize the formulation and evaluate its efficacy in vivo.

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