ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

DEVELOPMENT AND CHARACTERIZATION OF NANOFIBROUS STRUCTURES FOR ATOPIC DERMATITIS TREATMENT

M.Sc. THESIS

Nursema PALA AVCI

Department of Textile Engineering

Innovative Technical Textiles Programme

JANUARY 2023

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ATOPİK DERMATİT TEDAVİSİ İÇİN NANOLİFLİ YAPILARIN GELİŞTİRİLMESİ VE KARAKTERİZASYONU

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Nursema PALA AVCI, a M.Sc. student of İTU Graduate School student ID 503191848, successfully defended the thesis/dissertation entitled "DEVELOPMENT AND CHARACTERIZATION OF NANOFIBROUS STRUCTURES FOR ATOPIC DERMATITIS TREATMENT", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my spouse,

FOREWORD

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Nursema PALA AVCI
(Textile Engineer)

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ABBREVIATIONS

AD	**:**Atopic Dermatitis
A. niger	**:** *Aspergillus Niger*
ATTC	**:** American Type of Culture Collection
BO	**:** Broge Oil
C. albicans	**:** *Candida Albicans*
ECM	**:** Extracellular Matrix
E. Coli	**:** *Escherichia Coli*
FTIR	**:** Fourier Transform Infrared Spectroscopy
FLG	**:** Flaggrin
GLA	**:** Gamma Linolenik Acid
HPO	**:** Hypericum Perforatum Oil
PBS	**:** Phosphate Buffer Saline
PCL	**:** Poly (ε-caprolactone)
PSMs	**:** Plant secondary metabolites
PVP	**:** Polyvinylprolidone
PVA	**:** Polyvinilalcohol
S. aureus	**:** *Staphylococcus Aureus*
SC	**:** Stratum Corneum
SCORAD	**:** Scoring Atopic Dermatitis
Std. Dev.	**:** Standard Deviation
SEM	**:** Scanning Electron Microscopy
UV-vis	**:** Ultraviolet-visible
TEO	**:** Thyme Essential Oil
TPC	**:** Total Phenolic Method
TEWL	**:** Transepidermal Water Loss
WWT	**:** Wet Wrap Therapy

SYMBOLS

°C	**:** Celcius
kV	**:** Kilovolt
rpm	**:** Rotation per munite
wt	**:** Weight/weight
v/v	**:** Volume/volume
MPa	**:** Megapascal
µS/cm	**:** Microsiemens per cantimeter

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DEVELOPMENT AND CHARACTERIZATION OF NANOFIBROUS STRUCTURES FOR ATOPIC DERMATITIS TREATMENT

SUMMARY

Atopic dermatitis (AD) is a chronic, itchy, and inflammatory skin disease that causes dry skin, rashes, and inflammation. Since AD has no definitive treatment so far, moisturizers and softening creams are used to control the disease, whose severity changes periodically; Topical or oral anti-inflammatory drugs and systemic corticosteroids are applied to control exacerbations and to minimize the risk of infection by relieving itching. However, since long-term use of synthetic drugs causes many side effects, it is not recommended for young children and infants, who make up the majority of people suffering from this disease. For this reason, various complementary treatments such as hydrogels, wet dressings or natural oil-added wound dressings have been developed as an alternative to drug therapy.

Wound dressings are structures that protect the wounds against various bacteria and infections, support cell proliferation in the injured area, provide the necessary oxygen circulation for the skin, and at the same time maintain the moisture balance of the wound environment. These structures can be woven fabrics (gauze), hydrogels or nanofiber surfaces. Wound dressings can be made functional by adding therapeutic agents on or inside the structure of dressings. When therapeutic agents are added to the surface of dressings post-production, they cannot be released efficiently. In order to achieve sustained release, additives must be trapped in the fibers by different methods during the fiber production phase. Thus, it is possible to adjust the dosage of therapeutic agents and transfer them to the skin surface in a controlled manner according to the healing process. For this reason, studies on nanofiber wound dressings produced by electrospinning method have increased in recent years.

Electrospinning is a widely used fiber spinning method for the preparation of nanofibers, versatile and easily adaptable to different materials. Electrospinning apparatus consists high voltage source, conductive polymer solution, syringe, syringe pump, needle/nozzle and collector plate. While the conductive polymer solution, which is fed from the pump and comes to the syringe tip, is drawn from one pole to the other by the electric field effect between the collector plate and the syringe tip, while the solvent in the polymer is removed just before it reaches the collector, and the polymer solidifies, accumulating on the collector plate in the form of nano or micro-sized fibers, forming a nanofiber surface. In electrospinning; Solution or polymer properties, the distance between the needle tip and the collector (collector plate), the amount of applied voltage, the collector movement or environmental factors such as humidity, pressure and temperature are the parameters that affect the nanofiber structure.

There are three different electrospinning methods commonly used for the controlled release properties of electrospinning surfaces. These are blend electrospinning, emulsion electrospinning and coaxial electrospinning. Two methods whose sustained release properties can be compared among these three methods are emulsion and coaxial electrospinning. Because the fibers produced by the blend electrospinning method do not show continuous release by making burst release.

In the emulsion electrospinning method, the oil phase and the aqueous phase form an emulsion with the help of surfactant added to the polymer solution, and fibers are produced with a single nozzle electrospinning mechanism. With this method, it is possible to encapsulate the additives in the fiber and provide continuous release. The fibers produced by the coaxial electrospinning method are in the core-shell structure. Therefore, sustained release for a long time can be achieved. The nozzle used to obtain this structure has two separate needles, one in the center of the other.

Essential oils (EOs) are oils extracted from plants that contain a variety of complex chemical compounds. Since prehistoric times, EOs have been widely used for a variety of medicinal purposes, including antibacterial, antiviral, insecticidal, and analgesic and anti-inflammatory. Because many EOs are volatile by nature, encapsulating oils into the fiber is a cost-effective way to protect them from evaporation and oxidation while also controlling their release. Thyme oil (TEO) used in this study shows high antibacterial activity thanks to components such as thymol and carvacol. Since S.aureus colonization accumulating on the skin surface in AD disease causes exacerbation of the disease, the production of nanofibers containing TEO has been considered as a solution to this problem. On the other hand, Hypericum Perforatum oil (HPO), which has been approved by the literature to contribute to wound healing by supporting cell proliferation thanks to its components such as hypericisin and hyperforin, was chosen as another nanofiber additive. Finally, nanofibers were produced with Borage oil (BO) with the highest Gamma Linolenic acid (GLA) content, which is one of the essential fatty acids that cannot be synthesized in the bodies of patients with AD due to the deficiency of the δ-6-desaturase enzyme. GLA is an important component in AD patients in terms of preventing water loss in the skin and protecting the barrier functions of the skin. In addition to additives, it is also important to use biocompatible and biodegradable polymers in nanofiber production due to their low toxicity and mimicry of the skin's extracellular matrix (ECM). Examples of these polymers are polyvinylalcohol (PVA), polycaprolactone (PCL), polyvinylprolidone (PVP) used in the thesis.

In this study, it was aimed to obtain essential oil loaded (hypericum perforatum oil, thyme oil and borage oil) nanofiber structures using emulsion and coaxial electrospinning methods using PVA, PCL and PVP polymers. The effects of the variables on nanofiber structures were evaluated by changing parameters such as polymer type, polymer concentration, essential oil type, essential oil ratio, surfactant type and surfactant ratio. In addition to the production and characterization tests of nanofibrous structures, the antibacterial properties of TEO-containing samples were investigated.

Firstly, nanofibers were produced by emulsion electrospinning method using hydrophilic PVA polymer and HPO. The effects of increasing amounts of surfactant used for emulsion formation in the prepared solutions on fiber morphology were observed. Then, emulsion nanofibers were produced with hydrophilic polymers PVP and hydrophobic PCL polymers separately. This time, the amount of surfactant was

kept constant and the effects of the increased amount of oil on the fiber structure were examined.

After studies on the effect of oil and surfactant in emulsion electrospinning method, nanofibers were produced from PVP/PCL polymers at different rates for the optimization of coaxial nanofibers. The morphologies of the produced fibers were examined, the hydrophilicity/hydrophobicity test was performed on the surfaces and the effect of polymer ratios on the surface properties was investigated. As a result of the studies, the most suitable polymer ratio was determined for oil loading. Oil-loaded fiber productions were made by adding TEO, BO and TEO:BO (1:1 v/v mixture) to the PVP polymer, which will form the core structure of the fiber in coaxial fibers. The results of SEM images, fiber diameter distributions, FTIR and antibacterial activity tests of the produced samples were analyzed and interpreted.

Finally, oil-loaded nanofibers with a hydrophobic PCL polymer core/shell structure were produced. While the shell polymer solution was kept constant at 10% wt PCL, the core polymer solution was prepared at 10% wt PCL and 8% wt PCL. TEO and TEO:BO mixed oils were added to the core solutions as oil additives. The obtained nanofibers were evaluated in terms of fiber morphology and surface hydrophilicity, the connections between the viscosity and conductivity values of the solutions and fiber structures were explained, and suggestions for future studies were presented.

ATOPİK DERMATİT TEDAVİSİ İÇİN NANOLİFLİ YAPILARIN GELİŞTİRİLMESİ VE KARAKTERİZASYONU

ÖZET

Atopik dermatit (AD), cildin kurumasına, döküntüler ve iltihaplanmaların meydana gelmesine sebep olan kronik, kaşıntılı ve inflamatuar bir deri hastalığıdır. AD hastalığının şu ana kadar kesin bir tedavisi olmadığından dolayı dönemsel olarak şiddeti değişen hastalığın kontrol altına alınabilmesi amacı ile nemlendiriciler ve yumuşatıcı kremler; alevlenmeleri kontrol etmek ve kaşıntının giderilerek enfeksiyon riskinin en aza indirilmesi için de topikal ya da oral anti-inflamatuar ilaçlar veya sistemik kortikosteroidler uygulanmaktadır. Fakat sentetik ilaçların uzun süreli kullanımlarının birçok yan etkiye sebebiyet vermesinden dolayı, özellikle bu hastalıktan muzdarip olan kişiler arasında büyük çoğunluğu oluşturan küçük çocuklar ve bebekler için tavsiye edilmez. Bu nedenle ilaç tedavisine alternatif olarak hidrojeller, ıslak sargılı pansuman, antimikrobiyel tesktiller ya da doğal yağ katkılı yara örtüleri gibi çeşitli tamamlayıcı tedaviler geliştirilmiştir.

Yara örtüleri yaraları çeşitli bakteri ve enfeksiyonlara karşı koruyarak yaralı bölgede hücre çoğalmasını destekleyen, cilt için gerekli oksijen sirkülasyonunu sağlarken aynı zamanda yara çevresinin nem dengesini koruyan yapılardır. Bu yapılar dokuma kumaşlar (gazlı bez), hidrojeller ya da nanolifli yüzeyler olabilir. Yara örtüleri üzerine ya da içerisine tedavi edici ajanlar eklenerek fonksiyonel hale getirilebilir. Tedavi edici ajanlar yara örtülerinin yüzeyine üretim sonrasında eklendiğinde, bu maddelerin salınımı verimli bir şekilde sağlanamaz. Sürekli salım elde etmek için, lif üretim aşamasında katkı maddelerinin farklı yöntemlerle liflerin içine hapsedilmesi gerekmektedir. Böylece tedavi edici maddelerin dozajını ayarlamak ve iyileşme sürecine göre kontrollü bir şekilde cilt yüzeyine aktarmak mümkündür. Bu nedenle son yıllarda elektrospinning yöntemi ile üretilen nanolifli yara örtüleri ile ilgili çalışmalar artmıştır.

Elektroeğirme, nanoliflerin hazırlanması için yaygın olarak kullanılan, çok yönlü ve farklı malzemelere kolayca uyarlanabilen bir lif çekme yöntemidir. Elektroeğirme düzeneği; yüksek voltaj kaynağı, iletken polimer çözeltisi, şırınga, şırınga pompası, iğne/düze ve toplayıcı plakadan oluşur. Pompadan beslenerek şırınga ucuna gelen iletken polimer çözeltisi toplayıcı plaka ile şırınga ucu arasındaki elektrik alan etkisi ile bir kutuptan diğerine doğru uzayarak çekilirken, toplayıcıya ulaşmadan hemen önce polimerdeki çözücü uzaklaşır ve polimer katılaşarak, nano veya mikro boyutlu lifler şeklinde toplayıcı plaka üzerinde birikerek nanolifli yüzey oluşturur. Elektro eğirmede; solüsyon veya polimer özellikleri, iğne ucu ile kollektör (toplayıcı plaka) arasındaki mesafe, uygulanan voltaj miktarı, kolektör hareketi ya da nem, basınç ve sıcaklık gibi çevresel etkenler nanolif yapısını etkileyen parametrelerdir

Elektroeğirme ile elde edilen yüzeylerin kontrollü salım özellikleri için yaygın olarak kullanılan üç farklı elektroeğirme yöntemi vardır. Bunlar karışım elektro eğirme, emülsiyon elektro eğirme ve koaksiyel elektro eğirmedir. Bu üç yöntem arasında sürekli salım özellikleri açısından değerlendirilebilecek iki yöntem emülsiyon ve koaksiyel elektroeğirmedir. Çünkü karışım elektroeğirme yöntemi ile üretilen lifler patlama salımı yaparak sürekli salım göstermezler.

Emülsiyon elektroeğirme katkılı nanolif üretiminde kullanılabilecek uygun maliyetli ve basit bir yöntemdir. Bu yöntemde polimer çözeltisine eklenen sürfaktan yardımıyla yağ fazı sulu faz içerisinde homojen bir şekilde dağılarak bir emülsiyon oluşturur ve tek düzeli elektroeğirme mekanizması ile lifler üretilir. Bu yöntemle katkı maddelerini lif içerisine hapsetmek ve sürekli salınım sağlamak mümkündür.

Yağların nanolif yapısı içerisine eklenmesi için kullanılan koaksiyel elektro eğirme yöntemiyle üretilen lifler çekirdek-kabuk yapısında olduğu için kontrollü salımın uzun süreli sağlanabileceği bir yöntemdir. Bu yapıyı elde etmek için kullanılan düzenekte, düze olarak biri diğerinin merkezinde bulunan iki ayrı iğne bulunmaktadır. Çekirdek yapıyı oluşturacak olan iç iğneden genellikle katkı maddesi içeren solüsyon, dış iğneden ise yapıya destek sağlayacak polimer solüsyonu geçer. Bu solüsyonların birbirine karışmayan solüsyonlar olması gerekmektedir. Eş eksenli lifler sayesinde sürekli salımın sağlanabilmesi, çekirdek lifindeki katkı maddesinin kabuk lifleri tarafından çevresel faktörlere karşı korunmasından ve patlama salımının engellenmesinden dolayıdır.

Uçucu yağlar (EO'lar), çeşitli karmaşık kimyasal bileşikler içeren bitkilerden ekstrakte edilen yağlardır. Tarih öncesi zamanlardan beri, EO'lar antibakteriyel, antiviral, insektisidal, analjezik ve antiinflamatuar dahil olmak üzere çeşitli diğer tıbbi amaçlar için yaygın olarak kullanılmaktadır. Pek çok EO doğası gereği uçucu olduğundan, yağları elyaf içine kapsüllemek, onları buharlaşma ve oksidasyondan korumanın ve aynı zamanda salınımlarını kontrol etmenin uygun maliyetli bir yoludur. Bu çalışmada kullanılan kekik yağı (TEO), timol ve karvakol gibi bileşenleri sayesinde yüksek antibakteriyel aktivite göstermektedir. AD hastalığında cilt yüzeyinde biriken S.aureus kolonizasyonu hastalığın alevlenmesine neden olduğundan TEO katkılı nanoliflerin üretim parametrelerinin belirlenmesi bu soruna çözüm olarak düşünülmüştür. Diğer bir nanolif katkı maddesi olarak ise hiperisisin ve hiperforin gibi bileşenleri sayesinde hücre çoğalmasını destekleyerek yara iyileşmesine katkı sağladığı literatür tarafından onaylanan Hypericum Perforatum yağı (HPO) seçilmiştir. Son olarak, δ-6-desatüraz enziminin eksikliği nedeniyle AD'li hastaların vücutlarında sentezlenemeyen esansiyel yağ asitlerinden biri olan Gama Linolenik asit (GLA) içeriği en yüksek Hodan yağı (BO) ile nanolifler üretilmiştir. GLA, AD hastalarında ciltte su kaybını önlemesi ve cildin bariyer fonksiyonlarını koruması açısından önemli bir bileşendir. Katkı maddelerine ek olarak nanolif üretiminde düşük toksisite ve cildin hücre dışı matriksini (ECM) taklit etmesi nedeniyle biyouyumlu ve biyolojik olarak parçalanabilen polimerlerin kullanılması da önemlidir. Biyopolimerler, mantar, bakteri, ağaç veya farklı kabuklardan, hayvanlardan elde edilen biyouyumlu doğal polimerlerdir. Doğal polimerlere örnek olarak kitosan, jelatin, selüloz, kolajen, zein, hyaluronik asit vb verilebilir. Biyouyumlu sentetik polimerlere verilebilecek örnekler arasında da polilaktik asit (PLA), poliglikolik asit (PGA), polivinilalkol (PVA), polikaprolakton (PCL), polivinilprolidon (PVP), poliüretan (PU), polilaktik-ko-glikolik asit (PLGA) yer almaktadır

Bu çalışmada, PVA, PCL ve PVP polimerleri ile emülsiyon ve koaksiyel elektrospinning yöntemleri kullanılarak uçucu yağ yüklü (hypericum perforatum yağı, kekik yağı ve hodan yağı) nanolif yapıların elde edilmesi amaçlanmıştır. Değişkenlerin nanolif yapıları üzerindeki etkileri, polimer tipi, polimer konsantrasyonu, uçucu yağ tipi, uçucu yağ oranı, yüzey aktif madde tipi ve yüzey aktif madde oranı gibi parametreler değiştirilerek değerlendirilmiştir. Nanolifli yapıların üretim ve karakterizasyon testlerine ek olarak, TEO içeren numunelerin antibakteriyel özellikleri incelenmiştir.

Çalışmada ilk olarak hidrofilik PVA polimeri ve HPO kullanılarak emülsiyon elektroeğirme yöntemi ile nanolifler üretilmiştir. Hazırlanan çözeltilerde emülsiyon oluşması için kullanılan yüzey aktif maddenin artan miktarlarda (ağırlıkça %2, %4 ve %6) kullanılmasının lif morfolojisine etkileri gözlenmiştir. Daha sonra hidrofilik bir polimer olan PVP ve hidrofobik PCL polimerleri ile ayrı ayrı emülsiyon nanolifler üretilmiştir. İki çözeltiye de hacimce 1:1 oranında karışım HPO:BO yağları eklenmiştir. Bu defa sürfaktan miktarı sabit tutularak ve artan yağ miktarının (hacimce %1, %2 ve %3) lif yapısına etkileri incelenmiştir. Eklenen yağların lif yapısına katılıp katılmadığının kontrol edilmesi için yağ yüklü nanoliflere FTIR analizleri yapılmıştır.

Emülsiyon elektroeğirme yönteminde yağ ve sürfaktan etkisi ile alakalı yapılan çalışmalardan sonra koaksiyel nanoliflerin optimizasyonu için farklı polimer oranlarında PVP/PCL polimerlerinden çekirdek/kabuk yapılı nanolifler üretilmiştir. Üretilen liflerin morfolojileri incelenmiş, yüzeylerin hidrofiliklik/hidrofobiklik özelliklerini tespit etmek için su temas açısı testi yapılmış ve polimer oranlarının yüzey özelliklerine etkisi incelenmiştir. Sonuçlara göre çekirdekteki hidrofilik PVP polimerinin konsantrasyonu azaldıkça çözelti viskozitesi de azaldığından dolayı kabuk yapısına doğru polimer göçünden kaynaklı yüzeylerin temas açılarında azalmalar olabileceği sonucuna ulaşılmıştır. Optimizasyon çalışmaları sonucunda yağ yüklemesi yapmak amacıyla en uygun polimer oranı belirlenmiştir. Koaksiyel liflerde lifin çekirdek yapısını oluşturacak olan PVP polimerine TEO, BO ve TEO:BO (1:1 karışım) eklenerek nanolif üretimleri yapılmıştır. Üretilen numunelerin SEM görüntüleri, lif çapı dağılımları, FTIR ve antibakteriyel etkinlik testlerinin sonuçları incelenerek yorumlanmıştır.

Son olarak, hem kabuk hem çekirdek yapısı hidrofobik PCL polimeri olan yağ katkılı koaksiyel nanolifler üretilmiştir. Kabuk polimer solüsyonu ağırlıkça %10'da sabit tutulurken, çekirdek polimer solüsyonu ağırlıkça %10 ve %8'de hazırlanmıştır. Çekirdek solüsyonlarına yağ katkı maddesi olarak TEO ve TEO:BO karışım yağları ve TX-100 yüzey aktif maddesi eklenmiştir. Elde edilen nanolifler, lif morfolojisi, yüzey hidrofilikliği ve salım davranışları açısından değerlendirilmiş, çözeltilerin viskozite ve iletkenlik değerleri ile lif yapıları arasındaki bağlantılar açıklanmıştır. Polimer çözeltisine eklenen TX-100 yüzey aktif maddesinin hidrofobik PCL polimerinden üretilen nanolifleri hidrofilleştirdiği gözlemlenmiştir. Tezin sonunda gelecek çalışmalar için öneriler sunulmuştur.

1. INTRODUCTION

Atopic dermatitis (AD) is a chronic, itchy, and inflammatory skin disease that causes skin dryness, rashes, and inflammation (Darsow, Eyerich, & Ring, 2004). AD can be caused by genetic characteristics as well as environmental factors. In pathophysiological studies, immunological irregularities such as T1/T2 imbalance and interleukin 31 as an important pruritic skin factor have been detected. These conditions cause dysfunctions that prevent moisture retention in the skin and dryness occurs in the skin (Raap et al., 2012). In addition, skin inflammation and acute exacerbations of AD occur as a result of weakening of the immune system, especially as a result of colonization of Staphylococcus Aureus bacteria on the skin (Leung, 2003). Compared to healthy individuals, it has been proven that AD patients have a higher amount of S. aureus colonization even in the lesion-free areas of their skin (Hauser et al., 1985). Also, the amount of bacteria in the lesioned areas of the skin is related to the severity of the disease (De Benedetto et al., 2009). AD is not a completely curable disease, but moisturizers and soothing creams relieve dryness for the control of the disease. Topical or oral anti-inflammatory drugs and systemic corticosteroids are also applied to control exacerbations and to minimize the risk of infection by relieving itching (Paller et al., 2012; Galli et al., 2016). However, corticosteroids are not recommended especially for young children, since their long-term use causes many different side effects (Farhi et al., 2010). In addition, moisturizers and antibiotics used during the treatment also create a great economic burden on the person. Therefore, various complementary therapies have been developed, such as antibacterial textiles, natural oil-added dressings (Metwally et al., 2021), hydrogels (Wang, 2017), and wet wrap therapy (Dabade et al., 2012).

The use of synthetic chemical drugs in the treatment of diseases poses a great threat to both human health and the environment every year. For this reason, researchers have started to look for alternative and more environmentally friendly solutions in the treatment of bacteria and viruses that cause diseases. Essential oils (EO) and natural plant extracts are studied and applied as alternatives to synthetic drugs due to their

antibacterial, antioxidant, anti-inflammatory properties (Mele, 2020). Thyme Essential oil (TEO) and Hypericum Perforatum oil (HPO), which have antibacterial properties, can be used as additives in the production of nanofiber surfaces, since one of the most important reasons for the increase in AD severity is the colonization of S.aureus bacteria on the skin surface. According to the literature, the thymol component in TEO and the hyperforin component in HPO show antibacterial properties against S.aureus bacteria (Kwon et al., 2018; Saddiqe, Naeem & Maimoona, 2010). Natural oils can be used in internal treatment as well as topically for an effective therapy for dry skin or as a supportive treatment in medical textiles (Foster, Hardy and Alany, 2010). One of the causes of skin dryness caused by AD is the deficiency of the δ-6-desaturase catalyst that enables the conversion of linolenic acid (LA), which is one of the essential fatty acids that cannot be synthesized by the human body and must be obtained from food, to its metabolite gamma linolenic acid (GLA). Therefore, it is stated that external and internal treatments with GLA are effective in AD disease (Krysiak et al.,2020). Natural oils containing the most GLA in their structure can be listed as borage oil (24%), black currant seed oil (15%) and evening primrose oil (7-9%) (Kanahera, Ohtani, Uede, & Furukawa, 2007).

Carrier surfaces are needed in order to control the absorption of natural oils by the skin and to protect the treated area against environmental factors. Wound dressings, which protect the wounds against various bacteria and infections, ensure the oxygen circulation required for cell regeneration in the wound area and maintain the appropriate moisture balance around the wound, can be used in topical applications by adding natural oils or extracts on or inside the carrier structure (Kurtoğlu and Karataş, 2009). Woven fabrics produced from different fibers (Kanahera, Ohtani, Uede and Furukawa, 2007), knitted fabrics (Zimniewska et al., 2019) or nanofiber surfaces (Metwally et al., 2021) can be used as carrier surfaces. As a result of the subsequent addition of natural oils to the surface of carrier textiles, the sustained release of oils cannot be achieved efficiently. In order to achieve sustained release, it is necessary to confine the additives into the fiber by different methods during the fiber production phase. Thus, it is possible to adjust the dosage of the treatment aids and transfer them to the skin surface in a controlled manner according to the healing process. For this reason, studies on nanofibrous wound dressings produced by electrospinning method have increased in recent years.

When the studies are examined, it is seen that different methods are used to add EOs and natural plant extracts to nanofiber surfaces. Some researchers have obtained oil-added nanofiber wound dressings by directly adding oils (physical adsorption) after forming the surface (Metwally et al., 2021). Thus, nanofibers with high surface area can be loaded with additives in large amounts, but with this method, the substances adsorbed by the surface are released from the surface very quickly. Therefore, these systems are more suitable for short-term drug release (Sill and Recum, 2008). However, with this method, the long therapy times required for AD cannot be provided and the oil added to the surface is transferred to the skin in a short time and is consumed. To avoid this, sustained release should be ensured in order to maintain the transfer of the therapeutic agents in the wound dressing applied to the lesioned area from the nanofiber surface to the skin surface for a long time. Drug dosage adjustments vary according to the type of diseases and the treatment method to be applied. In conventional dosing, drugs can be administered alone or in different combinations as a single dose. Such applications are ideal for antibiotics or drugs with pain-relieving effects that require rapid action. However, for some chronic conditions, drug release is required at periodic dosage intervals. Sustained release systems extend the time required for drug delivery, allowing the dosage amount required for treatment to be adjusted (Buzgo, Mickova, Rampichova, & Doupnik, 2018).

There are three different electrospinning methods commonly used for the controlled release properties of surfaces obtained by electrospinning. These are blend electrospinning, emulsion electrospinning and coaxial electrospinning. Blend electrospinning is the most widely used, simple and inexpensive method for the production of EO added nanofiber surfaces. After the polymer solution and drug solution are prepared separately, they are mixed and electrospun together. However, the disadvantages of the method are that the additives are close to the fiber surface, so long-term and sustained release cannot be achieved by burst release, and the bioactive agents are damaged in the presence of organic solvents (Jain, Shetty & Yadav, 2020). Emulsion electrospinning, which is another preferred production method in order to provide sustained drug release from nanofibers, is a cheap and simple as well as effective method. With the help of surfactant added to the polymer solution, oil phase and aqueous phase create an emulsion and fibers are produced with a single syringe electrospinning mechanism. With this method, it is possible to form core-shell

structured fibers and to provide sustained release. The last method is the coaxial electrospinning method. Since the fibers produced by this method are in the core-shell structure, it is a method in which sustained release can be achieved for a long time. In the apparatus used to obtain this structure, there are two separate needles, one in the center of the other, as a nozzle. The solution containing additives passes through the inner needle, which will form the core structure, and the polymer solution that will support the structure passes through the outer needle. Sustained drug release is possible thanks to the coaxial fibers, because the drug in the core fiber is protected against environmental factors by the shell fibers and the burst release is prevented.

Since AD is not a completely curable disease, complementary therapies are needed to improve the quality of patients' life by reducing the increasing severity of the disease. The aim of the methods used as a complementary treatment is to keep the skin moist by preventing water loss caused by the dysfunction of the skin barrier, to prevent the dry skin from flaking and itching, and to prevent the accumulation of S. aureus bacteria on the skin surface. For this reason, in this study, it is aimed to develop an electrospun nanofiber wound dressing with a sustained release feature, containing natural oil additives and biocompatible materials, with moisturizing and antibacterial properties, for the complementary treatment of Atopic Dermatitis. For doing so, an electrospun nanofiber wound dressings were designed by adding BO nanofibers in order to moisturize the skin and support collagen production and TEO against bacterial growth and HPO to support wound healing. Emulsion electrospinning and coaxial electrospinning methods were used as production methods in order to provide long-term and sustained release of the oils added to the structure.

2. THEORETICAL BACKGROUND

2.1 Atopic Dermatitis

Atopic dermatitis (AD) is a recurrent inflammatory skin disorder which is characterized by intense pruritus and erythema (See Fig. 2.1) (Leung & Bieber, 2003). About 2 to 5 percent of people worldwide suffer from AD and 90% of patients initially experience it in the first five years of life. Generally, types of involvement differ depending on age. While facial, scalp and extensor involvement is seen in infants and young children, predominant flexural involvement is seen in older children and adults (Lalan, Baweja & Misra, 2015),(Lyons, Miller & Stone, 2015).

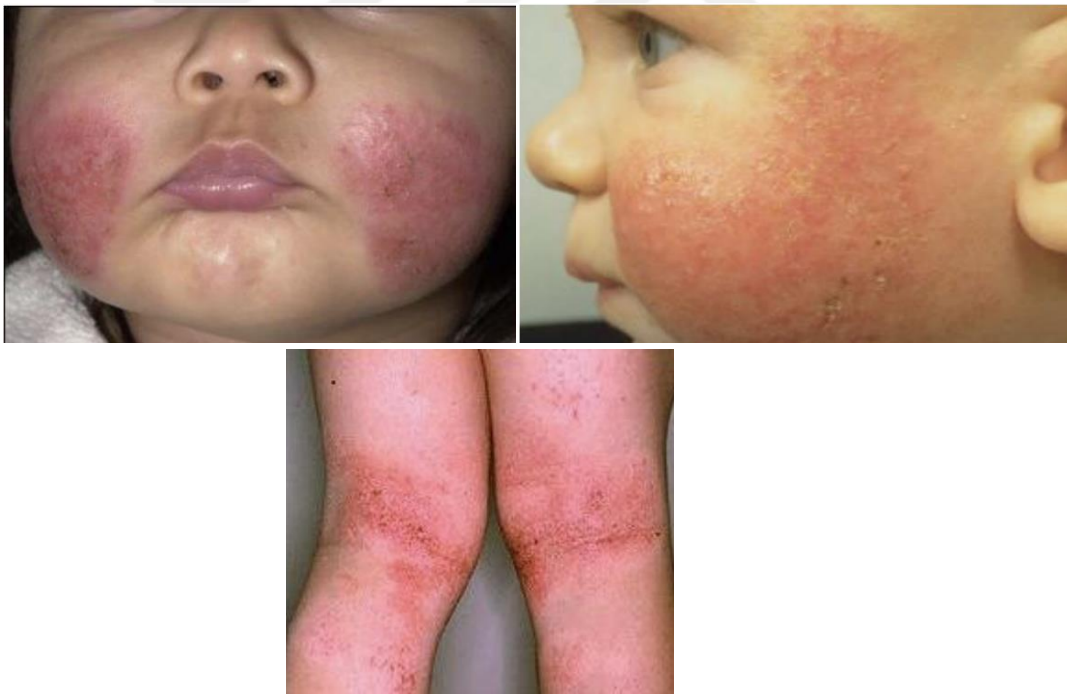

Figure 2. 1 : Visual skin appearance of AD (Ulutas, 2011).

The etiology and pathophysiology of AD are complicated and poorly understood. Impaired epidermal barrier, immune dysregulation, genetic factors, and environmental factors all together generate the usual clinical presentation (Leung, Jain & Leo, 2003). Clinically, extrinsic and intrinsic AD are the two types of the disease. About 80% of

the AD patient population exhibits extrinsic AD features, while about 20% exhibits intrinsic AD characteristics. Serum IgE levels and dominant immunological polarization are the main distinctions between the two clinical types of AD (Lalan, Baweja & Misra, 2015). High total IgE levels and presence of specific IgE for environmental and food allergens were observed in extrinsic AD patients, whereas total IgE values were normal and specific IgE was not observed in intrinsic AD patients (Tokura, 2010). Intercellular proteins, transglutaminases, filaggrin (FLG), and keratins are important proteins involved in epidermal function. In the top layer of skin, FLG works as a waterproof mortar between keratinocytes. When there are genetic mutations in these proteins, the skin becomes vulnerable to allergens and microbes (Kim, Kim & Leung, 2019).

2.1.1 Diagnosis

The diagnosis of AD is determined clinically and is based on historical features, the morphology and distribution of skin lesions, and related clinical indicators. In order to facilitate classification, a variety of organisations have collaborated to produce formal sets of criteria. The diagnostic criteria for AD used in the world today were first determined by Hanifin and Rajka in 1980 (Eichenfield et al., 2014).

Active AD cannot be diagnosed without problem of itching. Itching is a defining characteristic of AD. As a result of irritation caused by itching, skin sores occur. Symptoms might range from moderate to severe. It is believed that these symptoms are caused by a lowered itching threshold. Nassif et al. demonstrated that patients with active AD had a greater cutaneous sensitivity to irritants than patients without atopy. The leading causes of itching in persons with AD are sweating, wool, emotional stress, certain meals, alcohol, upper respiratory infections, and house dust mites (Nassif, Chan, Storrs & Hanifin, 1994).

Atopic dermatitis is classified as mild, moderate and severe according to the extent of the disease, the intensity of the itching, and the sleep loss it causes. In this classification, a scoring called the SCORAD (Scoring Atopic Dermatitis) index is used. The SCORAD scoring system, which took more than three years to develop, was released in 1993 by a task team of over 30 European experts (Fig 2.2). This technique takes into account both subjective (pruritus and loss of sleep) and objective signals (such as severity and extension). SCORAD is one of the finest verified systems and is

appropriate for clinical trials, but according to other authors, it is too time and labor-intensive for ordinary clinical use. The so-called TIS score (Three-Item Severity score), which is a condensed version of SCORAD, is based on the evaluation of erythema, oedema/papulation, and excoriation on a scale of 0 to 3. While the original SCORAD offers a more in-depth and thorough evaluation and is suggested for research purposes, this score is particularly appropriate for general practice, routine clinical use, and screening purposes in clinical trials (Gelmetti & Colonna, 2004)

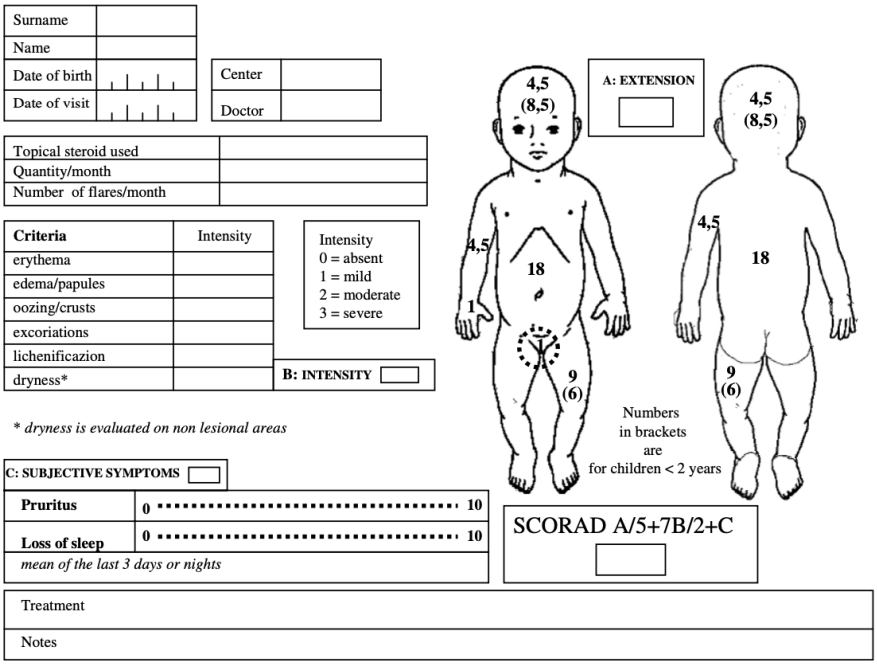

Figure 2. 2: SCORAD Index calculation form. Objective symptoms (A and B) and subjective symptoms (C) are considered and calculated to determine the final score (Gelmetti & Colonna, 2004).

2.1.2 Conventional treatment methods

The aim of the treatment of patients with AD is to reduce itching and inflammation and to provide adequate moisture to skin. Finding and removing the causes of attacks is another crucial step. Considerations for allergies, infectious agents, the physical environment, and sources of mental stress should be taken into account when developing treatment strategies for acute and chronic conditions (Kristal & Klein, 2000). The quality of life of people with AD is significantly impacted, thus this should be considered while selecting a course of treatment. Since AD is not a completely treatable disease, patients should be informed about this issue and they should be informed that their compliance with treatment is important in reducing symptoms.

Severe skin dryness is a typical symptom among AD patients. This is why the skin needs to be moisturized for 20 minutes, 2-3 times per day, with a warm bath. The bath water should be infused with oil if the skin is really dry. To avoid secondary infections, skin should be washed with soaps with a neutral pH and antibacterial cleaners should be applied. After washing, creams and greasy ointments should be applied to the skin to protect the stratum corneum barrier (Charman & Willams, 2003).

Among the topically applied drug treatments in AD, the most commonly used drugs are topical corticosteroids. Topical corticosteroids suppress inflammation and its severity, especially in the treatment of very itchy active skin manifestations after skin moisturisation. It also reduces the bacterial colonization present in AD and the density of Staphylococcus aureus on the skin. However, due to its side effects, corticosteroids with the lowest potency that can be effective should be preferred and should not be used more than 2 days a week. In addition, they should not be applied for longer than one week on the face, axilla and genital areas where the skin is thin (Hanifin & Tofte, 1999).

The majority of AD patients respond effectively to topical treatment. Generally, the use of systemic treatment only considered in severe AD patients not responding to topical therapy. Systemic corticosteroids can be included to the treatment if people with AD develop skin symptoms on 25% of the body surface. Severe itching and itching marks on the skin, super-infections, bleeding, lichenification, itchy nodules, sleep disturbances, and impaired quality of life are all problems that follow skin dryness in AD patients. These problems are treated with systemic antihistamines and anxiolytics (Baron et al., 2002)

2.2 Complementary Therapy for Atopic Dermatitis: Textile-Based Treatments

Since AD has complex pathophysiology and the causes of which cannot be determined exactly, it is not completely treatable. Therefore, improving the quality of life by relieving the symptoms, preventing acute exacerbations and enhancing the cosmetic appearance of the disease is the primary goal of AD treatment (Goldenberg, 2016). Because AD disease is a condition that fluctuates in severity, moisturizers and emollient creams are used to treat dryness; topical or oral anti-inflammatory medications and systemic corticosteroids are used to manage exacerbations and reduce the risk of infection by alleviating itching (Paller et al., 2012). However, long-term

and high doses of corticosteroids can cause severe side effects, so it is not recommended to be used especially in the treatment of young children (Wang, 2017). For this reason, various complementary therapies have been developed such as wet wrap dressings, hydrogels and antimicrobial textiles.

2.2.1 Wet wrap therapy

First reported by Nikol et al. in 1987 as a method for the treatment of AD, wet wrap therapy (WWT) is done by wrapping two layers of gauze or tubular dressings over the body after the application of topical medication to the skin to reduce itching and inflammation (Nicol, 1987). As can be seen in Figure 2.3, there are also studies that cover the body by dipping the first layer of the dressing directly into the drug medium as a different method. However, wrapping after applying the drug to the skin is the most preferred method since it saves time. WWT has been promoted during the past 20 years as a generally safe and efficient treatment option for kids with severe and/or refractory AD. Although there are still a lot of unresolved problems in this area, WWT is still used in AD patients (Devillers & Oranje, 2006).

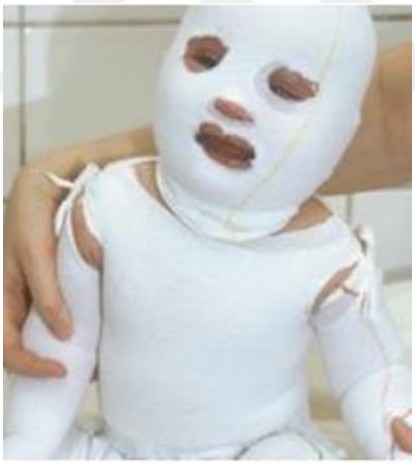

Figure 2. 3: Wet wrap dressing (Dabade et al., 2012).

Many studies have been conducted on the positive effects of WWT in the treatment of AD. Goodyear et al. showed that WWT was effective in the treatment of AD with their study on 30 children in 1991 (Goodyear, Spowart & Harper, 1991). Oranje et al., in their experiments on mice to observe the efficacy of WWT in the treatment of AD, found that WWT reduced transepidermal water loss (TEWL) and improved epidermal thickness (Oranje et al., 2009). WWT is the last topical therapy in the treatment of moderate-to-severe AD patients before the introduction of systemic drugs with high

side effects. The need for systemic treatment in moderate to severe AD reduces thanks to the wet dressings. In a study in which 72 patients were evaluated objectively with the SCORAD index, a significant decrease in the SCORAD index was observed in all cases with WWT applied for 2-16 days, thus systemic treatment was not required for any patient. According to the study, the more severe the AD at baseline, the more patients benefited from treatment (Nicol et al., 2014).

The WWT is thought to provide a cooling effect. A wet-wrap dressing's cooling impact may lower skin temperature and relieve itching and inflammation. The reduction of pruritus thereby controls sleep disruption. A wet-wrap bandage can not only rehydrate the skin and improve topical medicine absorption, but it can also act as a physical barrier to prevent scratching and speed up the healing of an injury (Shekariah, Kalavala & Alfaham, 2011). But besides all these advantages, there are also some disadvantages. The most dangerous side effect is an increase in cortisol levels in the body and systemic absorption of steroids used in dressings. In addition, these dressings can cause skin maceration, infection and folliculitis. Because WWT is a short-term therapy, the dryness of the dressings and the need for constant moisturization also increase the burden on caregivers and families (Oranje, 2006).

2.2.2 Hydrogels

AD disease can manifest in many different forms and there are several options for the treatment of the disease, but new treatments are still needed. The dermal application of treatments is also suitable for both local and immediate removal of AD skin lesions and to avoid the side effects of systemic drugs by transferring them from the skin to the body with the help of a carrier. The basic objective of applying a high-water content product to typical dry skin is also a good treatment principle for AD. As a result of their structure versatility, ability to carry all types of drugs, capacity to be modulated and responsive to stimuli, and high water content, hydrogels have piqued the scientific community's interest as excellent candidates for drug delivery carriers in skin research (Barbosa et al., 2021).

For example, desonide hydrogel is an excellent example of how a carrier surface can influence therapy efficacy and patient compliance. For the first time, a topical corticosteroid was made available in a hydrogel formulation, free of the irritants, alcohol and surfactants. The hydrogel formulation for desonide corticosteroid

treatment worked better in terms of moisturizing and barrier function (Gelbard & Hebert, 2009). Lee et al. studied a prednisolone hydrogel to reduce skin thinning associated with long-term prednisolone use. Polyvinyl alcohol and slightly cross-linked alginate were used as hydrophilic and biocompatible materials to encapsulate prednisolone, providing water retention and controlled drug delivery as well as reducing IgE levels and mast cell infiltrations (Lee et al., 2018).

It is beneficial to use the wound healing agents/drugs as a wound dressing compared to other usage methods (ointment, cream and tincture) and to apply them directly on the wound, and to reduce the side effects of the chemical compounds in these agents. The disadvantage of using drugs in the form of ointments or hydrogels is that they cannot maintain the stable form and maintain the moisture of the healthy tissue around the wound. If the ointment or hydrogels are not covered, they will dry out and cannot be used on crusted wounds (Pourhojat et al., 2018). For this reason, a layered structure should be formed by combining hydrogels with a protective surface.

2.2.3 Antimicrobial textiles

Despite the fact that meta-studies have not been able to detect any appreciable changes in eczemas, antibacterial clothes are categorized as a complementary therapy similar to antisepsis (Höfer, 2018). AD is a complex illness that is influenced by both environmental and genetic factors. Its pathogenesis includes immunological dysregulations that result in abnormalities in the function of the epidermal barrier, such as decreased water retention. The high rate of cutaneous colonization with S. aureus and S. epidermidis (up to 90% in moderate to severe eczema) may possibly be explained by the intricate network of immunological deregulations. Only S. aureus is capable to create super-antigens and other virulence factors, which worsens the skin irritation (De Benedetto, 2009). Textile materials with antibacterial properties are examined in order to reduce bacterial colonization accumulated on the skin surface and reduce the severity of the disease. There have been many studies that aim to develop antibacterial textile materials and examine the effects of these materials in the treatment of AD.

The increase in AD severity due to S. aureus bacteria may be due to immunomodulatory toxins secreted by S. aureus, which have superantigen properties that can stimulate the activation of T cells and macrophages, thereby inducing skin

inflammation and exacerbating AD (Leung et al., 2004). Therefore, antimicrobial textiles may be seen as a desirable alternative therapy to treat AD by killing S. aureus colonization. It has been reported that some metal nanoparticles and organic antibacterial agents develop antibacterial textiles for the treatment of AD due to their low toxicity and good compatibility. Examples of metal nanoparticles used for antibacterial purposes are titania nanoparticles, silver nanoparticles, zinc oxide nanoparticles and copper nanoparticles. Among these particles, silver nanoparticles are the most widely used ones in studies because they show low toxicity to humans, are effective against a wide range of microorganisms, and show high efficiency (Dhiman & Chakraborty, 2015). In a study in which the effects of wearing textiles made of silver-loaded seaweed-based cellulosic fiber on epidermal skin physiology of 37 patients with AD were analyzed , silver-containing fabrics were shown to reduce S. aureus in AD skin when compared to the conventional cotton fabrics and improve the course of AD. The tested silver-loaded seaweed fiber appears to be suitable for application in the garments of AD patients according to positive in vivo results (Fluhr et al., 2010). In another study investigating the antibacterial effects of metal nanoparticles, cotton nanofibers were coated by dipping into solutions of Ag, ZnO and TiO2 nanoparticles. According to the test results, it was observed that the nanoparticles showed a strong biological activity against gram positive and gram negative bacteria (Al-Dharob et al. 2022).

Bioactive compounds called plant secondary metabolites (PSMs) such as terpenes and phenols are abundant in natural extracts. Due to their antioxidant, anti-inflammatory, and antibacterial properties, these compounds are becoming an increasingly popular subject of investigation in the field of wound dressings. Many different phenolic monoterpenes in PSMs are responsible for their antibacterial effects. Reproduction of bacterias are prevented by PSMs because they disrupt the bacterial cell membrane, impair quorum sensing and kill bacteria (Al-jumaili et al., 2018). Wound dressings made of Thymol-loaded Polycaprolactone and polylactic acid (PCL/PLA) fibers produced by Karami et al. were subjected to antibacterial tests. Antibacterial assays showed inhibition against S. aureus and E. coli. In the results of the wound closure assay, the gauze and Comfeel Plus® showed 68% and 87% wound closure, respectively, while the thymol-containing samples accelerated wound closure by more than 92% in 14 days (Karami et al., 2013). The incorporation of Ajwain essential oil

into core-shell electrospun nanofibers has antibacterial and wound healing potential. In vivo tests on rats have shown that nanofibers have the potential to reduce bacterial infection and increase wound closure. In addition, according to histological results, it was shown that there was no inflammation and increased collagen deposition (Zare et al., 2021). Also, cinnamon-doped electrospun chitosan/gelatin membranes exhibited antibacterial activity against S. aureus and E. Coli (Ahmadi et al., 2021). There are many studies examining the antibacterial properties of natural substances in the therapy of AD. In Table 2.1, examples of materials with antibacterial properties such as essential oils and natural additives used in the literature to improve AD are given.

Table 2. 1: Antibacterial PSMs used in wound dressing studies.

PSMs	References
Curcumin	(Vollono et al., 2019)
Palmarosa Oil	(Lee & Lee, 2020)
Chamaecyparis Obtusa EO	(Yoo et al., 2020)
Citrus grandis Osbeck extract	(Yi, Hong & Yoo, 2010)
Hypericum Perforatum Oil	(Pakolkapçıl et al., 2021)
Tyhme EO	(Liu et al., 2019)
Clove EO	(Unalan et al., 2019)

2.3 Nanofiber Producing Techniques

Nanotechnology-created materials exhibit exceptional physical and chemical characteristics. Nanofibers are the most spectacular products of these nanomaterials because of their extremely high surface area/volume ratio and porosity. Nanofibers can be made from a variety of components, and they are employed in a variety of applications.

The term "nanofiber" can be divided into two parts: "nano" and "fiber." Fibers are defined by the textile industry as a filament, either natural or synthetic, such as cotton or nylon, that may be spun into yarn. Geometrically, a "fiber" is defined as a slender, elongated, threadlike structure. The term "nano" technically refers to a billionth of a unit scale. Nanofiber, in general, refers to fibers having diameters ranging from 50 to 300 nanometers. The large surface areas and small pore sizes of nanofibers give great importance to nanofibers.

Nanofiber technology is used in a variety of applications, including batteries and fuel cells, capacitors, transistors, and diodes, energy transfer systems, composites for aerospace structures, tissue engineering, drug delivery, and wound dressings.

Various production techniques, such as self-assembly, phase separation, template synthesis, meltblown and electrospinning have been used to produce micro to nano scale fiber surfaces (see Fig. 2. 4). In the template-based synthesis method, nanofibers can be synthesized or extruded using commercial nanoporous membrane templates. The fiber diameters are uniformly distributed and the fiber lengths are in the micrometer size. The phase separation method consists of five steps: raw material dissolving, phase separation and gelling, solvent extraction, freezing and freeze drying. This method is very time consuming and fiber diameters are in the range of 50-500 nm and fiber lengths are a few micrometers. In the production of nanofibers by self-assembly, atoms, molecules and molecular aggregates organize themselves through weak and non-covalent forces such as hydrogen bonds and electrostatic interactions to become stable and functional entities in meso or nanoscale. Disadvantage of the method is the need for longer preparation time. It also produces nanofibers in the 100 nm range. In the meltblown method, the polymer blend is extruded through small holes with high velocity heated air streams and fibers in the 150-1000 nm range are produced. Fiber diameters depend on the extrusion nozzle used, but it is difficult to obtain fibers smaller than 100 nm (Zhang et al., 2005). Among these production methods, the best way to produce nanofibers is by using electrospinning. The method results in fibers with dimensions between a few nanometers to a few micrometers. Researchers have been drawn to electrospinning because of its ease of use, low cost, and versatility in producing nanofibrous surfaces capable of imitating the morphological properties of the skin's extracellular matrix (ECM). Moreover, these nanofibrous meshes are also able to support cell adhesion, migration, growth and differentiation as well as angiogenesis, which are vital events for the occurrence of an effective wound healing process. In addition, bioactive molecules, various drugs, growth hormones, keratinocyte cells, metal ions or natural oils have also been incorporated into the electrospun nanofibres to improve the healing performance of these dressings.

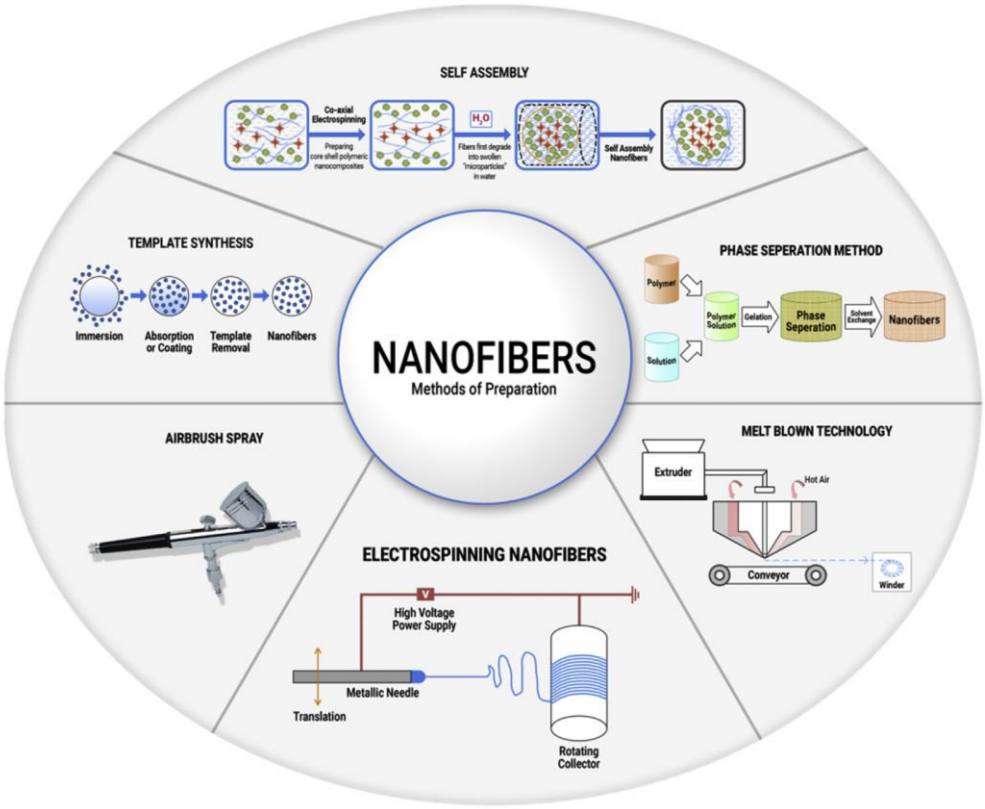

Figure 2. 4: Different nanofiber producing methods (Kamble et al., 2017).

Electrospinning is a widely used fiber spinning method for the production of nanofibers. It is versatile and easily adaptable to different materials. An electrospinning assembly as shown in Figure 2. 5 consists of a high voltage source, a conductive polymer solution, a syringe, a syringe pump, a truncated needle, and a collector plate. In this technique, the conductive polymer solution, which is fed from the pump comes to the syringe tip and is drawn from one pole to the other by the electric field effect between the collector plate and the syringe tip. When a high voltage (5–50 kV) is applied to the droplet, the surface of the droplet picks up a charge and becomes highly electrified. This phenomenon causes the droplet to change into a cone-shape also known as Taylor cone. Electrostatic force overcomes a droplet's surface tension when high voltage intensity increases. As a result, a jet of polymer solution is ejected from the droplet and drawn towards the collector plate. Solvent in the polymer is removed just before reaching the collector, and the polymer solidifies, forming nano or micro sized fibers (Khalf ve Madihally, 2017).

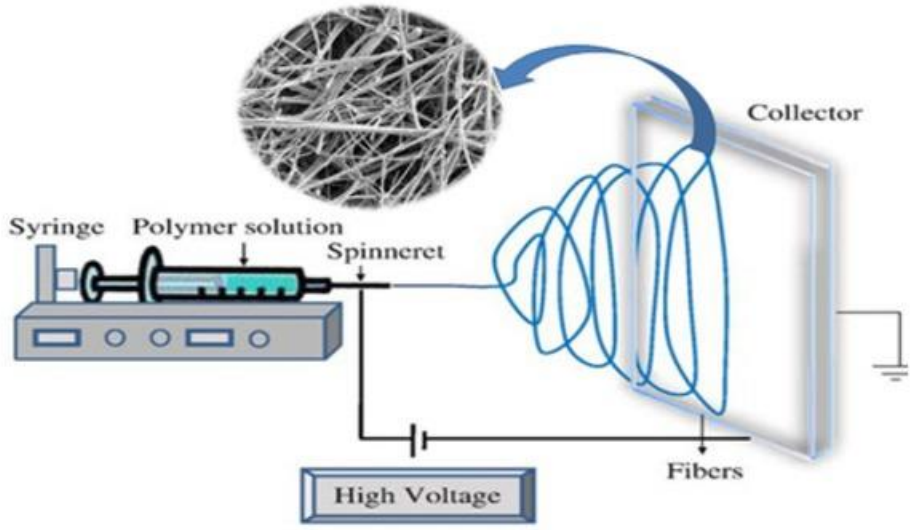

Figure 2. 5: Electrospinning apparatus (Faki, Gürsoy & Yılmaz, 2019).

In electrospinning; solution or polymer properties, the distance between the needle tip and the collector plate, the amount of applied voltage, the collector movement or environmental factors such as humidity, pressure and temperature are the parameters that affect the nanofiber structure (Bhardwaj and Kundu, 2010).

It is known that the spherical polymer droplet at the nozzle tip turns into a Taylor cone under electrical effects and starts to form nano-sized fibers after a critical voltage value. The critical voltage value may differ for each polymer. As the voltage increases, the fiber diameters become thinner, this is due to the stretching of the polymer solution in conjunction with the charge repulsion within the polymer jet (Sill & Von Recum, 2008). But if the applied voltage exceeds the critical voltage value, beads can occur. Differences in flow rate also cause changes in fiber morphology. For example, insufficient flow rate causes interruptions in the polymer jet, while excessive flow rate causes the production of thick fibers or bead formation. Another factor affecting the nanofiber structure, such as flow rate and voltage, is the viscosity of the solution. In low-viscosity solutions entangled polymer chains break into fragments before reaching the collector plate due to voltage and surface tension, and these fragments cause bead formation (Pillay et al., 2013). When polymer density in the solution increased, viscosity and polymer chain entanglement also increase (Haider et al., 2013). At the critical viscosity, polymer jet overcomes the surface tension and uniform beadless nanofibers occur. However, if the viscosity of the polymer solution exceeds the critical level, the solution does not flow properly from the needle and freezes at the needle tip, clogging the needle. Therefore, it causes non-uniform and beaded production.

2.4 Biomedical Applications of Nanofibers

2.4.1 Tissue engineering

The reason for the increase in the demand for organ transplantation every year is the high number of cases of tissue damage or organ failure. The aim of tissue engineering is to develop materials that will support the structure so that the injured tissue can self-repair in order to eliminate the disadvantages that may occur in conventional tissue transplantation. Therefore, knowledge of cell biology, chemistry, materials science, nanotechnology is required to ensure successful tissue regeneration. 3D scaffolds enriched with extracellular matrix (ECM)-like components are required for tissue formation. Many of the ECM molecules in tissues have intertwined fibrous structures that support cell adhesion and proliferation. Thus, producing ECM-like structures is the most important research topic of tissue engineering (Nemati et al., 2019).

Scaffolds with a nanofibrous architecture have been produced so far using phase separation, self-assembly, and electrospinning. Among them, electrospun nanofibers have started to attract a lot of attention in tissue engineering due to their three-dimensional porous structure, high surface area-to-volume ratio and variable mechanical properties. In order to achieve successful results in tissue engineering, natural scaffolds are needed in terms of chemical structure, morphology and surface functional groups (Li & Xia, 2014). Almost every extracellular matrix (ECM) of connective tissue, such as cartilage, bone, and skin, is based on nanofibrous structures, and these structures have shown an effect not only on cell-cell interaction but also on cell-ECM interaction (Osanloo, Arish & Serenthi, 2020). Therefore, electrospun nanofibrous structures are widely used in tissue engineering due to their high ability to mimic ECM. For example, in the development of artificial human skin from collagen nanofibers by Zhou et al., the effects of the structure on cell proliferation, mechanical and thermal properties were investigated. In the study, the tensile strength of 6.72 ± 0.44 MPa indicates that the structure is suitable for use as artificial human skin. In addition, adhesion, proliferation, and differentiation of cells were promoted by electrospun tilapia collagen nanofibers and have been shown to aid rapid wound healing (Zhou et al., 2016). In another tissue engineering study, a bionanocomposite scaffold based on poly(butylene adipate-co-terephthalate) (PBAT) and Hyaluronic acid (HA) nanoparticles was prepared by electrospinning. Then, human adipose stem

cells (hASC) were seeded on the composite material and their differentiation in osteoblasts and in vivo biocompatibility were evaluated. In this study, it has been shown that the composite material has a suitable morphology, structure and mechanical properties that enable hASC attachment, proliferation and differentiation in bone cells. The only drawback the structure showed was that it triggered a mild inflammatory response (Neto et al., 2015).

2.4.2 Wound dressing

When skin injury occurs, it is very important to ensure that the skin structure can perform its functions as soon as possible in order to protect the body. Although the skin is generally self-renewing, some types of wounds do not heal or take a long time to heal due to extensive lesions or chronic wounds. For this reason, wound dressings are being developed that can imitate the natural structure of the skin and attract cells that can secrete vital extracellular materials (for example, cytokines, collagen and growth factors) that help repair damaged tissues.

As shown in Figure 2. 6, the wound healing process is a complex process consisting of four main processes: homeostasis, inflammation, proliferation and remodeling. For this reason, wound dressings used to accelerate wound healing should provide certain properties. Recent advances in science and technology have made it possible to find the right materials for wound dressings for various types of wounds. To accomplish good wound healing, it is essential to know which substance is optimal for a certain wound. The characteristics of an ideal wound dressing are as follows: control of moisture around the wound, excellent transmission of gases, protection of the wound from infections and microorganisms, removal of excess exudates, ease of replacement and removal, biocompatibility, biodegradability, elasticity, nontoxicity, pain relief, and cost-effectiveness (Jain, Domb & Kumar, 2014). There are many different types and application areas of wound dressings. Various types of wound dressings can be listed as animal based, plant based, synthetic based, hydrogels, alginates, hydrocolloids, films, sponges, foams and electrospun nanofiber surfaces (Rezvani et al., 2019).

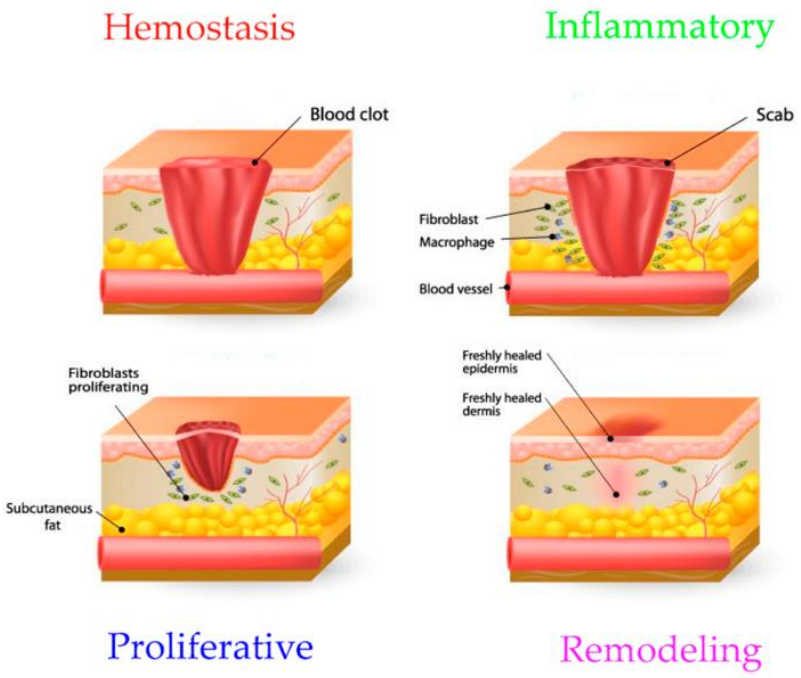

Figure 2. 6: Schematic represantation of wound healing stages (Gobi et al., 2021).

The most important dressings of the group of animal-derived dressings are collagen sponges with high porosity and high water absorption. The use of such dressings is limited because the production costs are high and they are difficult to maintain and store (Iordanskii, 2014).

Among the aforementioned production techniques, the electrospinning method is the most remarkable one. Electrospun mats provide enhanced exudate absorption and permeability for water and oxygen, thanks to their high specific surface area, high aspect ratio and high porosity. They also support cell proliferation in the injured area by imitating the extracellular matrix (ECM) and contributes to the rapid healing of the wound. Antibacterial and bioactive agents can be easily incorporated into fibers by in situ or post-electro-spinning modification. The release behavior of additives can be controlled by the choice of polymer and electrospinning parameters (Liang et al., 2022).

Akturk et al. formed nanofiber surfaces by the use of electrospinning method and combined collagen, an animal polymer, and PEO, a synthetic polymer, to be used as a wound dressing. Different ratios of gold nanoparticles (AuNPs) were added to the nanofiber solutions. As the amount of AuNP in the solution increased, the spinnability of the collagen solution also increased. After seeding the created surfaces with

keratinocytes and L929 fibroblast cells, it was shown that the cells adhered to the structure at the end of 1 day according to SEM images (Aktürk & Keskin, 2016). According to the literature, plants have played a significant role as herbal treatments for a broad variety of diseases. Traditional medicinal herbs are used to treat a wide variety of wounds and skin disorders. Herbal dressings are mostly harmless, therefore they can be used for an extended period of time with the correct treatment methods (Adamu et al., 2022). In a study by García-Salinas et al., essential oil components with antibacterial properties namely, thymol and tyrosol loaded polycaprolactone (PCL) nanofiber dressings were produced in order to reduce inflammation during wound healing and to inhibit bacteria that cause inflammation. Compared to conventional drugs, the natural ingredients showed similar effects both in reducing inflammation by reducing the size of inflammatory cells and in fighting bacteria (Garcia et al., 2020). Synthetic-based dressings, on the other hand, have started to be preferred frequently in recent years due to the ease of access to cheap raw materials, biocompatibility, biodegradability, good mechanical properties, low cytotoxicity, flexibility and gas permeability. Polyvinyl alcohol (PVA) is a hydrophilic synthetic polymer widely used in various biomedical fields. However, it has insufficient elasticity, a thick membrane, and imperfect hydrophilic elements, so it can be used in combination with different polymers rather than as a stand-alone wound dressing. For example, Ardekani et al., by using PVA, chitosan and gelatin polymers, produced a wound dressing with a natural additive with antibacterial properties (Ardekani et al., 2019). Examples of other biocompatible polymers used in the field of wound dressing are Polyvinylpyrrolidone (PVP), Polycaprolactone (PCL), Polylactic acid (PLA), Polyethylene glycol (PEG) and Polyurethane (PU) (Gobi et al., 2021).

2.4.3 Drug delivery

A very wide range of methods are utilized to transfer therapeutic agents into human or animal body through drug delivery. Principles from biology, physics, and mathematics are utilized to guide the methods employed to regulate the drug release from pharmaceutical systems. The aim of this field, which requires interdisciplinary cooperation, is to apply bioactive agents appropriately and to increase the effect of drug therapy in order to get the desired response from the body to the treatment. Controlled drug release systems are developed depending on the active substance used. It aims to control drug exposure over time, to help the drug overcome physiological

barriers, to protect the drug from premature elimination, and to minimize drug exposure in any part of the organism while directing the drug to the desired effect site. The chemical and biological mechanisms that control drug release can be listed as dissolution, diffusion, osmosis, cleavage, swelling, erosion, and targeting (Bruschi, 2015).

Drug delivery systems are constantly being improved to maintain the effectiveness of pharmaceutical ingredients. Oral delivery systems are a frequently preferred method and still continue to be preferred because of the effects that increase patient compliance such as different dosage adjustments, ease of use without pain, high safety, and self-use. However, orally taken drugs may lose their effectiveness or undergo chemical changes in the ways they pass until they reach the area where they need to be effective. For example, the drug may degrade due to stomach acid. In addition, the solubility of the drug in the intestine and the permeability of the intestinal membrane may also have adverse effects on the effectiveness of the drug. In order to overcome these disadvantages, intravenous injection method can be used. Thus, the drug is transported directly to the problem area where it should act, via blood vessels. However, this method also has disadvantages such as causing pain, low patient compliance and cost of disposal of penetrating-cutting instruments. In order to minimize all these disadvantages, transdermal drug delivery systems are being developed (Ramadon et al., 2022).

Transdermal drug delivery requires a surface on which drugs can be loaded. These surfaces, called modern wound dressings, can be films, foams, hydrogels or fibrous structures (Krysiak & Stachewicz, 2022). Among these surfaces, nanofiber surfaces are the most preferred recently. The use of nanofiber surfaces as drug carriers is due to their high surface area and higher solubility of drugs in this area (Osanloo, Arish & Seresthi, 2020). Additives that will positively affect the disease treatment process can be added to the nanofiber surfaces produced by electrospinning. These substances can be antibacterial, antifungal, antioxidant drugs, growth hormones, numerous kinds of DNA and RNA, keratinocyte cells, metal ions or natural oils (Tort and Acartürk, 2015). Various approaches have been used to develop electrospun nanofiber scaffolds that can function as a nanocargo carrier, such as integrating drug into electrospun nanofibers using methods such as blending, emulsion or coaxial electrospinning, or coating the drug on the surface of electrospun nanofibers. For example, Rahmani et al.

developed a nanofiber transdermal drug delivery system for use in pain treatment. Buprenorphine loaded poly(vinyl pyrrolidone) (Bup/PVP) and buprenorphine loaded poly(vinyl alcohol)/poly(vinyl pyrrolidone) (Bup/PVA/PVP) nanofiber surfaces were produced by electrospinning process. When the release times were evaluated, the Bup/PVP samples reached the maximum drug release amount in the first 1.5 hours, while the Bup/PVA/PVP samples reached the maximum drug release amount after 12 hours (Rahmani et al., 2021). There are several parameters that affect drug release from nanofibers. These are drug/polymer ratio, fiber diameter, fiber morphology, porosity. In addition, methods such as crosslinking of nanofibers or functionalization by surface grafting polymerization affect drug release (Zhang et al., 2005). Moreover, loading various drugs into/on the nanofibers increases therapeutic efficacy and reduces toxicity because only a small fraction of ordinary drugs can act on the lesion and cause toxic side effects once it enters the body. So far, many studies have investigated nanofiber drug delivery systems. Today, many researchers in the world are working on electrospinning and nanofibers about drug delivery (Zhou et al., 2022).

2.5 Sustained Drug Release of Nanofibers

Nanofiber surfaces produced by the electrospinning method are one of the most popularly researched and studied areas today. Electrospun surfaces are used in many fields such as filters, sensors, tissue engineering and wound dressings. Among the biomedical uses, developments in drug delivery are one of the most striking areas. Using electrospun fibers in drug delivery provides advantages such as high drug loading rate up to 60%, high encapsulation efficiency, providing physico-chemical compatibility with different substances thanks to polymer diversity, and being able to provide sustained release. In addition, it is a simple method and cost-effective (Chou, Carson & Woodrow, 2015).

Polymer selection according to the intended application is one of the most important parameters in order to achieve sustained release from electrospun fibers. Many synthetic or natural polymers can be used for this purpose. In the literature, the most common biodegradable polymers used are polylactic acid (PLA), polyglycolic acid (PGA), poly (lactic-co-glycolic) acid (PLGA) and polycaprolactone (PCL), and non-biodegradable polymers are polyurethane (PU), polycarbonate and nylon- 6. Since natural polymers such as silk, collagen, gelatin, alginate, and chitosan are difficult to

be electrospun alone, their combination with synthetic polymers has been studied for sustained drug release (McDonald et al., 2010).

Factors such as polymer degradation, drug dissolution, and drug dispersion in the fiber may affect drug release from nanofibers. In fibers produced from non-degradable polymers, the release of drug from the fibers depends on a fixed geometry, whereas in fibers consisting of degradable polymers, the fiber's geometry will change depending on the degradation, so the diffusion amount of the drug also changes depending on the polymer degradation rate (Chou, Carson & Woodrow, 2015).

Unlike burst-release fibers, there are different parameters to consider for the design of sustained-release fibers. Burst-release fibers generally dissolve quickly in aqueous media and discharge their additives to the area where they are applied. The design of burst-release fibers is therefore simpler. Polymer selection, drug-polymer compatibility, drug physico-chemical properties and electrospinning parameters are important in sustained-release fiber design and can affect drug release (Fredenberg et al., 2011).

2.6 Methods for Producing Electrospun Drug Delivery Nanofibers

Blend electrospinning, emulsion electrospinning and coaxial electrospinning methods are commonly used for the drug delivery and sustained release properties of nanofiber surfaces.

2.6.1 Blend electrospinning

Blend electrospinning is one of the simplest methods for drug loading into nanofibrous structures. After the polymer solution and drug solution are prepared separately, they are electrospun together (Fig. 2. 7). The formation of the drug release pattern is related to polymer-drug compatibility and drug solubility. The compatibility of physicochemical structures with each other can increase the sustained release time. Hydrophobic polymers and hydrophobic drugs, or hydrophilic polymer / hydrophilic drug structures can provide a constant release by affecting the distribution and release kinetics of the drug on the nano surface. For this reason, studies have focused on the hydrophobic/hydrophilic structure differences between drug and polymer. For example, to provide good encapsulation in nanofibers, hydrophobic polyesters with hydrophobic drugs such as rifampicin and paclitaxel; hydrophilic polymers such as

gelatin, PEG (polyethylene glycol) and PVA (polyvinyl alcohol) with hydrophilic drugs such as doxorubicin can be blend electrospun (Vlachou, Siamidi & Kyriakou, 2019).

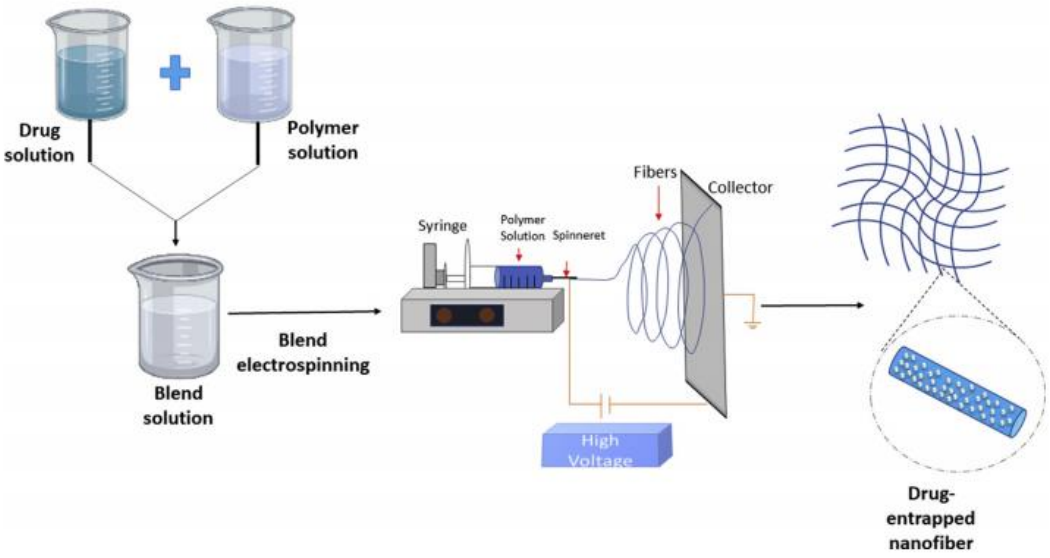

Figure 2. 7 : Blend electrospinning method (Jain, Shetty & Yadav, 2020).

The disadvantage of the method is uncontrolled release of the drug due to incompatible drug/polymer matrices or dissolution of biodegradable parts when organic solvents are used. Therefore, if unstable biological agents are to be encapsulated, this method is not suitable because the fibers are obtained with a single phase (Manuel, Jesus & Aracely 2016). Numerous papers in the literature describe the use of blending electrospinning to load a drug or therapeutic aids into a polymeric delivery system. Lu et al. successfully blended poly (N-isopropyl acrylamide) (PNIPAAm) fibers with ethyl cellulose (EC) using electrospinning, and added ketoprofen (KET), a nonsteroidal antiinflammatory medication, to the system. The resultant complexes exhibited generally smooth and cylindrical fibers with no evidence of phase separation. The drug was discovered to be present in the fiber matrix in its amorphous physical state, and considerable intermolecular interaction between KET and the polymeric scaffold was observed. In cell culture, these systems were found to be non-toxic and biocompatible (Hu et al., 2015). In another study, Kenawy et al. investigated the release of tetracycline from electrospun mats composed of PEVA, PLA, and a 50/50 blend, and they discovered that electrospun PEVA and 50/50 PLA/PEVA mats provided relatively smooth release of drug electrospun fibers over a 5-day period (Kenawy et al., 2002).

2.6.2 Emulsion electrospinning

Emulsion electrospinning is a cost-effective and simple method used to achieve controlled drug release (See Fig. 2. 8) Using the emulsion electrospinning method, nanofibrous structures can be produced with immiscible EO (or drug) and aqueous polymer solution. The most important factor in this method is the preparation of the emulsion. The basis of emulsion electrospinning is the dispersion of two or more immiscible liquids to form an emulsion liquid. Emulsions consist of two phases, a continuous phase and a dispersed phase. The dispersed phase or oil phase should be homogeneously dispersed in the continuous phase. In this technique, an emulsifying agent is added to the solution of natural oils and polymer, and the solution is vortexed to form an emulsion. The emulsion stability and release behavior of oils are affected by three different phases, namely the oil phase, the aqueous phase, and the surfactant used to form an emulsion (Jain, Shetty, & Yadav, 2020). The difference from blend electrospinning is that instead of dispersing the additive on the surface of the fiber, it forms nano-droplet structures in the fiber thanks to the emulsifying agents added to the solution.

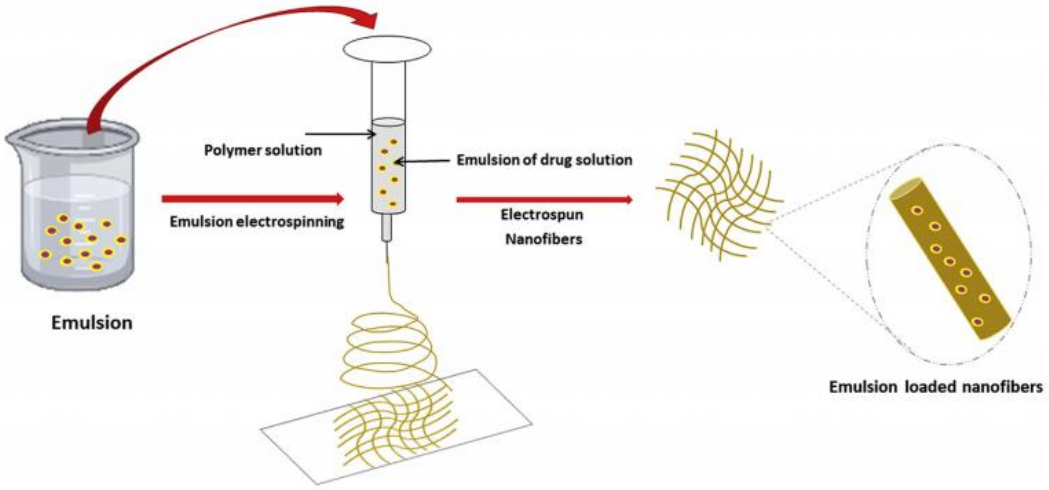

Figure 2. 8: Emulsion electrospinning(Jain, Shetty & Yadav, 2020).

Çallıoğlu, Güler and Çetin (2019) obtained nanofiber surfaces by emulsion electrospinning method by adding thyme essential oil and surfactant to PVP and gelatin polymers in order to facilitate emulsion formation. After dissolving the PVP polymer in 12% (wt) and gelatin in 6% (wt) distilled water, four solutions containing thyme essential oil at 1, 3, 5 and 7 (wt) ratios were prepared. The surfactant ratio was fixed at 3% (wt) in all solutions. It has been observed that the antibacterial properties

of the produced surface are high and the antibacterial properties increase as the thyme oil content increases. The best fiber diameter distribution and droplet-free surface were obtained by adding 3% oil. While a sticky surface was obtained at 5% oil, no fiber was drawn at 7% oil. In addition, the produced nanofibers showed antibacterial activity for a period of 192 hours when stored at 24 ºC and 37 ºC.

2.6.3 Coaxial electrospinning

In the electrospinning method, blend and emulsion methods are often preferred because they are both cheap and easy to load the therapeutic agents into the fibers. The fabrication of monolayer nanofiber membranes using the drug (or additive) blending approach is simple and reproducible, but because the drug is dispersed on the nanofiber with a large specific surface area, it quickly leads to the burst release of drugs from the nanofiber surface and cannot provide sustained release for a long period (Hu et al., 2015). In a study by Ullah et al., cellulose acetate nanofibers containing different amounts (10%, 15% and 20%) of BB (Blumea Balsemifera) oil were produced for use as a natural oil-added wound dressing. Surface morphology, antibacterial and antioxidant properties, and release behavior of the produced nanofiber surfaces were investigated. Surfaces with antibacterial properties against Gram + and - bacteria showed burst release in the range of 0-12 hours at all oil ratios (Ullah et al., 2021).

Coaxial electrospinning method has been developed in order to minimize the burst release in blend electrospinning and to load some sensitive additives such as proteins, growth hormones etc. negatively affected by organic solvents into the fiber without being damaged. In this method, two conductive polymer solutions are fed through using a special nozzle with two needles, one of which is in the center of the other. The solution containing additives passes through the inner needle, which will form the core structure, and the polymer solution that will support the structure as shell layer passes through the outer needle (Fig. 2. 9). These solutions should be immiscible solutions (Rubert et al., 2014). Sustained drug release is possible thanks to the coaxial fibers, because the drug in the core fiber is protected against environmental factors by the shell fibers and the burst release is prevented. For example, hydrophobic and slowly degrading shell polymers such as PCL prevent rapid diffusion of the drug-loaded core (Song et al., 2013). In the method used to create multiple drug delivery systems, first, the core polymer swells and pores form in the shell that releases the drug. The

advantage of this method is that the core-shell arrangement protects the encapsulated drug or biomolecule, thereby increasing its functionality. It also prevents direct contact of bioactive parts with the external environment. Taking advantage of this benefit, Cheng et al. (2018), used coaxial electrospinning to load biological molecules into core-shell nanofibers. Platelet-rich plasma was incorporated into polycaprolactone/PVA/silk fibroin nanofibers. They also looked at the release profile and discovered that the polycaprolactone/PVA/silk fibroin nanofibers were released for 30 days.

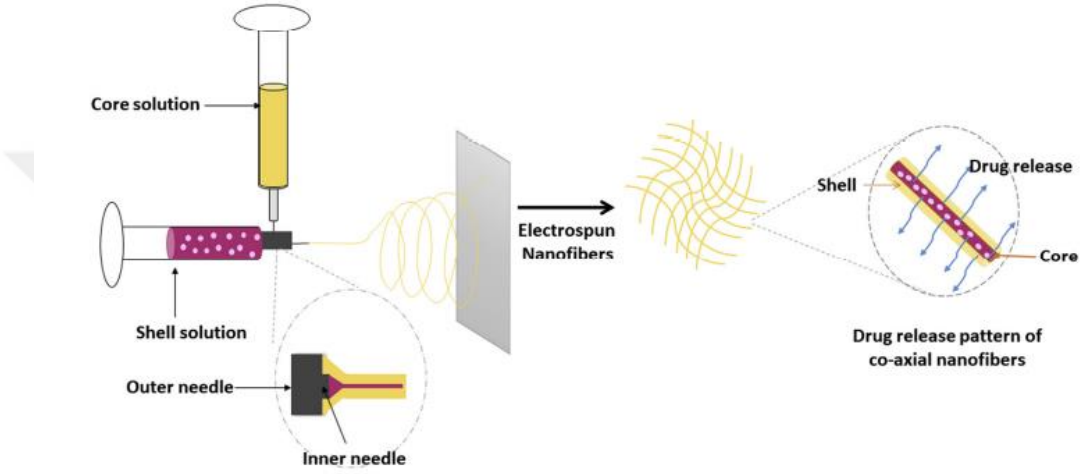

Figure 2. 9: Coaxial electrospinning (Jain, Shetty & Yadav, 2020).

The fluid fed from the core need not always be conductive or be a polymer solution. Additives can also be fed directly from the inner needle. Although existing studies show that the spinnability of the core fluid itself is not as important as the shell fluid, when the viscosity of the core fluid is too low, fiber formation problems occur. Therefore, the core fluid must also have a certain minimum viscosity if it is to be entrained continuously without breaking (Moghe & Gupta, 2008). Just like in the single syringe method, the polymer liquid forms a Taylor cone with the effect of the electrostatic field. It is assumed that the Taylor cone is formed by the interaction of the surface tension of the solution, the electric charges formed in the solution and the electrostatic force acting on it. When the applied electrostatic force is increased to the point where the solution overcomes the surface tension, a fine jet of solution emerges. Before the elongated thinning polymer solution reaches the collector plate, the solvents evaporate and accumulate on the collector plate as solid coaxial micro-nanofibers (Sun et al., 2003).

Electrospun nanofibers made from a variety of biocompatible and biodegradable polymers have shown a great potential for usage as efficient wound dressings. For instance, due to their outstanding electrospinning capabilities and biocompatibility, poly(e-caprolactone) (PCL) and polyvinylpyrrolidone (PVP) are frequently utilized as scaffolds and controlled drug delivery systems. PCL is a semi-crystalline polyester that is simple to work with, has strong mechanical qualities, and is very compatible with many different kinds of polymers. However, its hydrophobic feature restricts its use as a scaffold in tissue engineering since it may influence cell adhesion. By combining PCL with the proper hydrophilic polymer, it is possible to modify its hydrophobicity (Varsei et al., 2021). Suganya et al. (2011) they produced PCL/PVP nanofiber wound dressings containing herbal medicine in their study. The PCL used in the study powers the matrices and the PVP works as an excellent drug delivery medium. Then the fabrication the antibacterial activities of the surfaces were tested. According to the study, it has been determined that nanofiber wound dressings are at a level that can show high effectiveness against pathogenic microorganisms in the hospital environment. In some studies, coaxial nanofibers with PCL/PCL structure are also produced in order to extend the release time and obtain stronger structures. Repanas and Glasmacher (2015), produced nanofibers with different amounts of drug loading by changing the polymer ratios of both the shell structure and the core structure. As the amount of drug added to the structure increased, the fibers became thinner, the amount of encapsulation decreased and the structure remained hydrophobic. As both the core and the shell polymer ratio increased, the encapsulation amount of the drug increased and the structure became hydrophilic.

2.7 Essential Oil Loaded Nano Fibrous Structures for Treatment of Atopic Dermatitis

Essential oils (EOs) are liquid oils extracted from plants that contain a complex variety of chemical components. EOs act as natural antioxidants and antibacterials. More than 85% of the total content of EOs consists of over 70 components, including polyphenol terpenes, monoterpenes and sesquiterpenes (Viuda-Martos et al., 2010). These are called phenolic compounds. Hydro-distillation using a Clevenger-type device is the most often used process for extracting essential oils from various plant components, including the foret, roots, leaves, peel, bark, stem, and fruits. Additionally, plant oils

are a significant renewable raw material source for surfactants, cosmetics, lubricants, flooring materials, coatings, and resin products (Bakkali et al., 2008). Because all EOs are very volatile and easily degradable, encapsulating them in nanocarriers should increase their stability and antibacterial effectiveness (Saporito et al., 2018). EOs derived from aromatic plants contain a variety of constituents, including phenols, terpenoids, volatile oils, and aliphatic compounds (Bilia et al., 2014). Since prehistoric times, EOs have been utilized extensively as antibacterial, antiviral, insecticidal, and a variety of other medical purposes, including analgesic and anti-inflammatory.

To prevent bacterial infections, antibacterial compounds such as silver, zinc oxide, titanium dioxide, tellurium, and copper oxide nanoparticles are combined with nanofiber mats. These compounds, however, frequently induce toxicity in healthy skin (Shiekh, Singh & Kumar, 2020). To mitigate this harmful effect, wound dressings might contain secondary metabolites of antibacterial plant compounds. Nanofiber mats, in particular, when mixed with plant extracts such as EOs, have been shown to improve hemostasis, anti-inflammatory response, wound closure, and re-epithelialization. EOs have been used in a variety of biomedical applications by researchers (Sofi et al., 2019). Nonetheless, EOs are highly volatile and have a poor solubility in water. Encapsulation of EOs is a cost-effective way to protect them against evaporation and oxidation while also controlling their release. Zhang et al., for example, inserted thymol EO, an antibacterial agent, into core–shell nanofibers to control the release of the compound (Zhang et al., 2019b). It has been suggested that encapsulating even a small amount of EOs into electrospun nanofibers could result in increased antimicrobial activity due to bacterial cells penetrating the fibrous matrix and coming into direct contact with antimicrobial compounds due to the nanofibers' high exposed surface area (Liakos et al., 2015).

It has been observed that EOs may perturb the lipid portion of bacterial membranes, resulting in alterations in membrane permeability and leaking of the bacterial membrane's internal biological macromolecules. Additionally, the synthesis of DNA, RNA, proteins, and polysaccharides in fungal and bacterial cells has been found to be hindered by EOs (Böhme et al., 2014).

The majority of EOs are more powerful against gram-positive bacteria than they are against gram-negative bacteria. This difference could be explained by a difference in the composition of the cell membrane. On average, 90%–95% of the gram positive

cell membrane is composed of peptidoglycans, which enables hydrophobic compounds to permeate the cells and act on the cell wall and cytoplasm. Phenolic chemicals, which are components of EOs, permeate the cell walls of Gram positive bacteria and exert their antibacterial activity either by blocking energy-producing enzymes or by denatureing the proteins in the cell wall, so weakening the cell wall barrier (Nazzaro, Fratianni & Martino, 2013).

There are two important problems that need to be solved in order for AD complementary therapy to be considered successful. The first of these is the drying of the skin by losing water (TEWL) due to the inability of the skin barrier to perform its functionality properly. Another problem is the S.aureus bacteria that form colonies on the skin surface. Dry skin causes itching and as a result of scratching, the skin barrier is damaged, and the proliferation of S.aureus bacteria, which causes an increase in the severity of the disease, is facilitated in the open wound. For this reason, EOs have been researched to prevent the skin from losing moisture and to prevent bacterial growth by showing antibacterial properties.

Thyme (Thymus vulgaris) plant has been known as a medicinal plant since ancient times and is used for therapeutic purposes thanks to its ingredients such as thymol and carvacrol, which play an important role as antioxidant and antimicrobial agents. Among these components thymol, 2-isopropyl-5-methylphenol, is a phenolic monoterpene and main active compound of TEO. According to the study by Burt et al. (2005), it has been reported that the main components of Thyme Essential Oil (TEO) are carvacrol, thymol, p-cymene and γ-terpinene (See Fig. 2. 10). In addition to its antibacterial properties, these components also enable TEO to show high antioxidant properties. Also, thymol, one of the most important components of TEO, plays an important role in controlling inflammation caused by infections in wound treatment. Inflammation in wounds disrupts collagen synthesis in the skin, causing infection and wound growth. For this reason, it has been seen as important in the field of wound treatment that thymol disrupts the function of the cytoplasmic membranes of bacteria that cause inflammation, and has an antimicrobial effect by preventing the transport of nutrients to the cell membrane of the bacteria (Braga et al., 2006). In a study conducted by Young Mi and Seon Hee to evaluate the effects of thyme and lavender oils on immunity and skin condition in mice with AD, these essential oils were shown to be effective in reducing AD symptoms (Young Mi & Seon Hee, 2015).

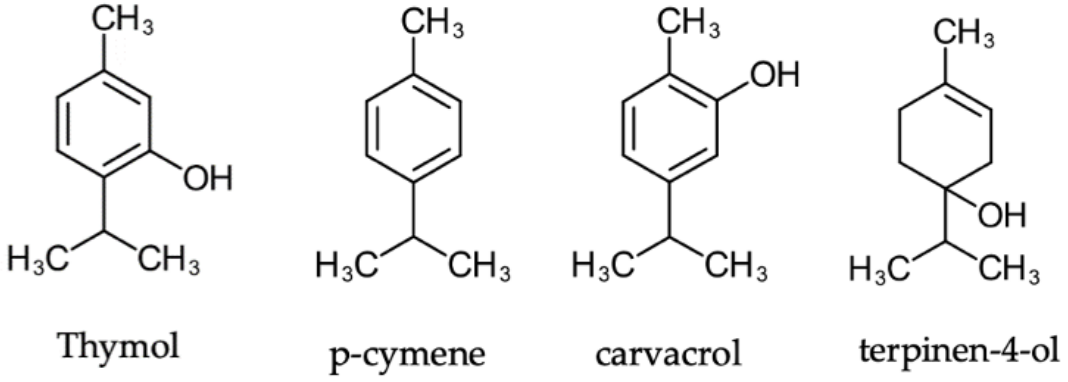

Figure 2. 10 : Thyme Essential Oil main compounds (Coetzee, Militky & Venkataraman, 2022).

Recently, Hypericum Perforatum Oil (HPO) (St. John's Wort), which has anti-inflammatory, antimicrobial, antioxidant and pain reliever properties, has started to attract attention as one of the most used medicinal plants in the world. It is studied in detail in the pharmaceutical industry due to its components such as hypericins, hyperforins and flavonoids (See Fig. 2. 11). Hyperforin is the main ingredient that provides antibacterial properties and helps skin epithelialization (Saddiqe, Naeem & Maimoona, 2010). HPO, which is frequently used in alternative treatment methods in Turkey, has a wide range of applications in the treatment of different wounds and burns (Gunes & Tihminlioglu, 2017). Süntar et al. (2010) reported that HPO increases infection resistance, has an anti-inflammatory effect that will contribute to rapid healing of the wound by providing fibroblast migration and collagen deposition . There are several studies about electrospun wound dressings with HPO content. In one of these studies, PCL/Gelatin electrospun wound dressings with HPO were formed and in vivo tests were done. Consistent with the literature, it has been stated that HPO has significant effects on wound healing (Guleken et al., 2021). In another one, HPO encapsulated in PEG fibers showed a controlled release for 14 days upon contact with the wound and it was stated that electrospun structures containing HP showed activity against gram positive and gram negative bacteria (Egri & Erdemir, 2019). In the study published by Schempp et al (2003) on the use of HP in the treatment of AD, it was shown that the phloroglucin derivative hyperforin component of HP has a visible antibacterial property against S. aureus bacteria, which is one of the causes of AD. In addition to electrospun surfaces, different dressing studies where HPO was loaded were also carried out. Bölgen et al. (2020) fabricated HPO-loaded chitosan cryogel

surfaces and tested the antibacterial and antioxidant properties of these surfaces. As a result of this study, it was concluded that HPO-loaded chitosan cryogel scaffolds with antimicrobial and antioxidant properties can be used as wound dressings for exudative and long-healing wounds in tissue engineering applications.

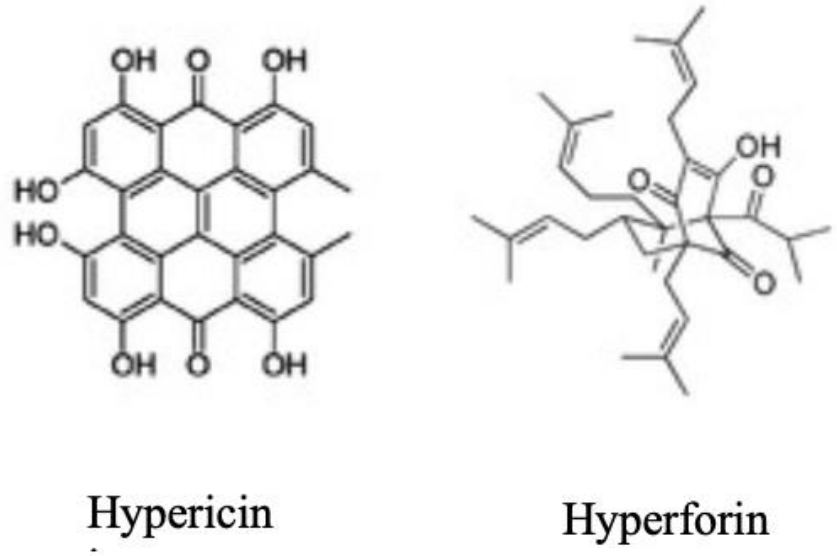

Figure 2. 11: Hypericum Perforatum oil compounds (Wölfe, Seelinger & Schemp, 2014).

While investigating the causes of AD, abnormalities in the metabolism of polyunsaturated fatty acids were found in most patients. These fatty acids are structural components of cell membrane phospholipids and are very important in maintaining the fluidity of cell membranes. Since the multiple fatty acids linolenic acid (LA) and α-linolenic acid (ALA) cannot be synthesized by the human body, they must be taken with external supplements. It has been shown that oral or skin intake of gamma linolenic acid (GLA), a metabolite of LA taken from the outside, has positive effects on AD. Since AD patients are presumed to have a deficiency of the δ-6-desaturase catalyst that converts LA acid to GLA, they have to take GLA from outside. The natural oil containing the most GLA in nature is borage oil (24%). (Kanehara, Ohtani, Uede & Furukawa, 2007)

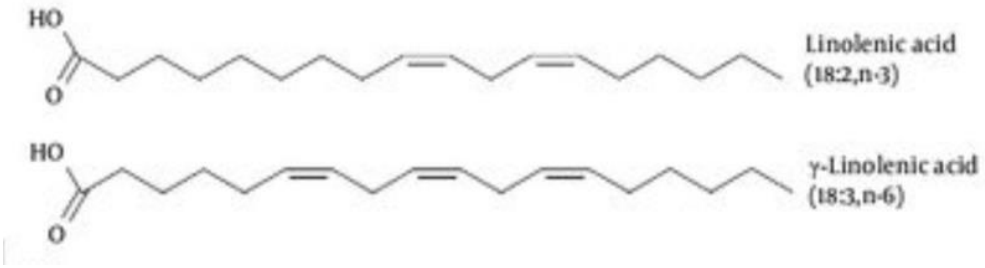

Figure 2. 12: Chemical structure of Linolenic acid (LA) and Gamma Linolenic Acid (GLA) (Vahdat et al., 2015).

In studies examining the applications of borage oil for the treatment of AD, it was observed that the barrier function of the skin, as measured by transepidermal water loss (TEWL), was normalized in infants and children with seborrheic and atopic dermatitis. In addition, an 11% reduction in transepidermal water loss occurred when healthy elderly people were provided with 360 or 720 mg of GLA daily with borage oil capsules (Foster, Hardy & Alany, 2010).

Among the studies on the treatment of atopic dermatitis with topical applications with borage oil there is one study in which borage oil was coated on cotton underwear with chemical methods and tested on pediatric patients with atopic dermatitis for 14 days. Clinical symptoms of erythema, pruritus, papules, erosion, scaling, and lichenification were all quantified by scoring on a 4-point scale (0-3, representing grades of "none", "mild", "moderate", and "severe") to evaluate outcomes. A transepidermal water loss test was also performed before and after a 2-week test period. There was improvement in itching and erythema values in children who used underwear containing borage oil. A statistically significant decrease was also observed in transepidermal water loss ($p= 0.0480$) (Kanehara, Ohtani, Uede & Furukawa, 2007).

In another study, in which the electrospun dressing and borage oil were used together, to help moisturize the atopic skin, vegetable oils with high GLA values (borage, black cumin seeds, evening primrose) were dripped onto the electrospun patches to help moisturizing the atopic skin, water vapor transmission rates (WVTR), hydration ability (FUA), and skin hydration were tested. Hydrophobic polystyrene and hydrophilic PA6 (Nylon 6) polymers were used as polymers. In order to combine the hydrophilic and hydrophobic properties of both polymers, a PS-PA6 composite structure and a PS + PA6 sandwich structure were produced. When the results are examined, the

hydrophilic PA6 polymer shows that the oil spreads more and provides more moisturizing on the skin, while the importance of the use of hydrophobic polymers for long and controlled oil release in the scenario where two separate surfaces produced with PS and PA6 polymers are used by forming a sandwich structure. (Krysiak et al., 2021)

3. EXPERIMENTAL WORKS & ANALYSIS

3.1 Materials

The polymers used for electrospinning, namely, Polyvinylpyrrolidone (PVP) (Mw: 360.000 g/mol), Polycaprolactone (PCL) (Mw: 80.000 g/mol) and PVA (Mw: aprrox. 70.000g/mol) were obtained from Sigma-Aldrich. Triton X-100 (Sigma-Aldrich) and Kolliphor® RH 40 (BASF) were used as the surfactants. Formic acid (100%), glacial acetic acid (100%) and ethanol were purchased from Merck. Distilled water was used as one of the solvents. TEO was obtained Bioterra, HPO was obtained from Zade Vital and BO was obtained from Botalife.

Poly(ε-caprolactone) (PCL) are biocompatible, biodegradable, aliphatic polyesters widely used for biomedical and pharmaceutical applications (See Fig. 3. 1). It is semi-crystalline and its melting temperature is between 59-64 ◦C but its Tg is -60 ◦C. PCL can be biodegraded by microorganisms as well as hydrolytically degraded to non-toxic products under physiological conditions. The fact that it does not produce toxic products has increased the preference of PCL in short and medium-term biological applications. Compared to natural polymers, polyester biopolymers degrade more slowly. Since PCL has five repeating hydrophobic -CH2 units in its chemical structure, it degrades in a long time compared to other biodegradable polyesters (Abedelwafa et al., 2013). Based on its molecular weight, PCL has better mechanical properties compared to other biodegradable polymers . In addition, there are studies showing that properties such as strength and deformability of electrospun nanofiber structures affect cell proliferation, migration and cell morphology in biomedical applications (Carnegie & Cabaca, 1993).

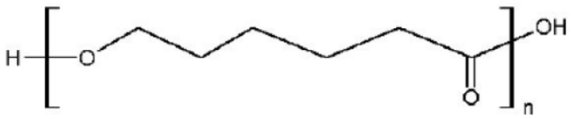

Figure 3. 1: Poly(ε-caprolactone) (PCL) chemical structure (Mahapatro & Singh, 2011).

Polyvinylprollidone (PVP) is a biocompatible, water-soluble synthetic polymer that is generally used as a drug dissolution enhancer. It is also called polyvidone or povidone and consists of linear 1-vinyl-2-pyrrolidone groups. As shown in the Figure 3.2, the polymer has a carbon chain containing an amide group at the co-substituent and having a poly-N-vinylamide structure. The Tg value varies between 100-175 ◦C depending on the molecular weight of the polymer (Teodorescu & Bercea, 2015). The rapid dissolution of PVP can be slowed down by making it composite in other polymers. Especially since the surface/volume ratio is high in the fibers produced by electrospinning, PVP fibers can dissolve very quickly in a wet environment. This causes burst release in drug loaded nanofibers. For this reason, new application areas for PVP fibers can be found with methods to delay wet environment PVP dissolution (Contardi et al., 2021).

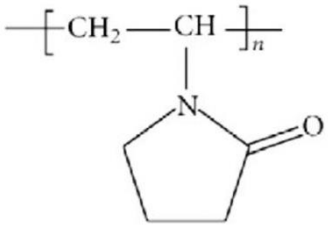

Figure 3. 2: Polyvinylprollidone (PVP) chemical structure (Sreekanth et al., 2019).

Polyvinyl alcohol (PVA) is a synthetic, semi-crystalline, water-soluble, biocompatible polymer produced by hydrolysis of poly(vinyl acetate). The physicochemical and mechanical properties of PVA vary according to the number of hydroxyl groups in its structure. Its melting temperature is 230 ◦C and Tg 80 ◦C. It is preferred in tissue engineering because it has tissue-like elasticity and strength. However, since poor water resistance limits its use in the medical field, its use in composites with different materials can increase the variety of application areas (Lin, Yu & Yang, 2006).

Triton X-100 is a nonionic surfactant. Nonionic surfactants contain both hydrophilic and lipophilic groups in their structure. The size and strength balance of these opposing groups is also called the hydrophilic-lipophilic balance (HLB). The surfactant to be used to form the emulsion is selected depending on the emulsion type. It is appropriate to use surfactants with an HLB value of less than 6 in water-in-oil (w/o) emulsions and between 6-20 in oil-in-water (o/w) emulsions. Triton X-100 has an HLB value of 13.6.

In the chemical structure of Triton X-100, unlike ionic surfactants, hydrophilic polyoxyethylene (POE) has a longer chain length than the hydrophobic part. In addition, nonionic surfactants are generally considered to be less toxic to living organisms than ionic surfactants (Jin et al., 2008; Stoleru & Brebu, 2021).

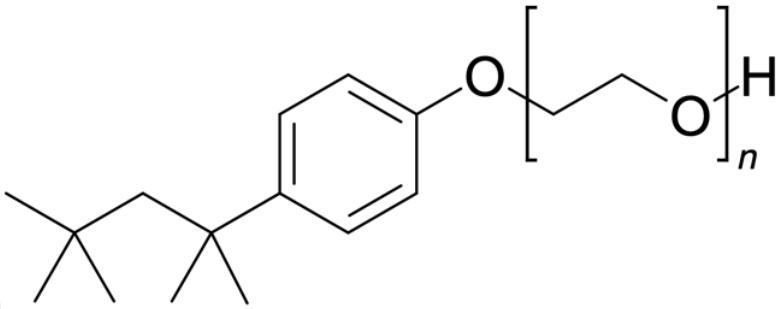

Figure 3. 3: Triton X-100 non-ionic surfactant chemical structure.

Cremophor RH 40, also known as Polyoxyl 40 hydrogenated castor oil, is a non-ionic surfactant obtained by reacting hydrogenated castor oil with ethylene oxide. The main component of this product is glyceryl polyethylene glycol oxystearate. They form the hydrophobic part of the substance together with fatty acid glyceryl polyglyceryl esters (Rachmati et al., 2017). Since they have a branched alkyl structure, their penetration into oil is greater than the linear chain alkyl structure. With an HLB value of 14-16, Cremophor RH 40 has a higher emulsifying capacity than Cremophor EL because there is 40 moles of ethoxy per mole of castor oil for Cremophor RH 40. This is 35 mol for Cremophor EL (Weerapol et al., 2014).

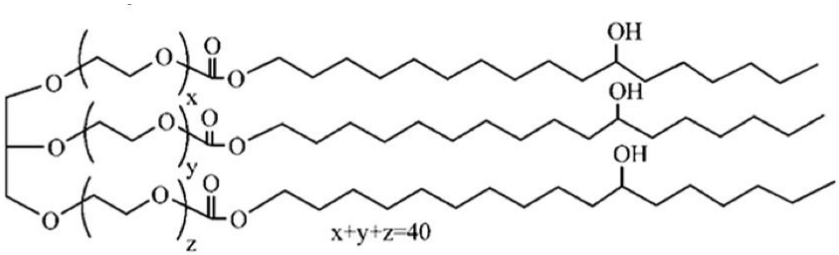

Figure 3. 4: Cremophor RH 40 non-ionic surfactant chemical structure (Weerapol et al., 2014).

3.2 Methods

In this section, all the methods used in the production, characterization and testing of nanofibers are given. Data such as electrospinning parameters and polymer ratios were given under the studies.

3.2.1 Electrospinning

Two different methods were used in the production of nanofibers. These are emulsion electrospinning and coaxial electrospinning. All production was done with the NanoSpinner NE300 model electrospinning device (Fig.3.5). A single nozzle was used for emulsion electrospinning as shown in Figure 3.6(a). The coaxial nozzle shown in the Figure 3.6(b) was used for coaxial production. The coaxial nozzle has an outer syringe diameter of 2 mm and an inner syringe diameter of 1.15 mm. Inovenso IPS-14 double syringe pump was used to feed the polymer solutions that will form the shell and core structures at different feed rates.

Figure 3. 5: NanoSpinner NE300 model electrospinning device.

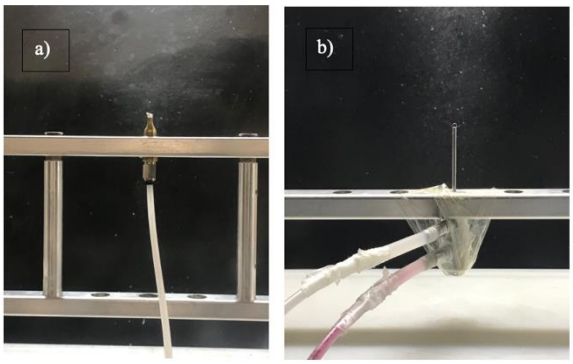

Figure 3. 6: Single nozzle (a), Coaxial nozzle (b).

3.2.2 Scanning electron microscopy (SEM)

In the studies, nanofiber morphology was examined with SEM device belonging to two different brands. Nanofibers were carbon coated with the Leica EM ACE200 coating device in preparation for imaging in studies using the ZEISS EVO 40 device. In studies using TESCAN - VEGA4 - W source and EDX Scanning Electron Microscopes (SEM), the samples were coated with Au/Pd with Quorum Sputter Coater. Fibre diameters were measured with the ImageJ software by selecting 100 different fibres from SEM images.

Figure 3. 7: SEM devices used for nanofiber image analysis ZEISS EVO 40 a), TESCAN - VEGA4 - W source and EDX b), respectively.

3.2.3 Fourier transform infrared spectroscopy (FT-IR)

The nanofiber surfaces were characterized by Perkin Elmer Spectrum 100 branded Fourier transform infrared spectroscopy (FT-IR) to check the presence of chemical bonding between polymers and oils and the surface molecular interactions of the samples. The tranmittances of all samples were analyzed in the frequency range of 500 - 4000 cm-1.

3.2.4 Water contact angle (WCA)

The contact angle measurement system - KSV - CAM 101 was used to determine the hydrophilic/hydrophobic properties of oil-loaded nanofiber surfaces. Deionized water (0.25 L) was dropped on the nanofiber surfaces and the contact angles of the samples were measured. All measurements were repeated 3 times for reliability.

3.2.5 Viscosity measurements

The viscosities of all electrospinning polymer solutions were measured with the Brookfield DV-E Viscometer. Measurements were made with S21 spindle at 100 rpm.

3.2.6 Conductivity measurements

The conductivity measurements of the solutions were made in an Endress+Hauser brand conductivity measuring device at room temperature. The unit of conductivity is given as μS/cm for all solutions.

3.2.7 UV-Vis spectrophotometer

Total phenolic method was applied to examine the release behavior of nanofiber samples with added thyme oil. The nanofibers, whose release behavior will be examined, were kept in 1 ml of PBS solution for each 5 mg of nanofibers in an incubator at 37 °C. After 5 μL samples were taken from the PBS medium at 0, 30 min, 1, 2, 4, ,8, 24, and 48 h intervals, fresh PBS was returned to the medium as much as the amount taken. Total phenol content (TPC) was determined using Folin-Ciocalteu reagent (Singleton & Rossi, 1965). Thyme oil was diluted with 80% methanol at different dilutions, 10,100,1000,10,000,100,000 times. Samples from PBS medium after 30 min,1,2,4,8,12,24 incubation hours were added to test tubes in 0.3 ml volume without dilution, followed by 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with water) and 2 ml of sodium carbonate (7.5% w/v) was added. Tubes were vortexed, sealed with parafilm and incubated for 1 hour at room temperature. Absorption at 765 nm was measured with an HP 8451 spectrophotometer (Hewlett-Packard, Cambridge, UK) and compared with a gallic acid calibration curve. Results are expressed as mg gallic acid equivalents (GAE)/l sample. Each assay was performed in triplicate.

3.2.8 Antibacterial tests

Disk diffusion method was used for antibacterial activity analysis of TEO-containing samples. Nanofiber samples were cut into 6 mm diameter circles. Then, 100 μl of 0.5 Mc Farland turbidity of S.aureus (ATCC 25923), E.coli (ATCC 25922), C.albicans (ATCC 10231) and A. niger suspensions was spread in petri dishes and incubated aerobically at 35 ± 2 °C for 24 hours. Inhibition zone diameters were measured with a ruler to calculate the antibacterial effect after incubation. The absence of an inhibition zone was interpreted as insufficient antibacterial effect.

3.3 Experimental Works

3.3.1 Emulsion electrospinning of PVA nanofibers containing HPO – effect of surfactant amount

The pioneering work of the thesis studies has been the emulsion electrospinning of PVA nanofibers containing HPO. PVA, which is a hydrophilic polymer that is frequently used in the medical field, was used in emulsion electrospinning to examine its interaction with oil and surfactant. In the absence of surfactant in aqueous solutions, oils do not disperse homogeneously in the solution. For this reason, it is aimed to produce nanofibers with the optimum fiber morphology and a solution that can remain in the emulsion for the longest time by changing the amount of surfactant by keeping the oil ratio constant. Since the production of nanofibers by electrospinning takes a long time, it is important that the solution remains in emulsion. The effect of the surfactant on fiber morphology was investigated to form a basis for the studies aiming to provide controlled release of essential oil trapped in the fiber by emulsion electrospinning method, especially during the treatment of skin disorders.

For fabricating nanofibrous structure using PVA as the polymer and HPO as the additive, distilled water was used as the solvent and Kolliphor® RH 40 (BASF) was used as the non-ionic surfactant. Before the electrospinning process, emulsions containing PVA, HPO and Kolliphor® RH 40 were prepared. A solution containing 12% (w/w) PVA was prepared by dissolving the polymer in distilled water for 2 hours at 70 °C. Amount of HPO added was 2% (v/v). Kolliphor® RH 40 was added at 2%, 4%, and 6% (w/w) ratios to PVA-HPO emulsion in order to observe the effect of surfactant ratio on nanofiber structure. Viscosity measurements of the emulsions were carried out prior to spinning. During electrospinning the feed rate was set as 0.3 mL/hour. The system voltage was fixed at 25 kV, the distance was 15 cm and the roller speed was determined as 200 rpm. The TESCAN - VEGA4 - W source, EDX Scanning Electron Microscope (SEM) was used for image analysis of nanofiber surfaces produced in this part of the study. FT-IR analysis was also conducted.

Table 3.1 shows the content of the pristine PVA nanofiber and oil loaded PVA electrospun fibers (HP-C2, HP-C4, and HP-C6) and the measured viscosities of polymer solutions for each sample.

Table 3. 1: The content and the viscosity values of polymer solutions for each sample group.

	PVA/solvent (w/w)	The amount of surfactant (w/w)	The amount of oil (v/v)	Viscosity (cP)
Pristine PVA	12%	-	-	91.5
HP-C2	12%	2%	2%	184.5
HP-C4	12%	4%	2%	270.8
HP-C6	12%	6%	2%	328

Since the aim has been to examine the effect of the amount of surfactant on the nanofiber morphology, the amount of surfactant was increased by keeping the amount of oil constant at 2% (v/v). The addition of surfactant to polymer solution can affect the viscosity of solutions by modulating the polymer/solvent and polymer/surfactant interactions via electrostatic, hydrophobic, and hydrogen bonding interactions (Kriegel et al., 2009). The results in Table 3.1 showed that the viscosity of the solution increased as the amount of surfactant increased in the polymer solution.

The SEM images at 20,000 magnification and fibre diameter distributions of electrospun fibres are shown in Figure 3. 8. The viscosity of the solution is a parameter that has an effect on the morphological structure and average diameter of the nanofibers (Liao et al., 2015). As shown in the SEM images and fibre diameter distribution graphs (Fig. 10), the diameters of the fibers have thickened due to the increased viscosity (Table 2) caused by the addition of surfactant at 2%, 4%, and 6% (w/w) ratios. According to the results, the average diameter size of electrospun fibres were calculated as 82 ± 17 nm, 131 ± 47 nm, 176 ± 59 nm, and 208 ± 105 nm for the pristine, HP-C2, HP-C4, HP-C6 samples, respectively.

In the literature, the increase in the diameter of the fibers has been accepted as an indicator of the encapsulation of more oil in the fiber (Zhang & Zhang, 2018). In addition to the increase in fiber diameters, the fibers tended to coalesce and lose their fiber morphology in some parts (Figure 3. 9). The reason for these morphological changes can be attributed to the amount of surfactant and viscosity.

In the FT-IR analysis, peaks similar to those of H.Perforatum oil were observed for the PVA nanofibers with oil content. Peaks due to C-H stretching are observed in the region of 2850-3000 cm^{-1} in both H.Perforatum oil and PVA nanofiber doped with oil (Fig. 3. 10). This is promising since it indicates that HPO was successfully incorporated into the fibre structure.

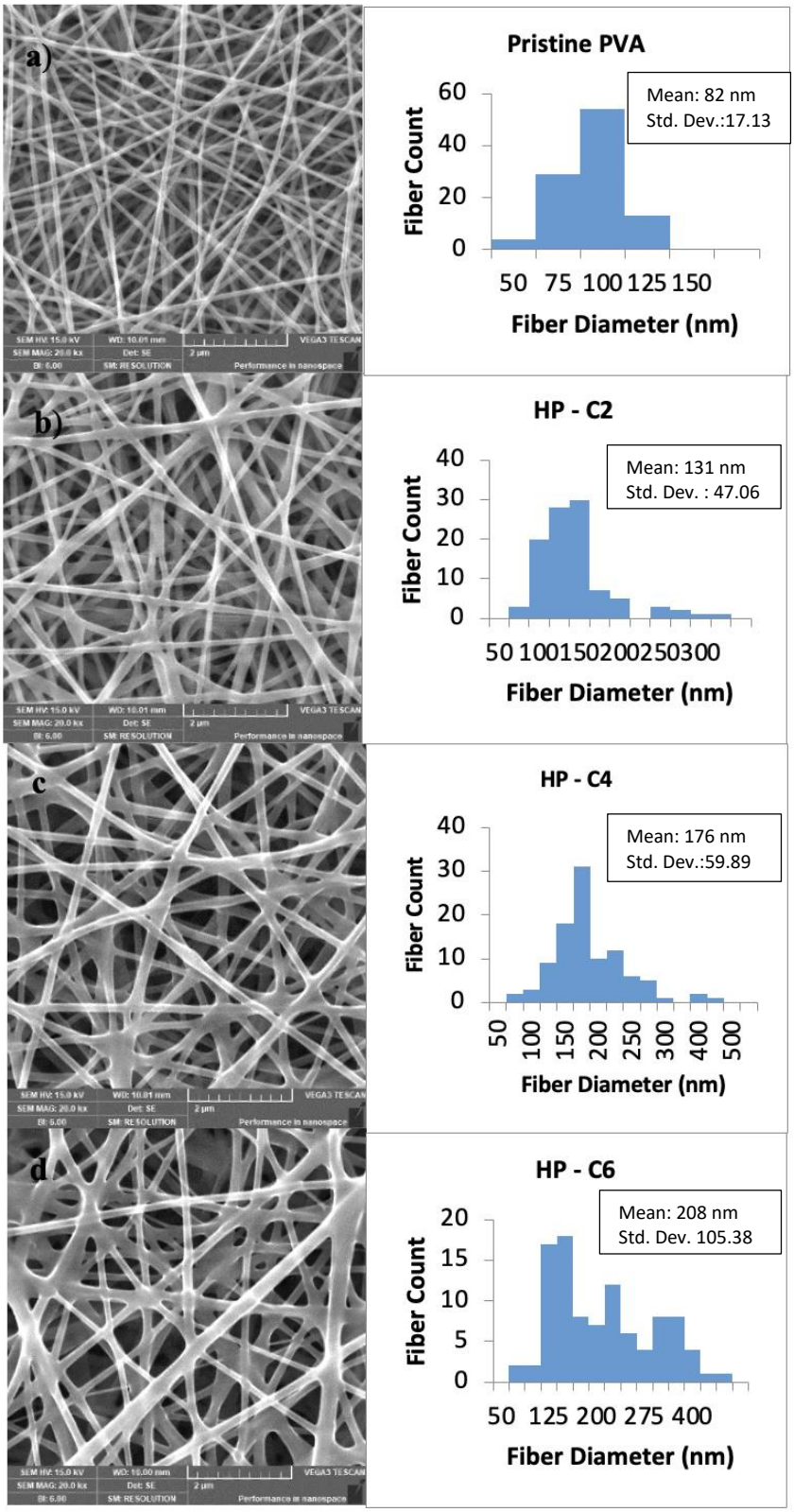

Figure 3. 8: SEM images (at x20.000 magnifications) and fibre diameter distribution graphs of pristine PVA nanofiber (a), HP-C2 (b), HP-C4 (c), HP-C6 (d) samples, respectively.

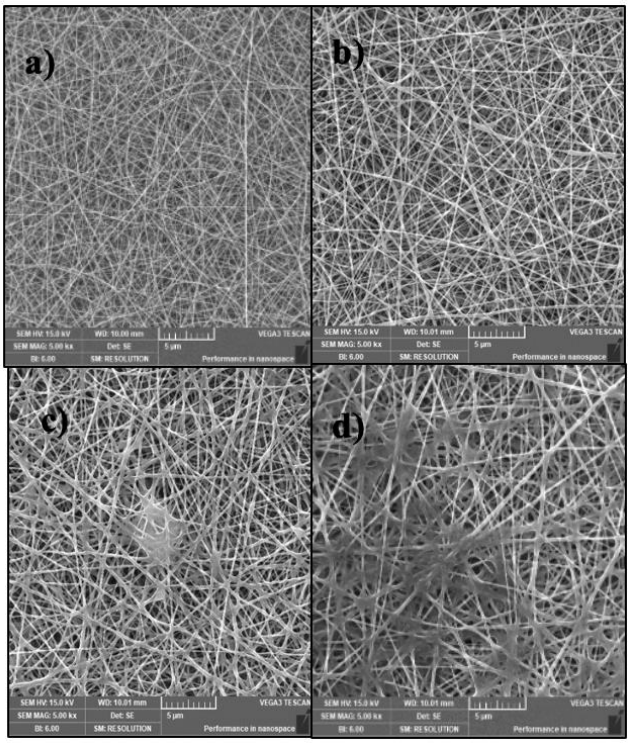

Figure 3. 9: SEM images of pristine PVA nanofibers (a), HP-C2 (b), HP-C4 (c), HP-C6 (d) samples at x5.000 magnifications.

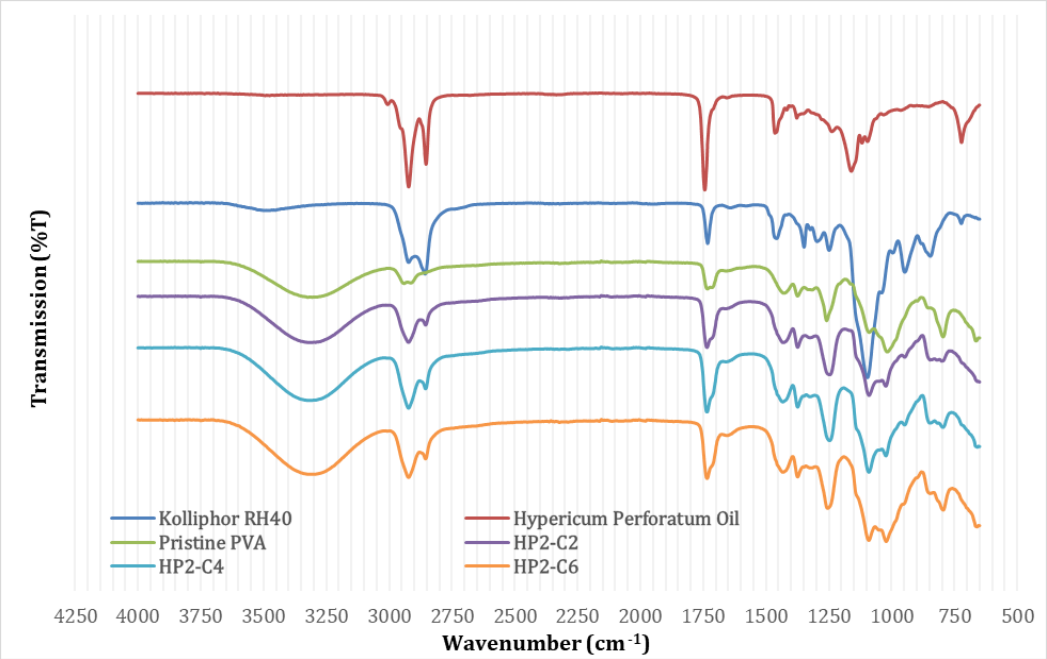

Figure 3. 10: FT-IR Spectrum of nanofibers, Kolliphor RH40, and Hypericum Perforatum Oil.

3.3.2 Emulsion electrospinning of PCL and PVP nanofibers containing BO and HPO

In this part of the study the aim has been to observe the nanofiber diameter and uniformity depending on different oil ratios, and thus determine suitable formulations for electrospun wound dressing. For doing so, fiber morphology and surface properties were investigated by adding increasing proportions of BO and HPO mixture (1:1) to hydrophilic PVP and hydrophobic PCL polymer solutions seperately.

Polyvinylpyrrolidone (PVP), Polycaprolactone (PCL), formic acid (100%), glacial acetic acid (100%), ethanol and distilled water were used for preparing solutions where Kolliphor® RH 40 (BASF) and Triton X-100 (Sigma-Aldrich) were used as surfactants. During electrospinning the feed rates were set as 1.5 mL/hour for PVP solution and 1.62 mL/hour for PCL solution. The system voltage was fixed at 26.6 kV for PVP and 24 kV for PCL. The distance was 15 cm and the roller speed was determined as 200 rpm for both polymer solutions.

PCL solution was prepared by dissolving PCL polymer (10% wt) in acetic acid/formic acid (1:1 w/w) under magnetic stirring for 2 hours at room temperature. After that 3% (wt) Triton X-100 was added as the surfactant. To prepare the PVP solution, PVP (10% wt) was dissolved in distilled water/ethanol (1:1 w/w) solvent mixture for 12 hours at room temperature. After dissolution is complete, 3% (wt) Kolliphor® RH 40 was added as a surfactant and HPO+BO mixture (volume ratio of 1:1) was added at varying ratios to both solutions (1%, 2%, 3% v/v). After all solutions were vortexed at 3000 rpm for 2 minutes with vortex mixer nanofibers were electrospun. In order to compare the oil-added fibres with the pristine ones, surfactant and oil-free fibres were also produced from PCL and PVP solutions. ZEISS EVO 40 Scanning Electron Microscope (SEM) was used for image analysis of nanofiber surfaces. Fibre diameters were measured with the ImageJ software by selecting 100 different fibres from SEM images. Fourier Transform Infrared Spectroscopy (FT-IR) analysis and the contact angle measurements were also carried out for the samples.

Since the effects of oil additives on the morphological structure of nanofibers were investigated in the study, first, the hydrophilicity/hydrophobicity states of the polymers were determined to confirm the use of polymers with two different properties. As shown in Figure 3.11, PCL is a hydrophobic polymer because its contact

angle is larger than 90 degrees, whereas PVP is a hydrophilic polymer since its contact angle is less than 90 degrees.

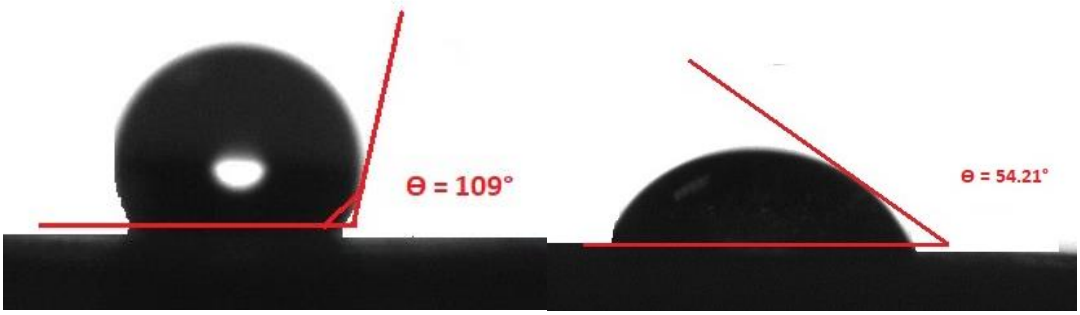

Figure 3. 11: Contact angle measurement images of nanosurfaces produced from pristine polymer solutions a), PCL b), PVP.

Figure 3.12, shows the fibre diameter distribution and SEM images for PVP fibres where PVP-O1, PVP-O2 and PVP-O3 stand for 1%, 2% and 3% (v/v) of HPO+BO mixture (volume ratio of 1:1) added, respectively. When the results were examined, the increase in the amount of oil caused the fibre diameters to thicken up to a certain level. After the addition of 3% oil, the fibre diameters became thinner again. This could be because of insufficient surfactant required for micelle formation as the amount of oil increases, and the thinning of the fibre diameters of the non-micelle-forming oil in the solution. As a result, bead-free and smooth fibres were obtained in all oil ratios, although there were changes in fibre diameters with increasing oil content.

As demonstrated in Figure 3.13**,** the same increment at the fibre diameters was observed for PCL fibres (O1, O2 and -O3 stand for 1%, 2% and 3% (v/v) of HPO+BO mixture added, respectively). However, as the amount of oil in PCL fibres increased, deterioration in the nanofiber structure occurred. Fibres tended to merge and lose their fibre morphology in some parts.

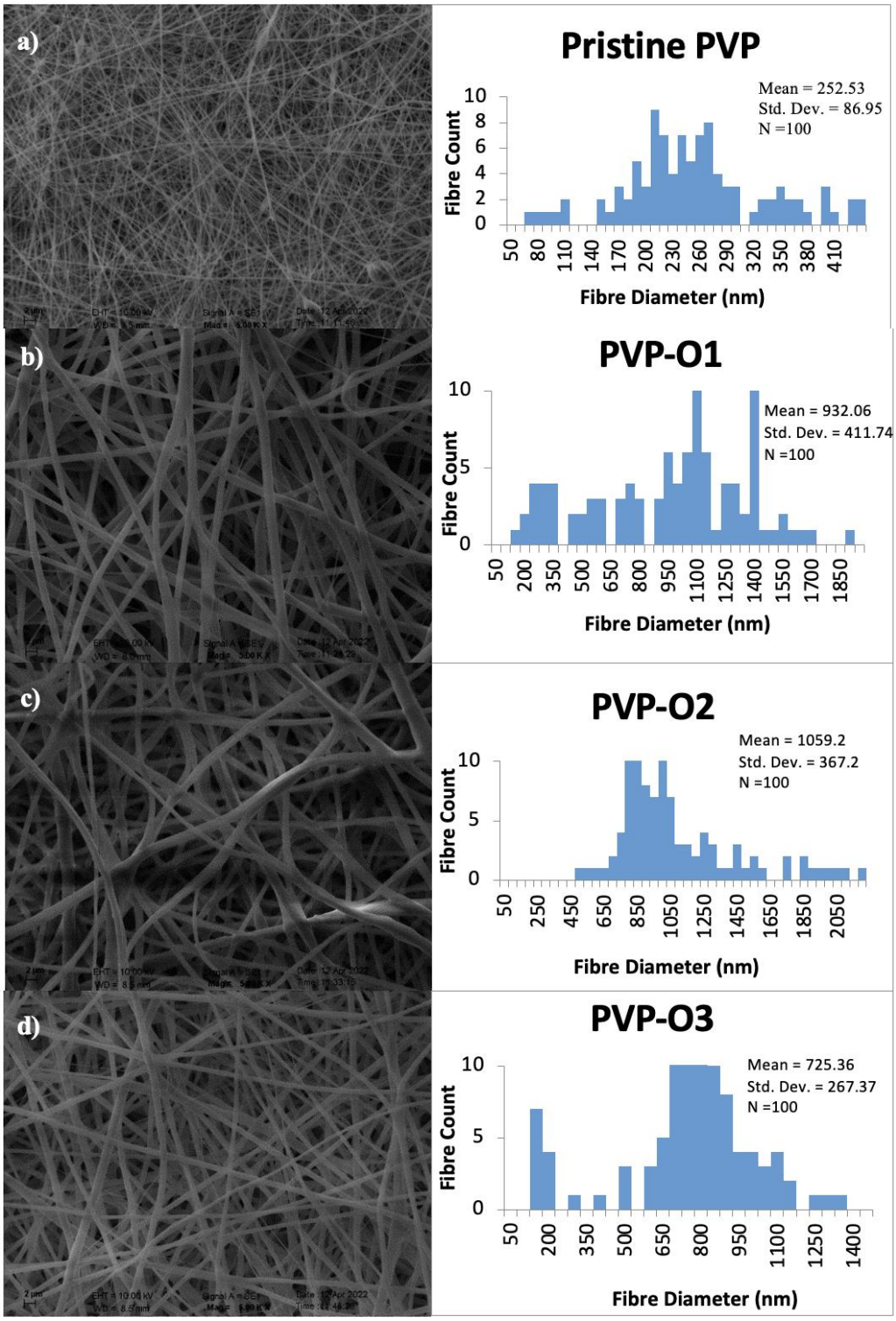

Figure 3. 12: SEM images (at x5.000 magnifications) and fibre diameter distribution graphs of pristine PVP nanofiber a), PVP-O1 b), PVP-O2 c), and PVP-O3 d samples, respectively.

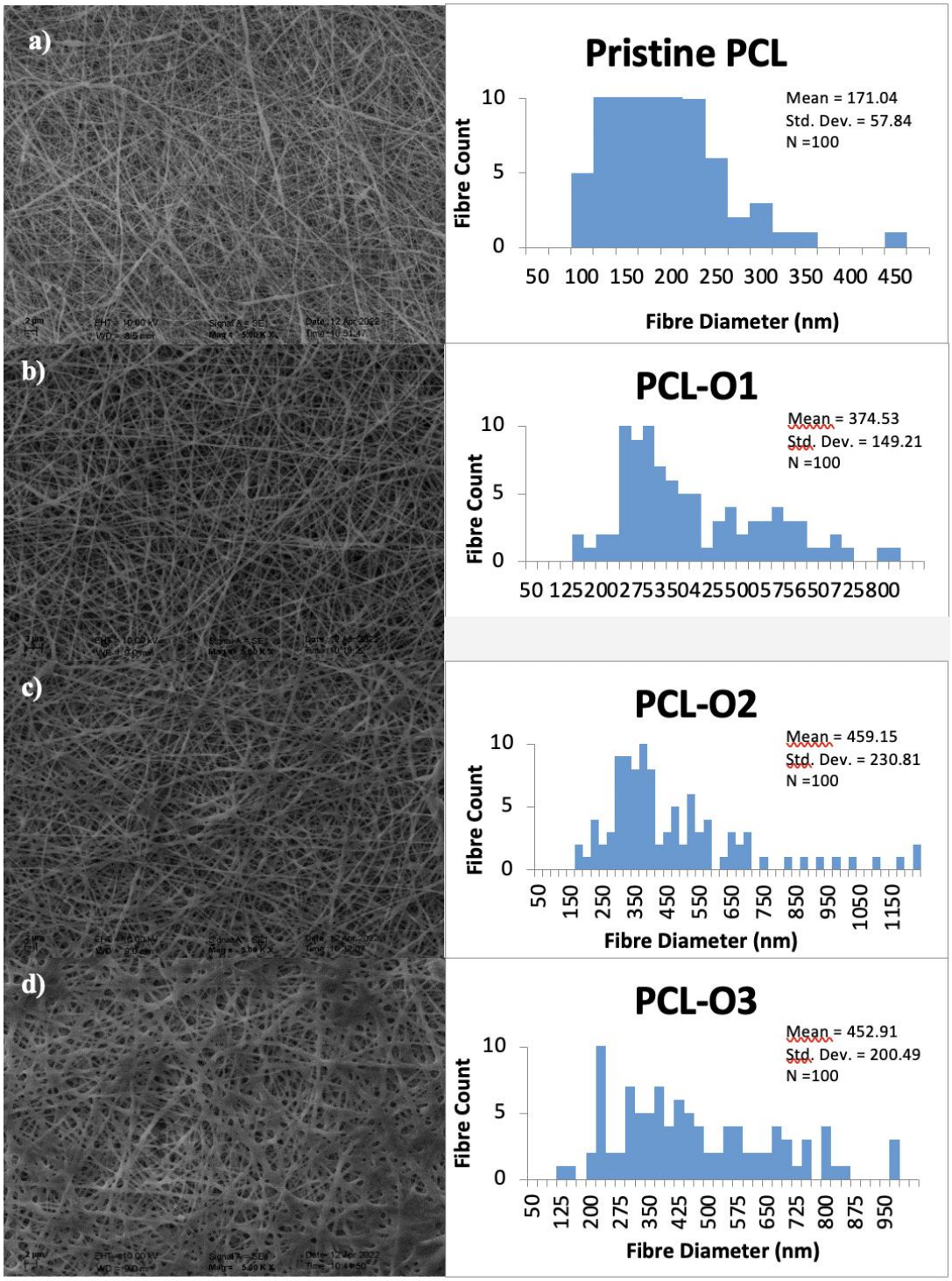

Figure 3. 13: SEM images (at x5.000 magnifications) and fibre diameter distribution graphs of pristine PCL nanofiber a), PCL-O1 b), PCL-O2 c), PCL-O3 d samples, respectively.

In the FT-IR analysis, peaks similar to those of the oil mixture were observed for the PVP nanofibers with oil content. C-H stretching peaks are observed in the region of 2850-3000 cm^{-1} in both oil mixture and PVP nanofiber doped with oil (Fig. 3.14). This is promising since it indicates that PVP nanofibers contain oil and surfactant in their structure. Also, the hygroscopic nature of pristine PVP was indicated by a C=O stretching band at 1647 cm^{-1} as in the literature (Rahma et al., 2016). In addition, the O-H bonds in the surfactant structure in the region of 3500-3300 cm^{-1} overlap with the O-H bonds in the doped PVP nanofibers and create stronger peaks. In the Pristine PCL spectrum (Fig. 3. 15), 1726 cm-1 (carbonyl stretching), 1294 cm-1 (C–O and C–C stretching), 1238 cm-1 (asymmetric C–O–C stretching), and 1162 cm-1 (symmetric C–O–C stretching) which were similar to other researchers (Liverani & Boccaccini, 2016). Additionally, peaks due to C-H stretching are observed in the region of 2850-3000 cm^{-1} in both oil mixture and PCL nanofiber doped with oil.

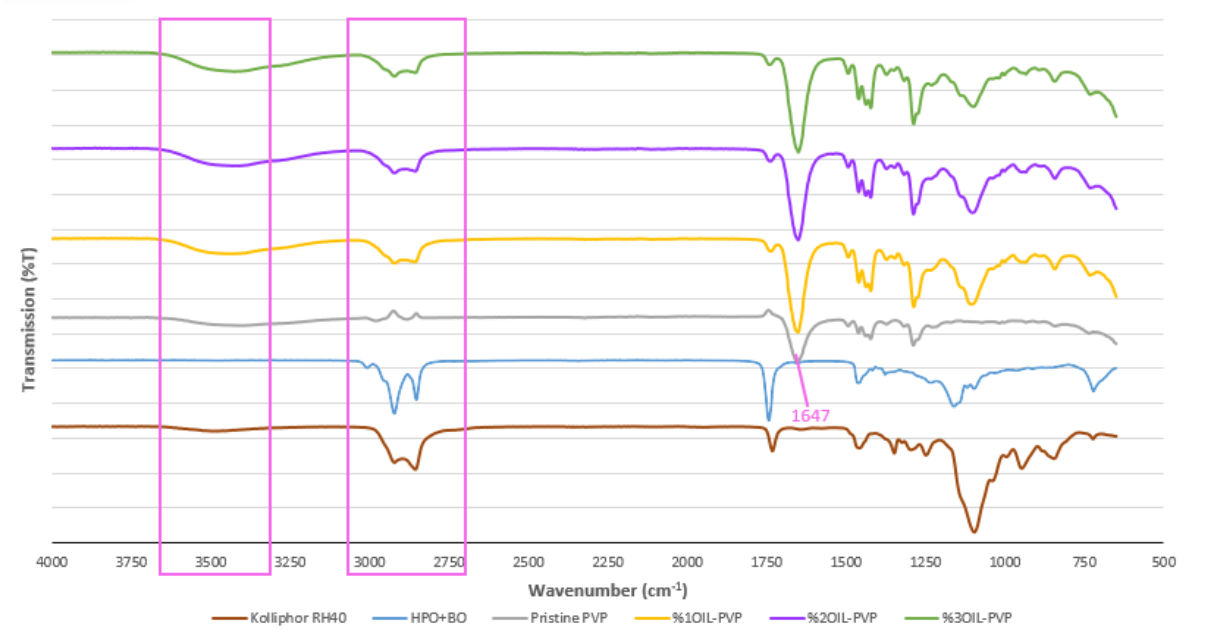

Figure 3. 14: FT-IR Spectrum of PVP nanofibers, Kolliphor RH40, and HPO/BO mixture.

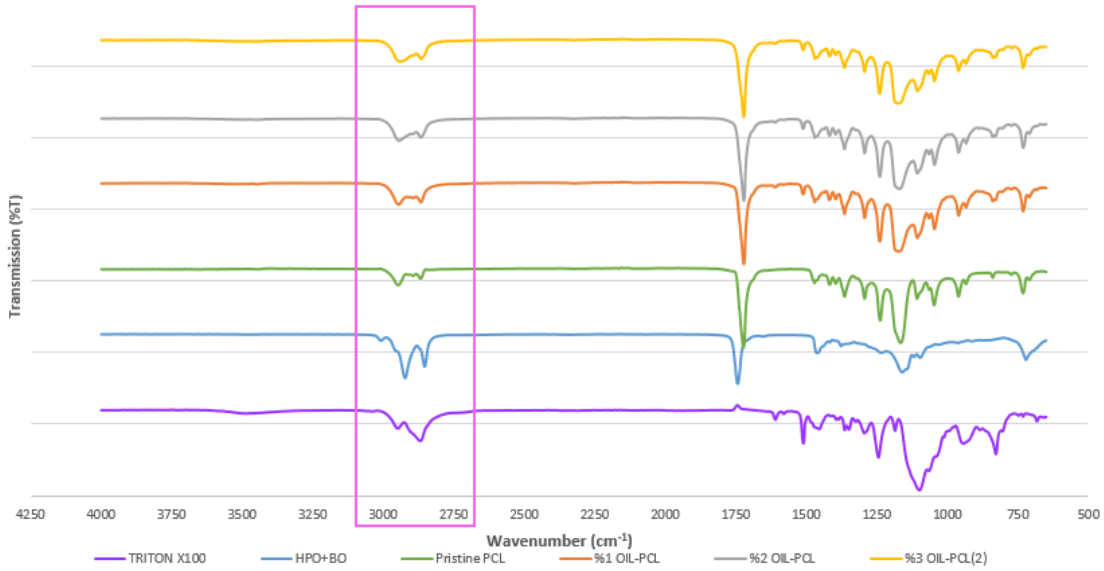

Figure 3. 15: FT-IR Spectrum of PCL nanofibers, Triton X100, and HPO/BO mixture.

Observation of surfactant and oil peaks in FTIR analysis indicates that the oils are successfully incorporated into the fibre structure by forming micelles with surfactants. In further studies in the thesis, the sustained release behavior of oil-loaded polymers and the antibacterial properties of additives are investigated.

3.3.3 Production of PVP/PCL nanofibers in different polymer ratios by coaxial electrospinning method

In this part of the study, nanofibers were produced from different ratios of PVP/PCL polymers by coaxial electrospinning method in order to optimize the core-shell nanofibers to be loaded with oil. The morphologies of the produced fibers were examined, the hydrophilicity/hydrophobicity test was performed on the surfaces and the effect of polymer ratios on the surface properties was investigated.

PCL polymer solutions were prepared by dissolving PCL at different polymer ratios (10, 8, 6 % wt) in asetic acid/formic acid (1:2 w/w) under magnetic stirring for 3 hours at room temperature. To prepare the PVP solutions, PVP (10, 8, 6 % wt) was dissolved in distilled water/ethanol (1:1 w/w) solvent mixture for 12 hours at room temperature. PCL solutions were used as shell, PVP solutions were used as core solutions. To examine the effect of polymer ratios on nanofiber morphology and hydrophilicity/hydrophobicity of surfaces, nanofiber surfaces were fabricated by coaxial electrospinning method at different polymer ratio combinations given in Table 3.2.

Table 3. 2: Polymer ratios of nanofiber surfaces fabricated by coaxial electrospinning method.

	PCL (Shell)	PVP (Core)
S1	10%	10%
S2	8%	8%
S3	6%	6%
S4	10%	8%
S5	10%	6%
S6	8%	6%

During coaxial electrospinning of the nanofibers an Inovenso IPS-14 double syringe pump was used to feed the polymer solutions that will form the shell and core structures at different feed rates. Optimum electrospinning parameters were adjusted as in Table 3.3 by making changes in the parameters during production and the same production parameters were used for all samples.

Table 3. 3: Electrospinning production parameters of PVP/PCL coaxial nanofibers.

Feed Rate (mL/h)	Voltage (kV)	Distance (cm)	Collector Speed (rpm)
0.8 for PCL 0.2 for PVP	24.4	20	200

In order to investigate the change in hydrophilic/hydrophobic surface properties of nanofibrous surfaces formed with different polymer ratios, surfaces were formed by coaxial electrospinning method using PCL polymer, which is known to be hydrophobic, to form the shell structure of the fibers, and PVP polymer, which is hydrophilic, for the core structure, at different rates. SEM images and fiber diameter and distributions of all samples are presented in Figure 3.16.

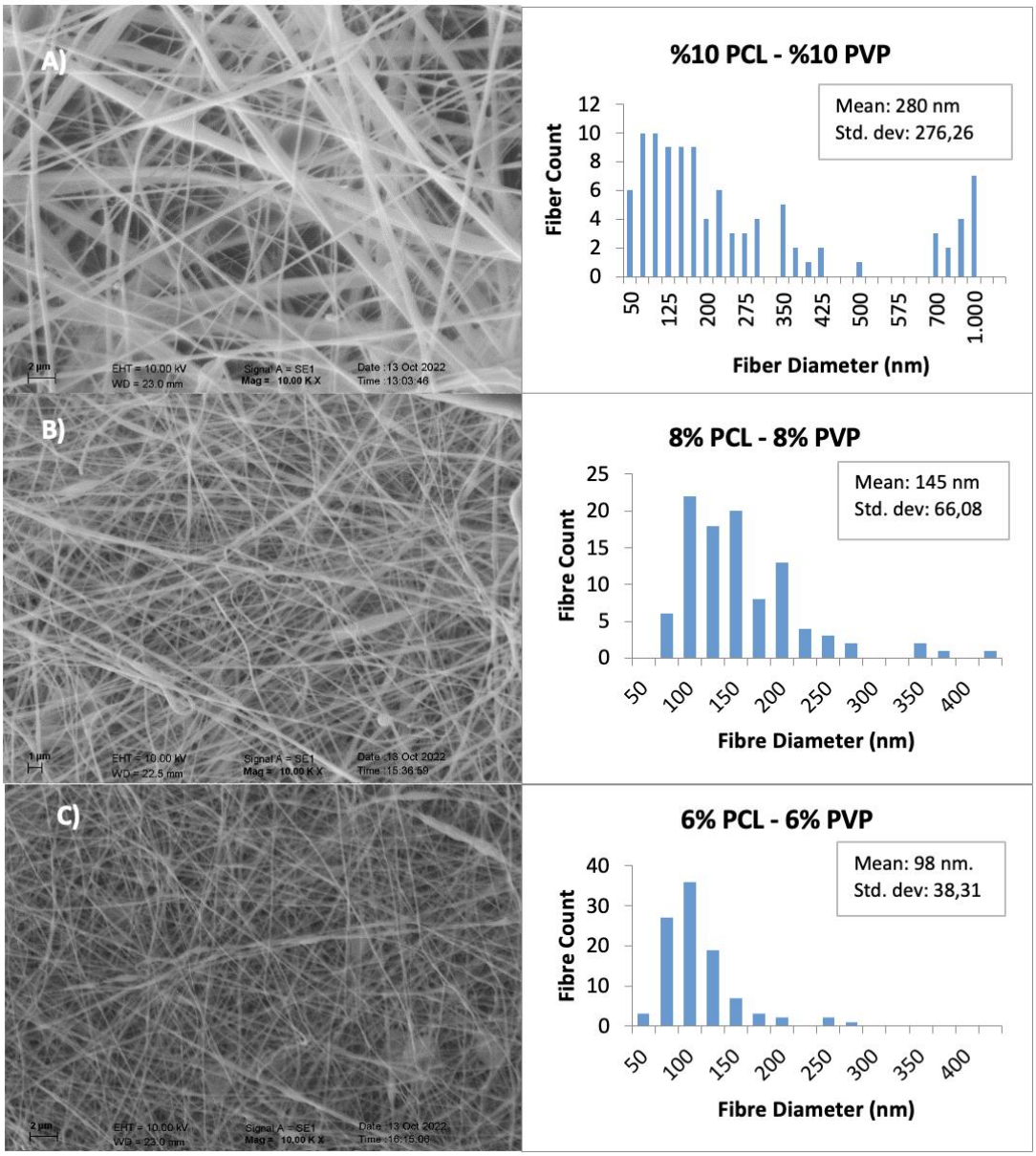

Figure 3. 16: SEM images (at x10.000 magnifications) and fibre diameter distribution graphs of coaxial nanofibers 10% PCL – 10% PVP A), 8% PCL – 8% PVP B), 6% PCL – 6% PVP C), 10% PCL – 6% PVP D), 10% PCL – 8% PVP E), 8% PCL – 6% PVP F) samples, respectively.

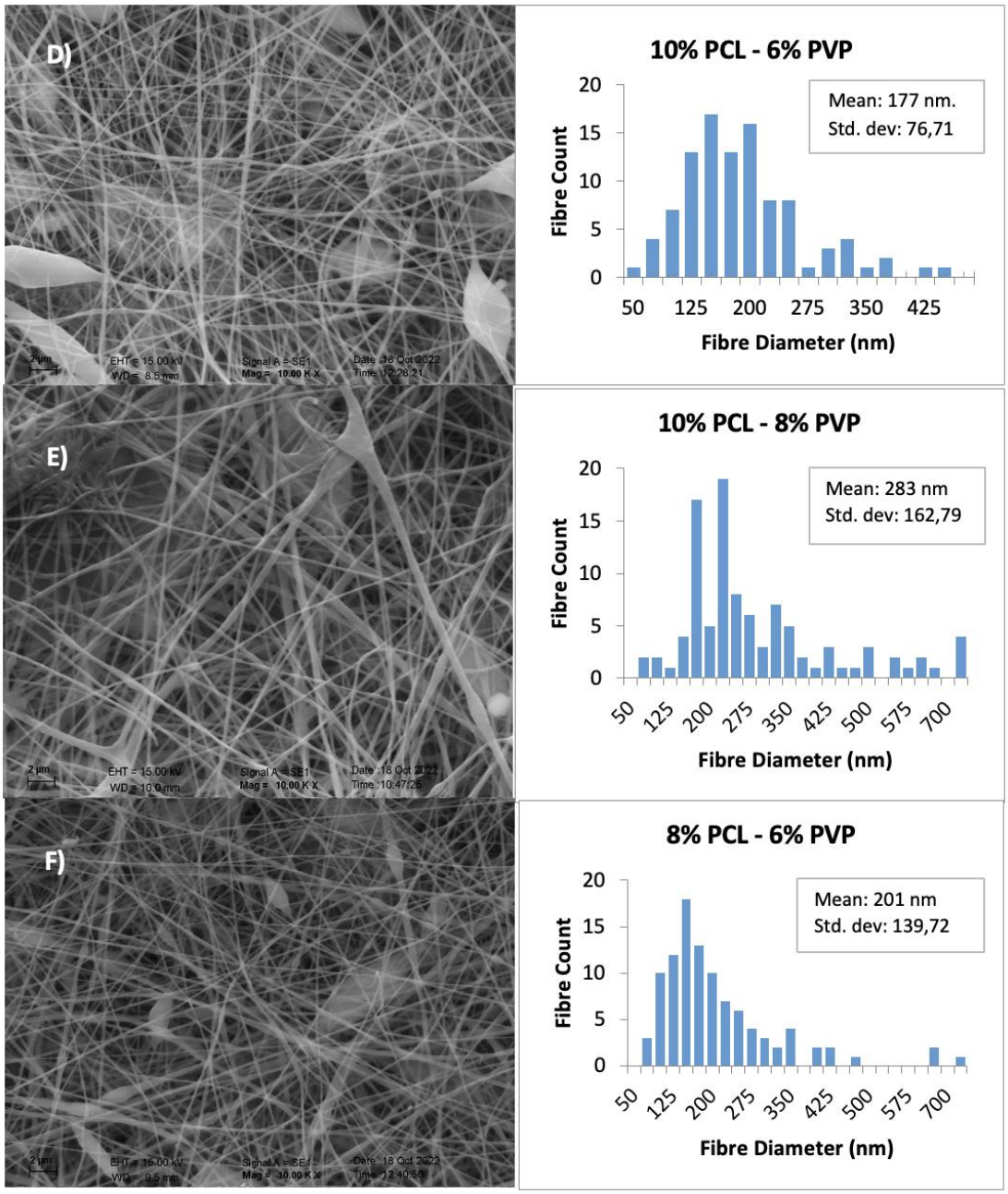

Figure 3. 17 (continued): SEM images (at x10.000 magnifications) and fibre diameter distribution graphs of coaxial nanofibers 10% PCL – 10% PVP A), 8% PCL – 8% PVP B), 6% PCL – 6% PVP C), 10% PCL – 6% PVP D), 10% PCL – 8% PVP E), 8% PCL – 6% PVP F) samples, respectively.

When the first three SEM images (A, B, C) are examined, it is clearly seen that there was a decrease in fiber diameters as the concentration decreased in both the shell and core polymer. The average fiber diameters are 280 ± 276.26 , 145 ± 66.08 and 98 ± 38.31 nm, respectively. Since there were problems in jet formation during the production of sample A, the diameter distribution was not homogeneous and the

standard deviation was high, as can be seen from the SEM images. The reason for the changes in fiber diameters can be explained by the decrease in the viscosity of the polymer solutions with the decrease in concentration. Viscosities of polymer solutions are given in Table 3.4. According to the literature, viscosity of the solution is a parameter that has an effect on the morphological structure and average diameter of the nanofibers (Liao et al., 2015). When the other SEM images (D, E, F) were examined, it was observed that the bead formation in the fibers increased as the PVP concentration in the core decreased. This may be due to the fact that the viscosity values of the shell and core structures are different from each other. After examining all the images and diameters, it was decided to continue with the 8% PCL - 8% PVP sample, which has the most beadless and the best diameter distribution for the production of oil-added fibers.

Table 3. 4: Viscosities of PCL and PVP polymer solutions.

Polymer Type	Polymer Ratio	Viscosity (cP)
PCL	6%	110
PCL	8%	301.5
PCL	10%	666
PVP	6%	144.5
PVP	8%	300
PVP	10%	593.3

To assess the surfaces' hydrophilicity and hydrophobicity, a WCA test was conducted. It has been demonstrated that the contact angle of the pure PCL polymer, which is known to be hydrophobic, is 109°, and the contact angle of the pure PVP polymer, which is known to be hydrophilic, is 54.21°. Two different test groups were formed in order to separately examine the effect of decreasing the polymer ratios in both the shell and core fluids at the same time and the effects of the decrease in the polymer ratio of the core fluid while keeping the polymer ratio of shell fluid constant. Figure 3.17 shows the WCA measurements of the samples when the core/shell polymer ratios were reduced at the same rate. As the viscosity decreases with the decrease of the polymer ratio, it could be easier for the hydrophilic polymer in the core to migrate towards the shell and the enclosure of the core polymer might be more difficult.

When the results in Figure 3.18 are examined, as in the first group, as the viscosity of the core polymer decreases, there may be polymer migration from the core to the shell. Contact angles decreased less in the second group compared to the first group. The reason for this might be due to the fact that core enclosing performance of the shell polymer did not change since the PCL ratio (and the viscosity) was kept constant. Thus, the hydrophilicity of the surfaces increased and the contact angles decreased. In a study by Koushki et al., it was found that PCL had a water contact angle of 110° and PVA/PCL coaxial electrospun nanofibers had a water contact angle of 83°. The outcomes demonstrated that adding PVA to PCL nanofibers decreased the final core-shell structure's hydrophobicity. PVA is generally known to be very hydrophilic due to the presence of hydroxyl groups (O-H) and hydrogen bands; hence, adding PVA to a hydrophobic polymer like PCL can enhance the hydrophilicity of the final structure (Koushki, Bahrami & Ranjbar Mohammadi, 2018). In addition, in the study conducted by Huang, Zhang and Ramakhrishna (2005), the WCA values of pure PCL polymer and shell-core PCL-GEL nanofibers were compared and the contact angle of coaxial fibers was found to be lower. To the best of our knowledge, no study has been found in the literature that tests the changes in the contact angle depending on the viscosity change in the core polymer. In our study, surrounding PVP fibers with PCL fibers helped to combine the different properties of the two polymers and eliminated the disadvantages of individual polymers in the production of wound dressings.

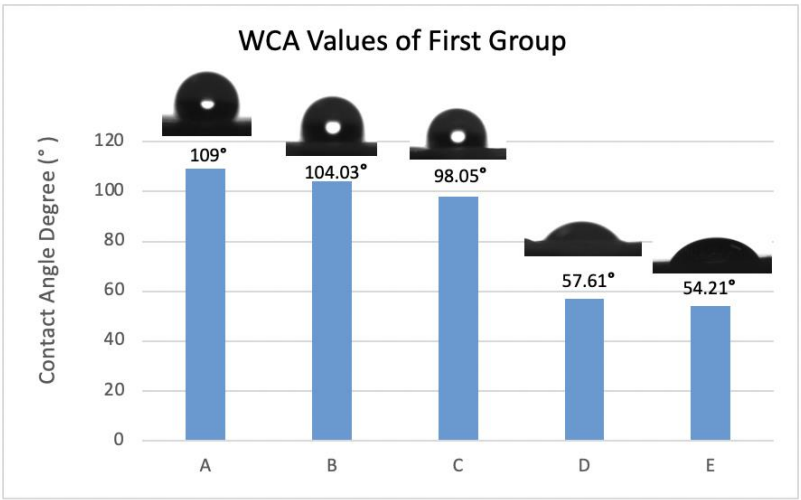

Figure 3. 18: WCA values of monolitic PCL - PVP nanofibers and coaxial nanofibers with different polymer ratios; %10 PCL A), %10 PCL - %10 PVP B), %8 PCL - %8 PVP C), %6 PCL - %6 PVP D), %10 PVP E), respectively.

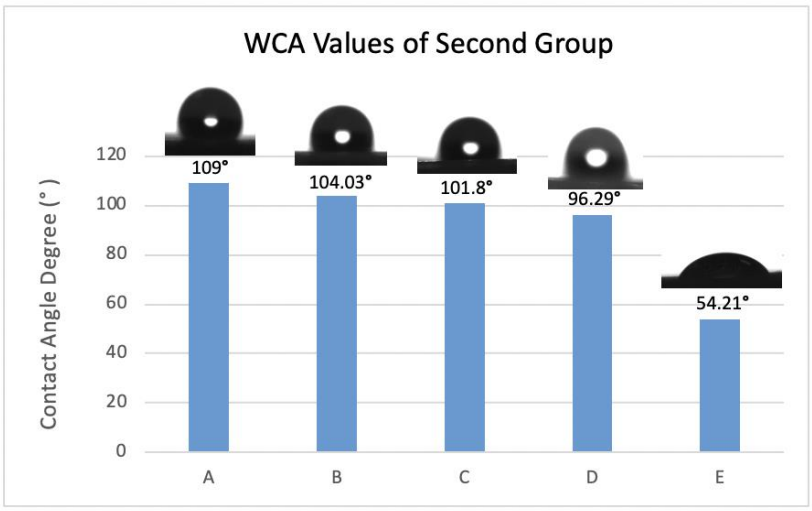

Figure 3. 19: WCA values of monolitic PCL - PVP nanofibers and coaxial nanofibers with different polymer ratios; 10% PCL A), 10% PCL – 10% PVP B), 10% PCL – 8% PVP C), 10% PCL – 6% PVP D), 10% PVP E), respectively.

After this study, which was carried out to determine the optimum polymer ratio before the production of oil-loaded coaxial fibers, it was decided to continue the production of coaxial fibers with 8% PVP / 8% PCL.

3.3.4 Production of EO doped PVP/PCL nanofibers with coaxial electrospinning method

In the previous study, the most suitable polymer ratios for the core-shell structure were determined before oil loading on the coaxial nanofibers. In this part of the thesis, it is aimed to examine the fiber morphology, release behaviour and the antibacterial effect of nanofibers by loading oil in the core parts of the fibers. Therefore, PCL polymer solutions were prepared by dissolving PCL at 8 % wt in asetic acid/formic acid (1:2 w/w) under magnetic stirring for 3 hours at room temperature. To prepare the PVP solutions, PVP (8 % wt) was dissolved in distilled water/ethanol (1:1 w/w) solvent mixture for 12 hours at room temperature. After polymer dissolution, 3% v/v TEO, 3% v/v BO and 3% v/v TEO+BO were added separately into 8% wt PVP core polymer solution to produce EO doped core/shell fibers. TX-100 was added to all oil-added solutions at a rate of 3% wt as a surfactant for homogeneous dispersion of the oils in the polymer solution. Electrospinning parameters given in Table 3.3 were used in the productions.

When the SEM images and diameter distribution graphs given in Figure 3.19 were examined, it was observed that the additives of oil and surfactant caused an increase

in fiber diameters. In parallel with previous studies, the increase in fiber diameters is directly proportional to the increase in the viscosity of polymer solutions. The fact that BO-doped fibers are thicker than TEO-doped fibers may be due to the different conductivity of the solutions. Because solutions with high conductivity cause finer fibers to be obtained in electrospinning (Angammana & Jayaram, 2011). Viscosity and conductivity values of the solutions used in production are given in Table 3.5.

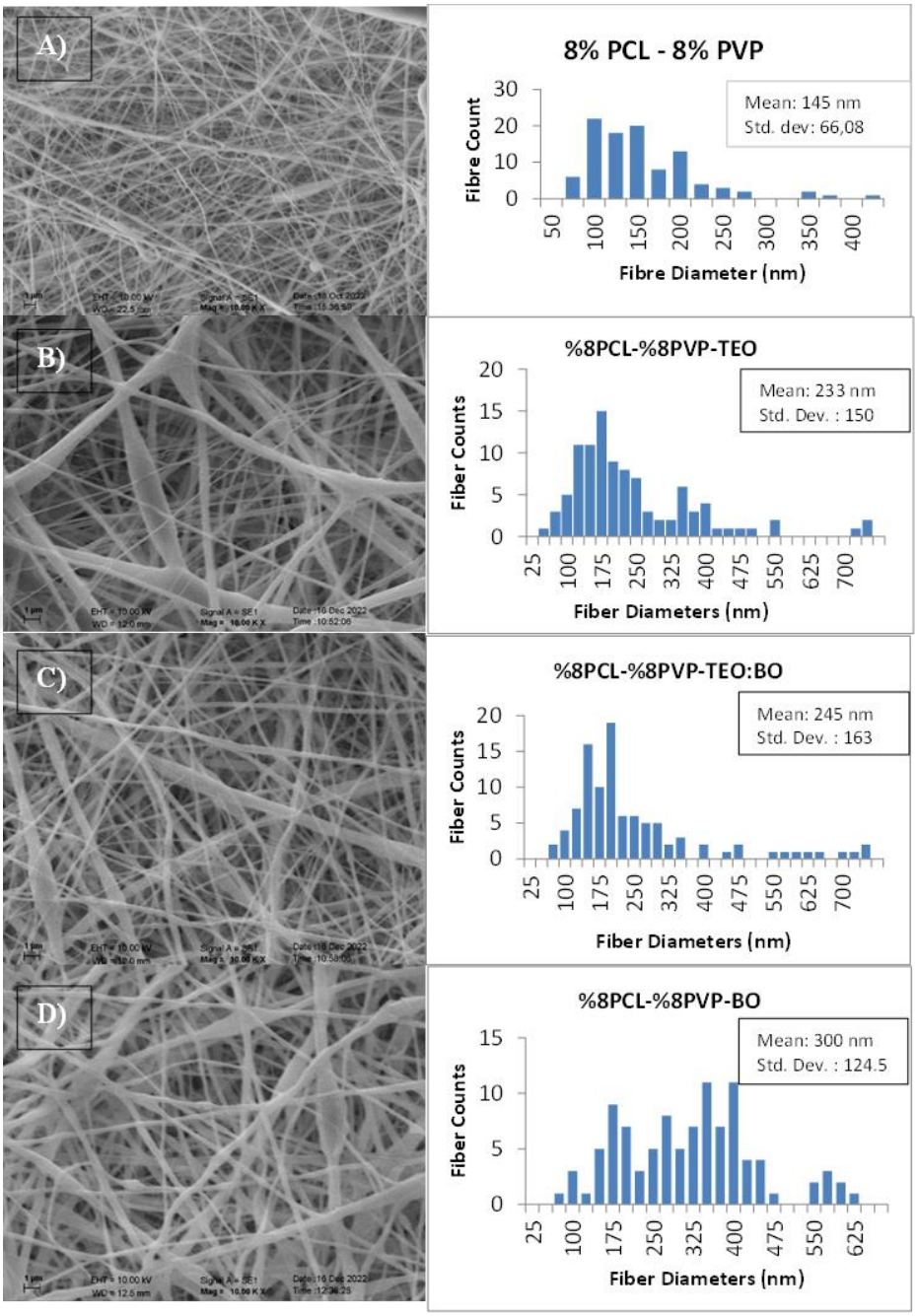

Figure 3. 20: SEM images (at x10.000 magnifications) and fibre diameter distribution graphs of EO doped coaxial nanofibers 8% PCL – 8% PVP A), 8% PCL – 8% PVP-TEO B), 8% PCL – 8% PVP-TEO:BO C), 8% PCL – 8% PVP-BO D) samples, respectively.

Table 3. 5: Viscosity and conductivity values of PCL, PVP and oil-added PVP solutions.

Polymer Solution	Viscosity (cP)	Conductivity (µS)
%8 PCL	301.5	14
%8 PVP	300	16.6
%8 PVP + TEO	328	19.3
%8 PVP + TEO:BO	333.5	18
%8 PVP + BO	349	16.4

The FTIR spectra of pristine and oil added PVP/PCL nanofiber samples, TEO, and BO were shown in Figure 3.20. As shown in Fig. 3.20 the spectrum of TEO showed characteristic peaks at 3525 (O H vibration of hydroxyl group of thymol), 2962 (C–H stretching of methyl and isopropyl groups on the phenolic rings of thymol). The peaks at 1590 cm−1 and 1425 cm−1 were attributed to the C=C skeletal vibration of benzene ring in TEO. Also peaks at 1250 cm−1 and 811 cm−1 were assigned to the -C-O- stretching vibration and CH wagging vibrations respectively (Lin, Zhu & Cui, 2018). The hygroscopic nature of PVP, indicated by a C=O stretch band at 1647 cm-1, appears in the spectrum of the undoped PVP/PCL sample. In addition, O-H peaks in the 3300-3500 cm-1 region, which are not normally found in the PCL structure but found in PVP, show that PVP cannot be fully confined to the core of the fiber. Also characteristic bands that belongs PCL spectrum such as 1726 cm-1 (carbonyl stretching), 1294 cm-1 (C–O and C–C stretching), 1238 cm-1 (asymmetric C–O–C stretching), and 1162 cm-1 (symmetric C–O–C stretching) are showed on the FTIR spectrum graph.

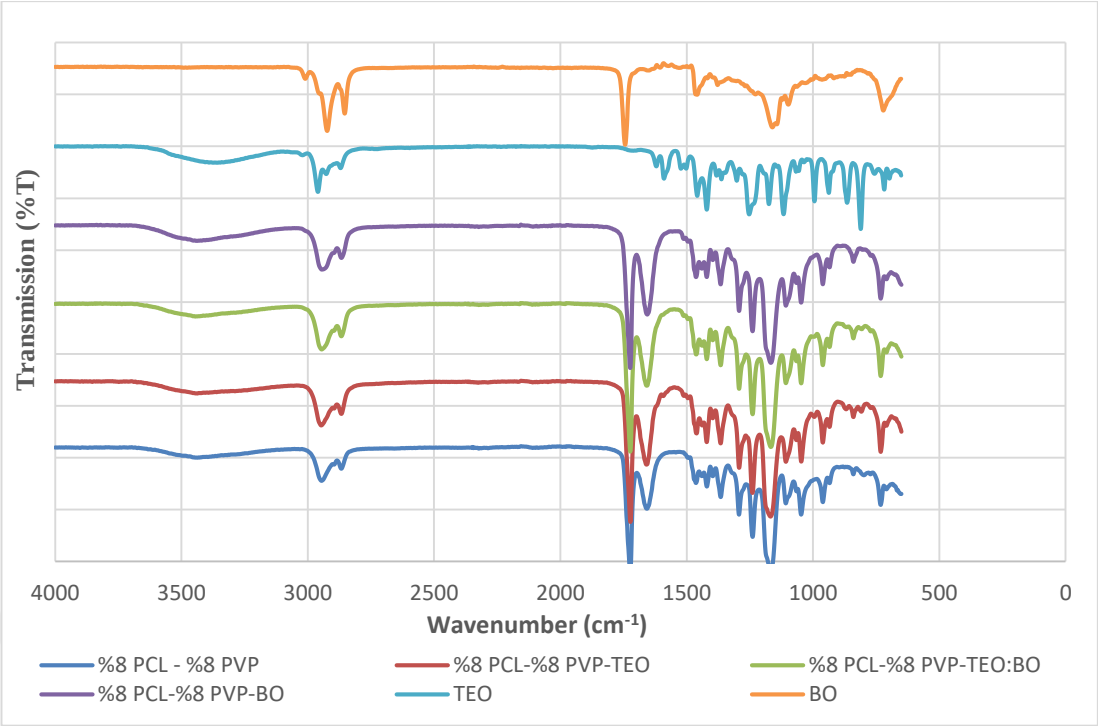

Figure 3. 21: FTIR spectrum of PVP/PCL coaxial nanofibers and EOs.

Although it is seen from the FTIR graph that PVP is not trapped inside by forming the core of the fiber, it was checked whether the oils were successfully added to the structure of the fibers by performing antibacterial tests of the oil loaded nanofiber surfaces (Figure 3. 21). No effect was observed as a result of the effect tests performed by agar diffusion method against S.aureus, E.coli bacteria, C.albicans fungus and A.niger yeast. This may be due to insufficient amount of TEO or because it is volatile because it is not trapped in the fiber core, it moves away from the structure.

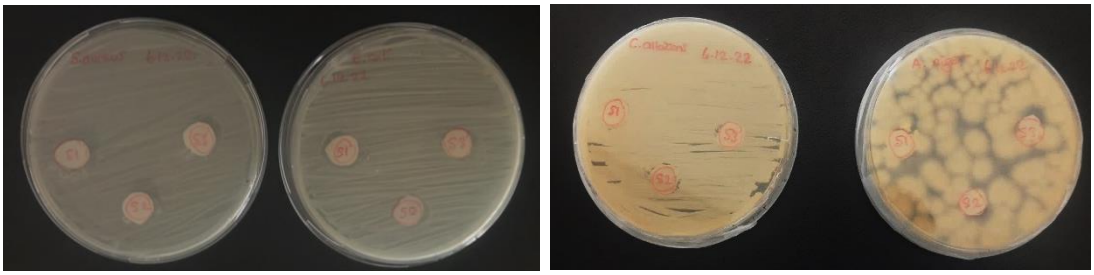

Figure 3. 22: Antibacterial tests results of PVP/PCL coaxial nanofibers against S.aureus a), E.coli b), C.albicans c), and A.niger d), respectively. Sample codes: %8PVP/%8PCL/TEO S1, %8PVP/%8PCL/BO S2, %8PVP/%8PCL/TEO:BO S3.

3.3.5 Production of EO doped PCL/PCL nanofibers with coaxial electrospinning method

PCL, a hydrophobic, durable, biocompatible and biodegradable polymer, which is frequently preferred in biomedical fields recently, was preferred for this study to compare the fiber morphologies, release behaviors and contact angle values of oil-loaded fibers produced by the coaxial method when the core/shell structure is both hydrophobic. In the study, the polymer solutions that will form the core of the fibers were studied at two different polymer ratios, 10% wt and 8% wt. In the method, PCL solutions were prepared separately for the core and the shell. In order to form the shell structure, 10% wt of PCL polymer was dissolved in a 2:1 formic acid/acetic acid (w/w) solvent combination for 3 hours at room temperature with magnetic stirring. PCL solution to be used as core and was dissolved in %10 wt and 8% wt ratio of 1:1 formic acid/acetic acid (v/v) solvents for 3 hours at room temperature. The reason for using the combination of two organic acids as solvents for PCL in the study is that organic acids are generally better tolerated by living tissues than organic solvents commonly used for electrospinning, and a more biocompatible production can be achieved (Hudecki et al., 2017). The reason for increasing the amount of formic acid in the solution that will form the shell structure is to provide nanofiber spinning stabilization due to the high conductivity of formic acid (Hudecki et al., 2017). For the production of oil-added fibers, 3% v/v TEO and 3% v/v TEO:BO (1:1 mix) were added to core solutions, seperately. In order for the oils to be homogeneously dispersed in the solution and form an emulsion, TX-100 was added as surfactant at a rate of 3 wt% to all oil-added solutions and mixed in a vortex mixer at 3000 rpm for 2 minutes to homogenize.

Table 3.6 below gives the parameters used for coaxial electrospinning method. Since all oil-added samples are produced with the same production parameters, they are not given in the table.

Table 3. 6: Electrospinning parameters of PCL/PCL nanofiber production with coaxial method.

	Polymers	Feed Rate	Voltage (kV)	Distance (cm)	Collector Drum Speed (rpm)
Shell	10% PCL	0.9 mL/h	24	20	200
Core	8% PCL, 10% PCL	0.3 mL/h			

When the SEM images of the fibers produced by coaxial method were examined, the addition of oil and surfactant caused an increase in fiber diameters in both polymer ratios. Since additives increase solution viscosities and change conductivity, they have important effects on fiber diameters. As shown in the Figure 3.22, the diameters of 10%PCL/8%PCL, 10% PCL/8%PCL/TEO, and 10% PCL/8%PCL/TEO:BO fibers are 138 ± 41, 218 ± 93, 217 ± 103 nm, respectively. On the other hand, average fiber diameters of 10% PCL/10% PCL, 10% PCL/10% PCL /TEO and 10% PCL/10% PCL /TEO:BO fibers are 231 ± 96.56, 239 ± 109.4, 248 ± 70.2, respectively (See Fig. 3. 23). When average fiber diameters are compared in the two production, 10%PCL/10%PCL fibers are thicker than 10%/8% PCL fibers, which may be due to the higher polymer concentration of core solution. In addition, the viscosity of the polymer solutions increased with the addition of surfactant and oil. Also, the amount of conductivity varies according to the added oils. When TEO is added, the conductivity of the solutions is higher compared to the TEO:BO mixture. For this reason, it should be considered that all natural oils can create unique effects in solutions. All viscosity and conductivity values are given in Table 3.7.

Table 3. 7: Viscosity and conductivity values of PCL polymer solutions.

Polymer Solution	Viscosity (cP)	Conductivity (μS/cm**)**
10% PCL (Shell)	660	13.7
10% PCL (Core)	630	12.6
10% PCL/TEO	584.2	14.2
10% PCL/TEO:BO	739	13.2
8% PCL	298	12
8% PCL/TEO	265	13.4
8% PCL/TEO:BO	318	12.5

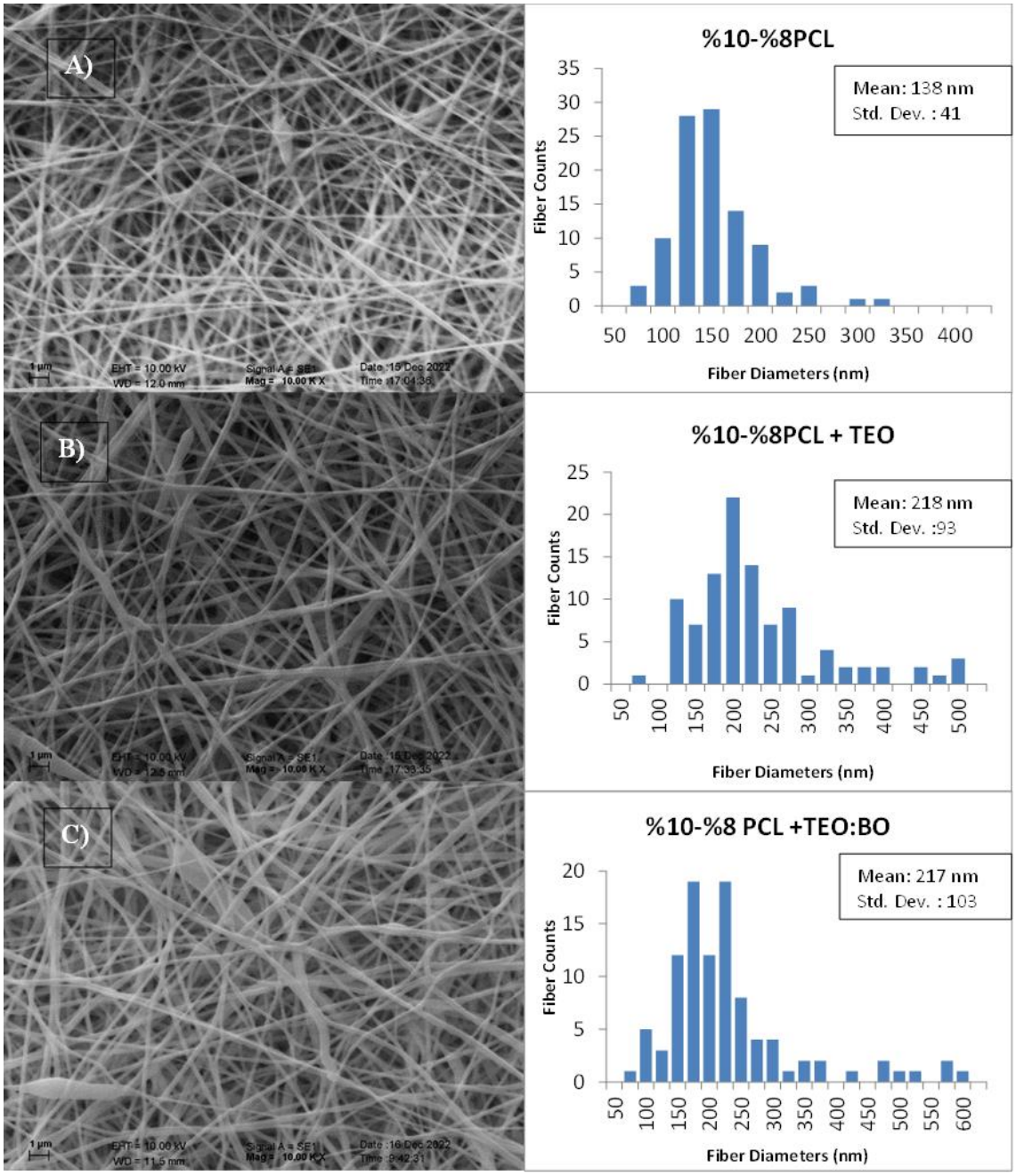

Figure 3. 23: SEM images (at x10.000 magnifications) and fibre diameter distribution graphs of EO doped coaxial nanofibers 10% PCL – 8% PCL A), 10% PCL – 8% PCL-TEO B), 10% PCL – 8% PCL-TEO:BO C) samples, respectively.

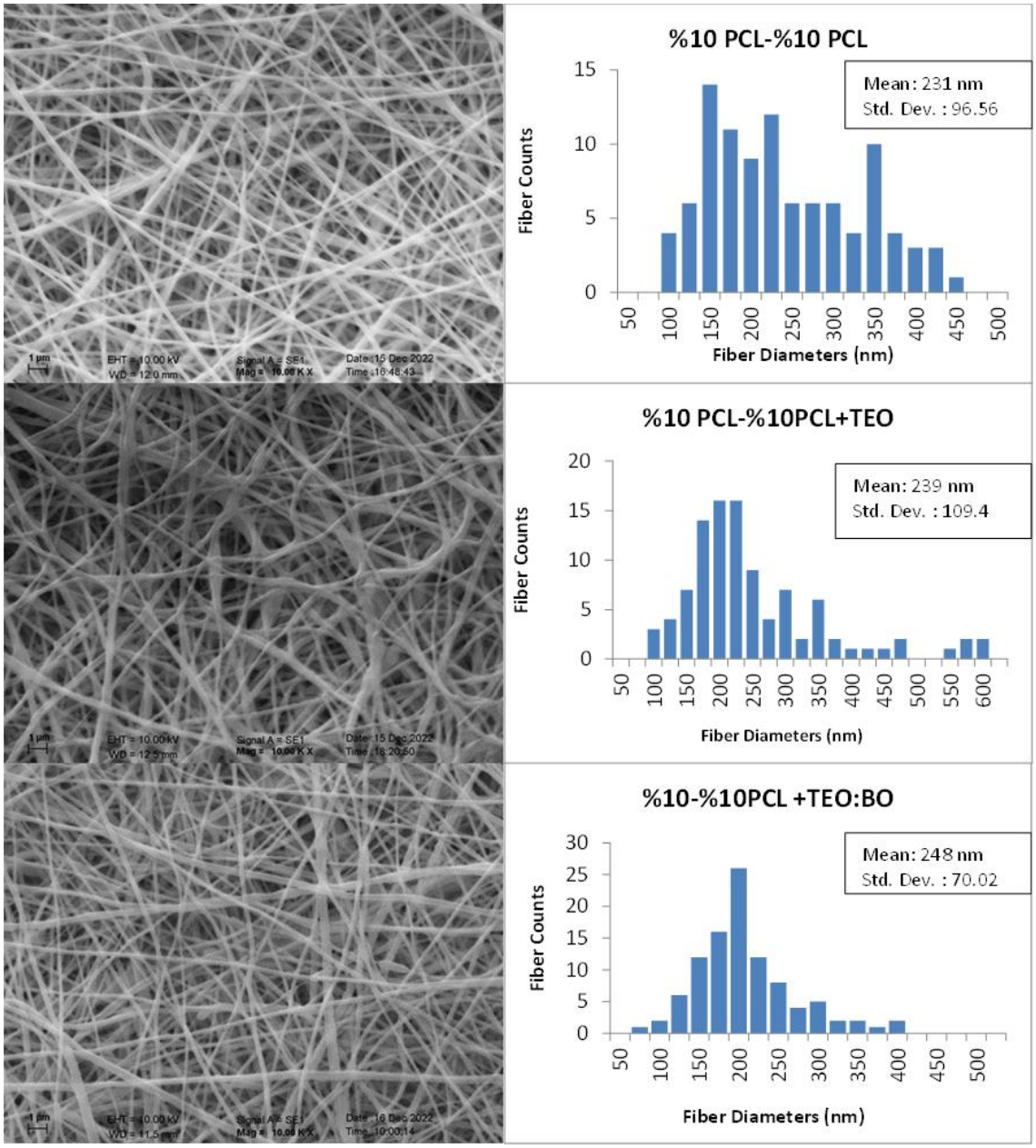

Figure 3. 24: SEM images (at x10.000 magnifications) and fibre diameter distribution graphs of EO doped emulsion nanofibers 10% PCL- 10 %PCL A), 10% PCL- 10 %PCL -TEO B), 810% PCL- 10 %PC -TEO:BO C) samples, respectively.

In order to examine the effect of oil and surfactant additives on nanofibers produced from hydrophobic polymers against the hydrophilic/hydrophobic properties of nanofiber surfaces, WAC test was performed on the samples (See Fig. 3.24). While the contact angles of the PCL samples without additives were 109°, the samples with oil and surfactant added a hydrophilic structure and the contact angles were measured

as 0°. According to the literature, TX-100, a nonionic surfactant, has a hydrophilic poly(ethylene oxide) chain and an aromatic hydrocarbon lipophilic or hydrophobic group. Therefore, surfaces produced from hydrophobic polymers become hydrophilic (Chen et al., 2021). In addition, the OH groups in the structure of the oils or the degradation of the polymers by the oils may also be the cause of the hydrophilization of the structure (Unalan et al., 2019).

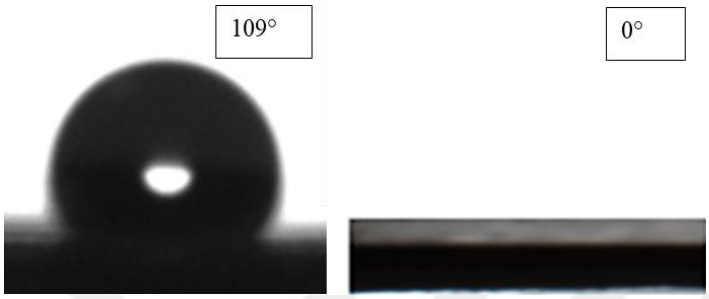

Figure 3. 25: WCA test results of pristine PCL fibers a), and oil/TX-100 added PCL nanofibers b), respectively.

Finally, the release behavior of coaxial samples was tested by taking samples at regular intervals for 48 hours. Samples that showed rapid release in the first hour had a constant amount of release up to the 24th hour on average. The release rate in the 10% PCL / 8% PCL sample showed a rapid increase from the 24th hour compared to the other sample. The reason for this may be that since the polymer content is lower in the core, the polymer degrades faster than the other sample over time and releases the oil in it.

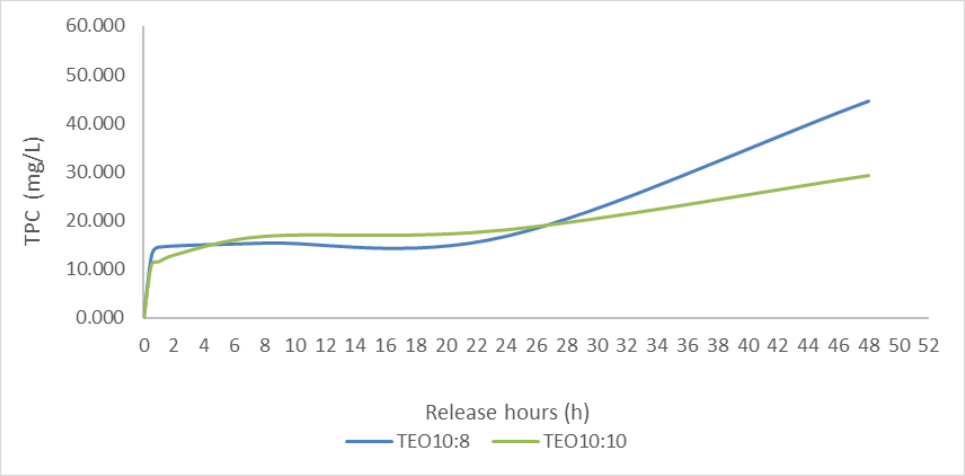

Figure 3.25 : Release behaviours of TEO loaded 10% PCL/ 10% PCL and 10% PCL/ 8% PCL coaxial nanofibers.

4. CONCLUSION & RECOMMENDATION

In this study, essential oil loaded (hypericum perforatum oil, thyme oil and borage oil) nanofiber structures were obtained by emulsion and coaxial electrospinning methods using PVA, PCL, and PVP polymers. The effects of the variables on nanofiber structures were evaluated by changing parameters such as polymer type, polymer concentration, essential oil type, essential oil ratio, surfactant type and surfactant ratio. In addition, two different electrospinning methods were used and essential oil loaded, beadless nanofibers within a range of approximately 150-500 nm diameters were obtained with both methods.

In the studies with emulsion electrospinning, hypericum perforatum oil was added to PVA and the effect on nanofiber production was evaluated by changing the ratio of surfactants used to form emulsions. In the results, increment in nanofiber diameters was observed when the essential oil ratio was kept constant (2% v/v) and the surfactant ratio increased from 2% to 6%. Therefore, it was concluded that the amount of surfactant used in the emulsion electrospinning method has an effect on fiber formation and diameter distribution.

Essential oil loaded PCL and PVP nanofibers were also obtained by using emulsion electrospinning method and 1:1 mixtures of hypericum perforatum oil and borage oil. The studies were carried out with two different polymers, hydrophilic PVP and hydrophobic PCL, and the effect of polymer type was evaluated. By the loading of 1%, 2% and 3% essential oils to 10% PCL and 10% PVP solutions, beadless nanofiber structures were obtained. On the other hand, under the same production conditions, thinner nanofibers were obtained with PCL compared to PVP, and the average fiber diameters in each group increased with the addition of essential oil. As a result, it was observed that the polymer type and the essential oil ratio have a significant effect on the nanofiber structure.

Nanofiber structures have been obtained by the coaxial electrospinning method, which is widely used in drug release studies and provides core-shell structured nanofibers. In the first part of nanofiber forming by coaxial electrospinning method, the effect of polymer type and concentration on the formation of shell-core nanofibers was evaluated. Hydrophobic PCL and hydrophilic PVP polymers were obtained by using

two different feeding units, using PCL in the shell part and PVP in the core part. Both the PCL concentration in the shell was changed as 6%, 8% and 10%, and six different nanofibers were obtained by changing the PVP concentration at the same rates. In the results of the study, it was observed that the contact angles decreased from 104.3° to 57.61° depending on the polymer concentration.

In the continuation of the coaxial electrospinning studies, it was aimed to obtain mechanically strong, essential oil-loaded nanofibers by using PCL polymer in both the shell and the core. 10% wt PCL was used in the shell part, while two different concentrations were tried with 10% wt and 8% wt PCL in the core part. Thyme oil at a ratio of 3% v/v and a mixture of thyme and borage at a ratio of 1:1 at 3% v/v were added to the core part, and samples were obtained by emulsion with TX1-00 at a ratio of 3% wt. While the average diameter of the unloaded nanofibers was around 150 nm, it was observed that the nanofiber diameters increased to around 200 nm with the loading of thyme oil and thyme oil + borage oil. In addition, while the contact angle of unloaded PCL/PCL samples is 109°, the essential oil loaded samples gained a highly hydrophilic character. It is thought that, this is caused by the surface propagation of the TX 100 surfactant used in the core and hydrophilizing the structure. Finally, the release behavior of TEO-loaded PCL coaxial fibers with different core polymer ratios in PBS was tested according to the TPC method. After the samples had rapid release in the first hour, they made constant release until the 20th hour. Then the release rate of the 10% PCL/8% PCL sample increased compared to the 10% PCL/10% PCL sample. The reason for this was interpreted as the fact that the ratio of the core polymer is low, it undergoes rapid polymer degradation and releases the oil in it to the environment faster.

In conclusion, by using emulsion and coaxial electrospinning methods, it is possible to achieve a kind of encapsulation of different essential oils within nanofiber structures, as it is aimed in the thesis. On the other hand, there are factors that need to be considered in the loading of hydrophobic essential oils in liquid form into nanofibers. The results of this study showed that, the parameters such as polymer type, concentration, surfactant ratio, oil type and oil ratio change have effect on the contact angles and release characteristics of nanofibers, especially diameters and fiber formation. Hypericum perforatum oil which is effective in wound healing and cell regeneration, thyme oil which is known for its very strong phenolic substance content and high antimicrobial character, and borage oil containing a high level of GLA are

useful essential oils in AD treatment. The study showed that it is possible to obtain nanofiber structures by using these essential oils both individually and in mixtures. It should be also noted that, the differences in conductivity and viscosity values as well as the contents of the essential oils created differences in properties and the formation of nanofibers.

In future studies, to increase the oil ratios from 3% to higher levels in order to improve the antimicrobial effects of oil-added nanofiber structures and to adjust the production parameters for this purpose can be investigated. In addition, in order to reduce moisture loss on the skin surface, which is an important health problem for AD patients , to evaluate the effect of oil-added nanofibers studies can be conducted with volunteers and also transepidermal water loss measurements can be carried out. In this way, the effects of nanofibers doped with oils with different properties on cells and tissues can be evaluated in detail.

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PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

- **Pala, N., Aral, N., Nergis, B.** 2022 : Emulsion Electrospinning of PVA Nanofibers Containing Hypericum Perforatum Oil. International Congress – Innovative Textiles (ICONTEX2022), May 18-19, 2022 Online.
- **Pala, N., Aral, N., Nergis, B.** 2022 : Borage Oil and Hypericum Perforatum Oil Containing Nanofibers for Atopic Dermatitis Treatment. International Congress – The International Graduate Research Symposium (IGRS'22), June 1-3, 2022 Online.
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- Özen, M. S., **Pala, N.,** Ertek, D. A., Akyıldız, S. H., & Usta, İ., (2021). Agro Textiles - I. Uluslararası Nonwoven Teknik Tekstil Teknoloji Dergisi , vol.2, no.71, 58-62.