ORGANOLEPTIC AND SUN PROTECTING FACTOR (SPF) TEST OF NANOCREAM PREPARATION WITH ACTIVE INGREDIENTS OF *Centella asiatica* AND *Moringa oleifera* IN VIVO

THESIS

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MALANG
2025

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MASTER OF BIOLOGY STUDY PROGRAM
FACULTY OF SCIENCE AND TECHNOLOGY
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ORGANOLEPTIC AND SUN PROTECTION FACTOR (SPF) TEST OF NANOCREAM PREPARATION WITH ACTIVE INGREDIENTS OF *Centella asiatica* AND *Moringa oleifera* IN VIVO

Master's Program in Biology, Faculty of Science and Technology,
State Islamic University of Maulana Malik Ibrahim Malang

ABSTRACT

Centella asiatica and *Moringa oleifera* are well-known for their antioxidant properties, making them suitable active ingredients in topical sunscreen formulations. Modern pharmaceutical preparations increasingly use nanotechnology to improve the penetration of active compounds into target skin cells. Among various nanotechnology approaches, nano-creams are widely used to enhance the efficacy of topical delivery. One common method to produce these creams is through homogenization, where parameters such as rotational speed and duration influence the particle size and physical quality of the nano-cream. This experimental study aimed to investigate the effect of homogenization duration on the physical characteristics and efficacy of sunscreen creams containing nanoparticle extracts of *C. asiatica* and *M. oleifera*. The study employed a Completely Randomized Design (CRD) with five replicates per group. Animal subjects (rats) were divided into three main groups: a positive control group exposed to UVB radiation and treated with a commercially available sunscreen; A negative control group exposed to UVB radiation without treatment; and treatment groups exposed to UVB radiation and treated with nano-creams prepared with different homogenization times (no homogenization, 45 minutes, 70 minutes, and 95 minutes). All cream formulations contained 10% combined extracts incorporated into a skin-friendly base. Physical characterization included organoleptic tests (color, odor, texture) and skin irritation assessment. The in vivo effectiveness was evaluated by comparing the degree of sunburn on rat skin across groups. Results showed that all nano-creams significantly protected the skin against UVB damage ($p < 0.05$). The formula homogenized for 70 minutes (Formula B) was the most effective, with a sunburn score of 0.00, indicating superior protection. Homogenization duration influenced physical properties organoleptic but did not affect irritation responses. Extended homogenization improved cream performance and sensory attributes, suggesting 70 minutes as the optimal homogenization time for maximum sun protection.

Keywords: Nano-cream, Particle size, Sunscreen, *Centella asiatica*, *Moringa oleifera*.

UJI ORGANOLEPTIK DAN FAKTOR PERLINDUNGAN MATAHARI (SPF) PADA SEDIAAN NANOCREAM DENGAN BAHAN AKTIF *Centella asiatica* DAN *Moringa oleifera* SECARA VIVO

Program Magister Biologi, Fakultas Sains dan Teknologi,
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ABSTRAK

Centella asiatica dan *Moringa oleifera* terkenal karena sifat antioksidannya, sehingga menjadikannya bahan aktif yang cocok dalam formulasi tabir surya topikal. Sediaan farmasi modern semakin banyak menggunakan nanoteknologi untuk meningkatkan penetrasi senyawa aktif ke dalam sel kulit target. Di antara berbagai pendekatan nanoteknologi, krim nano banyak digunakan untuk meningkatkan kemanjuran pengiriman topikal. Salah satu metode umum untuk memproduksi krim ini adalah melalui homogenisasi, di mana parameter seperti kecepatan putar dan durasi memengaruhi ukuran partikel dan kualitas fisik krim nano. Studi eksperimental ini bertujuan untuk menyelidiki pengaruh durasi homogenisasi terhadap karakteristik fisik dan kemanjuran krim tabir surya yang mengandung ekstrak nanopartikel *C. asiatica* dan *M. oleifera*. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan lima ulangan per kelompok. Subjek hewan (tikus) dibagi menjadi tiga kelompok utama: kelompok kontrol positif yang terpapar radiasi UVB dan diobati dengan tabir surya yang tersedia secara komersial; Kelompok kontrol negatif yang terpapar radiasi UVB tanpa pengobatan; dan kelompok perlakuan yang terpapar radiasi UVB dan diobati dengan krim nano yang disiapkan dengan waktu homogenisasi yang berbeda (tanpa homogenisasi, 45 menit, 70 menit, dan 95 menit). Semua formulasi krim mengandung 10% ekstrak gabungan yang dimasukkan ke dalam basis yang ramah kulit. Karakterisasi fisik meliputi uji organoleptik (warna, bau, tekstur) dan penilaian iritasi kulit. Efektivitas in vivo dievaluasi dengan membandingkan tingkat sengatan matahari pada kulit tikus di seluruh kelompok. Hasil penelitian menunjukkan bahwa semua krim nano secara signifikan melindungi kulit terhadap kerusakan UVB ($p < 0,05$). Formula yang dihomogenisasi selama 70 menit (Formula B) adalah yang paling efektif, dengan skor sengatan matahari 0,00, yang menunjukkan perlindungan yang unggul. Durasi homogenisasi memengaruhi sifat fisik organoleptik tetapi tidak memengaruhi respons iritasi. Homogenisasi yang diperpanjang meningkatkan kinerja krim dan atribut sensorik, menunjukkan 70 menit sebagai waktu homogenisasi optimal untuk perlindungan matahari maksimum.

Kata kunci: Nano-krim, Ukuran partikel, Tabir surya, *Centella asiatica*, *Moringa oleifera*.

إختبار الحسية وعامل الحماية من الشمس (SPF) لمستحضر كريم نانوي يحتوي على مكونات فعالة من نبات السنتيلا الآسيوية والمورينجا أوليفيرا في الجسم الحي

برنامج الماجستير في علم الأحياء، كلية العلوم والتكنولوجيا،
جامعة مولانا مالك إبراهيم الإسلامية الحكومية، مالانج

خلاصة

تشتهر السنتيلا الآسيوية والمورينجا أوليفيرا بخصائصهما المضادة للأكسدة، مما يجعلهما مكونين فعالين مناسبين في تركيبات واقي الشمس الموضعي. تستخدم المستحضرات الصيدلانية الحديثة بشكل متزايد تقنية النانو لتحسين اختراق المركبات الفعالة في خلايا الجلد المستهدفة. من بين العديد من مناهج تقنية النانو، تُستخدم كريمات النانو على نطاق واسع لتعزيز فعالية التوصيل الموضعي. إحدى الطرق الشائعة لإنتاج هذه الكريمات هي من خلال استعمال تقنية التجانس، حيث تؤثر معاملات مثل سرعة الدوران والمدة على حجم الجسيمات والجودة الفيزيائية لكريم النانو. هدفت هذه الدراسة التجريبية إلى التحقيق في تأثير مدة التجانس على الخصائص الفيزيائية وفعالية كريمات واقي الشمس التي تحتوي على مستخلصات الجسيمات النانوية من السنتيلا الآسيوية والمورينجا أوليفيرا. استخدمت الدراسة تصميمًا عشوائيًا كاملاً (CRD) بخمس مكررات لكل مجموعة. تم تقسيم الحيوانات (الجرذان) إلى ثلاث مجموعات رئيسية: مجموعة ضابطة إيجابية تعرضت لإشعاع UVB وعولجت بواقي شمس تجاري؛ مجموعة ضابطة سلبية تعرضت للأشعة UVB دون الخضوع لعلاج؛ ومجموعات العلاج التي تم تعريضها للأشعة UVB وعولجت بكريمات نانوية محضرة بأوقات تجانس مختلفة (بدون تجانس، 45 دقيقة، 70 دقيقة، و95 دقيقة). احتوت جميع تركيبات الكريم على 10% من المستخلصات المركبة المدمجة في قاعدة صديقة للبشرة. تضمن التوصيف الفيزيائي اختبارات حسية (اللون والرائحة والملمس) وتقييم تهيج الجلد. تم تقييم الفعالية في الجسم الحي من خلال مقارنة درجة حروق الشمس على جلد الفئران عبر المجموعات. أظهرت النتائج أن جميع كريمات النانو تحمي البشرة بشكل كبير من أضرار الأشعة فوق البنفسجية ($p < 0.05$). كانت التركيبة المتجانسة لمدة 70 دقيقة (التركيبة B) هي الأكثر فعالية، بدرجة حروق الشمس 0.00، مما يشير إلى حماية فائقة. أثرت مدة التجانس على الخصائص الفيزيائية الحسية ولكنها لم تؤثر على استجابات التهيج. أدى التجانس الممتد إلى تحسين أداء الكريم والخصائص الحسية، مما يشير إلى أن 70 دقيقة هي وقت التجانس الأمثل للحصول على أقصى حماية من الشمس.

الكلمات المفتاحية: كريم نانوي، حجم الجسيمات، واقي الشمس، سنتيلا اسياتيكا، المورينجا اوليفيرا.

FOREWORD

Assalamu alaikum Wr. Wb.

Bismillah Arahmani Arahim, all praise be to Allah SWT. The Lord of the universe because of His blessings and mercy, the author can complete this final assignment entitled "Organoleptic and Sun Protection Factor (SPF) Test of Nano-cream Preparation with Active Ingredients of *Centella asiatica* And *Moringa oleifera* in Vivo". Also, do not forget to convey prayers and greetings to the great Prophet Muhammad SAW. who has upheld the Islamic religion which is embedded until the end of time. Amin.

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May the good deeds that have been given to the author be rewarded by Allah SWT. This thesis has been written carefully and as well as possible, but if there are any shortcomings, suggestions and constructive criticism are highly expected by the author.

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Malang 26 June 2025

Writer

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CHAPTER I

INTRODUCTION

1.1 Background

The human face reflects various aspects, including emotions, identity, aesthetic appeal, and health. Therefore, the health of the face and skin is very important for individuals. However, many people suffer from skin issues such as premature aging, sunburn, skin cancer, pigmentation, tissue damage, and dry skin. All of these problems are caused by ultraviolet (UV) rays, which lead to skin damage. Consequently, there has been an increased awareness in recent years about the importance of using sunscreen, resulting in a variety of products available in the market that address this issue (Arie *et al*., 2024).

Most sunscreens contain chemical ingredients, making them more harmful and less beneficial, causing skin irritation and side effects. They also have limitations, such as the need for frequent reapplication, decreased effectiveness of some types with prolonged exposure, skin reactions and allergies, undesirable texture and appearance, potential health effects of some ingredients, and insufficient protection against all types of UVA and UVB rays. This has led to a need to search for safer alternatives, and a shift toward using natural materials and exploiting the biologically active components of medicinal plants.

Among the plants that have been used for medicinal purposes since ancient times and in modern times are *M. oleifera* and *C. asiatica*. There are many studies that have developed formulations made from natural and safe ingredients, including the use of *M. oleifera* and *C. asiatica* (Arie *et al*., 2024).

قال الله تعالى: ﴿أَوَلَمْ يَرَوْا إِلَى الْأَرْضِ كَمْ أَنبَتْنَا فِيهَا مِن كُلِّ زَوْجٍ كَرِيمٍ﴾ سورة الشعراء الآية 7

Allah reminds people of His signs in the universe and calls them to contemplate His creation to increase their faith in Him.

﴿أَوَلَمْ يَرَوْا﴾ That is, have they not witnessed and contemplated with their eyes and insights. ﴿إِلَى الْأَرْضِ﴾means at its creatures and plants. ﴿كَمْ أَنبَتْنَا فِيهَا﴾ means how many diverse plants and crops We have caused to grow on the earth. ﴿مِن كُلِّ زَوْجٍ كَرِيمٍ﴾means of every Allah and beneficial kind. (الزوج) in the language refers to a class or type, while (كَرِيمٍ) denotes good creation and good benefit. Indeed, every living thing on this Earth was not created in vain. Many plants around the world have different bioactive compounds. Each plant has certain advantages and functions. For example, *C. asiatica* and *M. oleifera* plants. Allah grows many good plants, not only physically good, but also beneficial to humans, such as medicine. Some plants that have the potential to be medicine are *C. asiatica* and *M. oleifera*.

Moringa oleifera is known for its therapeutic properties and active compounds. It contains triterpenoids, flavonoids, saponins, and alkaloids, which enhance the antioxidant activity of *M. oleifera* extracts (Lazaely *et al*., 2021). The *M. oleifera* plant contains 46 types of antioxidants and 36 anti-inflammatory compounds that can act as sun protectants, making it effective for sun protection. Ultraviolet (UV) rays generate free radicals (Desi *et al*., 2023), and flavonoid compounds can serve as antioxidants and sun protectants due to the presence of chromophore groups that can absorb UV rays, reducing skin exposure (Ika *et al*., 2021). Studies indicate that nearly all phenolic and flavonoid compounds possess photoprotective properties due to their ability to absorb ultraviolet radiation (Melati *et al*., 2022). Flavonoids, in particular, contain conjugated double bonds capable of absorbing UVB radiation. The absorbed UVB energy is released at a much lower intensity, with most

of the energy being converted into harmless thermal energy, thus preventing or reducing erythema caused by UV exposure. Additionally, flavonoids exhibit anti-inflammatory activity by influencing the arachidonic acid pathway. They inhibit COX-2 expression, thereby suppressing prostaglandin synthesis, which plays a crucial role in UV-induced erythema development (Melati *et al*., 2022).

C. asiatica is a perennial, creeper, faintly aromatic and a valuable medicinal herb of both Old World and the New World. It is widely distributed throughout tropical and subtropical regions of World. Its potential antioxidant, antimicrobial, cytotoxic, neuroprotective and other activities have been widely claimed in many reports and basically is very much related to its properties and mechanism of action of the plant's bioactive constituents namely the triterpenic acid (asiatic acid madecassic acid), triterpenic saponin (madecassoside and asiaticoside), flavanoids and other phenolic compounds (Yanni *et al*., 2023). The active compounds include pentacyclic triterpenes, mainly asiaticoside, madecasosside, asiatic acid and madecassic acid. Is used also as an ingredient of cosmetics (Yanni *et al*., 2023, Afifah *et al*., 2024).

The scientific studies have proved a variety of biochemical components i.e. secondary metabolites have been found in *C. asiatica*. The active compounds include pentacyclic triterpenes, mainly Asiatic-o-side, madecassoside, Asiatic and madecassic acids. The chemical constituents of *C. asiatica* plant have a very important role in medicinal applications and it is believed due to its biologically active components of triterpenes saponins (Lazaely *et al*., 2024). The triterpenes of *C. asiatica* are composed of many compounds including Asiatic acid, madecassic acid, Asiaticosside, madecassoside, brahmoside, brahmic acid, brahminoside, thankiniside, isothankunisode, centelloside,

madasiatic acid, centic acid, and cenellicacid (Rasangani, 2023). Apart from its terpenoid content, it is notably rich in total phenolic compounds, primarily contributed by flavonoids such as quercetin, kaempferol, catechin, rutin, apigenin, and naringin, as well as volatile oils including caryophyllene, farnesol, and elemene (Nora *et al*., 2017).

Evaluation on *C. asiatica* has found its chemical composition consist of four triterpenes, namely madecassoside, asiaticoside, madecassic acid and asiatic acid. The significantly stimulated collagen synthesis. *C. asiatica* has been incorporated into skincare products due to its collagen-stimulating properties, which contribute to restoring skin firmness and elasticity while enhancing overall skin appearance (Harun *et al*., 2023). Preliminary UV study suggested that the *C. asiatica* could be a potential natural protection against UVB damage and THIS activity might be due to its triterpene component(s) (Dong *et al*., 2024).

Nanotechnologies utilize materials at a nanoscale, altering their properties compared to their larger counterparts. An intriguing application of this technology is in nano-cosmetics, where nanomaterials enable the creation of innovative products. Additionally, the development of new excipients designed for nano-formulations presents opportunities for creating delivery systems with enhanced efficiency, minimized irritancy, and reduced toxicity (Kale *et al*., 2021).

Nano-formulations play a crucial role in delivering active compounds to and through the skin for various therapeutic applications. They are efficient, cost-effective, and relatively straightforward to produce, offering substantial advantages over traditional coarse emulsions. Nanotechnology serves as a pivotal innovation, enabling the development of advanced and novel products (Vaishali *et al*., 2020). In recent years, there

has been growing interest in utilizing topical vehicle systems to enhance drug permeation through the skin. Nano-cream can be formulated using high-energy techniques such as ultrasound generators, high-pressure homogenizers, or high-shear stirring methods (Vaishali *et al*., 2020).

Consequently, their small droplet size, typically ranging from 100 to 600 nm, makes them highly effective for use in cosmetics and personal care products. This nanoparticle-sized range ensures uniform and smooth application of the cream on the skin surface, facilitating the efficient release of active drug ingredients contained within the formulation (Vaishali *et al*., 2020).

Several factors, including particle size, log P, ionic strength, hydrogen bonding capability, and the physicochemical properties of the vehicle, influence both the rate and extent of drug transport through the stratum corneum. Microemulsions and nano-emulsions offer significant advantages due to their effectiveness as delivery systems for topical drugs (Rahul *et al*., 2024).

Although previous studies have extensively investigated formulations involving natural plant extracts, particularly in the form of nano-emulsions, limited research has explored the development of creams incorporating active ingredient nano-cream, specifically from *C. asiatica* and *M. oleifera*. Most existing research primarily emphasizes optimizing the concentration of active compounds without addressing the crucial role of formulation techniques such as homogenization. Furthermore, the impact of homogenization duration on important product attributes—including organoleptic qualities, skin irritation potential, and sun protection factor (SPF)—has been largely overlooked. Additionally, many studies have focused on the general phytochemical

benefits rather than systematically optimizing the physical properties and performance of the final cream formulation. Therefore, this study aims to fill these gaps by developing a cream based on nano-cream of *C. asiatica* and *M. oleifera*, and investigating how variations in homogenization duration influence its sensory properties, safety, and SPF effectiveness through comprehensive in vivo evaluations.

1.2 Problem Formulation

Drawing from the provided background, the problem statement can be articulated as follows:

1. How does the duration of homogenization influence the organoleptic (sensory) properties of nano-cream formulations as assessed by trained panelists?
2. How does the duration of homogenization affect the potential for skin irritation in animal models?
3. How does the duration of homogenization impact the Sun Protection Factor (SPF) values observed in animal skin models?

1.3 Research Objectives

Based on the problem formulation above, the objectives of this research are as follows:

1. To evaluate the effect of different homogenization durations on the organoleptic (sensory) properties of nano-cream formulations using trained panelist assessments.
2. To determine the impact of homogenization duration on the potential for skin irritation in animal models.

3. To assess how variations in homogenization duration influence the Sun Protection Factor (SPF) values in animal skin models.

1.4 Benefits of the Research

The anticipated benefits derived from this research include the following:

1. The public can gain scientific information about the benefits of *Centella asiatica* and *Moringa oleifera* leaves, which can be used as ingredients to create nano-cream formulations for sun protection.
2. For educational institutions, it can serve as an educational resource to enhance the awareness and knowledge of teachers and students regarding the benefits of natural ingredients like *Centella asiatica* and *Moringa oleifera* leaves, which can be used as components in sun protection formulations, and can act as a reference for further qualitative research.
3. For researchers, this adds insight and knowledge in the field of biology, serves as a data source in research preparation, and can enhance understanding and learning materials for researchers regarding the effects of differences in speed and duration of homogenizer use, as well as the benefits of natural ingredients such as *Centella asiatica* and *Moringa oleifera* leaves, These findings can serve as a foundation for utilizing raw materials in the production of sunscreen formulations, contributing to advancements in the cosmetics industry.

1.5 Limitation of the Problem

The scope of the problem in this research is as follows:

1. This research is limited to the formulation of nano-cream s using an oil-in-water (O/W) emulsion system
2. Physical characterization focuses on organoleptic properties and irritation potential
3. In vivo testing will be conducted on rats to assess photoprotective efficacy, with SPF as a key evaluation parameter

CHAPTER II

LITERATURE REVIEW

2.1 Importance of Skincare and Sun Protection

2.1.1 Sunburn

Erythema (sunburn) is an acute inflammatory skin response triggered by excessive UV exposure. This inflammation is primarily caused by the dilation of cutaneous blood vessels. UVB-induced erythema leads to the release of inflammatory mediators such as histamine and prostaglandins in the affected skin, resulting in increased blood flow, leukocyte infiltration, and ultimately, visible redness (Melati *et al*., 2022).

Sunburn is an acute inflammatory skin reaction occurring due to extended exposure to ultraviolet (UV) rays from the sun or artificial sources such as tanning beds. Sunburns can be quite painful, and in most cases, conservative treatment is all that is necessary. However, seeking hospital evaluation and treatment may be necessary in severe cases. Every year, over a third of the population experiences sunburn, which can have lasting consequences (Karla *et al*., 2023).

The severity of sunburn depends primarily on the duration and intensity of exposure to UV rays. Overall, the number of sunburns experienced in individuals correlates with an increased risk of skin cancer (Karla *et al*., 2023).

2.1.2 The Protecting the Skin from UV Rays

Strong evidence supports the role of daily photoprotection and regular sunscreen use in prevention (Sara *et al*., 2024). In a study involving 46 patients randomly assigned to either a vehicle or daily use of sunscreens with UVA and UVB protection for 24 months,

significant histological differences in solar elastosis were observed between the vehicle group and the treatment group (Nicole *et al*., 2022).

Moreover, in a study involving 12 subjects, each participant was exposed to a single minimal erythemal dose of simulated solar radiation on three areas of buttock skin—unprotected skin, vehicle-treated skin, and skin treated with a day cream offering UVA and UVB protection—along with a control area that received no exposure (Linna *et al*., 2021). The unprotected skin exhibited significant melanization, thickening of the stratum corneum and stratum granulosum, increased tenascin expression, reduced type I procollagen levels, and slight elevations in lysozyme and alpha-1 antitrypsin. These changes were effectively mitigated by the day cream with sunscreen protection (Linna *et al*., 2021).

In a prospective study, 32 participants were instructed to apply a broad-spectrum, photostable sunscreen (SPF 30) daily for 52 weeks. By the end of the study, significant improvements were noted in skin texture, clarity, and both mottled and discrete pigmentation. Notably, all participants (100%) exhibited improvements in skin clarity and texture (Randhawa *et al*., 2016).

2.1.3 Sunscreen

Sunscreen is a substance designed to protect the skin from the harmful effects of the sun's rays. It works by reflecting, absorbing, and scattering ultraviolet (UV) radiation, specifically UVA and UVB, to offer protection against both types. However, the regulatory definition of sunscreen may vary depending on the specific country (NCI dictionary of cancer terms 2019). As defined by the Sunscreen Innovation Act (SIA, 2019), the term “sunscreen active ingredient” refers to a component specifically intended for application to human skin to absorb, reflect, or scatter ultraviolet radiation.

In vivo SPF testing involves exposing protected (with sunscreen) and unprotected skin areas to artificial light from a solar simulator. After approximately 24 hours, pigmentation and erythema are assessed. Although testing methods vary among regulatory agencies, they are harmonized by ISO standards. Creams and lotions are the main market products. Emulsions, or water-oil systems, form the basis of a wide variety of formulations, incorporating various ingredients, particularly photo-protective agents due to their lipophilic nature. These emulsions are compatible with skin physiology, remaining on the surface while allowing evaporation and perspiration (Lucas *et al*., 2022).

The effectiveness of sun protection depends on the product type, method of application, and quantity used. Studies show that sunscreens are often applied incorrectly and in insufficient amounts, reducing their protective effect. Sunscreens come in various forms such as sticks, sprays, creams, lotions, oils, tanning products, and makeup. Effectiveness is influenced by exposure conditions, SPF level, application amount, reapplication timing, product type, required layer thickness, coverage, and skin absorption. Literature suggests that the formulation vehicle is crucial for creating effective sunscreens. However, ensuring proper application by consumers remains a challenge, and there is still no consensus on the ideal sunscreen (Lucas *et al*., 2022).

2.1.4 Sunscreen Standards

According to the International Organization for Standardization (ISO) 24443 guidelines, a minimum UVA protection factor to SPF ratio of 1:3 is required for all marketed sunscreens (ISO 24443:2012). In a study evaluating 20 sunscreens against both FDA guidelines and ISO 24443 standards, 19 out of 20 sunscreens complied with the

critical wavelength (CW) requirements set by the FDA, while only 11 out of 20 met the ISO 24443 standard (Wang *et al*., 2017).

To address this discrepancy, the FDA proposed a new sunscreen regulation in 2019, which emphasized a requirement for the ratio of UVA1 (340–400 nm) to total UVA and UVB (290–400 nm) radiation to be ≥0.7. However, the FDA has yet to finalize this rule. (Wang *et al*., 2019). It is evident that further global standardization is necessary to better protect and guide consumers. It is important to emphasize that effective photoprotection includes seeking shade when outdoors and applying a broad-spectrum tinted sunscreen with a sun protection factor (SPF) of 30 or higher to exposed areas (Wang *et al*., 2019).

While alternative methods for assessing the efficacy of UVA filters have been proposed, the FDA currently relies on critical wavelength (CW) determination. According to this method, sunscreen products can be labeled as "broad spectrum" if 90% of their UV absorbance occurs at wavelengths ≥370 nm (Wang *et al*., 2017). In Europe, the International Organization for Standardization (ISO) 24443 guidelines require a minimum ratio of UVA protection factor to SPF of 1:3 for all marketed sunscreens (ISO 24443:2012). While various systems exist for calculating, testing, and labeling the SPF factor, the outcomes of these assessments often result in updates to the regulatory annexes. These annexes are essential considerations when formulating sunscreen products (Giulio *et al*., 2020). As mentioned earlier, providing comprehensive information on sunscreen product regulations within a few pages is not feasible, as each geographic region has its own specific requirements and nuances (Giulio *et al*., 2020).

2.2 The Effect of UV Rays on the Skin

Solar ultraviolet radiation (UVR) is composed of UVA (320–400 nm), UVB (280–320 nm), and UVC (100–280 nm). UVA can be further divided into UVA1 (340–400 nm) and UVA2 (320–340 nm). UVC, having the shortest wavelength, is considered the most harmful form of UVR. However, it is entirely absorbed by the ozone layer and does not reach the Earth's surface (Young *et al*., 2017). UVB constitutes the primary component of UV radiation responsible for causing sunburn or UV-induced erythema and is significantly more erythema-genic than UVA (Young *et al*., 2017).

For instance, in individuals with skin phototype I, the minimal erythema dose (MED) for UVB ranges from 20–40 mJ/cm², whereas for UVA, it is considerably higher at 20–40 J/cm². Although UVB represents only about 6% of the UVR that reaches the Earth's surface, it is notably more cytotoxic than UVA (Pei-Wen *et al*., 2019). UVB radiation is mainly absorbed by the epidermis of the skin, while UVA, due to its longer wavelength, penetrates deeper into the dermis. Despite having lower energy compared to UVB, UVA is about 20 times more prevalent in the Earth's atmosphere and can pass through glass. The ratio of UVB to UVA fluctuates with seasonal changes. Research on skin models has shown that UVA exposure induces apoptosis in dermal fibroblasts and elevates levels of MMPs, enzymes responsible for collagen breakdown (Pei-Wen *et al*., 2019).

Recent research explores the effects of UVA and UVB ultraviolet rays on the skin, highlighting significant differences between the two types. UVB (280–320 nm) and UVA (320–400 nm) reach the surface and biomass. UVB rays primarily affect the skin's surface layers (epidermis), leading to sunburns and playing a crucial role in stimulating vitamin D production. However, UVB rays pose a greater risk of skin cancer development is absorbed

by the skin and accounts for injury, inflammation (erythema), immunosuppression, photoaging, melanoma-, non-melanoma skin cancer and eye damage. UVA rays penetrate deep into the skin's dermis, contributing to premature aging signs such as wrinkles and dark spots (Kátia *et al*., 2025).

2.2.1 The Importance of Sun Protection Factor (SPF) in Evaluating Sunscreen

The widespread popularity of sunscreens today stems from a consensus in medical recommendations. However, selecting the most suitable product can be challenging for consumers. To simplify and standardize sunscreen products, criteria were established for measuring the in vivo sun protection factor (SPF) using standardized scientific protocols (Young *et al*. 2017).

The Sun Protection Factor (SPF) is a globally recognized measure that evaluates a product's effectiveness against UVB radiation, the primary cause of erythema (sunburn). Protection against UVA radiation, on the other hand, is determined through in vivo or in vitro testing (Young *et al*., 2017). The efficacy of sunscreen products has been assessed using SPF, as outlined by guidelines from COLIPA/JCIA/CFTA-SA (2006) and ISO 24444:2010, providing a quantitative method to measure the level of protection offered by sunscreens against solar radiation (Young *et al*., 2017).

The SPF value does not provide a clear indication of the level of protection against UVA1 (340–400 nm). It is derived from an in vivo test that assesses protection against sunburn or erythema, a biological response primarily caused by UVB (290–320 nm) and partially by UVA2 (320–340 nm). In August 2007, the U.S. FDA proposed renaming SPF to "Sunburn Protection Factor" to better reflect its focus on erythema; however, this

proposal was rejected. In 2011, the FDA finalized its ruling, retaining the term “Sun Protection Factor” (Young *et al*., 2017).

2.3 *Centella asiatica*

2.3.1 Classification of *Centella asiatica*

C. asiatica, commonly referred to as *Centella asiatica* or *Indian pennywort*, is a perennial herbaceous plant within the *Apiaceae* family (historically known as *Umbelliferae*, a name retained under the "Nomina Conservanda" rules of the ICBN). Its taxonomic classification is outlined as follows (Madathilparambil *et al*., 2017):

- Kingdom: *Plantae*
- Clade: *Angiosperms*, *Eudicots*, *Asterids*
- Order: *Apiales*
- Family: *Apiaceae*
- Genus: *Centella*
- Species: *Centella asiatica*

Historically, *C. asiatica* was classified under the genus Hydro-cotyle due to morphological similarities. However, detailed studies, including light microscopy of chromosomes and scanning electron microscopy of pollen and fruit morphology, have established its distinctiveness from Hydro-cotyle sibthorpoides, leading to its reclassification under the genus *C. asiatica* within the *Apiaceae* family (Madathilparambil *et al*., 2017).

2.3.2 Morphology and General Benefits of *Centella asiatica*

C. asiatica was describe as having a rounded apex leaves, deeply cordate stipulate base and petiole of height of about 20 cm. There are reports that there are up to 20 different

species of *C. asiatica* with each having their peculiar features. *C. asiatica* is a prostrate, faintly aromatic, stoloniferous, perennial, creeping runner belongs (Musavvara *et al*., 2021).

C. asiatica has been used as a medicine in India since ancient time drug for enhancing cognitive function by revitalizing the nerve and brain cells (Musavvara *et al*., 2021). The plant has also been used extensively as a traditional remedy for a wide spectrum of ailments such as leprosy, psoriasis, eczema, dermatitis, ulcerous skin, wound burn, scar management and as adaptogenic and cardiotonic agents. Most of the biological actions of *C. asiatica* have been ascribed to the pent-acyclic triterpene compounds mainly Asiatic acid, madecassic acid and triterpene saponin-asiatico-side, madecassoside (Madathilparambil *et al*., 2017).

C. asiatica is also a good source for several micronutrients, iron, phosphorus, sodium, vitamin C, vitamin A, carotene and dietary fibres. This ancient Asian herbal remedy is now becoming popular in the West (Madathilparambil *et al*., 2017). *C. asiatica* is often considered as a 'panacea' for several ailments, hence the plant species have extensively been inves-tigated by various workers for the phytochemical, pharmacological, microbiological and physiological studies. Data on many recent experi-mental studies have scientifically validated the traditional uses of the extract of *C. asiatica* (Sharma *et al*., 2022).

These findings endorse that the plant having multiple therapeutic potentials, which were amply and vividly demon-strated by different animal models such as wound-healing model in rats, streptozocin induced Alzheimeir's model, zebrafish Parkinson's model, adriamycin–induced cardiomyopathy model, nitroglycerine and bradykinin induced

hyperalgesia (migraine) model, radiation-induced dermatitis model, memory impairment induced by cerebral ischemia-reperfusion and also having anxiolytic, neuronal dendritic growth and nerve stimulating effects, suppression of scars in diabetic patients and reverse mutation assay for non-toxicity, safety and non-mutagenicity of the extract, protection of healthy cells against radiation-induced damages (Sharma *et al*., 2022).

2.3.3 Active Compounds as Sun Protecting in *Centella asiatica*

C. asiatica commonly known as mandukparni is a valuable medicinal herb of belonging to family *Apiaceae*. The plant has been used since ancient time to cure various ailments and sufferings all around the world. The curative properties of the herb have been documented in traditional system of medicine (Buranasudja *et al*., 2021). In Ayurvedic system of medicine *C. asiatica* has mentioned as one of the main herbs for rejuvenation of nerves and brain cells. As per various texts available the plant could be used for blood purification, treating high blood pressure, brain tonic for memory enhancement, succored longevity, anti-aging and antioxidant potential (Buranasudja *et al*.,2021).

C. asiatica described as an important medicinal herb used from ancient time to relieve various symptoms. activity of *C. asiatica* extracts conferred. The significant sun protection efficiency was found with the SPF value of 1.275, 1.461, 1.582 against 40 µg/mL, 50µg/mL and 60µg/mL respectively (Abhinay *et al*., 2022).

2.4 *Moringa oleifera* (*Kelor*)

2.4.1 Classification of *Moringa oleifera*

M. oleifera belongs to the genus kelor, which is the only genus in the family *Moringaceae*. However, *M. oleifera* is one of 14 species within the *Moringaceae* family, native to regions including Africa, Arabia, India, Southeast Asia, South America, and the

Pacific and Caribbean Islands. It is known locally as "Okwe Oyibo" in Igbo, "Zogale" among Hausa-speaking communities in Nigeria, and "Ewe Ile" in Yoruba (Rong *et al*., 2022).

M. oleifera is also referred to by various names in different regions and languages, including "Nuggekai" in Kannada, "Sonjna" in Marathi, "Murungai" in Tamil, "Mashinga Sanga" in Malayalam, and "Muringa" in Konkani (Olagbemide *et al*., 2019). *Moringa oleifera* is one of the 14 species belonging to the " *Moringa*ceae" family. Its taxonomic classification is as follows (Ashutosh *et al*., 2023):

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Brassicales*

Family: *Moringaceae*

Genus: *Kelor*

Species: *Oleifera*

2.4.2 Morphology and General Benefits of *Moringa oleifera.*

M. oleifera belongs to the *Moringaceae* family and has many names, such as kelor, *Centella asiatica*, kerol, marangghi, moltong, kelo, keloro, kawano, and ongge. This plant grows in both lowland and highland areas, reaching a height of 7-11 meters. The leaves of M. oleifera are oval-shaped and small, arranged in compound clusters on a single stem (Irda *et al*., 2022).

The part of the *M. oleifera* plant those functions as a natural sunscreen is the *M. oleifera* leaf. The phytochemical content in *M. oleifera* leaves is in the form of flavonoids,

which have the ability to act as sunscreens because there are chromophore groups that work by gives color to plants (Putri *et al*., 2019). *M. oleifera* is a rapidly growing, evergreen, deciduous tree that reaches a height of 10–12 meters. Its leaves are typically bipinnate or, more commonly, tripinnate, measuring up to 45 cm in length. They are alternately and spirally arranged along the twigs (Satish *et al*., 2022).

The flowers are bisexual, fragrant, and encircled by five unequal, thinly veined yellowish-white petals. The fruits are pendulous, linear, three-sided pods with nine longitudinal ridges, typically ranging from 20 to 50 cm in length, though they can occasionally grow up to 1 meter or more, with a width of 2.0 to 2.5 cm (Satish *et al*., 2022). The pods, typically containing up to 26 seeds each, are dark green during development and require approximately three months to mature after flowering. Upon reaching maturity, the pods turn brown and split open longitudinally along their three angles, releasing the dark brown, triangular seeds (Masitlha *et al*., 2024).

M. oleifera tree of average height, ranging from 15 to 20 feet, can produce hundreds to thousands of seed pods annually, resulting in a substantial yield of seeds each year. When the bark is wounded, it secretes a gum that is initially white but gradually turns reddish-brown or brownish-black upon exposure to air (Masitlha *et al*., 2024). Trees grown from seeds develop a deep and sturdy taproot accompanied by a wide-spreading system of thick, tuberous lateral roots. Propagation can also be achieved using cuttings that are at least 1 meter in length and have a minimum diameter of 4 cm. For successful growth, at least one-third of the cutting should be buried in the soil (Ashutosh *et al*., 2021).

M. oleifera is a highly nutritious and medicinal plant, widely recognized for its benefits, particularly in enhancing milk production in nursing mothers. The leaves are rich

in essential nutrients, including Vitamin C, Calcium, Vitamin A, Potassium, and Protein. Research has demonstrated that nearly every part of the plant contains various bioactive compounds with significant biological functions (Ashutosh *et al*., 2021).

Some of these compounds act as antioxidants, aiding in free radical scavenging, reducing oxidative stress, and offering potential protective effects against cancer (Ashutosh *et al*., 2021). *M. oleifera* also exhibits a wide range of medicinal properties, including antibacterial, antiviral, antifungal, anti-inflammatory, antispasmodic, and diuretic effects, among others. These benefits are attributed to its diverse chemical composition (Harshika *et al*., 2024).

The leaves are particularly notable for their high-quality protein, as they contain all essential amino acids in balanced proportions. Additionally, *M. oleifera* is abundant in carbohydrates, vitamins, minerals, fatty acids (both essential and non-essential), and phytochemicals. Numerous applications of M. oleifera have been documented, reflecting its versatility and therapeutic potential (Harshika *et al*., 2024).

M. oleifera serves a variety of purposes, including as a food source for humans and forage for animals. It also contributes to improving soil fertility, producing biogas, acting as a cleaning agent, and generating gum. Additionally, *M. oleifera* is utilized in the production of animal feed, pulp, and for water purification, among many other applications. (Harshika *et al*., 2024).

2.4.3 Active Compounds as Sun Protecting in *Moringa oleifera*

The part of the *M. oleifera* plant those functions as a natural sunscreen is the *M. oleifera* leaf. The phytochemical content in *M. oleifera* leaves is in the form of flavonoids, which have the ability to act as sunscreens because there are chromophore groups that work

by gives color to plants (Putri *et al*., 2019). Increasing the concentration of the active ingredient from *M. oleifera* leaf extract increases the SPF value (Desi *et al*., 2023). The findings indicated that *M. oleifera* extract demonstrated potential as an effective active ingredient in sunscreen formulations, offering moderate antioxidant activity (Phuong *et al*., 2024). *M. oleifera* extract has been shown to have a synergistic effect in protecting the skin against UVB radiation. This is achieved through both the absorption of UVB radiation and the supply of antioxidant agents, which work together to prevent UVB oxidative damages to the skin (Phuong *et al*., 2024). The use of natural sunscreen active ingredients is becoming increasingly popular in the cosmetic industry, in order to promote public health and protect the environment. The polyphenol-enriched extract from *M. oleifera* leaves shows promise as a potential candidate for this purpose (Phuong *et al*., 2024).

2.5 The Potential Use of *Centella asiatica* and *Moringa oleifera* in Topical Formulations

Asiatic-o-side in *Centella asiatica* and β-sitosterol in *Moringa oleifera* exhibit antioxidant properties, making them potential active ingredients in anti-aging formulations. Moreover, Mo *M. oleifera* ringa plants, particularly their leaves, contain high antioxidants, including crucial phenolic bioactive compounds like flavonoids (quercetin, kaempferol, isorhamnetin, and apigenin). Research has indicated that *M. oleifera* leaves contain β sitosterol at 90 mg/g, total phenolics at 8 µg/mL, flavonoids at 27 µg/mL, and high antioxidant activity (69.72±1.15%) of the ethanol extract. The *C. asiatica* herb extract and *M. oleifera* leaf extract has antioxidant activity. Combining the two extracts has the potential to increase their antioxidant activity. Studies have shown that combining extracts increases antioxidant activity compared to each extract alone (Suena *et al*., 2024).

In a study conducted by Muchtaromah *et al*., the study highlights the potential of combining *C. asiatica* and *M. oleifera* extracts in a stable, effective herbal for anti-inflammatory (Muchtaromah *et al*., 2024).

2.6. Nanotechnology in Cosmetics

Nanotechnology has advanced significantly in the skincare industry, providing innovative delivery systems for active ingredients in topical formulations. Nano-cream serve as carriers that facilitate the delivery of these ingredients, improving their skin penetration and potentially enhancing their effectiveness (Aziz *et al*., 2019). The application of nanotechnology in skincare has been extensively explored in numerous studies, highlighting its potential to improve the performance of cosmetic products. By enabling the formulation of smaller nano-cream, nanotechnology facilitates better absorption of active ingredients into the skin, aiding in damage repair and enhancing overall product efficacy. A range of nanocarriers, including liposomes, noisomes, solid lipid nano-cream, nano-capsules, ratslles, dendrimers, and metal nano-cream, have been utilized to elevate the quality and effectiveness of cosmetic formulations (Cardoza *et al*., 2022).

The effectiveness of nanotechnology-based cosmetic products is influenced by factors such as molecular size, lipophilicity, degree of ionization, and the barrier properties of the skin. Formulations utilizing lipid nano-cream are particularly promising, as they possess the ability to effectively penetrate the skin, thereby enhancing product efficacy (Manikanika *et al*., 2021). Nanotechnology has become a valuable asset in the skincare industry, providing advanced delivery systems for active ingredients and enhancing the overall effectiveness of cosmetic products (Tsvetelina, 2024). Nanotechnology has been

increasingly investigated and applied in the cosmetic industry to enhance the efficacy and performance of products. The integration of nanomaterials into cosmetics offers several advantages, including improved product performance, longer retention, enhanced appearance, and increased safety of formulations. These benefits align with consumer demands for higher-quality and more effective cosmetic products (Tsvetelina, 2024).

Nano-cream is utilized in cosmetics to enhance the delivery of active ingredients, improve product texture and appearance, and function as UV filters, showcasing the wide-ranging applications of nanotechnology in cosmetic formulations (Tsvetelina, 2024). Nanotechnology is nothing but the fundamental study about how materials or particles react or works at the nanoscale in the development and use of structures, devices, and systems having unique characteristics and purposes. Nano-formulations have a definite place in the delivery of the active compounds to and through the skin for a range of therapeutic purposes. These are elegant, relatively simple and inexpensive to make and offer significant delivery advantages over coarse emulsions (Vaishali *et al*., 2020).

Nanotechnology facilitates the penetration of active ingredients through skin layers due to its small droplet size. The nanoparticle size range of 20-500 nm offers a significant advantage in nano cream formulations, as it enhances the absorption of active substances into the skin (Ika *et al*. 2021).

2.7 Sun Protecting Factor (SPF)

The concept of topical photoprotective products dates back to ancient Egyptian times around 4000 BC; however, the first commercial sunscreens did not emerge until the 1920s–1930s (Ma *et al*., 2021). During that period, knowledge of UV radiation was limited, with primary focus placed on UVB protection. As sunscreen usage grew in popularity over

the years, the need for standardizing photoprotection against UVB radiation was recognized and implemented (Ma *et al*. 2021).

The FDA recognized SPF as the standard for measuring sun protection in 1978. UV-induced erythema is primarily caused by UVB radiation, with a smaller contribution from UVA2. For many decades, SPF—based on UV-induced erythema as the endpoint—remained the sole metric for evaluating sun protection. This persisted despite advances in UV radiation research indicating that UVA also plays a significant role in photoaging (Ma *et al*. 2021, Lim *et al*. 2019).

In 1992, The Boots Company in the UK introduced the UVA star rating system; however, it was not widely adopted. While alternative methods for assessing the efficacy of UVA filters have been proposed, the FDA currently relies on critical wavelength (CW) determination as the standard measure (Wang *et al*. 2017) Using this method, sunscreen products can be labeled as "broad spectrum" if 90% of their UV absorbance occurs at wavelengths ≥370 nm (Wang *et al*., 2017). In Europe, the International Organization for Standardization (ISO) 24443 guidelines require a minimum ratio of UVA protection factor to SPF of 1:3 for all marketed sunscreens (ISO 24443:2012).

2.8 Nano-cream

Nano-cream is one of several delivery system technology innovations in cosmetic products. Nano-cream is a semisolid preparation, which is a stable emulsion and has a diameter of about 20-500 nm. Nano-cream preparations are easier to use and spread over the skin area easily and comfortably. Another advantage of nano-cream as topical preparations is that they increase the absorption of active substances in the skin. So that,

people prefer cosmetic products in cream dosage forms rather than other cosmetic dosage forms (Sumaiyah *et al*. 2021).

Nano-cream or nano-emulsion is a topical pharmaceutical preparation that is applied to the skin topically. Nano-cream is a drug delivery system consisting of an oil and an aqueous phase combined by a surfactant and cosurfactant. Nano-cream has a particle size with a scale of 100-600 nm (Ika *et al*., 2021). The small nanoparticle size of 20-500 nm is an advantage of the nano cream dosage form because it can increase the absorption of active substances in the skin. Nano-cream can also contain many components of the active substance so that it becomes an option in cosmetic formulations (Ika *et al*., 2021).

Nano-cream is one of several delivery system technology innovations in cosmetic products. Nano-cream is a semisolid preparation, which is a stable emulsion. Nano-cream preparations are easier to use and spread over the skin area easily and comfortably. Another advantage of nano-cream as topical preparations is that they increase the absorption of active substances in the skin. So that, people prefer cosmetic products in cream dosage forms rather than other cosmetic dosage forms (Sumaiyah *et al*., 2021).

Nano-cream formulations can incorporate various active ingredients, making them a versatile choice for cosmetic applications. These formulations are designed to meet the essential physical properties of nano-cream, such as pH, nano-cream type, dispersion, adhesion, and percentage transmittance (Rahman *et al*. 2021).

The term "nanotechnology" describes the development and utilization of materials that exhibit unique physicochemical properties at the nanoscale, differing from those of their bulk counterparts. These innovative materials undergo internal structural

reorganizations, resulting in an increased surface area and modified interactions with biological systems (Saurav *et al*. 2023).

The development of nanoproducts has enabled the incorporation of active pharmaceutical ingredients (APIs) into nano-sized drug delivery systems, fostering innovation in product design. Several nanoproducts are already available for treating skin conditions, including atopic dermatitis, skin cancer, burns, wound healing, and UV protection. These nanotechnology-based solutions, such as nano-pharmaceuticals and nano-cosmeceuticals, have demonstrated significant effectiveness in delivering APIs to the skin (Saurav *et al*. 2023).

2.9. The Effect of Rotational Speed of Homogenization

The process of mixing (homogenization) is undertaken and required in many food, cosmetic, pharmaceutical, and other industries. Developments in the research into emulsion continue to grow as emulsion offers benefits, among other things is in the pharmaceutical industry in which emulsion formation can reduce odor and unpleasant taste of oil (Zahra *et al*., 2021).

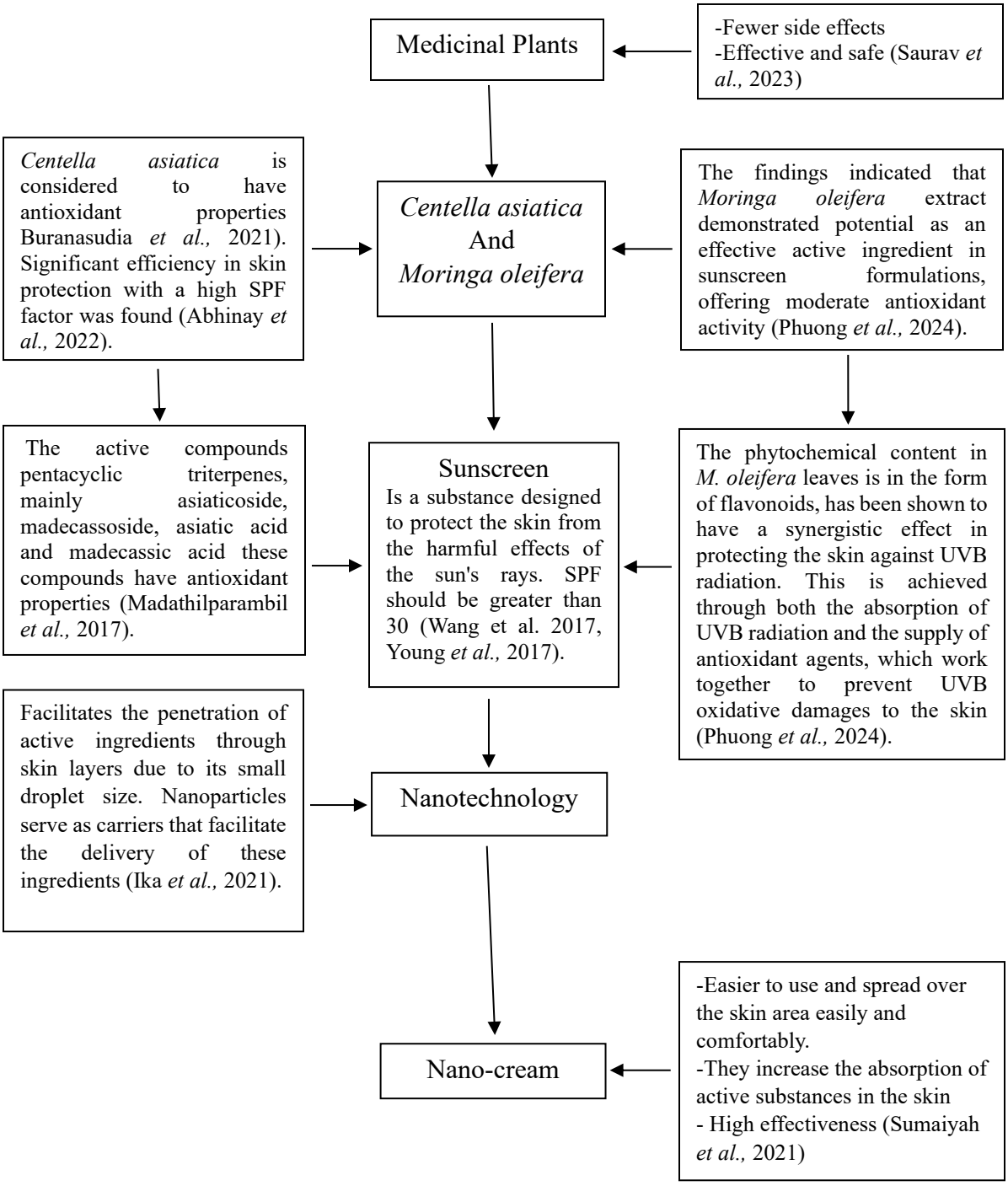
Homogenization is the process of converting two fluids that are immiscible (cannot be mixed) into emulsion. Homogenization in mixing, emulsification, and suspension technologies is known as an operation which essentially consists of two stages: first, droplet size reduction in the inner part phase and second, the simultaneous distributing of droplets into the continuous phase. factors that influence the droplet size generated by homogenization include the type of emulsion used, the temperature, characteristics of the phase components, and the input energy. The intensity and duration of the mixing process

depends on the time it takes to dissolve and distribute the materials to be mixed evenly (Zahra *et al*., 2021).

The effect of speed and length of time at the time of homogenization process increases the homogenization that plays a role in the formation of emulsions. The higher the stirring speed and the longer the stirring time, the density and viscosity values will decrease. However, the increased stirring speed and the longer the stirring time will prolong the separation time of the oil emulsion in water. density and viscosity increase as solid concentrations increase (Zahra *et al*., 2021).

The results of a study conducted by Zahra showed that the greater the stirring speed and time, the smaller the density and viscosity value produced and the greater the stability value obtained. The greater the concentration of solids, the greater the density, viscosity, and stability of the emulsion (Zahra *et al*., 2022).

2.10 Conceptual Framework



2.1 Figure: Conceptual framework of the research

From Figure 2.1 regarding the conceptual framework, it can be summarized that illustrates, the figure illustrates the role of medicinal plants, such as *C. siatica* and *M. oleifera*, in enhancing the effectiveness of sunscreens through the use of nanotechnology. These plants contain active compounds, such as madecassoside and Asiatic acid, which possess antioxidant properties that help protect the skin from the harmful effects of ultraviolet rays (Abhinay *et al*., 2022, Phuong *et al*., 2024). Studies have shown that *M. oleifera* extract contributes to improving sunscreen efficacy due to its synergistic phytochemical content, which enhances skin protection (Phuong *et al*., 2024). Studies have shown that *C. asiatica* the active compounds pentacyclic triterpenes, mainly asiaticoside, madecassoside, Asiatic acid and madecassic acid these compounds have antioxidant properties (Madathilparambil *et al*., 2017). Nanotechnology is applied in the production of these products (Ika *et al*., 2021), utilizing nanotechnology as an efficient means to deliver active ingredients to the skin, increasing their effectiveness and ease of use. Nano-cream easier to use and spread over the skin area easily and comfortably. They increase the absorption of active substances in the skin, high effectiveness (Sumaiyah *et al*., 2021). Consequently, this technology enables the development of innovative and effective skincare products with enhanced sun protection (Ika *et al*., 2021).

CHAPTER III

RESEARCH METHODS

3.1 Types and Research Design

The study titled " Organoleptic and Sun Protecting Factor (SPF) Test Of Nano-cream Preparation with Active Ingredients of *Centella asiatica* and *Moringa oleifera* in Vivo " is experimental in nature and utilizes a Completely Randomized Design (CRD) with three main groups, each consisting of five replications. The groups are as follows: (1) the normal control group, which includes rats exposed to UVB radiation without treatment using the nano-cream —serving as a baseline to assess the effects of UVB exposure; (2) the positive control group, in which rats are exposed to UVB radiation and treated with a commercially available sunscreen product—used as a benchmark for evaluating the efficacy of the test cream; and (3) the treatment groups, comprising rats exposed to UVB radiation and treated with the formulated nano-cream —used to assess the protective effects of the cream under different homogenization durations.

The study is conducted in four main stages:

1. Extraction of PEF-MUAE from a combined mixture of *Centella asiatica* and *Moringa oleifera* leaves.
2. Formulation of the Nano-cream using the extracted active compounds.
3. Evaluation of the cream's physical characteristics, including organoleptic assessment and skin irritation testing.
4. In vivo testing of the cream's sun protection effectiveness by measuring erythema levels in treated animal models.

3.2 Time and Place of Research

This study was conducted from October 2024 to March 2025. The extraction of PEF was performed in the Agricultural Technology Laboratory at Universitas Brawijaya. The MUAE process took place in the Thermodynamics Laboratory and the Materials Laboratory of the Physics Study Program. The preparation, physical characterization tests, and effectiveness assessments of the nano-cream were carried out in the Animal Physiology Laboratory of the Biology Study Program at the Faculty of Science and Technology, State Islamic University of Malang.

3.3 Tools and Materials

3.3.1 Tools

The tools utilized in this research: 100 ml Graduated Cylinder (IWAKI), 500 ml Beaker (Pyrex®), 250 ml Beaker (Pyrex®), 250 Mesh Filter (Gilson), Spatula (Fisherbrand), Volumetric Pipette (IWAKI®), Analytical Balance (Sartorius), Microwave (Samsung), Ultrasonic Cleaner (Baku BK-2000), Freeze Dryer (Christ), Hot Plate (Thermolyne Cimarec), Mortar and Pestle, Stirring Rod (Fisherbrand), Micropipette (Select BioProduct), Homogenizer (IKA T25 Digital), Cage, UVB Lamp (Exottera Daylight Basking Spot), Ruler.

3.3.2 Materials

The materials used include simplicia of *C. asiatica* and *M. oleifera* leaves, Stearic Acid, Olive Oil, Tween 80, Propylene Glycol, Potassium Sorbate, Sodium Benzoate, Distilled Water (Aquades), Transparent plastic and adhesive tape, Experimental rats (for in vivo studies for Irritation test and Evaluation of nano-cream in Experimental Animals).

3.4 Research Procedure

3.4.1 Preparation of Simplicia from *Centella asiatica* and *Moringa oleifera*

The simplicia of *C. asiatica* leaves was sourced from the Center for Research and Development of Medicinal Plants and Traditional Medicines in Tawangmangu, while the simplicial of *M. oleifera* leaves was obtained from the Herbal Laboratory at UPT Materia Medica in Batu, and the rats were obtained from Iwan farm, from pakishaji.

3.4.2 Preparation of Extract

The simplicia of *C. asiatica* and *M. oleifera* leaves were treated with PEF at a frequency of 2,000 Hz and a voltage of 2,000 volts for 3 seconds (Wahyuni *et al*., 2022). This was followed by extraction using a microwave, in which 475 grams of simplicial from *C. asiatica* and *M. oleifera* (in a 1:1 ratio) were dissolved in 19,000 mL of distilled water (1:40 g/mL) at a power of 300 watts for 4 minutes (Zhang *et al*., 2023; Tengku *et al*., 2019).

The extraction continued with an ultrasonic cleaner operating at a frequency of 40 kHz and a power of 120 watts for 9 minutes and 30 seconds. The results of the MUAE extraction were then freeze-dried to obtain the extract in powder form, which was subsequently filtered using a 250-mesh sieve (Harsha *et al*., 2017).

3.4.3 Formulation Design of Nano-cream Preparation

Table 3.1 Formulation Design of Nano-cream Preparation

Composition	Function	Concentration (%)
Combination Extract	Active Ingredients	10
Stearic Acid	Solid Lipid	10
Olive Oil	Liquid Lipid	9
Tween 80	Surfactant	10
Propylene Glycol	Humectant	0,9
Potassium Sorbate	Preservative	0,06
Sodium Benzoate	Preservative	0,15
Aquades	Aqueous Phase	Ad 50

3.3.4 Preparation of Nano-cream Formulation

The Nano-cream formula with active ingredients from *M. oleifera* leaves and *C. asiatica* refers to the research conducted by Sumaiyah and Suntorn, Oana, which has been modified. The cream preparation was made using the oil-in-water (o/w) method, totaling 50 g with three replications for each formulation. The oil phase includes stearic acid and olive oil, heated with a hot plate at 70˚C (Suntorn *et al*., 2021, Oana *et al*., 2022).

Similarly, the water phase, consisting of Tween 80, potassium sorbate, sodium benzoate, propylene glycol, and distilled water, is heated on a hot plate at the same temperature. The oil phase is then gradually added to the water phase while stirring until a homogeneous cream mass is achieved. The extract is subsequently incorporated and mixed using a magnetic stirrer. Finally, the mixture of the extract and the cream base is

homogenized using a homogenizer with three different homogenization time variations (Table 3.2).

Table 3.2 Variations of Homogenization Time

Formula	Speed (rpm)	Time (min)
A	15.000	45
B	15.000	70
C	15.000	95
D	Without homogenizer treatment	

3.4.5 Physical Characterization Test of Nanoparticle Cream

The physical characterization test of nano-cream is conducted on each replication, including organoleptic tests, Irritation test.

1. Organoleptic Test

Organoleptic evaluation includes assessing the shape, color, and odor of the cream formulation through visual and sensory analysis with a panel of 20 volunteer (10 males and 10 females) (Elsha *et al*., 2023).

2. Irritation test

This test was conducted to evaluate the effect of the nanoparticle sunscreen on the skin of rats. Twenty rats were prepared by shaving the fur in a 3 × 3 cm area on their backs, which was then divided into two equal sections: one designated as the test area and the other as the control area (Yang *et al*., 2024).

After 24 hours, 0.25 grams of the nanoparticle sunscreen was applied to the test area. The area was covered with a transparent plastic layer, secured with adhesive tape, and left for

24 hours. Subsequently, the adhesive tape and plastic were removed, and the area was rinsed with water. Observations were conducted periodically to assess skin condition and irritation at specific time points: 0, 1, 24, 48, and 72 hours after cream removal (Pallavi *et al*., 2015, Yi Yang *et al*., 2024). Observations were conducted to assess the occurrence of erythema and edema in the test animals (Nanda *et al*., 2023). The evaluation of irritation involves assigning scores to each tested area, ranging from 0 to 4, as specified in Table 3 (Helena, 2023).

Table 3.3 Erythema and Edema Score

Value	Erythema Formation	Edema Formation
0	No Erythema	No Edema (no swelling)
1	Very Slight Erythema (barely perceptible), edges of area not well defined	Very Slight Edema (mild swelling)
2	Slight erythema (pale red in color and edges definable)	Slight Edema
3	Moderate to severe erythema (defined in color and area well defined)	Moderate to severe Edema (severe swelling)
4	Severe erythema (beet to crimson red) to slight eschar formation (injuries in depth)	Severe edema (severe swelling)

The Primary Dermal Irritation Index (PDII) is determined using the following equation (Yuda *et al*., 2023):

$$PDII = \frac{\sum \text{Erythema Score} + \sum Edema\ Score}{\sum Total\ Rats + \sum Observation\ Time}$$

The calculated PDII value is then compared to the standards provided in ISO10993, as indicated in Table 3. This comparison is essential to draw conclusions regarding the results of the testing. The ISO 10993 standard serves as a reference point to determine whether the level of irritation caused by the tested formulations falls within acceptable limits for safety and human use (Helena, 2023; Angga *et al*., 2023; Yuda *et al*., 2023).

Table 3.4 Classification of Irritation Response (ISO 10993)

Average PDII	Irritation Classification.
0-0,4	No significant irritation observed.
0,5-1,9	Mild irritation with minimal impact.
2,0-4,9	Moderate irritation with noticeable effects.
5,0-8,0	Severe irritation with significant impact.

3.4.6 In Vivo Effectiveness Testing of Optimized Nano-cream Formulation on Animal Models

The effectiveness test of nano-cream is conducted in vivo using rats as test animals on the nano-cream formulation with optimal physical characterization results. Thirty rats obtained from the Physiology Laboratory were acclimatized for 15 days before starting the treatment. The treatment received approval from the ethics committee at Maulana Malik Ibrahim State Islamic University Malang in accordance with the guidelines established by research ethics commission Faculty of Science and Technology UIN Maulana Malik Ibrahim Malang; nomor 48/EC/KEP-FST/2025.

3.4.6.1 Treatment Groups

The effectiveness testing of the sun protecting nano-cream formulation was conducted using UVB exposure on the backs of the rats. The rats were allocated into three groups as follows:

1. Normal control group, consisting of rats exposed to UVB but not treated with the nano-cream.
2. Positive control group (+), consisting of rats exposed to UVB and treated with a commercial Sun protecting product.
3. Treatment group, consisted of rats exposed to UVB radiation and treated with the Nano-cream formulation that demonstrated optimal physical test results.

3.5 In Vivo UVB Exposure Testing of Nanoparticle Sunscreen Cream on Rats Skin

3.5.1 In Vivo Sunscreen Testing

The in vivo testing of the nano-creams sunscreen effectiveness was performed on rats exposed to UVB radiation. UVB radiation was selected for this study due to its potent ability to induce immediate and severe sunburn by primarily affecting the epidermal layer. It is also approximately 1,000 times more effective than UVA radiation in causing sunburn and is more genotoxic, making it a primary focus in sunscreen research (Liyan *et al*., 2022).

3.5.2. Animal Model and Preparation

Male rats as the test subjects because they are easier to handle, and provide reliable research results. Weighing 200 grams, as male rats are generally more manageable and exhibit greater physiological stability, unaffected by estrous cycles and pregnancy, which can influence experimental outcomes. The animals were allowed a 15-day acclimatization

period prior to the experiment. One day before the experimental procedure, the backs of all rats were saved using a razor blade, exposing an area of approximately 3×4 cm of skin to ensure uniform exposure to UVB radiation (Dian *et al*., 2020; Heru *et al*., 2020).

3.5.3 Application of Nano-cream

Approximately 1 gram of the nanoparticle sunscreen cream was applied to the shaved skin area and left for 1 hour. After this period, the rats were subjected to UVB exposure using a UVB lamp positioned 30 cm above the skin (Melati *et al*., 2020). The exposure continued for 24 hours. Following the UVB exposure, the erythema (redness) was assessed by measuring the diameter and degree of redness (Aprilita *et al*., 2018; Heru *et al*., 2020).

3.5.4 Experimental Groups

The rats were randomly divided into six experimental groups, with five rats per group:

1. Normal Control Group: Exposed to UVB radiation without treatment.
2. Positive Control Group: Exposed to UVB radiation and treated with a commercial sunscreen (Sun Battle).
3. Treatment A: Exposed to UVB radiation and treated with a nano-cream homogenized for 45 minutes.
4. Treatment B: Exposed to UVB radiation and treated with a nano-cream homogenized for 70 minutes.
5. Treatment C: Exposed to UVB radiation and treated with a nano-cream homogenized for 95 minutes.

6. Negative Control (Treatment D): Exposed to UVB radiation and treated with a nano-cream without homogenization.

3.5.5 Outcome Measures

The primary outcome measures included the assessment of erythema (redness) in the skin, quantified by measuring the diameter and degree of redness following UVB exposure. This data was used to evaluate the protective effects of the nano-cream formulations.

3.5.6 Erythema Area Calculation

The area of erythema is measured using a ruler. The erythema scoring system used is as follows (Table 3.5) (Melati *et al*., 2020; Aprilita *et al*., 2018):

Table 3.5 The erythema scoring system (Melati *et al*., 2020**)**

00	Indicates no erythema
01	Indicates very slight erythema with a diameter ≥ 25 mm
02	Indicates clearly defined erythema with a diameter of 25.10 – 30.00 mm
03	Indicates moderate to severe erythema with a diameter between 30.10 – 35.00 mm
04	Indicates erythema forming crusts and bright red with a diameter ≥ 35.10 mm

3.6 Data Analysis

The results of the optimal formulation can be observed from the outcomes that meet the standards in physical character tests and the effectiveness evaluation of the sun-protecting nano-cream. For qualitative tests, which can be described descriptively, such as organoleptic tests and irritation tests, the data are presented in the form of tables and figures (Elsha *et al*., 2023). Data SPF obtained from in-vivo assays were analyzed using Kruskal-

Wallis Test (nonparametric test), followed by Mann-Whitney Test test determine significant differences ($p < 0.05$) between treatment groups using SPSS software version 26 (Melati *et al*., 2020; Helena, 2023).

CHAPTER IV

RESULTS AND DISCUSSION

5.1 Physical Characterization Results of the Nano-cream

5.1.1 Organoleptic Test

A sensory assessment of the cream (Figure 5.1) was conducted using 20 participants aged between 17 and 29 years. The evaluation was carried out through a questionnaire to gather direct feedback. The questionnaire included four questions related to the physical attributes of the cream, as outlined in Table (5.1), covering color, scent, appearance, and texture (Figure 5.1) (Elsha *et al*., 2023), each with four response options.

The results of the sensory test, based on feedback from 20 respondents, showed that the color of formulations A and D was green, while formulations B and C had a darker green shade. Among them, formulation B was the most preferred in terms of color. Regarding scent, formulations A, C, and D had a mild fragrance, whereas formulation B was found to be the most favored scent. In terms of texture, formulations B, C, and D were rated as very smooth, while formulation A was described as simply smooth.

Formulation B emerged as the most preferred in terms of texture. As for the overall appearance, formulation D had the most preferred cream consistency, while formulations A, B, and C were rated as having a good appearance. That is differences in homogenization duration influenced the physical characteristics of the cream—such as color, scent, and texture—which affected participants' sensory perceptions and preferences.

Figure 5.1 Nano-cream

Table 5.1 Results of organoleptic observation of the cream formulations

Formula	Color	Fragrance	Texture	Appearance
A	Green	Mild Fragrance	Smooth	Good
B	Dark Green	No Fragrance	Very Smooth	Good
C	Dark Green	Mild Fragrance	Very Smooth	Good
D	Green	Mild Fragrance	Very Smooth	Excellent

5.1.2 Irritation Test

Skin sensitivity test results in rats (Figure 5.2) showed no significant allergic reactions to creams containing *M. oleifera* and *C. asiatica* extracts at different homogenization times. One hour after application, mild redness was observed in some rats (rats 3 and 4 in formulation A, rat 1 in formulation B, rat 5 in formulation C, and rat 3 in formulation D). However, these symptoms completely disappeared after 24 hours, and no redness or swelling was recorded during the 72-hour observation period. Based on the irritation test results (Table 5.3), no redness or edema reactions were observed on the skin of the test animals for any of the formulations. The primary irritation index (PDII) was zero

for all formulations, confirming their safety and lack of skin irritation according to ISO 10993 standards. In other words, differences in homogenization time did not affect the skin irritation responses in the animal models.

Table 5.3 Irritation Test Results

Time / Formula	After 1 hour	After 24 hours	After 48 hours	After 72 hours
A	No irritation	No irritation	No irritation	No irritation
B	No irritation	No irritation	No irritation	No irritation
C	No irritation	No irritation	No irritation	No irritation
D	No irritation	No irritation	No irritation	No irritation

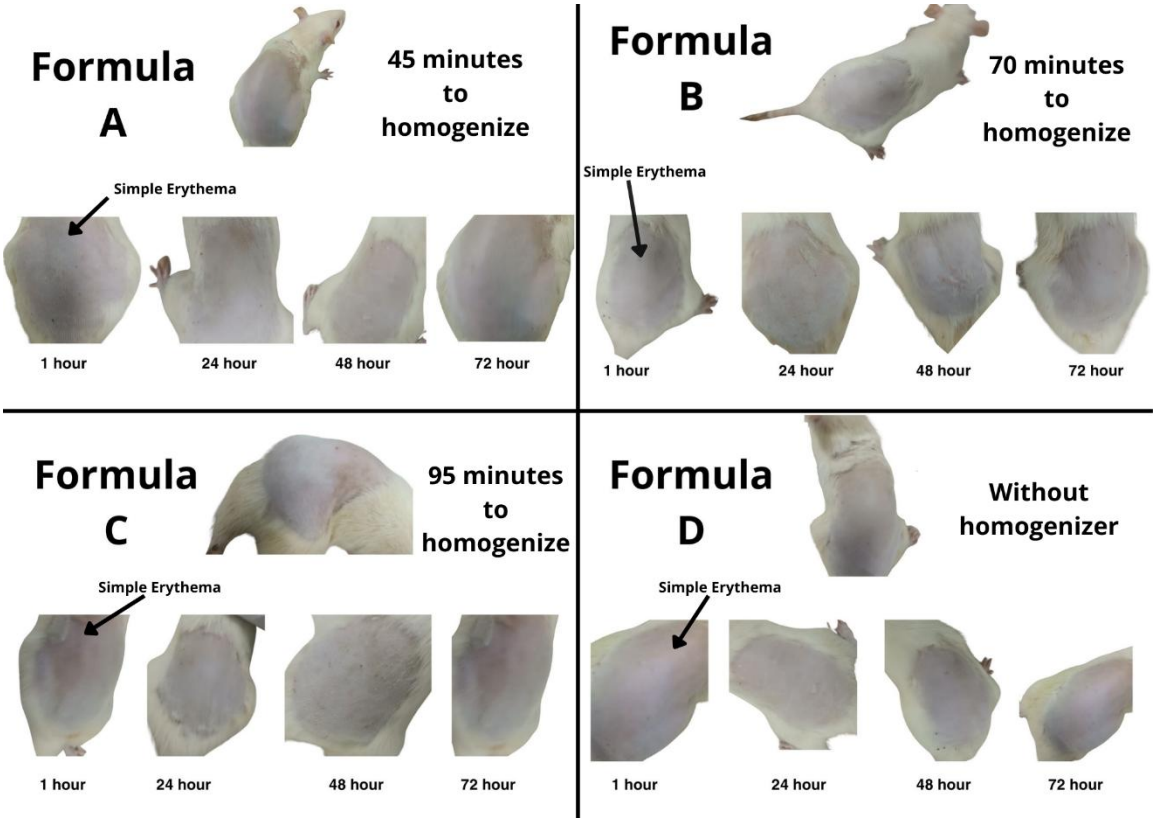


Figure 5.2 Visual Irritation test results

The figure 5.2 illustrates the visual outcomes of the irritation test conducted on animal models following the topical application of the nano-cream formulations. The groups treated with the nano-cream s, demonstrated little to no visible irritation. These observations support the quantitative findings and confirm the creams' safety for topical use, as no adverse dermal reactions were recorded. The results visually reinforce the conclusion that the formulations are well-tolerated and non-irritating.

5.2 Evaluation of Erythema in Experimental Animals Exposed to UVB

The test was conducted by observing erythema (Figure 5.3) on experimental animals exposed to ultraviolet (UVB) radiation. Erythema severity was recorded on a scale from 0 to 4 (Table 3.5). Erythema was observed across all groups, with varying diameters (Figure 5.3).

The erythema evaluation revealed distinct patterns across treatment groups. In the normal control group, severe reactions were observed, with rat 1 scoring 4 (crusting erythema ≥35.10mm), rats 2 and 5 scoring 3 (moderate-severe erythema 30.10-35.00mm), rat 3 scoring 2 (defined erythema 25.10-30.00mm), and rat 4 showing slight erythema (score 1, ≥25mm). The positive control group exhibited mixed responses: rat 4 scored 4, rat 5 scored 3, rats 2-3 scored 2, and rat 1 showed slight erythema (score 1).

Notably, in the treatment group, Formula B showed no visible signs of erythema in all rats, with each subject scoring 0, indicating complete photoprotective effectiveness, while Formula A and C showed consistent mild protection (all rats scored 1, very slight erythema ≥25mm). Formula D approached Formula B efficacy, with 4 rats scoring 1 and one rat (Rat 1) scoring 0. These results suggest that Formulas B and D offered superior UVB protection, with Formula B achieving 100% prevention of erythema formation.

Nano-creams containing active ingredients of *C. asiatica* and *M. oleifera* with different homogenization duration showed significant efficacy in protecting the skin of rats from UVB exposure. Statistical analysis revealed a highly significant difference ($p < 0.05$) between all treatment groups. The data obtained were analyzed using Kruskal-Wallis Test to compare erythema diameters, yielding a p-value of 0.000 ($p < 0.05$), indicating significant differences among treatment groups.

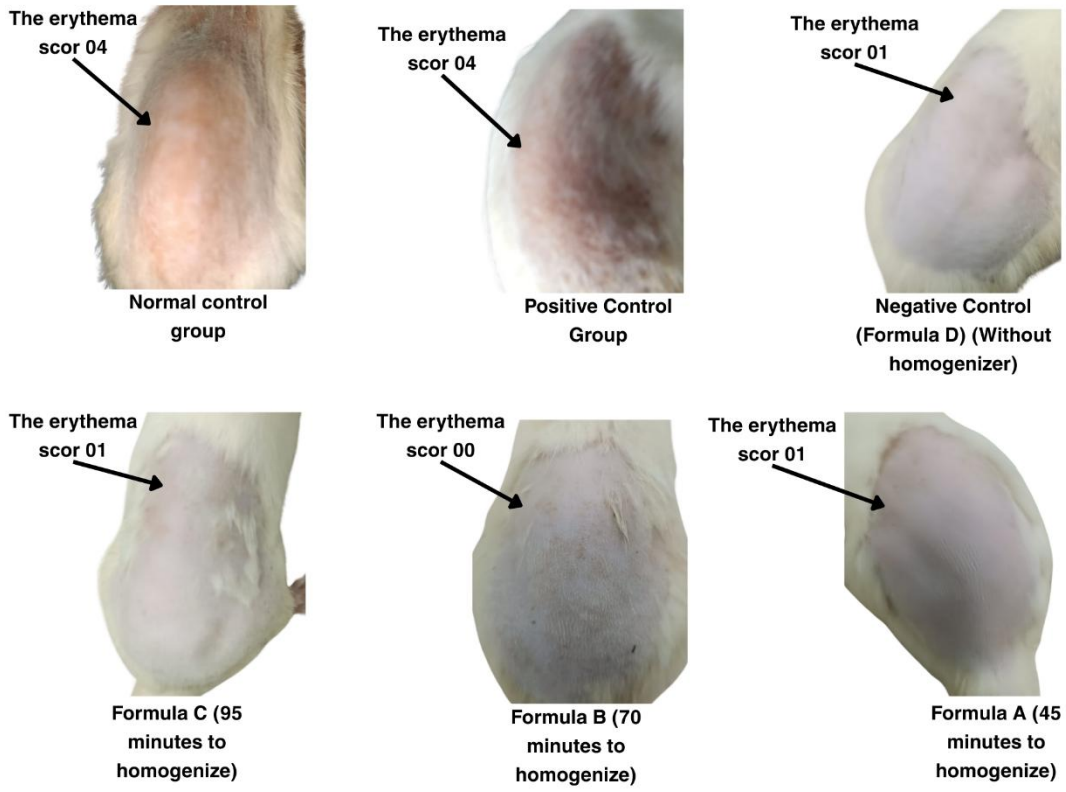
The Kruskal-Wallis test (Mean Rank) showed that the most protected group was Formula B (70 minutes of homogenization). To further identify the groups that showed the most significant differences, a comparison was performed between Formula B and the other groups (Normal Control Group; Positive Control Group; Formula A; Formula C; Formula D). A post-hoc James-Howell test revealed a significant difference in Formula B compared to all other groups (Normal Control Group; Positive Control Group; Formula A; Formula C and Formula D), with probability values less than 0.05. This indicates that Formula B showed a statistically significant difference in Sun Protection Factor (SPF) compared to all other treatments, confirming its superior behavior in the experimental setting. This indicates that the treatment application effectively inhibited redness in the experimental animals. The highest redness inhibition was observed in Formula B (70 min of homogenization), while the negative control group showed minimal redness inhibition, similar to the positive controls. This means that varying homogenization durations had a significant impact on the Sun Protection Factor (SPF) values recorded in the animal skin models.

Although Formula B exhibits the largest particle size (392.99 nm) compared to Formula A (66,92 nm), Formula C (32,89), this property enhances its effectiveness as a

sunscreen. According to studies conducted by Indriarini et al. and by Venny and Sri, the optimal particle size range for sunscreen formulations was found to be between 261.75 nm and 404.53 nm (Venny and Sri,2020; Indriarini *et al*., 2021).

As noted by Dhea *et al*. (2022), larger particles tend to increase both the absorption and scattering of UV radiation. Furthermore, the relatively large particle size in Formula B may limit dermal penetration, promoting surface-level protection—a desirable attribute for topical sunscreen formulations designed to remain on the skin's surface and serve as a physical barrier against UVB radiation (Dhea *et al*., 2022).

Figure 5.3: Erythema in Experimental Animals Exposed to UVB



5.3 Islamic Perspective on Research Results

Human societies have been closely connected to their environments since the beginning of their formation, using its components to obtain food and medicine. Medicinal plants have been used as a medical resource in almost all cultures (Fatemeh *et al*., 2018). This is highlighted by Allah in Surah Abasa, ayat 31-32:

قال الله تعالى: ﴿ وَفَاكِهَةً وَأَبًّا (31) مَّتَاعًا لَّكُمْ وَلِأَنْعَامِكُمْ﴾ سورة عبس

He mentioned fruits and plants as a source of food and medicine for both humans and animals, allowing their use for healing and consumption.

According to the results of this study, which confirmed that the cream made from *C. asiatica* and *M. oleifera* is safe in terms of skin sensitivity (Table 5.3) and effective in protecting the skin from UVB (Figure 5.3), this is attributed to the active ingredients of the two plants. Allah mentioned this in the Qur'an in Surah An-Nahl ayat 69:

قال الله تعالى: ﴿ثُمَّ كُلِي مِنْ كُلِّ الثَّمَرَاتِ فَاسْلُكِي سُبُلَ رَبِّ ذُلُلًا يَخْرُجُ مِنْ بُطُونِهَا شَرَابٌ مُخْتَلِفٌ أَلْوَانُهُ فِيهِ شِفَاءٌ لِلنَّاسِ﴾ سورة النحل

The Ayat suggests that honey, which serves as a remedy for diseases (شفاء), is derived from plants. This emphasizes the therapeutic benefits of medicinal plants and their potential in treating and enhancing human health (شفاء للناس).

C. asiatica and *M. oleifera* are plants known for their diverse properties in both traditional and modern medicine. One of the components in *M. oleifera* and *Centella asiatica* is its active compounds, such as flavonoids and phenolic compounds, which have antioxidant and anti-inflammatory properties, as well as the ability to act as a natural sunblock (SPF), offering protection against UV radiation. The combination of Qur'anic Ayat and the Sun Protection Factor found in *M. oleifera* and *Centella asiatica* extract

reflects the wonders of Allah's creation, manifested through the variety of plants that grow on Earth. These plants, with their therapeutic properties, including UV protection, demonstrate the greatness of Allah in creating everything beneficial for humanity.

قال الله تعالى: ﴿فكلوا مما رزقكم الله حلالا طيبا واشكروا نعمة الله إن كنتم إياه تعبدون﴾ سورة النحل

The word "فكلوا" in this verse is interpreted as "طيبا", encompassing food, drink, and so on. A person enjoys whatever is available that is good and lawful. The Quran's instructions regarding goodness are in line with the upright and balanced human nature. The meaning of the word "حلال" is that something is lawful and the method of obtaining it is lawful. The word "اشكروا" refers to its benefits to the body, in terms of its nutritional content, vitamins, proteins, and other elements. This research represents a development in halal extraction using *Centella asiatica* and *Moringa leaves* with the addition of halal ingredients (Tafsir al-Tabari).

CHAPTER V

CONCLUSION

5.1 Conclusion

Based on the results of the research that has been conducted, it can be concluded that:

1. Differences in homogenization duration influenced the physical characteristics of the cream—such as color, scent, and texture—which affected participants' sensory perceptions and preferences.
2. Differences in homogenization duration did not result in skin irritation responses in animal models.
3. The duration of homogenization exhibited a notable impact on the Sun Protection Factor (SPF) values recorded in animal skin models, and the best homogenization duration was 70 minutes (Formula B).

5.2 Suggestions

Based on the research results, the following suggestions can be used to improve further research:

1. It is essential to evaluate the sun protection level of *Centella asiatica* extract at different homogenization durations to determine the optimal time for effective UV protection.
2. Further studies are needed on the active compounds of *Moringa oleifera* and *Centella asiatica* to assess their effectiveness as anti-inflammatory and antioxidant agents.
3. Additional research should be conducted on *Moringa oleifera* and *Centella asiatica* to explore their potential for development as a sunscreen product.
4. It is recommended to evaluate higher concentrations of *Centella asiatica* and *Moringa oleifera* extracts to assess their safety profile at maximum dosage levels.

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APPENDICES

Appendices 1. Ethical Clearance

KEMENTERIAN AGAMA REPUBLIK INDONESIA
UNIVERSITAS ISLAM NEGERI MAULANA MALIK IBRAHIM MALANG
FAKULTAS SAINS DAN TEKNOLOGI
KOMISI ETIK PENELITIAN
Jalan Gajayana No. 50 Malang 65144 Telp./ Fax. (0341) 558933
https://saintek.uin-malang.ac.id/

KETERANGAN KELAIKAN ETIK
(*ETHICAL CLEARANCE*)
Nomor. 48/EC/KEP-FST/2025

KOMISI ETIK PENELITIAN FAKULTAS SAINS DAN TEKNOLOGI UIN MAULANA MALIK IBRAHIM MALANG TELAH MEMPELAJARI DENGAN SEKSAMA RANCANGAN PENELITIAN YANG DIUSULKAN:

Judul	:	Organoleptic and Sun Protecting Factor (SPF) Test Of Nano-Cream Preparation With Active Ingredients Of Pegagan (*Centella Asiatica*) And Moringa (*Moringa Oleifera*) In Vivo
Peneliti	:	Hafidha Zekkour
Unit/ Lembaga	:	Program Studi Biologi Fakultas Sains dan Teknologi UIN Maulana Malik Ibrahim Malang
Tempat Penelitian	:	Laboratorium Fisiologi Hewan Program Studi Biologi Fakultas Sains dan Teknologi UIN Maulana Malik Ibrahim Malang

DENGAN INI MENYATAKAN BAHWA PENELITIAN TERSEBUT ***TELAH MEMENUHI SYARAT*** ATAU ***LAIK ETIK***

Malang, 31 Januari 2025
Ketua Komisi Etik

Prof. Dr. Hj. Bayyinatul Muchtaromah, M. Si
NIP. 19710919 200003 2 001

Appendices 2. Research Activities

THE PROCESS OF MAKING NANO-CREAM

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

(i)

(j)

(k)

(l)

(m)

(n)

(o)

(p)

(q)

(r)

Images: (a). Were treated PEF, (b). Distilled water, (c). Macrowiv, (d). Ultrasonic cleaner, (e). Extract, (f). Olive oil (oil phase), (g). Stearic acid (oil phase), (h). Tween 80 (water phase)**,** (i). Potassium benzoate (water phase), (j). Sodium benzoate (water phase), (k). Propylene glycol (water phase)**,** (l). Aquades (water phase)**,** (m). Water Phase**,** (n). Oil phase into the water phase**,** (o). Pouring the extract**,** (p). Inserting the magnetic stirrer**,** (q). Stirring speed**,** (r). Pouring the cream into the pot.

Appendices 3. Application of Nano-cream

(a)

(b)

(c)

(d)

(e)

(f)

(g)

Images: (a). Rat , (b). Preparation of the cage , (c). Nano-cream , (d). Nano-cream, (e). A shaved rat, (f). Apply nano-cream, (g). UVB rays.

Appendices 4. Data From the Results of Characterization of Particle Size Using PSA

CORDOUAN

Measurement Report ***Sample Name : Formula A***

Measurement Date: *21.02.2025 16:47* ***by:*** *DefaultExpert*
Report Date: *21.02.2025 16:54* ***by:*** *DefaultExpert*
comment:

SOP : DefaultSOP

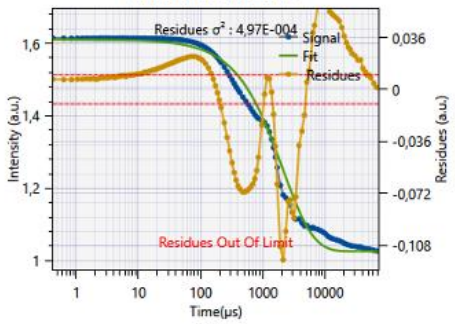
Solvent	*: Ethanol*	***Refractive index***	*: 1,36*	***Viscosity(mPa.s) at 24,79 °C***	*: 1,0613*
Device	*: AmeriQ*	***Wavelength***	*: 638 nm*	***Angle :*** *170°*	
Laser power	*: 10 %*	***Temperature set by***	*: Sensor*		
Algorithm	*: Cumulants/Pade Laplace/SBL*				
Scattering Model	*: Rayleigh*	***Particle refractive Index (a.u.)***	*: 1,56 (Real) 0,01 (Imaginary)*		
Experiment	*: Mono-Acquisition*				

Overview

From (hh:mm:ss)	To (hh:mm:ss)	Duration (hh:mm:ss)	T (°C)	Viscosity (mPa.s)	Laser Power (%)	Beta (a.u.)	CountRate (kcps)
00.2 s	06:03.8	06:03.6	24,79-25,08	1,0557 - 1,0613	10	0,61	371 - 1485

Cumulants **Formula A**

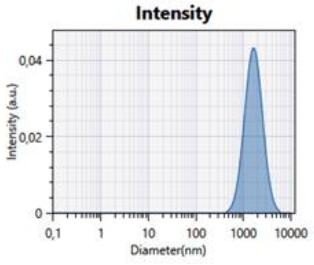
Fit and Residues



From (hh:mm:ss) : 00.2 s **To (hh:mm:ss) : 06:03.8** **Duration (hh:mm:ss) : 06:03.6**
Beta (a.u.) : 0,61

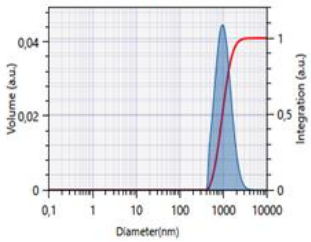
Z average (nm)	PDI (a.u.)	Std Dev (%)	Diffusion Coeff (m²/s)	Decay Rate (s-1)
1248,94	0,1999	0,45	236,42	33,09029E-014

Intensity



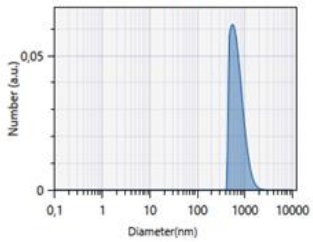
Di 10% : 946,03 nm Di 50 % : 1647,66 n
Di 90 % : 2869,67 nm Mean : 1792,73 n

Volume



Dv 10% : 595,8 nm Dv 50 % : 990,8 nm
Dv 90 % : 1647,66 nm Mean : 1069,68 nm

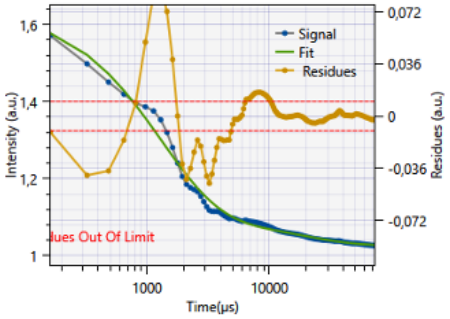
Number



Dn 10% : 472,82 nm Dn 50 % : 653,52 nm
Dn 90 % : 1037,68 nm Mean : 715,22 nm

Pade Laplace ***Formula A***

Fit and Residues σ^2 = 3,59E-005

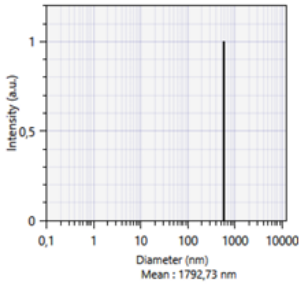


From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 06:03.8 Duration (hh:mm:ss) : 06:03.6
Tau (µs) : 161,602029024195 Channel : 452
Beta (a.u) : 0,61

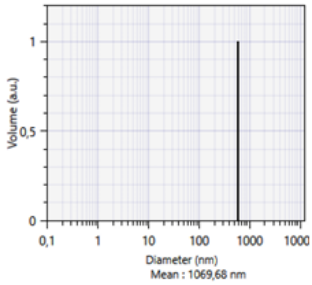
Detected Size

Size (nm)	Intensity (a.u)	Volume (a.u)	Number (a.u)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
580,24	0,65	1	1	508,88	71,22555E-014
11205,07	0,35			26,35	36,88296E-015

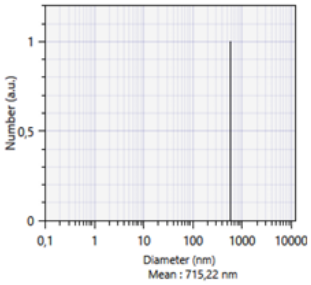
Intensity



Volume

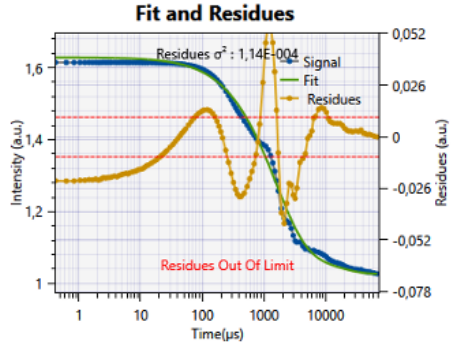


Number



SBL-Intensity **Formula A**

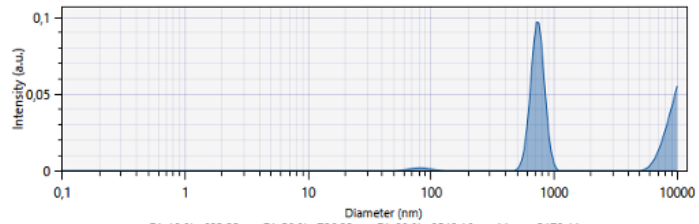
Fit and Residues



From (hh:mm:ss) : 00.2 s To (hh: mm:ss) : 06:03.8 Duration (hh: mm:ss) : 06:03.6
Beta (a.u): 0,61

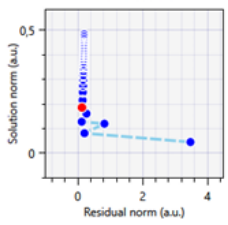
Mode (nm)	Mean (nm)	Std Dev (%)	Intensity (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
81,59	82,18	19,26	1,99	3618,87	50,65168 E-013
716,84	737,22	13,11	61,32	411,91	57,65258 E-014
9116,74	8223,55	11,33	23,13	32,39	45,3316 E-015
10000	9591,4	3,74	13,56	29,53	41,32763 E-015

Intensity



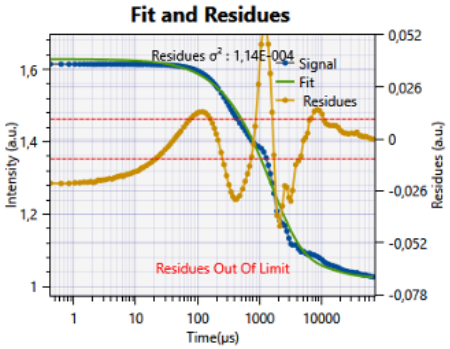
Di 10 % : 623,99 nm Di 50 % : 786,29 nm Di 90 % : 9548,16 nm Mean : 3179,44 nm

L-CURVE



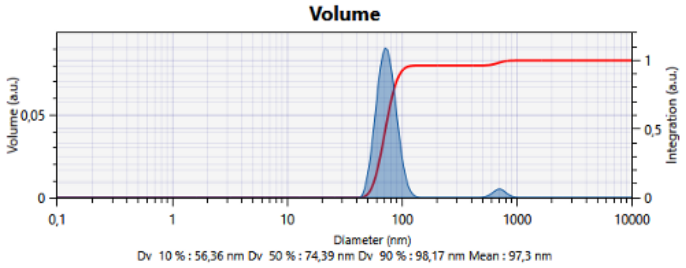
Best Solution : 6

SBL-Volume **Formula A**

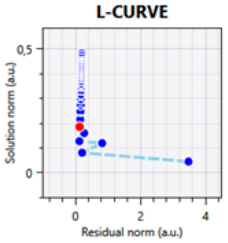


From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 06:03.8 Duration (hh:mm:ss): 06:03.6
Beta (a.u): 0,61

Mode (nm)	Mean (nm)	Std Dev (%)	Volume (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
71,02	74,18	19,59	96,34	4157,32	58,18819 E-013
716,84	705,72	13,61	3,66	411,91	57,65258 E-014

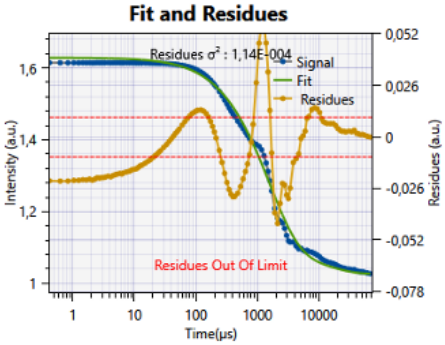


Dv 10 % : 56,36 nm Dv 50 % : 74,39 nm Dv 90 % : 98,17 nm Mean : 97,3 nm



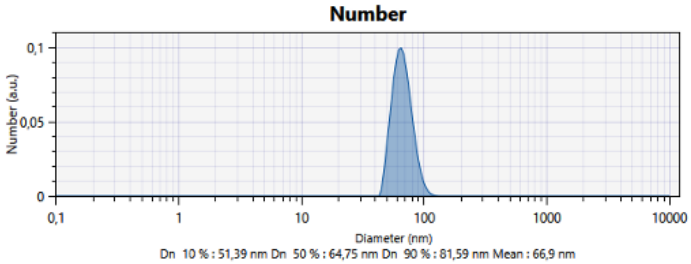
Best Solution : 6

SBL-Number **Formula A**

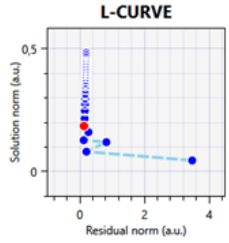


From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 06:03.8 Duration (hh:mm:ss) : 06:03.6
Beta (a.u) : 0,61

Mode (nm)	Mean (nm)	Std Dev (%)	Number (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
64,75	66,92	18,73	100	4560,1	63,82568 E-013



Dn 10 % : 51,39 nm Dn 50 % : 64,75 nm Dn 90 % : 81,59 nm Mean : 66,9 nm



Best Solution : 6

CORDOUAN Technologies

Measurement Report — *Sample Name : Formula B*

Measurement Date: *21.02.2025 16:33* ***by:*** *DefaultExpert*
Report Date: *21.02.2025 16:39* ***by:*** *DefaultExpert*
comment:

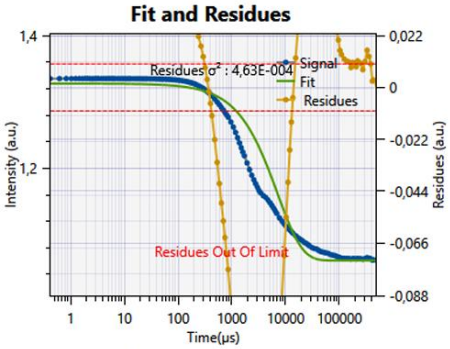
SOP : DefaultSOP

Solvent *: Ethanol* ***Refractive index*** *: 1,36* ***Viscosity(mPa.s) at 25,02 °C*** *: 1,0568*
Device *: AmeriQ* ***Wavelength*** *: 638 nm* ***Angle :*** *170°*
Laser power *: 10 %* ***Temperature set by*** *: Sensor*
Algorithm *: Cumulants/Pade Laplace/SBL*
Scattering Model : *Rayleigh* ***Particle refractive Index (a.u.) :*** *1,56 (Real) 0,01 (Imaginary)*
Experiment *: Mono-Acquisition*

Overview

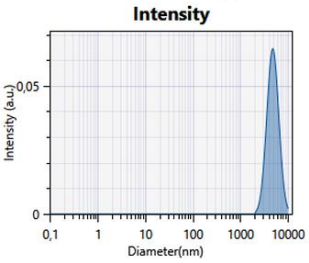
From (hh:mm:ss)	To (hh:mm:ss)	Duration (hh:mm:ss)	T (°C)	Viscosity (mPa.s)	Laser Power (%)	Beta (a.u.)	CountRate (kcps)
00.2 s	05:17.4	05:17.2	24,96-25,08	1,0557 - 1,058	10	0,33	679 - 3406

Cumulants — Formula B

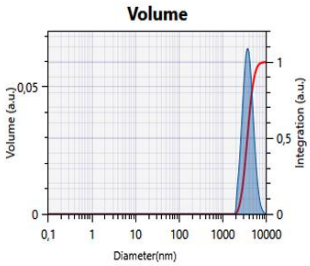


From (hh:mm:ss) : 00.2 s **To (hh:mm:ss) : 05:17.4** **Duration (hh:mm:ss) : 05:17.2**
Beta (a.u.) : 0,33

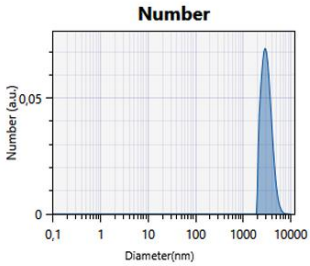
Z average (nm)	PDI (a.u.)	Std Dev (%)	Diffusion Coeff (m²/s)	Decay Rate (s-1)
4203,68	0,085	0,29	70,25	98,32643E-015



Di 10% : 3296,65 nm Di 50 % : 4772,16
Di 90 % : 6908,08 nm Mean : 4930,72 n

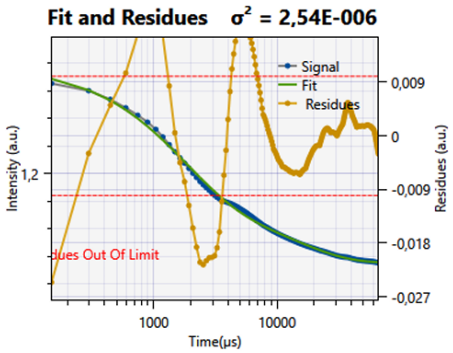


Dv 10% : 2616,2 nm Dv 50 % : 3787,16 nm
Dv 90 % : 5482,21 nm Mean : 3922,31 nm



Dn 10% : 2277,35 nm Dn 50 % : 3005,46 n
Dn 90 % : 4350,65 nm Mean : 3177,99 nm

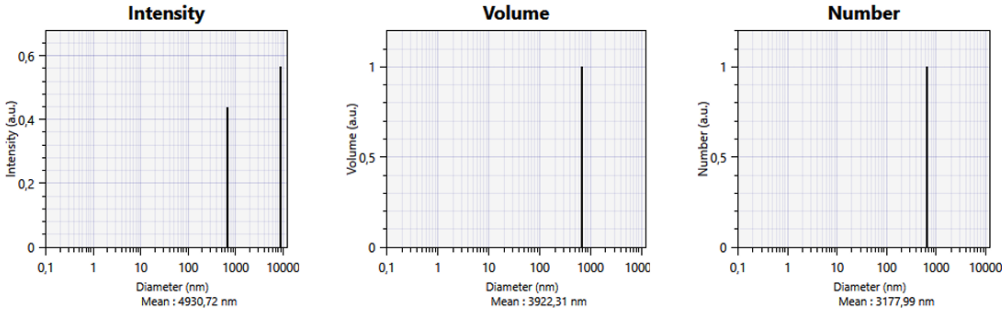
Pade Laplace ***Formula B***



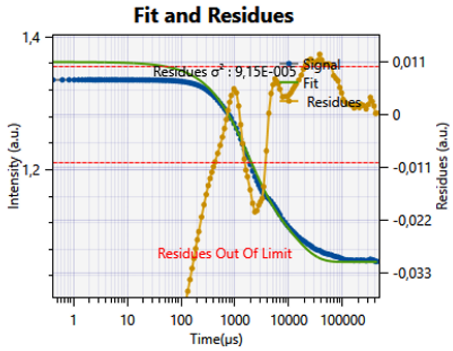
From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 05:17.4 Duration (hh:mm:ss) : 05:17.2
Tau (µs) : 148,486070407668 Channel : 448
Beta (a.u) : 0,33

Detected Size

Size (nm)	Intensity (a.u)	Volume (a.u)	Number (a.u)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
651,94	0,44	1	1	452,97	63,40059E-014
8541,87	0,56	5,5e-4	2,3e-7	34,57	48,38907E-015

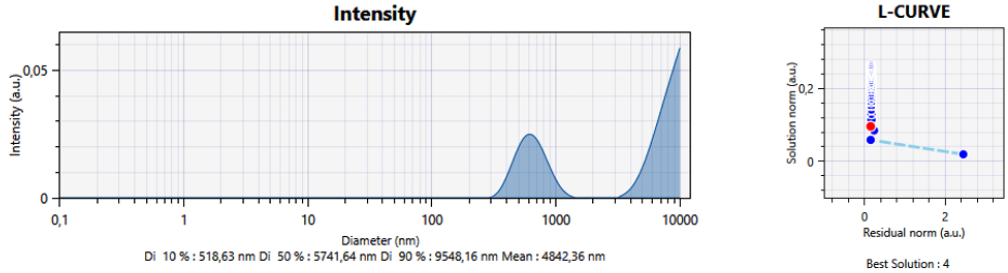


SBL-Intensity **Formula B**

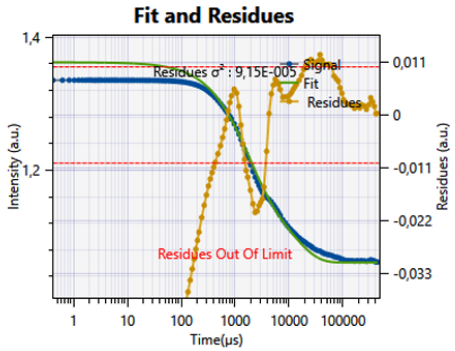


From (hh:mm:ss) : 00.2 s To (hh: mm:ss) : 05:17.4 Duration (hh: mm:ss) : 05:17.2
Beta (a.u): 0,33

Mode (nm)	Mean (nm)	Std Dev (%)	Intensity (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
623,99	650,45	28,76	38,01	473,26	66,23987 E-014
10000	7757,79	21,19	61,99	29,53	41,33331 E-015

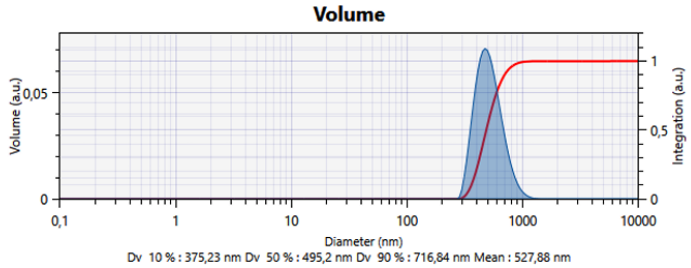


SBL-Volume **Formula B**

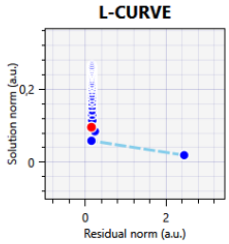


From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 05:17.4 Duration (hh:mm:ss): 05:17.2
Beta (a.u): 0,33

Mode (nm)	Mean (nm)	Std Dev (%)	Volume (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
472,82	523,33	27,31	100	624,57	87,4181 E-014

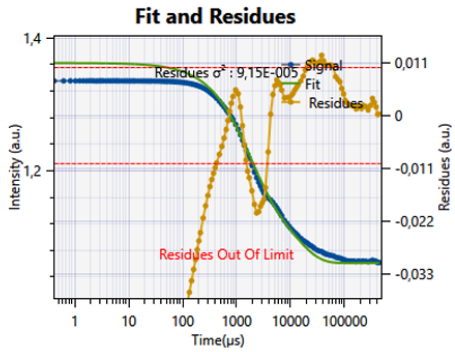


Dv 10 % : 375,23 nm Dv 50 % : 495,2 nm Dv 90 % : 716,84 nm Mean : 527,88 nm



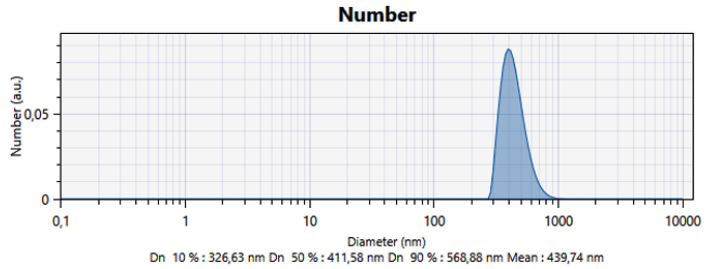
Best Solution : 4

SBL-Number **Formula B**

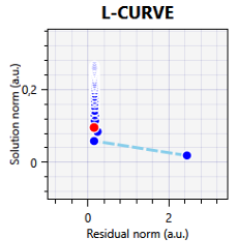


From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 05:17.4 Duration (hh:mm:ss) : 05:17.2
Beta (a.u) : 0,33

Mode (nm)	Mean (nm)	Std Dev (%)	Number (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
392,99	440,05	23,24	100	751,45	10,51775 E-013



Dn 10 % : 326,63 nm Dn 50 % : 411,58 nm Dn 90 % : 568,88 nm Mean : 439,74 nm



Best Solution : 4

CORDOUAN Technologies

Measurement Report — Sample Name : Formula C

Measurement Date: 21.02.2025 16:04 **by:** DefaultExpert
Report Date: 21.02.2025 16:17 **by:** DefaultExpert
comment:

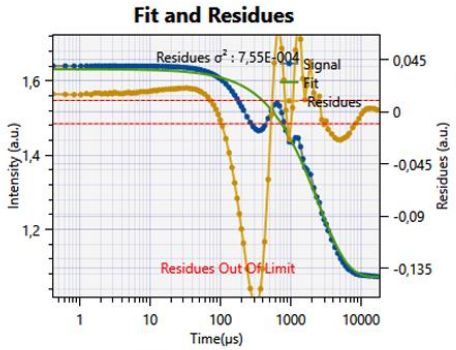
SOP : DefaultSOP

Solvent : Ethanol **Refractive index** : 1,36 **Viscosity(mPa.s) at 25,02 °C** : 1,0568
Device : AmeriQ **Wavelength** : 638 nm **Angle** : 170°
Laser power : 10 % **Temperature set by** : Sensor
Algorithm : Cumulants/Pade Laplace/SBL
Scattering Model : Rayleigh **Particle refractive Index (a.u.)** : 1,56 (Real) 0,01 (Imaginary)
Experiment : Mono-Acquisition

Overview

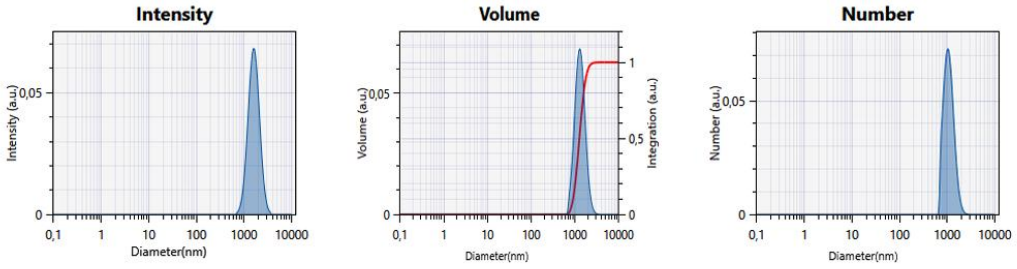
From (hh:mm:ss)	To (hh:mm:ss)	Duration (hh:mm:ss)	T (°C)	Viscosity (mPa.s)	Laser Power (%)	Beta (a.u.)	CountRate (kcps)
00.2 s	05:28.6	05:28.4	24,96-25,08	1,0557 - 1,058	10	0,66	271 - 1391

Cumulants — Formula C



From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 05:28.6 Duration (hh:mm:ss) : 05:28.4
Beta (a.u.) : 0,66

Z average (nm)	PDI (a.u.)	Std Dev (%)	Diffusion Coeff (m²/s)	Decay Rate (s-1)
1442,04	0,0758	0,28	204,77	28,66024E-014

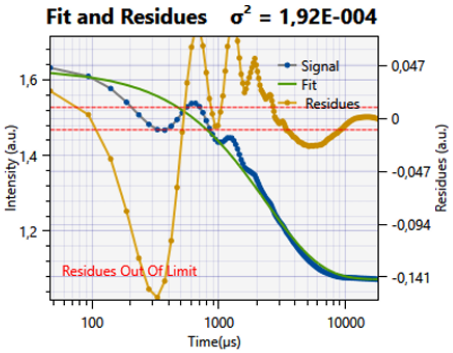


Di 10% : 1138,22 nm Di 50 % : 1573,21
Di 90 % : 2277,35 nm Mean : 1667,71 n

Dv 10% : 946,03 nm Dv 50 % : 1307,57 nm
Dv 90 % : 1807,29 nm Mean : 1353,58 nm

Dn 10% : 786,29 nm Dn 50 % : 1086,79 nm
Dn 90 % : 1502,13 nm Mean : 1112,55 nm

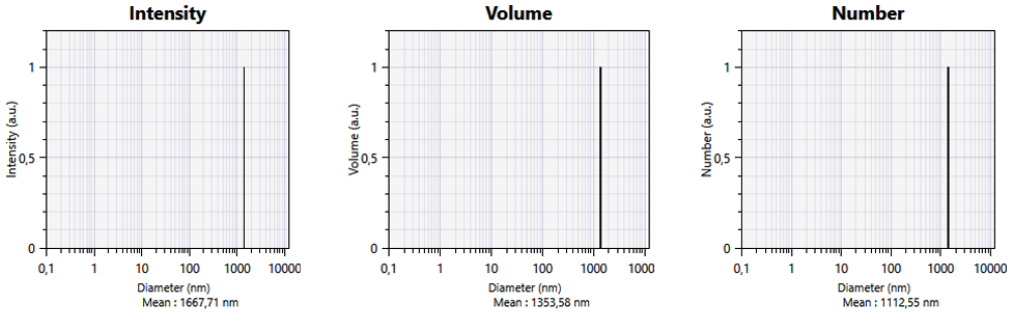
Pade Laplace ***Formula C***



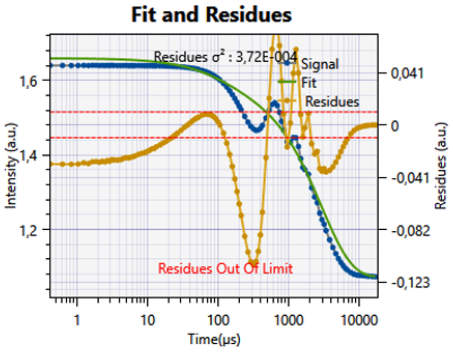
From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 05:28.6 Duration (hh:mm:ss) : 05:28.4
Tau (µs) : 46,7516644577999 Channel : 388
Beta (a.u) : 0,66

Detected Size

Size (nm)	Intensity (a.u)	Volume (a.u)	Number (a.u)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
1387,39	1	1	1	212,83	29,78926E-014

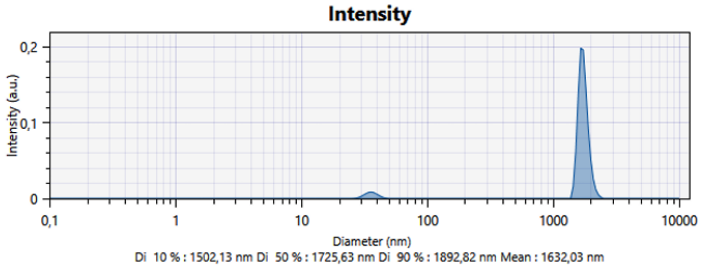


SBL-Intensity **Formula C**

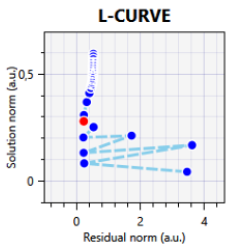


From (hh:mm:ss) : 00.2 s To (hh: mm:ss) : 05:28.6 Duration (hh: mm:ss) : 05:28.4
Beta (a.u): 0,66

Mode (nm)	Mean (nm)	Std Dev (%)	Intensity (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
35,5	35,74	12,52	5,77	8318,33	11,64279 E-012
1725,63	1730,94	9,91	94,23	171,12	23,95025 E-014

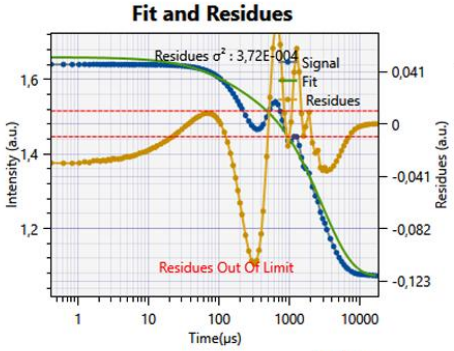


Di 10 % : 1502,13 nm Di 50 % : 1725,63 nm Di 90 % : 1892,82 nm Mean : 1632,03 nm



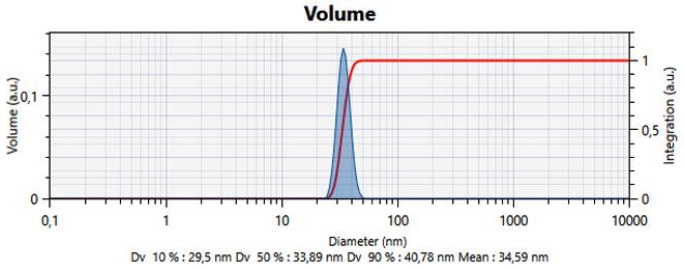
Best Solution : 8

SBL-Volume **Formula C**

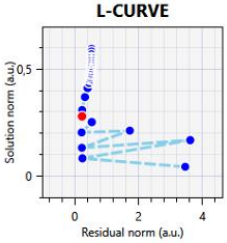


From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 05:28.6 Duration (hh:mm:ss): 05:28.4
Beta (a.u): 0,66

Mode (nm)	Mean (nm)	Std Dev (%)	Volume (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
33,89	34,39	12,93	100	8711,97	12,19375 E-012

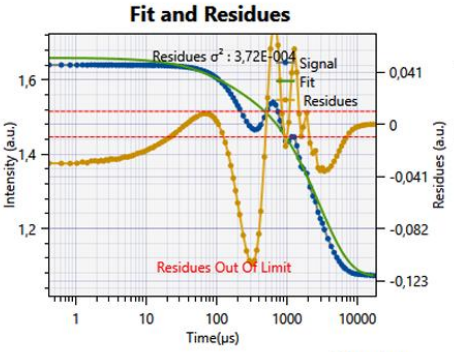


Dv 10 % : 29,5 nm Dv 50 % : 33,89 nm Dv 90 % : 40,78 nm Mean : 34,59 nm



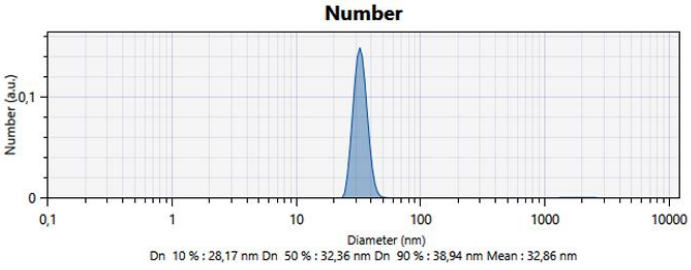
Best Solution : 8

SBL-Number **Formula C**

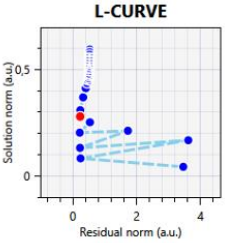


From (hh:mm:ss) : 00.2 s To (hh:mm:ss) : 05:28.6 Duration (hh:mm:ss) : 05:28.4
Beta (a.u) : 0,66

Mode (nm)	Mean (nm)	Std Dev (%)	Number (%)	Decay Rate (s-1)	Diffusion Coeff (m²/s)
32,36	32,89	12,73	100	9124,24	12,77079 E-012



Dn 10 % : 28,17 nm Dn 50 % : 32,36 nm Dn 90 % : 38,94 nm Mean : 32,86 nm



Best Solution : 8

KEMENTERIAN PENDIDIKAN,
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Telp. +6282132101344
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www.labftp.ub.ac.id

LAPORAN HASIL ANALISA
Report of Analysis

No. LU.LT/049/XII/2024

Pemakai Jasa : Noviza Mawar S
Customer
Instansi : UIN Maulana Malik Ibrahim malang
Institute
Nama Sampel : Krim (4 sampel)
Sample Name
Jenis Pengujian : Ukuran Partikel
Test Type
Alat Uji : PSA (Shimadzu SALD-7500nano)
Instrument
Tanggal Diterima : 27 Desember 2024
Receiving Date
Tanggal Uji : 31 Desember 2024
Date of Test
Kemasan Sampel : pot 10 gram
Sample Packaging
Lampiran : 8 Halaman
Attachment
Hasil/ *Report* :

Kode Sampel	Median (µm)	Mean (µm)	Modul (µm)	Absorbansi	Std Dev
D	5,526	5,938	3,356	0,497	0,633
	5,546	5,928	3,356	0,508	0,624

Malang, 02 Januari 2025
Laboran

Pipit Elok Nikmatus S, S.T
NRK. 20240199112923001

Mengetahui
Kepala Laboratorium Terpadu

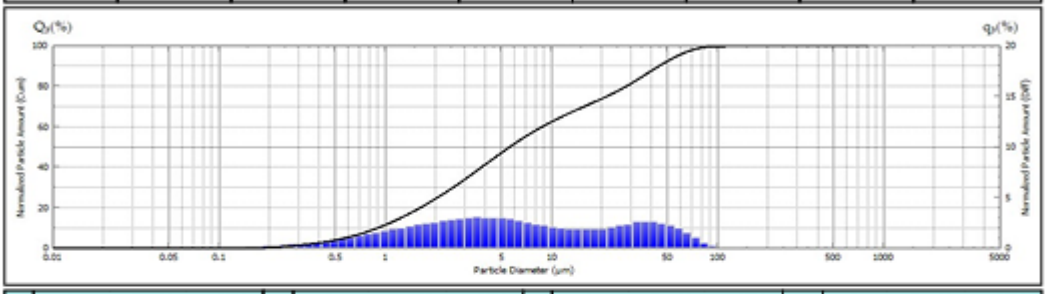
Lutfi Mahfudzah, S.Si
NIP. 19880502001122001

LABORATORUM TERPADU FTP UB

Shimadzu SALD-7500(WingSALD II:Version 3.4.11) Flow Cell with Sampler

File Name	D-2			Meas Date	2024/12/30 12:00:28(+0700)
Sample ID	D		Sample No.	D	
Comment	----				
Median D	5.526	Mean V	5.938	Absorbance	0.497
Modal D	3.356	Std Dev	0.633	----	----

10.000 %D	20.000 %D	30.000 %D	40.000 %D	50.000 %D	60.000 %D	70.000 %D	80.000 %D	90.000 %D
0.875	1.592	2.513	3.731	5.526	8.656	15.410	27.598	43.816



	Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)
1	800.000	100.000	0.000	27	38.727	87.335	2.651	53	1.875	23.323	2.435	79	0.091	0.000	0.000
2	712.049	100.000	0.000	28	34.469	84.685	2.597	54	1.669	20.888	2.305	80	0.081	0.000	0.000
3	633.768	100.000	0.000	29	30.680	82.088	2.379	55	1.485	18.583	2.154	81	0.072	0.000	0.000
4	564.092	100.000	0.000	30	27.307	79.709	2.216	56	1.322	16.429	2.013	82	0.064	0.000	0.000
5	502.077	100.000	0.000	31	24.305	77.493	2.028	57	1.177	14.416	1.881	83	0.057	0.000	0.000
6	446.879	100.000	0.000	32	21.633	75.465	1.917	58	1.047	12.535	1.729	84	0.051	0.000	0.000
7	397.750	100.000	0.000	33	19.255	73.548	1.868	59	0.932	10.807	1.554	85	0.045	0.000	0.000
8	354.022	100.000	0.000	34	17.138	71.680	1.843	60	0.830	9.253	1.395	86	0.040	0.000	0.000
9	315.102	100.000	0.000	35	15.254	69.838	1.846	61	0.738	7.857	1.248	87	0.036	0.000	0.000
10	280.460	100.000	0.000	36	13.577	67.992	1.895	62	0.657	6.610	1.096	88	0.032	0.000	0.000
11	249.627	100.000	0.000	37	12.084	66.097	1.999	63	0.585	5.514	0.935	89	0.028	0.000	0.000
12	222.183	100.000	0.000	38	10.756	64.098	2.107	64	0.521	4.579	0.802	90	0.025	0.000	0.000
13	197.757	100.000	0.000	39	9.573	61.991	2.230	65	0.463	3.777	0.689	91	0.022	0.000	0.000
14	176.016	100.000	0.000	40	8.521	59.761	2.376	66	0.412	3.088	0.589	92	0.020	0.000	0.000
15	156.665	100.000	0.000	41	7.584	57.384	2.560	67	0.367	2.499	0.507	93	0.018	0.000	0.000
16	139.441	100.000	0.000	42	6.750	54.824	2.718	68	0.327	1.992	0.432	94	0.016	0.000	0.000
17	124.111	100.000	0.000	43	6.008	52.107	2.892	69	0.291	1.560	0.368	95	0.014	0.000	0.000
18	110.467	100.000	0.077	44	5.348	49.215	2.978	70	0.259	1.192	0.308	96	0.013	0.000	0.000
19	98.322	99.923	0.220	45	4.760	46.237	2.970	71	0.230	0.883	0.255	97	0.011	0.000	0.000
20	87.513	99.703	0.535	46	4.236	43.267	2.992	72	0.205	0.628	0.203	98	0.010	0.000	0.000
21	77.892	99.169	1.080	47	3.771	40.275	3.037	73	0.183	0.425	0.152	99	0.009	0.000	0.000
22	69.328	98.088	1.513	48	3.356	37.237	3.004	74	0.162	0.273	0.110	100	0.008	0.000	0.000
23	61.707	96.576	1.955	49	2.987	34.233	2.902	75	0.145	0.164	0.076	101	0.007	0.000	0.000
24	54.923	94.621	2.259	50	2.659	31.331	2.798	76	0.129	0.088	0.048				
25	48.885	92.362	2.449	51	2.366	28.534	2.665	77	0.115	0.040	0.027				
26	43.510	89.913	2.578	52	2.106	25.868	2.545	78	0.102	0.012	0.012				

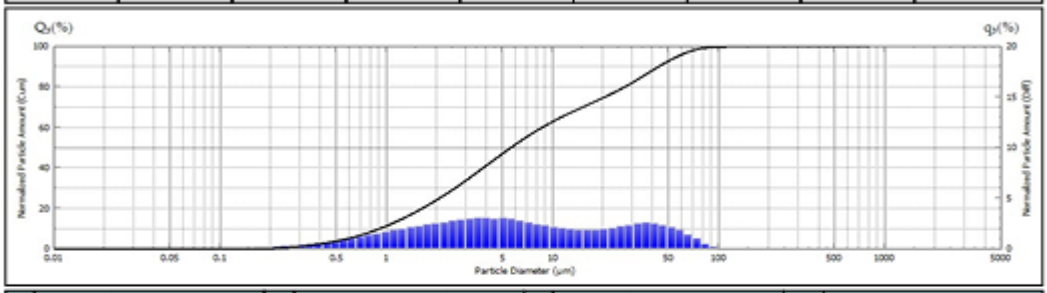
Condition File Name	----			Emulation Table	None		
Ref Index	1.70-0.20i			Calc Mode	Normal mode		
Averaging Count	128	Accumu	1	Interval	2	Sensor Starting Point	1
D Range for Analysis	OFF			Side/Backward Sensor		Enable	
Decentralized Solvent	Pure water			Dispersing Agent	----		
Denst of Disper Agent	----			Dispersion Method	External ultrasonic bath 2 min		
Pump Speed	5	----	----	Time for Sonicator	0		
Sample Preparation	----			Sequence File Name	----		
Dim of Particle Amount	Volume	Cum Distribution		Undersize		Diff Distribution	q
Distribution Function	None			Smoothing Level	0	Data Shift	0

LABORATORUM TERPADU FTP UB

Shimadzu SALD-7500(WingSALD II:Version 3.4.11) Flow Cell with Sampler

File Name	D-1				Meas Date	2024/12/30 12:00:05(+0700)	
Sample ID	D			Sample No.	D		
Comment	----						
Median D	5.546	Mean V	5.928	Absorbance	0.508	----	----
Modal D	3.356	Std Dev	0.624	----	----	----	----

10.000 %D	20.000 %D	30.000 %D	40.000 %D	50.000 %D	60.000 %D	70.000 %D	80.000 %D	90.000 %D
0.891	1.615	2.546	3.771	5.546	8.557	14.858	26.661	42.880



	Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)
1	800.000	100.000	0.000	27	38.727	87.875	2.583	53	1.875	23.018	2.418	79	0.091	0.000	0.000
2	712.049	100.000	0.000	28	34.469	85.292	2.545	54	1.669	20.600	2.288	80	0.081	0.000	0.000
3	633.768	100.000	0.000	29	30.680	82.747	2.351	55	1.485	18.312	2.145	81	0.072	0.000	0.000
4	564.092	100.000	0.000	30	27.307	80.396	2.206	56	1.322	16.167	2.007	82	0.064	0.000	0.000
5	502.077	100.000	0.000	31	24.305	78.190	2.032	57	1.177	14.160	1.874	83	0.057	0.000	0.000
6	446.879	100.000	0.000	32	21.633	76.158	1.936	58	1.047	12.286	1.723	84	0.051	0.000	0.000
7	397.750	100.000	0.000	33	19.255	74.222	1.900	59	0.932	10.563	1.550	85	0.045	0.000	0.000
8	354.022	100.000	0.000	34	17.138	72.323	1.887	60	0.830	9.014	1.393	86	0.040	0.000	0.000
9	315.102	100.000	0.000	35	15.254	70.436	1.903	61	0.738	7.621	1.247	87	0.036	0.000	0.000
10	280.460	100.000	0.000	36	13.577	68.533	1.963	62	0.657	6.374	1.096	88	0.032	0.000	0.000
11	249.627	100.000	0.000	37	12.084	66.570	2.074	63	0.585	5.278	0.934	89	0.028	0.000	0.000
12	222.183	100.000	0.000	38	10.756	64.496	2.189	64	0.521	4.344	0.803	90	0.025	0.000	0.000
13	197.757	100.000	0.000	39	9.573	62.306	2.320	65	0.463	3.541	0.697	91	0.022	0.000	0.000
14	176.016	100.000	0.000	40	8.521	59.987	2.468	66	0.412	2.844	0.600	92	0.020	0.000	0.000
15	156.665	100.000	0.000	41	7.584	57.518	2.650	67	0.367	2.244	0.519	93	0.018	0.000	0.000
16	139.441	100.000	0.000	42	6.750	54.869	2.802	68	0.327	1.726	0.439	94	0.016	0.000	0.000
17	124.111	100.000	0.000	43	6.008	52.066	2.966	69	0.291	1.287	0.364	95	0.014	0.000	0.000
18	110.467	100.000	0.068	44	5.348	49.100	3.042	70	0.259	0.923	0.293	96	0.013	0.000	0.000
19	98.322	99.932	0.199	45	4.760	46.058	3.025	71	0.230	0.630	0.223	97	0.011	0.000	0.000
20	87.513	99.733	0.498	46	4.236	43.033	3.034	72	0.205	0.407	0.164	98	0.010	0.000	0.000
21	77.892	99.235	1.021	47	3.771	39.999	3.063	73	0.183	0.243	0.113	99	0.009	0.000	0.000
22	69.328	98.214	1.439	48	3.356	36.936	3.019	74	0.162	0.130	0.071	100	0.008	0.000	0.000
23	61.707	96.775	1.871	49	2.987	33.917	2.909	75	0.145	0.059	0.039	101	0.007	0.000	0.000
24	54.923	94.904	2.171	50	2.659	31.009	2.797	76	0.129	0.019	0.016				
25	48.885	92.733	2.361	51	2.366	28.211	2.659	77	0.115	0.003	0.003				
26	43.510	90.372	2.497	52	2.106	25.552	2.534	78	0.102	0.000	0.000				

Condition File Name	---			Emulation Table	None		
Ref Index	1.70-0.20i			Calc Mode	Normal mode		
Averaging Count	128	Accumu	1	Interval	2	Sensor Starting Point	1
D Range for Analysis	OFF			Side/Backward Sensor			Enable
Decentralized Solvent	Pure water			Dispersing Agent	----		
Densi of Disper Agent	---			Dispersion Method	External ultrasonic bath 2 min		
Pump Speed	5	---	---	Time for Sonicator	0		
Sample Preparation	---			Sequence File Name	----		
Dim of Particle Amount	Volume	Cum Distribution		Undersize		Diff Distribution	q
Distribution Function	None			Smoothing Level	0	Data Shift	0

Appendices 5. Irritation Results

Formula	Number Rat	After 01 hour	After 24 hours	After 48 hours	After 72 hours
Formula (A) homogenized for 45 min	1	0	0	0	0
	2	0	0	0	0
	3	1	0	0	0
	4	1	0	0	0
	5	0	0	0	0
Formula (B) homogenized for 70 min	1	1	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
Formula (C) for homogenized 95 min	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	1	0	0	0
Formula (D) without homogenization	1	0	0	0	0
	2	0	0	0	0
	3	1	0	0	0
	4	0	0	0	0
	5	0	0	0	0

Appendices 6. SPSS Results

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Group	Statistic	df	Sig.	Statistic	df	Sig.
Data	1.00	.237	5	.200*	.961	5	.814
	2.00	.237	5	.200*	.961	5	.814
	3.00	.	5	.	.	5	.
	4.00	.	5	.	.	5	.
	5.00	.	5	.	.	5	.
	6.00	.473	5	.001	.552	5	.000

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Test of Homogeneity of Variance

		Levene Statistic	df1	df2	Sig.
Data	Based on Mean	7.583	5	24	.000
	Based on Median	2.925	5	24	.034
	Based on Median and with adjusted df	2.925	5	10.039	.070
	Based on trimmed mean	7.288	5	24	.000

Kruskal-Wallis Test

Ranks

	Group	N	Mean Rank
Data	1.00	5	24.40
	2.00	5	23.80
	3.00	5	14.50
	4.00	5	3.50
	5.00	5	14.50
	6.00	5	12.30
	Total	30	

Test Statistics[a,b]

	Data
Kruskal-Wallis H	23.405
df	5
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Group

Mann-Whitney Test

Ranks

	GROUP	N	Mean Rank	Sum of Ranks
DATA	1.00	5	8.00	40.00
	4.00	5	3.00	15.00
	Total	10		

Test Statistics[a]

	DATA
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.795
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[b]

a. Grouping Variable: GROUP

b. Not corrected for ties.

Ranks

	GROUP	N	Mean Rank	Sum of Ranks
DATA	2.00	5	8.00	40.00
	4.00	5	3.00	15.00
	Total	10		

Test Statistics[a]

	DATA
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.795
Asymp. Sig. (2-tailed)	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[b]

a. Grouping Variable: GROUP

b. Not corrected for ties.

Mann-Whitney Test

Ranks

	GROUP	N	Mean Rank	Sum of Ranks
DATA	3.00	5	8.00	40.00
	4.00	5	3.00	15.00
	Total	10		

Test Statistics[a]

	DATA
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-3.000
Asymp. Sig. (2-tailed)	.003
Exact Sig. [2*(1-tailed Sig.)]	.008[b]

a. Grouping Variable: GROUP

b. Not corrected for ties.

Mann-Whitney Test

Ranks

	GROUP	N	Mean Rank	Sum of Ranks
DATA	4.00	5	3.00	15.00
	5.00	5	8.00	40.00
	Total	10		

Test Statistics[a]

	DATA
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-3.000
Asymp. Sig. (2-tailed)	.003
Exact Sig. [2*(1-tailed Sig.)]	.008[b]

a. Grouping Variable: GROUP

b. Not corrected for ties.

Mann-Whitney Test

Ranks

	GROUP	N	Mean Rank	Sum of Ranks
DATA	4.00	5	3.50	17.50
	6.00	5	7.50	37.50
	Total	10		

Test Statistics[a]

	DATA
Mann-Whitney U	2.500
Wilcoxon W	17.500
Z	-2.449
Asymp. Sig. (2-tailed)	.014
Exact Sig. [2*(1-tailed Sig.)]	.032[b]

a. Grouping Variable: GROUP

b. Not corrected for ties.

LIFE STORY

I am Hafidha Zekkour from Algeria, born on December 12, 2001. I obtained my bachelor's degree from the University of Batna 2 Algeria in 2022, Faculty of Natural and Life Sciences. Now I am studying for a master's degree at Maulana Malik Ibrahim University of Malang, Indonesia, Faculty of Science and Technology, Biology study Program. I will complete my final assignment as a master's student entitled research **"Organoleptic and Sun Protecting Factor (SPF) Test of Nano-cream Preparation with Active Ingredients of *Centella asiatica* and *Moringa oleifera* in vivo."**