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Formulation, evaluation and comparative study of liquid crystal emulsion

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Abstract
The main purpose of this study was to prepare and evaluate Liquid Crystal Emulsion.
The comparative evaluation of liquid crystal emulsions containing an API and herbal oil demonstrated that both formulations were physically stable and suitable for topical application, with zeta potential values of −53.3 mV (API) and −56.5 mV (herbal oil), indicating strong colloidal stability. However, the herbal oil-based emulsion showed slightly greater stability and better resistance to aggregation. Both emulsions maintained an optimal skin-compatible pH and exhibited well-defined lamellar liquid crystal structures under microscopy. While the API formulation had higher viscosity and uniform droplet distribution, the herbal oil emulsion excelled in sensory properties, offering a smoother texture and pleasant natural aroma. Most notably, the herbal oil formulation showed superior anti-aging activity by enhancing skin hydration, elasticity, and providing antioxidant protection. These findings suggest that the herbal oil-based liquid crystal emulsion is more stable and effective for cosmoceutical and anti-aging applications compared to the API-based formulation.

Keywords: Liquid crystal emulsion, zeta potential, API, Herbal oil, Anti-aging

Introduction

Liquid Crystal Emulsion

A unique state of matter, called liquid crystal exists in case of certain substances that is located between solid and liquid state. Liquid crystals are typically elongated organic molecules with an uneven distribution of electrical charges along their axes (dipole). This gives rise to a special physical characteristic to which liquid crystals owe their name, between the crystalline and liquid states. They possess both structural order and mobility. Liquid crystalline structures exhibit anisotropy, having optical direction and the characteristic properties of solids and liquids. They exhibit a further state of aggregation, namely the liquid crystalline or mesophase indicating the unique structure intermediate between that of a true liquid and a solid crystal phase. Liquid crystals are found to be birefringent, due to their anisotropic nature. So they demonstrate double refraction (having two indices of refraction). When liquid crystalline structure is viewed under crossed Nicole prism of a polarizing microscope, intense color bands and birefringence are seen. Anisometric molecular shape, associated with the polarizability is the basic requirement for the formation of liquid crystalline phases. Even drugs that include organic acids or basic salts with anisometric molecular shape might fulfil the requirements for liquid crystal formation. On the basis of molecular structure, a compound may pass through one or many different liquid crystalline phases depending upon its order and symmetry, before transforming into a truly isotropic fluid (the liquid phase). Liquid crystals are categorized into two generic classes i.e. thermotropic and lyotropic mesophases. Thermotropic liquid crystalline phases are formed by a change of temperature, whereas lyotropic phases are formed when mixed with aqueous phase. The phase transitions of thermotropic liquid crystal are temperature-dependent, while those of lyotropic liquid crystals depend on both temperature and concentration [1].

Molecules in a crystal are highly ordered, while molecules in a liquid are free to diffuse in a random way. Thus, molecules in liquid crystal phases diffuse like the molecules of a liquid but they contain some degree of orderness. Hexagonal and cubic mesophases are particularly of high interest in the drug delivery field due to their exceptional potential as drug vehicles. They are highly investigated for their ability to control or sustain the release of both hydrophilic and hydrophobic drug molecules having wide range molecular weights.

Drugs can be incorporated in these gel-like phases, additionally they have non-toxic, biodegradable and bio adhesive characteristics, which cause significant value addition in drug delivery [1]. These characteristics of hexagonal and cubic phases made them one of the favorable means for the researchers to deliver drug through different routes of administration e.g. buccal, gastrointestinal, intravenous, lung, nasal, oral, rectal and vaginal.

This review attempted to provide a comprehensive insight highlighting the multidisciplinary aspect of liquid crystal system. Owing to the wide applicability of liquid crystal system in different areas of science and technology, this review tried to investigate the current status of research to the application of cubic and hexagonal phases in the drug delivery context.

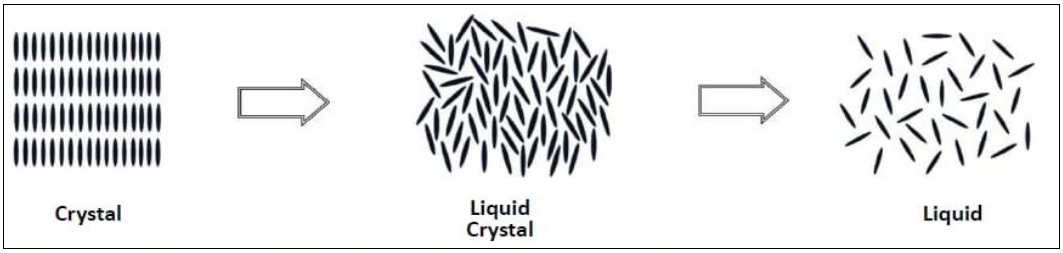


Fig 1: Formation of liquid crystal phase between crystal and liquid state of aggregation

Thermotropic liquid crystals

Most of the thermotropic liquid crystals are composed of rod-like molecules and classified into three types, nematic, smectic and cholesteric. They are formed on heating the crystalline solid or cooling the isotropic liquid. Nematic phase (thread-like) is the simplest liquid crystalline phase, where the molecules maintain long-range orientation. There exists no positional order. Liquid crystals used in electronic display are primarily of the nematic type. When viewed under a polarizing microscope the defect regions linking these domains appear as dark threads. Smectic phase (soap-like) a name that was coined by Friedel from a Greek word, meaning 'grease or slime'. The smectic structure is stratified as the molecules are arranged in layers with their long axes approximately normal to the plane of the layers with a well-defined interlayer spacing. Cholesteric phase is also known as chiral nematic liquid crystal. The arrangement of cholesteric phase can be described as a combination of the nematic and smectic, where some layers which resemble the smectic phase are incorporated in the nematic layers. Due to the helical structure, it exhibits an interesting phenomenon like optical rotation, selective reflection and two-color circular polarization [1].

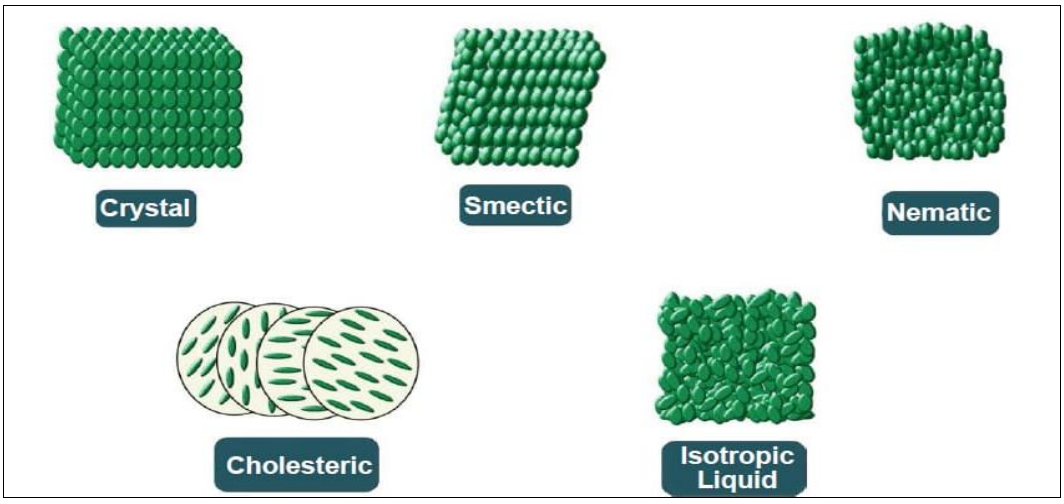


Fig 2: Thermotropic liquid crystal phases—crystal, smectic, nematic, cholesteric.

Fig 2: Thermotropic liquid crystals: crystal; smectic; nematic; cholesteric; isotropic liquid nematic phase (thread like) is the simplest liquid crystalline phase, where the molecules maintain long-range orientation. smectic (soap-like) structure is stratified, with molecules arranged in layers and their long axes approximately normal to the plane of the layers, exhibiting a well-defined interlayer spacing. cholesteric phase (also known as *chiral nematic* liquid crystal) combines features of nematic and smectic arrangements. It incorporates smectic-like layers within the nematic layers.

Lyotropic liquid crystals: Lyotropic liquid crystals, briefly called lyotropic or lyomesophases are mixtures of amphiphilic molecules in a solvent at a given temperatures and relative concentration. The driving factors for the formation of lyotropic mesophases are the temperature, the structure of the organic molecule, and the water/ amphiphile ratio. Lyotropic liquid crystals are further classified as lamellar, hexagonal and cubic phase. Lyotropic liquid crystal systems of surfactant molecules can absorb water from the environment. Depending upon the water content of the environment, it triggers spontaneous phase-transitions resulting lamellar, hexagonal and cubic phases. Three

properties of surfactant (s) are found to be affecting the formation of lyotropic liquid crystalline phase, these are, the magnitude of the repulsive forces between adjacent head groups at the interface of surfactant and water, degree of contact between water and alkyl chain, and conformational disorders in the alkyl chains.

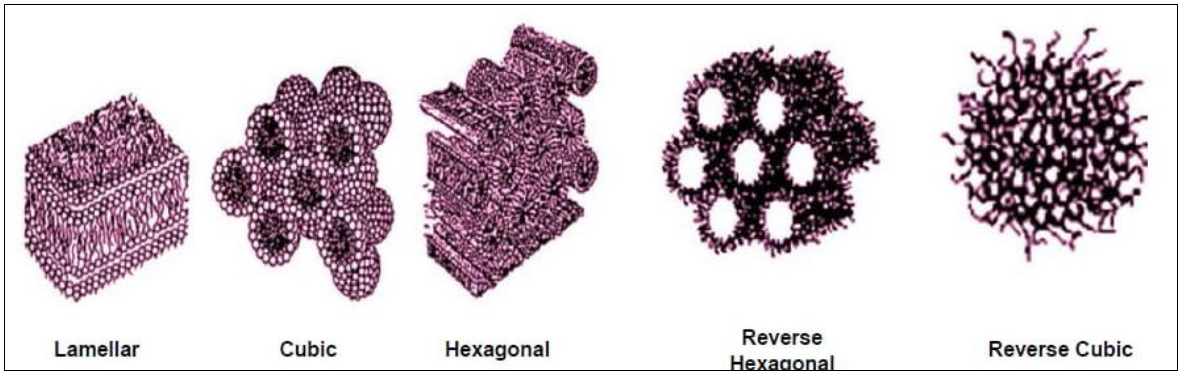


Fig 3: Lyotropic liquid crystal phases—lamellar, cubic, hexagonal structures.

Fig 3: Lyotropic liquid crystal systems: lamellar; cubic; hexagonal; reverse hexagonal; reverse cubic. Lamellar phase or neat phase has double layers of surfactant molecule consisting of polar head groups, which protrudes in the intervening layers of aqueous interface. Cubic phase structure consists of continuous curved bilayers and a pair of interpenetrating non-intersecting aqueous channels separated by each other. Cubic phase has spherical packing and the ionic portion of the molecule on the surface of the sphere and the non-polar portion (water-insoluble) directed toward the center of the sphere. Hexagonal phase or middle phase can be seen as an array of hexagonally close packed water layers sheltered by a surfactant monolayer: The long range order is two dimensional

Lamellar phase or neat phase has double layers of surfactant molecule consisting of polar head groups, which protrudes in the intervening layers of aqueous interface. The hydrocarbon chains are in a dynamic disordered state that is similar to paraffin in the liquid state and the surfactant bilayers are separated by water layers. A notable feature of the neat phase is its relative fluidity in spite of high surfactant content and thus the lamellae can glide easily over one another.

Hexagonal phase or middle phase can be seen as an array of hexagonally close packed water layers sheltered by a surfactant monolayer. The long range order is two dimensional. Two types of hexagonal mesophase can be encountered: normal and reverse mesophase, respectively in aqueous and anhydrous organic solvents. In anhydrous organic solvents (low water content), the structure is reversed and the hydrophilic polar groups now form the inner core and are shielded from the non-aqueous environment by a layer of hydrocarbon chains. These are called reverse hexagonal phase or reverse middle phase. Also when large quantities on non-ionic surfactant are added to the system, a hexagonal structure and the solution containing reversed micelles may be formed. The hexagonal phase is much stiffer than the lamellar phase.

Cubic phase structure consists of continuous curved bilayers and a pair of interpenetrating non-intersecting aqueous channels separated by each other. Cubic phase has spherical packing and the ionic portion of the molecule on the surface of the sphere and the non-polar portion (water-insoluble) directed toward the center of the sphere. The interfacial area of cubic phase is comparatively larger than other phases. The unique microstructure of a cubic phase offers advantageous properties for controlled drug release. The close packing of the micelles accounts for the marked flow-resistance of the phase, which is stiffer than the mesophase. The cubic phase is formed spontaneously in excess water. Furthermore, the structure provides a slow release matrix for drugs of varying polarity and size because of its dual polar nonpolar nature. Additionally, release of the drug from within the cubic phase is controlled to certain degree because of its unique microstructure. Subsequently, the cubic phase displays bio adhesive properties. Thus, the structure is appropriate for gastrointestinal, pulmonary, nasal, oral, buccal, rectal, and vaginal drug delivery. As a whole, the cubic mesophase is stable *in vitro*.

Emulsion

An emulsion in pharmacy is a biphasic liquid dosage form that consists of two immiscible liquids, typically oil and water, in which one liquid is dispersed as microscopic droplets within the other. These two phases are thermodynamically incompatible, meaning they do not mix naturally and will separate over time. To maintain a stable dispersion, emulsifying agents (emulsifiers or surfactants) are incorporated into the formulation. These agents reduce the interfacial tension between the oil and water phases and help to stabilize the emulsion by preventing the droplets from coalescing or separating.

Pharmaceutical emulsions are designed to deliver drugs that are either oil-soluble or water-insoluble, and they offer several therapeutic and practical advantages. By dispersing the drug in either the aqueous or oily phase, emulsions can enhance the solubility, bioavailability, and sometimes the taste masking of certain active pharmaceutical ingredients (APIs). Emulsions also provide a versatile platform for drug delivery via multiple routes, including oral, topical, parenteral, and rectal administration [2].

Types of Emulsions

Oil-in-water (O/W)

Oil droplets dispersed in water. Suitable for oral, intravenous, or topical use. Example: Castor oil emulsion (laxative).

Water-in-oil(W/O)

Water droplets dispersed in oil. More often used for topical or intramuscular preparation.

Example: Creams for dry skin conditions.
Uses in Pharmacy: Oral emulsions: Improve the taste and bioavailability of oily drugs (e.g., cod liver oil). Topical emulsions: Deliver drugs through the skin with improved absorption and cosmetic acceptability. Parenteral emulsions: Used in intravenous nutrition (e.g., lipid emulsions like Intralipid®□). Controlled drug delivery: Certain emulsions can be used to control the release rate of active pharmaceutical ingredients.

Emulsifying Agents: These are surface-active agents (surfactants) that stabilize emulsions: Natural: Acacia, gelatin. Synthetic: Polysorbates (e.g., Tween 80), sodium lauryl sulfate. Finely divided solids: Bentonite, magnesium hydroxide. Stability Challenges: Pharmaceutical emulsions are thermodynamically unstable, and may undergo: Creaming: Droplets move to the top or bottom. Coalescence: Droplets merge to form larger ones. Phase separation: Complete separation of oil and water phases.

Feature	Liquid Crystal Emulsion (LCE)	Normal Emulsion
Structure	Ordered, gel-like, crystalline structure	Disordered, liquid phase dispersion
Stability	Highly stable, less prone to phase separation	Less stable, prone to separation over time
Release of Active Ingredients	Controlled, sustained release	Fast release
Bioavailability	Enhances bioavailability, especially for poorly soluble drugs	Moderate bioavailability
Skin Penetration	Better penetration, suitable for sensitive skin	Less effective penetration
Viscosity/Texture	Gel-like, smooth texture	Liquid or cream-like
Irritation	Gentle, less irritation	May cause irritation depending on formulation
Formulation Complexity	More complex, requires specific conditions	Easier to formulate
Applications	Controlled drug release, cosmetics, high-end skincare	Everyday skincare, food, and pharmaceuticals

Liquid crystal emulsion: A liquid crystal emulsion (LCE) is a specialized type of emulsion system used in cosmetic and pharmaceutical formulations, known for its unique structural and functional properties. It combines the characteristics of both emulsions and liquid crystals, offering enhanced stability, texture, and delivery of active ingredients [1].

Key Features of Liquid Crystal Emulsions

1. **Structure:** Liquid crystal emulsions form organized mesophases (typically lamellar or hexagonal) where surfactants and water/oil phases arrange themselves in a repeating pattern. These structures are intermediate between liquid and solid states, giving them unique visual and textural properties.
2. **Enhanced Stability:** LCEs are more stable than conventional emulsions due to the ordered structure which resists coalescence and phase separation.
3. **Improved Skin Compatibility:** The lamellar structure mimics the stratum corneum (outermost layer of the skin), leading to better skin hydration and mildness.
4. **Controlled Release of Actives:** The ordered matrix allows sustained release of active pharmaceutical or cosmetic ingredients over time.
5. **Better Sensory Feel:** They offer a smooth, non-greasy feel, making them highly suitable for skin care and cosmetic products.

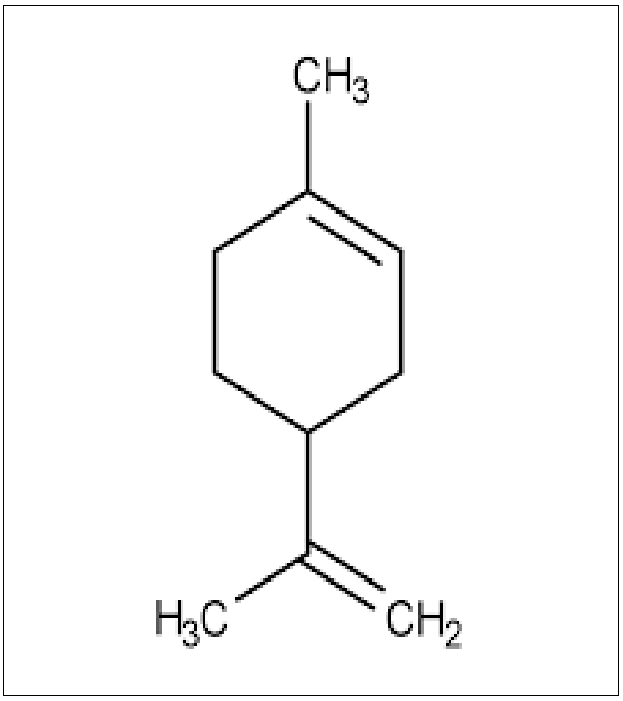
Applications in Cosmetics and Pharmaceutics

- Moisturizers and anti-aging creams
- Sunscreens
- Topical drug delivery systems
- Hair care products
- Transdermal patches

Selection of API
Combination of three OILS

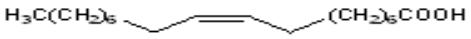
1. Orange peel oil
2. Avocado oil
3. Rosemary oil

1. Orange peel oil: Orange peel oil is derived from the flavedo of the fruit of *Citrus sinensis* (L.) Osbeck, commonly known as Sweet Orange, belonging to the Rutaceae family. The major component of orange peel oil is D-limonene, a monocyclic monoterpene with the molecular formula C10H16, known for its characteristic citrus odor.
Structure of D limonene: Responsible for anti-aging property.



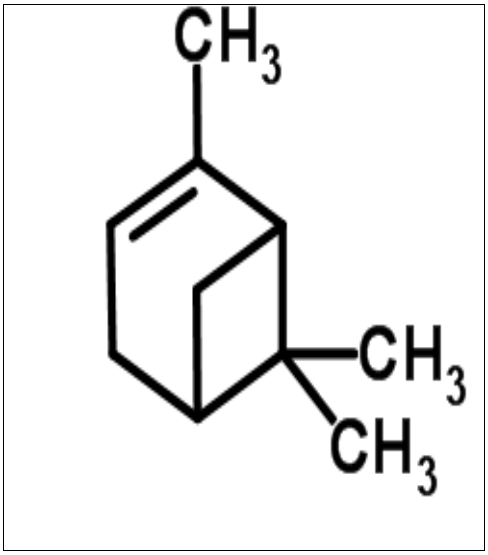
2. Avocado oil
Avocado oil is extracted from the pulp of the fruit of Persia americana Mill, commonly known as avocado, which belongs to the family Lauraceae. It is a nutrient-rich oil containing a high concentration of oleic acid, along with other valuable components such as palmitic acid, linoleic acid, palmitoleic acid, vitamin E (tocopherols), phytosterols (like β-sitosterol), squalene, lecithin, and carotenoids such as lutein.
Oleic acid, a monounsaturated omega-9 fatty acid with the molecular formula $C_{18}H_{34}O_2$, is the main constituent and plays a vital role in skin hydration and barrier repair. Vitamin E acts as a powerful antioxidant, protecting the skin

from oxidative damage and signs of aging. Phytosterols and squalene stimulate collagen production and improve skin elasticity, helping to reduce the appearance of wrinkles.
Structure of Oleic Acid: Responsible for anti-aging property:

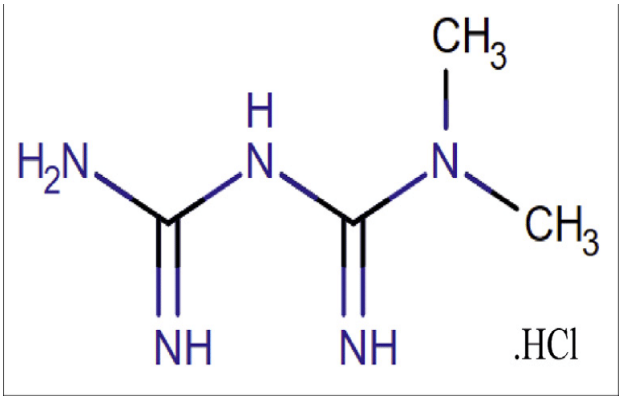


3. Rose Mary oil
Rosemary oil is obtained from the leaves of Rosmarinus officinalis (now classified as Salvia rosmarinus), a member of the Lamiaceae family. Its major active compounds include carnosic acid, rosmarinic acid, 1,8-cineole, camphor, and α-pinene.
Among these, carnosic acid and rosmarinic acid have strong antioxidant and anti-inflammatory effects. They help reduce oxidative damage, support collagen production, and protect the skin from UV-induced aging. Rosemary oil also improves circulation, which enhances skin tone and helps maintain a youthful appearance.

Structure of α-pinene: Responsible For anti-aging



Selection of API (Metformin)
Metformin is a widely used oral antidiabetic drug, primarily prescribed for type 2 diabetes. Its molecular formula is $C_4H_{11}N_5$. Recent studies suggest that metformin may also have anti-aging effects.
Metformin works by activating AMPK (AMP-activated protein kinase), which enhances cellular energy balance, reduces oxidative stress, and improves mitochondrial function. It also reduces chronic inflammation and may slow cellular senescence (aging of cells). These actions help protect tissues from age-related damage and may extend lifespan and health span.



Physio -chemical Property
1. Chemical name: 1,1-Dimethyl biguanide hydrochloride
2. Molecular formula: $C_4H_{11}N_5$
3. Molecular weight: 129.16 g/mol
4. Appearance: White crystalline powder
5. Solubility: Freely soluble in water; practically insoluble in acetone, ether, and chloroform
6. Melting point: ~223°C (decomposes)

Material and Method
Procedure for formulation of liquid crystal emulsion containing combination of three oils:

- **Oil phase:** Type-A, Type-B, Type-C oil + span 80 heat at 60°c.
- **Aqueous phase:** Methyl paraben dissolved in water + heat, after cooling add glycerin.

The water phase was added to swelled sodium CMC while continuously stirring. Then oil phase also added stirring and mixing quickly until the homogenous emulsion is obtained [3].

Ingredients	F_1	F_2	F_3	Role of Ingredients
Combination of orange, avocado, rosemary oil.	6ml	7.5ml	9ml	Anti-aging, antioxidant, anti-inflammatory, moisturizing
Span 80	4g	5g	6g	Surfactant
Tween 80	4g	5g	6g	Surfactant
Methyl paraben	0.2ml	0.4ml	0.6ml	Preservative
Glycerine	5ml	5ml	5ml	Humectant, preservative
Sodium CMC	1g	1g	1g	Thickening agent, binding agent
Distilled water	q.s	q.s	q.s	Vehicle

Procedure for formulation of liquid crystal emulsion containing API

Oil phase

Stearyl alcohol + dimithicone + API, heat the mixture at 80^{o}c and add stearic acid.

Aqueous phase: Methyl paraben was dissolved in distilled water and heat. This solution was cooled down and then glycerin was added.

Aqueous phase was added to oil phase while stirring on magnetic stirrer for 4hrs until a homogenous mixture obtained [3].

Ingredients	F1	F2	F3	Role of ingredients
Stearyl alcohol	3ml	3ml	3ml	Emollient, emulsifier
API	5g	5g	5g	Anti-aging
Tween 80	5g	4g	3g	Surfactant
Span 80	6g	5g	4g	Surfactant
Stearic acid	0.2ml	0.4ml	0.6ml	Thickener, emulsifier
Dimethicone	5ml	5ml	5ml	Moisturizer, emollient
Glycerin	5ml	5ml	5ml	Humectant, moisturizer
Methyl paraben	0.2ml	0.2ml	0.2ml	Preservative
Distilled water	q.s	q.s	q.s	Vehicle

Extraction of orange peel oil

Citrus oil, especially from orange peel, is a volatile essential oil found in the oil glands of the citrus peel. The major component is d-limonene (around 90-95%), which gives the oil its characteristic orange scent and therapeutic properties.

Citrus Oil Extraction from Orange Peel: Procedure

1. Set up the distillation apparatus: The distillation setup includes a distillation flask, basket heater, Clevenger apparatus, horizontal condenser, and a conical flask.

2. Prepare the sample: Weigh 100 g of orange peel and place it into the distillation flask. Add 200 mL of water to the flask.

3. Start the distillation: Supply heat to the distillation unit using a temperature-controlled basket heater.

4. Maintain appropriate conditions: Conduct the distillation at a temperature of 88°C for 60 minutes.

5. Collect the distillate: The distillate is collected in the Clevenger apparatus and conical flask. It separates into two layers:

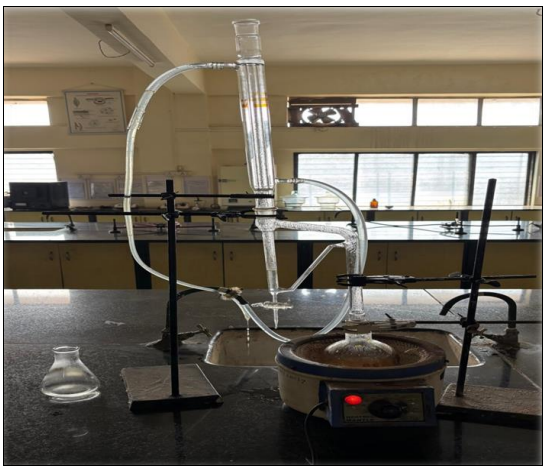
- A dense lower layer (citrus oil)
- A less dense upper layer (water)

6. Separate the oil

Use a separating funnel to isolate the citrus oil (lower layer) from the water.

7. Store the extracted oil

Transfer the separated citrus oil into clean glass bottles for storage.



Phytochemical tests of orange peel oil extract
Key constituents - Limonene, Hesperidine
Bromine test: A dilute Bromine-water solution is prepared and taken in a test tube. To that citrus oil extracted from orange peels is added. If limonene is present in the oil extracted, the color of the Bromine water gets changes from red brown to pale yellow. This is because of the fact that the Bromine present in the Bromine water solution occupies the space between the two double bonds present in limonene.

Shinoda test: Dissolve a small amount of the test solution (containing the suspected flavonoid) in ethanol. Add magnesium ribbon to the ethanolic solution. Dropwise add concentrated hydrochloric acid (HCI) to the solution. A bright violet color indicates the presence of flavonoids.

2. Avacado Oil Extraction
Extraction of Avocado Oil Using Reflux: Step-by-Step Procedure

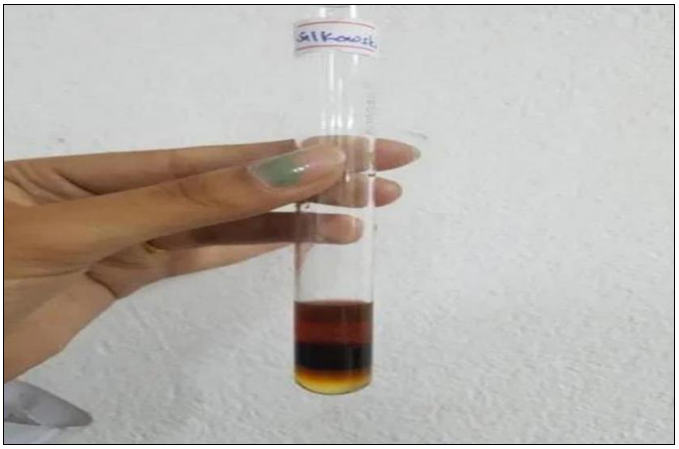
1. Preparation of Avocado Pulp
Wash, peel, and remove the seeds from ripe avocados.
Dry the pulp at 50-60°C, then grind it into a fine powder to ensure better extraction.

2. Reflux Extraction Setup
Place the dried avocado powder into a round-bottom flask.
Add n-hexane as the solvent.
Set up a reflux condenser.
Heat the mixture at 50-70°C for 2-4 hours to extract the oil.

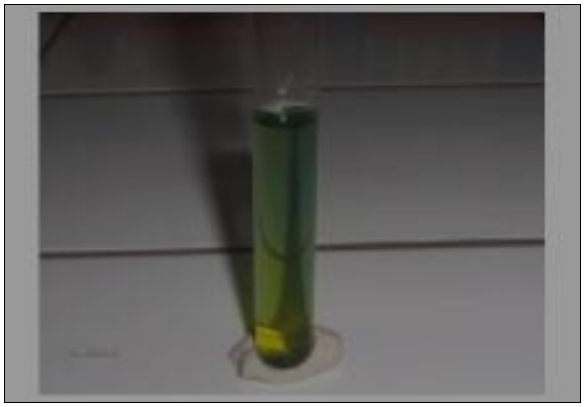
3. Filtration
After the mixture has cooled, filter it using gravity filtration to separate the oil-rich solvent from the solid residue.

4. Purification and Storage
Wash the extracted oil with distilled water to remove impurities.
Store the purified oil in an amber-colored bottle in a cool, dry place to maintain its quality.

Phytochemical test of avocado oil extract
Key constituents - Phytosterols
Salkowski test: involves mixing an extract with chloroform and concentrated sulfuric acid, with a reddish-brown coloration at the Interface indicating a positive result.



Liebermann-Burchard test: Mix oil with acetic anhydride and concentrated sulfuric acid. Positive result Blue green color indicates sterols.



3. Rose Mary Oil Extraction

Solvent Extraction of Rosemary Oil: Step-by-Step Procedure

1. Preparation

Grind the dried rosemary leaves to increase their surface area, which enhances solvent penetration.

2. Extraction

Soak the crushed rosemary leaves in a suitable solvent (e.g., n-hexane) inside a sealed glass container.

Allow the mixture to stand for 24 to 48 hours, shaking it occasionally to aid extraction.

3. Filtration and Evaporation

Filter out the solid plant material using a suitable filter method.

Remove the solvent from the filtrate by gentle heating to obtain concentrated rosemary oil.

4. Collection and Storage

Collect the concentrated rosemary oil.

Store it in a dark glass bottle in a cool, dry place to preserve its potency and prevent degradation.

Phytochemical tests of rosemary oil extract

- **Key constituents: Phytosterols, terpenoids**
- **Salkowski test:** Involves mixing an extract with chloroform and concentrated sulfuric acid, with a reddish-brown/ green coloration at the Interface indicating a positive result.

In this study, we extracted three herbal oils and subsequently performed phytochemical tests to confirm the presence of active constituents with potential anti- aging properties in each oil.

Result and discussion

Organoleptic Properties of Liquid Crystal Emulsion

Organoleptic properties refer to the sensory attributes of a formulation that can be perceived by the senses such as appearance, color, odor, texture, and feel. For liquid crystal emulsions, these properties are important to assess both consumer acceptability and formulation quality [2].

Oil	F_1	F_2	5F3
Color	Yellowish white	Milky white	Milky white
Odour	Pleasant	Pleasant	Pleasant

Drug	F_1	F_2	F_3
Color	Transparent	Milky white	Milky white
Odour	Pleasant	Pleasant	Pleasant

pH

pH is a measure of the acidity or alkalinity of a formulation and is expressed on a scale from 0 to 14, where 7 is neutral. The pH of liquid crystal emulsions is a crucial parameter because it affects.

Oil	Drug
F1-4.3	F1-4.46
F2-4.4	F2-5.2
F3-5.5	F3-5.3

Viscosity determination

Viscosity is a measure of a fluid's resistance to flow. In liquid crystal emulsions, viscosity is a critical parameter because it influences.

The emulsion sample is placed in the sample holder. With a spindle no.2 rotates at a speed of 60rpm within the sample, and the resistance to rotation is measured. The instrument gives a viscosity reading typically in centipoise (cP) or milli Pascal-seconds (mPa·s).

Oil	Drug
F1- 250 cp	F1- 198 cp
F2- 332.7 cp	F2- 608.4 cp
F3- 650.8 cp	F3- 559.3cp

Centrifugation test

The centrifugation test is used to assess the physical stability of emulsions, including liquid crystal emulsions. It helps predict the tendency of an emulsion to undergo phase separation, creaming, or sedimentation under accelerated conditions.

Procedure

Place a sample of the emulsion into suitable centrifuge tubes. Load the tubes into the centrifuge balanced properly. Centrifuge the samples at a specified speed (e.g., 3000-5000 rpm) for a set time (typically 15-30 minutes). After centrifugation, inspect the samples for any signs of: Phase separation (oil and water layers) Creaming or sedimentation Changes in appearance or texture

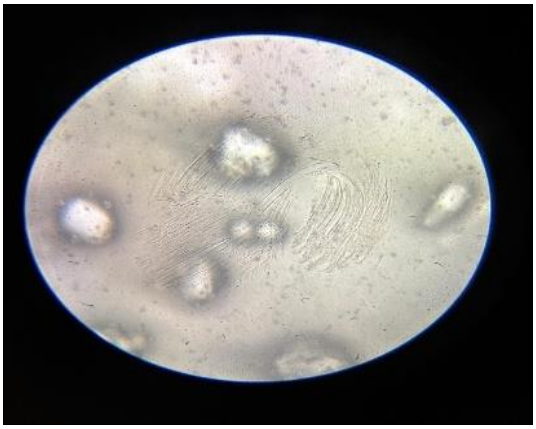
Oil	Drug
F1- Phase separation	F1- Phase separation
F2- Phase separation	F2- No separation
F3-No separation	F3- No separation

Microscopic evaluation

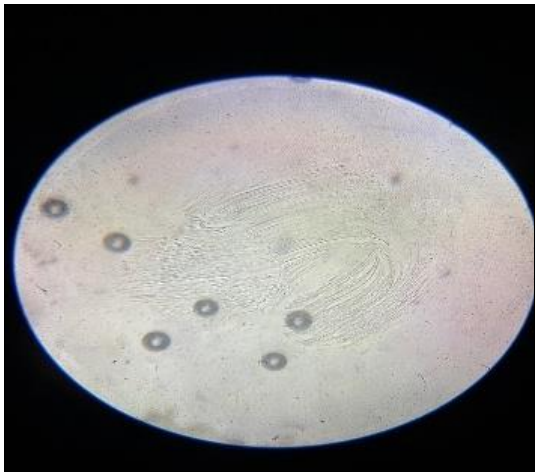
Microscopic evaluation is an essential technique used to observe the microstructure and morphology of liquid crystal emulsions. It helps in understanding the internal organization, droplet size, shape, and the presence of liquid crystal phases, which directly impact the stability and performance of the formulation [6].

Procedure

Prepare a thin sample of the emulsion on a clean microscope slide. Cover with a coverslip to avoid evaporation. Observe under a polarized light microscope or phase contrast microscope, as liquid crystals exhibit birefringence (unique light patterns) under polarized light. Capture images and analyze droplet size and arrangement [2].



Liquid Crystal containing oil



Liquid Crystal containing Drug

Zeta Potential

Zeta potential is the electrical potential at the slipping plane around dispersed particles or droplets in an emulsion. It reflects the surface charge of these particles and is a key indicator of the emulsion's physical stability [3, 1].

Measurement

Zeta potential is measured using electrophoretic light scattering instruments (e.g., Zetasizer).

A sample of the emulsion is diluted appropriately and placed in a specialized cell.

An electric field is applied, and the velocity of droplet movement (electrophoretic mobility) is measured to calculate zeta potential.

Importance

A higher absolute value of zeta potential (positive or negative) usually means stronger electrostatic repulsion between droplets, preventing aggregation or coalescence.

Low zeta potential values can lead to particle flocculation and phase separation, indicating instability.

It helps predict the long-term stability of emulsions and suspensions.

Stable Emulsions

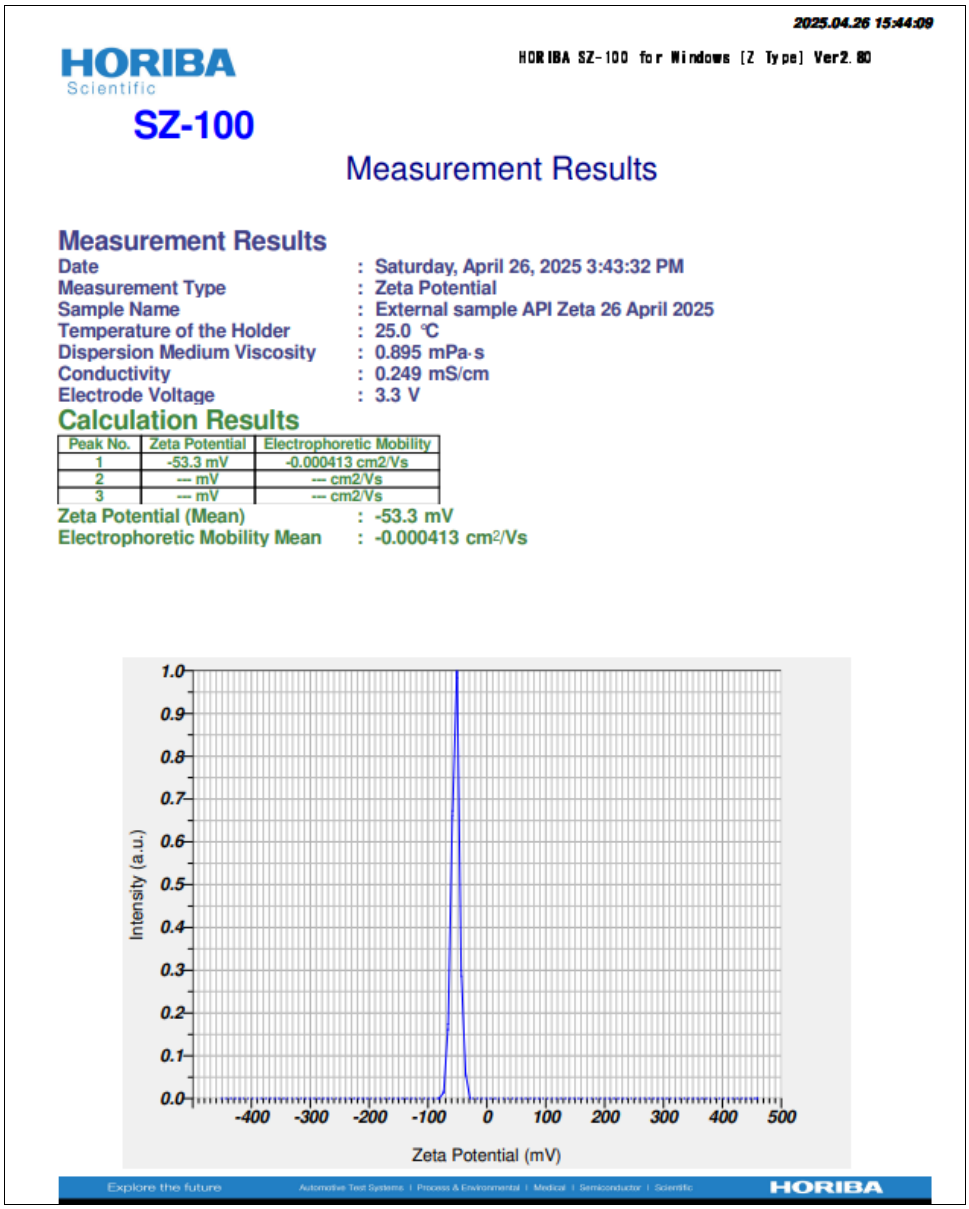
Zeta potential values greater than ±30 mV (either +30 mV or -30 mV) indicate strong electrostatic repulsion between droplets, leading to good physical stability and resistance to aggregation or coalescence.

Unstable Emulsions

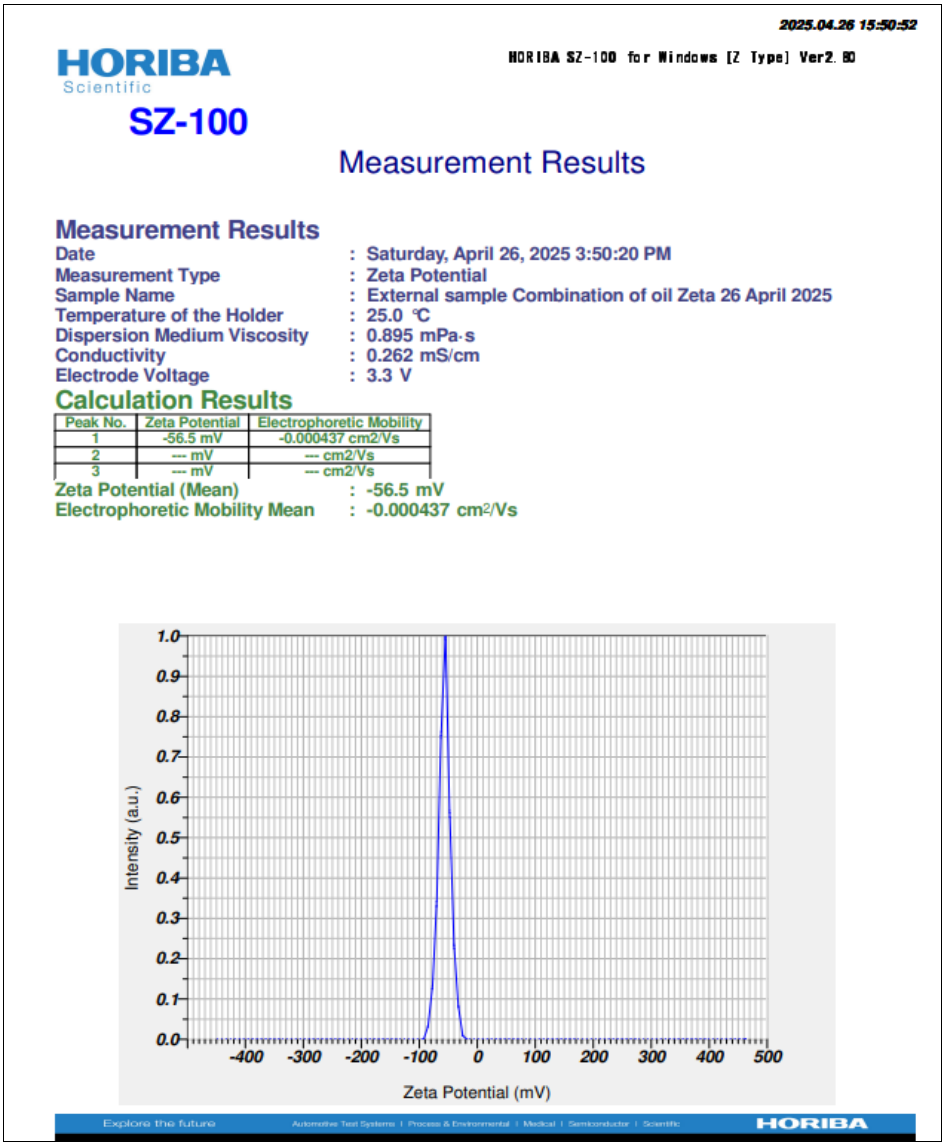
Zeta potential values close to 0 mV suggest weak or no repulsive forces, which can result in droplet flocculation, aggregation, and phase separation, indicating poor stability.

The liquid crystal emulsion of oil-based combination showed a zeta potential of -56.5 mV, indicating high colloidal stability. And liquid crystal emulsion containing API showed a zeta potential of -53.3 mV. This stability ensures even distribution of active ingredients, reducing aggregation and improving product performance. Additionally, the presence of oil contributes to anti-aging properties by enhancing skin hydration, improving elasticity, and offering antioxidant protect

Liquid Crystal containing Drug



Liquid Crystal containing Oil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HORIBA SZ-100 for Windows [Z Type] Ver2.80

HORIBA Scientific

SZ-100

Measurement Results

Measurement Results

Date : Saturday, April 26, 2025 3:50:20 PM
Measurement Type : Zeta Potential
Sample Name : External sample Combination of oil Zeta 26 April 2025
Temperature of the Holder : 25.0 ℃
Dispersion Medium Viscosity : 0.895 mPa·s
Conductivity : 0.262 mS/cm
Electrode Voltage : 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-56.5 mV	-0.000437 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -56.5 mV
Electrophoretic Mobility Mean : -0.000437 cm²/Vs

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7. Moisture content determination by Skin Analyzer

Moisture determination by a skin analyzer gives information about the hydration level of the stratum corneum, which is the outermost layer of the skin. Here's what this information typically includes [7, 15].

1. Skin Moisture Content (%)

Indicates how much water is present in the outer skin layer. Normal skin hydration usually ranges between 45-55%.

2. Skin Type Assessment

Helps determine if the skin is dry, normal, or oily based on moisture and oil content.

3. Skin Condition Evaluation: Detects dehydration, which can lead to roughness, flakiness, or sensitivity.

4. Effectiveness of Skincare Products

Measures changes in skin moisture before and after product application (e.g., moisturizers or emulsions).

5. Real-time Monitoring: Provides instant feedback on skin hydration, which is useful in both clinical and cosmetic research.

Skin analyzers often use bioimpedance, capacitance, or conductance technology to measure moisture non-invasively.

API	F_1	F_2	F_3
M.C before application of emulsion	40%	44%	45%
M.C after application with emulsion	50%	62%	55%

OIL	F_1	F_2	F_3
M.C before application of emulsion	42%	45%	47%
M.C after application with emulsion	55%	57%	65%

Conclusion

Based on the comparative evaluation, both the API-based and herbal oil-based liquid crystal emulsions demonstrated favorable physicochemical properties, structural integrity, and stability. However, the herbal oil formulation proved to be more stable, as indicated by a higher absolute zeta potential value and better resistance to aggregation. Additionally, it exhibited enhanced anti-aging benefits, including improved skin hydration, elasticity, and antioxidant activity, along with superior sensory attributes such as a smoother texture and pleasant aroma. Therefore, while the API-based emulsion may be more suitable for

targeted therapeutic use, the herbal oil-based emulsion is the preferred choice for cosmeceutical and anti-aging applications due to its greater stability and multifunctional skin benefit

Future scope

- Liquid Crystal emulsion suspension has superior physiochemical stability compared to conventional emulsion
- Liquid Crystal emulsion can encapsulate vitamins, peptides and anti-oxidants for sustained release
- Improved sensory profile: Non greasy, moisturizing, elegant skin feels
- Used for different skin types and conditions (eg. Anti-aging, acne, hydration)

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