**T.C.**

**YEDITEPE UNIVERSITY**
**INSTITUTE OF HEALTH SCIENCES**
**DEPARTMENT OF DRUG AND COSMETIC PRODUCTION TECHNOLOGIES**

# DEVELOPMENT OF FORMULATION CONTAINING EXTRACT OF ORANGE PEEL AND D-LIMONENE

**MASTER OF SCIENCE THESIS**

**ÖVGÜ ULUN**

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**SUPERVISOR**
**ASSIST. PROF. DR. GÜLENGÜL DUMAN**

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 16/07/2019 tarih ve 2019/12-25 sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Sağlık Bilimleri Enstitüsü Müdürü

## DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

27/06/2019

Övgü ULUN

## DEDICATION

I would like to dedicate this work to my family, my best friends and my love…

## ACKNOWLEDGEMENTS

First of all I would like to acknowledge God, for giving me the great opportunity to step in the wonderful world of science, for providing me every day the enthusiasm and wisdom to proceed successfully and above all for offer me the chance to meet amazing people who were the clue for the development of this thesis.

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## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| AOS | Alpha-Olefin Sulfanates |
| CMC | Carboxy Methyl Cellulose |
| CPE | *Citrus Sinensis* Peel Extract |
| DL | D-Limonene |
| FC | Formulation Code |
| G | Glycerol |
| GC-MS | Gas Chromotography-Mass Spectrometry |
| GL | Glycolipid |
| HA | Sodium Hyaluronate |
| IRS | Inner Root Sheath |
| MC | Methyl Cellulose |
| MCFA | Medium Chain Fatty Acid |
| ORC | Outer Root Sheath |
| PDI | Poly Dispersity Index |
| PW | Purified Water |
| PVA | Polyvinyl Alcohol |
| SL | Soy Lecithin |
| SLS | Sodium Laureth Sulfate |
| SLES | Sodium Lauryl Ether Sulfate |
| VCO | Virgin Coconut Oil |

**ABSTRACT**

**Ulun, Ö. (2019). Development of Formulation Containing Extract of Orange Peel and D-limonene. Yeditepe University, Institute of Health Science, Department of Drug and Cosmetic Production Technologies, MSc thesis, İstanbul.**

Nowadays finding personal cleansing products which have safe and natural based ingredients in their formulation is very hard. Most of cleansing products in the market have harmful chemicals. These chemicals can damage to hair and create skin irritation what can lead systemic illness and cancer.

The purpose of this thesis is formulation dissolvable film personal cleansing product which contains natural ingredients such as *citrus* peel extract ( CPE ), virgin coconut oil ( VCO ), glycolipid ( GL ) and sodium hyaluronate (Na-HA). Different amount of glycolipid and CPE are used in different formulations to decide most effective optimized formulation. CPE (d-limonene (DL)) used because of its cleansing and stress relieving effects. GL used as natural surfactant and have no harmful effect to human hair and skin. Na-HA used as carrier to give conditioning effect.

The reason of using dissolvable film form is increasing product stability and safeteness without using synthetic surfactants. The formulation which contains GL, CPE ( DL ), Na-HA and VCO is quite useful for cleaning and conditioning skin and hair such as reviving human hair and scalp from years of using product that contains harsh chemicals. Our formulation is better other many formulations because it is using benefits of natural oil and extracts and non-ionic natural surfactant like GL instead of using chemicals that is harmful for hair and skin.

Final formulation ( F1 ) was determined by examining different formulations. The final formulation was choosen because of its low mechanical properties, efficient cleaning and conditioning actions, non preservative usage, containing natural origin surfactants and base and gives no skin irritancy. In addition to that properties, F1 formulation also has antimicrobial properties.

More over dissolvable film formulation with natural ingredient gives user ease of carrier and help environment by letting biodegredable substances go down the drain instead of chemicals.

**Key Words:** Dissolvable film, biosurfactants, sodium hyaluronate, *citrus sinensis* peel extract, d-limonene, personel care products

**ÖZET**

**Ulun, Ö. (2019). Portakal Kabuğu Ekstresi ve D-limonen İçeren Formülasyon Geliştirilmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, İlaç ve Kozmetik Üretim Teknolojileri ABD., Master Tezi. İstanbul.**

Günümüzde, güvenli ve doğal kaynaklı ham maddeleri içeren kişisel temizlik ürünleri bulmak oldukça zordur. Marketlerde bulunan çoğu kişisel temizlik ürünleri zararlı etkileri olan kimyasallar içermektedir. Bu kimyasal maddeler saçı ve deriyi tahrip ederek iritasyona hatta devam etmesi durumunda sistemik hastalıklara sebep olarak kansere bile neden olabilmektedir.

Tezimizin amacı, turunçgil kabuk ekstraktı ( TKE ), organik hindistan cevizi yağı ( OHCY ), glikolipid ( GL ), sodyum hyaluronat (Na-HA) gibi doğal maddeleri içeren çözülebilen film formulüasyonunda kişisel temizlik ürününün elde edilmesidir. Nihai ürünün optimize edilebilmesi için farklı miktarlarda glikolipid ve TKE denenmiştir. TKE (d-limonen (DL)), temizleme ve stres azaltıcı etkileri nedeniyle kullanılmıştır. GL, doğal bir surfaktan olduğu ve saça ve cildi zararlı hiç bir etkisinin olmaması nedeniyle kullanılmıştır. Na-HA ise taşıyıcı olarak ve nemlendirme etkisinden dolayı kullanılmıştır.

Nihai ürünün stabilitesinin ve güvenliğinin sentetik surfaktanlar kullanılmadan arttırılabilmesi için çözülebilen film formu seçilmiştir. Formülasyonda kullanılan GL, Na-HA, TKE ( DL ) ve OHCY özellikle saç ve derinin temizlenmesinde ve nemlendirilmesinde etkili ve doğal oldukları için tercih edilmişlerdir. Ürünümüzün diğer zararlı kimyasal içeren ürünlerden farklı doğal yağ, ekstrakt ve surfaktanlar içererek saçta ve ciltte temizleme ve nemlendirme etkisi yaratmasıdır.

Nihai formülasyona ( F1 ) farklı film preperatları değerlendirilerek karar verilmiştir. Film formülasyonun, düşük mekanik özellikleri olması, etkili temizleme ve bakım sağlaması, koruyucu içermemesi, doğal kaynaklı surfaktanlar içermesi ve cilde tahriş yapmamasına dikkat edilerek seçilmiştir. Ayrıca F1 formülasyonunun antimikrobiyel özelliklere de sahip olduğu gösterilmiştir.

Çözülebilen film formülasyonun kullanıcıya taşıma kolaylığı sağlamasının yanı sıra çevreye de tahliye edildiğinde zararlı kimyasallar içermediği için olumlu etkileri bulunmaktadır.

**Anahtar Kelimeler:** Çözülebilen film, doğal surfaktanlar, sodyum hyaluronat, portakal kabuğu ekstraktı, d-limonen, kişisel temizlik ürünleri

## 1. INTRODUCTION

Today's consumers want to know ingredients of their products to be sure about their safeteness. Their concerns make manufacturers to find safer formulations. Over the years, herbal extracts alone or as a whole used for skin, hair and overall appearance in order to cleaning, perfuming, mousturing, changing appearance, get rid of bad odours and keeping them in good conditions ( 1 ). Herbal extracts are put in to the cosmetic formulations because of their properties such as antioxidant, anti-inflammatory, antiseptic and anti-microbial effect.

Recently, natural cosmetics have gained importance all over the world. Herbal cosmetic products promise to have efficacy and natural favoured in order to used in daily routine. Because of their natural origin, no side effect was observed which are commonly seen in other synthetic cosmetic products ( 2 ). Herbal products like extracts; essential oils and powders have been used in cosmetics as either active ingredients or as excipients.

Essential oils are added in formulations as flavouring agents, scent and anti-microbial agents. Many essential oils are considered completely safe. Essential oils are mixtures of mainly low molecular weight chemical substances which are combined with their lipophilicity, results in an ability to pass across membranes very efficiently ( 3 ). *Citrus* oil is an essential oil found in the peel of a *citrus* fruit such as lemon, orange, sweat lime etc. *Citrus* essential oils are one of the most popular used fragrances in natural skin, bath and body care products where they add benefits to formulations. These oils comprise a mixture of volatile compounds like terpenes and oxygenated derivatives such as aldehydes ( citral ), alcohols and esters, their main component being d-limonene. It is constituting up to 90-95 % in orange peel oil and plays an important role in the field of flavour and fragrances due to its physicochemical properties ( 4 ). Another properties of d-limonene is cleaning so it is appropriate essential oil for the formulation of personal care products such as shampoo and soap.

Cosmetic formulators face a number of challenges in the selection of preservatives, choosing skin-friendly and safe solutions that are effective and economically viable for the formulation of herbal cosmetics. One of the modern delivery systems is, which has gained the popularity among the pediatric and geriatric

patients as orally dissolvable film usage ( 5 ). This formulation gained importance because it has many advantages over other formulations.

In our thesis we aim to formulate dissolvable film formulation consisting herbal extracts such as VCO , CPE ( DL ) for usage as personel cleansing products. This solid product will disintegrate when consumer pour water on it. This formulation will provide safe and effective usage, ease of handling and proper dosage use.

## 2. GENERAL INFORMATION

Today, herbs are widelyused for development of new products in order to used in cosmeceutical and pharmaceutical areas. Natural cosmetics are considered the products in which plants are used raw or extract form ( 6 ). Herbal cosmetics are free from harsh synthetic chemicals which are mostly toxic and give irritancy to the skin. Instead of traditional synthetic products, different plant parts and extracts are used in cosmetic products which gives same effect. They also consist of natural nutrients like Vitamin E that keeps skin healthy, glowing and good condition ( 7 ). Especially essential oils play an important role for protecting our body from viral, parasitic, and bacterial fungal contamination from the world. Essential oils can be used in cosmetic lotions, bath soaps, hair rinses, perfumes and room sprays formulations ( 8 ). Essential oils can be obtained by many different methods such as distillation, solvent extraction, cold press, supercritical fluid extraction, microwave extraction and ultrasound assisted extraction ( 9 ).

### 2.1 SKIN AND HAIR STRUCTURE

The skin is considered an organ which creates border between organism and environment. Skin is important for preventing dehydration, blocking penetration of detrimental materials and microorganisms, maintaining a constant body temperature and etc. Skin should be treated appropriately for performing its duties. Skin is composed of three layers: the epidermis, the dermis and the subcutaneous tissue. The epidermis consits of many layers such as prickle layer, granular layer and stratum corneum and seperated from the dermis by basement membrane. The dermis consists of sweat duct, sebaceous gland, sweat gland, blood vessel, connective tissue, sebocytes, hair follicle and capillary ( 10 ).

The hair follicle is described as a tubelike pocket of the epidermis which surrounds a small section of the dermis at its base. Hair follicle has different stuructures such as outer root sheath ( ORC ), inner root sheath ( IRS ), hair shaft, dermal papilla and hair matrix ( Figure 2.1. ). Dermal papilla is important for hair follicle development and after ( 11 ). Hair shaft comprises cuticle, cortex and medulla. Medulla is innermost layer and has high lipid content more than rest of the fibre ( 12 ). Cuticle is outer layer

and has long chain fatty acids which are the reason of hydrophobicity of hair surface ( 13 ). Cortex is the main component of the hair and has cortical cells which are filled with long keratin filaments. The keratin chains have many sulfur-containing cysteine residues. These residues are attached to keratin filaments and make covalent disulfide bonds between adjacent keratin chains. These disulfide bonds give shape, stability and texture for hair ( 14 ).

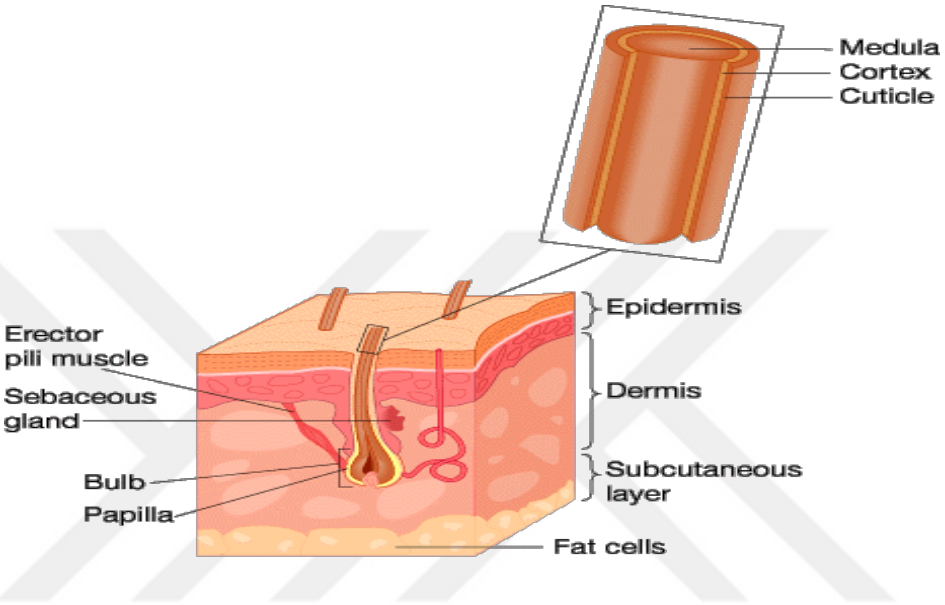


**Figure 2.1.** The Structure Of Hair Follicle (15)

For a successful cosmetic application, it is essential to change structure of hair shaft. The chemical reaction of hair operations such as coloring, perming or straightening occur in the hair cortex and they change properties of hair. Excessive chemical application, poor grooming, and environmental exposure can result hair breakage ( 14 ). In our thesis we aim to create formulation of hair product which is free of preservatives and consisting essential oils for giving strength to hair and preveting hair loss.

## 2.2 SHAMPOO AND CLASSIFICATION

Shampoo is the main hair care product which is generally in liquid form in order to used for cleansing scalp and excess sebum, dandruff, environmental dirtiness, and residues of hair care products ( 16 ). The main purpose of shampoo is removal of unwanted buildup without damaging hair scalp and conditioning ( 17 ).

In shampoo formulation surfactant usage is a must, because most of the dirtiness origin is water insoluble. For the efficiently removal of dirt, surfactants are used between 10-20 % proportions in formulations. Surfactanst have two ends, one of them is hydrophilic and the other is hydrophobic. The hyrophilic part favors aqueous phase, and hydrophobic part favors compatible with oil phase ( 18 ). Shampoo's conditioning effect is attained by placing conditioning ingredients on to the hair surfaces. These ingredients reduces fiber friction through charge interaction between the negatively charged hair and the positively charged conditioning agent for making the hair feel smooth and silk ( 17 ). Cleansing and conditioning  mechanism of shampoo is given in Figure 2.2.

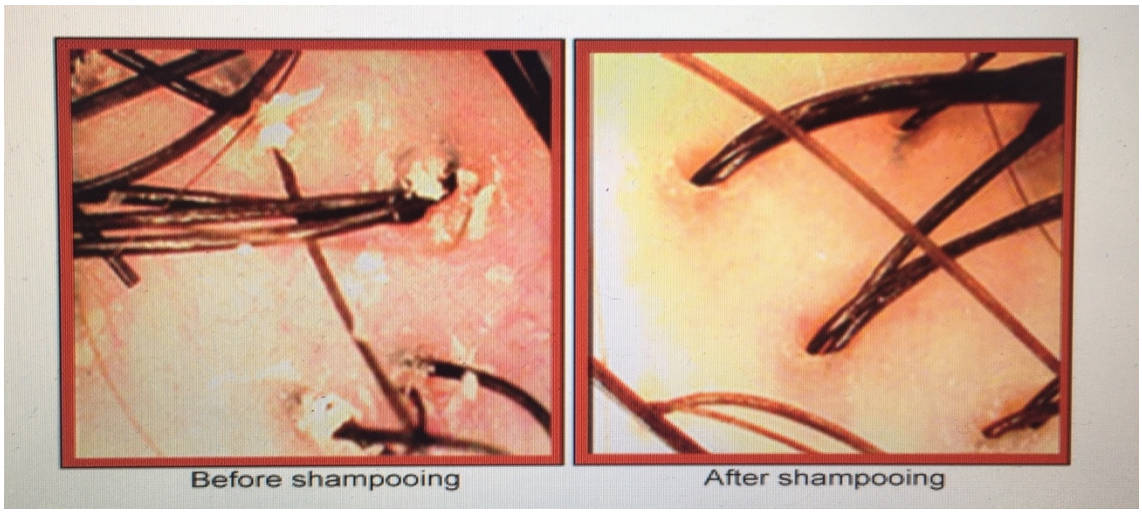


**Figure 2.2.** Cleansing and Conditioning Mechanism of Shampoo (16)

## 2.3 SURFACTANTS

Surfactants have ability to make connection between different phases. They are mainly used for emulsion and suspensions systems in order to as cleansing agents, emulsifying agents, foam boosters, hydrotropes, solubilizing agents and suspending agents ( 19 ). Surfactans contain at least one solvent-loving ( lyophilic ) group and solvent-fearing ( lyophobic ) group and opposing forces in the same molecule form adsorption and aggregation ( 20 ).

Surfactants are classified with respect to nature of hydrophilic head-group as; amphoterics, anionics, cationics and nonionics. Amphoteric surfactants have both negative and positive charge on their hydrophilic end. This property gives them zero

charge. They are derived from imidazoline and classified as Alkylamido Alkylamines. These surfactanst don't sting the eyes and used mostly in baby shampoos ( 21 ). Betaines and alkyl-substituted amino acids ( sodium lauraminopropionate ) are classified as amphoteric surfactants. Anionic surfactants are most commonly used surfactants in laundry detergents. They are obtained from oleochemical and petrochemical sources. They are classified as soaps ( the potassium and ammonium salts), alkyl and alkyl ether sulfates ( lauryl ( ammonium, sodium, triethanolamine, diethonolamine ) sulfate and laureth ( sodium and ammonium ) sulfate; SLS, SLES ), alpha-olefin sulfanates ( AOS ) and miscellaneous anionic surfactants ( sulfosuccinates and fatty glyceryl ether sulfonates ) ( 10, 21 ). Cationic surfactants have positively charged end and generally used in fabric softeners as a substitute in detergents. Cationic surfactants usage in shampoo is more limited than other surfactants (10, 21 ). Nonionic surfactants are other major class of surfactants. They have advantages over anionic surfactants; they have more hardness tolerant and more compatible with enzymes than anionic surfactants ( 21 ).

Today many pharmaceutical and cosmetics products contain surfactants and most of the commercial surfactants are chemically synthesized from petroleum derivatives. Many surfactants cause skin reactions such as irritant contact dermatitis or inflammation. In addition to that, they also cause environmental problems with long-term usage ( 22, 23 ). Natural origin surfactants which are called biosurfactants start to gain importance because of hazardous problems. They are produced by microorganisms and have properties such as biodegradability, low toxicity, ecological acceptability. Biosurfactants are classified into glycolipids, lipopetides, phosholipids, fatty acids and polymeric compounds ( 22 ). Glycolipids are mostly used biosurfactants owing to their physico-chemical properties and biological activities ( 24, 25 ). Sophorolipids, rhamnolipids and mannosylerythritol lipids are glycolipids used mostly in cosmetic industry. Rhamnolipids are most known and used biosurfactants which are produced from *Pseudomonas* bacteria. They are very effective biosurfactants and show antimicrobial activity against several microorganisms such as bacteria and fungi ( 22 ). The chemical structure of rhamnolipids was given in Figure 2.3.

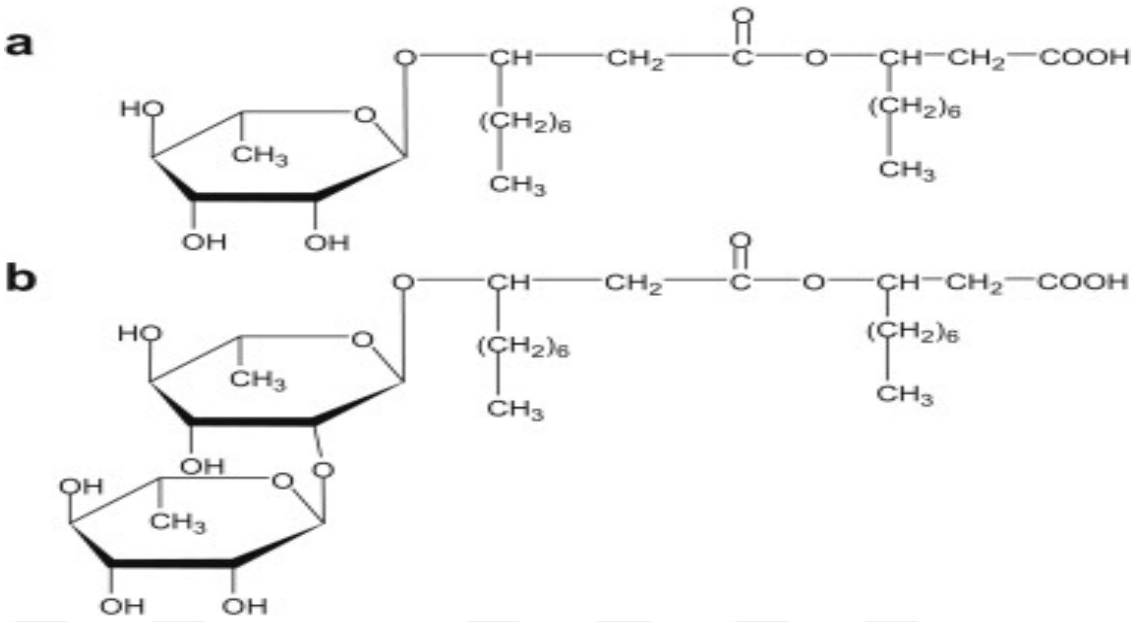


**Figure 2.3.** Chemical Structure of Rhamnolipids; (A) Mono-Rhamnolipid, (B) Di-Rhamnolipid (26)

Rhamnolipids are used in many cosmetics formulations; acne pads, anti-dandruff products, contact lens solutions, deodorants, nail care products, toothpastes etc. ( 27, 28 ). It is expected the increase of rhamnolipids usage in personel care products because of their high surface activities, low toxicity, skin compatibility, low skin irritation properties, benefit product efficiency, efficacy and the economy ( 22, 29, 30 ).

## 2.4 DISSOLVABLE FILM FORMULATION

Dissolvable films have launched in the market recently. This form promise to provide ease of transport and usage over other forms. This technology was developed recently for oral care markets in the form of breath strips and highly accepted by consumers. A fast dissolving film is defined as “an ultra-thin film containing active ingredient that dissolves or disintegrates in within few seconds ( 31 ). There are methods for formulation of dissolvable film. In our thesis, dissolvable film was developed by using solvent casting method ( Figure 2.4. ). In this method, water soluble ingredients are dissolved to form a clear viscous solution. Essential oil encapsulated by SL and mixed with GL and other agents and combined with the bulk. The mixture will be added to the aqueous viscous solution. The final solution poured to six well plate and allowed to dry

Aim of the thesis is creating a personal care formulation which is preservative and detergent free and consists natural origin ingredients in dissolvable film form. Solid

formulation is a novel formulation which provides safe and efficacy product with long shelf life and ease of handling.

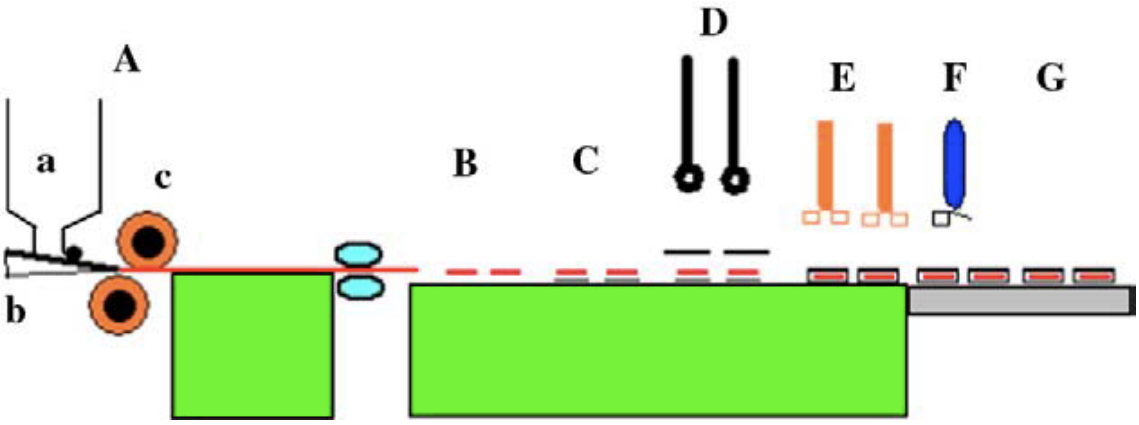


**Fig. 1. Schematic representation of a typical OST manufacturing unit. A – Formation of medicated film takes place. The rollers can be adjusted to get the desired film thickness. After formation of film, it is dried. a – Reservoir for the filmformingmaterials, b – deaerator and film applicator, c – rollers. B – The driedmedicated film is slit and cut into little strips of desiredsize. C – Strips are placedinto lower packagingweb.D– Laser printer prints on upper packagingweb. E – Sealing head seals the strips into single dose sachets. F – Introduction of tear-notch/slit/cut off to sachet. G – Quality control conveyer to final packaging.**

**Figure 2.4.** Schematic Presentation of Dissolvable Film Manufacturing Process (32)

## 2.5 API AND OTHER INGREDIENTS

### 2.5.1 *Citrus* Peel Extract and D-limonene

*Citrus* types are the most economically applicable and broadly grown fruit tree crops in the world and in Turkey. Their fruits are an important source of secondary metobolites for nutrition, health, and industrail implementations. *Citrus* fruits consist lipids ( oleic, linoleic, palmitic, stearic acids, glycerol, phytosterol ), sugars ( glucose, fructose, sucrose ), acids ( citric, malic, tartaric, benzoic, oxalic, succinic acid ), insoluble carbonhydrates ( cellulose, peçtin ), enzymes, flavonoids ( hesperidin, naringin, peel oil ( d-limonene ), volatile constituents, pigments, vitamins and minerals ( 33 ). Their smell is consequence of a complex combination of soluble and volatile compounds and consisting mostly of mono- and sesquiterpenes which are gathered in specialized oil glands in the peel ( flavedo ) and oil bodies in the juice sacs ( 34 ).

Because of *citrus sinensis* peels containing plenty fragrant substances, they are used for extracting essential oils. These extracts are important raw materials for flavoring foods, beverages, perfumes, cosmetics, etc. ( 35 ). For hair care *citrus* species are used for potential source of vitamin C, reducing skin itching and nourishment ( 36 ).

The major constituent in several *citrus* essential oil is *d-limonene* or (R)-(+)-4-iso-proprenil-1-metilcicloesene, a monocyclic mono-terpene listed in the Code of Federal Regulation as flavouring agent. *Limonene* containing essential oils are used also in aromatherapy massage with no reported clinical toxicity ( 37 ).

### 2.5.2 Virgin *Coconut* Oil

Coconut, *Cocos nucifera L.*, is one of the most cultivated plant because of its nutritional and therapeutic properties such as antibacterial, antifungal, antiviral, antiparasitic, antidermatophytic, antioxidant, hypoglycemic, hepatoprotective and immunostimulant ( 38 ). Most commerical coconut oils obtained from copra by smoke drying, sun drying or combination of both methods. On the other hand, Virgin Coconut Oil (VCO) is extracted by a wet process from coconut milk under controlled temperature. VCO has more therapeutic properties than coconut oil which is obtained from copra ( 39 ). VCO consists 92 % of saturated fatty acids almost 70 % of them are lower saturated fatty acids known as medium chain fatty acid (MCFA), especially lauric acid ( C12 : 0 ) and myristic acid ( C14 : 0) ( 40, 41 ). VCO is best solvent for flavours, essences and emulsifiers because of its MCFA composition. Researchers found that coconut oil penetrates more readily into hair fibers because of its polar character ( 42 ). With respect to that we can say using virgin *coconut* oil in shampoo composition provides moisturizing, protecting and anti hair loss effects.

### 2.5.3 Sodium Hyaluronate

Sodium hyaluronate ( Na-HA ) is a naturally occuring non-sulfated glycosaminoglycan which has elasticity and other rheological properties ( 43 ). The structure of Na-HA is given in Figure 2.5. Sodium hyaluronate is found in human body; vitreous of the eye to extracellular matrix of cartilage tissues ( 45 ). Na-HA is not species specific, so there is no need test for skin allergency

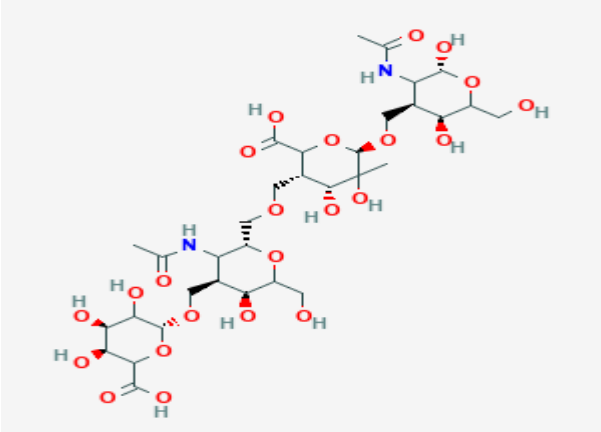

**Figure 2.5.** Chemical Structure of Sodium Hyaluronate (44)

( 46 ). Na-HA has many properties such as viscoelasticity, biocompatibility, hydration and lubrication. Because of these properties, Na-HA can be used food, medical and cosmetic industries ( 47, 48 ). We can say that Na-HA is promising carrier for the use of soap or shampoo formulations.

In our thesis hyalauronic acid purchased from Chemland Co. Ltd. which has molecular weight between 8.000 and 20.000 Da. Due to the Nanjing NutriHerb Biotech Co. Ltd company, hyalauronic acid which has molecular weight between 10.000 - 200.000 Da considered as low molecular weight sodium hyaluronate. Na-HA with low molecular weight penetration is given in Figure 2.6.

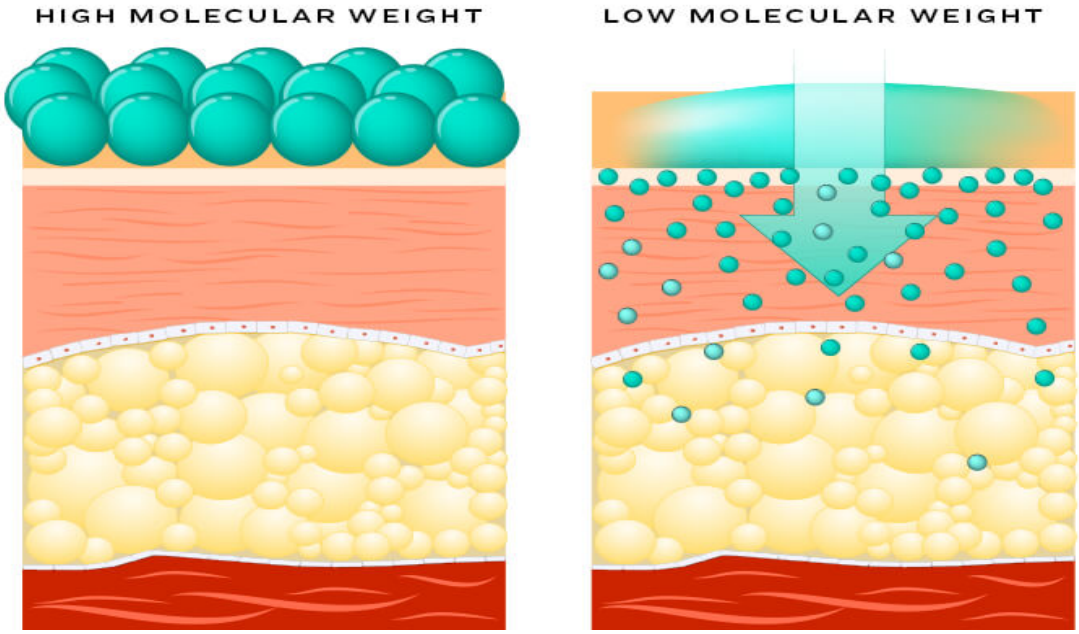


**Figure 2.6.** Penetration of Low Molecular Weight Sodium Hyaluronate (49)

### 2.5.4 Glycolipid

Glycolipid is a compound of fatty acids with a carbohydrate and found in the myelin sheath of nerves ( 50 ). Glycolipids have structural diversity and an ability to decrease surface tension between the surface and interface ( 51 ). Because of that glycolipids used as biosurfactant in many formulations in cosmetics, pharmaceuticals, food etc. Biosurfactants have gained attention because in contrast to other chemical surfactants, they derived from natural origin. Biosurfactants are classified in four categories; glycolipid types, fatty acid type, lipopeptide type and polymer type ( 51 ).

Glycolipid also classified into categories such as, rhamnolipids, sophorolipids, trehalolipids, suc- cinoyl-trehalolipids, lipids of cellobiose or ustilagic acid, mannosyl-erythritol lipids or ustilipids, xylolipids and lipids of oligosaccharides ( 52 ).

Rhamnolipids are best known glycolipids and produced generally by *P. Aeruginosa* yeast. They have higher emulsfying properties and biological activities such as hemolytic, anticancer, antifungal, antibacterial and antiviral ( 53 ).

### 2.6. MICROFLUIDIZER

For the production of nano emulsion, high pressure homogenization technique microfluidization was used. Smaller particle size can be obtained by microfluidizer compared to traditional equipments. The SL and CPE ( DL ) suspension was introduced to inlet resorvoir. Operating pressure of pump was adjested to 15.000 psi. After that the pump drives suspension to the interaction chamber where the suspension were seperated and collided with one another from opposite directions at high velocity and creates shearing action. The microfluidized suspension entered external cooling where ice was placed on the cooling jacket to decrease temperature caused by microfluidization process. After this step, the microfluidized suspension was re-circulated five times in continous mode and collected from outlet (54). Schematic presentation of working principle of Microfluidizer is given in Figure 2. 7.

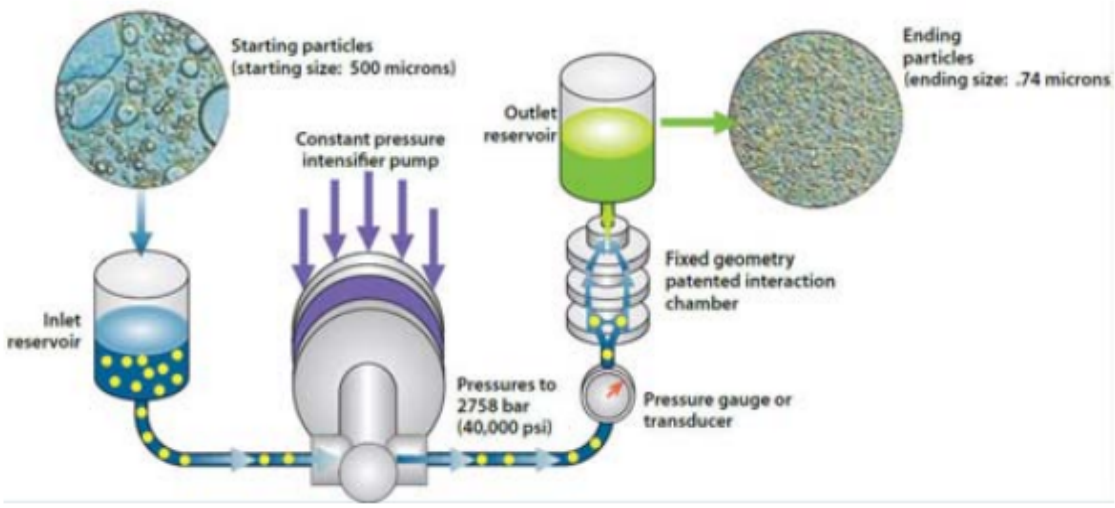


**Figure 2.7.** Working Principle of Microfluidizer (54)

## 2.7 REGULATIONS AND RESTRICTIONS

### 2.7.1 Limonene:

Limonene products are either used directly or further diluted or mixed with other components by industrial end users. Industrial products containing limonene are mainly hand cleaners, industrial cleaning/degreasing products and removers and strippers. The final concentration of limonene in industrial products varies widely and ranges from < 1 % to 95 %. The major use of limonene in consumer products is as flavouring and/or fragrance agents in food, pharmaceuticals and household and cosmetic products. The concentration of limonene in the final consumer products also varies largely and is predominantly low ≤ 1 %, but can be as high as 70 %.

Occupational exposure may occur during the use, transportation and disposal of limonene or limonene products. A high potential for worker exposure during use of limonene or limonene products has been identified due to widespread end uses, modes of applications and lack of control measures at some worksites. Workers are likely to be exposed by skin and eye contact during manual operations and cleaning of equipment, repacking, formulation and end use. There is also a potential for inhalation exposure, especially during use of limonene or limonene products in confined spaces and in places with limited ventilation, heated blending processes, high speed mechanical stirring, worksites with an open mixing process and no exhaust ventilation, and certain modes of applications such as spraying. Deliberate skin contact occurs during use of hand cleaners containing limonene.

Limonene is a flammable liquid and explosive vapour/air mixtures may be formed at temperatures above 48 °C. Its packing group ( III ) indicates that flammability risk is in the lower range. Rags or other combustible material that have been dipped or soaked in limonene may spontaneously combust. Distillation to dryness may also lead to concentration of peroxides and the risk of explosion. D-limonene is readily absorbed by inhalation and ingestion. Dermal absorption is reported to be lower than by the inhalation route. *d*-limonene is rapidly distributed to different tissues in the body, readily metabolised and eliminated primarily through the urine.

Limonene exhibits low acute toxicity by all three routes in animals. Limonene is a skin irritant in both experimental animals and humans. Limited data are available on the potential to cause eye and respiratory irritation. Autoxidized products of d-limonene

have the potential to be skin sensitisers. Limited data are available in humans on the potential to cause respiratory sensitisation.

Autoxidation of limonene occurs readily in the presence of light and air forming a variety of oxygenated monocyclic terpenes. Risk of skin sensitisation is high in situations where contact with oxidation products of limonene occurs.

Potential hazards relevant to public health are skin irritancy and sensitisation from use of consumer products, varying with the concentration of limonene in the product and, for sensitisation, with its oxidation status.

Surfaces of equipment may remain contaminated with limonene and/or limonene products as limonene has low volatility. This may result in dermal contact during handling of equipment. The EASE model that best describes this scenario is non-dispersive use with intermittent contact. According to the EASE model, intermittent contact is assumed to be 2 to 10 events per day involving exposure as part of a process. This results in an exposure of 0.1 to 1 mg/cm$^2$/d. Concentration of d-limonene in consumer products was given in Table 2.1 ( 55 ).

**Table 2.1 - Concentrations of essential oils in consumer products**

| Products | % of Essential Oils |
|---|---|
| Cosmetics and toiletries | 1-5 |
| Personal fragrances | 5-20 |
| Cleaning products | 1-10 |
| Deodorants | 1-10 |
| Food | Very low |
| Compounded essential oil products | |
| Massage oils | 4-5 |
| Products sold in supermarkets | < 2 |

There is no restrictions and regulations for other ingredients such as VCO, GL, Na-HA and SL.

# 3. MATERIALS and METHODS

## 3.1 MATERIALS

### 3.1.1 Raw Materials

*Citrus sinensis* was used in this thesis as plant material produced in Alata Horticultural Research Institue in Mersin. D-limonene standard ( 97 % ) purchased from Alfa-Aesar. Virgin Coconut oil were purchased from Aksuvital. Hyalauronic acid was purchased from Chemland Co. Ltd. Glycolipid ( Rheance® One ) was purchased from Evonik Nutrition & Care GmbH. Cellulose derivatives ( methyl cellulose ) and PVA used as film base materials and glycerol used as plasticizers. Soy lecithin water dispersion (2%) was prepared as nano particle by using Microfluidics equipment at 5 cycle and 15.000 psi and final dispersion was lyophilized. All raw materials used in experiments given in Table 3.1. Hyalauronic acid and glycolipid composition given in Table 3.2., Table 3.3.

**Table 3.1.** Raw Materials Used in the Experiments

| Name | Purpose of Use |
|---|---|
| *Citrus sinensis* Peel Extract (d-limonene) | Cleaning agent, scent |
| Virgin Coconut Oil | Conditioning agent, scent |
| Sodium Hyaluronate (*) | Film forming agent, viscosity modifier, moisturizing agent |
| Glycolipid (**) | Surfactant, cleaning agent, humectant |
| Soy Lecithin | Surfactant |
| Cellulose Derivatives and PVA | Film forming agent |
| Glycerol | Humectant |

**Table 3.2.** Composition of Na-HA as a Film Forming Agent (*)

| Name | Cas No. | % |
|---|---|---|
| Water | 7732-18-5 | 98.04 ± 2.50 |
| Sodium Hyaluronate | 9067-32-7 | 1.00 ± 0.10 |
| Phenoxyethanol | 122-99-6 | 0.90 ± 0.10 |
| Ethylhexylglycerin | 70445-33-9 | 0.06 ± 0.001 |

**Table 3.3** Composition of GL (**)

| Name | EC No. | % |
|---|---|---|
| Rhamnolipids: fermentation products of glucose with Pseudomonas bacteria | 943-175-7 | 40% - 50% |
| Benzoic acid, sodium salt | 208-534-8 | <= 1% |

### 3.1.2 Materials and Equipments

Materials consist of pH indicator paper, tissue, plastic wrap, aluminium foil, polyethylene container, six well plates

Microfluidics M-110EH-30 ( Figure 3.1. ) was used to prepare soy lecithin ( SL ) nano particles. The mechanical tests ( tensile strength ) were performed by using Instron® machine. CPE extract was prepared by using Clevenger apparatus. CPE was analyzed by gas chromatography-mass spectrometry ( GC-MS ). The film surface morphology was evaluated by microscope with AxioCam ERc5s. The particle size distribution of SL was determined by the Zetasizer Nano ( Malvern Instruments ). SL nano particles were lyophilized by vacuum lyophilization machine. Film formulations were mixed by tip sonicator. The SL dispersions were prepared by homogenizator.

## 3.2 METHODS

### 3.2.1 FORMULATION DEVELOPMENT

#### 3.2.1.1 Extraction of *Citrus* Peel Extract

*Citrus sinensis* peel extract was obtained by distillation method in Yeditepe University laboratory ( Figure 3.1. ). *Citrus sinensis* peel removed and cut into smaller pieces around 7 mm size by food chopper. After that *citrus* peels put into the baloon flask and add purified water up to ¾ of the container. Condensed water and oil mixture were collected in a beaker and oil part ( extract ) seperated from mixture. After that some anhydrous sodium sulfate was added to collect water to oil mixture to get rid of all water part.

**Figure 3.1.** Distillation Set-up for Obtaining *Citrus Sinensis* Extract

### 3.2.1.2 Preparation of Dissolvable Film

First of all solvent casting method was used in order to formulate films ( F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, F13, F14, F15 ). Formulations trials were composed by consisted cellulose derivatives ( methyl cellulose ( MC ), carboxy methyl cellulose ( CMC )) and polyvinyl alcohol ( PVA ). The other ingredients of the formulation were Na-HA as a film forming agent, glycerol as a humectant, glycolipid as a surfactant, CPE ( DL ) as a cleaning agent, VCO as a conditioning agent and purified water ( Table 3.1.).

Soy lecithin was prepared as a nano particle by using microfluidics device ( Figure 3.2.) and SL nano dispersions were lyophiilzed. Then CPE ( DL ) and GL were encapsulated by SL in proportions of ( 1:1:1 ). The mixture of CPE + GL (w / wo) + SL were reconstituted with purified water ( F1, F2, F3, F4, F5, F6 ). Mixture was mixed by tip sonicator after addition of other ingredients. Other formulations which were formulate without SL are directly mixed with other ingredients by tip sonicator (F7, F8, F9, F10, F11, F12, F13, F14, F15 ). MC, CMC, PVA dispersions were prepared using mixing method by homogenizator (at 20.000 rpm, 30 minutes ) (F2, F3, F4, F8, F9, F11, F12, F13 ). Those polymer dispersions were prepared at 1 %, 2 % and 5 % concentrations.

Solutions were poured in to the six well plate and put in oven at 50 $^{0}$C, 60 % RH over a night. Finally all mixtures turned to a film form.


**Figure 3.2.** Microfluidics Equipment

### 3.2.1.3 Formulation Optimization

**Table 3.4.** Formulation Trials

| **FC** **Formulation Code** | **MC** Methyl cellulose g | **CMC** Carboxy methyl cellulose g | **PVA** Polyvinyl alcohol g | **G** Glycerol g | **HA** Sodium hyaluronate (1%) g | **GL** Glycolipid g | **SL** Soy lecithin g | **CPE** Peel Extract (DL) g | **VCO** Virgin coconut oil g | **PW** Purified water ml |
|---|---|---|---|---|---|---|---|---|---|---|
| F1 | | | | | 97,4 | 0,2 | 0,2 | 0,2 | 2 | qs. 100 |
| F2 | 2 | | | | | 0,2 | 0,2 | 0,2 | 2 | qs. 100 |
| F3 | | 2 | | | | 0,2 | 0,2 | 0,2 | 2 | qs. 100 |
| F4 | | | 5 | | | 0,2 | 0,2 | 0,2 | 2 | qs. 100 |
| F5 | | | | | 99,8 | 0,2 | | | | qs. 100 |
| F6 | | | | | 97,6 | | 0,2 | 0,2 | 2 | qs. 100 |
| F7 | | | | | 100 | | | | | qs. 100 |
| F8 | 2 | | | 2 | | 0,2 | | | | qs. 100 |
| F9 | | 2 | | 2 | | 0,2 | | | | qs. 100 |
| F10 | | | 5 | 2 | | 0,2 | | | | qs. 100 |
| F11 | 2 | | | 3 | | 0,2 | | | | qs. 100 |
| F12 | | 2 | | 3 | | 0,2 | | | | qs. 100 |
| F13 | | | 5 | 3 | | 0,2 | | | | qs. 100 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| F14 | | | | | 99,6 | 0,4 | | | | qs. 100 |
| F15 | | | | | 99 | 1 | | | | qs. 100 |

### 3.2.2 QUALITY CONTROL TEST OF FILMS

The organoleptic tests, mechanical tests and physicochemical properties of film formulations were performed for the quality control of films. Organoleptic test consists of appearance, color, shape and texture. The texture of films were determined by surface morphology analysis. The pH value of films were determined. Cleaning properties, conditioning properties and the evaluation of eye irritancy tests were applied for quality control of dissolvable films. Tensile strength analysis was performed on the film by ( Instron device. The antimicrobial activity of the films were performed by microbiology analysis.

#### 3.2.2.1 Tensile Strength Analysis

The mechanical properties of the dissolvable film were measured by Instron Machine in Yeditepe University, which was equipped with a 100 N ( 10,2 kg ) load cell. Film strips were cut in to dimensions of 5 mm x 10 mm and held between of wood sticks. After put in machine, film strips were pulled by at a rate of 2 mm/min and the force and elongation were measured when the film strip broke. For comparing film formulations, results of PVA formulations and HA formulations were examined statistically by T-Test ( Student's T-Test ). Analysis procedure is given in Figure 3.3, Figure 3.4. and Figure 3.5.

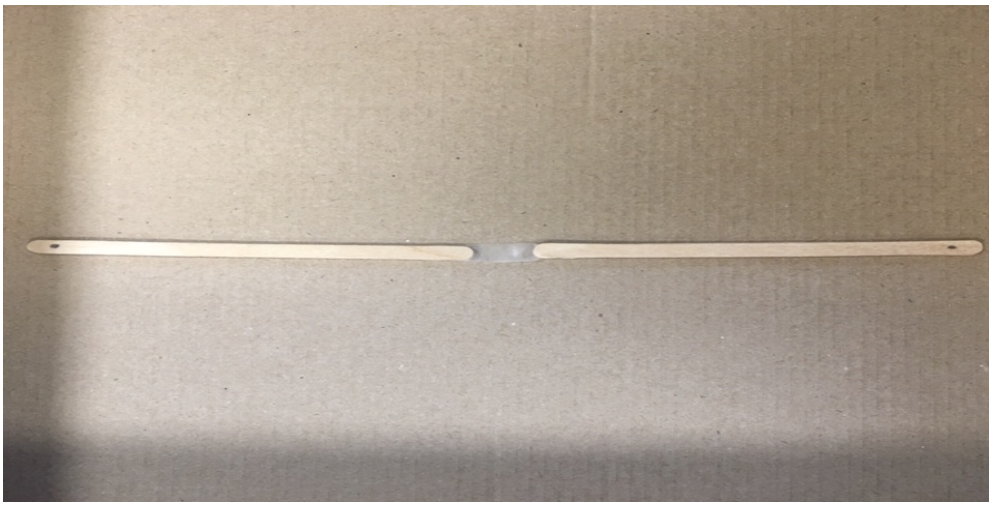

**Figure 3.3.** The Film Installation Process (with Using Wood Sticks)

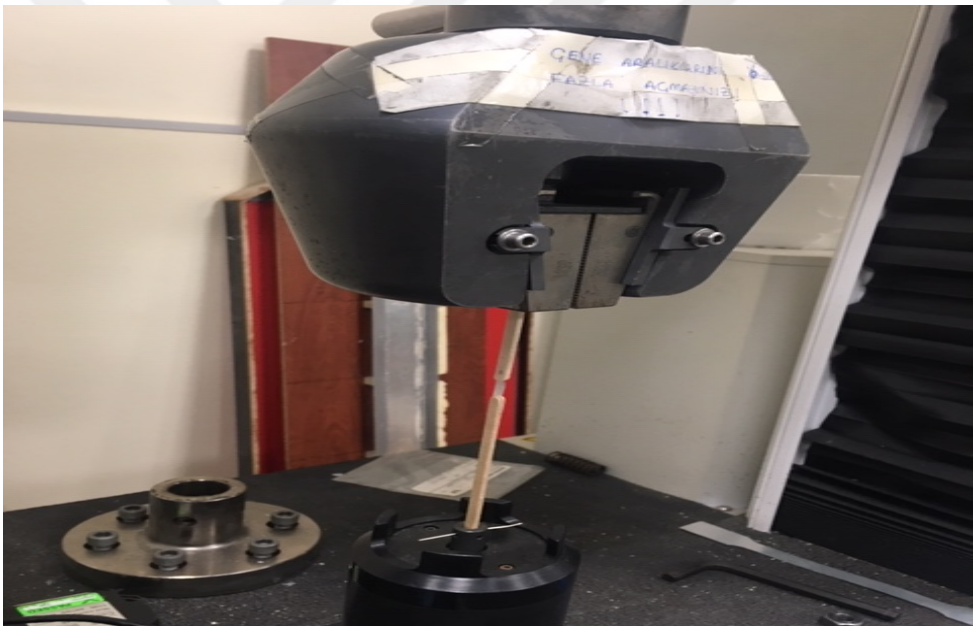

**Figure 3.4** Determination The Tensile Strength of the Film


**Figure 3.5.** Tensile Strength Measurement Process

### 3.2.2.2 Microbiology Analysis

Disc Diffusion ( Agar-Based ) Method was applied for the determination for antimicrobial activity of the formulation. Disc diffusion method was used to test the antibacterial activity of the compounds at 10 mg/ml concentration against *Candida albicans* ( ATCC 10231 ), *Escherichia coli* ( ATCC 10536 ), Pseudomonas aeruginosa ( ATCC 15442 ) and *Staphylococcus aureus* ( ATCC 6538 ) for five complexes. $H_2O$ was used as solvent for all compounds. Standard discs of Ofloxacin 5µg ( Antibacterial Agent) and Nystatin ( Antifungal Agent ) 100 units served as positive controls and references for

the agar-based disc diffusion method. The bacterial and fungal suspensions which achieved the turbidity of the 0.5 McFarland standards were inoculated to Mueller Hinton Agar (bacterial) and Sabouraud 2 % Dextrose Agar (fungal) with sterile ecuvion sticks. Blank paper discs with a diameter of 6.0 mm were impregnated with of the tested concentration of the stock solutions and placed on agar. The zones of growth inhibition around the discs were measured after 18 to 24 hours of incubation at 37 °C and 25 °C for microorganisms. The sensitivities of the microorganism species towards the compounds were determined by measuring the sizes of inhibitory zones on the agar surface around the discs. For disc diffusion, the zone diameters were measured with slipping calipers European Committee on Antimicrobial Susceptibility Testing ( EUCAST ) ( 56 ).

### 3.2.2.3 Surface Morphology Analysis

The surface morphology of the dissolvable film formulations were evaluated by using optical microscope ( ZEISS Axiocam ERc5S ) at 4x zoom. The Anaylsis procedure was shown in Figure 3.6 and Figure 3.7.

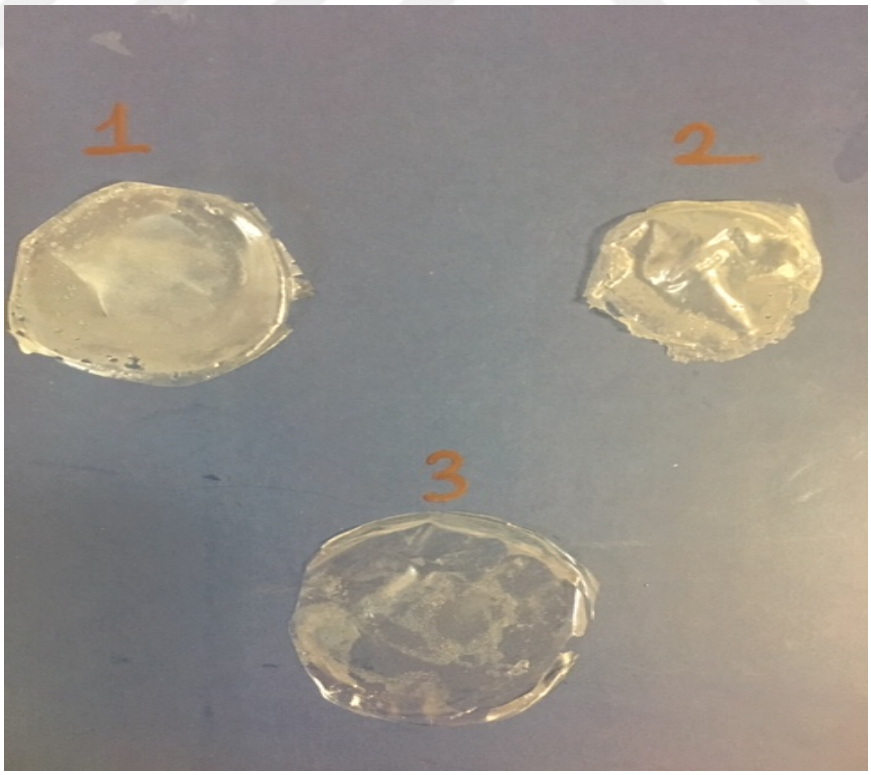


**Figure 3.6.** Film Formulations (***F1***:HA,GL,VCO,DL,SL,DW; ***F2***:HA,VCO,DL,SL,DW, ***F3***:HA,DW)


**Figure 3.7.** Microscobic Analysis

### 3.2.2.4 Measurement of Paricle Size of SL Nano Particle Dispersion

Particule size of SL and SL- CPE- d-limonene combination was determined by using Zetasizer Nano (Malvern Instruments).

### 3.2.2.5 Other Quality Control Studies

The other quality control tests were determination of pH value, cleaning action, conditioning action and skin irritancy tests. Cleaning action, conditioning action and skin irritancy tests was determined by feeling comparison to shampoo in Turkish market.

## 4. RESULTS AND DISCUSSION

### 4.1. FILM FORMULATION EVALUATION

Film formulations (F1-F15) are examined according to mechanical properties, tensile stregth, texture, appearance, conditioning propperties, skin irritancy and pH value ( Table 4.1.)

**Table 4.1** Evaluation of Film Formulations

| Formulation Code( FC) | Mechanical Properties (Tensile Strength (N)) | Film Texture | Cleaning prperties * | Conditioning Properties** | Skin Irritancy *** | pH |
|---|---|---|---|---|---|---|
| **F1** | **5,71±3129** | **homogeneous transparent, and smooth** | **1** | **1** | **3** | **6** |
| F2 | 15,85±4462 | Homogenous, not transparent and not smooth | 1 | 2 | 2 | 7 |
| F3 | 21,46±3781 | Homogenous, not transparent and not smooth | 1 | 2 | 2 | 7 |
| F4 | 41,25±2638 | Homogenous, not transparent and not smooth | 1 | 2 | 2 | 7 |
| F5 | - | homogeneous, transparent, and smooth | 2 | 3 | 3 | 6 |
| F6 | - | homogeneous, transparent, and smooth | 1 | 1 | 3 | 7 |
| F7 | - | homogeneous, transparent, and smooth | 3 | 2 | 3 | 7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| F8 | - | Not homogenous, not transparent and foamy | 2 | 3 | 1 | 7 |
| F9 | - | Not homogenous, not transparent and foamy | 2 | 3 | 1 | 7 |
| F10 | - | Not homogenous, not transparent and foamy | 2 | 3 | 1 | 7 |
| F11 | - | Not homogenous, not transparent and foamy | 2 | 3 | 1 | 7 |
| F12 | - | Not homogenous, not transparent and foamy | 2 | 3 | 1 | 7 |
| F13 | - | Not homogenous, not transparent and foamy | 2 | 3 | 1 | 7 |
| F14 | - | homogeneous, transparent, and smooth | 2 | 2 | 3 | 6 |
| F15 | - | homogeneous, transparent, and smooth | 2 | 2 | 3 | 6 |

***1:**Excellent, 2:Good, 3:Low

**1:Excellent, 2:Good, 3:Low

***1:High, 2:Mild, 3:Low

The final formulation was chosen by considering physicochemical properties, mechanical properties (tensile strength), film texture, cleaning properties, conditioning properties and skin irritancy tests. Quality control studies such as cleaning properties, condtioning properties and skin irritancy tests were evaluated by asking ten voluenteers

their observations. The evaluation for cleaning and conditioning properties consisted of three criteria which were 1 means excellent, 2 means good, 3 means low. For skin irritancy test the evaluation consisted of three criteria 1 means high, 2 means mild and 3 means low. The tensile strength results give the average and the standard deviation of 4 measurements (F1, F2, F3, F4). The tensile strength values of films (MC, CMC, PVA, Na-HA) varied with a range of (5,71±3129 - 41,25±2638).

As seen in Table 4.1; F1, F6 and F7 formulations have better physical and chemical properties then others. F2, F3, F4, F8, F9, F10, F11, F12, F13 formulations which are containing cellulose derivatives and PVA film texture were fragile, not transparant, not homogenous. Also their pH values were found around 7. In contrast film texture of formulations which are containing Na-HA base ( F1, F5, F6, F7, F14, F15 ) were generally homogenous, transparent and smooth. Their pH values were found around 6. Film texture of formulations which are containing glycerol ( F8, F9, F10, F11, F12, F13 ) were not homogenous and foamy. Their pH values were found around 7. Skin pH is around 6, so it is important to personal care product that have pH value around this number. pH value of formulations F1, F5, F6, F14 and F15 are 6 which is suitable for skin usage and pretty good feeling. Film texture is important for using product efficiently and fastly. Formulations which have low tensile strength are more convenient for usage as personal care products. GL and soy lecithin are natural origin surfactants and enable homogenous formulation. HA is more convenient for using as base material because it provides moisture effect besides its low tensile strength. CPE (DL) have better cleaning properties. VCO gives conditioning properties and nice odor to the formulation. F1 was choosen the final formulation because it had better properties than other formulations. The final formulation recepie is given in Table 4.2.

**Table 4.2.** The ingredients of a final personal care formulation (F1)

| **Primary Ingredients** | |
|---|---|
| Surfactants ( SL ) | 0,2 % |
| Base ( HA ) | 1 % |
| Purified Water | q.s. 100 ml |
| **Additional Ingredients** | |
| Cleaning agents ( CPE, GL) | 0,4 % |
| Conditioning agents ( VCO) | 2 % |

## 4.2 GC- MS RESULTS

GC-MS analysis results of *citrus sinensis* peel extract which were obtained by steam explosion and cold press methods given in Fig.10. GC-MS analysis performed in Alata Horticultural Research Institue in Mersin. In this thesis, *Citrus sinensis* peel extract which was obatined by steam explosion method contains 94.98 % d-limonene was used in the formulations Figure 4.1.

| RT | Bileşen | Distillation | Cold Press | Limonen Std. |
|---|---|---|---|---|
| 11,386 | α-pinene | 0,48 | 0,46 | 0,30 |
| 15,397 | Sabinene | 0,60 | 0,34 | 0,27 |
| 16,616 | δ-3-carene | 0,43 | | |
| 17,210 | Myrcene | 1,87 | 1,84 | 1,84 |
| 18,991 | Limonene | 94,98 | 96,52 | 97,13 |
| 19,330 | β-phellandrene | 0,32 | 0,31 | 0,25 |
| 31,932 | Linalool | 0,61 | 0,53 | |
| 32,250 | Octanol | 0,53 | | |
| 36,742 | α-terpineol | 0,18 | | |
| 38,179 | Carvone | | | 0,21 |

**Figure 4.1.** Results of GC-MS Analysis

## 4.3 TENSILE STRENGTH ANALYSIS RESULTS

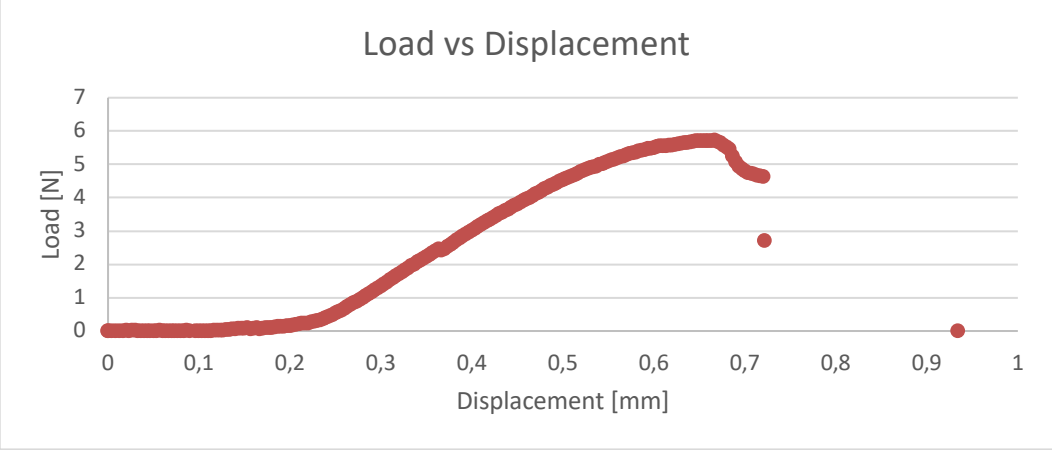


**Figure 4.2.** Load vs Displacement Graph

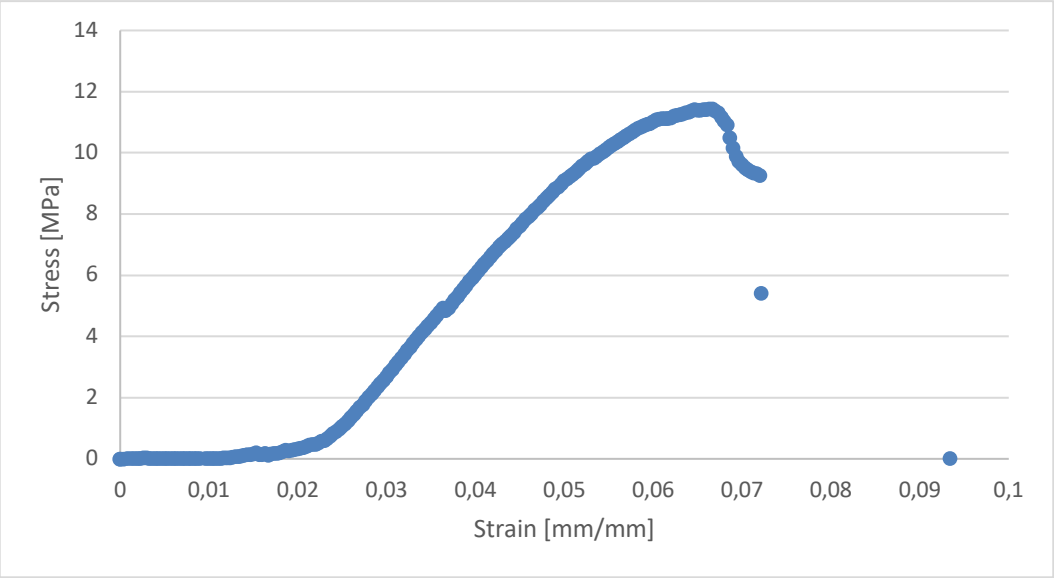


**Figure 4.3.** Stress vs Strain Graph

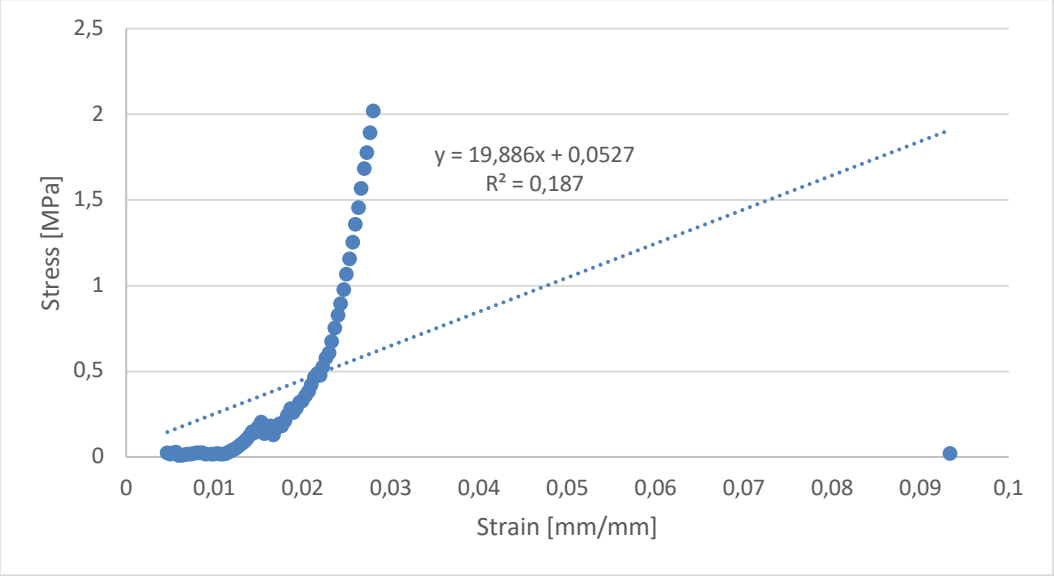


**Figure 4.4.** Stress vs Strain Graph (Slope Calculation)

A statistic T-Test ( Student's T-Test ) was applied in order to show the significant difference between the means of two groups PVA and Na-HA. As seen in Table 4.3, there are istatistically differences in both elongation and load variables of two groups. P value was calculated < 0,001. The average of PVA group is bigger tha Na-HA group that shows, tensile strength of PVA group is higher than Na-HA group.

Normally in dissolvable film formulations, tensile strength is appropriate if it has high endurance. Films which are prepared with cellulose derivatives and PVA have high tensile strength in comparison with Na-HA. Our product for personal cleaning usage so

we don’t want high tensile strength in contrast low tensile strength is more suitable for using as shampoo.

**Table 4.3. T-Test Results (P Value)**

| | Group | N | Mean | Std. Deviation | Std. Error Mean | P value |
|---|---|---|---|---|---|---|
| Elongation | PVA | 419 | 4,38222 | 2,691014 | ,131465 | 0,000* |
| | Na-HA | 218 | ,25594 | ,149301 | ,010112 | |
| Load | PVA | 419 | 32,64474 | 7,646517 | ,373557 | 0,000* |
| | Na-HA | 218 | 2,28873 | 1,668476 | ,113003 | |

## 4.4 MICROBIOLOGY ANALYSIS RESULTS

**Figure 4.5.** Antimicrobial Activity (Inhibition Zone Measurement)

Results for *S.aureus*

**1: PK: (control group)** Antibiotic ofloxacin

**2:** HA

**3:** Film formulation (Sodium hyaluronate + Glycolipid (GL)

+Virgin coconut oil (VCO) + Citrus peel extract (CPE- (D-limonene) + Soy lecithine SL

**4:** CPE (D-limonene)

**5:** Glycolipid (GL)

**6:** Virgin coconut oil (VCO)

**Table 4.4.** Antibacterial and Antifungal Activities of Compounds (inhibition zone)

| Compound | Conc. Of test Compound | Zone of Inhibition (diameter in mm) *C. albicans* ATCC 10231 | Zone of Inhibition (diameter in mm) *E. coli* ATCC 10536 | Zone of Inhibition (diameter in mm) *S. aureus* ATCC 6538 | Zone of Inhibition (diameter in mm) *P. aeruginosa* ATCC 15442 |
|---|---|---|---|---|---|
| Ofloxacin (Antibacterial Agent) | 5 µg | - | 32 | 40 | 35 |
| Nystatin (Antifungal Agent) | 100 units | 35 | - | - | - |
| Virgin Coconut Oil | 10 mg/ml | - | - | - | - |
| D-Limonene Standard (%97) | 10 mg/ml | 15 | 10 | 15 | 15 |
| Sodium Hyaluronate | 10 mg/ml | - | - | - | - |
| Glycolipid | 10 mg/ml | 15 | 25 | 25 | 25 |
| HA + GL +VCO + CPE (D-limonene) + SL | 10 mg/ml | 15 | 20 | 20 | 20 |

Antimicrobial activity of the formulation was performed against *E.coli*, *S.aureus* and *P. aeruginosa*. *E.coli* is a bacteria which is generally found in lower instestine warm-blooded organisms ( 57 ). *E.coli* is a gram-negative bacterium and some strains of them can cause gastroenteritis, meningitis and wound infections ( 58 ). *S.aureus* is a gram-positive bacterium and can grow both aerobically or anaerobically. It is found on the skin and mucous membranes. *S. aureus* doesn't cause infection on healthy skin but if it enters the bloodstream, it can cause serious infections ( 59 ). *P. aeruginosa* is a gram-negative bacterium found in soil and water. It can causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, gastrointestinal infections and a variety of systemic infections ( 60 ).

As seen in Table 2. F1 formulation has the antimicrobial activity for against *E.coli, S. aureus* and *P. aeruginosa*. It was found that there wasn't any antifungal effect according to microbiology test.

## 4.5 SURFACE MORPHOLOGY ANALYSIS RESULTS

Surface morphology analysis was applied to three optimized formulations ( F1, F6 and F7 ) which are shown in Figure 4.6, Figure 4.7 and Figure 4.8.

**Figure 4.6.** Surface Appearance of Formulation ***F1***

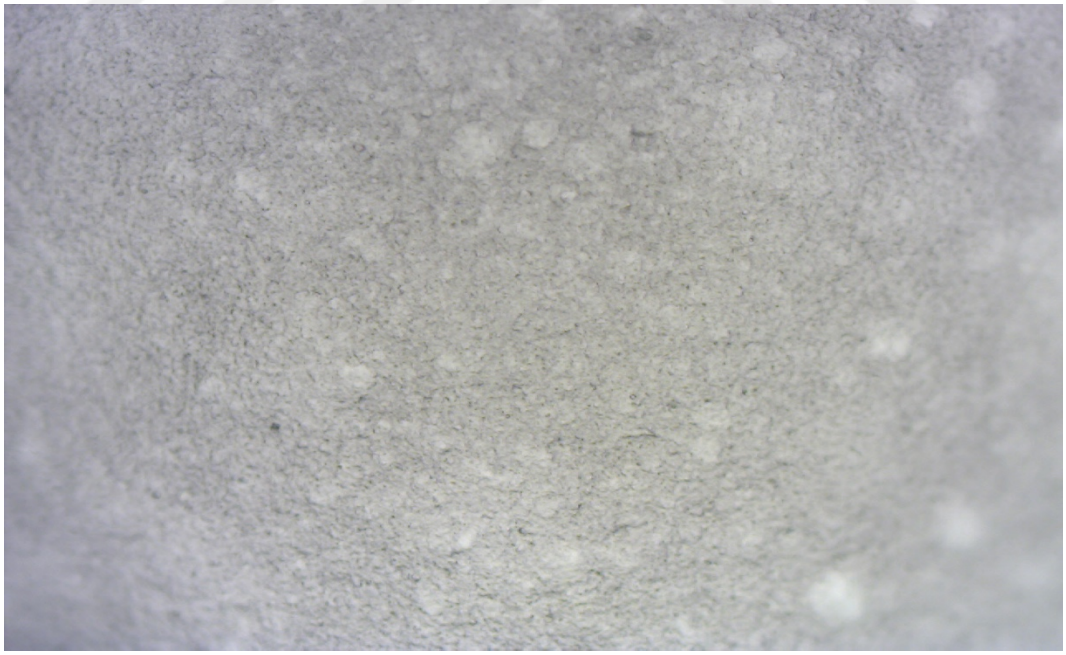

**Figure 4.7.** Surface Appearance of Formulation ***F6***

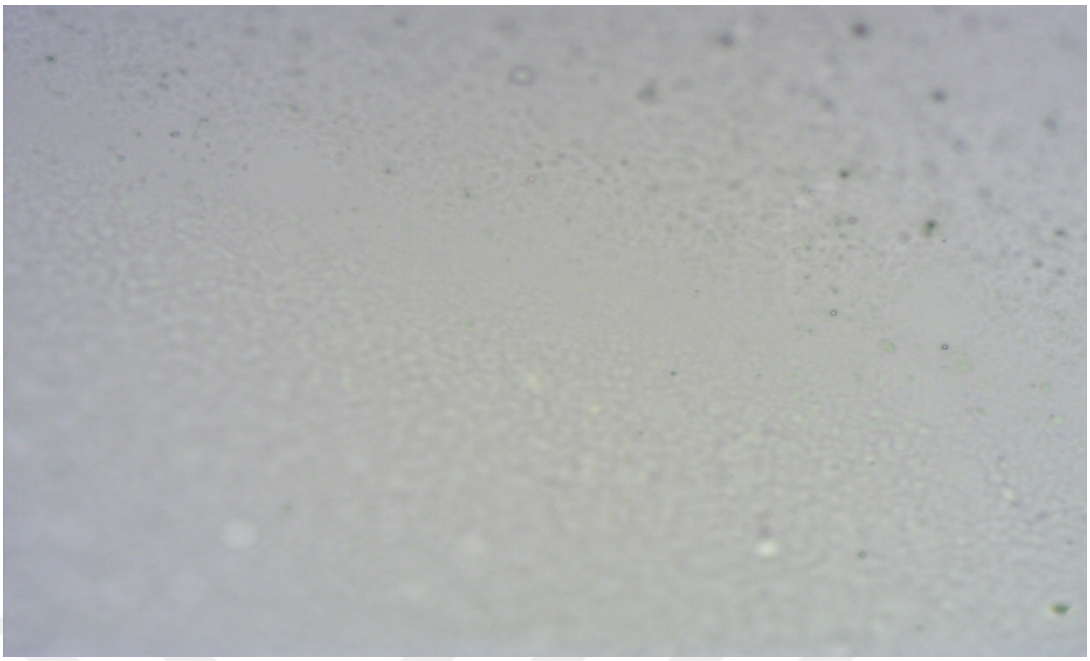

**Figure 4.8.** Surface Appearance of Formulation ***F7***

As seen in figures, all of three formulations had smooth surface with some pores, which is an indication of uniform distrubition of all ingredients.

## 4.6 MEASUREMENT OF PARTICLE SIZE RESULTS

**Results**

| | | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|---|
| **Z-Average (d.nm):** | 174,7 | **Peak 1:** | 285,5 | 93,5 | 189,0 |
| **PdI:** | 0,412 | **Peak 2:** | 29,51 | 4,6 | 8,019 |
| **Intercept:** | 0,930 | **Peak 3:** | 4359 | 1,9 | 938,1 |
| **Result quality :** | Good | | | | |

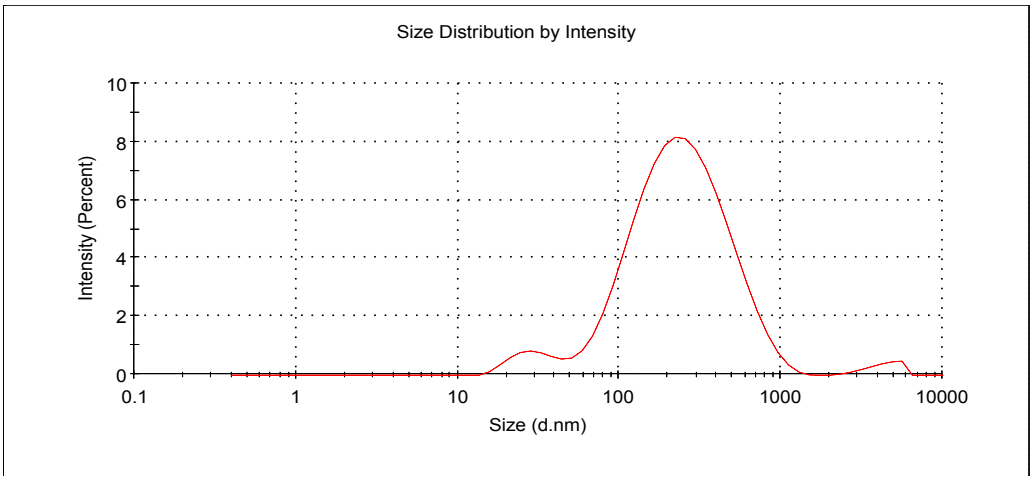


**Figure 4.9.** Particle Size Result of Soy Lecithin and Essential Oil

Results

| | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|
| Z-Average (d.nm): 103,4 | Peak 1: | 138,5 | 98,9 | 74,92 |
| PdI: 0,282 | Peak 2: | 4367 | 2,0 | 935,4 |
| Intercept: 0,938 | Peak 3: | 19,02 | 1,1 | 3,561 |

Result quality : Good

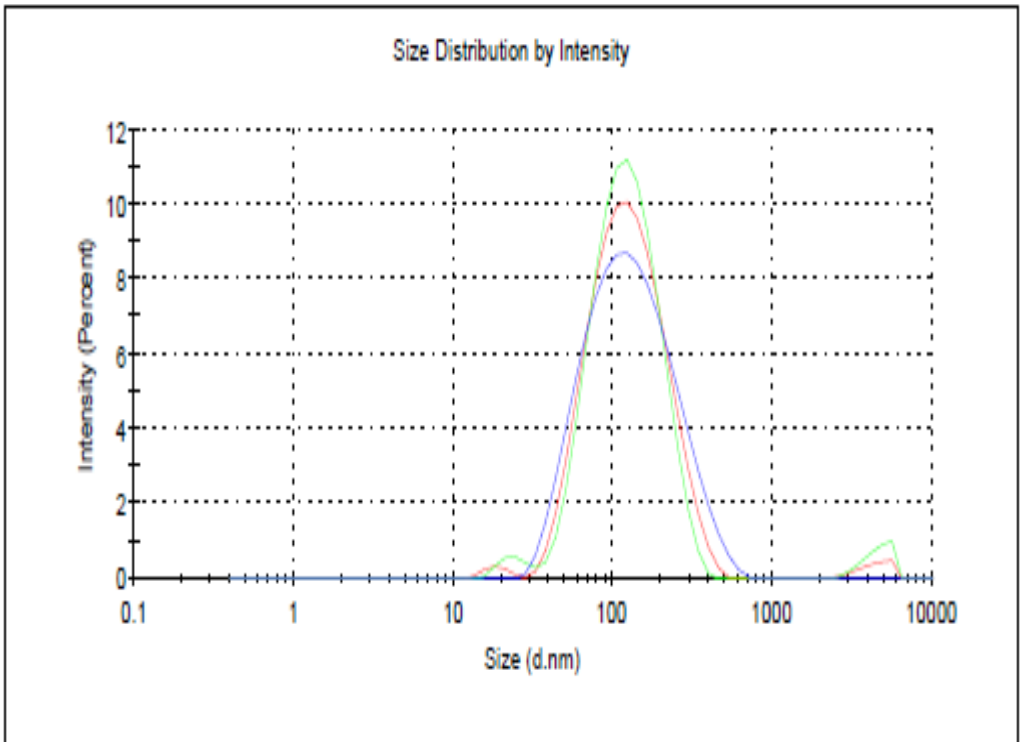


**Figure 4.10.** Particle Size Result of Soy Lecithin

Before using in formulations, SL was prepared as nano particle. After that CPE was encapsulated by SL for ease of incorporation to the HA base and increasing the solubility. As seen in figures, both SL and SL with CPE polydispersity index ( PDI ) values are smaller than 0,5 which shows its nano particle property. The encapsulation of CPE, size is obtained around 174,7 nm ( 93,5 % ) which shows homegeneous encapsulation ( Figure 4.9. ).

## 5. CONCLUSION

As a conclusion, there is need in the market for personel cleansing products which has natural origin ingredients and safe formulations. In our thesis we formulate dissolvable film product which contains natural origin oil, herbal extract, natural surfactants and carrier.

In optimized film formulation (F1), nano particle SL used for encapsulation of CPE. GL was used as natural and non-ionic surfactant, Na-HA was used for film forming agent, CPE ( DL ) was used for cleaning agent and VCO was used for conditioning agent. The final film formulation (F1) was decided with respect to quality control studies results. According to mechanical test results, the tensile strength of the F1 was evaluated 5,71±3129. According to surface morphology analysis results, F1 was homogenous, transparant and smooth. According to microbiology analysis results, F1 had mild antimicrobial activities against *E.coli*, *S.aureus* and *P. aeruginosa*. According to pH results, F1 had pH value 6. According to cleaning properties results which was observed by using 10 volunteers, F1 had 1 which was excellent. According to conditioning properties results which was observed by using 10 volunteers, F1 had 1 which was excellent. According to skin irritancy test results, F1 had 3 which was low skin irritancy.

Usage of natural ingredients like VCO and CPE ( DL ) help to revive human hair and scalp which was damaged by using chemical treatments. More over because of using natural surfactants ( GL, SL ) same efficieny was attained instead of using harsh chemicals. Human hair comprises mainly keratin. When personal care products that contain harsh chemicals is used, the structure of hair is damaged and these chemicals also lead the development of cancer or other serious illness. In this thesis product stability and safeteness was improved by using dissolvable film form and natural surfactants . The form also provides ease of transportation and help environment that contain natural ingredients. It is important to provide formulation which is effective as other products that contain harmful chemicals.

It is expected that natural origin personal care products will spread in future. Awaraness of harm effects of synthetic chemicals to human body and environment will change buyer choices. By developing technologies, we can see personel care products in different forms which are preservative free.

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## 7. APPENDIX A

### 7.1. RESUME

**Kişisel Bilgiler**

| | | | |
|---|---|---|---|
| **Adı** | Övgü | **Soyadı** | ULUN |
| **Doğum Yeri** | K.Maraş | **Doğum Tarihi** | 19.04.1990 |
| **Uyruğu** | TC | **TC Kimlik No** | 31540697252 |
| **E-mail** | ovguulun@gmail.com | **Tel** | (538) 829 37 47 |

**Öğrenim Durumu**

| Derece | Ala | Mezun Olduğu Kurumun | Mezuniyet |
|---|---|---|---|
| **Doktora** | - | - | - |
| **Yüksek** | İlaç ve Kozmetik Üre. | Yeditepe Üniversitesi | - |
| **Lisans** | Kimya ve Biyoloji | Koç Üniversitesi | 2013 |
| **Lise** | Anadolu Lisesi | Ted Mersin Koleji | 2008 |

| **Bildiği Yabancı Dilleri** | **Yabancı Dil Sınav Notu (□)** |
|---|---|
| İngilizce | YDS:72, TOEFL: 543 |
| | |

□ Başarılmış birden fazla sınav varsa(KPDS, ÜDS, TOEFL; EELTS vs), tüm sonuçlar yazılmalıdır

**İş Deneyimi (Sondan geçmişe doğru sıralayın)**

| Görevi | Kurum | Süre (Yıl - |
|---|---|---|
| Ar-Ge Proje Yöneticisi | Lamas Biyoteknoloji | 2018- |
| Ar-Ge Proje Yöneticisi | BMT Teknoloji ve Proses | 2014-2017 |

**Bilgisayar Bilgisi**

| Program | Kullanma becerisi |
|---|---|
| Microsoft Office Programları | Çok İyi |
| Mat Lab | Orta |
| Aspen HYSYS | Orta |

*Çok iyi, iyi, orta, zayıf olarak değerlendirin

**Bilimsel Çalışmaları**

| |
|---|
| |
| |

**SCI, SSCI, AHCI indekslerine giren dergilerde yayınlanan makaleler**

**Diğer dergilerde yayınlanan makaleler**

| |
|---|
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**Uluslararası bilimsel toplantılarda sunulan ve bildiri kitabında (*Proceedings*) basılan bildiriler**

| |
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**Hakemli konferans/sempozyumların bildiri kitaplarında yer alan yayınlar**

| |
|---|
| |
| |

**Diğer (Görev Aldığı Projeler/Sertifikaları/Ödülleri)**

| |
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