



Caffeine - Loaded NLC Gel for Periorbital Skin Care, Anti-Cellulite and Anti-Aging Application.

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ABSTRACT

Periorbital skin is particularly susceptible to premature aging due to its thin structure, reduced barrier function, and increased vulnerability to oxidative stress. Caffeine, a naturally occurring methylxanthine, has demonstrated potential for improving microcirculation, reducing puffiness, enhancing lipolysis, and providing antioxidant and anti-aging benefits. However, its incorporation into topical formulations may be limited by its hydrophilicity and relatively rapid diffusion from conventional delivery systems. The present study aimed to develop and characterize a caffeine-loaded nanostructured lipid carrier (NLC) gel for targeted periorbital skin care with potential anti-aging and anticellulite activity. Caffeine-loaded NLCs were prepared using a combination of solid and liquid lipids and optimized for particle size, polydispersity index, zeta potential, entrapment efficiency, and drug loading. The optimized NLC dispersion was incorporated into a suitable gel base and evaluated for pH, viscosity, spreadability, homogeneity, drug content, in vitro drug release, skin permeation, and stability. The formulation was further assessed for its potential antioxidant and anti-aging/anticellulite effects using appropriate in vitro models. The developed NLC gel is expected to provide sustained release and enhanced topical delivery of caffeine, thereby improving its residence time and therapeutic efficacy at the skin surface. The proposed formulation may offer a promising nanotechnology-based approach for managing periorbital puffiness, early signs of skin aging, and localized cellulite-related cosmetic concerns. Further in vivo and clinical studies are warranted to establish its long-term safety, efficacy, and consumer acceptability.

Keywords: Caffeine; nanostructured lipid carrier; NLC gel; periorbital skin; anti-aging; anticellulite; topical delivery; skin permeation.

1. INTRODUCTION

1.1 TOPICAL DRUG DELIVERY

Topical drug delivery system is one of the oldest and most widely used methods of drug administration, with a history spanning more than a thousand years. In ancient times, ointments and salves prepared from plant, animal, and mineral extracts were commonly used for the treatment of various skin disorders. These preparations were administered through the topical route and were widely employed by ancient civilizations such as the Chinese, Egyptians, and Babylonians.

The skin acts as a protective barrier that regulates the entry and exit of many chemicals, prevents excessive moisture loss, and helps maintain body temperature and overall homeostasis. Topical drug delivery systems are beneficial because they avoid drug degradation in the gastrointestinal tract and bypass hepatic first-pass metabolism, thereby improving the bioavailability of suitable drugs. Furthermore, these formulations deliver the drug directly to the site of action, resulting in effective local therapy with reduced systemic side effects ^{3,12,14,17,24,34}

In other words, a topical drug delivery system can be defined as a dosage form that is applied to the surface of the skin or mucous membranes to produce a localized therapeutic effect or, in some cases, a systemic effect through transdermal absorption. These formulations are available in various dosage forms, including creams, ointments, gels, lotions, pastes, foams, sprays, powders, and transdermal patches, depending on the nature of the drug and the desired therapeutic outcome.

VARIOUS TOPICAL FORMULATIONS AVAILABLE IN MARKET ARE LISTED BELOW: -

Semi -Solid preparations: -

Eg: - Gels, Paste, Creams, Ointments.

Liquid Preparations: -

Eg: - Lotions, Solutions, Liniments.

Solid Preparations: -

Eg: - Powders.

Other Preparations: -

Eg: - Transdermal patches, Foams, Sprays, etc.

Benefits of Topical Drug Delivery System:

- Avoidance of First-Pass Metabolism.
- Targeted, Localized Therapy.
- Improved Patient Compliance.
- Reduced Systemic Side Effects.
- Patient-Friendly Formulations.
- Lower Dose and Enhanced Efficacy.
- Non-Invasive and Easily Reversible.
- Controlled or Sustained Release Possibilities.

1.2 Anatomy and Physiology of Skin

The skin is the largest organ of the human body, accounting for approximately 15% of the total body weight in adults. It serves as the primary protective barrier against external physical, chemical, and biological agents while preventing excessive trans epidermal water loss and maintaining body homeostasis. In addition, the skin plays important roles in thermoregulation, immune defense, sensory perception, and vitamin D synthesis. Structurally, the skin consists of three major layers: (1) Epidermis, (2) Dermis, and (3) Hypodermis (Subcutaneous Tissue/Fascia). Each layer possesses distinct anatomical and physiological characteristics that influence drug permeation and topical drug delivery ^{17,34,37,44,45}

1.2.1 Epidermis

The epidermis is the outermost layer of the skin and is composed of five distinct strata: stratum Basale, stratum spinosum, stratum granulosum, stratum lucidum (present only in thick skin), and stratum corneum. The principal cell type of the epidermis is the keratinocyte, along with melanocytes, Langerhans cells, and Merkel cells, each contributing to pigmentation, immune surveillance, and sensory functions.

1.2.2 Dermis

The dermis lies beneath the epidermis and is connected to it by the basement membrane. It is composed of two connective tissue layers: the papillary dermis and the reticular dermis. The papillary dermis is the superficial, thin layer composed of loose connective tissue that interdigitates with the epidermis, whereas the reticular dermis is a deeper and thicker layer consisting predominantly of dense irregular connective tissue rich in collagen and elastin fibers. These structural proteins provide the skin with mechanical strength, elasticity, and resilience.

1.2.3 Hypodermis

The hypodermis, also known as the subcutaneous tissue or superficial fascia, is the deepest layer of the skin. It is primarily composed of adipose tissue and loose connective tissue and contains large blood vessels, lymphatic vessels, sensory nerves, and the deeper portions of hair follicles. This layer functions as an energy reserve, thermal insulator, and shock absorber while providing structural support to the overlying skin.

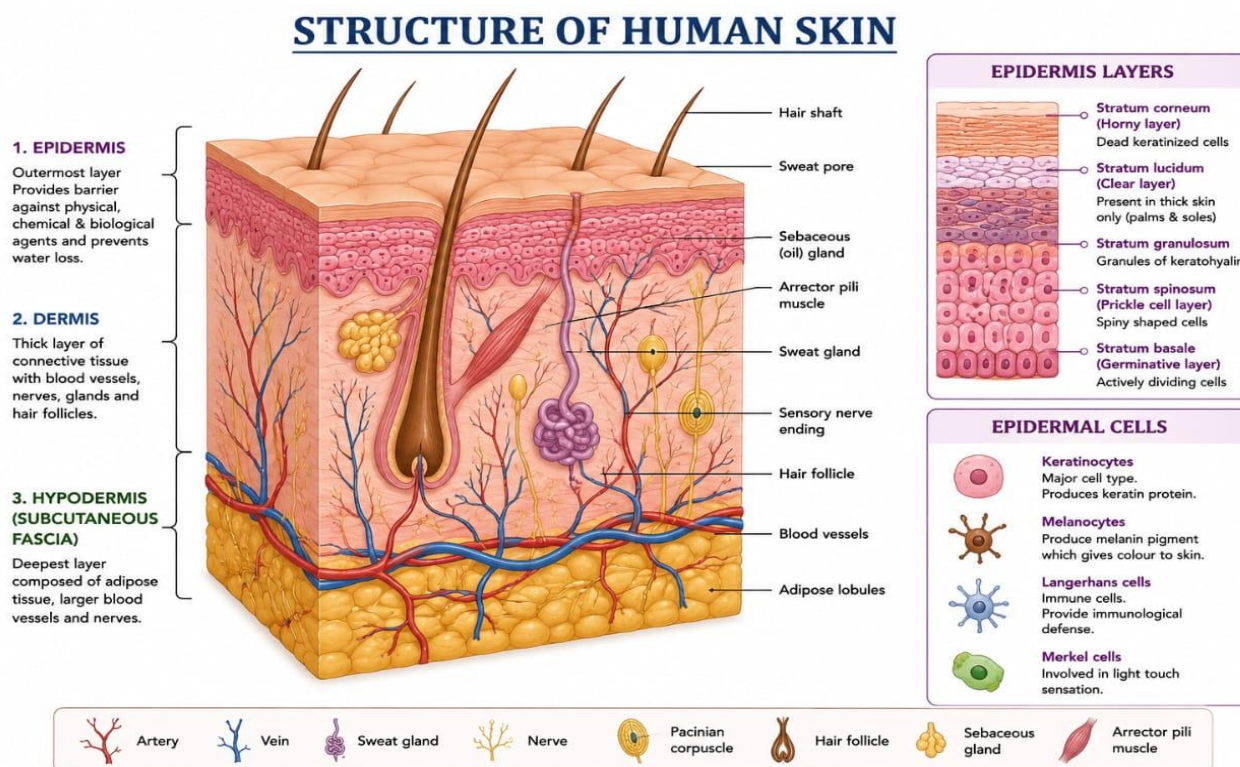


Fig. 1.01: Structure of Skin

The anatomical structure and physiological properties of the skin significantly influence the permeation and delivery of drugs through the topical route. The stratum corneum, the outermost layer of the epidermis, serves as the principal barrier to percutaneous absorption. It is composed of approximately 40% lipids, 40% proteins, and 20% water, forming a highly organized lipid matrix that favors the permeation of lipophilic molecules. Consequently, lipophilic drugs generally exhibit better skin penetration than hydrophilic drugs. Due to the relatively low water content of the stratum corneum, hydrophilic drugs encounter considerable resistance during permeation. In addition to the transcellular and intercellular pathways, hair follicles and sweat gland ducts provide appendageal routes that facilitate the absorption of certain drugs, particularly nanoparticulate formulations such as nanostructured lipid carriers (NLCs).

1.3 Skin Aging

Skin aging is a complex and progressive biological process characterized by gradual structural and functional deterioration of the skin. It results from the interaction of intrinsic (chronological) and extrinsic (environmental) factors, leading to visible and physiological changes in skin appearance and function

2,4,32

1.3.1 Intrinsic Skin Aging

Intrinsic aging, also known as chronological aging, is a natural process that occurs with advancing age. It is primarily influenced by genetic and physiological factors, including:

- Genetic predisposition
- Hormonal changes
- Cellular senescence
- Decreased collagen and elastin synthesis

Characteristics of intrinsic aging include:

- Fine wrinkles and expression lines
- Thinning of the epidermis
- Dry and rough skin
- Reduced skin elasticity and firmness

1.3.2 Extrinsic Skin Aging

Extrinsic aging is caused by external environmental and lifestyle-related factors that accelerate the natural aging process. The major contributing factors include:

- Ultraviolet (UV) radiation
- Visible blue light (high-energy visible light)
- Air pollution
- Cigarette smoking
- Poor nutritional habits
- Psychological stress

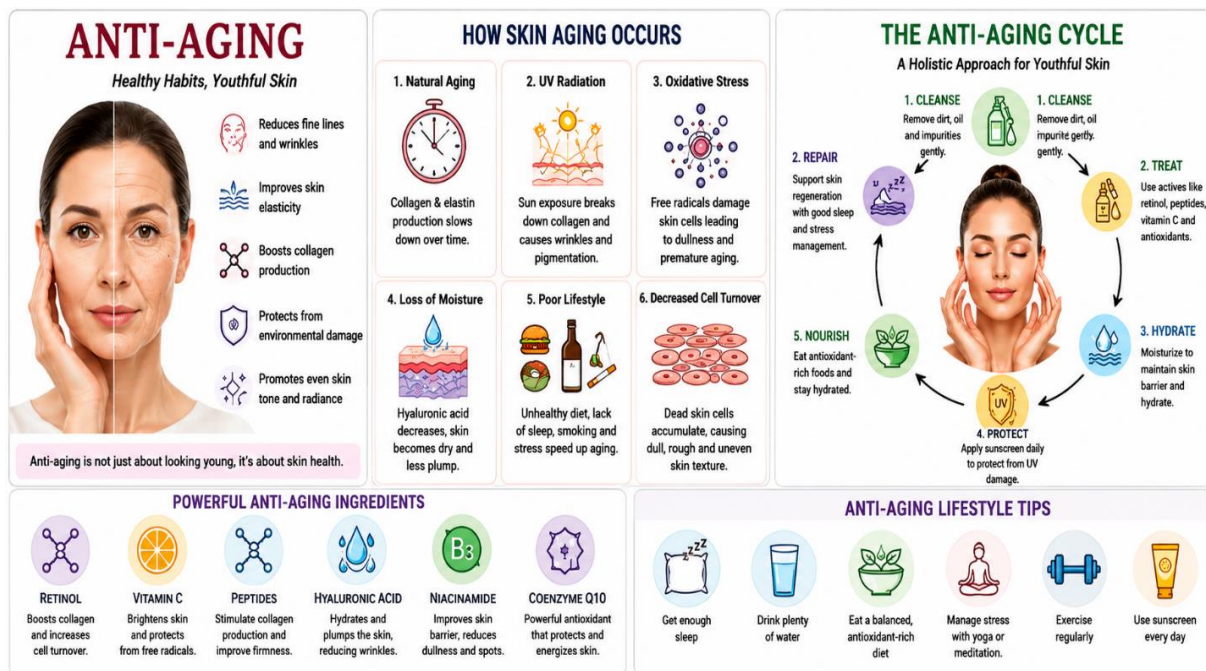


Fig. 1.02: Anti-aging

1.4 Cellulite

Cellulite is a common structural and cosmetic alteration of the skin characterized by an uneven, dimpled or “orange-peel” appearance. It is most commonly observed on the thighs, buttocks and hips and occurs predominantly after puberty. Scientific reviews describe cellulite as a multifactorial condition, meaning that its development cannot be explained by a single cause. Interactions between the skin, subcutaneous adipose tissue, connective-tissue septa, microcirculation, hormones, genetics and aging are thought to contribute to its appearance.

Cellulite is extremely common among postpubertal females. Published reviews commonly estimate that approximately 80–90% of women experience some degree of cellulite, although precise prevalence estimates vary between studies because of differences in definitions and assessment methods. Importantly, cellulite can occur in individuals of different body sizes and is not simply a consequence of excess body weight. Its development is strongly influenced by the anatomical organization of connective tissue and fat beneath the skin ^{4,15,20,21,33}

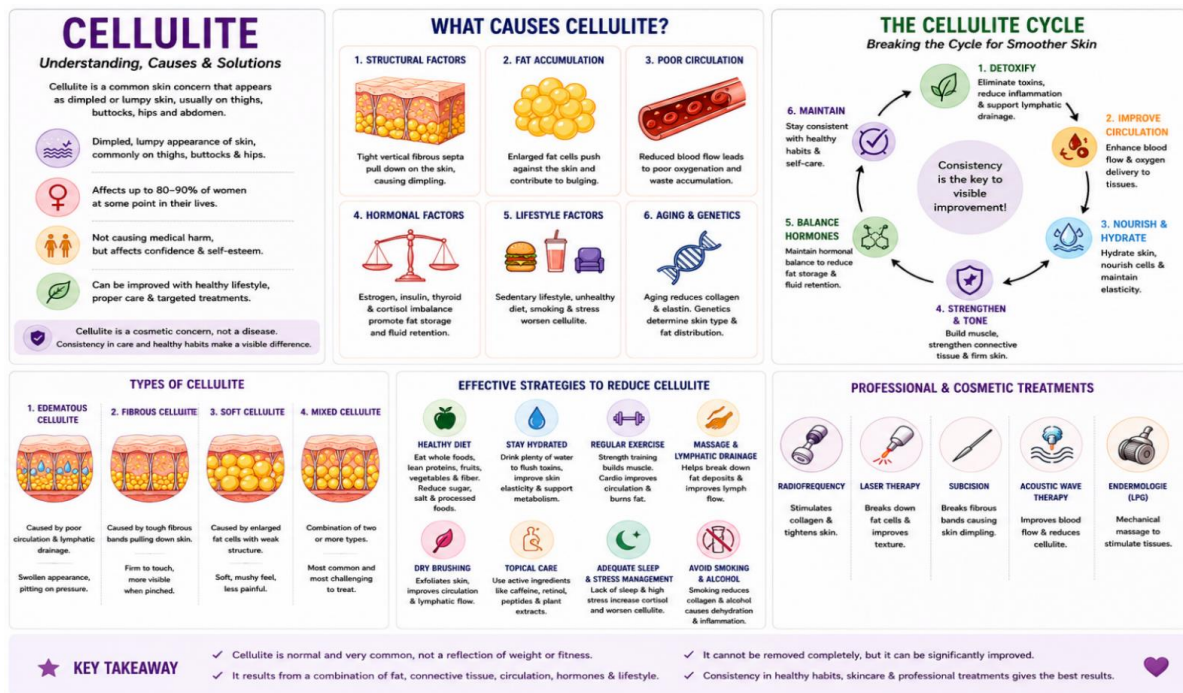


Fig. 1.03: Cellulite

1.4.1 Pathophysiology of Cellulite

The exact pathophysiology of cellulite is still being investigated. One important explanation focuses on the arrangement of fibrous connective-tissue septa beneath the skin. These septa connect the dermis to deeper tissues and provide structural support. In cellulite-affected areas, differences in the arrangement and mechanical behavior of these septa can allow superficial fat lobules to push upward while the fibrous bands pull portions of the skin downward. This opposing force can create the characteristic depressions and dimples seen on the skin surface.

Other proposed mechanisms include changes in microcirculation, tissue-fluid balance and inflammation. Alterations in small blood vessels and the surrounding connective tissue have been proposed to influence fluid movement and tissue pressure. However, current research indicates that cellulite is complex, and no single mechanism completely explains all cases.

1.4.2 Concept of Anti-Cellulite

The term anti-cellulite refers to approaches intended to reduce the visible appearance of cellulite and improve the smoothness and structural appearance of the skin. Since cellulite has several contributing mechanisms, research has investigated treatments directed at different tissue levels, including the dermis, subcutaneous fat and fibrous connective-tissue septa.

Anti-cellulite approaches have included topical preparations, massage and mechanical techniques, energy-based procedures and treatments that specifically address fibrous septa. Research suggests that different approaches may work through different mechanisms, and the degree and duration of improvement can vary considerably. A major challenge is that cellulite is a structural condition with multiple contributing factors, so no single treatment can be expected to provide the same result for everyone.

1.4.3 Role of Topical and Cosmetic Approaches

Topical anti-cellulite preparations commonly contain ingredients such as caffeine, retinoids and botanical extracts. Proposed mechanisms include supporting the appearance of the skin, influencing local microcirculation and promoting changes in dermal structure. However, reviews emphasize that clinical evidence for many topical products remains limited, with studies often being small or having relatively short follow-up periods.

1.4.4 Current Perspective

Current research shows that cellulite is common, multifactorial and complex. It is not simply a problem of excess fat, and its presence does not necessarily indicate poor health or an unhealthy body. The scientific understanding of cellulite has progressed from theories focused mainly on fat and circulation toward a broader anatomical model involving the dermis, adipose tissue and especially the fibrous connective-tissue septa.

Therefore, the modern anti-cellulite approach focuses on understanding the individual's underlying tissue structure and selecting appropriate methods according to the severity and characteristics of the cellulite. Although several treatments can improve its appearance, current reviews emphasize that results vary and that cellulite cannot reliably be considered permanently eliminated. Continued research is needed to better understand its biological mechanisms and develop treatments that provide safer and longer-lasting improvement

1.5 Gels

According to the United States Pharmacopeia (USP), gels are semisolid systems consisting of a liquid phase immobilized within a three-dimensional network formed by organic or inorganic substances. They are commonly formulated for application to the skin and other suitable sites³⁵

Gels generally possess a relatively rigid structure due to the presence of physical or chemical cross-linking within the gelling system. A typical gel contains a natural or synthetic polymer that forms a three-dimensional matrix throughout a hydrophilic liquid or dispersion medium. This network entraps and immobilizes the liquid phase, resulting in a semisolid preparation with suitable consistency for topical application.

1.5.1 Advantages of Gels

- Gels can be designed to provide controlled or prolonged drug release.
- They can accommodate both hydrophilic and, with suitable formulation approaches, lipophilic drugs.
- They are generally easy to prepare and administer compared with several other semisolid dosage forms.
- Many gel-forming materials exhibit good biocompatibility and biodegradability.
- Topical gels are generally easy to remove by washing and can provide a non-greasy application.
- They may provide prolonged contact or retention at the application site, improving topical drug delivery.
- They usually possess good spreadability, allowing uniform distribution over the skin.
- Aqueous gels can produce a cooling and refreshing sensation after application.

1.5.2 Disadvantages of Gels

- Certain drugs may undergo chemical or physical instability because of interactions with the polymer or other formulation components.
- Incompatible ingredients may cause precipitation or phase separation, affecting gel stability.
- Improper formulation can result in flocculation or aggregation, leading to changes in the gel structure.
- Loss of solvent or water during storage may cause drying, shrinkage, or changes in gel consistency.
- Some gelling agents, preservatives, or other excipients may cause skin irritation or sensitivity in susceptible individuals.
- The relatively high water content of many aqueous gels can support microbial or fungal growth if an appropriate preservative system and packaging are not used.
- Some gels may have poor stability at extreme temperatures or pH conditions, resulting in changes in viscosity or appearance.

1.6 Caffeine (1,3,7-trimethylxanthine)

Caffeine is a naturally occurring methylxanthine alkaloid widely present in coffee, tea, cocoa, and several other plant sources. Chemically, caffeine is known as 1,3,7-trimethylxanthine and possesses various pharmacological and biological activities, including antioxidant, anti-inflammatory, and lipolytic effects. Owing to these properties, caffeine has gained considerable interest in topical and cosmetic formulations, particularly for skin-care applications.

Caffeine has attracted attention in cosmetic and dermatological formulations because of its potential effects on microcirculation, antioxidant protection, and lipid metabolism. Topically applied caffeine may influence cutaneous blood flow and has been investigated for its potential to reduce the appearance of puffiness and improve the overall appearance of the periorbital region. Its antioxidant properties may also help counteract oxidative stress, which is an important contributor to premature skin aging. In addition, Incorporation of caffeine into an NLC-based gel combines the advantages of a nanostructured lipid carrier with those of a topical gel. The NLC system can serve as a carrier for caffeine, while the gel provides suitable viscosity, spreadability, ease of application, and prolonged residence at the site of application. Such a formulation may therefore provide a promising approach for the topical application of caffeine for periorbital skin care, anti-aging, and improvement in the appearance of cellulite ^{2,4,8,9,15,19,20}

1.6.1 Benefits of Caffeine in Anti-Aging

- Antioxidant activity: Helps neutralize reactive oxygen species (ROS), which contribute to oxidative damage and premature skin aging.

- Protection against photoaging: May help reduce cellular damage associated with environmental and UV-induced oxidative stress.
- Supports skin microcirculation: Caffeine can influence cutaneous blood flow.
- Anti-inflammatory potential: May help reduce inflammatory responses associated with environmental stress and skin aging.
- Helps improve skin appearance: Topical caffeine may contribute to a smoother and more refreshed appearance of the skin.
- Potential support for collagen preservation: By reducing oxidative and inflammatory stress, caffeine may indirectly help protect the skin extracellular matrix.

1.6.2 Benefits of Caffeine in Anti-Cellulite Applications

- Lipolytic activity: Caffeine inhibits phosphodiesterase (PDE), increasing intracellular cyclic AMP (cAMP), which can promote lipolysis in adipocytes.
- May reduce the appearance of cellulite: Increased lipid mobilization may contribute to a smoother appearance of areas affected by cellulite.
- Improves microcirculation: Caffeine may enhance local blood flow, supporting nutrient and oxygen delivery to the skin.
- Supports fluid movement: Improved microcirculation and lymphatic function may help reduce the appearance associated with local fluid accumulation.
- Antioxidant activity: Helps counter oxidative stress that can contribute to alterations in skin structure.
- Skin-firming potential: Through its effects on microcirculation and skin appearance, caffeine may contribute to temporarily improved skin smoothness and firmness.

1.7 Nanostructured Lipid Formulations (NLCs)

Nanostructured lipid carriers (NLCs) are advanced lipid-based nanocarrier systems developed as an improved generation of solid lipid nanoparticles (SLNs). NLCs are composed of a combination of solid and liquid lipids, stabilized by suitable surfactants, which form a nanostructured lipid matrix capable of incorporating active pharmaceutical or cosmetic ingredients. The presence of liquid lipid produces an imperfect or less ordered lipid matrix, providing additional space for drug incorporation and potentially improving loading capacity compared with conventional solid lipid systems^{1,3,5,6,12,14,18}

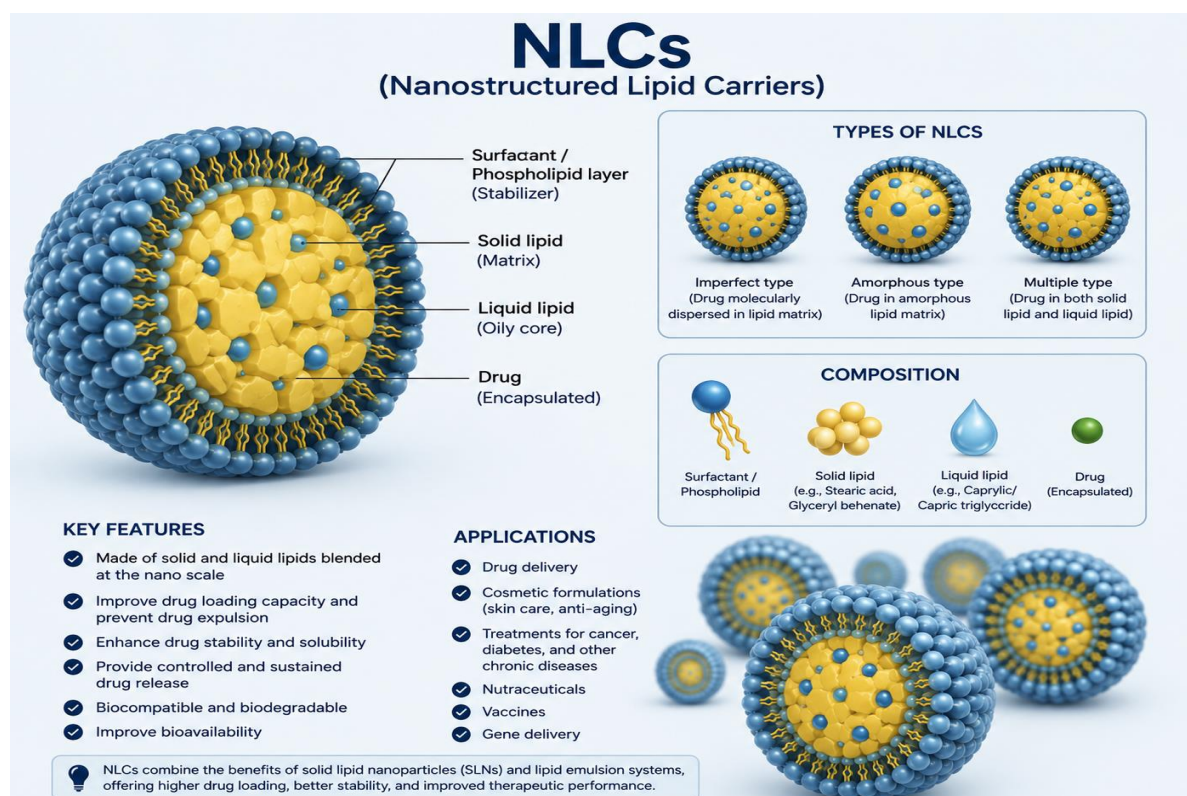


Fig. 1.04: NLC

1.7.1 Relevance of NLCs for Caffeine Delivery

Caffeine is a multifunctional cosmetic active that has been investigated for applications including periorbital skin care, improvement in the appearance of signs of skin aging, and reduction in the visible appearance of cellulite. However, conventional topical formulations may not always provide optimal residence and delivery of the active ingredient at the skin surface. Incorporating caffeine into an NLC system provides an opportunity to improve its topical delivery by entrapping the active within a lipid-based nanostructured matrix.

For anti-aging applications, the potential antioxidant and skin-supportive properties of caffeine, together with the moisturizing and occlusive characteristics of the lipid carrier, may contribute to an improved appearance of fine lines, dryness, and tired-looking skin. In cellulite-focused formulations, NLC-based delivery may provide prolonged topical exposure to caffeine, whose proposed effects on phosphodiesterase activity, intracellular cAMP, lipolysis, and local microcirculation have made it an interesting cosmetic active for improving the appearance of cellulite.

1.7.2 Benefits of Caffeine-Loaded NLC Gel

The incorporation of caffeine-loaded NLCs into a topical gel combines the advantages of the nanocarrier with the desirable properties of a gel vehicle
12,16,34,37,39,44,45

- Enhanced topical delivery
- Prolonged residence
- Controlled release of caffeine
- Improved skin hydration
- Potential anti-aging support
- Periorbital skin-care application
- Potential anti-cellulite application
- Improved formulation acceptability

1.7.3 NLC Preparation Methods ^{5,6,12,25,26,36}

➤ High-pressure homogenization (HPH)

High-pressure homogenization (HPH) is one of the most widely employed and scalable techniques for the preparation of nanostructured lipid carriers (NLCs). The method is particularly suitable for industrial-scale production because it offers reproducibility, high encapsulation efficiency, and the ability to produce nanoparticles with a relatively narrow particle-size distribution. In this technique, the lipid phase containing the drug is melted above its melting temperature and mixed with a heated aqueous surfactant solution to form a coarse pre-emulsion. The pre-emulsion is then forced through a narrow homogenization gap under high pressures, typically ranging from 100 to 2000 bar. HPH can be performed using either hot homogenization or cold homogenization techniques. Hot homogenization is generally preferred for thermostable drugs because the lipid remains in the molten state throughout processing. In contrast, cold homogenization is more suitable for thermolabile compounds, as it minimizes thermal degradation by processing the solidified lipid at lower temperatures.

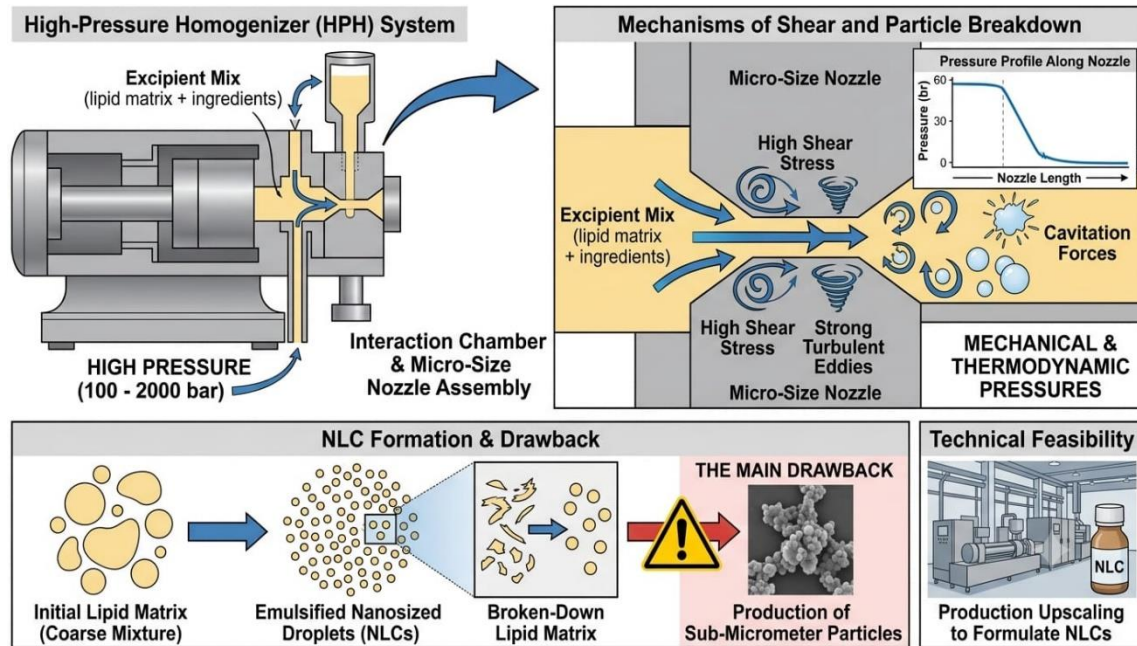


Fig. 1.05: HPH Process

➤ High-shear homogenization (HSH) and ultrasonication

High-shear homogenization (HSH) combined with ultrasonication is a simple, cost-effective, and widely used technique for the preparation of nanostructured lipid carriers (NLCs), particularly in laboratory-scale research. The method provides efficient particle-size reduction and facilitates the production of stable lipid nanoparticles suitable for topical drug delivery. Initially, the solid lipid is melted approximately 5–10°C above its melting point, and the drug is dissolved or dispersed within the molten lipid phase. A heated aqueous phase containing surfactants is then added while high-speed homogenization is performed to produce a coarse emulsion. The resulting emulsion is subsequently subjected to ultrasonication, which further decreases droplet size through cavitation, producing nanoparticles with improved homogeneity. The final dispersion may be concentrated by ultracentrifugation or other suitable separation techniques before incorporation into topical formulations.

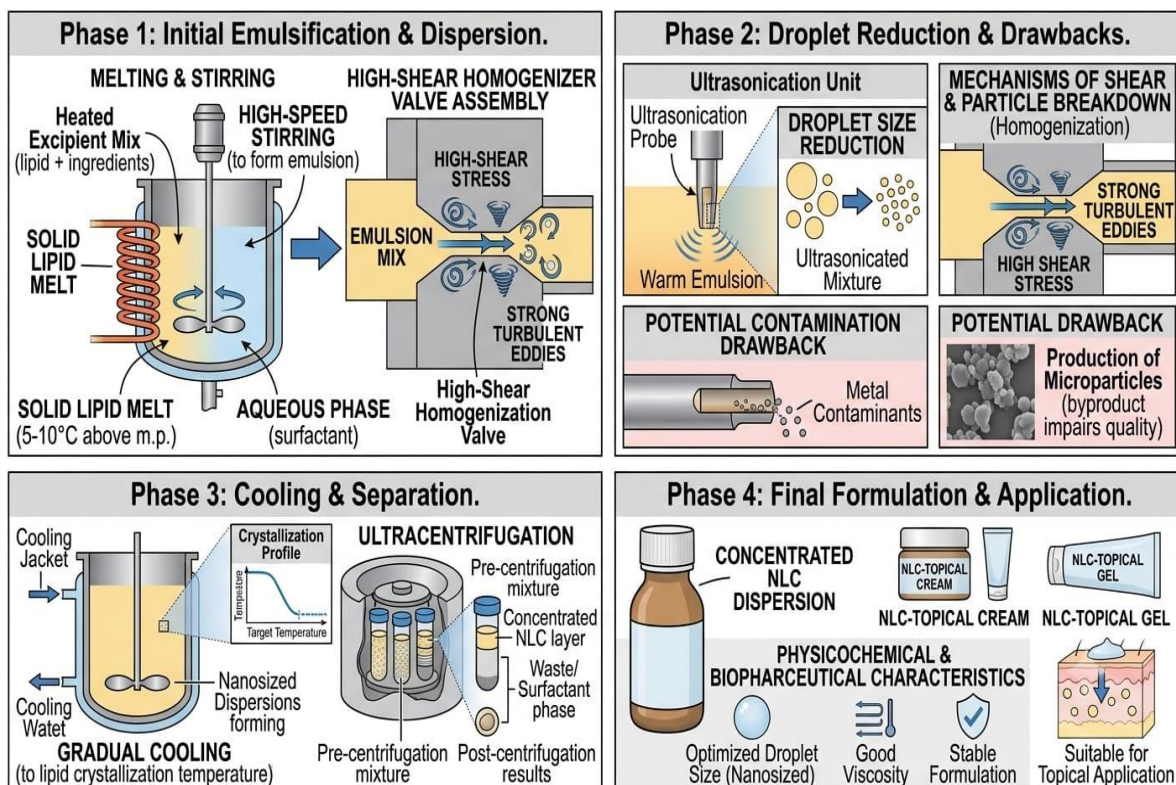


Fig. 1.06: HSH and Ultrasonication process

➤ Microemulsion

The microemulsion technique is a simple and reproducible approach for preparing nanostructured lipid carriers (NLCs). It is based on the formation of a thermodynamically stable oil-in-water microemulsion, followed by rapid cooling to induce lipid crystallization and nanoparticle formation. In this method, the lipid phase is heated approximately 10°C above its melting point, after which the drug is incorporated into the molten lipid. The heated lipid phase is mixed with an aqueous surfactant/co-surfactant solution under continuous stirring to produce a transparent microemulsion. Rapid cooling in an ice bath or by dispersion into cold water causes lipid crystallization, resulting in the formation of nanostructured lipid carriers. The microemulsion method is appreciated for its ease of preparation, reproducibility, and ability to produce relatively uniform nanoparticles. However, the requirement for elevated processing temperatures may limit its applicability for thermosensitive drugs,

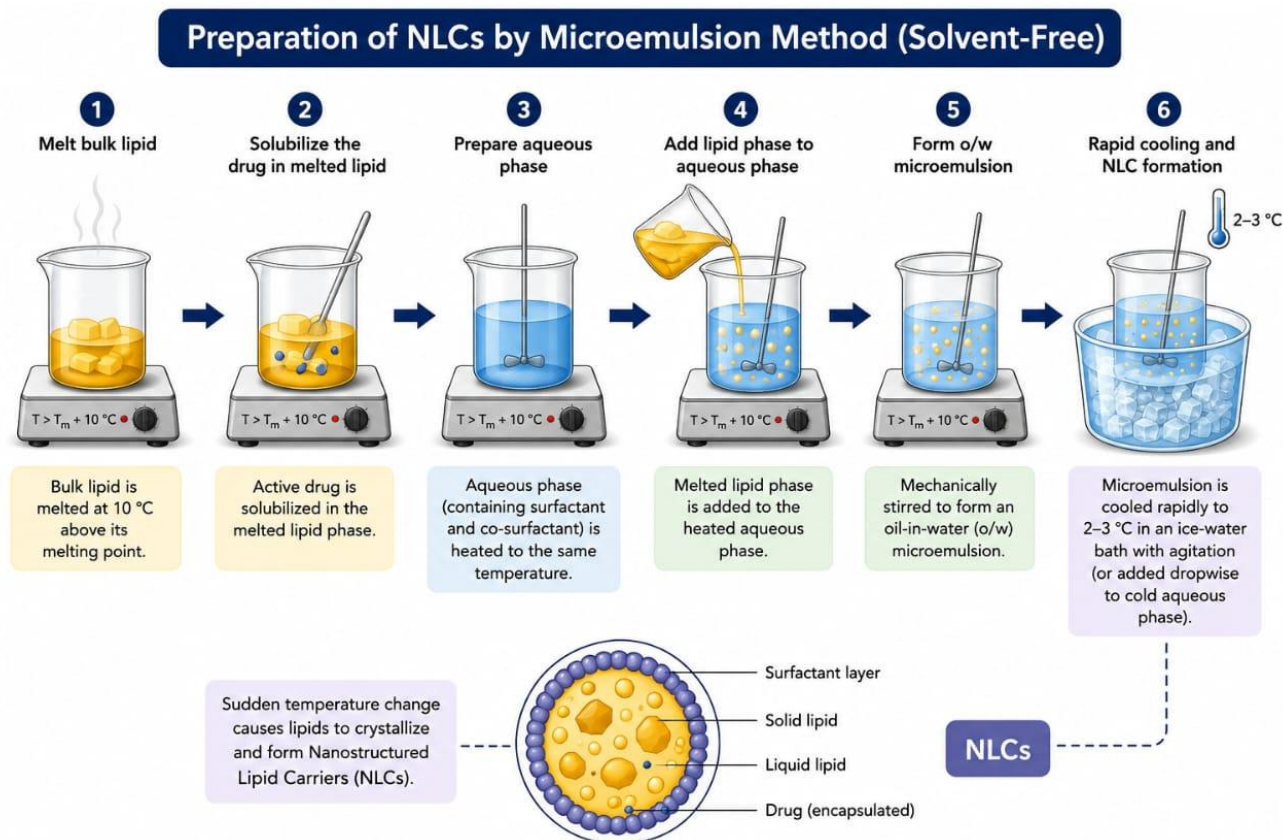


Fig. 1.07: Preparation of NLC by Microemulsion Method

➤ Solvent emulsification/evaporation

The solvent emulsification/evaporation technique is particularly useful for drugs that are sensitive to elevated temperatures. In this method, lipids are dissolved in a water-immiscible organic solvent and subsequently emulsified within an aqueous surfactant solution to form an oil-in-water emulsion. Following emulsification, the organic solvent is removed by evaporation under reduced pressure or continuous stirring, resulting in precipitation of lipid nanoparticles. Compared with thermal methods, this approach minimizes heat-induced drug degradation and is therefore suitable for thermolabile compounds. Despite these advantages, residual organic solvents may remain in the final formulation if solvent removal is incomplete. Consequently, careful solvent selection and complete solvent evaporation are essential to ensure formulation safety and regulatory compliance.

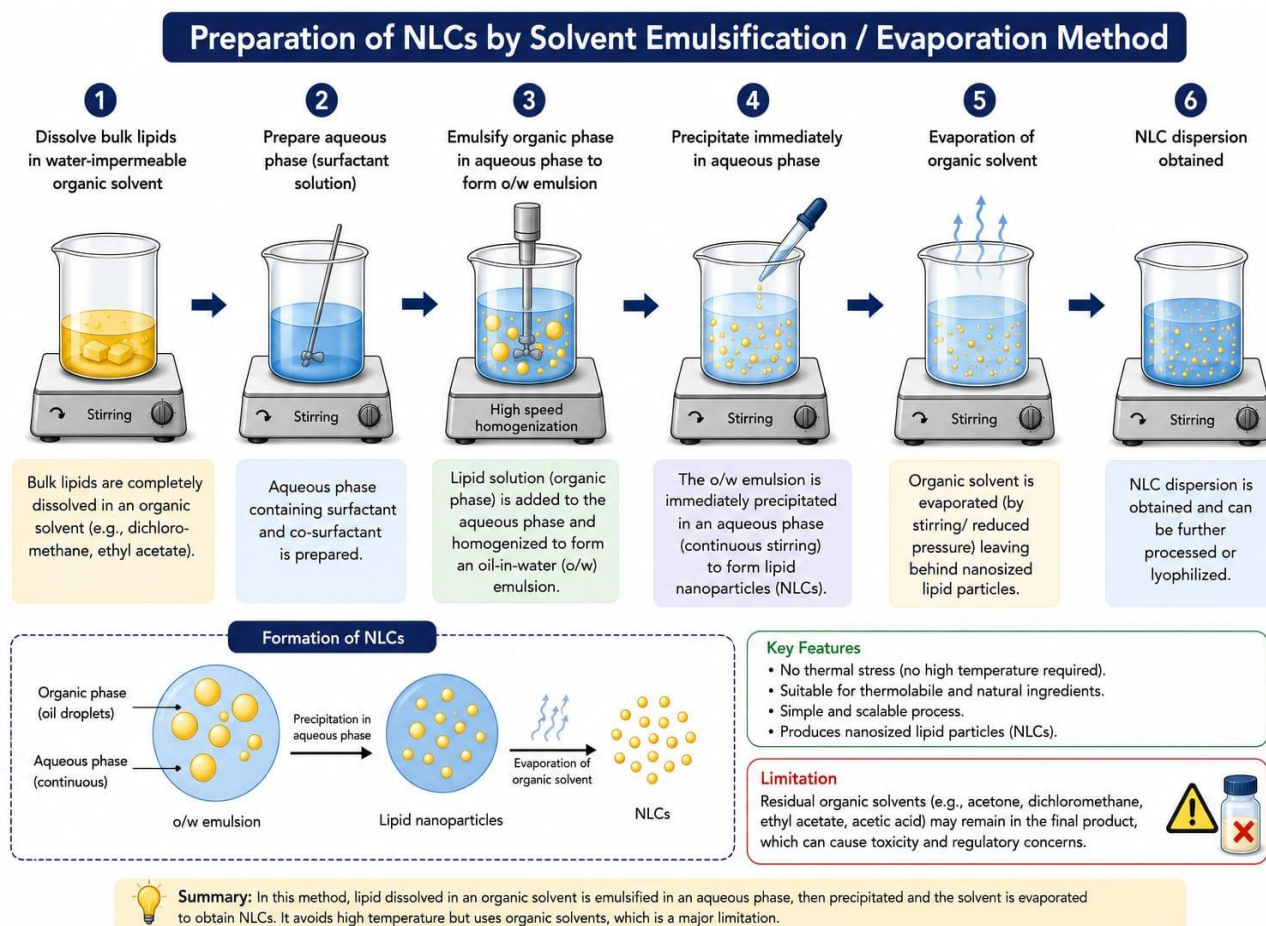


Fig. 1.08: Solvent Emulsification / Evaporation Method

2. AIMS & OBJECTIVES

2.1 Aim

The main of this work is to prepare and evaluate a caffeine loaded nanostructured lipid carrier gel for periorbital skin care, anti-aging anti-cellulite application.

2.2 Objectives

The key objectives of the study are:

- To perform pre-formulation studies for caffeine drug.
- To formulate and evaluate the nanostructured lipid carrier using suitable excipients.
- To incorporate the caffeine into the nanostructured lipid carrier and formulate the gel.
- To evaluate the nanostructured lipid carrier gel.

2.3 Need of the study

Caffeine is naturally occurring methyl xanthine alkaloids over the year there has been extensive demonstration which state that caffeine has anti-oxidant, anti-inflammatory, vasoconstrictive and lipolytic properties. In skin-care caffeine has major application due to its ability to protect skin cells from oxidation stress, improve blood circulation reduce puffiness under eye and minimize fine line and wrinkles. However the incorporation of caffeine remains a challenge due to its poor affinity for lipophilic matrices and the barrier properties of stratum corneum. Poor water solubility of drugs gives rise to poor dissolution, absorption, and subsequently low bioavailability and therapeutic efficacy. By encapsulating caffeine in lipid nanoparticles (NLCs) and suspending them in a gel base, it shows massive boost in skin penetration, antioxidant property, making it ideal for treating under-eye puffiness and

periorbital wrinkles. This is done by decreasing the particle size of the drug to reach a nano size ranging from 10 to 100 nm. So, nanoparticles are considered advantageous drug delivery system than the conventional one.

3. METHODOLOGY

The chemicals that were used are AR/LR grade or the best possible grade available were used as supplied by manufacturer without further purification or investigation.

Equipment's list

Sl.no	Equipment's	Model/Company
1	Electronic Balance	Systronics, Gujarat
2	Magnetic stirrer	REMI Electrotechnics Ltd., Mumbai, India
3	Digital pH meter	Aczet Pvt, Ltd
4	Viscometer	Brookfield DV-II+Pro
5	UV Spectrophotometer	Jasco Int.co.Ltd, Japan

Table no. 1: List of Equipment's

List of chemicals:

Ingredients	Source
Tea powder	TATA CONSUMER PRODUCTS LTD
Carbopol 934	Molychem, Mumbai
Stearic acid	Thermofisher Scientific India Pvt.Ltd.
Tween 80	Thomas Baker [Chemicals]Pvt.Ltd.
Oleic acid	Sd-fine chem Limited
Triethanolamine	Thomas Baker [Chemicals]Pvt.Ltd.
Sodium benzoate	NICE CHEMICALS [P] LTD
Propylene Glycol	Triveni Interchem Pvt. Ltd.
Calcium Carbonate	Molychem, Mumbai
Chloroform	Thermofisher Scientific India Pvt.Ltd.
Distilled water	Spurthy Collage of Pharmacy

Table no 2: List of chemicals

3.1 Extraction of caffeine:

- Weigh tea powder and transfer it to a beaker.
- Add hot/distilled water and stir or heat gently for several minutes to extract the water-soluble components.
- Filter the mixture to remove the tea leaves/insoluble material.
- Cool the filtrate and perform liquid-liquid extraction with an appropriate organic solvent in a separating funnel, following your laboratory's approved solvent/SOP.
- Separate the organic layer containing caffeine and repeat the extraction if required.
- Combine the organic extracts and dry them using an appropriate drying agent.
- Remove the solvent under controlled laboratory conditions to obtain crude caffeine.
- If higher purity is required, recrystallize the crude caffeine using a suitable solvent system.

3.2 Pre-Formulation STUDIE FOR CAFFEINE EXTRACT

1. **Authentication of Crude Drug:** The authentication is based on supplier information provided with the tea material.
2. **Organoleptic Evaluation:** The Caffeine extract was evaluated for its physical characteristics, including colour, odour, appearance, and texture. Organoleptic evaluation provides preliminary information regarding the quality, identity, and acceptability of the extract before formulation.
3. **Solubility Study:** The solubility of Caffeine extract was determined in different solvents to identify a suitable solvent system for formulation development. Solubility influences drug loading, entrapment efficiency, and release characteristics of the final dosage form.
4. **pH Determination:** The pH of the Caffeine extract solution was measured using a calibrated digital pH meter. Determination of pH is important to ensure compatibility of the extract with excipients and to maintain skin-friendly characteristics in the topical formulation.
5. **Moisture Content:** The moisture content of the extract was determined by the loss-on-drying method. Moisture determination is essential to evaluate the stability and storage characteristics of the extract, as excess moisture may lead to degradation or microbial growth.
6. **Partition Coefficient:** Take 10 mL of Caffeine extract and 10 mL of benzene in a separating funnel. Shake gently and allow the layers to separate. Collect the aqueous and benzene layers separately. Take a measured aliquot of the aqueous layer, add distilled water and phenolphthalein, and titrate with standardized 0.1 N NaOH until a persistent pale-pink endpoint is obtained. Determine the concentration of the extract in both layers and calculate the partition coefficient.

$$\text{Partition coefficient} = \frac{[\text{Solute}]_{\text{non polar}}}{[\text{Solute}]_{\text{polar}}}$$

7. **Murexide Test:** The murexide test was performed as a qualitative chemical test to confirm the presence of caffeine in the isolated extract. Caffeine, a purine alkaloid containing nitrogen atoms, undergoes oxidation to form characteristic products that react with ammonia to produce a purple or violet colour, indicating a positive murexide reaction.

3.3 Preparation of NLC

Homogenization Method

- **Preparation of lipid phase:** Accurately weigh stearic acid and oleic acid and heat them together at approximately 70–75°C, or sufficiently above the melting point of the stearic-acid phase, until a uniform lipid phase is obtained.
- **Preparation of aqueous phase:** Dissolve the required amount of caffeine in a suitable portion of warm distilled water and add Tween 80. Maintain the aqueous phase at approximately the same temperature as the lipid phase.
- **Emulsification:** Add the hot aqueous phase gradually to the molten lipid phase with continuous stirring to form a coarse emulsion.
- **Homogenization:** Homogenize the coarse emulsion using a suitable laboratory high-shear homogenizer to reduce the lipid-droplet size.
- **Cooling:** Continue stirring while allowing the dispersion to cool to room temperature. Cooling solidifies the stearic-acid component and forms the nanostructured lipid carrier dispersion.
- **Final adjustment:** Make up the dispersion to the required final weight with distilled water and mix uniformly.

Ingredients	F1	F2	F3	F4
Caffeine	0.125	0.125	0.125	0.125
Stearic acid	1.00	1.25	1.50	1.75
Oleic acid	0.28	0.42	0.52	0.70
Tween 80	1.18.	1.18	1.18	1.18
Distilled water	22.625	22.375	22.125	21.875

Table no. 3: Formulation Chart of NLC

3.4 EVALUATION OF NLC's:

1. Zeta potential

The zeta potential (ZP) is a determining factor of the nano dispersion's stability, describing the surface charge and showing long-term stability. Furthermore, ZP is calculated using electrophoretic mobility of the particle in aqueous media. At higher values, particle aggregation due to electrostatic

repulsion has a lower probability of occurrence. Meanwhile, at lower ZP, there is a higher possibility for dispersions to coagulate or flocculate, potentially reducing stability. For electrostatically stable NLCs, the ZP of dispersion should be less than -30 mV or above +30 mV. The value of ZP can be measured using electrophoretic light scattering.

2. Entrapment efficiency

Entrapment efficiency (EE) is defined as the ratio of entrapped drug weight to total drug weight added to the dispersion. Subsequently, an ultrafiltration-centrifugation method is used to determine the amounts of drugs encapsulated per unit weight of the NLCs. A known NLC dispersion is prepared, and centrifugation is carried out in a centrifuge tube fitted with an ultrafilter. After suitable dilution, an appropriate analysis method is used to determine the amount of free drug supernatant. Entrapment efficiency formula of an NLC gel is:

$$EE \% = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Total amount of drug}} \times 100$$

3. Transmission Electron Microscopy (TEM)

The morphology of the prepared NLC formulation was examined using transmission electron microscopy (TEM). A small quantity of the NLC dispersion was suitably diluted with distilled water. A drop of the diluted sample was placed onto a carbon-coated copper grid and allowed to remain for a suitable period to facilitate adsorption of the nanoparticles onto the grid. Excess sample was carefully removed, and the specimen was allowed to dry. The prepared grid was then examined under a transmission electron microscope at suitable accelerating voltage and magnification. The TEM images were obtained to evaluate the morphology, shape, surface characteristics and approximate size of the NLC particles.

3.5 Preparation of Caffeine Extract NLC gel:

- **Preparation of Carbopol dispersion:** Accurately weigh 0.40 g of Carbopol 934 and slowly sprinkle it into a portion of distilled water with continuous stirring to avoid lump formation. Allow it to hydrate completely.
- **Preparation of aqueous phase:** Dissolve 0.08 g sodium benzoate in a suitable quantity of distilled water. Add 2 g propylene glycol and mix until a uniform solution is obtained.
- **Incorporation of NLC:** Add 20 g of the prepared caffeine-loaded NLC suspension to the aqueous phase with gentle continuous stirring to obtain a uniform dispersion.
- **Gel formation:** Add the hydrated Carbopol 934 dispersion slowly to the NLC dispersion with continuous gentle stirring until a homogeneous mixture is obtained.
- **Neutralization:** Add triethanolamine dropwise with continuous stirring until the Carbopol is adequately neutralized and the desired gel consistency and suitable pH are achieved.
- **Final adjustment:** Add distilled water q.s. to 40 g and mix gently to obtain a uniform caffeine-loaded NLC gel.
- The prepared gel was allowed to stand to remove entrapped air bubbles and evaluated for appearance, homogeneity, pH, viscosity, spreadability, drug content, and stability.

Ingredients	Formulation
NLC suspension	20 g
Triethanol amine	q.s
Propylene glycol	2.0 g
Carbopol 934	0.40
Sodium benzoate	0.08 g
Distilled water	q.s

Table no. 4: Formulation chart of Caffeine Extracted NLC gel (40 gm)

3.6 Evaluation of NLC gel:

1. Visual inspection
2. PH determination
3. Spreadability
4. Skin Irritation Test

5. Viscosity

Visual inspection: Caffeine Loaded NLC gel underwent visual inspection to assess their uniformity, texture, absence of phase separation, and any signs of aggregation.

pH determination : A specific quantity of gel, measured in grammes, was precisely weighed and subsequently dispersed in 25 ml of distilled water. pH of dispersion was determined using a digital pH meter.

Spreadability: The Spreadability of the gel formulation was determined by using sliding plate apparatus and by measuring the diameter of 1 gm of gel between horizontal plates after 1 minute. The standardized weight tied on the upper plate was 20 gm. An excess of gel is placed between two glass slides and a 1000 gm weight is placed on them for 5 minutes, to compress the sample to a uniform thickness. The bottom slide is anchored to the apparatus and weights are placed in the pan. The time in seconds needed to separate the two slides is taken as a measure of spreadability. A shorter time interval indicates better spreadability.

$$\text{Formula} = \text{Spreadability} = \frac{M \times L}{T} \times 100$$

Where,

M = weight tied to the upper slide.

L = length of the glass slide.

T = Time taken to separate two slides (sec).

Skin Irritation Test : Apply the gel on the skin and leave for some time to check the irritation or inflammation

Viscosity: The viscosity of prepared gel was measured using Brookfield viscometer at different RPM viscosity was measured and noted. The measurement was made over the whole range of speed settings from 5-100 rpm with 10 seconds between two successive speeds.

4. RESULTS

4.1 PREFORMULATION STUDY

Authentication of Crude Drug

The tea powder was produced by TATA CONSUMER PRODUCTS LIMITED

Organoleptic Evaluation

Parameter	Observation
Colour	Pale Yellowish
Odour	Characteristics odour
Appearance	Smooth
Texture	Smooth and Homogenous

Table no. 5: Organoleptic evaluation



Fig. 4.01: Extracted Caffeine powder

Solubility study

Solvent	Solubility
Purified water	Freely soluble
Propylene glycol	Soluble
Stearic acid	Slightly soluble
Oleic acid	Slightly soluble
Stearic acid + Oleic acid mixture	Slightly soluble

Table no. 6: Solubility Study**pH Determination**

Prepare a 1% w/v solution of the extract in distilled water and measure the pH.

pH = 6.55

**Fig. 4.02: pH Determination of Caffeine extract****Moisture Content**

Weight of empty dish (W_1) = 37.47 g

Weight of sample + petri dish (before drying) (W_2) = 42.47g

Weight of sample + petri dish (after drying) (W_3) = 42.27g

Moisture Content (%) = $[(42.47-42.27) / (42.47-37.47)] \times 100$

= $(0.2 / 5) \times 100$

= 0.04×100

= 4 %

Partition Coefficient

Trial	Phase	Initial (ml)	Final (ml)	V2 Titre (ml)	N2 (NaOH)	V1 Sample(ml)	N1 = N2V2/V1	Partition Coefficient (P)
1	Aqueous	0.00	1.10	1.10	0.1 N	10	0.0110 N	-
1	Octanol	0.00	0.80	0.80	0.1 N	10	0.0080 N	0.727
2	Aqueous	0.00	1.15	1.15	0.1 N	10	0.0115 N	-

Trial	Phase	Initial (ml)	Final (ml)	V2 Titre (ml)	N2 (NaOH)	V1 Sample(ml)	$\frac{N1}{N2V2/V1}$	Partition Coefficient (P)
2	Octanol	0.00	0.83	0.83	0.1 N	10	0.0083 N	0.722
3	Aqueous	0.00	1.20	1.20	0.1 N	10	0.0120 N	-
3	Octanol	0.00	0.88	0.88	0.1 N	10	0.0088 N	0.733

Average

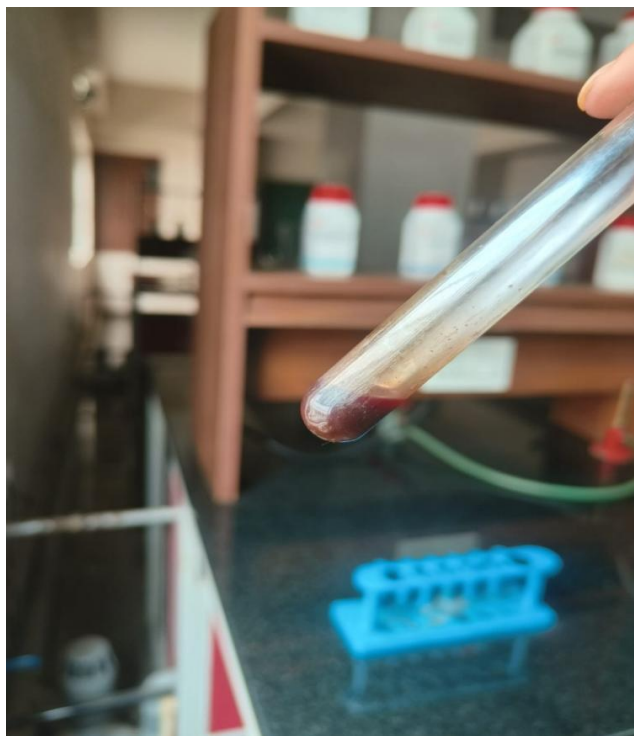
0.727

Table no.7: Partition Coefficient

Murexide Test: Place a small amount of your solid sample or concentrated extract on a porcelain dish, watch glass, or evaporating dish & add a tiny pinch of potassium chlorate and 1 to 2 drops of concentrated hydrochloric acid to the sample.

Heat the mixture gently on a water bath or steam bath until it evaporates completely to dryness, Expose the cooled yellowish-red residue to ammonia gas or add a drop of dilute ammonia solution.

Observation: Development of Purple colour, indicating the presence of caffeine

**Fig. 4.03: Murexide test**

4.2 EVALUATION OF NLC's

Morphological Study

The caffeine-loaded NLCs were predominantly spherical in shape with a smooth surface and distinct integrity. The particles showed no obvious aggregation and exhibited an approximate size range of 300–500 nm.

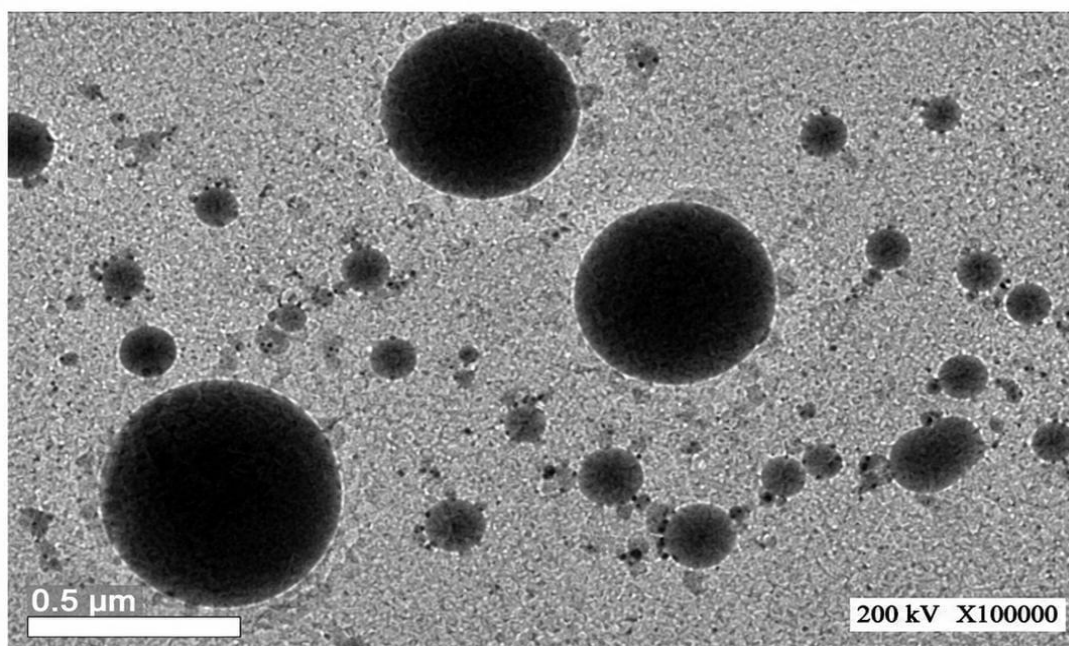


Fig. 4.05: TEM image of Caffeine loaded NLC's

Physical Inspection

Formulation code	Transparency /Opacity	Homogeneity	Clumping / aggregation	Lump formation
F1	Slightly translucent	Non-uniform	Yes	Yes
F2	Slightly translucent	Non-uniform	Yes	Yes
F3	Translucent	Uniform	No visible clumps	No Lumps
F4	Slightly translucent	Non-uniform	Yes	Yes

Table no. 8: Physical evaluation of NLC's

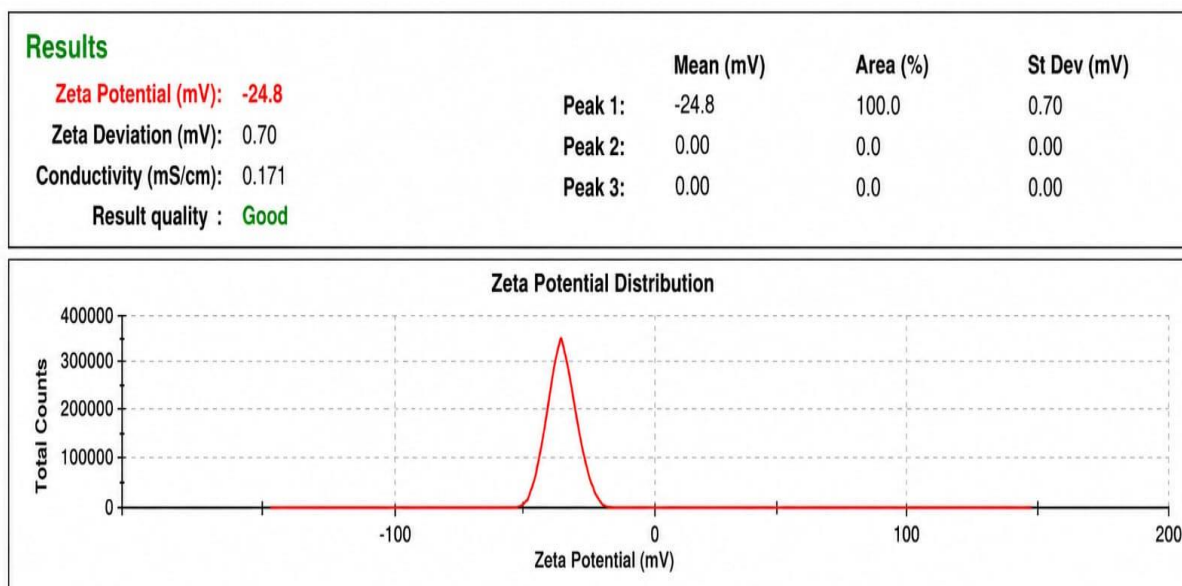
Zeta Potential

Fig. 4.06: Zeta potential of Caffeine loaded NLC

Entrapment efficiency

Serial Number	Formulation code	Entrapment Efficiency (%)
1	F1	60.13
2	F2	58.09
3	F3	72.55
4	F4	48.96

Table no. 9: Entrapment Efficiency**4.3 EVALUATION OF CAFFEINE LOADED NLC GEL****Visual Inspection**

Formulation code	Colour	Clarity	Consistency	Presence of Particulate
F3	Pale Yellowish	Translucent	Thick gel	No Particulate

Table no. 10: Visual inspection of NLC gel**Fig. 4.07: Final formulation of Caffeine loaded NLC Gel****pH determination****Fig. 4.08: pH determination of NLC gel**

Formulation	pH
F3	6.98

Table no. 11: pH determination of NLC gel

Spreadability

Formulation	Spreadability (g·cm/sec)
F3	18.88

Table no. 12: Spreadability determination

Skin irritation test

Formulation	Skin irritation
F3	No Irritation

Table no. 13: Skin irritation test

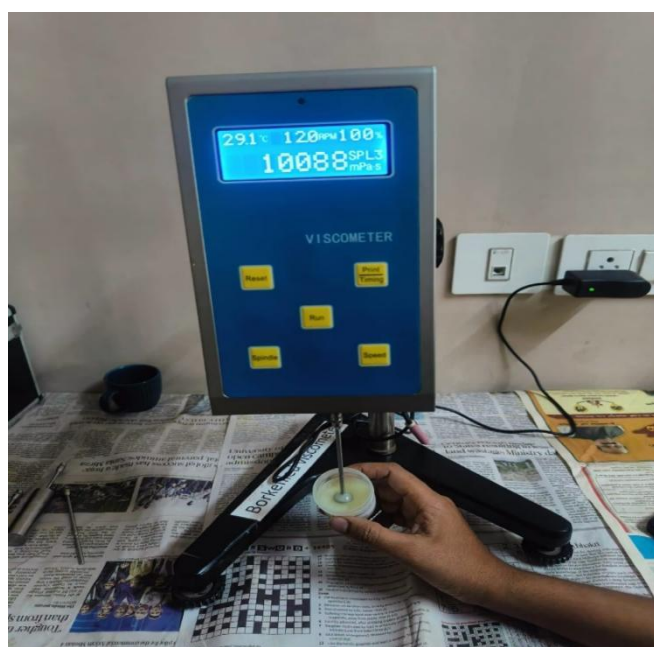
Viscosity determination

Fig. 4.09: Viscosity determination

Formulation	Viscosity(m.pa.s)
F3	10088

Table no. 14: Viscosity determination

5. DISCUSSION**5.1 Preformulation Studies**

The present study was undertaken to formulate and evaluate a caffeine-loaded nanostructured lipid carrier (NLC) gel intended for periorbital skin care with potential anti-ageing and anti-cellulite applications. Preformulation studies of the extracted caffeine showed a pale yellowish, smooth and homogeneous powder with a pH of 6.55. The extract was freely soluble in purified water and soluble in propylene glycol, indicating suitability for incorporation into the formulation. The moisture content was found to be 4%, while the partition coefficient was 0.727 ± 0.006 . The positive murexide and tannic acid tests further supported the presence of caffeine.

5.2 Evaluation of NLC suspension

Four NLC formulations were prepared by varying the concentrations of stearic acid and oleic acid. Among the formulations, F3 exhibited superior physical characteristics, showing uniformity without visible clumping or lump formation. The NLC particles were predominantly spherical with smooth surfaces and an approximate size range of 300–500 nm. F3 showed the highest entrapment efficiency of 72.55%, compared with F1, F2 and F4.

5.3 Evaluation of Caffeine loaded NLC gel

The optimized F3 NLC was successfully incorporated into a pale-yellowish translucent thick gel. The formulation showed a pH of 6.98, viscosity of 10088 mPa·s and spreadability of 18.88 g·cm/sec. No irritation was observed during the skin irritation evaluation. These findings indicate that F3 possessed satisfactory physical characteristics and was suitable for further consideration as a topical caffeine-loaded NLC gel.

6. CONCLUSION

The present study successfully developed and evaluated a caffeine-loaded nanostructured lipid carrier (NLC) gel for potential periorbital skin care, anti-ageing and anti-cellulite applications. Preformulation studies confirmed the suitability of the extracted caffeine for formulation development, with satisfactory organoleptic characteristics, solubility, pH and moisture content. Among the four NLC formulations prepared, F3 was identified as the optimized formulation based on its favourable physical appearance, uniformity and absence of visible aggregation.

F3 demonstrated the highest entrapment efficiency of 72.55%, indicating comparatively better incorporation of caffeine within the lipid carrier system. The optimized NLC showed predominantly spherical morphology with an approximate particle-size range of 300–500 nm. The NLC dispersion was successfully incorporated into a topical gel, producing a pale-yellowish, translucent and thick preparation. The final gel exhibited a pH of 6.98, viscosity of 10088 mPa·s and spreadability of 18.88 g·cm/sec, with no irritation observed during the skin irritation test.

Overall, the developed caffeine-loaded NLC gel demonstrated promising physicochemical characteristics for topical application. However, further detailed in-vitro, ex-vivo, stability and clinical studies are required to establish its long-term efficacy, safety and therapeutic potential.

7. SUMMARY

The present investigation focused on the formulation and evaluation of a caffeine-loaded nanostructured lipid carrier (NLC) gel for potential use in periorbital skin care, anti-ageing and anti-cellulite applications. Caffeine was extracted and subjected to preformulation studies including organoleptic evaluation, solubility, pH, moisture content, partition coefficient and qualitative identification tests. The extracted caffeine showed a pale yellowish, smooth and homogeneous appearance, a pH of 6.55 and 4% moisture content. The partition coefficient was found to be 0.727 ± 0.006 , while positive murexide and tannic acid tests supported caffeine identification.

Four caffeine-loaded NLC formulations, F1–F4, were prepared using varying concentrations of stearic acid and oleic acid. Evaluation revealed that F3 possessed the most favourable characteristics, including uniformity and absence of visible clumping. Its entrapment efficiency was 72.55%, the highest among the prepared formulations. The NLCs exhibited predominantly spherical morphology with an approximate size range of 300–500 nm.

The optimized F3 NLC was incorporated into a Carbopol 934 gel. The final formulation was pale yellowish, translucent and thick, with a pH of 6.98, viscosity of 10088 mPa·s and spreadability of 18.88 g·cm/sec. No irritation was observed. Thus, F3 was selected as the optimized caffeine-loaded NLC gel formulation.

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