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YEDİTEPE UNIVERSITY  
INSTITUTE OF HEALTH SCIENCES  
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TECHNOLOGIES

**DEVELOPMENT OF MICROEMULSION  
ALGINATE-BASED HYDROGEL FORMULATION  
CONTAINING ROSE HIP BY USING  
MICROFLUIDICS TECHNIQUE**

MASTER OF SCIENCE THESIS

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## THESIS APPROVAL FORM

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### APPROVAL

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## **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.

2021/06/15

Sibel Dinçer





*To the guardian angels of my life: my lovely mom and dad*

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## LIST OF SYMBOLS AND ABBREVIATIONS

|                   |                                |
|-------------------|--------------------------------|
| BC                | Bicontinuous                   |
| Ca <sup>2+</sup>  | Calcium ion                    |
| CaCl <sub>2</sub> | Calcium chloride               |
| C <sub>1</sub>    | Capric acid                    |
| CMC               | Critical Micelle Concentration |
| DLS               | Dynamic Light Scattering       |
| EtOH              | Ethyl alcohol                  |
| F                 | Formula                        |
| HLB               | Hydrophilic-Lipophilic Balance |
| kHz               | Kilohertz                      |
| La                | Lauric acid                    |
| Li                | Linoleic acid                  |
| μm                | Micrometer                     |
| mm                | Millimeter                     |
| mV                | Millivolts                     |
| M                 | Myristic acid                  |
| nm                | nanometre                      |
| O                 | Oleic acid                     |
| o/w               | Oil-in-water                   |
| PdI               | Polydispersity index           |
| PMMA              | Polymethylmethacrylate         |
| S                 | Sample                         |
| S <sub>1</sub>    | Stearic acid                   |
| SC                | Stratum Corneum                |
| SEM               | Scanning Electron Microscope   |
| sj                | Subject                        |
| TLC               | Thin Layer Chromatography      |
| TEWL              | Transepidermal Water Loss      |
| UV                | Ultraviolet                    |
| w/w               | Weight per weight              |
| w/o               | Water-in-oil                   |

## ABSTRACT

**Dincer, S. (2021). Development of Microemulsion Alginate-based Hydrogel Formulation Containing Rose hip by Using Microfluidics Technique. Yeditepe University, Institute of Health Science, Department of Drug and Cosmetic Production Technologies. MSc Thesis, Istanbul.**

Nowadays cosmetic products that contain natural ingredients, compatible with the skin, non-toxic, not synthetically derived are in great demand. In addition, it is preferred the products that guarantee contribute to biodiversity preservation and more sustainable sourcing. This study was therefore designed to develop a microemulsion formulation containing natural rose hip, virgin coconut oil, and shea butter and incorporate them into the alginate-based hydrogel produced by a T-junction microfluidic technique. Thereby, it was designed to penetrate gradually an efficient amount of vitamin C, fatty acids, and phospholipids that were included in the beneficial natural ingredients to the skin via hydrogel without degradation. In the formula, rose hip, known for its high vitamin C content and natural oils which are rich content, compatible with the skin due to their structure and edible were preferred. A study was conducted with the rose hip used in the formula and the vitamin C content was investigated by using the thin layer chromatography (TLC) to decide the most efficient rosehip extract. The formula containing rose hip, virgin coconut oil, shea butter, and lecithin was developed by using the probe-sonicator and the formula obtained in nano-sized particles was characterized by utilizing the dynamic light scattering (DLS). The hydrogel structure prepared by the rosehip formula incorporated with monodispersed alginate microbubbles produced by utilizing the T-junction microfluidic technique was characterized by Scanning Electron Microscopy (SEM). In addition, application studies with a volunteer were conducted to examine the efficiency of the prepared hydrogel on the moisture and oil content of the skin. It was concluded that there was a significant difference in the study conducted with the volunteer ( $p < 0.05$ ). The obtained result showed that the hydrogel generated with the microfluidic system is a promising carrier for natural ingredients to be penetrated into the skin.

**Key Words:** Rose hip, vitamin C, alginate-based hydrogel, virgin coconut oil, shea butter, microfluidic method, microbubble

## ÖZET

**Dinçer, S. (2021). Mikroakışkan Tekniği Kullanılarak Kuşburnu İçeren Mikroemülsiyon Aljinat-bazlı Hidrojel Formülasyonunun Geliştirilmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, İlaç ve Kozmetik Üretim Teknolojileri Anabilim Dalı. Yüksek Lisans Tezi, İstanbul.**

Günümüzde doğal içerikli, ciltle uyumlu, toksik olmayan, sentetik türevli olmayan kozmetik ürünler büyük talep görmektedir. Ayrıca biyoçeşitliliğin korunmasına ve daha sürdürülebilir kaynak kullanımına katkı sağlamayı garanti eden ürünler tercih edilmektedir. Bu nedenle bu çalışma, doğal kuşburnu, saf hindistancevizi yağı ve karite yağı içeren bir mikroemülsiyon formülasyonu geliştirmek ve bunları bir T-bağlantılı mikroakışkan tekniği ile üretilen aljinat bazlı hidrojel içine dahil etmek için tasarlanmıştır. Bu sayede faydalı doğal içeriklerde bulunan C vitamini, yağ asitleri ve fosfolipitlerin etkin miktarda hidrojel yoluyla kademeli olarak cilde bozulmadan nüfuz etmesi için tasarlanmıştır. Formülde yüksek C vitamini içeriği ile bilinen kuşburnu ve zengin içeriğe sahip, yapısı gereği cilt ile uyumlu ve yenilebilir doğal yağlar tercih edilmiştir. Formülde kullanılan kuşburnu ile bir çalışma yapılmış ve en verimli kuşburnu özüne karar vermek için ince tabaka kromatografisi (TLC) kullanılarak C vitamini içeriği araştırılmıştır. Kuşburnu, saf hindistan cevizi yağı, karite yağı ve lesitin içeren formül, prob-sonikatör kullanılarak geliştirilmiş ve nano boyutlu partiküllerde elde edilen formül, dinamik ışık saçılımı (DLS) kullanılarak karakterize edilmiştir. T-bağlantı mikroakışkan tekniği kullanılarak üretilen tekil dağılımlı aljinat mikrokabarcıkları ile birleştirilmiş kuşburnu formülü ile hazırlanan hidrojel yapısı, Taramalı Elektron Mikroskobu (SEM) ile karakterize edilmiştir. Ayrıca hazırlanan hidrojelin cildin nem ve yağ içeriği üzerindeki etkinliğini incelemek için gönüllü ile uygulama çalışmaları yapılmıştır. Gönüllü ile yapılan çalışmada anlamlı farklılık olduğu sonucuna varılmıştır ( $p<0.05$ ). Elde edilen sonuç, mikroakışkan sistem ile üretilen hidrojelin, doğal bileşenlerin cilde nüfuz etmesi için umut verici bir taşıyıcı olduğunu göstermiştir.

**Anahtar Kelimeler:** Kuşburnu, C vitamini, aljinat bazlı hidrojel, sızma hindistan cevizi yağı, shea yağı, mikroakışkan yöntem, mikro kabarcık

## 1. INTRODUCTION AND PURPOSE

In recent years, there has been growing interest in natural products. Although it has recently become significant to develop high-impact products in cosmetics, it also has grown into being of the essence for the products to have qualities such as environmentally friendly, natural, and sustainable. Products containing non-toxic, biodegradable, natural, compatible components have been increasingly preferred. Natural products supply not only human wellbeing but also protection of the environment and biodiversity.

Developing technologies have been greatly influencing and changing cosmetics, and so they are aimed to overcome previously impeding conditions such as insufficient skin permeability, low stability, and incompatibility. Multi-disciplinary studies have been contributing to the development of the cosmetics industry and cosmetic products. The interest in nanoscale systems has noticeably grown in recent years. Nanoscale emulsions like microemulsions have constituted a highly promising technology to boost enhanced skin absorption in topical applications. Recent searches show that nanoscale cosmetics formulations present advantages like an effective release profile of ingredients and escalated skin penetration comparing to other conventional formulations. It also makes it possible to develop more stable cosmetics formulations and ease of product preparation with oil and water-soluble ingredients.

Microfluidics technique allows for precise control and manipulation of fluids on a small scale. It has had a raised interest in recent years due to its potential applications in different fields such as biotechnology, biochemistry, nanotechnology, and pharmaceutical. The microfluidic technique provides the production of monodisperse microbubbles, thus it facilitates the generation of regular porous hydrogels.

The aim of the study explained in this thesis is to develop a hydrogel generated by the T-junction microfluidic technique containing a rose hip formula and investigate its moisture effect on the skin. It was intended to develop a natural product in terms of processes, formulations, and ingredients while developing a formula. Also, it was desired to design not only a beauty product but also a product that is in perfect harmony with the skin and nature.

In this study with considering this information, microbubbles produced by a T-junction microfluidic method were incorporated with the natural ingredients formula including rose hip, virgin coconut oil, and shea butter, and then the porous hydrogel was developed and investigated. In addition, the developed formula and the generated hydrogel were characterized and the effect of the hydrogel on the skin was researched by conducting a study with the volunteer.

For this purpose:

- Rose hip extracts were analyzed.
- The formula including rose hip, virgin coconut oil, shea butter, and lecithin was developed and characterized.
- Microbubbles by the T-junction microfluidic technique to be used for hydrogel formation were produced and examined.
- The hydrogel incorporated with the developed rose hip formula was produced and characterized.
- Studies with the volunteer were carried out to observe the efficiency of the prepared hydrogel on moisture and oil content of the skin.

## 2. LITERATURE REVIEW

### 2.1. Microemulsions

Colloidal systems which are made by two immiscible liquid where one phase is dispersed as droplets in the other phase are called an emulsion. Ordinary emulsion, also called conventional emulsion or macroemulsion, is thermodynamically unstable and its average droplet size between 0.1 - 100  $\mu\text{m}$ . It has a turbid appearance. Microemulsion and macroemulsion terms seem similar but in point of fact, they are quite different. A microemulsion is specified as a thermodynamically stable emulsion [1]. The microemulsion concept was first introduced in the 1940s by Schulman and Hoar. They used this term when they obtained a translucent system from the turbid unstable ordinary emulsion by adding alcohol [2].

Microemulsions are thermodynamically stable mixtures of oil, water, and amphiphile. In addition to these, sometimes medium-chain alcohol, a polyhydroxy compound, and another surfactant can be added to the formulation, they called a cosurfactant [3]. Different from the macroemulsion, the droplet size of the dispersed phase in a microemulsion is in nanoscale. They are smaller than 500 nm. The size of droplets depends on some parameters such as surfactant type and concentration changes in the different nanoscale range. Due to the small droplet size range, they scatter visible light weakly and the globules seem invisible to the naked eye [4].

Another main characteristic separation between ordinary emulsion and microemulsion is their stability. Microemulsion systems present great thermodynamic stability as a result phase separation is not likely observed whereas phase separation could eventually arise in macroemulsions since it is a thermodynamically unstable system. Different from emulsions, which need external energy required for formation, microemulsions are required no energy in their formation. Emulsification forms spontaneously without mechanical shear. In addition to these, in contrast to macroemulsion, some problems like flocculation, sedimentation are not observed in microemulsion systems. And also, microemulsions are generally low viscosity systems [5]. The main differences between microemulsions and ordinary/conventional emulsions (macroemulsion) are shown in Table 2.1.

**Table 2.1.** Comparison of Microemulsion with Macroemulsion (Conventional Emulsion)

| Property                   | Microemulsion                    | Macroemulsion              |
|----------------------------|----------------------------------|----------------------------|
| <b>Interfacial tension</b> | Ultra-low                        | High                       |
| <b>Droplet size</b>        | <500 nm                          | 0.1 - 100 $\mu\text{m}$ .  |
| <b>Stability</b>           | Thermodynamically stable         | Thermodynamically unstable |
| <b>Preparation</b>         | Spontaneously or slight stirring | Require external energy    |
| <b>Viscosity</b>           | Low                              | High                       |

### 2.1.1. Interfacial Tension in Microemulsions

The interfacial tension is low in microemulsions. The ultra-low interfacial tension is the driving force for the thermodynamic stability of microemulsion formulations. The adsorption of surfactant on the surface of the globules or droplets reduces the interfacial tension between oil and water to low values. In a two-phase system, the phase that is dispersed in the form of droplets is called the dispersed or internal phase or discontinuous phase, and the other phase in which the droplets are distributed is called the continuous or external phase [6].

### 2.1.2. Types of Microemulsions

Microemulsions can be categorized by three basic types depending on their composition and these are water-continuous, oil-continuous, or bicontinuous. Water to oil ratio and preferred surfactant type usually affect this formation. When water is the continuous phase, oil is dispersed in water, and oil in water microemulsion (o/w) is formed. On the other hand, water droplets are dispersed in the continuous oil phase; the systems are named as water dispersed in oil (w/o). These are droplet microemulsions. Bicontinuous microemulsions are formed when the mixture includes a roughly equal volume of oil and water and the microstructure of the mixture alters constantly from one

to extreme [7, 8]. Schematic representation of different types of the microemulsion can be shown simply like in Figure 2.1.

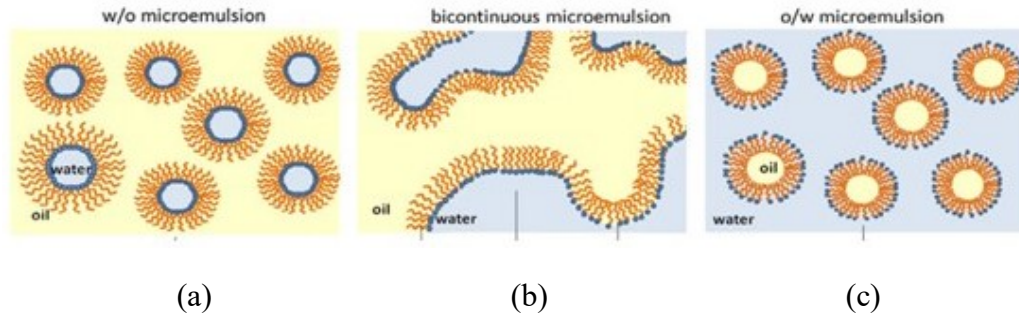


**Figure 2.1.** Types of the microemulsion: (a) water-in-oil, (b) bicontinuous, (c) oil-in-water; figure courtesy [9]

Moreover, as an example, in Figure 2.2, freeze-fracture electron microscopy images of oil-in-water, bicontinuous, and water-in-oil systems that consist of toluene, 1-butyl-3-methylimidazolium tetrafluoroborate, and ethanol was shown by Hou et al [10]. In addition, the type of microemulsion can be characterized by conductivity methods. Oil-in-water (o/w) microemulsion produces positive conductivity but water-in-oil microemulsion does not [11].

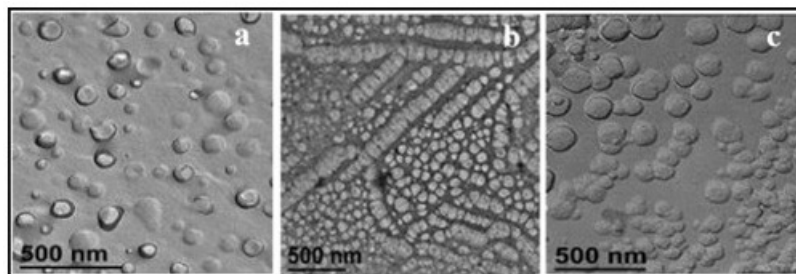


**Figure 2.2.** Freeze-fracture electron microscopy images of (a) O/W, (b) bicontinuous, (c) W/O; figure courtesy [10]

### 2.1.3. Characterization of Microemulsions

After completing the preparation of microemulsion, the characteristics of the system which was made can be identified. To do this, various identification methods can be performed. To characterize the microemulsion some features can be observed such as the droplet size, viscosity, turbidity, phase separation, density. There are various experimental methods for the characterization of microemulsions, for instance, conductivity, phase inversion studies, droplet diameter, polydispersity index, and rheological properties can be applied [12].

The size of droplets of the dispersed phase of a microemulsion is one of the primary characteristics that identify the physical behavior and functionality. One of the most widely used methods for characterizing particle size is Dynamic Light Scattering (DLS), also known as Photon Correlation Spectroscopy (PCS). In this technique, a sample is exposed monochromatic wave light beam, such as a laser (Figure 2.3). The Brownian motion of molecules, which is the random movement of particles because of the bombardment by the solvent molecules that surround them, are measured, and then this motion is associated with the size of particles in the sample. This technique also gives information about the size distribution or polydispersity [13].

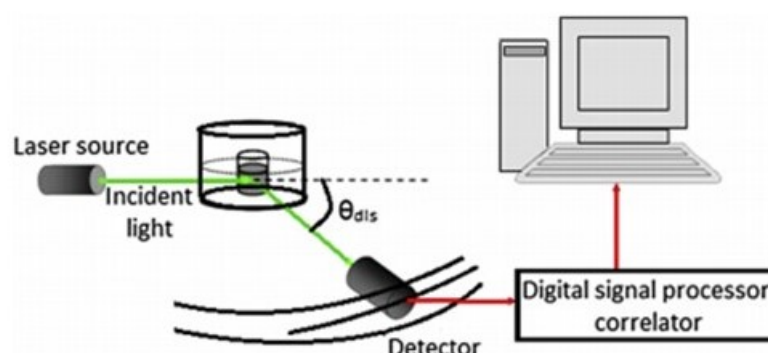


**Figure 2.3.** Schematic illustration of Dynamic Light Scattering method; figure courtesy [14]

#### **2.1.4. The Applications of Microemulsions**

Since microemulsion and its special properties were identified, the utilization of microemulsion systems has gained importance in various areas such as basic research and industry. Due to their characteristic properties, such as low interfacial tension, thermodynamic stability, large interfacial area, ability to solubilize lipophilic and hydrophilic compounds, widely uses and applications of microemulsions are in existence especially in pharmaceuticals, food, fuel, biotechnology, and cosmetic fields. This system can be used successfully for the delivery of both water-soluble and water-insoluble ingredients. In other words, the dispersed phase can be a repository for lipophilic or hydrophilic ingredients when the continuous phases are used inversely, aqueous or oil-based phase, respectively.

Microemulsion applications in topical delivery of drugs cosmetic or cosmeceutical skincare formulation have increased recently because of their good appearance, stability, high solubilizing potential, and ease of preparation. Microemulsion formulations offer thermodynamically stability and thus they have a longer shelf-life tendency in comparison with macroemulsions. Another benefit of microemulsions is that they ensure better cutaneous penetration. Low surface tension provides efficient contact with the skin. The droplet size of microemulsions can pass through the stratum corneum so that the actives can easily reach the sub-layers of the skin. These nano-structured carrier systems present surpassing skin permeation of active ingredients in comparison with conventional delivery systems [15]. To present better permeability effects, pleasant aesthetic appearance, and ease of application are another reason to use microemulsions in skincare products [16, 17].

Nowadays microemulsions are preferred on account of their unique properties in topical skin delivery formulations especially in pharmaceutical, cosmetic/cosmeceutical, and personal care products. For instance, the solubilization of lycopene which is an important antioxidant was demonstrated to enhance jojoba oil microemulsion for cosmetic or pharmaceutical uses [18]. The study demonstrates that water in oil microemulsion boosted the skin penetration of the antioxidant quercetin as a topical carrier system both in vitro and in vivo [19]. Microemulsion could offer a suitable carrier system for the formulation, solubilization, and protection of ascorbic acid for

different topical applications [20]. Microemulsions can be developed as transparent vehicles for waterproof, non-sticky, and easy-spreading sunscreen formulations [21]. Higher stability could be provided with microemulsion for cosmetic active ingredients like arbutin and kojic acid, naturally occurring whitening agents [22]. W/O microemulsions which can be applied as efficient cleaning agents to remove oily make-up cosmetics from the skin could be prepared [23].

Concisely, there are several advantages of the microemulsion system over the other dosage forms. It increases the rate of absorption and bioavailability. It presents rapid and efficient penetration of the ingredients. It provides protection from hydrolysis and oxidation. It requires less amount of energy. It can demonstrate a fine solubilization capacity for both hydrophilic and lipophilic components.

## **2.2. Microfluidics**

Microfluidics is defined as both the science that interdisciplinary studies to behaviors of fluids through micro-channels to manipulate and control, and the technology which manufacture, formulate, and design devices and processes at a micrometric scale with a small volume of fluids. There has been growing development and interest in academic research and practical applications in fields of physics, chemistry, and biology in recent years [24].

Microfluidic devices utilize the chemical and properties of liquids and gases to manipulate and control fluids at a micro-scale thus the make it possible to arrange the shape and size of bubbles and droplets. Because microfluidics offers a uniform distribution and controlling particle size reduction so that to improve quality and stability, it is a good potential technique for the production of emulsions, encapsulations, dispersions, and other formulations. In recent years, there are fields of usage in different industries like medical, pharmaceutical, cosmetic, chemical and food [25, 26, 27].

Microfluidics can propose different techniques, especially for pharmaceuticals and cosmetics. Microfluidics is considered a facilitating technology for the formulation of complex and sensitive active ingredients for drug development processes and the

cosmetics industry. Droplet or bubble generation with microfluidics can provide active ingredients that can be protected up to the moment of application. For instance, microfluidic devices have been used in the production of highly mono-dispersed emulsions at studies in recent years because they allow individual droplets to be formed [28]. Thanks to new developments in microfluidic techniques size and shape of micro-droplets or micro-bubbles can be controlled to alter the structure of a formulation so that the texture, appearance, intensity, distribution can be controlled, shelf life can be prolonged [29].

Microfluidics which is based on controlling fluids on a small ratio makes it possible to study with two or more immiscible or partially miscible fluids so that discrete droplets or bubbles can produce and manipulate. Channel-based microfluidics and planar surface methods are approaches to produce droplets or bubbles with microfluidic techniques. In channel-based methods, continuous and dispersed phases interact with each other into micro-channels so that single micro-droplets or micro-bubbles are generated [30]. In this method, T-junction, Y-junction, co-flowing, flow focusing, membrane are some of the used geometries to produce droplets or bubbles in microfluidic systems. A T-junction microfluidic device with a micro-capillary to produce monodisperse droplets with fluids has received a great deal of attention [31].

Different microfluidic techniques are designed for droplet or bubble generation mechanisms, T-junction micro-channel is one of them that is generally produced from polymers such as polymethylmethacrylate (PMMA) and polydimethylsiloxane (PDMS) is one of them [32]. Gas and liquid are joined in a T-shaped junction with geometry that exerts shear forces to break off fluids to generate stable and homogeneous flows of mono-disperse bubbles [28]. In this system, two immiscible phases called continuous and dispersed phases are driven into micro-channels by means of a separately controlled volume flow and pressure. By using a gas pressure regulator to control the gas phase and a syringe pump providing constant volumetric flow rates the system is conducted. While the fluids are flowing through micro-channels, the dispersed phase penetrates the continuous phase at the junction of the micro-channels that merge via the T-junction micro-device. A bubble begins to grow, it is drifting through the micro-channel due to flow and pressure, and a slug forms inside the channel, and then bubble breakage occurs

at the downstream side of the micro-channel so that a single bubble is generated [33]. It is depicted in Figure 2.4.

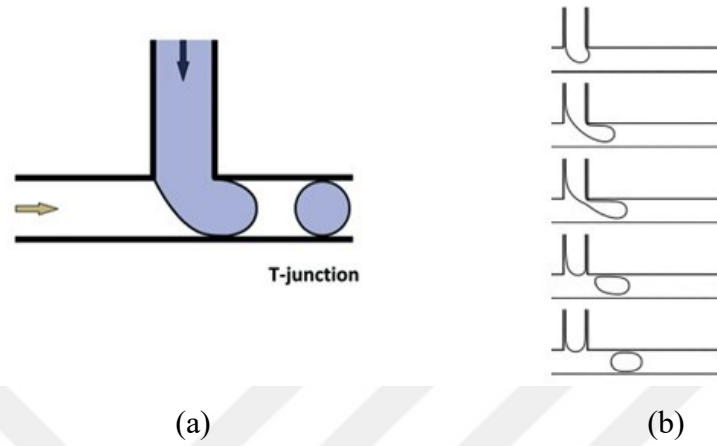


**Figure 2.4.** (a), (b) Schematic representation of micro-bubble formation in a T-junction microfluidic device; figure courtesy [34, 35].

Since microfluidic devices are enabled to study by giving control of small volumes of fluids and select multiphase flows required, especially T-junction method become a preferred system for mono-disperse gas bubble production [28]. By changing the flow rate of the fluids, the gas pressure, the channel dimensions, the dimension of the microbubbles obtained can be controlled and manipulated [36].

### 2.3. Rose hip

Rose hip (*Rosa* spp.) is a plant in the *Rosa* genus which belongs to the Rosaceae family which spread extensively and grows wild in certain regions of Europe, Northwest Africa, and East Asia. The fruit of rose hip, usually named rose hip or dog rose, is typically red to orange in color, shown in Figure 2.5 [37].



**Figure 2.5.** Rose hip plant and fruits; figure courtesy [38]

The entire rose hip fruit is called a pseudo-fruit. The pseudo-fruit of rose hip consists of red fleshy pulp (hypanthium) and numerous achenes containing single seeds (Figure 2.6) [39]. It will be mentioned as rose hip in this study.

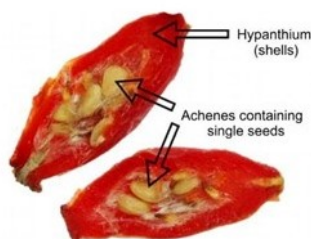


**Figure 2.6.** Main components of the pseudo-fruit of rose hip; figure courtesy [39]

Generally, it has been consumed by making jams, jellies, marmalades, herbal teas for centuries [37]. Fresh and dried fruit has been used as a remedy for curing infections, pain, inflammations and for therapy of cold in traditional folk medicine [40]. Rose hip includes plenty of bioactive constituents and their composition can alter slightly depending on regional and seasonal variations like all other vegetables and fruits. Rose hip has rich content in terms of beneficial organic and inorganic substances,

like vitamins, phenolic compounds, carotenoids, organic acids, minerals, sugars [41, 42]. Rose hip primarily is rich in flavonoids, carotenoids, fatty acids, and high content of vitamins. Especially, it is well known to have fairly high vitamin C content. It has been shown that it has antioxidant, anti-inflammatory, and anti-tumor properties [43]. It has exceptionally total antioxidant concentration compared with many fruits. It has a strong ability to scavenge reactive oxygen species with its constituents, especially vitamin C and polyphenolics [44].

Rose hip is well known for its rich vitamin C content. Rose hip possesses quite a number amount vitamin C content and it is the one of the fruit which have the highest vitamin C content among any other fruit and vegetable commonly known and easily accessible [45]. Although there is a slight dissimilarity in values in the studies done with products from different regions, Vitamin C is an essential constituent of rose hip [46]. It has been known that in the past people used rose hip as a remedy for protection against scurvy by not knowing that this disease arose from vitamin C deficiency [47]. Nowadays it is used as a natural vitamin C ingredient in natural vitamin preparations or supplements. The strong antioxidant activity of rose hip can be ascribed to high vitamin C content as well as phenolic compounds in its constituent vitamin C is widely known water-soluble antioxidant within the body but also skin. It helps to defend the skin against free radical damage. It is an essential compound in collagen formation and it supports to boost collagen production to provide smooth, firm, bright skin. It is known that dietary and topical application of vitamin c has beneficial effects on the skin. Therefore, it is one of the favored ingredients for many topical products [48].

Carotenoids are organic pigments that are found in plants and give the plant yellow, orange, or red color by absorbing wavelengths in the 400–550 nm range (violet to green light). They are effective scavengers of reactive oxygen species so it can be said that they can indicate efficient antioxidant properties. Rose hip includes a quite high amount of carotenoids. Carotenoids composition of rose hip fruits was studied and beta-carotene, lycopene, rubixanthin, zeaxanthin, and lutein were notified as main carotenoids [49].

Based on studies, it can be said that various phenolic compounds are identified in rose hip like phenolic acids, flavonoids, tannins, and anthocyanidins [50]. The anti-

inflammatory effect of rose hip was studied and indicated in vivo as well as its antioxidant activity. Being various fatty acids in rose hip have been mentioned in many researches. Especially, linoleic acid,  $\alpha$ -linolenic acid, and galactolipid have been indicated to have anti-inflammatory properties [51]. It is studied that the seeds of rose hip involve saturated and unsaturated fatty acids in various proportions, like palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid [52]. It was researched that the galactolipid and triterpene acids isolated from rose hip have an anti-inflammatory property with inhibitory effects and these have a capability to reduce symptoms related to inflammatory diseases, such as osteoarthritis and arteriosclerosis. [53]. Moreover, tocopherols, the major form of vitamin E, were documented as one of the constituents of rose hip, and  $\alpha$ -tocopherol was indicated as the main tocopherols. They present strong antioxidants activity [40]. Moreover, as shown in various studies, rose hip also contains several minerals such as Ca (Calcium), Mg (Magnesium), K (Potassium), and Na (Sodium) [52].

Rose hip has become a highly attractive natural raw substance for topical applications in pharmaceutical, cosmetic/ cosmeceutical products. By means of its major constituents, which include phenolic compounds, ascorbic acid, and healthy fatty acids, rose hip has strong antioxidant properties and anti-inflammatory actions and these features cause rosehip to have efficient anti-aging effects. It has valuable potential to promote healthy skin and improve cell longevity and prevent skin aging [54].

Rose hip has the properties of being a promising ingredient for skin applications with all of these desirable constituents. In a study, it was shown that quercetin isolated from rose hip has the potential to melanogenesis-inhibiting effect in B16 mouse melanoma cells. Although melanin is a pigment in the skin that gives its characteristic color and protects the skin against ultraviolet radiation, it is known that excessive synthesis can cause skin disorders [55]. In another study, the effects of rose hip on skin wrinkles and red blood cells longevity were studied, it was expressed that diminishing in the depths wrinkles, enhancing in moisture, increasing in skin elasticity, and improving in cell longevity were observed [54]. The anti-inflammatory effect of some components of rose hip and the strong antioxidant effect of rose hip which is attributed to high vitamin C content and phenolic compounds indicate that it is a very beneficial natural plant for skin health.

## 2.4. Virgin Coconut Oil

Oils derived from natural resources are generally considered as safe, biocompatible, and non-toxic, therefore they are widely selected as the oil phase of topical formulations. These compositions predominantly are accepted to be non-irritant to the skin, inert in nature, and generally regarded as safe (GRAS). Natural products like fruits, herbs are generally biodegradable, mild, and have low toxicity. Coconut oil is one of them [56]. The coconut tree (*Cocos nucifera* L.) is an important member of the palm tree family (Arecaceae), which grow up widely throughout tropical countries [57]. Coconut fruit of coconut palm tree consists of a kernel (meat), water, shell. Schematic illustration of layers of a coconut fruit can be depicted as in Figure 2.7 [58]. Although all coconut oil is derived from the coconut fruit, based on the way of preparation, it takes different names like virgin coconut oil, copra coconut oil, and refined coconut oil. They have been observed slight differences in their composition and biological effects [59]. To obtain copra coconut oil dry method is used. The fresh coconut meat of mature coconuts is dried by using heat or sunlight. The dried meat or kernel of the coconut is called copra. The copra is pressed to produce the oil. On the other hand, virgin coconut oil is extracted from fresh coconut milk or meat by using a wet method. Fresh coconut meat is pressed and the oil is separated from the extracted milk by boiling, centrifugation, or fermentation methods. Since some chemical processes like refining, bleaching, or deodorizing methods are not applied, the nature of extracted virgin coconut oil is not changed [60].

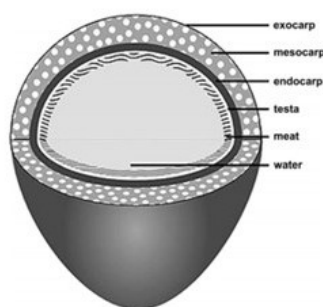


**Figure 2.7.** Layers of mature coconut fruit; figure courtesy [58]

Coconut oil is edible oil and known that it is beneficial for human health. However, too much consumption of coconut oil is not recommended because of its high levels of saturated fat. Virgin coconut oil is rich in essential fatty acids and this provides it a firm texture, and thus it is desirable material in pharmaceutical, nutritional and cosmetic applications. Marina et al demonstrated that the fatty acid composition of virgin coconut oil is namely caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (18:1), linoleic acid (C18:2). According to some studies, the fatty acid composition of virgin coconut oil is presented in Table 2.2 [61, 62].

**Table 2.2.** Fatty acid composition of virgin coconut oil [61, 62]

| Name of the fatty acid | Designation | Formula            | Nature      |
|------------------------|-------------|--------------------|-------------|
| Caproic acid           | C6          | $C_5H_{11}COOH$    | Saturated   |
| Caprylic acid          | C8          | $C_7H_{15}COOH$    | Saturated   |
| Capric acid            | C10         | $C_9H_{19}COOH$    | Saturated   |
| Lauric acid            | C12         | $C_{11}H_{23}COOH$ | Saturated   |
| Myristic               | C14         | $C_{13}H_{27}COOH$ | Saturated   |
| Palmitic acid          | C16         | $C_{15}H_{31}COOH$ | Saturated   |
| Stearic acid           | C18         | $C_{17}H_{35}COOH$ | Saturated   |
| Oleic acid             | C18:1       | $C_{17}H_{33}COOH$ | Unsaturated |
| Linoleic acid          | C18:2       | $C_{17}H_{31}COOH$ | Unsaturated |

The predominant fatty acid in virgin coconut oil is lauric acid; it was shown that it is about 40% - 50% [57, 61, 62]. The antimicrobial effects of lauric acid are notified and it is known that the antibacterial and anti-inflammatory properties of virgin coconut oil were reported [63]. Natural plant oils that contain fatty acid especially linoleic acid have been used traditionally for their wound healing properties and enhancing skin barrier function [64]. Moreover, it is shown in some studies that linoleic acid is

qualified for improving the barrier function of the skin [65]. Virgin coconut oil has a rich composition in regard to medium-chain triglyceride. It is recorded that the dominant triglyceride compositions of virgin coconut oil are LaLaLa, C1C1La, C1LaLa, LaLaM and LaMM with La, C1 and M are lauric, capric and myristic acids, respectively and Table 2.3 demonstrates this composition. Due to its medium-chain triglycerides composition, it has good solubility property and self-emulsification capability [57, 66].

**Table 2.3.** Major triglyceride composition of virgin coconut oil [57, 66]

| Designation | Triglyceride Compositions         |
|-------------|-----------------------------------|
| LaLaLa      | Lauric - Lauric - Lauric acid     |
| C1C1La      | Capric - Capric - Lauric acid     |
| C1 LaLa     | Capric - Lauric - Lauric acid     |
| LaLaM       | Lauric - Lauric - Myristic acid   |
| LaMM        | Lauric - Myristic - Myristic acid |

Polyphenols are another group of bioactive compounds that exist in the virgin coconut oil composition. It contains phenolic compounds like caffeic acid, p-coumaric acid, vanillic acid, syringic acid, ferulic acid, and these phenolic components provide the antioxidant capacity to the oil [67]. In addition, virgin coconut oil contains a small amount of  $\alpha$ -Tocopherol.  $\alpha$ -Tocopherol which is one of the groups of Vitamin E that include four tocopherols and four tocotrienols is a fat-soluble compound and is the most common and biologically active form of Vitamin E. It has a strong antioxidant effect as well as many health-promoting functions [63].

Coconut oil is traditionally used edible oil owing to its rich composition like polyphenols, many kinds of medium-chain triglycerides, and vitamin E. At the same time, the potentially beneficial properties of virgin coconut oil make it a significant raw material in topical formulations. Virgin coconut oil has been utilized traditionally as a

home remedy to enhance skin health and to heal some topical problems on the skin. It has been preferred to improve and moisturize skin and hair [63].

It was studied that virgin coconut oil has the capacity to demonstrate several biological effects like antioxidants. Moreover, Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil were studied and its inhibitory effects were indicated [68]. In a study, the effect of coconut oil was studied and antimicrobial and emollient effects of virgin coconut oil have been demonstrated in adult atopic dermatitis [69]. The beneficial effect of the topical application of coconut oil on the wound environment and healing process owing to its various biologically active components has been demonstrated [70].

It is known that virgin coconut oil has been traditionally utilized for centuries as a substance to enhance beauty, to support the growth of hairs, and to soothe and moisturize skin. Studies have revealed its effective benefits clinically by showing the composition composed of polyphenols, many kinds of medium-chain triglycerides and vitamin E. Virgin coconut oil is non-toxic to humans, it is known for its biodegradability and eco-friendly nature. Therefore due to its content, it provides convenience and confidence in many formulations. All of these unique properties of virgin coconut oil have been drawn attention recently, they have become making it a significant raw material in topical formulations.

## **2.5. Shea Butter**

The shea butter is extracted from the kernel of the nut of the shea tree (known *Vitellaria paradoxa*, synonym *Butyrospermum parkii*, *Butyrospermum paradoxum*) which is a member of Sapotaceae family and is indigenous to the wide savanna belt in the sub-Africa continent. It is also known as kernel fat or karité [71]. Shea fruit consists of layers; an outer layer which is a green epicarp, a pulp which is the mesocarp, and a shell which is a hard endocarp containing shea kernel. The fruit, its pulp, and its nut are demonstrated in Figure 2.8. The sweet pulp is consumable as a dietary contribution by people when ripe. However, it can be said that the most valuable product of the shea tree is the shea butter. The shea butter is derived from the kernel of the nut [72]. This butter has been found to have a high level of beneficial constituents for both health and

skin. It has been used for centuries traditionally as a skin treatment such as a medicinal ointment base or an emollient that is applied topically on the skin. Shea butter is edible oil and it has been utilized traditionally in culinary preparations as well [73].



**Figure 2.8.** Shea fruit, pulp and nut; figure courtesy [72]

Shea butter primarily is composed of fatty acid triglyceride and a high amount of unsaponifiable matter. Shea butter is rich in fatty acids. The fatty acid composition is characterized as 16 various saturated and unsaturated fatty acids in different percentages. Five of them are indicated as the most dominant. The main fatty acids content of the shea butter is shown in Table 2.4. These are oleic acid (C18:1), stearic acid (C18:0), palmitic acid (C16:0), linoleic acid (C18:2), and arachidic acid (C20:0) [74]. Various samples from different countries were studied and it was showed that the samples have similar profiles but different proportions, probably depend on temperature and rainfall conditions [71]. Although oleic acid is dominant in butter in a country whereas stearic acid is dominant in samples of another country, it is showed that oleic acid and stearic acid is predominant in shea butter and together composes about 85-90% of the total fatty acids [74, 75]. The high oleic acid content provides the liquid and soft texture to shea butter while the dominant percentage of stearic acid influences its solid consistency [74]. Shea butter was found to melt at 37.8 °C and so it can be said that that it is solid at room temperature and melts at body temperature [76].

**Table 2.4.** Main fatty acids content of the shea butter [77]

| <b>Fatty acid</b> | <b>Designation</b> | <b>Nature</b> |
|-------------------|--------------------|---------------|
| Palmitic acid     | C16:0              | Saturated     |
| Stearic acid      | C18:0              | Saturated     |
| Oleic acid        | C18:1              | Unsaturated   |
| Linoleic acid     | C18:2              | Unsaturated   |
| Arachidic acid    | C20:0              | Saturated     |

Three groups of triglycerides are identified in shea butter: polyunsaturated, diunsaturated, and monounsaturated. According to studies, although the proportion of triglycerides in shea butter varies depending on the origin of the fruit, SOS, SOO, OOO are the main triglycerides; S<sub>1</sub>: stearic acid, O: oleic acid, respectively. Table 2.5 demonstrates the main triglycerides content of the shea butter [71].

**Table 2.5.** Main triglycerides content of shea butter [77]

| <b>Triglycerides</b>         | <b>Designation</b>              |
|------------------------------|---------------------------------|
| Stearic - Oleic - Stearic    | S <sub>1</sub> OS <sub>1</sub>  |
| Stearic - Oleic - Oleic      | S <sub>1</sub> OO               |
| Oleic - Oleic - Oleic        | OOO                             |
| Palmitic - Oleic - Stearic   | POS <sub>1</sub>                |
| Stearic - Linoleic - Oleic   | S <sub>1</sub> LiO              |
| Stearic - Linoleic - Stearic | S <sub>1</sub> LiS <sub>1</sub> |

Maranz et al. expressed that shea butter contains 5-15% unsaponifiable, containing tocopherols, phytosterols (campesterol, stigmasterol,  $\beta$ -sitosterol, and  $\alpha$ -spinosterol), triterpenes (cinnamic acid esters,  $\alpha$ - and  $\beta$ -amyrin, parkeol, butyrospermol,

and lupeol), hydrocarbons such as karitene [78, 79]. It is mentioned that shea butter consists of various unsaponifiable components and antioxidant and anti-inflammatory properties of shea butter are attributed to these components and so it is stated that it is a desired active ingredient for topical applications in pharmaceutical and cosmetic/cosmeceuticals products [75]. Akihisa et al. expressed that a high level of unsaponifiable components of shea butter makes it a valuable ingredient in cosmetic and skincare product formulations. It is stated that the main unsaponifiable component of shea butter is triterpene alcohols and triterpene alcohols and their derivatives possess diverse biologically beneficial properties like anti-inflammatory and chemo-preventive effects [75].

In addition, another reason to be said that shea butter has natural antioxidant features is the tocopherol content of the butter. Tocopherols are defined as vitamin E and classified as a significant antioxidant. It is indicated that there are  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -tocopherols in relative proportions but  $\alpha$ -tocopherol is the main tocopherol content with about two-thirds ratio and it is usually ascribed by the highest antioxidant activity among the tocopherols [80]. Moreover, phenolic compounds found in shea butter like catechin, catechin family compounds, quercetin give it natural antioxidant property [78].

Some unsaponifiable constituents attract significant interest in skincare and cosmetic product formulations because of their beneficial effects on the skin such as anti-inflammatory, antioxidant effects. Being high levels of these constituents in shea butter is known. In addition, its natural origin and fair melting point (ranging from 34 - 42 °C) make it a good candidate as an ingredient [73]. The rich content and inherently good formulating properties provide a number of possibilities to use in various skincare and cosmetic product formulations [74, 75]. In a study, effects of topical use of shea butter were researched and it was stated emollient, anti-inflammatory, anti-aging, sun-screening properties of shea butter [81].

Actually, the shea butter has been utilized traditionally over the years for many various purposes like medicinal, consumption as a food, skincare. Nowadays, all of these features of shea butter have made it a highly preferable ingredient in various skincare applications products such as aging, damaged, dry, cracked skin [82].

## 2.6. Alginate

Alginate, which is sodium, magnesium, and calcium salt of alginic acid, is a naturally existing hydrophilic polysaccharide derived from the cell wall of brown seaweed known as Phaeophyceae. According to their structure, alginates are grouped as anionic polysaccharides. Alginates are natural linear copolymers consisting of  $\beta$ -(1–4) linked d-mannuronic acid and  $\alpha$ -(1–4)-linked l-guluronic acid block structures linked by a glycosidic bond in variable ratios and sequential arrangements that may show variation depending on the source and species. Besides, alginate is known to be a water-soluble biopolymer [83]. The molecular structure of alginate biopolymers is shown in Figure 2.9.

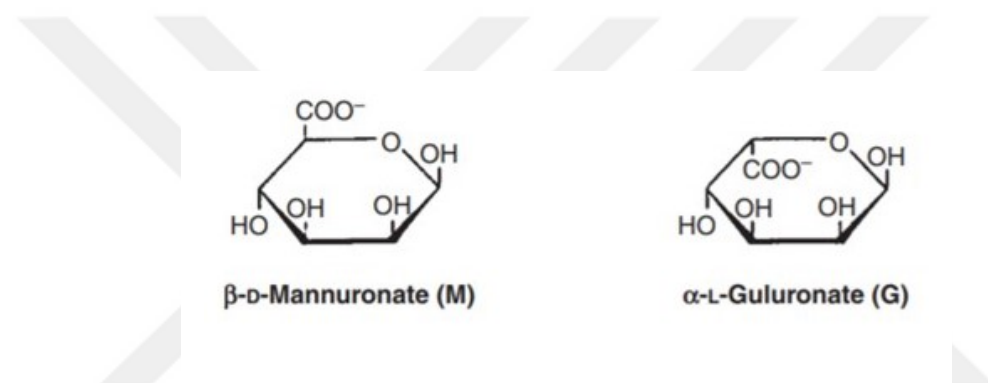


**Figure 2.9.** Molecular structure of alginate biopolymers; figure courtesy [83]

By means of their physical and chemical features like their biocompatibility, biodegradability, low toxicity, and chelating ability, these biopolymers have been extensively investigated and have wide usage in drug delivery, microencapsulation of cells, some biomedical applications as scaffolds [84, 85]. Besides, alginates can cross-link and form a network with their strong gelling properties. By the addition of divalent cations such as  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cu}^{2+}$  (calcium, cadmium, barium, copper ions, respectively), they make intermolecular and intramolecular cross-linking and form in three-dimensional networks hydrogels. The structure in which the divalent cations bind to alginate chains is known as the “egg-box” model [86]. Alginate gelation with  $\text{Ca}^{2+}$  ions is depicted in Figure 2.10. They offer a wide range of uses by providing convenience for modification, processing, and diversification [87].

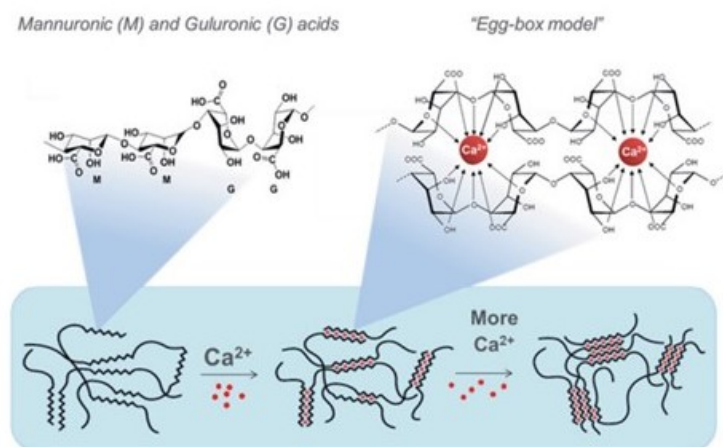


**Figure 2.10.** Structure of alginate gelation in the existence of calcium ions ( $\text{Ca}^{2+}$ ); figure courtesy [88]

Some features of alginate hydrogels such as the mechanical resistance and the structure depend on several factors containing the divalent ion concentration, the kind of cross-linker ion, the ratio of guluronic-mannuronic units in the alginate chains, the exposure time of alginate to divalent ion [89]. Since alginates are known as natural, biodegradable, and environmentally friendly substances, they have been so favored to study cosmetic purposes in recent years. Sodium alginate was utilized to develop microparticles as an abrasive agent that exfoliates dead skin cells. It has been shown that regular, spherical shapes with a smooth surface to minimize the risk of skin irritation were obtained [90]. There are various studies in different types of structures containing alginates like hydrogels, biopolymeric films, and gels in wound healing, tissue engineering applications. Besides, utilizing alginate-based biomaterials is reported to provide wide facilities to develop advanced innovative ideas in different areas like drug delivery, tissue engineering, cosmetic and personal care products [91].

There has been growing development and interest in academic research and practical applications to produce scaffold with microfluidics technique by generating bubbles in recent years. This technique can be taken place in two stages. After

generating micro-bubbles by utilizing a microfluidic device, three-dimensional foam-gel-like structures are formed by the gelation process [92, 93].

## 2.7. Surfactants

Surfactants, also called amphiphiles or surface-active agents, are unique substances that have two different functional groups with different affinity within the same molecule. They contain both a hydrophilic group that has an affinity to water and a hydrophobic group that does not show an affinity to water. They have different chain lengths. There is a hydrophilic group on the one side that is called the head and a hydrophobic group on the other side that is called the tail [94]. The schematic illustration of surfactant molecules can be shown in Figure 2.11.

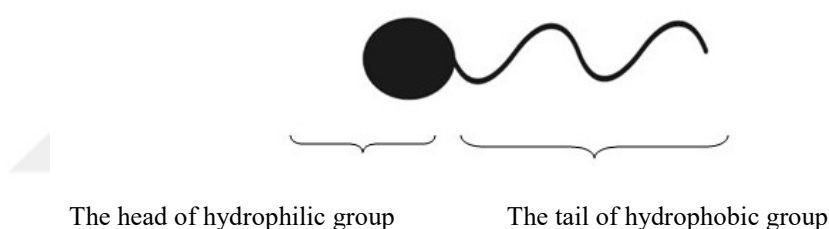


**Figure 2.11.** Schematic illustration of surfactant molecules

Thereby, a surfactant is soluble in water by way of the hydrophilic head group while the hydrophobic tail group enables the surfactant to be soluble in oils. Because of this molecular composition, surfactants reduce surface tension at the interface between immiscible phases like two liquids or a liquid and a solid. When surfactant molecules are distributed in the aqueous phase, they form self-assembled clusters in this phase and they adsorb to the interface between the phases such as water and oil and thus they lower the surface tension. These organized structures are called micelles. In a water phase, the hydrophilic head parts orient on the outer side of spherical micelles and they contact with the surrounding water phase and meanwhile the hydrophobic tail parts flock to the interior. On the contrary, when the head groups aggregate at the center and

the tails combine in the outer, these are called reverse micelle or water-in-oil micelle [95]. The schematic diagram of the micelles is shown in Figure 2.12.

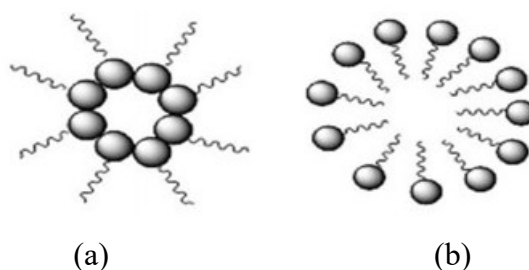


**Figure 2.12.** The schematic representation of the micelles: (a) micelle, (b) reverse micelle; figure courtesy [96]

The surfactant concentration at and above which micelles are formed is defined as critical micelle concentration (CMC). After surfactant is added to the solutions, the surface tension starts to decrease up to the CMC, and after this point, it remains almost constant. Surfactants may be used as emulsifiers, wetting agents, detergents, and dispersants in a variety of applications. They may be added to the formulations for different functions in cosmetics, e.g. as cleansing agents, emulsifiers, solubilizers, conditioning agents [97].

### 2.7.1. Hydrophilic-Lipophilic Balance (HLB)

Surfactants can be classified with hydrophilic-lipophilic balance value. The HLB concept was proposed by Griffin in 1949. The hydrophilic-lipophilic balance (HLB) indicates the balance between hydrophilic and lipophilic groups of amphiphilic compounds. It is commonly admitted that low HLB surfactants are preferred for water-in-oil microemulsions (w/o) and higher HLB surfactants are favoured for the formation of oil-in-water (o/w) microemulsion systems. Bicontinuous structures usually form when HLB is  $\sim 10$ . It is not an absolute predictor but a helpful method to select a surfactant suitable for an application [98].

### 2.7.2. Types of Surfactants

In addition, surfactants are classified according to the charge feature of the hydrophilic head group. There are four different groups.

**1. Anionic surfactants:** They have a negative charge on their hydrophilic head group.

**2. Cationic surfactants:** They are those that have a positive charge on their hydrophilic head group.

**3. Non-Ionic surfactants:** They involve no specific charge.

**4. Zwitterionic surfactants:** They are amphoteric surfactants and they have the potential to have both positive and negative charges.

The type of surfactant notably can affect the efficacy and safety of topical microemulsions. Surfactants that are used for microemulsion formation can be obtained from natural or synthetic resources [99].

### 2.7.3. Lecithin

Lecithin, naturally found in animal and plant tissues, is a complex mixture that contains various phospholipids. The primary phospholipid components of lecithin are Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol, and Phosphatidic acid [100]. It can be extracted from egg yolk, soybeans, corn, sunflower, and some plant seeds. Usually, for topical applications lecithin from soybean is preferred. Lecithin is one of the surfactants which are accepted as long-chain or high molecular weight. Through its amphiphilic structures, it is a naturally zwitterionic surfactant. It is added to formulations as an emulsifier to help to form emulsions by reducing the surface tension. Moreover, it can be used as an emollient, softens and smooths the skin [101].

It shows admirable compatibility with the skin and it does not produce skin irritation contrary to some other surfactants that have irritation potential so that it enhances the tolerance of the formulations. In favor of its natural origins, it is biodegradable and ecologically safe. Also, it is almost toxicologically safe. Because of its natural and valuable composition and desirable properties for topical applications,

lecithin is widely used in pharmaceuticals, skincare, and cosmetic products. The type of surfactant notably can affect the efficacy and safety of topical microemulsions. Surfactants that are used for microemulsion formation can be obtained from natural or synthetic resources [102].

## **2.8. Some Natural Compounds**

### **2.8.1. Antioxidants**

The free radicals, both the reactive oxygen species and reactive nitrogen species, are products that are normally generated as a product of cellular metabolism in the human body. They are unstable and highly reactive. When the body is exposed to overproduction of free radicals or external factored oxidant substances such as chemicals, ultraviolet radiation, air pollutants, drugs, pesticides, industrial solvents, the balance between free radical generation and antioxidant defenses are broken. Oxidative stress arises. This can affect the cell functions and irreversible damage or changes in the cell functions can emerge. Some problems can be observed like damages in the valuable biomolecules such as lipids, nucleic acids, carbohydrates, and proteins in the body. Their damaging effects can be associated with some disorders of the structure and function of the skin. Acceleration in skin pigmentation can be observed. Oxidized lipids, proteins, or nucleic acids can cause some changes in skin conditions. Due to increased reactive oxygen species, oxidative stress can affect the collagen, elastin, and connective tissues. Skin firmness and elasticity can get harmed and thus it can cause dry and wrinkled skin. The reactive oxygen species are known to have a noteworthy role in aging which is described by wrinkles and atypical pigmentation [103, 104].

Antioxidants are substances that can inhibit the oxidation reaction and act as a free radical scavenger. Generally, antioxidants are categorized as natural and synthetic. Fruits, vegetables, seeds, cereals, and edible plants have a wide variety of antioxidants and they demonstrate strong antioxidant activities. Natural antioxidants are polyphenols, tocopherols (Vitamin E), ascorbic acid, and carotenoids which are organic pigments in plants. Phenolic acids which are included in the plants naturally, such as caffeic acid, ferulic acid, p-coumaric acid, have significant antioxidant effects. Flavonoids present a powerful antioxidant action as well as anti-inflammatory effects. Tocopherol is known

as an active chain-breaking antioxidant. Carotenoids have quite good defensive functions against oxidative damages [105].

As there are many various antioxidant components in fruits and vegetables, not separately the cumulative synergistic effect of these antioxidants is the indicator of the antioxidant capacity of a plant. Therefore, generally, total antioxidant capacity is measured to determine the possible antioxidant benefits of a product. Because of their beneficial effects and activities against free radicals, antioxidants are among the desired ingredients in topical delivery applications in both pharmaceutical and cosmetics products [106].

### **2.8.2. Phenols**

Phenols are chemical compounds consisting of a hydroxyl group ( $\text{—OH}$ ) that is attached directly to an aromatic ring or phenyl ring. Depends on the number of phenol units and the structural elements that join the rings in the molecule, phenolic compounds can be classified. It can be said that flavonoids, phenolic acids, and polyphenols are the largest group of plant phenolic compounds [107].

Since phenolic compounds are found in plants as normal plant metabolites, they are widely found in the plant tissue. They are included in pigments and structural elements of a cell. They are known associated with color, taste, aroma, and flavor. It has been known that phenolic compounds existing naturally in plants are among the most valuable natural antioxidant compounds. Phenolic antioxidants can react with free radicals, blocking the chain reaction. Phenolic acids, that are derived from hydroxycinnamic acid (caffeic acid, ferulic acid, p-coumaric acid, etc.) and hydroxybenzoic acid (syringic acid, vanillic acid, p-hydroxybenzoic, etc.), distributed widely in many plant species and they have different antioxidant capacity depending on their structure. Flavonoids are the most abundant phenolic compounds and they are strong natural antioxidants against oxidative damage diseases. Furthermore, phenolic substances are protectants against bacterial pathogens and it is known that their antimicrobial and anti-inflammatory effects. In addition, they have protective action against ultraviolet radiation damages. Because of all of these beneficial properties of

phenolic compounds, they are among compounds that are requested to be contained in the formulation of the topical products [107, 108, 109].

### **2.8.3. Fatty acids**

Fatty acids are kind of a carboxylic acid compound with a long aliphatic hydrocarbon chain. They usually contain a straight chain of an even number of carbon atoms. They can be classified based on the number of double bonds. Fatty acids that have no double bond in the structure are called saturated fatty acids such as palmitic acid, stearic acid, and lauric acid. They are ordinarily solid at room temperature. When there is one double bond in the structure, they are referred to as monounsaturated fatty acids such as oleic acid. When there are multiple double bonds, like linoleic acid, are named polyunsaturated fatty acids. Unsaturated fatty acids are usually liquid at room temperature. Fatty acids are a significant structural element of lipids, that are energy storage of living cells, in plants, animals, and microorganisms. It is known that fatty acids have several biological effects. They can be found in organisms in the form of triglycerides, diglycerides, monoglycerides, phospholipids, cholesterol esters, and free fatty acids [110].

Fatty acids take place among the significant ingredients for topical delivery applications in pharmaceutical and cosmetic/cosmeceutical products. Since they have various beneficial properties, they are chosen for many different purposes. Fatty acids are primarily utilized in emulsion systems to reduce interfacial tension. Besides, they are added as surface-active agents so as to cleansing in soaps, detergent, and cosmetic/cosmeceutical products. In addition, topical applications of fatty acids have a good potential impact on the skin. They help to maintain healthy and youthful skin. They act as an emollient and they prevent water loss from the skin and help to make the skin smooth and soft. In addition, certain fatty acids exhibit antimicrobial property which inhibited several pathogenic microorganisms like *Listeria monocytogenes* in media [F-5]. They can be an ingredient in preparations or a part of carriers for active substances in formulations. Products that contain fatty acids are normally mixtures of varieties of fatty acids [111].

Commonly, oils that are derived from vegetable and fruit plants are not irritants or sensitizers for the skin. They are usually composed of monoglycerides, diglycerides, triglycerides, free fatty acids, natural antioxidants, and vitamins. Thus, uses of plant-derived fatty acid oils on the skin are generally accepted to be safe. Moreover, fatty acids are categorized as readily biodegradable [110].

#### **2.8.3.1. Triglycerides**

Fatty acids don't generally prefer to exist in a free state in nature, commonly, three fatty acids choose to be found together in combination with glycerol in a different form. These three fatty acids molecules bounded by glycerol are an ester named triglyceride. Triglycerides are an important component of fat in animal and plant tissues they are commonly characterized by their fatty acid composition. Thus, they have the capability to be an excellent vehicle and solvent for so many lipophilic active ingredients. Triglycerides can be expressed with both the average number of carbon atoms in the triglyceride and the iodine value which shows a measure of the unsaturated bonds present in the oil. It helps to understand the composition and properties of oil [112].

#### **2.8.4. Vitamin C**

Vitamin C, also known as L-Ascorbic acid or ascorbate, is one of the most essential natural compounds for the human body's functions. It is a water-soluble vitamin. Although vitamin C has significant functions in the body, unlike most plants and animals humans body is incapable of synthesizing it. Taking orally high doses of vitamin C does not result in a high increase in skin levels. Vitamin C is a skin constituent which is existed in both the epidermis and dermis. Therefore, topical application of this vitamin is recommended to support the skin with vitamin C and it has been widely used in dermatology and cosmetics [113, 114].

Environmental pollutants and sun exposure promote free radicals that lead to damage to the skin, therewithal UV lights impairs the skin's normal capability to neutralize free radicals. Thus the skin exploits antioxidants that help neutralize free radicals to protect itself from that damages. Vitamin C is known that is a very effective

antioxidant against reactive oxygen species and free radicals. It is one of the most substantial antioxidants in the skin as an electron donor. It provides protection like other skin antioxidants containing Vitamin E, glutathione, niacinamide, vitamin A. It is known that vitamin C is especially competent in neutralizing the superoxide anion, hydroxyl radical, and singlet oxygen by donating an electron. UV radiation exposure provokes a number of degenerative changes in the skin cells. UV lights both induce to raise free radicals and damage structural constituents in the skin. Thus visible changes occur on the skin surface including wrinkling, dry appearance, impaired pigmentation, slackening. Vitamin C might support to restrain these damages [115,116].

Vitamin C has a significant function as a co-factor in the skin and it helps to regulate the synthesis of the structural protein collagen that provides the skin its strength, toughness, turgor, and hydration. It has a significant role in the formation of collagen in the skin; also it contributes to maintenance. Studies have demonstrated the requirement of vitamin C for hydroxylation and secretion of procollagen [117]. It is known that the proliferation rate of younger cells declines with aging, and also vitamin C might be effective in enhancement the proliferative capacity of elderly dermal fibroblasts. It acts an effective role to enhance the dermal matrix by both decreasing collagen degradation and boosting collagen synthesis [118]. Wrinkling and sagging are signs of skin aging. Studies have shown that long-term regular use of vitamin C in topical applications helps to reduce wrinkles and decline roughness of the complexion, thus contributing to some positive structural changes in the epidermis and dermis. In addition, vitamin C is necessary for promoting the formation of the epidermal barrier, supporting the synthesis of epidermal lipids, and restoring the stratum corneum structure [119]. Moreover, studies show that topical application of vitamin C has an inhibitory effect on melanogenesis, acts as a reducing agent for melanin, thus it provides regulation of pigment production and it has mild skin lightening effects [120]. Vitamin C content of the skin reduces with aging in the progress of time. Even though the topical application is recommended, Vitamin C is easily affected when exposed to heat, oxygen, and light and might degrade in time. Moreover, it has low skin permeability in topical applications. Thus it has been studied to develop formulations that maintain stability and boost permeability recently. Some kinds of delivery systems such as nanosuspensions and microemulsions might be used to deliver to the skin vitamin C

with topical application by providing to stabilize the formulation [121]. Ultimately, all of these properties make topical vitamin C a valuable cosmeceutical ingredient.

## **2.9. Size and Structure Characterization Techniques**

### **2.9.1. Optical Microscope**

The optical microscope also called a light microscope is a kind of microscope to observe magnified images of small objects. It is an imaging technique that provides a specimen to be examined with the unaided eye by using light and lenses. It generally utilizes visible light, which allows analysis with limited size and resolution. An optical microscope has an eyepiece, also called an ocular lens, to bring the image into focus for the eye. It can have either a single eyepiece or dual eyepieces, also known as a stereomicroscope. There are two types of optical microscopes, according to the type of light exposure; reflected or transmitted light microscope. The reflected light microscope is utilized to analyze the surface of a specimen. The structural data of the sample can be analyzed by using a beam of light reflected off the sample surface. On the other hand, the transmitted light microscopy necessitates an optically transparent or translucent sample because of using a beam of light passes through the specimen. The optical microscope has objective lenses that generate different magnified images of an object specimen and stay in focus when one changes to another. Besides, optical microscopes can be connected to a computer to display and record the resulting image directly on a computer screen simultaneously [122].

### **2.9.2. Scanning Electron Microscope (SEM)**

An instrument that grounds on microscopy technique using a beam of accelerated electrons as a source of illumination to examine specimens is called an electron microscope. A scanning electron microscope (SEM) is a kind of electron microscope that is widely used for the examination in nanoscale imaging characterization of objects. It generates images of an object by scanning the surface of the sample with a focused high-energy beam of electrons. In this technique, electrons are used instead of light to create an output image. By this means, they have a high resolving power and thus they enable to examine detailed the structure of objects. A

scanning electron microscope component contains an electron optical column, electronics, a vacuum system, and software in general. The electron beam emerges from the electron gun which is used as a light source at the top of the column, is focused into a fine point, and scans over the specimen surface under vacuum. As a result of interactions between the beam electrons and the object, various signals are produced to acquire information about the surface of the specimen and collected by detectors, and then the monograph is displayed on the computer screen. Particle shapes, sizes, surface morphology, purity, and size distributions can be analyzed by the SEM images [123].

## **2.10. Skin**

The skin is the outer covering and the largest organ of the body. The thickness of the skin varies between 0.5 mm and 4.0 mm depending on which region of the body in; the thickest in the feet, and eyelids are thinnest. The skin renews every 28 days so it has the capability to rapidly recover itself. It is waterproof, pliable, and has a low permeability [124].

### **2.10.1. Functions of the Skin**

The skin serves numerous significant functions for the body [125].

- **Protection**

The skin has a protective barrier function against external aggressions.

- Physical protection against trauma
- Thermal barrier to heat injury
- Impermeability against harmful agents
- Protective barrier against harmful effects of ultraviolet radiation
- Resistant barrier against excessive loss of moisture

- **Thermoregulation**

- Regulating the body temperature with the secretion and evaporation of perspiration from the surface of the skin

- **Sensation**

- Sensing stimuli like warmth, cold, pressure, touch, pain, itching, and touch with sensory receptors

- **Maintaining fluid balance**

- Excretion of waste products and salt with perspiration
- Preventing absorption of excess water
- Preventing excess drying with sebum

- **Metabolic function**

- Storage for blood, fat, electrolytes, water
- Participating in vitamin D synthesis
- Producing metabolic materials for the restoration of skin integrity

### **2.10.2. Structure of the Skin**

The skin consists of three layers: the epidermis, the dermis, and the subcutaneous tissue (hypodermis). The layers of the skin were depicted in figure 2.13 [126]. The epidermis is the outermost, elastic, continually being regenerated layer of skin. It is composed of four or five layers depending on which area of the body in. Hairless and smooth skin is referred to as thick skin and it is situated on the soles of the feet and the palms of the hands. It consists of 5 layers from deep to superficial: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum, respectively in Figure 2.14 [127]. The remaining part of the skin is classified as hairy skin and called thin skin. Unlike hairless skin, it does not contain stratum lucidum and consists of 4 layers. The stratum basale, also called stratum germinativum, is the bottom layer of the epidermis. Comparing to the other layers, any blood vessels are not contained. The epidermis includes several types of cells that have different functions.

The stratum basale, also called stratum germinativum, is the bottom layer of the epidermis. The cells of the basal layer attach the epidermis to the membranes in the epidermal-dermal cell junction. Keratinocytes cells are first produced from this single layer of cells continuously. They are the main cells of the epidermis. New cells are generated by cell division at the base, and then they move towards the upper steadily. Keratinocytes also produce a protein called keratin which supplies endurance to the skin. While they move upwards, cells undergo structural changes: the cells die gradually, they lose their nuclei, they change shape and become flattened.

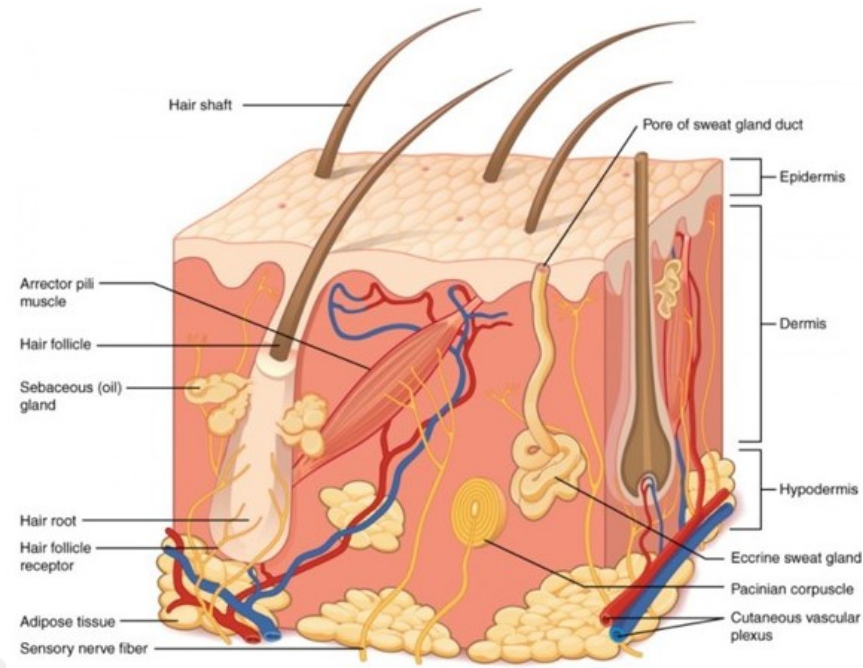


**Figure 2.13.** Layers of skin; figure courtesy [126]

Moreover, the base layer contains melanocytes and merkel cells. Melanocyte is defined as specialized skin cells that are located in sparse and regular intervals between keratinocytes along with the basal layer of the epidermis. Melanosomes which are organelles in the cells synthesize storage and transport melanin pigments. Melanin is primarily responsible for giving color to the skin. Moreover, the melanin absorbs light so that it provides protection to the skin from the damage from ultraviolet (UV) radiation. Merkel cells are specialized cells in the basal layer of the epidermis known to function mainly as touch receptors and they are responsible for stimulating sensory nerves. Keratinocytes are pushed towards the surface and they pass to the stratum spinosum which comprises 8-10 layers of cells from the stratum basale. This layer's cells are spiny-shaped cells. The cells in these layers attached to each other via a structure called a desmosome and contain large pale-staining nuclei. Glycolipid which helps make the skin relatively waterproof and prevent water loss from the skin is released from the cells in this layer. Langerhans cells which are interspersed among the keratinocytes in this layer are other specialized dendritic cells that are distributed in different layers of the epidermis. It is known that they have a role in immunologic

function. They help to protect the skin by responding against infections and by activating allergic reactions. From this layer, the keratinocytes move toward the stratum granulosum layer. The cells which contain three to five layers in the stratum granulosum layer have a granular appearance. While cell organelles and nuclei are disintegrating and their cell membranes are thick, they become flatter. The keratinocytes of the stratum granulosum are pushed into the stratum lucidum in the digits, the soles, and the palms. This layer has smooth and dead cells. The last and most superficial layer of the epidermis is the stratum corneum which contains 15 to 30 layers of cells.

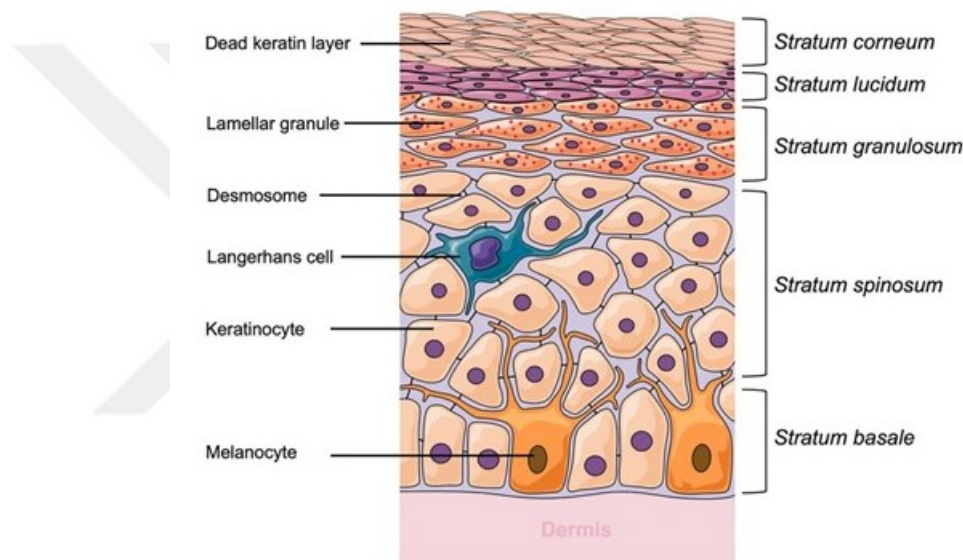


**Figure 2.14.** Layers of epidermis; figure courtesy [127]

When keratinocytes arrive at the uppermost layer of the epidermis, they are flattened and dead. This keratin-filled structure is termed corneocytes. These dead cells constitute the outer layer of the skin that is termed the horny layer or the stratum corneum. This layer also helps to restrain dehydration of the underlying tissues and penetration of microorganisms. This dry, tough, scaly layer flakes off daily so that the cycle of epidermal renewal occurs. This cycle is completed in a period of about 4 weeks [128,129, 130, 131].

The barrier function of the stratum corneum is likened to a brick and mortar structure like a brick wall. The corneocytes are made of tough protein and they form the brick structure of the stratum corneum. A layer of lipids composed of fatty materials constitutes the mortar. This structure allows water to percolate through thus this barrier makes the skin semi-permeable. One of the factors that affect the firmness, correct functioning of the stratum corneum, and elasticity is the moisture content of the skin. Retention of water can also affect the skin's barrier properties. When the skin dehydrates, the surface of the skin becomes rough, flaky, and thick; also the corneocytes are not shed as normal. The skin loses its elasticity with time, cracking on the skin is observed. Exposure to an excess alkaline environment may disrupt the acidic nature of the skin, which may affect skin protection. Although the skin is semi-permeable to water, it is a strong barrier for contaminants and micro-organisms. The surface film on the epidermis is slightly acidic and prevents harmful substances from penetrating the skin [132].

The dermis is the thickest layer of the skin that exists above the subcutaneous layer and beneath the epidermis and gives the skin its strength and flexibility. It includes blood vessels, nerve endings, sweat glands, hair follicles, sebaceous glands, lymphatic vessels. It is composed of two layers of connective tissue: The more superficial papillary dermis and the reticular dermis that is found deeper. The papillary layer is the thinner superficial layer of the dermis that extending into the stratum basale of the epidermis in the form of finger-like. It contains elastin fibers, some collagen, and loose connective tissue including capillaries. It supplies nutrients to the avascular epidermis via vessels and provides thermoregulation. The reticular dermis that constructs around 80% of the dermal thickness is dense irregular connective tissue and underlies the papillary layer. It includes vessels, fibroblasts, nerves, mast cells, epidermal appendages, interlaced thicker bundles of collagen, and elastic fibers and gives the skin its overall elasticity and strength. The fibroblast cells which synthesize elastin, collagen, and the viscous gel within the dermis are the main dermal cells. Collagen that gives the skin its strength, toughness, turgor, and hydration by binding water is the main structural protein. Elastin that provides the skin its elasticity and resilience is another protein found in the dermis.

The dermis contains appendages of the skin. The sweat glands found in the dermis are directly open to the upper layer of the skin. They help to regulate body temperature in response to heat and stress by producing odorless perspiration. Also, apocrine sweat glands found in the armpits, nipples, wings of nostrils, genital region are open into the hair follicles and secrete thick, oily perspiration that gives a characteristic odor when is digested by the skin bacteria. The sebaceous glands produce sebum which softens and lubricates the skin into hair follicles not directly to the outer surface. Sebum makes up a barrier to defend the skin against foreign substances and maintains the skin hydrated. Sebum mostly composed of triglycerides, fatty acids, and esters. The hair follicles are anchor-like for each hair shaft and regulate various types of hair growth. The cells in the hair bulb are nourished by blood vessels. Hair help to regulate body temperature, enhance sensation, protect against external factors. The main function of the blood vessels in the dermis is to supply nutrients to the skin and maintain the body temperature constant by enlarging and constricting. The nerve endings are responsible for touch and sense pain [128, 129, 130, 133].

The hypodermis also called the subcutaneous layer lie beneath the dermis and connects the skin to the fibrous tissue surrounding the muscles. This layer of fat provides the structural support for the skin, protective padding against external impact for underlying organs, aids to insulate the body from external cold and heat, and stores fat to supply energy [133].

### **2.10.3. Skin Hydration**

It is known that the water content of the dermis is about 70% and of the epidermis is about 15–30% and also this rate more drops in the stratum corneum [134]. In addition, the skin is not completely impermeable. Although the stratum corneum is a protective barrier by means of its structure, some substances might penetrate either through direct absorption or the openings of the sweat gland and hair depending on the lipid content and the path length of the stratum corneum. Ceramides, cholesterol, and long-chain saturated fatty acids are the main components forming the lipid barrier of the skin. The water content and lipids of the stratum corneum play together important role in skin hydration [135]. The bridge-mortar structure of SC is a significant formation and orderly arranged the stratum corneum intercellular lipids provide an effective, tight

barrier against transepidermal water loss (TEWL). Water is tightly bound by hygroscopic molecules and lipids in the stratum corneum.

The water content of the skin is imperative for regular functioning. Skin hydration plays a significant role in maintaining healthy skin and stimulating its natural barrier function. Hydrated skin is radiant, smooth, and resilient. On the other hand, dehydrated skin lacks water and it appears dull, dry, uneven, and flaky. Climate, lifestyle, nourishment, age, and the products used on the skin might contribute to the disruption of the water balance of the skin. Maintaining skin hydration balance might prevent early skin aging and protect the skin against insults of the environment [136].

#### **2.10.4. Skin Aging**

Skin aging is a complex, cumulative, gradual, and inevitable biological process, and this process is associated with the accumulation of physical, physiological, natural changes. Young individuals have smooth, supple, soft, plumper, and firmer skin, with the progress of time the changes in the skin become noticeable with signs of aging: loosening of the skin, loss of elasticity, the development and deepening of wrinkles, the appearance of age spots, leading to a paler skin tone, rough-textured appearance. These structural and functional changes occur in all layers of the skin. Skin aging is classified as chronological aging and photoaging. Chronological aging, also called intrinsic aging, is termed for changes due to the natural aging process. Some changes occur with the natural passage of time like the energy of the cells decreases, hormonal mechanism changes; skin regeneration decelerates due to the effect of genetic factors. With age, skin thins, and its subcutaneous fat support gradually lost. The term photoaging also called extrinsic aging, refers to the changes due to environmental factors, the leading factor is ultraviolet radiation. UV radiation stimulates harmful reactive oxygen species synthesis. Oxygen-free radicals accelerate the process of aging in the systems and that decelerates cell renewal, therefore, reducing the body's self-repair capabilities. The skin loses its ability to retain moisture and so the skin becomes drier [137]. As the number of melanocytes in the skin decreases with age, the protective effect of the skin acting as a barrier against UV radiation declines, this causes irregular pigmentation. Intrinsic and extrinsic factors perform synergistically, and so the structural changes occur in the skin, e.g. diminishing epidermal thickness, the flattening of dermal-epidermal junction,

reduction in the levels of elastin, collagen, and their molecular composition and architecture, reduced continuous formation of new cells [138, 139]. Due to lack of ability to retain adequate moisture, results from declined collagen and elastin production, and collapse in the organization of epidermal-dermal junction, Wrinkling becomes visible in the skin. Natural active ingredients could be widely used in topical applications against skin aging [140].



### **3. MATERIAL AND METHODS**

#### **3.1. Plant Materials**

Ripen self-grown rose hip harvested from Ankara province at the beginning of the autumn were used in the extraction experiments. After roses had bloomed and the petals had dropped, the hips were collected at the full ripening stage in late summer through early autumn. The identification of rose species was followed according to the European Pharmacopoeia 8.0 [141]. Half of the fruits were dried outdoor under mild temperatures (about 22-25 °C) and then dried fruits were stored at -80 °C until analysis. On the other hand, the other half of the handpicked rose hip was put in a freezer and kept at -80 °C for subsequent use.

#### **3.2. Preparation of Extracts**

In all processes, some precautions were performed like preferring amber glass bottles and using aluminum foil to wrap containers to avoid photodegradation.

##### **3.2.1. Air-dried Rose hip**

About 500.0 g of the air-dried fruits were weighed by using a laboratory balance produced by Ohaus, Switzerland, mixed with 1 L ethyl alcohol (EtOH) that is absolute for analysis produced by Merck (Darmstadt, Germany), and then crushed into small pieces and blended with IKA T50 Ultra-Turrax. The mixture in a brown bottle was put into IKA HS 501 shaker to shake for 24 hours at room temperature. The obtained mixture was filtered through a filter paper and the clear extract solution was gained. The solvent was then evaporated in a rotary evaporator at +40 °C and reduced pressure by using The Heidolph Laborota 4001 Rotary Evaporator. When all solvent was evaporated, the solid residue was redissolved in a small amount of distilled water and kept frozen at -20 °C and then the sample was lyophilized by using Christ Alpha 1-4 LSC Freeze dryer. The obtained dry residue was weighed and then it was stored at +4 °C for subsequent use.

On the other side, about 500.0 g of the air-dried fruits were weighed, mixed with 1L distilled water, and then broken into small pieces and blended with IKA T50 Ultra-Turrax. The mixture in a brown bottle was shaken using IKA HS 501 shaker for 24 hours at room temperature. After the shaking of the mixture for 24 hours, a cloudy mixture appeared. In order to get clear extraction, the obtained mixture was filtered and it was then centrifuged at 4500 rpm for 10 minutes by using Sigma 3-16PK and the supernatant was filtered through a filter paper the clear light orange-red mixture was obtained and then the solution was kept frozen at  $-20^{\circ}\text{C}$ . The sample was lyophilized by using Christ Alpha 1-4 LSC freeze dryer and the obtained dry residue was weighed, and finally, the extract was kept at  $+4^{\circ}\text{C}$  for further experiments.

Moreover, a fair of air-dried fruits was milled using a coffee grinder and passed through a mesh sieve to get a fine powder. The obtained hips powder was kept apart for further experiments.

### **3.2.2. Fresh-frozen Rose hip**

About 500.0 g of the fresh frozen rose hip were weighed and mixed with 1 L ethyl alcohol (EtOH) that is absolute for analysis, and then crushed into small pieces and blended. The mixture was put into the shaker for the maceration process for 24 hours at room temperature. Precautions against light were watched out. After maceration, the mixture was filtered through a filter paper to obtain a clear extract solution. The rotary evaporator was utilized to evaporate the excess solvent at reduced pressure and mild temperature. After the evaporation process, the solid residue was redissolved in a small amount of distilled water and kept frozen at  $-20^{\circ}\text{C}$  and then the extract was lyophilized. The orange-red dry extract was gained and it was stored in the fridge for further use.

On the other hand, a fair amount of the fresh frozen rose hip was weighed and they were lyophilized directly by using the freeze dryer. The lyophilized rosehip fruits were milled using a coffee grinder and passed through a mesh sieve to get a fine powder. An amount of the obtained hips powder was kept apart for further experiments.

Besides the rest of the obtained hips powder was mixed with a small amount of water and the sample was lyophilized by using a freeze dryer and then the prepared powder was stored in the fridge for further experiments.

### **3.3. Thin Layer Chromatography (TLC)**

The thin layer chromatographic method was used for the determination of L-ascorbic acid in the extracts of rose hip.

Preparation of mobile phase: The mobile phase was prepared by mixing Ethyl acetate: Acetone: Formic Acid: Water (produced by Sigma-Aldric, Missouri, United States; Isolab, Wertheim, Germany; Merck, Darmstadt, Germany, respectively) in the proportion of 5:3:1:1 v/v in the fume hood.

Preparation of reference solution: The standard L-ascorbic acid by Supelco (Bellefonte, PA, USA) was directly dissolved in a solution that was a mix of Ethanol: Distilled Water in the proportion of 3:2 v/v according to the European Pharmacopoeia 8.0. About 15 mg of ascorbic acid was added to 7.5 ml of the prepared solution.

#### **3.3.1. Air-dried Rose hip**

Preparation of sample solutions: The obtained extract from the ethyl alcohol process, the obtained extract from the distilled water process, and the powder gained from air-dried fruits were used for the thin-layer chromatographic method respectively. All samples were directly dissolved in the solution that was a mix of Ethanol: Distilled Water in the proportion of 3:2 v/v. All prepared solutions were placed for 30 minutes in an ultrasonic bath and then were filtered through a 0.45  $\mu\text{m}$  filter (Göttingen, Germany).

Process of Thin Layer Chromatography: In a fume hood, the mobile phase mixture was poured into a glass TLC developing chamber to cover the bottom and covered with a lid, and then the covered chamber was allowed to stand to provide in the saturation of the TLC developing chamber with solvent vapors while preparation of the TLC plate. A Merck HPTLC Silica gel 60 F254 Glass plate (10.0 cm x 10.0 cm), (Darmstadt, Germany) was prepared to fit the available developing chamber. 2.0  $\mu\text{L}$  of

the reference ascorbic acid solution and 20.0  $\mu\text{L}$  of each sample solution were spotted automatically by using CAMAG Linomat 5 (Muttentz, Switzerland). After drying, the prepared plate was inserted into the saturated developing chamber carefully by leaning the top of the plate against the chamber wall. The chamber was covered with the lid and then the eluent was allowed to move up by capillary force through the plate was observed cautiously to advance uniformly. When the eluent reached the upper part of the plate, the plate was taken out of the developing chamber and then it waited for a while to evaporate the residual solvent completely in the fume hood. The color development on the TLC plate was visualized under 254 nm (short-wave UV) light and 365 nm (long-wave UV) light.

### **3.3.2. Fresh-frozen Rose hip**

Preparation of sample solutions: The obtained extract from the ethyl alcohol process, the powder gained from lyophilized fresh-frozen fruits, the lyophilized powder obtained from lyophilized fresh-frozen fruits were prepared to analyze with thin layer chromatography. Each sample was prepared as mentioned before. After the mixtures were shaken in the ultrasonic bath, they were filtered through a Sartorius RC 0.45  $\mu\text{m}$  filter, and then the samples were kept carefully to protect them from light.

Process of Thin Layer Chromatography: The same processes were followed as mentioned before in the air-dried rose hip process.

### **3.4. Preparation of Microemulsion**

Organic Shea Butter (L'Occitane en Provence, France) and organic Virgin Coconut Oil (The Life Co, Turkey) were purchased from the market. Also, the lyophilized powder obtained from lyophilized fresh-frozen rose hip fruits and soybean lecithin (Sigma-Aldric, Missouri) were used to prepare microemulsion. 100.0 mg lecithin was directly dissolved in 10 g sterile distilled (Polifarma, Turkey) water in an amber flacon at room temperature. The amber flacon was submerged in an ice-water bath to avoid overheating and minimize temperature-driven side effects. The probe-sonicator probe (Bandelin Sonopuls Germany) was immersed directly into the flacon until about 1 cm from the bottom without touching the container walls. Then 100.0 mg

the powder of the prepared freeze-dried fresh rosehip fruit powder was added gradually into the amber flacon, it was sonicated for about 8 minutes in total. The prepared product was stored in the fridge. The frozen sample was lyophilized directly by using the freeze dryer and stored in the fridge for further experiments.

The obtained rose hip extract-lecithin (1:1, w/w) sample was distributed into an oil phase composing of virgin coconut oil and shea butter by using the probe-sonicator. The 6 different formulas were designed to obtain the most homogenous dispersion by changing the ratio of oil and water phase. The ratios of the designed formulas were demonstrated in Table 3.1.

**Table 3.1.** The content of pre-formulation dispersions

| Formulas  | Contents (w/w)        |             |                                      |             |
|-----------|-----------------------|-------------|--------------------------------------|-------------|
|           | Oil Phase             |             | Active +                             | Water Phase |
|           | (%)                   |             | Surfactant (%)                       | (%)         |
|           | Virgin<br>Coconut Oil | Shea Butter | Rose hip extract<br>– Lecithin (1:1) | Water       |
| <b>F1</b> | 25                    | 2           | 1                                    | 72          |
| <b>F2</b> | 30                    | 2           | 1                                    | 67          |
| <b>F3</b> | 35                    | 2           | 1                                    | 62          |
| <b>F4</b> | 40                    | 2           | 1                                    | 57          |
| <b>F5</b> | 45                    | 2           | 1                                    | 52          |
| <b>F6</b> | 48                    | 2           | 1                                    | 49          |

### 3.5. Nano-sized Dispersion Characterization

Dynamic Light Scattering (DLS) optical technique was conducted to analyze the particle sizes and size distribution of the prepared nano-sized particles. The prepared rose hip extract - lecithin sample was examined by this technique. A folded capillary

zeta cell that was suitable to examine particle size and zeta potential was filled with the specimen. The sample was inserted into the cell area and the average size and zeta potential of the sample was measured at 25 °C by using a Malvern Zetasizer Nano (Worcestershire, United Kingdom) with zetasizer software version 7.12.

### **3.6. Preparation of Hydrogel Manually**

The sodium alginate produced by Sigma-Aldric, Missouri was weighed and it was dissolved in a fair amount of distilled water to prepare a 1 % alginate solution by using a magnetic stirrer (Hei-Standard, Heidolph, Germany). When the solute was completely dissolved at room temperature, 0.25 % polyethylene glycol (PEG) provided from Sigma-Aldric, Missouri was added to the alginate solution and then filled up with distilled water. The solution was let stirring until getting completely clear. The solution was stirred on a magnetic stirrer to form a uniform aqueous solution. On the other side, calcium chloride (CaCl<sub>2</sub>) produced by Sigma-Aldric, Missouri was dissolved in distilled water to prepare a 1% CaCl<sub>2</sub> solution by using a magnetic stirrer. 2.5 g of alginate solution was mixed with 2.5 g of each prepared microemulsion specimen separately on a magnetic stirrer to obtain a 1:1 uniform mixture and then prepared mixtures were transferred into six different petri dishes. 5 g CaCl<sub>2</sub> was added to each petri dish gradually by using a hypodermic syringe. After the gelation process was completed, the prepared hydrogels were put into a vacuum oven (CLRC-17C, CLS Scientific, Turkey) for about 1 hour at 30 °C and 30 mbar pressure. The prepared six different hydrogels were lyophilized directly by using the freeze dryer.

### **3.7. Production of Sodium Alginate Micro-bubbles**

In this study, a T-junction microfluidic system was used to produce micro-bubbles. The experiment setup scheme is demonstrated in Figure 3.1. Two immiscible phases were utilized to produce bubbles, alginate solution as the continuous phase and nitrogen gas as the dispersed phase. T-junction microfluidic device with perpendicular rupturing produced from polymethylmethacrylate (PMMA) polymer was used in the experiment set up. Alginate solution prepared as mentioned before was fed into the system at a constant flow rate by using a 10 ml plastic syringe (Becton Dickinson).

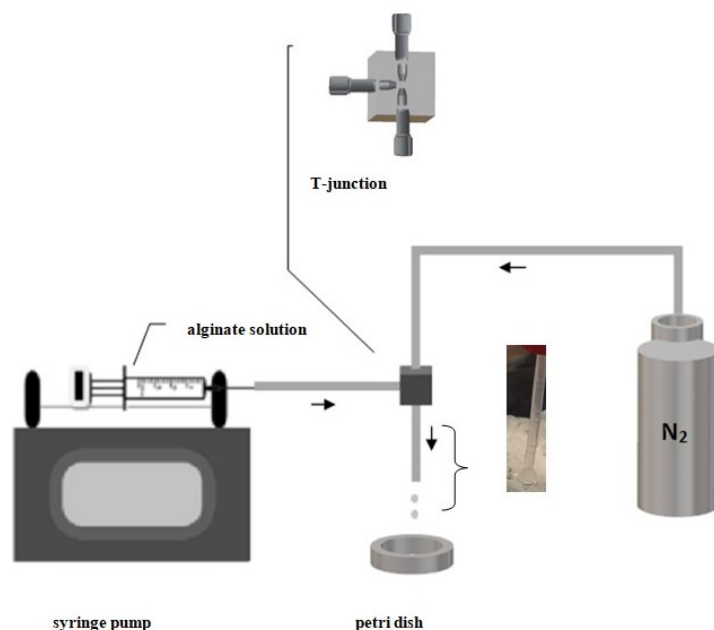


**Figure 3.1.** Schematic diagram of the experimental setup

A microfluidic syringe pump was used to pump the one phase, alginate solution, into the flow micro-channel and the microfluidic device was connected to this syringe for the alginate feed (IPS-14RS, Inovenso, Turkey). The flow rate was set at 200  $\mu\text{L}/\text{min}$ . Nitrogen at room temperature was used for the gas phase. The other 200  $\mu\text{m}$  diameter micro-channel was connected to the nitrogen gas tank. The pressure was controlled by a pressure indicator. Nitrogen gas was at constant pressure, 0.5 bar. Micro-bubbles were formed through the junction of the micro-channels. After waiting for arranging the rates of flow, allowed the system to produce uniform series bubbles, and then they were gathered at the outlet of the micro-channel. The bubbles clustered on the outer edge of the micro-channel were self-propelled due to gravitational force and they dropped in a glass petri dish.

### **3.8. Characterization of Size and Structure of the Obtained Sodium Alginate Micro-bubbles**

The size and distribution of the micro-bubbles were visualized by an optical microscope (AxioCam MRc 5, Zeiss, Germany). A small amount of the freshly

prepared alginate bubbles were collected on the microscope slides. The size of the micro-bubbles was measured immediately after generation in the micro-channel. The 5X and 10X objective lenses were used to observe different magnification. Micro-bubble diameters were determined and the images were recorded.

### **3.9. Preparation of Hydrogels**

While the alginate bubbles were generating and collecting in the petri dish, the selected microemulsion sample was carefully injected into the bubbles gradually with a hypodermic needle to incorporate. When this process was completed, the prepared  $\text{CaCl}_2$  solution was slowly injected manually. On average 4.0 g of the prepared microemulsion, 4.0 g of the sodium alginate solution, and the same amount of  $\text{CaCl}_2$  as the alginate solution were used up to prepare the hydrogel. The hydrogels in the dish were waited to reach a steady-state and then the excess solution was discarded. The hydrogels were prepared for the skin application studies with volunteers and they were kept in the fridge. Also, a sample was put into a vacuum oven and it was lyophilized for characterization experiments.

### **3.10. Characterization of the Hydrogels by Scanning Electron Microscope (SEM)**

The microstructures of the obtained hydrogels were examined with a scanning Electron microscope (FEI (Philips) XL30 SFEG) after drying in the lyophilizer. The monographs of the samples were displayed at different magnifications.

### **3.11. Skin Application Studies with the Volunteer (a case study)**

The measurements were conducted with a volunteer subject on different days. (n=3, a case study). Moisture and oil content of the skin were obtained from the face area of the subject by using a skin moisture and oil content analyzer (OEM Sk-8). Measurements were taken and recorded before and after the administration numerically.

### **3.12. Statistical Analysis**

All measurements were accomplished in triplicates. The obtained results were subjected to statistical analysis by using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The significance of differences between the means was commented by utilizing Dunnett's Multiple Comparison Test and Turkey's Multiple Comparison Test.



## 4. RESULT AND DISCUSSION

### 4.1. Determination of Vitamin C (L-ascorbic Acid) in Rose Hip Samples

It is known that rose hips contain as well as vitamin C (L-ascorbic acid) many other beneficial bioactive compounds such as flavonoids, phenolic acids, carotenoids [142]. In this study, merely the qualitative determination of vitamin C content in the rose hip samples was stipulated. Since thin layer chromatography is one of the chromatographic methods applied for the separation and determination of substances especially for qualitative analysis of chemical components in complex mixtures like herbal samples, beverages, it is widely preferred for determination methods of vitamins. Moreover, the TLC method is recommended for the qualitative determination of vitamin C in rose hips in European Pharmacopoeia 8.0. Therefore the TLC method was conducted to determine the existence of the vitamin C in the extracts of rose hip samples in this study [141, 143, 144].

There are several extraction methods available to determine the vitamin C content of rose hips [41, 145, 146]. In this study, two procedures were followed to preserve the rose hips that were harvested according to the ripening period and their color. Air-dried and freeze-dried rose hips were analyzed separately. Firstly, the air-dried rose hip samples were analyzed by the TLC method. In the beginning, ethanol and water were used for the extraction of plant material. Air-dried rose hips were separately extracted with ethanol and water, the obtained dry residue was weighed; it was obtained about 41.50 g and 126.50 g, respectively, and then they were lyophilized. In addition, some of the naturally air-dried rose hips were ground and sieved to obtain the powder of the fruit. The prepared samples were applied onto the layer of the sorbent plate as a band. By using a mixture of ethyl acetate: acetone: water: formic acid (5:3:1:1, v:v:v:v) to develop the TLC plates, the samples compared with standard Vitamin C [147]. After development and drying, the plate was observed in UV light at  $\lambda = 254$  nm and  $\lambda = 366$  nm [148]. The chromatograms were shown in Figure 4.1. The air-dried samples tested were not provided comparable and significant fingerprints for L-ascorbic acid; any significant band was not detected as expected [149]. This result indicated that the vitamin C content of the air-dried rose hip samples was reduced and these samples did not contain significant vitamin C (Figure 4.1.a-b-c).

On the other side, it was studied with the collected and freshly frozen rose hips. Some of the fresh plant material was extracted with ethanol by following the same method. The obtained dry residue was weighed; it was obtained about 54.49 g. Moreover, freshly frozen rose hips were lyophilized for the freeze-drying method, and then freeze-dried rose hip samples were ground and sieved. The prepared samples for the TLC method were applied onto the layer of the sorbent plate as a band and the same process was followed. Separation of vitamin C on the plate was detected [150]. After the chromatographic separation, the plate was observed under ultraviolet light to visualize the separated bands. The presence of vitamin C in rose hip samples was ascertained by comparing it with the standard. The chromatograms were shown in Figure 4.1. It was examined that ethanol extract of the fresh frozen fruit was not also provided significant fingerprints for Vitamin C (Figure 4.1.d). On the other hand, ground freeze-dried rose hip samples shown that the fruit had significant vitamin C content (Figure 4.1.e).

Both ethanol extract of air-dried and freshly frozen rose hip samples did not provide a significant band to detect vitamin C content as expected. This result showed that the ethanol extracts of the fruit did not contain a desirable amount of L-ascorbic acid. When the ground samples of air-dried and freeze-dried were compared, while no significant vitamin C was detected in air-dried fruit, the vitamin C content of freeze-dried rosehip samples was observed. This comparative study showed that freeze-dried rose hips contain more vitamin C than air-dried rose hips. There are studies that state that dried rose hips comparing to fresh rose hips possess a lower amount of vitamin C [44, 47]. As a result of this comparative study, it was determined that freeze-dried rose hips are more suitable for future studies.

Furthermore, the ground freeze-dried rosehip samples were lyophilized again for optimum conditions. The Vitamin C study with the TLC method was conducted for this prepared sample to determine the vitamin C content of the ground freeze-dried rosehip samples (Figure 4.1.f). When the chromatograms of the samples before and after lyophilization were compared, it was observed that the lyophilized rosehip powder gave a more pronounced band than before lyophilization. Although rose hips are declared to be one of the most plentiful natural sources of vitamin C, it is known that parameters

like soil, climatic conditions, altitude, ripening, and harvesting time, drying methods can affect the level of vitamin C [47, 151, 152]. These results again indicated that the

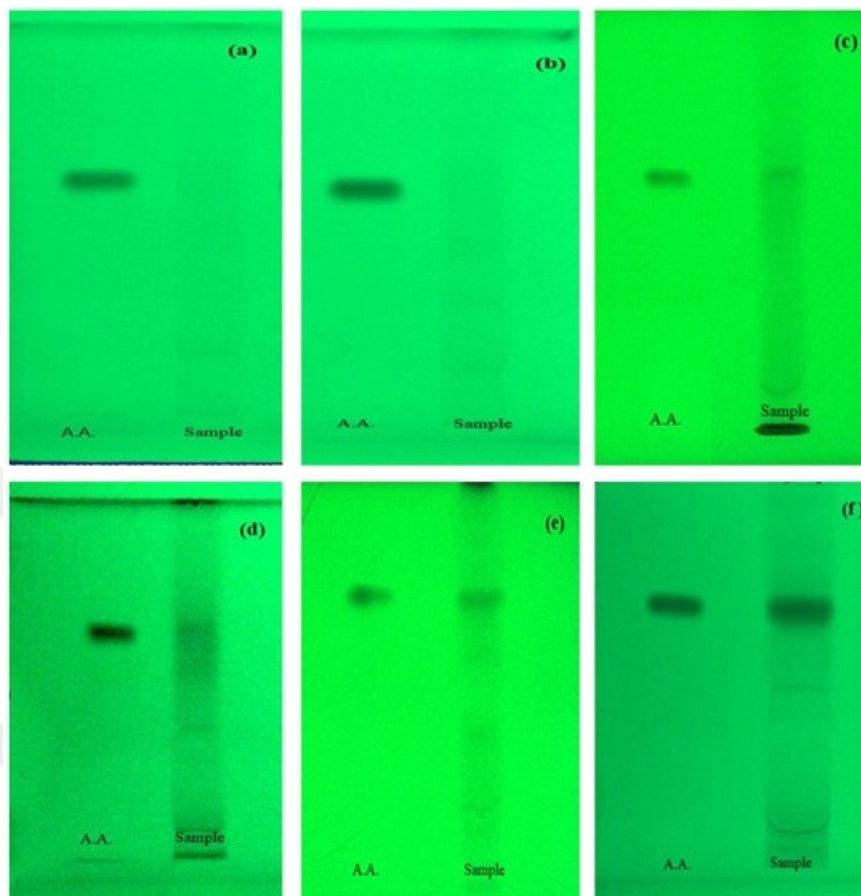


**Figure 4.1.** TLC Chromatogram of Vitamin C of rose hip samples, 254 nm UV, (a) Air-dried - ethanol extract, (b) Air-dried - water extract, (c) Air-dried powder, (d) Fresh-frozen - ethanol extract, (e) Freeze-dried - Fresh-frozen fruit, (f) Fresh-frozen - freeze-dried powder; (A.A.= Vitamin C ( L-Ascorbic Acid))

amount of vitamin C in rose hips depends on not only growing conditions but also on drying, processing, and storage conditions [153]. All of these results indicated that the use of lyophilized freeze-dried rosehip powder was more suitable. Thus, it was continued with lyophilized freeze-dried rosehip extract for further studies.

## 4.2. Preparing and Characterization of the Rose Hip Microemulsion

### Formula

Microemulsion formulas have been extensively used in topical drug delivery systems and cosmetic applications [154]. Emulsion systems are the suitable medium for hydrophilic and hydrophobic constituents being together. After the rose hip sample that would be used for the further experiment was prepared and examined, basic microemulsion preparation studies were carried out. Rose hip, virgin coconut oil, shea butter, and lecithin were used to prepare the final disperse system. While deciding the content of the formula, natural-origin ingredients were preferred. Rose hip was selected because it contains vitamins, phenolic compounds, carotenoids, organic acids, minerals that are known benefits for the skin. Especially it is well known for its high vitamin C content [45]. It is known that vitamin C is one of the vital molecules for skin health since its role in collagen synthesis and its antioxidant properties. Although the main ingredient in this prepared formula actually was rosehip, the other ingredients were added to the formula mainly as a carrier. However, virgin coconut oil and shea butter are known to be also natural ingredients beneficial for the skin. Shea butter contains valuable substances for the skin such as fatty acids, polyphenols, and a high amount of unsaponifiable matter; also virgin coconut oil does, like fatty acids, polyphenols, and  $\alpha$ -Tocopherol [155, 156]. Moreover, there are studies shows that virgin coconut oil is favorable for topical emulsion formulas and it is a suitable carrier [157, 158]. A semi-solid formula was prepared since it was known that vitamin C is a sensitive substance and it could be destroyed over time when exposed to heat and light [159]. As it is well known, solid and semi-solid systems are more stable compared to liquids [160]. In this study, microemulsion was preferred because it was thought to provide more penetration to the skin and be obtained a stable product [154, 159] while preparing the formula. It was researched that microemulsion was favorable carriers to deliver and stabilize ascorbic acid derivatives for topical applications [161]. Besides, it was planned that the active ingredients reach the deeper layers of the skin by passing through the stratum corneum.

Dynamic Light Scattering (DLS) optical technique was conducted to analyze the particle sizes and size distribution of the prepared nano-sized particles. It is one of the most well-known techniques that are used to measure the particle size and size

distribution of dispersed systems from nanometer to micrometers in diameter [162,163]. The polydispersity index (Pdl) is a measure of homogeneity of particles in a dispersed system and this is shown from 0.0 to 1.0 range, it is generally preferred below 0.7. The polydispersity index is one of the prominent criteria to figure out the uniformity of the particle size dispersion because aggregation or flocculation could occur in nonuniform systems [164]. In addition, Zeta potential is a scientific term that describes a measure of the magnitude of attraction and repulsion charges between particles or droplets in other words electrokinetic potential in colloidal systems. The Electrophoretic Light Scattering technique is used to measure the zeta potential and the zeta potential unit is usually millivolts (mV) [165]. This measurement helps to provide info about fundamental parameters known to affect stability. An absolute value of zeta potential above 30 mV usually is accepted to provide favorable stability in the colloidal systems [166]. These techniques were utilized to characterize the prepared nano-sized particles in this study.

First of all, as a pre-formulation study, rose hip extract and lecithin were mixed in a 1: 1 (w/w) ratio. It was known that the stability of rose hip content was weak. It was first examined to see if a homogeneous distribution was obtained with the probe-sonicator (70.0 kHz). The characterization of the nano-sized particles formed was examined with DLS (Figure 4.2). The size distribution of the rose hip extract -lecithin droplets was characterized by DLS at 25<sup>0</sup>C and the average value was 333.2 nm. It was examined that there were 88.1 % particles of 345.8 nm size. Pdl value which was a significant value to indicate the uniformity of the system was 0.530. Moreover, the zeta potential of the rose hip extract - lecithin sample was measured at -15.9 mV (Figure 4.3). These pre-formulation results gave an idea that a nano-sized homogeneous formula could be obtained with this preparation method and could be added to the decided carrier system.

For the next stage, rose hip and lecithin microemulsion was dispersed into the decided carrier system (virgin coconut oil and shea butter). For this purpose, 6 different samples with different ratios that were shown in Table 4.1 were performed as a pre-formulation study. The obtained rose hip extract - lecithin (1:1, w/w) sample was distributed into an oil phase composing of virgin coconut oil and shea butter by using

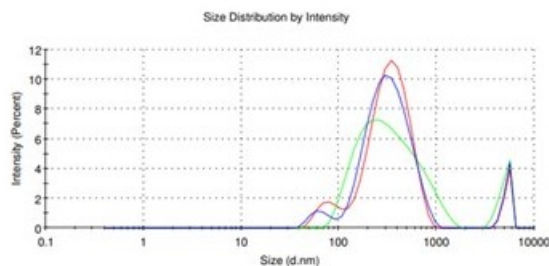


**Figure 4.2.** Particle size distribution by intensity rose hip extract - lecithin (1:1, w/w) at 25 °C

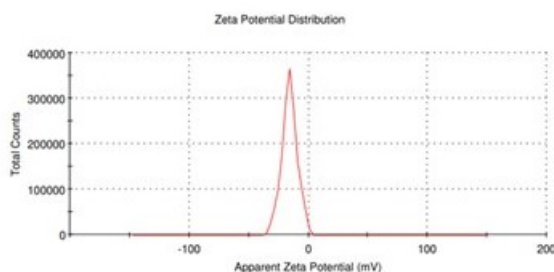


**Figure 4.3.** Zeta potential distribution of rose hip extract - lecithin (1:1, w/w) at 25 °C

the probe-sonicator (70.0 kHz) for 9 minutes in total. In order to protect both the structure of the oils in the formula and the vitamin C in the rosehip, the temperature was taken into consideration during the preparation of the microemulsion formula and it was developed under optimum conditions. The environment was conducted so that the temperature remained in equilibrium. Virgin coconut oil has a more liquid texture. Thus, the ratios of water and virgin coconut oil were changed while the other the ratios of the other components were fixed [167].

Since the obtained dispersion would be incorporated with a hydrogel, the ratios of the phases were changed attentively to provide stability and keep the active ingredients in optimum grade. Physical appearance is one of the emulsion system evaluation parameters. The developed samples were checked visually by the fluidity and uniform appearance. It was observed that as the amount of water in the prepared

**Table 4.1.** The content of pre-formulation dispersions and the appearances of the formulas

| Formulas  | Contents (w/w) |            |                |             | Appearance                      |
|-----------|----------------|------------|----------------|-------------|---------------------------------|
|           | Oil Phase      | Active +   |                | Water Phase |                                 |
|           | (%)            | Surfactant |                | (%)         |                                 |
|           | Virgin         | Shea       | Rose hip       | Water       |                                 |
|           | Coconut Oil    | Butter     | extract –      |             |                                 |
|           |                |            | Lecithin (1:1) |             |                                 |
| <b>F1</b> | 25             | 2          | 1              | 72          | Phase separation, heterogeneous |
| <b>F2</b> | 30             | 2          | 1              | 67          | Phase separation, heterogeneous |
| <b>F3</b> | 35             | 2          | 1              | 62          | Phase separation, heterogeneous |
| <b>F4</b> | 40             | 2          | 1              | 57          | Heterogeneous dispersion        |
| <b>F5</b> | 45             | 2          | 1              | 52          | Heterogeneous dispersion        |
| <b>F6</b> | 48             | 2          | 1              | 49          | Homogenous dispersion           |

formulas increased, the homogenous appearance deteriorated, in other words, the stability decreased. It was supposed that the small size of particles and lipophilic content of the formula could help the enhancement of penetration into the skin. Therefore, the formula that contained the uttermost oil phase was more uniform and it could be seen as more suitable for further studies. The developed formula containing

virgin coconut oil, shea butter, rose hip extract - lecithin, water in ratios 48, 2, 1, 49 (%) respectively, called formula 6, was observed to be stable and have a homogeneous phase at room temperature. Also, these observations showed that this was the most proper formula for further study.

#### **4.3. Preparation and Characterization of the Alginate-based Hydrogels containing the Rose hip Formula**

Alginate is a naturally derived polysaccharide that has found a wide range of applications in drug delivery and biomedical science. It has favorable properties like biocompatibility, non-toxicity, bioadhesive, and ease of gelation. Alginate has crosslinking and gelation properties in the presence of a divalent cation like the calcium ion. Alginate hydrogels have been used in applications related to the skin like drug delivery, tissue engineering, and wound healing in recent years [168, 169]. Hydrocolloids like alginate have been one of the natural materials that have taken attraction in the design of a controlled-release product for many years, for instance of the controlled release of proteins [170]. Due to all of these properties, alginate hydrogels were used to support to penetrate the active ingredients to the skin in this study.

We aimed to integrate the prepared rose hip formula with the alginate hydrogel. After the rose hip formulas were developed, the alginate hydrogels were manually produced with the 6 different rose hip formulas prepared within the scope of the pre-formula. Each prepared rose hip formula was mixed with the alginate solution at a ratio of 1:1. It was known that alginate generates three-dimensionally cross-linked networks in the presence of calcium ions and becomes hydrogel form. The mixture was taken to the glass petri dishes and then  $\text{CaCl}_2$  solution was added slowly to let the gelation process. After the gelation process was completed, the samples were lyophilized to observe the structure. The manually prepared rose hip hydrogel samples were depicted in Figure 4.4. These six samples were observed visually. The color and texture differences between them were noticeable. As a result of both the observation made after this study and the evaluations made after the microemulsion characterization study, rose hip formula named formula 6 (F6) was considered to be the most suitable formula for further experiments.



**Figure 4.4.** The samples of alginate-based hydrogel prepared manually containing rose hip formula

M. Zhang et al studied to develop double emulsions encapsulated by Ca-alginate hydrogel containing ascorbic acid for topical delivery. They stated that it might be a promising delivery system for topical applications by indicating release characteristics, skin permeation, storage stability, and antioxidant activity properties [171]. In the next step, alginate hydrogels were prepared with the selected formula using the microfluidic technique. It was aimed to be penetrated to the skin by facilitating the release of the active ingredients from the hydrogel. For this purpose, T-junction microfluidic device was used to generate the uniform micro-bubble. Micro-bubbles were generated with the prepared alginate solution by T-junction microfluidic device and the formed micro-bubbles were observed under the optical microscope. Bubble formation was generated at the outlet of the micro-channel of the T-junction microfluidic device [172]. The images of the micro-bubbles at 10X magnification were shown in Figure 4.5. The constant gas stream was passed through the micro-channel and the alginate solution was given to the system with the pump. The gas encountered the liquid in the T-junction device, and then the gas constricted the passage of the fluid flow and breaking up into bubbles at the outlet. The liquid flow rate and the gas pressure determined the size of the bubbles [173]. When the gas pressure was not high enough, there was only liquid flow, thus the liquid flow rate and the gas stream were arranged to create constant bubble generation. The bubbles were collected in the glass petri dish by being attentive

that they were not burst at the exit of the outer orifice. The micro-bubbles monitored at 10x magnification were observed that were equal dimensions. This was shown that uniform alginate bubbles could be obtained by this method. Generating bubbles of equal size is effective for forming homogeneous hydrogels structures. The bubbles were not so stable in the air and could burst in time [174].

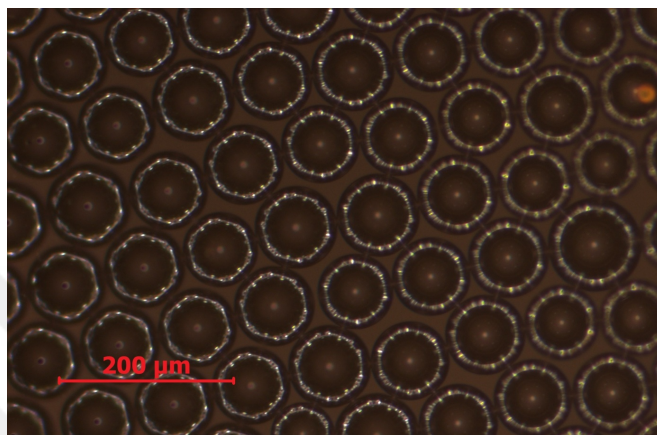


**Figure 4.5.** Optical microscopy image of the mono-dispersed alginate microbubbles generated using the T-junction microfluidic device (10X magnification). The scale bar= 200 μm.

After the production of equal-sized alginate micro-bubbles with the microfluidic method, the rose hip formula which was selected with the pre-formulation study was incorporated with alginate bubbles at a ratio of 1:1 (w/w) [167]. While alginate micro-bubbles were producing, the rose hip formula was incorporated gradually and then  $\text{CaCl}_2$  was added slowly for the cross-linking process. Besides, the rose hip formula was added to the hydrogel that had been already prepared alginate micro-bubbles and  $\text{CaCl}_2$  solution. Thereby, the difference between adding the rose hip formula before and after the formation of the hydrogel was observed.

The structures of all prepared hydrogels were viewed with the scanning electron microscope (SEM) and the SEM images were shown in Figure 4.6. The micrograph of the hydrogel sample (S4) that the micro-bubble was generated with the microfluidics

method and they were cross-linked with  $\text{CaCl}_2$  was depicted in Figure 4.6 (S4). It was observed regular bubbles were formed. The structure of the alginate-based hydrogel sample (S1) which contained the micro-bubble generated by microfluidics incorporated with rose hip formula was viewed with SEM. As seen in the SEM micrograph, a porous spongy structure was tried to be formed. It was obtained the highly porous structures with interconnected pores (Figure 4.6, (S1)). It could be clearly seen the dispersion of prepared formula in the alginate hydrogel in scanning electron microscopic image. It was detected that the prepared rose hip formula was filled in the cavities inside the micro-bubbles. By the means of the porous structure of prepared hydrogel, the release of the formula was facilitated. The expectation was to get the release of the formula from the bubbles. Thus, it was purposed to have more penetration into the skin. On the other hand, the micro-bubble was generated with the microfluidic method and they were cross-linked with  $\text{CaCl}_2$ , and then rose hip formula was incorporated. The micrograph of the sample (S3) was shown in Figure 4.6, (S3)). Although it could be seen that there were regular bubbles, the formula was dispersed irregularly, the rose hip formula remained on the surface and did not fill in the cavities. Unlike the sample S1, it was seen that the formula remained at surface edges, not inside the cavities in the sample S3. This application was not suitable for the purpose of the study. The aim was to incorporate the formula without being lost the bubbles. It clearly appeared that incorporation after the hydrogel had been made did not provide a favorable sample [175].

The hydrogel prepared manually with rose hip formula and alginate solution was also examined in SEM (Figure 4.6 (S2)). The sample S2 was observed that the cavities were not uniform. However there was a porous structure, homogeneous distribution was not seen, content uniformity was not provided. Unlike the sample S1 which was produced with microfluidics, there was a rather dispersed distribution and separated structures formed. The micrograph of the manually prepared hydrogels which just contained the alginate solution and  $\text{CaCl}_2$  solution was shown in Figure 4.6 (S5). The cavities formation of the manually prepared hydrogel sample (S5) was also examined and it was seen foliate structures. However, it did not regular cavities like the hydrogel samples (S4) prepared with microfluidics.

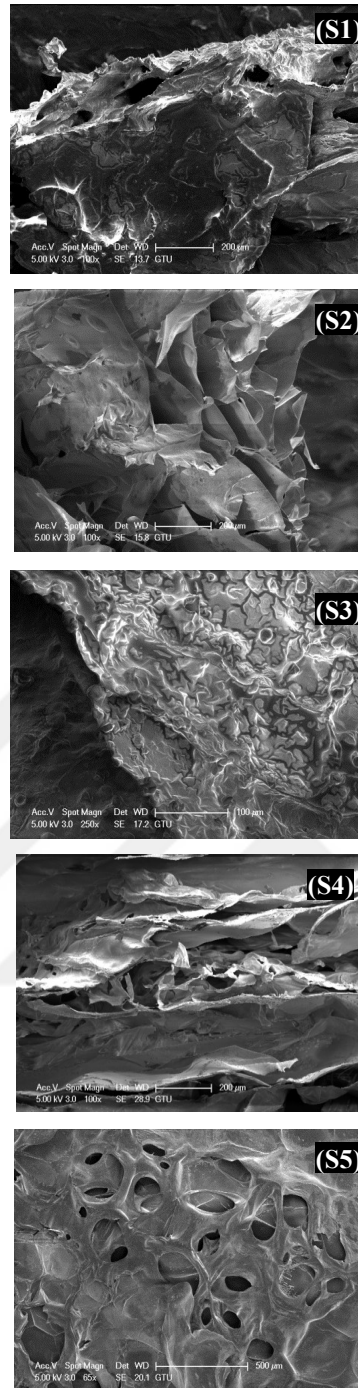


**Figure 4.6.** The SEM images of prepared hydrogels (S1: the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics; S2: the alginate-based hydrogel prepared manually with the rose hip formula; S3: the alginate-based hydrogel composing of the micro-bubble generated by microfluidics incorporated with rose hip formula after the gelation; S4: the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics; S5: the blank alginate-based hydrogel prepared manually).

#### 4.4. The Skin Application of the Alginate-based Hydrogels containing the Rose hip Formula

The skin application study was performed on a volunteer with the samples prepared. Rose hip formula, which was incorporated with micro-bubbles obtained at 200  $\mu\text{l}$  / min flow rate and 0.5 bar pressure values from alginate solution, was converted to hydrogel by cross-linking, and then this hydrogel mask prepared with optimum values was applied to the volunteer's skin. In addition, the study with the volunteer was performed on hydrogels obtained manually and also without rose hip formula. Briefly, the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics (S1), the alginate-based hydrogel prepared manually with the rose hip formula (S2), and the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics (S4) were applied in this study. The visual appearance of prepared samples was also displayed in Figure 4.7.

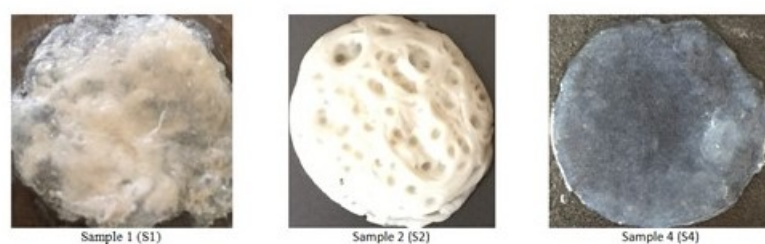


**Figure 4.7.** The appearance of the prepared hydrogels (S1: the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics; S2: the alginate-based hydrogel prepared manually with the rose hip formula; S4: the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics)

An efficacy test was carried out on a subject with the three prepared hydrogel samples. The prepared hydrogel samples were applied to the face area of the subject. The applications were performed on healthy, middle-aged (44), and undamaged skin on different days. Moisture and oil content of the volunteer's skin were measured

numerically before and after the administration with the digital skin moisture and oil content analyzer device. Measurements were taken at the 0<sup>th</sup> minute, 15<sup>th</sup> minute, 30<sup>th</sup> minute, 45<sup>th</sup> minute, and 60<sup>th</sup> minute, respectively. Each was measured in triplicate. Firstly, measurements were taken before the prepared hydrogels were placed on the subject's skin. Then, the hydrogels were applied in the application area and the measurements were taken at designated time intervals (15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, and 60<sup>th</sup> minutes). At the end of the 60<sup>th</sup> minute, the hydrogels were removed and the measurements were taken again at the 120<sup>th</sup> minute. The percentage values of water and oil content obtained from the skin surface by contacting the device to the skin were recorded. The changes of water content values in the applied skin with different hydrogels depending on time were examined in detail in the graphic in Figure 4.8.

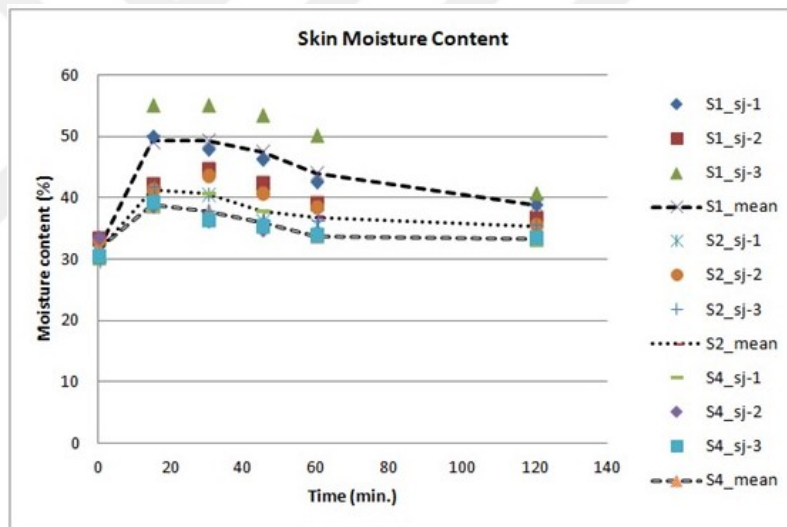


**Figure 4.8.** The moisture content ratios of the subject's skin that were performed each application with prepared hydrogels (S1: the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics; S2: the alginate-based hydrogel prepared manually with the rose hip formula; S4: the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics; sj-1/sj-2/sj-3: 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> day application to the volunteer, respectively)

The micro-bubble generated by microfluidics incorporated with rose hip formula hydrogel (S1) was applied first. The mean of the initial values of the moisture content of the skin was 31.4%. The mean values at 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, and 60<sup>th</sup> minutes were 49.3%, 49.3%, 47.5%, and 44.0%, respectively. As could be seen in the graph, the moisture content increased by 57% in the 15<sup>th</sup> minute and reached 49.3%. It was measured that after application with sample 1 (S1), the water percentage of the skin increased with time in the beginning. In the following periods, the moisture content started to decrease gradually. By the 60<sup>th</sup> minute, the value of 44.0% was obtained. Although this value was a lower percentage than the 15<sup>th</sup> minute, compared to the initial value, the 40% increment was observed in the moisture content of the skin. Then, the hydrogel sample was removed from the skin. When the measurement was applied from the same point after 120 minutes, it was observed that there was an increase in the moisture content of 24% compared to the initial value. This showed that the prepared sample provided a fairly enough permanent moisture increase in the skin. Also, this result indicated that the content of the prepared formula was penetrated efficiently by the skin. The skin observation was performed to visually confirm the skin hydration effect of prepared hydrogels. It was observed that after the application it leaves the skin more soft, resilient, supple, and radiant.

On the other hand, measurements were taken of the hydrogel prepared manually with rose hip formula (S2) and the blank alginate micro-bubble generated by microfluidics (S4). When the skin was examined at the 15<sup>th</sup> minute, there was a 32% raise with sample 2 (S2), but it provided a smaller enhancement compared to S1. On the other side, the increment with sample 4 (S4) lagged behind the other two samples and was measured as 22%. It was observed that the measurements taken with S2 at the 60<sup>th</sup> minute declined compared to the 15<sup>th</sup> minute, but there was a 17% upsurge in moisture by comparison with the initial value. In the measurement taken 1 hour after the removal of the hydrogel (S2), an average 13% increase in humidity in comparison to the initial value was measured. When the value both at the 15<sup>th</sup> minute and 120<sup>th</sup> minute were compared with S1, it was observed that the moisture content boosted less than S1. Although a 22% increase in moisture content was measured at the 15<sup>th</sup> minute with S4, it was observed that there was a dramatic decrease in the 60<sup>th</sup> minute because the hydrogel could not provide its moisture during the application as it dried quickly. Also, when the measurement at the 120th minute was examined, it was seen that the skin

moisture content almost returned to the initial value. Besides, the changes of moisture content values according to the type of prepared hydrogels were compared in detail at the 15<sup>th</sup> and 120<sup>th</sup> minute in the graphic in Figure 4.9.

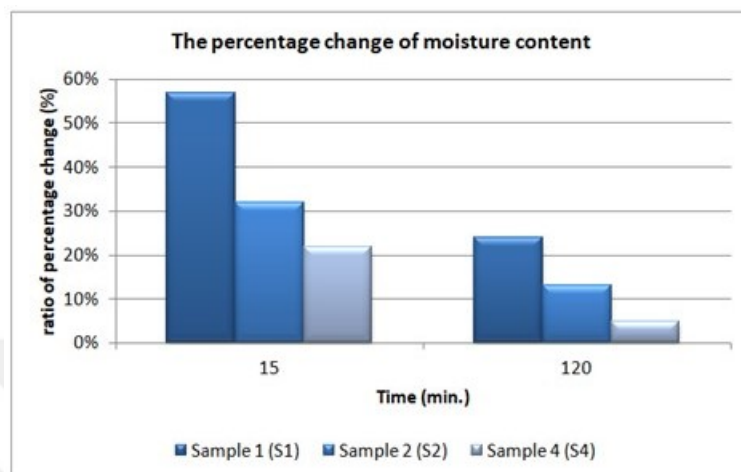


**Figure 4.9.** The percentage change of the subject's skin moisture content at 15<sup>th</sup> and 120<sup>th</sup> min. (S1: the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics; S2: the alginate-based hydrogel prepared manually with the rose hip formula; S4: the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics)

The values were also evaluated statistically by utilizing GraphPad Prism Dunnett's Multiple Comparison Test. When S1 was compared to the skin in the 15<sup>th</sup> minute statistically, it was observed that there was a significant difference in the moisture content of the skin ( $p < 0.0001$ ) (Figure 4.10). At the 15<sup>th</sup> minute, S1 was compared with S2 for the moisture content by using Turkey's Multiple Comparison Test, there was a significant difference between them ( $p < 0.05$ ). It was observed that there was a significant difference when S1 was contrasted to S4 at the 15<sup>th</sup> minute. Moreover, at the 120<sup>th</sup> minute, S1 was compared with S2, there was a significant difference between them ( $p < 0.05$ ). It was displayed that there was a significant difference when S1 was contrasted to S4 at the 120<sup>th</sup> minute. Although there was a significant difference in both S1 and S2 in the 15<sup>th</sup> minute, it was seen that S2 could not

maintain the same significant difference in moisture content increase at the 120<sup>th</sup> minute ( $p < 0.05$ ). However, it was seen that the significant difference continued at the 120<sup>th</sup> minute ( $p < 0.0001$ ) in the application with S1. In addition, in the S4 application, no significant difference was observed at the 120<sup>th</sup> minute as to the 0<sup>th</sup> minute in the moisture content of the skin.

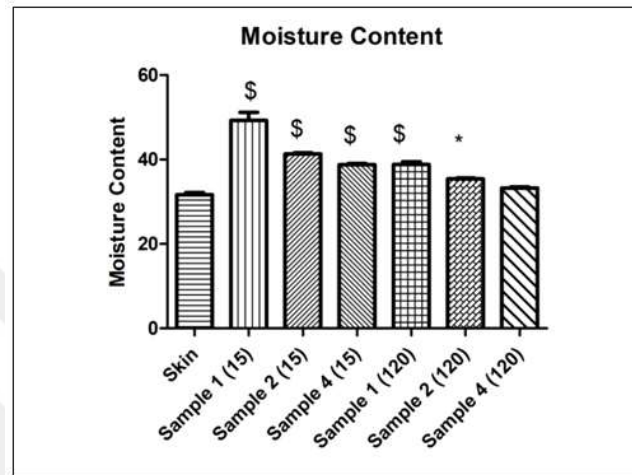


**Figure 4.10.** Comparison of prepared hydrogel samples according to moisture content variation at 15th and 120th minute. (S1: the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics; S2: the alginate-based hydrogel prepared manually with the rose hip formula; S4: the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics), (\$: statistically difference when compared with the skin ( $p < 0.0001$ ), \*: statistically difference when compared with the skin ( $p < 0.05$ )).

The result showed that blank alginate hydrogel appeared to provide an instant upsurge in moisture but did not provide actual moisture development in the skin. Meanwhile, it was observed that the skin surface treated with the hydrogel filled with the prepared formula was more hydrated than that of non-filled samples. As a result, it has been found that the micro-bubble generated by microfluidics incorporated with rose hip formula hydrogel (S1) provided a higher rate of moisture increase on the skin and showed a longer permanence.

In addition, the skin's oil content was also measured at the same time with the analyzer while on application. The changes of oil content values in the applied skin with different hydrogels depending on time were examined in detail in the graphic in Figure 4.11. The average initial oil content value was gauged 14.0% and then sample 1 was applied in this area. In the 15<sup>th</sup> minute, the oil content increased by a mean of 58% and reached a mean 22.0% ratio. By the time of progress, the oil content started to decrease compared to the 15<sup>th</sup> minute. At the 60<sup>th</sup> minute, an average value of 19.2% was measured and this was indicated that the application side was a mean 38% higher oil content than the value before placing sample 1. When the value taken at the end of the 120<sup>th</sup> minute was considered, it was seen that the product provides a 24% upsurge in the skin oil content by comparison with the value before the application. This result showed that with sample 1 both the oil content of the application area increased during the application and also the effect of the product continued after the passage of time.

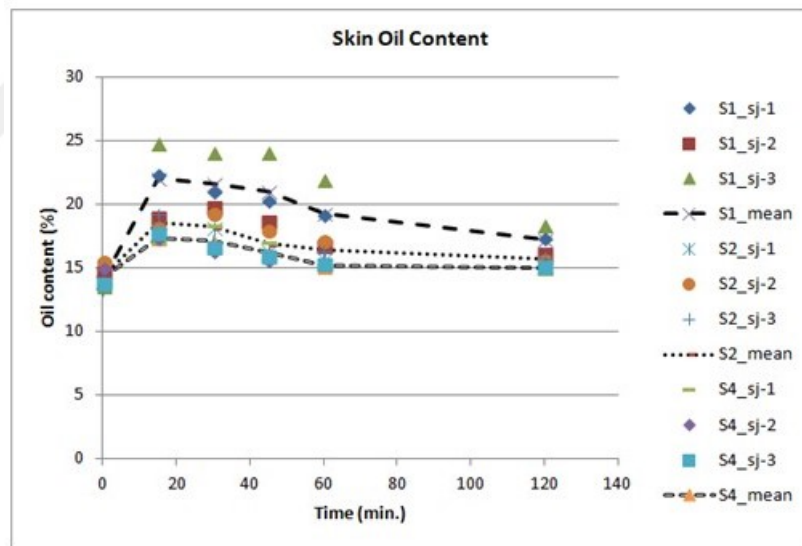


**Figure 4.11.** The oil content ratios of the subject's skin that were performed the application with prepared hydrogels (S1: the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics; S2: the alginate-based hydrogel prepared manually with the rose hip formula; S4: the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics; sj-1/sj-2/sj-3: 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> day application to the volunteer, respectively)

On the other hand, the oil content escalation at the 15<sup>th</sup> minute where S2 applied area was measured as 30%. This rate declined in the progress of time and increased by 15% in the 60<sup>th</sup> minute, reaching 16.4% from the initial average value of 14.2%, and this value reached a 10% raise at the end of the 120<sup>th</sup> minute. It was observed that these values were lower by comparison with S1, and S2 performed less in oil content enhancement of the skin. The changes of oil content values according to the type of prepared hydrogels were compared in detail in the graphic in Figure 4.12. In the application with S4, the first average value was 14.2%, while the average values of 22%, 7%, and 6% were measured at the 15<sup>th</sup>, 60<sup>th</sup>, and 120<sup>th</sup> minutes, respectively. Although there was an instant upsurge in the first application, the oil content reduced rapidly in the process of time with the rapid drying of the hydrogel. The oil value of the skin did not show a significant enhancement compared to the initial condition. In this study, it was shown that the developed formula was effective on the skin and the microfluidic method could have an advantage for producing more effective hydrogels compared to the manual method. These results indicated that the micro-bubble generated by microfluidics incorporated with rose hip formula (S1) provided a higher and long-lasting oil content rate on the skin.

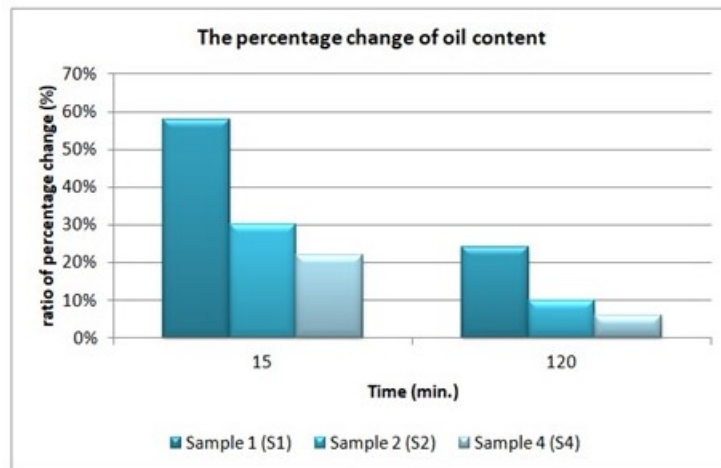


**Figure 4.12.** The percentage change of the subject's skin oil content at 15<sup>th</sup> and 120<sup>th</sup> min. (S1: the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics; S2: the alginate-based hydrogel prepared manually with the rose hip formula; S4: the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics)

The values were also evaluated statistically by utilizing Dunnett's Multiple Comparison Test. When S1 was contrasted to the skin at 15<sup>th</sup> minutes statistically in oil content measurement, it was seen that there was a significant difference ( $p < 0.0001$ ). (Figure 4.13). At the 15<sup>th</sup> minute, S1 was compared with S2 for the oil content of the skin, there was a significant difference between them ( $p < 0.05$ ). It was seen that there was a significant difference when S1 was contrasted to S4 at the 15<sup>th</sup> minute. Moreover, at the 120<sup>th</sup> minute, S1 was compared with the skin, there was a significant difference between them ( $p < 0.05$ ). It was concluded that the significant difference continued at the 120<sup>th</sup> minute according to the 0<sup>th</sup> minute in the oil content of the skin.

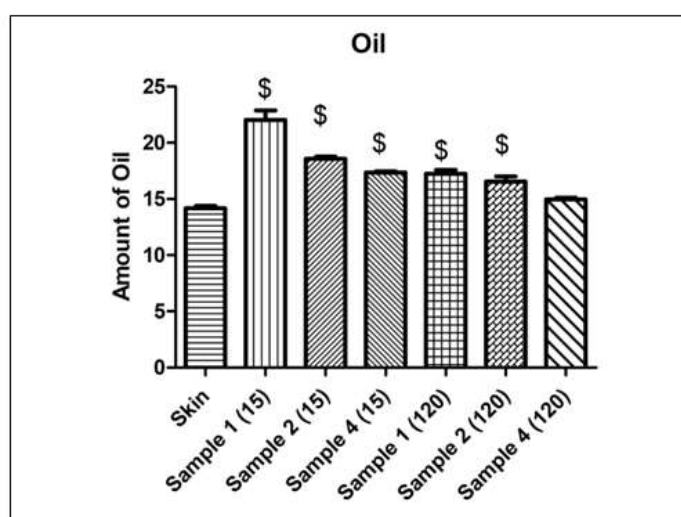


**Figure 4.13.** Comparison of prepared hydrogel samples according to oil content variation at 15<sup>th</sup> and 120<sup>th</sup> minute (S1: the alginate-based hydrogel composing of the rose hip formula incorporated with the micro-bubble generated by microfluidics; S2: the alginate-based hydrogel prepared manually with the rose hip formula; S4: the blank alginate-based hydrogel composing of micro-bubble generated by microfluidics), (\$: statistically difference when compared with the skin ( $p < 0.0001$ )).

In this study, it was considered that as the exposure time of active ingredients was prolonged, the penetration of them into the skin could be raised. Besides, it was regarded that more intensive application would be provided with hydrogel and would

extend the retention time in the applied area, a hydrogel incorporated with a microemulsion formula was prepared, and thus, it was aimed to improve the effectiveness of the ingredients. It is known that nano-sized formulations have been preferable in cosmetic products to enhance skin absorption recently [176]. Nano-size droplets were expected to improve active ingredients' penetration into the skin.

The results suggested that moisture and oil levels of the skin surface treated with the hydrogel filled with the prepared formula enhanced and it resulted in visual changes of the stratum corneum, which in turn skin permeation of active ingredients was boosted. While deciding the active ingredients for the content of the formula, it was attentive that not to use any synthetic chemical-based synergists and natural products were preferred. Moreover, it was known that both lipid and water provide moisture to the skin [177]. Therefore, the content of the prepared formula was selected accordingly. It was known that topically applied vitamin C showed a beneficial effect on the skin. The research demonstrated that topical application of 5% vitamin C provided improvement in the appearance of photodamaged skin and it was claimed a prolonged topical treatment with an appropriate vehicle may support activating a dermal synthesis of elastic fibers [178]. Although the ascorbic acid content of rose hips was focused in this study because antioxidant properties of ascorbic acid are well known and rose hip is one of the fruits that become prominent with its ascorbic acid content; it was preferred because rose hip possesses antioxidant properties due to not only its ascorbic acid content but also its phenolic components [44, 149]. In addition, it was known that shea butter and virgin coconut oil are also capable of nourishing the skin with their rich content [179, 180]. In another research reported by Nevin al. studied to reveal that wounds healed much faster with topical application of virgin coconut oil. It was shown that pepsin-soluble collagen and fibroblast proliferation boosted significantly [70]. Another research investigated the efficacy, patient acceptability, and bio-physiological effects of products for childhood atopic dermatitis and they showed that the cream consisting of shea butter had the same efficacy and acceptability as a ceramide-precursor product [181]. Besides, the formulas contained nano-sized particles that helped to enhance penetration into deeper layers of the skin by overcoming the stratum corneum barrier.

Since it was known that the mask could contain more intensive content and it was easier to keep the ingredients more stable in the mask, the hydrogel mask product was preferred in this study. Alginate hydrogels have been using as a dressing in recent years. In a study, amoxicillin-loaded chitosan/poly-glutamic acid nanoparticles were incorporated into alginate/calcium hydrogels that form a matrix in which depots releasing to treat *Helicobacter pylori* infection [182]. Three-dimensional (3D) hydrogels-based scaffolds have been researched for the treatment of skin injuries in recent years and they could be seen as promising products. Ehterami et al. developed vitamin E-loaded porous cross-linked hydrogel composed of alginate, chitosan, calcium chlorides in wound healing. They reported the prepared hydrogel was capable to accelerate wound healing with the antioxidant activity of vitamin E [183]. In another research reported by Yu et al. studied to develop wound dressings contain alginate hydrogel which simvastatin-mesoporous hydroxyapatite microspheres were incorporated into. They indicated in vivo study that the formation of new blood vessels boosted and re-epithelialization of the cutaneous wounds improved with the prepared wound dressing [184]. It was observed in this study that the ratio of moisture content increased rapidly within 15 minutes. This was a favorable result for the instant application of the mask.

## 5. CONCLUSION

As a consequence, it is known that products developed with natural ingredients are becoming more preferred today. The use of products with natural ingredients is placed emphasis in terms of both skin health and nature compatibility and sustainability. Besides, utilizing natural and herbal raw materials in cosmetics is evolving into a strong alternative due to their potential to overcome the side effects of synthetic materials. At the same time, it is purposed to increase the effectiveness of the products with the developing technologies and to evolve more stable, more compatible, and high-impact products in cosmetics.

In this thesis, the formulation was developed by preferring natural ingredients. This developed formula was incorporated with the microbubbles produced with microfluidics techniques and then the hydrogel was produced. The formula consisting entirely of natural ingredients was prepared with rose hip, virgin coconut oil, and shea butter, and it was desired to benefit from the beneficial components of these products by penetrating the skin. This contributed to the favorable results of the prepared formulation. It is known that the beneficial properties asserted to rose hips have been especially attributed to vitamin C. Therefore, the vitamin C of the rose hip was studied in detail. Moreover, it achieved the goal to develop a product that was compatible with the skin and nature since the alginate used in hydrogel production was also a natural product.

The objective of this research work was to develop a micro emulsion-based controlled-release hydrogel containing rose hip extract and natural oils by microfluidics technique. The T-shaped microfluidic device was used to produce microbubbles in the study. After incorporating the microbubbles with the rose hip formula, the formula was expected to gradually penetrate the skin from the hydrogel. The characterization study of the developed formula and the produced hydrogel were investigated. Characterization studies demonstrated that the projected product could be obtained with these methods.

Studies were carried out with the volunteer to evaluate the effectiveness of the product. The skin moisture and oil content were measured quantitatively before and after application and the results of prepared hydrogel were compared with the hydrogel

prepared with the manual method and placebo hydrogel. It was evaluated that a statistically significant difference was obtained. Moreover, in the studies with the volunteer, the sustainability of the effectiveness was evaluated and this was demonstrated that the hydrogel prepared with these methods could have the potential to design effective cosmetics. It was concluded that this positive upsurge was attributed to the porous structure of the produced hydrogel. In addition, the natural pleasant fragrance and user-friendly structure of the hydrogel were evaluated as another positive achievement. Under favor of this study, it was demonstrated that hydrogel could be developed with a different effective method with natural ingredients.

The results of the study could be evaluated as promising methods to generate different hydrogel masks in cosmetics that provide a slow-release delivery system, enhanced bio-efficacy, and protection of the active constituents. In the next stages, the effectiveness of the formula and hydrogel might be investigated in more detail with various skin tests. Besides, it could be prepared by altering the amount of the active ingredients. Even, different ingredients might be utilized to observe the skin penetration.

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## 7. APPENDICES

### 7.1. Voluntary Information and Consent Form

#### Gönüllü Denek Bilgilendirme ve Onay Formu

##### Gönüllünün isim ve adresi:

Bahar Ramzi, Kozapark site Kinali blok 58 Esenyurt / İstanbul

**Herhangi bir sorun olduğunda gönüllünün ya da yakınının ilişki kurabileceği hekimin bilgileri:** Doç. Dr. Asuman Cömert Erkılınç. Yeditepe Üniversitesi Hastanesi, Deri ve Zührevi Hastalıkları Bölümü

##### Araştırmanın amacı, yöntemi, araştırmadan beklenen tıbbi yararlar, yan etkiler ve olası tehlikeler:

Mikro-akışkanlar tekniği kullanılarak kuşburnu içeren mikroemülsiyon aljinat esaslı hidrojel formülasyonunun geliştirilmesi çalışması yapılmıştır. Alginat bazlı hidrojeller T- bağlantılı mikroakışkan tekniği kullanılarak üretilmiştir. Gönüllü ile uygulama yapılacak hidrojellerde doğal kaynaklı aktif bileşenler kullanılmıştır. Yardımcı olarak kullanılan tüm ürünler ve cihazlar onaylı, laboratuvar sınıfı ürünlerdir. Aktif bileşenlerin cilde nüfus etmesi ile deride nem, esneklik, canlılık artışı beklenmektedir. Doğal aktif bileşenler kullanıldığından ve doz ayarlaması yapıldığından herhangi bir yan etki beklenmemektedir. Aktif bileşenlere karşı herhangi bir alerjisi olmadığını belirten gönüllünün sağlıklı cildi üzerine uygulama yapılmaktadır.

##### Onay:

Araştırmaya gönüllü olarak katılım sağladım. Araştırmanın amacı, yöntemi, araştırmadan beklenen yararlar, olabilecek yan etkiler hakkındaki bilgilendirmeler tarafıma yapıldı. Anladım ve kabul ediyorum.

Gönüllünün istediği anda araştırmacıya haber vererek çalışmadan çekilebileceği ya da araştırmacı tarafından gerek görüldüğünde araştırma dışı bırakılabileceği hakkında bilgilendirme tarafıma yapıldı, anladım ve kabul ediyorum.

Gönüllünün araştırmayı kabul etmemesi durumunda veya herhangi bir nedenle çalışma programından çıkarılması veya çıkması halinde, hastalığı ile ilgili tedavisinde bir aksama olmayacağı dair bilgilendirme tarafıma yapıldı, anladım ve kabul ediyorum.