EMULSION ELECTROSPINNING OF PVA/ROSEMARY NANOFIBERS

Original scientific paper
DOI: 10.5937/CT_ITI24013G

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ABSTRACT: *The purpose of this study was to develop a nanofibrous wound dressing material through the emulsion electrospinning method utilizing polyvinyl alcohol (PVA) polymer and rosemary essential oil (REO). The study involved the preparation of PVA/REO emulsions at varying REO ratios (0, 2, 4, 6, 8 wt%). First, the emulsion properties, such as viscosity, conductivity, and surface tension, were determined. The incorporation of REO resulted in a discernible enhancement in viscosity, accompanied by a minor reduction in conductivity. There was no appreciable alteration in surface tension. The emulsion electrospinning approach was used to produce the nanofiber composite material from these emulsions. Subsequently, the surface morphology of these nanofiber composites was investigated via the scanning electron microscope (SEM). Although REO increased the diameters of the nanofibers produced, it was observed that the nanofibers were smooth, beadless, and exhibited uniform structural properties. Besides, gas chromatography-mass spectrometry (GC-MS) was used to identify chemical compounds in rosemary essential oil. According to GC-MS analysis, 25 components were identified in REO, and 1.8-cineole (eucalyptol) was identified as the major component (58.7%). The study found that the addition of REO had a significant effect on the emulsion properties and morphology of the nanofibers. The emulsion electrospinning approach was got through to be a promising method for producing composite nanofibers from two immiscible liquids, such as an essential oil and an aqueous polymer solution.*

Keywords: *Polyvinyl alcohol, Rosemary, Essential oil, Nanofibers, Wound dressing, Emulsion electrospinning.*

EMULZIJA ELEKTROSPINING NANOVLAKNA PVA/RUZMARINA

APSTRAKT: *Svrha ovog istraživanja je bila da se razvije nanofibrozni materijal za zavoje rana metodom emulzije elektrospinovanja korišćenjem polimera polivinil alkohola (PVA) i eteričnog ulja ruzmarina (REO). Studija je uključivala pripremu PVA/REO emulzija u* *različitim REO odnosima (0, 2, 4, 6, 8 tež.%). Prvo su određena svojstva emulzije, kao što su viskoznost, provodljivost i površinski napon. Ugradnja REO je rezultirala uočljivim povećanjem viskoziteta, praćeno manjim smanjenjem provodljivosti. Nije bilo značajnih promena u površinskom naponu. Za proizvodnju kompozitnog materijala od nanovlakna od ovih emulzija korišćen je pristup emulzionog elektropredenja. Nakon toga, morfologija površine ovih kompozita od nanovlakna je ispitana pomoću skenirajućeg elektronskog mikroskopa (SEM). Iako je REO povećao prečnike proizvedenih nanovlakna, primećeno je da su nanovlakna bila glatka, bez perli i da su pokazala ujednačena strukturna svojstva. Pored toga, gasna hromatografija-masena spektrometrija (GC-MS) je korišćena za identifikaciju hemijskih jedinjenja u eteričnom ulju ruzmarina. Prema GC-MS analizi, 25 komponenti je identifikovano u REO, a 1,8-cineol (eukaliptol) je identifikovan kao glavna komponenta (58,7%). Studija je otkrila da dodatak REO ima značajan uticaj na svojstva emulzije i morfologiju nanovlakna. Pristup emulzionog elektropredenja postao je obećavajući metod za proizvodnju kompozitnih nanovlakna iz dve tečnosti koje se ne mešaju, kao što su eterično ulje i vodeni rastvor polimera.*

Ključne reči: *polivinil alkohol, ruzmarin, eterično ulje, nanovlakna, zavoj za ranu, elektrospinovanje emulzije.*

1. INTRODUCTION

Essential oils are natural aromatic volatile oils extracted from plants. They have been used for centuries in various cultures around the world. Today, essential oils are utilized in traditional and complementary medicine, aromatherapy, massage therapy, cosmetics, perfumes, and the food industry. They exhibit a range of activities including antiparasitic, antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory, anti-carcinogenic, anti-aging, and neuroprotective properties [1-2].

Rosemary (*Rosmarinus officinalis L.)* is a medicinal and aromatic plant species that grows on the northern and southern coasts of the Mediterranean, as well as in the lower regions of the Himalayas. It has been found to possess antioxidant, antimicrobial, antibacterial, anti-cancer, anti-inflammatory, and pesticide removal properties. Due to these characteristics, rosemary is utilized for medicinal purposes, as well as in perfumes, cosmetics, and aromatherapy [3].

Nanofibers are being increasingly explored and utilized in the field of wound dressings due to their unique features and advantages over traditional materials. Nanofiber-based wound dressing represents a promising advancement in wound care technology, offering a range of benefits that can contribute to faster healing, reduced infection risk, and improved patient outcomes [4-5].

Electrospinning is a technique used to produce microscale to nanoscale materials from polymeric solutions [6]. Emulsion electrospinning is a novel and efficient method for the easy production of functional nanofiber structures from two immiscible liquids, such as an essential oil and an aqueous polymer solution. This approach allows for the preparation of homogeneous and stable emulsion solutions, making the production of nanofibers much

easier compared to conventional electrospinning systems. Additionally, emulsion electrospinning does not require any extra apparatus [7].
The objective of this study was to fabricate a nanofiber material for wound dressing via emulsion electrospinning method using polyvinyl alcohol (PVA) polymer and rosemary essential oil (REO). The present study demonstrates an innovative approach to the production of PVA/REO nanofibers by emulsion electrospinning method, which has the potential as a wound dressing.

2. MATERIALS AND METHODS

2.1 Materials

PVA (Mw 89,000-98,000, 99+ % hydrolyzed) was used as a polymer, and distilled water was used as a solvent. To prepare emulsion; a surfactant (PEG-40 hydrogenated castor oil) was used as an emulsifier and rosemary (*Rosmarinus officinalis L.*) essential oil was used as an additive. PVA polymer was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA); surfactant was supplied by Ersa Chemistry, Izmir, Turkey; and the rosemary essential oil was acquired from Botalife, Isparta, Turkey. All chemicals were analytical grade and used without further purification.

2.2 Methods

The study began with emulsion preparation for nanofiber production via emulsion electrospinning. Emulsion preparation occurred in two stages. Initially, the polymer solution was created by mixing PVA polymer in distilled water. Afterwards, various ratios of rosemary oil and surfactant were added to the PVA solution to obtain the emulsions. The emulsion was stirred for approximately 24 hours at room temperature. Subsequently, the emulsion properties were determined, including conductivity (SelectaCD2005), viscosity, and surface tension (measured by the Wilhelmy Plate technique with Biolin Scientific Sigma 702). The surfactant was used to create an oil-in-water emulsion due to the immiscibility of the polymer solutions and REO. Table 1 presents the concentration of polymer, surfactant, and REO used in the study.

Table 1: The concentration of PVA, surfactant, and REO

Sample Codes	PVA (wt%)	Surfactant (wt%)	REO (wt%)
PVA	10	0	0
PVA/REO-0	10	3	0
PVA/REO-2	10	3	2
PVA/REO-4	10	3	4
PVA/REO-6	10	3	6
PVA/REO-8	10	3	8

The nanofibers were fabricated through the emulsion electrospinning process. The electrospinning process parameters (Table 2), including applied voltage, distance between the needle and collector, and solution flow rate, were optimized and kept constant during the spinning process. The emulsion electrospinning process was conducted under the same ambient conditions for all samples, and the nanofibers were collected on an aluminum sheet for 15 minutes.

Table 2: Process parameters of emulsion electrospinning

Voltage (kV)	Distance Between Electrodes (cm)	Feed Rate (mL/h)	Humidity (%)	Temperature (°C)
21.5	18	0.250	38 (± 2)	20 (± 2)

In the last part of the study, the morphology of the nanofibers, including the fiber diameter, the uniformity of the diameter, and the surface structure of the web, was analyzed using the SEM. Fiber diameters were calculated using Image J analysis software. Statistical analysis of the morphological parameters, such as the fiber diameter histogram, was performed using the SPSS statistical program. The formula below was used to calculate the average fiber diameter uniformity coefficient values:

$$A_n = \frac{\sum n_i d_i}{\sum n_i} \quad \text{(Number average)} \tag{1}$$

$$A_w = \frac{\sum n_i d_i^2}{\sum n_i d_i} \quad \text{(Weight average)} \tag{2}$$

ni=fiber number *di=fiber diameter*

The fiber diameter uniformity coefficient was calculated by the ratio of Aw to An. The closer the average fiber diameter uniformity coefficient is to 1, the more uniform the fibers [8]. Additionally, the identification of chemical compounds in rosemary essential oil was accomplished using gas chromatography coupled with mass spectrometry (GC-MS).

3. RESULTS AND DISCUSSION

Table 3 presents the conductivity, viscosity, and surface tension values of polymer solutions containing REO.

Table 3: Properties of polymer solutions of PVA with various concentrations of REO

Sample Codes	Conductivity (µS/cm)	Viscosity (Pa·s)	Surface Tension (mN/m)
PVA	309	2.43	41.73
PVA/REO-0	323	4.28	40.13
PVA/REO-2	314	6.46	40.19
PVA/REO-4	300	8.43	37.87
PVA/REO-6	296	9.41	37.03
PVA/REO-8	292	12.61	37.69

The conductivity value of the solution prepared with only a PVA polymer was measured as 309 µS/cm. The addition of a surfactant to the solution resulted in a slight increase in conductivity. However, the subsequent addition of REO to the solutions caused a decrease in conductivity. As the REO content in the solutions increased, the trend of decreasing conductivity persisted. Furthermore, various studies have demonstrated that certain essential oils can reduce conductivity [7, 9].

As anticipated, the viscosity values of the emulsions exhibited an increase in accordance with the REO ratio in the solutions. It is essential to acknowledge that the surfactant also contributed to an elevation in the viscosity of the emulsions to a certain extent. The viscosity of the PVA polymer solution without REO was measured as 2.43 Pa·s. Upon the addition of only surfactant to this solution, the viscosity of the PVA/REO-0 solution was determined to be 4.28 Pa·s. It was also determined that the viscosity continued to increase with the addition of REO to the solutions.

The surface tension values was analyzed to determine the impact of REO addition to polymer solutions. The results indicated that the addition of REO did not significantly alter the surface tension values.

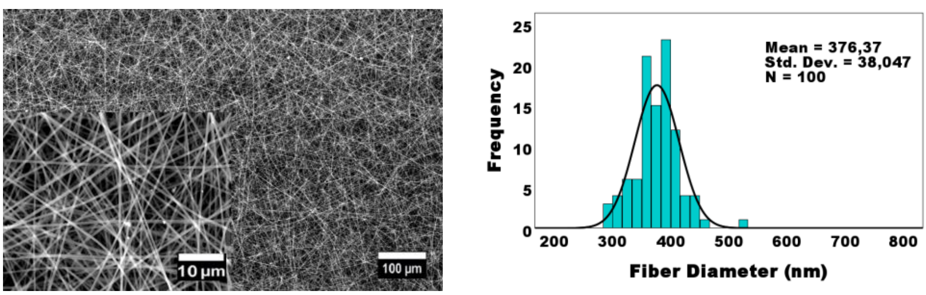
The essential oil of rosemary was subjected to analysis by gas chromatography-mass spectroscopy (GC–MS). A total of 25 components, representing 99.06% of the total oil, were identified in Table 4. The major components were identified as 1.8-cineole (eucalyptol) (58.7%), camphor (9.66%), α-pinene (8.69%), 2-α-pinene (4.08%), borneol (3.55%), camphene (2.96%), α-terpineol (2.49%), respectively. The findings of other studies yielded comparable results [10].

Table 4: Components of REO by GC-MS analysis

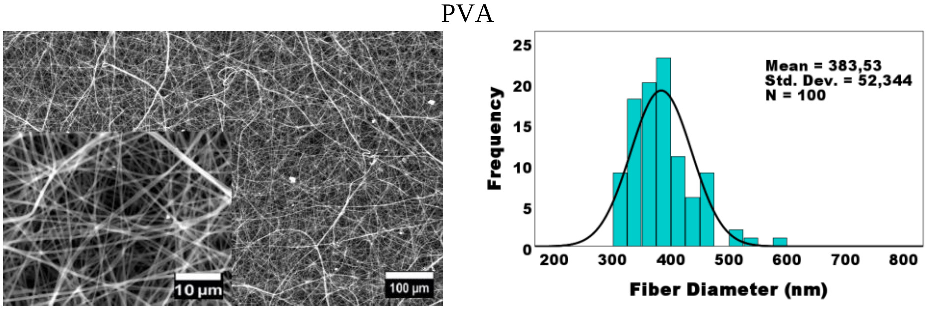
Component Name	% Area
Tricyclene	0.03
α-Thujene	0.01
α-Pinene	**8.69**
Camphene	**2.96**
2-α-Pinene	**4.08**
α-Myrcene	0.89
I-Phellandrene	0.33
α-Terpinene	0.21
o-Cymene	1.98
1.8-Cineole (Eucalyptol)	**58.7**
cis-ocimene	0.02
ç-Terpinene	1.70
α-Terpineolene	0.10
α-Linalool	0.41

Camphor	**9.66**
Borneol	**3.55**
4-Terpineol	0.61
α-Terpineol	**2.49**
Exobornyl acetate	1.11
α-Gurjunene	0.02
trans-Caryophyllene	0.77
Aromadendrene	0.52
Alloaromadendrene	0.11
Ledene	0.07
Caryophyllene oxide	0.04

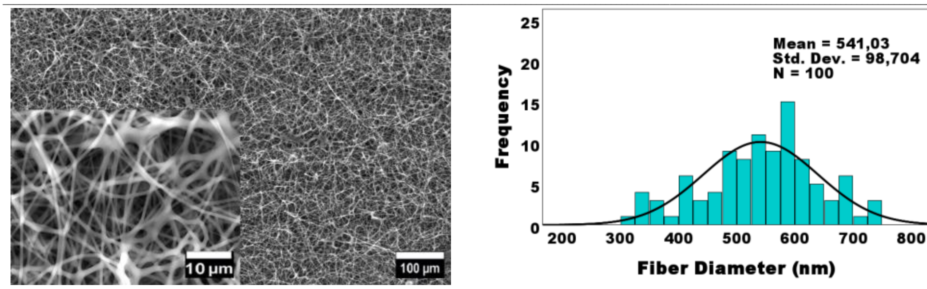
The SEM images and fiber diameter histograms of the PVA/REO nanofiber samples, produced with varying REO concentrations, are presented in Figure 1. These images and histograms are provided at two magnifications: 1.000x and 10.000x.



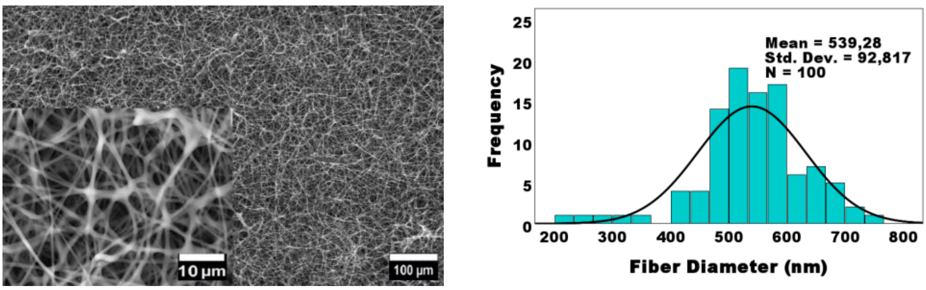
PVA



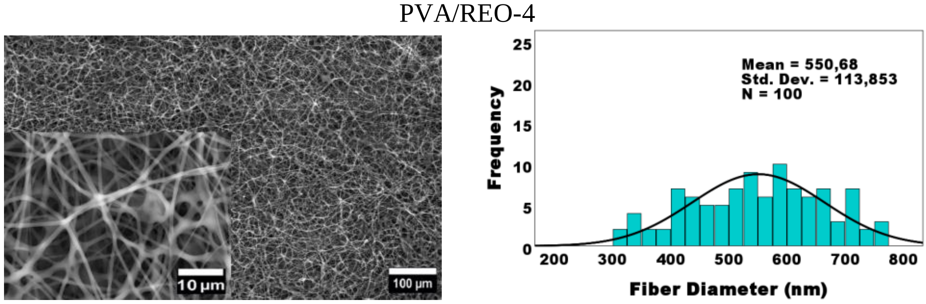
PVA/REO-0



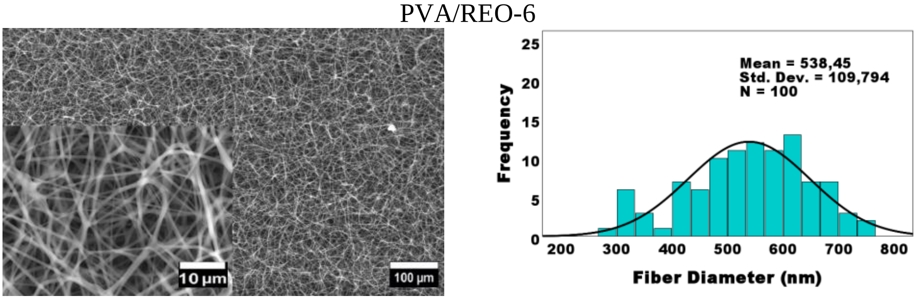
PVA/REO-2



PVA/REO-4



PVA/REO-6



PVA/REO-8

Figure 1: SEM images and fiber diameter histograms of PVA/REO nanofiber samples produced with various REO concentrations (1.000x, 10.000x).

Upon examination of the SEM images of the nanofibers produced at the same process parameters, it was observed that the nanofibers produced exclusively with PVA polymer exhibited thin, smooth, bead-free, and uniform structural characteristics. It was determined that the nanofibers produced by the addition of surfactant to the PVA solution exhibited a uniform structure and that the fineness of the nanofibers was comparable to that of the PVA polymer. The histogram curves of both nanofibers exhibited a single peak, indicating a normal distribution of fiber fineness. Upon examination of the SEM images of the nanofibers obtained by adding REO to the solutions, it became evident that the fibers exhibited a more adhesive appearance. The histograms derived from the SEM images revealed that the standard deviation of the nanofibers containing REO was higher, indicating the formation of nanofibers with disparate fiber diameters (ranging from 200 to 800 nm).
The influence of REO concentration on the average fiber diameter and fiber diameter uniformity coefficient is illustrated in Figure 2.

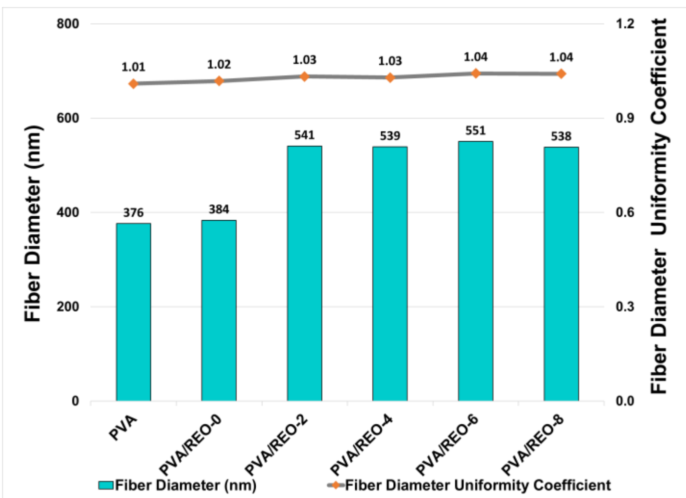


Figure 2: The effect of REO concentration on the average fiber diameter and fiber diameter uniformity coefficient of PVA nanofibers

Figure 3 illustrates that the nanofibers produced with PVA polymer have an average fiber diameter of 376 nm. The average fiber diameter of the nanofibers obtained with the addition of surfactant has an average fiber diameter of 384 nm. Upon examination of the average fiber diameters of the nanofibers obtained by adding REO to the solutions, it was observed that the fiber diameters increased. The average fiber diameter of the nanofibers obtained by adding REO was found to be between 538 and 551 nm. It was determined that the increasement of the concentration of REO did not cause a significant change in the average fiber diameter.
Upon examination of the graph of fiber diameter uniformity coefficients, it was found that the addition of REO increased fiber non-uniformity. The fiber diameter uniformity

coefficient of nanofibers without REO was 1.01, while this value increased to 1.04 when REO was added. These results also support the SEM images.

3. CONCLUSION

In this study, REO-loaded PVA-based nanofibrous material (PVA/REO) was fabricated by emulsion electrospinning process. The GC-MS analysis identified 25 components in rosemary oil, with 1.8-cineole (eucalyptol) representing the most abundant (58.7%). According to the obtained results; it was observed that viscosity, spinning performance, fiber morphology, and average fiber diameter were affected by the REO concentration in emulsions. The addition of REO resulted in a noticeable increase in viscosity, while a slight decline in conductivity was observed. There was no significant change in surface tension. The study revealed that it is possible to produce nanofibers from two immiscible liquids, namely rosemary essential oil and an aqueous polymer solution (PVA), using the emulsion electrospinning method. It was noticed that, although REO increased the diameters of the nanofibers produced, the nanofibers exhibited smooth, beadless, and uniform structural properties. Considering the superior properties of rosemary such as being antibacterial and antimicrobial along with those of nanofibers, the composite material produced can be used as a wound dressing.

ACKNOWLEDGMENT

The authors would like to thank Osman BODUR, and Taner ERKAYMAZ, and Süleyman Demirel University Natural Products Application and Research Center (SUDUM) for their contributions during GC-MS analysis.

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