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THE EFFECT OF ESSENTIAL OIL ON FIBER MORPHOLOGY AND SURFACE PROPERTIES IN COAXIAL NANOFIBERS

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Abstract: In this study, core-shell nanofibers were produced by using hydrophilic polyvinylpyrrolidone (PVP) polymer in the core and hydrophobic poly(e-caprolactone) (PCL) polymer in the shell. Essential oil added nanofiber structures were developed by adding thyme oil (TEO) and borage oil (BO) in the PVP core part by using Triton X 100 (TX-100) as the surfactant. 8% PVP-8% PCL nanofibers were produced by adding TEO, BO and a 1:1 volume/volume mixture of these two (TEO:BO) to the PVP solution. Addition of essential oil and surfactant to the solutions resulted in different conductivity and viscosity values. SEM images were analyzed and it was observed that nanofiber diameters increased when essential oil and surfactant were added to the core of the coaxial nanofibers. Pristine, TEO-added, TEO:BO added and BO-added nanofibers were calculated as 145 ± 66, 233 ± 150, 245 ± 165 and 300 ± 124 nm, respectively. Besides, water contact angle measurements showed that TX-100 and essential oil additives caused high hydrophilization of nanofiber by changing the hydrophobic nature of PCL. While the contact angle of the 8% PVP-8% PCL sample without additives were 98°, the contact angle of the oil and surfactant containing samples were measured as 0°. In conclusion, it was observed that the nanofiber morphology and surface properties changed when different essential oils and surfactant were added to the core-shell nanofibers.

Keywords: Nanofiber, Essential Oil, Coaxial Electrospinning, Polycaprolactone, Polyvinylpyrrolidone

Koaksiyel Nanoliflerde Esansiyel Yağların Lif Morfolojisi ve Yüzey Özellikleri Üzerindeki Etkisi

Öz: Bu çalışmada, çekirdekte hidrofilik polivinilpirolidon (PVP) polimeri ve kabukta hidrofobik poli(e-kaprolakton) (PCL) polimeri kullanılarak çekirdek-kabuk nanolifler üretilmiştir. Yüzey aktif madde olarak Triton (TX-100) kullanmak suretiyle PVP çekirdek kısmına kekik yağı (TEO) ve hodan yağı (BO) eklenerek esansiyel yağ katkılı nanolifli yapılar geliştirilmiştir. PVP çözeltisine TEO, BO ve bu ikisinin (TEO:BO) 1:1 hacim/hacim karışımı eklenerek %8 PVP-%8 PCL nanolifler üretilmiştir. Çözeltilere esansiyel yağ ve yüzey aktif maddenin eklenmesi, farklı iletkenlik ve viskozite değerleri ile sonuçlanmıştır. SEM görüntüleri analiz edilmiş ve koaksiyel nanoliflerin çekirdeğine esansiyel yağ ve yüzey aktif madde eklendiğinde nanolif çaplarının arttığı gözlenmiştir. Katkısız ve TEO, TEO:BO ile BO katkılı nanoliflerin çapları sırasıyla 145 ± 66, 233 ± 150, 245 ± 165 and 300 ± 124 nm olacak şekilde ölçülmüştür. Ayrıca su temas açısı ölçümleri, TX-100 ve esansiyel yağ katkı maddelerinin PCL'nin hidrofobik yapısını değiştirerek nanoliflerin yüksek oranda hidrofilizasyonuna neden olduğunu göstermiştir. Katkısız %8 PVP-%8 PCL nanoliflerin temas açıları 98° iken, yüzey aktif madde ve yağ içerikli numunelerin temas açılarının 0° olduğu tespit edilmiştir. Sonuç olarak, çekirdek-kabuk yapısındaki nanoliflere farklı yağlar ve yüzey aktif maddeler eklendiğinde nanolif morfolojisinin ve yüzey özelliklerinin değiştiği gözlemlenmiştir.

Anahtar Kelimeler: Nanolif, Esansiyel Yağ, Koaksiyel Elektroeğirme, Polikaprolakton, Polivinilpirolidon

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1. INTRODUCTION

Electrospinning method is a frequently used method for the production of wound dressings by loading functional substances into nanofibers. The fabrication of monolayer nanofiber membranes using the drug/oil and polymer blending approach is simple and reproducible, but because the additive is dispersed on the nanofiber with a large specific surface area, it quickly leads to the explosive release of additive from the nanofiber surface and sustained release cannot be achieved for a long period (Chen et al., 2022). Emulsion electrospinning and coaxial electrospinning methods are preferred to provide long-term release.

An emulsion is formed by mixing two immiscible liquids, typically an aqueous solution and an organic solvent or oil phase. The emulsion contains tiny droplets of one liquid dispersed within the other (Zhang, Feng & Zhang, 2018). Surfactants, also known as emulsifying agents, play a crucial role in emulsion and coaxial electrospinning. Surfactants are molecules with hydrophilic (water-attracting) and hydrophobic (water-repelling) portions. When added to the emulsion, surfactants help stabilize the droplets of one liquid within the other, preventing them from coalescing or separating. In electrospinning, surfactants serve several important purposes such as emulsion stabilization, core-shell structure formation, controlled release and enhanced fiber properties (Stoleru and Brebu, 2021).

Essential oils are oils extracted from plants that contain a variety of complex chemical compounds. Since prehistoric times, essential oils have been widely used for a variety of medicinal applications, including antibacterial, antiviral, insecticidal, and analgesic and anti-inflammatory purposes. Essential oils are highly volatile and have poor water solubility. Therefore, encapsulation of essential oils in nanofibers is a cost-effective way to protect them against evaporation and oxidation, as well as to control their release.

In the coaxial electrospinning method, two 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 (See Fig. 1). In their study Zhang et al. inserted thymol, an antibacterial agent, into core–shell nanofibers to control the release of the compound. All of the unencapsulated thymol in the study evaporated in 19 hours. On the other hand, 36% of thymol encapsulated in core-shell nanofibers with PLGA polymer evaporated within 72 hours. (Zhang et al., 2019). It has been suggested that encapsulating even a small amount of essential oils into electrospun nanofibers could result in increased antimicrobial activity since bacterial cells could penetrate the fibrous matrix and come into direct contact with antimicrobial compounds due to the nanofibers' high exposed surface area (Liakos et al., 2015).

One of the hot research topics in recent years has been the encapsulation of extracts, which can be used as antimicrobial agents, in carriers such as nanofibers and nanoparticles, and their transportation to the area to be treated in order to prevent the increase of pathogens that cause the disease or prevent the increase of their severity (Miguel et al., 2019, Ambekar and Kandasubramanian, 2019; Sabra et al., 2020). Thyme (Thymus vulgaris) plant, being one of the natural extracts examined for its use in pharmaceutical and cosmetic fields, 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 is the main active compound of Thyme essential oil (TEO).

According to the study by Burt et al. (2005), it has been reported that the main components of TEO are carvacrol, thymol, p-cymene and γ-terpinene. 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. In the study by Çallıoğlu, Güler and Çetin (2019), antibacterial activities of nanofibers were tested by adding thyme oil at different concentrations to Poly polyvinylpyrrolidone (PVP) / Gelatine (GEL) polymers. It has been observed that the structures exhibit an antibacterial effect for more than 192 hours. Also, Ansarifar and Moradinezhad (2021) showed that thyme oil-added electrospun zein nanofibers have a significant effect on prolonging the shelf life and maintaining quality of strawberries, as they delay biochemical changes and show antimicrobial ability. Koushki et al. (2018), produced PCL/PVP structured coaxial nanofibers with thyme extract loaded into the core structure. They examined the effects of polymer concentration, thyme concentration and electrospinning parameters on nanofibrous surfaces. They concluded that thyme extract additive reduced the nanofibrous mats strength and increased the pore size.

Although nanofiber studies examining the antimicrobial effects of thyme oil are frequently included in the literature (Kang et al., 2022; Gelmetti, 2009), simply reducing inflammation may not be sufficient in the treatment of dermatitis types such as atopic, seborrheic, contact, and skin diseases such as psoriasis and lichen planus since these types of skin diseases cause transepidermal water loss (TEWL).

Borage oil (BO), on the other hand, is a vegetable oil that contains the highest amount of Gamma linolenic acid (GLA). GLA, one of the essential fatty acids, is a structural component of cell membrane phospholipids and is very important in maintaining the fluidity of the cell membrane. Since GLA cannot be synthesized by the body in diseases such as atopic dermatitis (AD), it must be taken externally. In the literature, water vapor transmission rates (WVTR), fluid uptake ability (FUA) and skin hydration were tested by dripping borage oil on PS and PA6 electrospun nanofiber surfaces to help moisturize atopic skin (Krysiak et al., 2021). In another study, the spreading behavior of borage oil was tested by dripping on the PVB electrospun wound dressing (Krysiak et al., 2020).

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 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). In the literature, there are different studies in which PCL and PVP polymers are used in core-shell nanofiber production. Zhu et al., produced core-shell nanofibers for synergistic therapy targeting melanoma skin cancer. The nanofibers consisted of a chitosan (CS)-loaded PCL shell and a 5-fluorouracil (5-FU)-loaded PVP core. These nanofibers demonstrated high drug encapsulation efficiency along with strong mechanical properties (Zhu et al., 2019). Nanofiber-based drug delivery systems offer a solution to minimize the side effects of drugs in the treatment of diseases. In the study in which PCL/PVP core-shell nanofibers containing multi-walled carbon nanotubes (MWCNT) as carriers of 5FU, a common cancer drug, were produced, the addition of MWCNTs to the shell phase improved the tensile properties, while higher PVP content increased the degradability of nanofibrous surfaces. The release behavior exhibited sustained and prolonged drug release (Nasari et al., 2020).

The aim of this study has been to obtain coaxial nanofibers containing two different types of essential oil used therapeutically in various skin diseases and to examine the properties of nanofibers depending on the types of essential oil and the use of surfactant. For this purpose, Thyme and Borage oils with proven efficiency against various skin problems as mentioned in the literature are employed. Nanofibers were developed by coaxial electrospinning method using hydrophobic PCL in the shell and hydrophilic PVP loaded with TEO, BO and TEO:BO (1:1) in the core. The selection of PCL polymer as the outer shell serves the dual purpose of enhancing the tensile strength of nanofibrous mats and prolonging the release duration by mitigating the burst release of essential oils contained within the hydrophilic core. The choice of PVP polymer for the core is motivated by the objective of achieving a harmonious balance in the hydrophobic nature of the PCL polymer, while ensuring compatibility with the skin. The effects of two different essential oils, their mixtures and surfactant added to the PVP solutions were examined. The study mainly focuses on investigating how the nanofiber structure changes depending on the type and properties of oil additives and aims to to create a guiding starting point for further studies.

2. EXPERIMENTAL

2.1. Materials

In this study, Polyvinylpyrrolidone (PVP) (Mw: 360.000 g/mol) and Polycaprolactone (PCL) (Mw: 80.000 g/mol) were obtained from Sigma-Aldrich. 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 from Bioterra and BO was obtained from Botalife. Triton X-100 (TX-100) (Sigma-Aldrich) was used as the surfactant.

2.2. Coaxial Electrospinning of Nanofibers

In the study, PCL polymer solutions were prepared by dissolving 8% wt PCL in acetic 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. In this study, PCL solutions were used as shell, PVP solutions were used as core solution, nanofiber surfaces were fabricated by coaxial electrospinning method (Figure 1) with different oil additives given in Table 1.

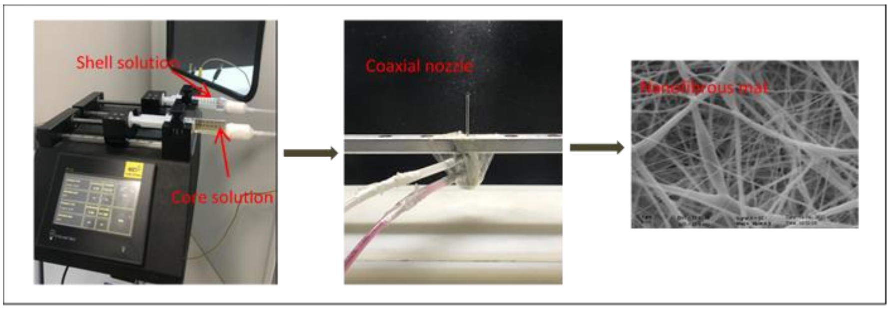

Figure 1: *Coaxial electrospinnig method*

Table 1. Polymer ratios of nanofibrous mats fabricated by coaxial electrospinning

	PCL Solution Concentration (%) (Shell)	PVP Solution Concentration (%) (Core)	Additive (Core)
Pristine	8	8	-
TEO added	8	8	TEO+TX-100
TEO:BO added	8	8	TEO:BO + TX-100
BO added	8	8	BO + TX-100

To produce essential oil added core/shell fibers, 3% v/v TEO, 3% v/v BO and 3% v/v TEO:BO were added separately to the 8% PVP solution to form the core. In order to ensure homogeneous distribution of the oils in the polymer solution, TX-100 as a surfactant was added at a rate of 3% by weight to all the oil-added solutions.

To produce nanofibers, NanoSpinner NE300 model electrospinning device was used. Inovenso IPS-14 double syringe pump was employed to feed the polymer solutions that will form the shell and core structures at different feed rates. The coaxial nozzle has an outer syringe diameter of 2 mm and an inner syringe diameter of 1.15 mm. Optimum electrospinning parameters were adjusted as in Table 2 by making changes in the parameters during production and the same production parameters were used for all samples.

Table 2. Electrospinning production parameters

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

2.3. Characterization of Electrospun Nanofibers

The viscosities of polymer solutions were gauged using the Brookfield DV-E Viscometer to examine how polymer ratio and viscosities influenced fiber morphology and surface properties. For conductivity assessment, solutions were measured using an Endress+Hauser conductivity device at room temperature, with conductivity presented in µS/cm for all solutions. The ZEISS EVO 40 Scanning Electron Microscope (SEM) was utilized to analyze the nanofiber surfaces. For SEM imaging, gold/palladium coating was applied to nanofiber surfaces using the Leica EM ACE200 coating device. Images at 10000x magnification were captured to determine fiber diameters. Using ImageJ software, 100 different fibers from SEM images were selected for diameter measurement. Also, surface porosity and average pore sizes were calculated with ImageJ software by using SEM images. 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 transmittances of all samples were analyzed in the frequency range of 500 - 4000 cm^{-1}. The KSV - CAM 101 contact angle measurement system was used to determine the hydrophilicity traits of nanofiber surfaces. A droplet of deionized water (0.25 µl) was placed on the nanofiber surfaces to measure contact angles. All measurements were conducted in triplicate for reliability.

3. RESULTS AND DISCUSSION

The viscosity and conductivity values of the essential oil and TX-100 surfactant added solutions are presented in Table 3. As can be seen from the table, presence of the oils and surfactant add different conductivity and viscosity properties to the polymer emulsion and the change in those properties seemed to jointly influence the diameters of the nanofibers produced.

Table 3. Viscosity and conductivity values of PCL, PVP and oil and surfactant added PVP emulsion

Polymer Solution/Emulsion	Viscosity (cP)	Conductivity (µS)
8% PCL	301.5	14.0
8% PVP	300.0	16.2
8% PVP + TEO + TX-100	328.0	19.3
8% PVP + TEO:BO + TX-100	333.5	18.0
8% PVP + BO + TX-100	349.0	16.6

According to the SEM images and diameter distribution graphs presented in Fig. 2, it was observed that addition of oil and surfactant into the nanofiber structure caused an increase in fiber diameters. When the fiber diameter distributions examined, the mean diameters and standard deviations of pristine, TEO-added, TEO:BO added and BO-added nanofibers were calculated and given at Table 4. It was also observed that thickening in the diameters of the nanofibers with additives depended on the type of oil (TEO, BO or their mixture TEO:BO). The average diameter of the TEO-containing sample was thinner than that of the BO-containing one where the average diameter of the TEO:BO containing sample was in between.

Table 4 : Fiber diameters of nanofibrous mats

Sample Codes	Pristine	TEO added	TEO: BO added	BO added
Fiber Diameters (nm)	145 ± 66	233 ± 150	245 ± 165	300 ± 124

In the literature, solutions with high conductivity cause finer fibers to be obtained in electrospinning (Angammana and Jayaram, 2011). In the study conducted by Maroufi et al. (2021), TEO was added to PLA nanofibers by blend electrospinning method. The addition of TEO to the solution increased the conductivity and caused thinning of the fiber diameters. In this study, the PVP solution, which forms the core of the coaxial fibers, is in the form of emulsion thanks to the TX-100 surfactant. According to the literature, nanofiber diameters should be thinned due to the fact that the addition of oil increases conductivity. However, TX-100 added to the PVP solution increases the viscosity of the solution. In the study by Aykut et al., due to the hydrophobic interaction between the hydrophobic tail of TX-100 and the carbon chains of PAN and the presence of hydrogen bonds between the CN groups of PAN and TX-100, when the nonionic surfactant TX-100 is included in the polymer solution the increase in viscosity and decrease in conductivity leads to an increase in mean fiber diameter (Aykut et al., 2013). While the addition of surfactant increased the viscosity, fiber diameters differed because TEO and BO added different conductivity values to solutions. The reason why different oils add different conductivity to the polymer solution is the chemical bonds in their structures. As the number of ions in the solution increases, the conductivity of the solution increases (Aras et al., 2019).

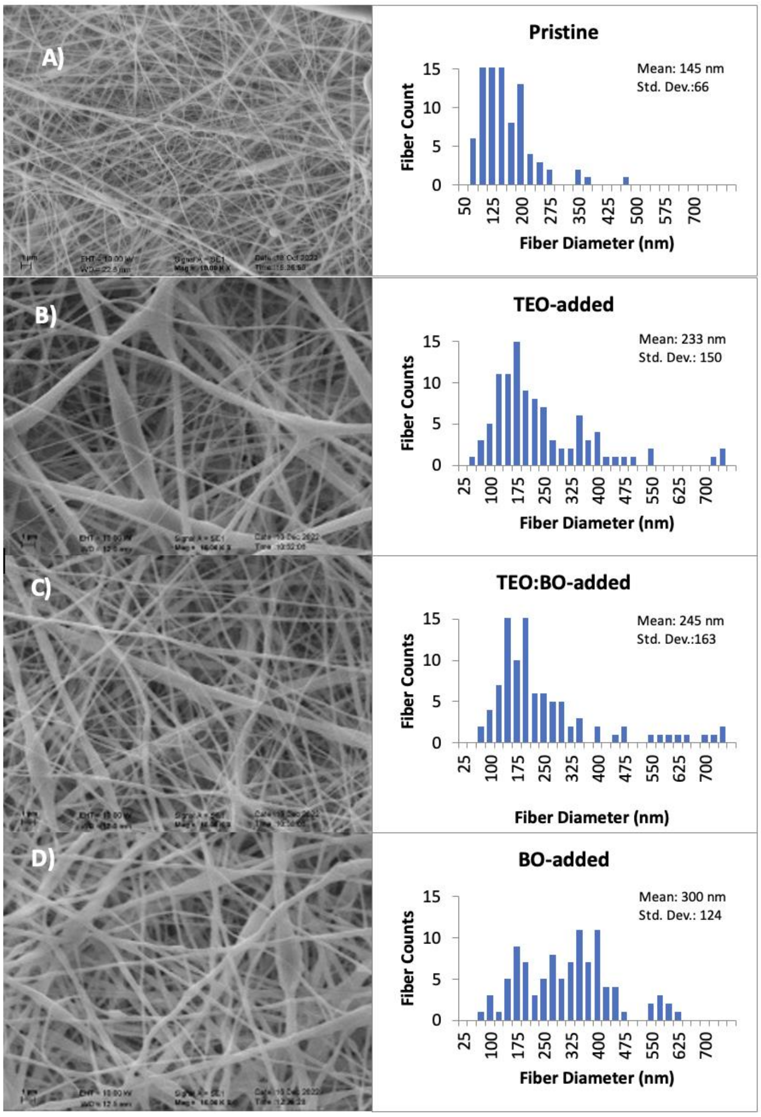

Figure 2: *SEM images (at x10.000 magnifications) and fibre diameter distribution graphs of pristine and EO added coaxial nanofibers: PCL8/PVP8 A), PCL8/PVP8-TEO B), PCL8/PVP8-TEO:BO C), PCL8/PVP8-BO D) samples, respectively.*

Table 5 : Average size of pores and surface porosity of nanofibrous mats

	Pristine	TEO-added	TEO: BO-added	BO-added
Average Pore Size (µm)	0.246	0.339	1.242	2.517
Surface Porosity (%)	27.48	28.38	32.65	34.48
Threshold values	120	80	110	125

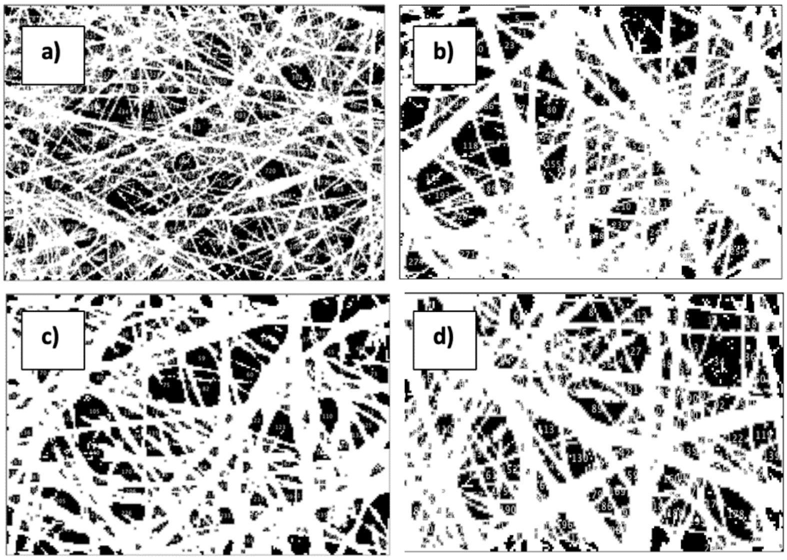

Figure 3: *Calculation of surface porosity and numbering of pores based on SEM images; pristine a), TEO-added b), BO-added c) and, TEO:BO-added d), respectively*

Black pixels (considered empty space) in segmented images as shown in Figure 3 were analyzed in ImageJ using the Analyze Particles command, which counts the number of pixels in individual black pixel clusters. Threshold values were set manually for each sample, as the contrast values in the images of different samples differ. Since pore depth analysis could not be performed with the program, only surface porosity analysis and the average values of these pore sizes were calculated using SEM images. Calculated values are given in Table 5. When the surface porosity values are examined, it is seen that the surface porosity values increase in parallel with the thickening in the nanofiber diameters with the addition of oil and surfactant into the coaxial nanofiber. In the literature, studies in which the surface porosities of nanofibrous mats were calculated using SEM images have shown that nanofiber diameters are directly proportional to the average surface porosity. (Morais et al., 2023). In addition, the pore sizes for all three samples are larger than those of pristine nanofiber.

The FTIR spectra of pristine and oil added PVP/PCL nanofiber samples, TEO, BO and TX-100 were shown in Figure 4. As shown in the figure, 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 pristine 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 both polymers are present in the fiber structure produced. Also characteristic bands that belongs to 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. The characteristic bands of both polymers can be seen together in coaxial nanofibers consisting of PCL and PVP.

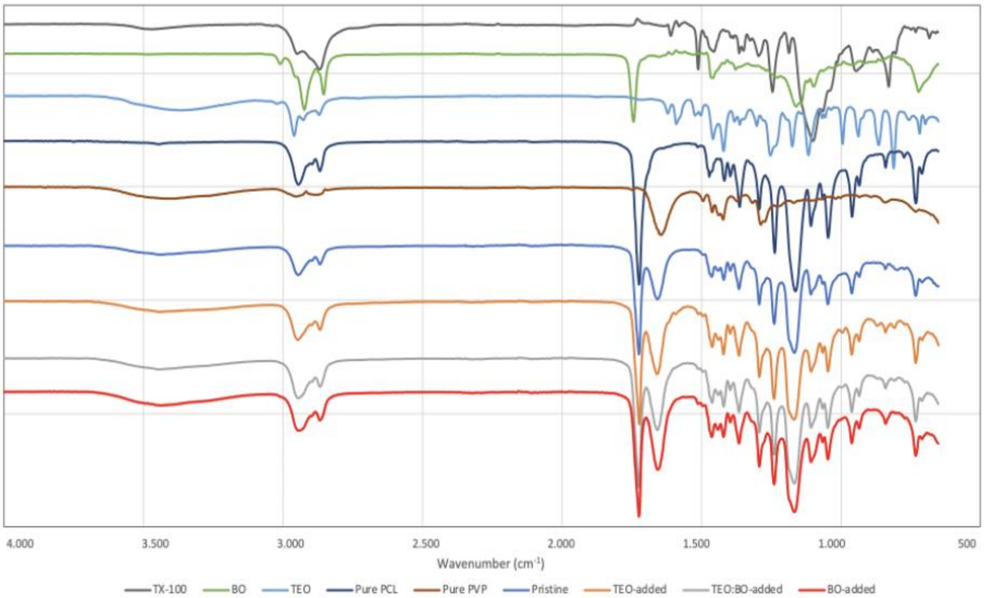

Figure 4: *FTIR graph of oils, surfactant, pristine and oil-added nanofibers*

Finally, water contact angle (WCA) tests were performed on the samples to examine the effect of oil and surfactant additives on the hydrophilicity properties of nanofiber surfaces (See Fig. 5). While the contact angle of the 8% PVP-8% PCL sample without additives were 98°, the contact angle of the oil and surfactant containing samples 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, OH groups in the structure of oils or the breakdown of polymers by oils can cause hydrophilization of the structure (Unalan et al., 2019).

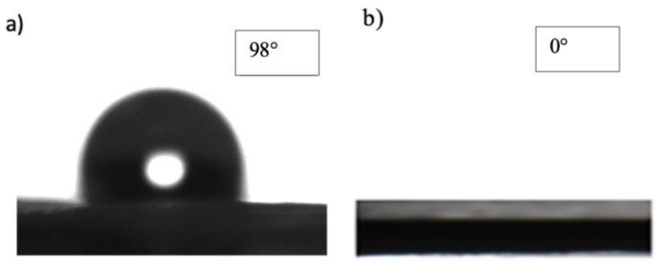

Figure 5: a. *WCA test results of pristine nanofibers,* ***b.*** *all oil and TX-100 added nanofibers, respectively.*

4. CONCLUSION

The use of essential oils externally is a common approach in skin disease treatment, and wound dressings can be developed by adding oils to nanofibers. On the other hand, the viscosity and conductivity values obtained depending on the chemical structures of the essential oils used are effective on nanofiber formation. In the study, the effects of TEO, BO and TX-100 on nanofiber structures were investigated by preparing PVP based emulsions and fed in the core of the PVP/PCL core-shell fibers. It was determined that TEO, BO and TX-100 additives changed the conductivity values of the solutions at different levels and the nanofiber diameters are increased by the addition of essential oil and surfactant and porosity ratios are also changed. According to the results, one factor to be considered regarding the encapsulation of essential oils in nanofibers is that the essential oils affect the viscosity and conductivity values to a different extent. The water contact angle measurements showed that TX-100 and oil additives caused high hydrophilization of nanofiber surfaces by changing hydrophobic nature of PCL which is a biodegradable and mechanically durable polymer. The results emphasize that when essential oils and surfactants are used in wound dressing studies, the effect of these additives on fiber formation should be taken into account. In future studies, the release behavior and antibacterial properties of optimized oil-loaded core-shell nanofibers will be investigated. Also, TEM measurements will be performed to confirm that the nanofibers form a core-shell structure.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Banu NERGIS: Determination and management of the conceptual design process, literature review and critical review. Nursema PALA: Literature review, experimental design, data collection, data analysis and interpretation. Nebahat ARAL: Experimental design and management, data analysis and interpretation, critical review.

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