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Production of lavender oil loaded antibacterial polymeric membranes

Özlem EĞRİ [1,*]

[1] *Department of Genetic and Bioengineering, Faculty of Engineering and Natural Sciences, Tokat Gaziosmanpaşa University, Turkey*

Abstract

Antibacterial polymeric membranes were produced within the context of this study. PCL polymer solution and PEG solution that was mixed with lavender oil (*Oleum lavendula angustifolia*) was electrospun concurrently from opposite directions. Thus lavender oil used as antibacterial agent was entrapped within PEG fibers. Antibacterial polymeric membranes were produced by collecting electrospun fibers on rotating mandrel. Interaction of the solvent with the polymer determined with Fourier Transform Infrared Spectroscopy (FTIR). Surface morphology of the membrane and fiber diameters were investigated by using Scanning Electron Microscopy (SEM) images. Mechanical strengths of the membranes were evaluated by standard tensile tests. Hydrophilicity of the membranes was characterized by contact angle measurements and water uptake capacity was characterized by swelling tests. In vitro controlled release of the lavender oil by dissolution of PEG fibers in aqueous medium was investigated under in vitro conditions. Antibacterial activity of the membranes on gram positive and gram negative bacteria species was observed by disk diffusion tests.

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1. Introduction

Lavandula (common name lavender) is a flowering plant in the mint family, *Lamiaceae* [1]. Volatile essential oils of the plants are obtained by vapor distillation of flowers and peduncles. Lavender oil is an essential oil that can be obtained from many lavender plant species. *Lavandula latifolia, Lavandula angustifolia, Lavandula stoechas* and *Lavandula intermedia* are the most common species used for oil production. *Lavandula angustifolia* amongst these species is the most frequently commercially used speices [2]. It is one of the most traded essential oils. Linalool ve linalil acetate are the common components found in the volatile essential oil. Ratio of linalil acetate component determines the quality of the oil produced [3].

It is known that lavender essential oil is used in various range of applications and gives benefit. It is used in aromatherapy with different forms, lotions, soaps, candles and baby products because of its aroma. It is known that lavender oil supports healing of burns and slashes. Lavender oil was shown to be effective on wound healing in a clinical study, in which postpartum lavender oil was applied after episiotomy [4-6]. Studies exist in literature, that indicate healing of skin tissues and increasing the collagen synthesis by lavender oil treatment [7]. Besides many characteristic properties, it also exhibits neuroprotective effect [8]. It was used in aromatherapy as stress and anxiety reliever, and also reported to assist therapies of insomnia, anxiety, chronic pains [9-11]. It is used in complementary medicine to support therapy of respiratory tract problems (flu, tussis, asthma, cold, strep throat, bronchitis, etc.) [12].

It is known that oil itself and even its vapor exhibit antiviral and antifungal effect [13,14]. Lavender oil exhibits as a strong antibacterial agent against foodborne bacteria (*Escherichia coli* and *Enterobacter cloacae*) because of its basic components of linalool and linalil acetate [15]. Other components of the oil (limonen, a-pinen ve β-pinen) exhibit antibacterial activity against several human pathogenic bacterias [16]. Besides, lavender oil was determined to be also effective on, *Staphylococcus aureus* resistive to methicillin and *Enterococcus faecium* resistive to vancomycin, by in vitro studies [17].
Antibacterial effect is important for avoiding infection of the environment that microorganisms exist. In many scientific studies which antibacterial substances were worked with, generally micro/nano particles of metals such as zinc, silver and copper were frequently used. Use of nanoparticles, especially silver etc. over a

*Corresponding author. *Email address: ozlm.gnrl@gmail.com*

certain dose in the medical field carries the risk of toxicity [18,19]. For this reason there is an orientation through the use of herbal sourced substances recently. It was reported in literature that some volatile essential oils exhibit antibacterial activity thus they protect the plants they exist in, from microorganisms [20]. Antibacterial activity may occur as a result of interaction of the active main components present in the volatile oils with each other. Lavender oil exhibits antiseptic property due to its components [21].

Antibacterial agents can be added to the structure of polymeric membranes in order to be used in certain fields (wound dressing materials, meshes for hernia repair, etc.) [22-24]. It was reported by one of the studies that antibacterial activity was exhibited by Copaiba oil which was loaded into electrospun PLA/PVP nanofibers [25]. In an another study, oil obtained from the shell of *Tecomella undulata* plant was loaded into polymeric membranes of PCL/PVP and it is concluded that it can be used as therapeutic agent of dermal bacterial infections [26]. It was reported that *H. perforatum* (centaury) oil loaded PEG/PCL membranes exhibited both antibacterial activity and support cell proliferation *in vitro* [27].

In this study it was aimed to provide antibacterial property to the membranes by including the lavender oil into the electrospun polymeric membranes. Chemical structure, morphology, density, porosity and mechanical properties of the membrane was characterized and wettability, hydrophilicity, mass loss in time and oil release from the membrane was evaluated under *in vitro* conditions. Antibacterial activity of commerical oil used in this study, plain and lavender oil loaded membranes on *S. aureus* and *E. coli* which are gram (+) and gram (-) beacterias, were evaluated separately.

2. Materials and Methods

2.1. Materials

Polyethyleneglycol (PEG) (M_w:10000 Da) purchased from Merck, Germany and Polycaprolactone (PCL) (M_w:80000 Da) purchased from Sigma-Aldrich, UK. Lavender oil (*Oleum lavendula angustifolia*) (Awe Cemre Laboratories, Turkey) was used as antibacterial agent. Chloroform (Merck, Germany) and Methanol (Merck, Germany) were used as solvent. *Esherichia coli* and *Staphylococcus aureus* bacterial strains were supplied by Gaziosmanpasa University, Faculty of Medicine, Biochemistry Laboratories and Mueller Hinton Bouillon (MHB), Luria-Bertani broth (LB), Mueller Hinton Agar (MHA), Eosine Metilen Blue (EMB) Agar mediums (Merck, Germany) were used for antibacterial activity tests.

2.2. Methods

2.2.1. Membrane production

PCL solution and PEG solution mixed with lavender oil (*Oleum lavendula angustifolia*) was electrospun concurrently and from opposite directions. Thus lavender oil that was used as antibacterial agent, was entrapped in PEG fibers. Polymeric membranes were produced by collecting electrospun fibers on a rotating mandrel. For this purpose PEG/chloroform solution (4% w/v) was prepared in 40 mg/ml concentration. 10% (v/v) lavender oil was added into this solution dropwise. Homogeneous solution was transferred into a syringe with 20G needle and the fibers were collected on a rotating mandrel while the solution was electrospun at a rate of 3ml/h using a syringe pump. The gap between the needle and the mandrel was 8 cm, and the voltage applied was 16kV. PCL (10% w/v) was dissolved in chlroroform:methanol mixture (4:1) and it was electrospun concurrently and from opposite directions with the lavender oil containing PEG solution. PCL solution was also transferred to an another syringe with 20G needle and electrospun at a rate of 1 ml/h. The gap between the needle and the mandrel was 10 cm, and the voltage applied was 16kV. Antibacterial polymeric membranes were placed in a vacuum desicator for 1 day in order to avoid residual solvent and then stored at +4°C with proper package.

2.2.2. Chemical structure and morphology of the membranes

Chemical structure analyses were conducted comparatively by taking FTIR (Perkin Elmer, Spectrum 100, USA) spectra of membrane (with/without oil), polymers forming membrane and oil separately (Figure 1). Analyses were carried out with FTIR-ATR unit. Measurement data were collected by recording the infrared (IR) beam intensity reflected from the crystal while IR beam was absorbed by the components of the membrane sections that were fixed on diamond discs having approximately 2 mm diameter.

Membrane sections were fixed on SEM sample platform and conductive surfaces were created by coating with gold (Quorum K150RS, UK) for 15

minutes under vacuum, and SEM (Tescan Mira3 XMU, Czechia) images were collected for evaluation of membrane morphology (Figure 2).

2.2.3. Density and porosity

Volume (cm^3), density (g/cm^3), specific volume (V_m) values were determined by measuring the thickness of the membrane sections with 2.0 x 2.0 cm dimensions and membrane porosities(%) were calculated using the equation 1 [28].

$$\text{Porosity (\%)} = [(V_m - V_o) / V_m] \times 100 \quad (1)$$

Where V_m and V_o are the specific volume of electrospun membrane and the pure film (prepared by polymer blend) respectively. Calculations were made for 10 different mambrane (with/without oil) sections and average values were presented in Table 1.

2.2.4. Mechanical properties

Membrane elasticity was determined by applying forces between 1.5 and 3.5 N onto 10 cm long and 5 mm wide membrane sections. Tensile test instrument (Dvt Bp D Nu, Turkey) was used to take measurements under ASTM (ASTM D 882-02, 2002) standards. Extension (%) values were recorded against applied tensile force and elongation at break (mm), yield point (N/mm2) and modulus of elasticity (MPa) values were calculated (Table 2).

2.2.5. Hydrophilicity and wettability

Hydrophilicity of the membranes was tested using a contact angle measurement device (Attension, Biolin Scientific, Sweden) with compliance to related standards (ASTM D 7334, 2013). Measurements were taken under air atmosphere. 4 µl of deionized water was dropped on the membrane surface and the image of the droplet on the surface was captured using a camera. Contact angles between the droplet and the membrane was measured from the images transferred to the computer. 3 measurements from each surface were used to calculate average values and membrane hydrophilicity was evaluated.

In order to determine wettability of the membranes, attention was paid to have equal weights for each circular polymeric membrane sections with 7.5 mm diameter. Membranes kept in phosphate buffer solution (PBS) with a pH value of 7.4 and at 37°C temperature, they were taken out at different periods of time (3, 6, 9, 12, 15, 20, 25, 30, 45, 60 minutes) and weighed. Obtaned values were used to calculate swelling ratio using equation 2 [24].

$$\text{Swelling Ratio(\%)} = [(W_t - W_o) / W_o] \times 100 \quad (2)$$

Where W_o is initial weight; W_t weight measured at each observation period. Swelling ratio (%) was sketched with respect to time using the calculated values and wettability of the membranes was evaluated (Figure 3).

2.2.6. Mass loss in time

Membranes with 2.0 x 2.0 cm dimensions were weighed and kept in phosphate buffer solution (PBS) with a pH value of 7.4 and at 37°C temperature for different periods of time (2,4,6,8,10,12,14 days). Membranes were taken out at observation periods and weighed after 3 days of drying. Weight remaining (%) was calculated using equation 3 and mass loss values of membranes in time were evaluated (Figure 4) [29].

$$\text{Weight remaining(\%)} = (W_{after} / W_{before}) \times 100 \quad (3)$$

Where W_{before} and W_{after} dry weights of the membranes before and after incubation in PBS, respectively.

2.2.7. Controlled release of lavender oil

In vitro release studies were conducted by investigation of lavender oil release from polymeric membranes in PBS (pH 7,4; 37ºC). Lavender oil containing circular sections of membranes with 7.5 mm diameter were kept in PBS for 14 days. Released oil amount was determined by measuring the absorbance values at λ:225 nm using a spectrophotometer (BOECO S-30 Spectrophotometer, Germany) each 12 hours. Experiments were repeated 5 times and values were calculated as average. Thus lavender oil release graph was sketched and evaluated (Figure 5).

2.2.8. Antibacterial activity

Lavender oil loaded and no oil containing membranes were tested against *E. coli* and *S. aureus* which are gram negative and gram positive bacteria respectively, by disc diffusion test. Membranes (circular sections with 0.75 mm diameter) and lavender oil was treated with UV beam for 20 minutes to sterilize. *E. coli* and *S. aureus* species in solid culture media were inoculated into MHB liquid culture media. Organisms in MHB liquid culture media were incubated at 37C° in an incubator for 24 hours of pre-activation and then 18 hours for activation. Then liquid culture was diluted with sterile water and adjusted to give 0.2 absorbance

at 660 nm wavelength using a spectrophotometer. This absorbance value was accepted to suit up to 0.5 McFarland standard which was determined for the cultures to be used in antibacterial activity test by disc diffusion method. Samples taken from *E. coli* and *S. aureus* liquid cultures with turbidity values suitable to this standard were cultured to MHA media by using sterile swab. Sterile membranes and 10 µl lavender oil embedded sterile discs were placed with appropriate distances in petri dishes of culture. Samples were left for incubation at 37°C for 24 hours. Antibacterial activities of membranes and the lavender oil on *E. coli* and *S. aureus* species were evaluated by measuring the zone diameters after incubation period. Experiments were repeated 3 times at different periods and average values were calculated (Figure 6), (Table 3).

3. Results and Discussion

3.1. Chemical structure (FTIR Spectra) of polymers and electrospun membranes

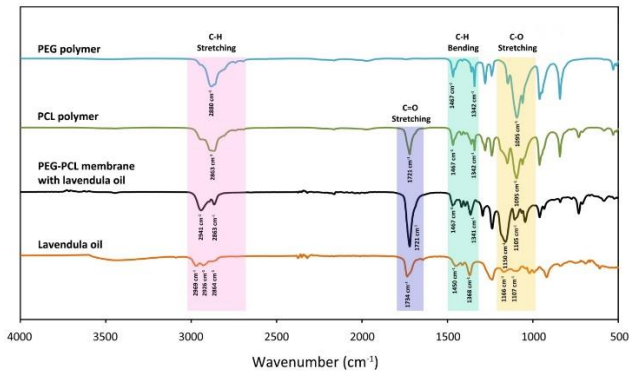

Figure 1. FTIR spectra for polymers (PEG and PCL), PEG-PCL membrane with oil and lavender oil.

C-H stretching peak of CH_2 groups existent in the PEG structure was observed at wavenumber 2880 cm^{-1} and C-H stretching peak of symmetrical CH_2 groups existent in the PCL structure was observed at wavenumber 2683 cm^{-1} (Figure 1). C-O stretching peaks of PEG and PCL polymers were observed at wavenumber 1095 cm^{-1}. Peaks observed on spectra between wavenumbers 1467cm^{-1} and 1341 cm^{-1} belong to C-H bending. Peak splitting occurs due to different stretching and bending vibrations of the carbon atoms that belong to different groups. C=O stretching peak of carbonyl groups existent in PCL structure was observed at wavenumber 1721 cm^{-1} and ester bond induced C=O stretching peak of lavender oil was observed at wavenumber 1734 cm^{-1}. Three carbon atoms found in the structure of glycerol which was formed by ester bonding of fatty acids, have different stretching vibrations. Triple splitting of C-H stretching peak at wavenumbers 2969 cm^{-1}, 2926 cm^{-1} and 2864 cm^{-1} observed on the spectrum that belongs to lavender oil is due to this. Specific peaks for both polymers and lavender oil were existent on the FTIR spectrum of the PEG-PCL membrane produced by electrospinning of PCL and lavender oil containing PEG solutions from opposite directions. C=O stretching peaks of PCL and lavender oil overlapped at wavenumber 1721 cm^{-1} and a wider and stronger peak was observed for membranes with oil. It can be conluded that the lavender oil was successfully included in the membrane. When FTIR spectra of the polymers forming the membrane, oil and oil containing membranes were compared, it was concluded that no chemical structure change occurred for PEG and PCL polymers due to solvent interactions and electrospinning process (under high voltage).

3.2. Morphology (SEM)

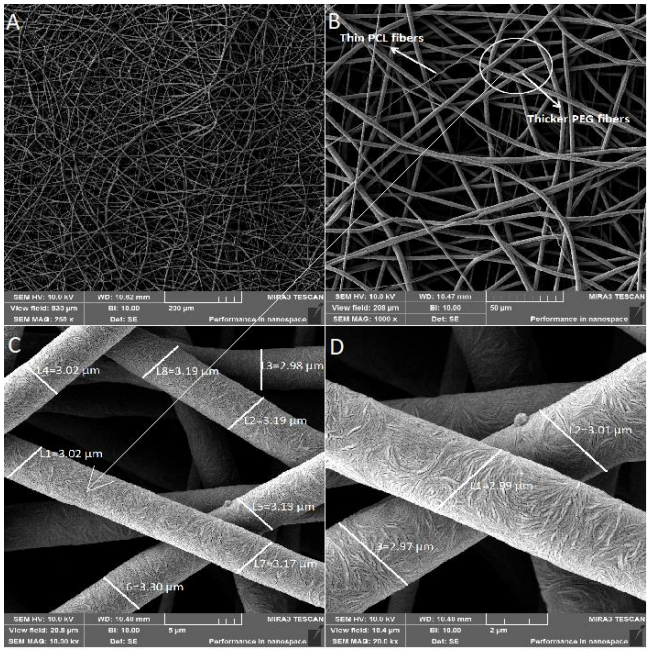

Figure 2. SEM images of %10 (v/v) lavender oil containing PEG/PCL membrane: (A)x250 magnification, (B)x1000 magnification (thin PCL fibers and thicker PEG fibers), (C)x10.000 magnification and (D)x20.000 magnification (with diameters of PEG fibers).

Thick PEG and thin PCL fiber structures that were concurrently electrospun from opposite directions, were evident on the SEM images of lavender oil containing PEG/PCL membranes (Figure 2A, 2B). Flow rates of the PCL and lavender oil containing PEG solutions were 1 ml/h and 3 ml/h respectively. It is thought that thin PCL fibers and thick PEG fibers were formed due to this reason. Average fiber diameter of the PEG fibers that seem to be almost identical, was 3.13±0.1µm (Figure 2C). Dense cracks were observed on the fibers (Figure 2D). Membranes were kept under

vacuum while they were conductive coated and SEM images were taken. It is thought that these cracks were formed while fast evaporation of lavender oil encapsulated in PEG fibers due to the loss of atmospheric pressure at vacuum conditions.

3.3. Density & Porosity

Density (g/cm^3), specific volume of membranes (V_m, cm^3/g) and porosity (%) values of the plain and lavender oil loaded PEG/PCL membranes were presented on Table 1. Density, specific volume and porosity(%) values of plain membrane were 0.870 g/cm^3, 0.291 cm^3/g and 75% respectively. Density, specific volume and porosity(%) values of lavender oil loaded membrane were 0.935 g/cm^3, 0.394 cm^3/g and 63% respectively. Density of the membranes increased while specific volume (V_m) decreased by oil loading as expected. This resulted with a decrease in porosity of the membrane as well. Nevertheless, 75% and 63% are still high porosity values. Creating structures with high porosity in unit volume is one of the important advantages of electrospinning technique [30]. High porosity resulted with higher gas permeation, water uptake capacity and release rate of components entrapped within the membrane.

Table 1. Density (g/cm^3), specific volume (V_m, cm^3/g) and porosity (%) in plain and lavender oil loaded membranes.

Membranes	PCL %(w/w)	PEG %(w/w)	Lavender oil %(w/w)	Density (g/cm^3)	V_o (cm^3/g)	Vm (cm^3/g)	Porosity (%)
Plain	0.817	0.183	0	0.870	3.442	0.291	75
Lavender oil loaded	0.629	0.151	0.219	0.935	2.535	0.394	63

Table 2. Tensile test results of plain and lavender oil loaded membranes.

Membrane	Elongation at break (mm)	Ultimate Tensile Stress (N/mm^2)	Yield Point σ:F/A (N/mm^2)	Young's Modulus (MPa)
Plain	478.12	2.27	0.97	9.7
Lavender oil loaded	443.25	2.13	0.63	6.3

3.4. Mechanical properties (Stress-Strain)

Elongation at break and ultimate tensile stress values of plain and lavender oil loaded membranes were found to be 478.12 mm, 2.27 N/mm^2 and 443.25 mm, 2.13 N/mm^2 respectively (Table 2). Yield point and Young's modulus values were found to be 0.97 N/mm^2 and 9.7 MPa for plain membrane and 0.63 N/mm^2 and 6.3 MPa for lavender oil loaded membrane (Table 2). Tensile strength values of the membranes were quite good. The membrane length was extended to almost 4-5 fold around break point. Strength of the oil loaded membrane decreased a little. Besides, oil loaded in the membrane increased stiffness but decreased the elasticity. It can be concluded that polymeric membranes exhibited sufficient elasticity. Mechanical stability of the membrane structure formed by nonwoven fibers is one of the advantages provided by electrospinning technique [27].

3.5. Hydrophilicity (contact angle measuremets) and wettability (swelling ratio %)

Wetting is the freely spreading of liquid on the solid surface in order to create adhesion. Wetting level of the solid by the liquid, is relevant to the contact angle between them. Contact angle is defined to be the angle **(**θ) between the liquid droplet and the solid surface [31]. The solid surface is accepted to be wetted by the liquid (water) if the contact angle **(**θ) is smaller than 90° (hydrophilic material), and not wetted if it is greater than 90° (hydrophobic material). Average contact angle value of the lavender oil containing electrospun PEG/PCL membrane was 65.76° ± 0.42°. Measurements were also taken for the plain membranes and effect of oil on hydrophilicity was evaluated. Average contact angle value for plain membrane was 79.54° ± 0.56°. Hydrophilicity was decreased as expected by addition of oil with hydrophobic character to the membrane structure. Membrane surfaces can be said to have hydrophilic

(θ<90°) character. Surface properties of the biomaterials with the potential of use in medical field, are very important. Especially, interaction between tissue and material surface defines the biocompatibility of the material for *in vivo* applications. Hydrophilic character supports biocompatibility of the material since it resembles the *in vivo* conditions.

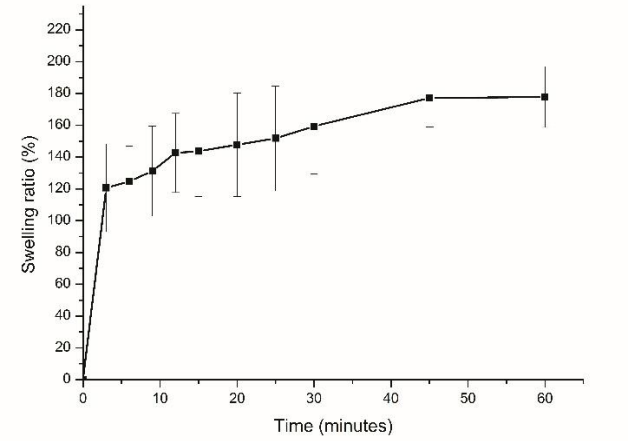

Figure 3. Degree of swelling ratios (%) of lavender oil containing membranes.

Wettability of the material surface gains importance together with water uptake capacities of the membranes in many applications (wound dressing membranes etc.). Water uptake capacity increases with high porosity (%) values in hydrophilic materials. Swelling ratios (%) were calculated for the membrane sections kept in buffer solution (PBS) using the weight measurements by taking out from solution medium in different time periods (3, 6, 9, 12, 15, 20, 25, 30, 45, 60 minutes) (Figure 3). Membranes rapidly soaked within the first three minutes (swelling ratio ~120 %). Similar swelling ratio values were reached at the end of 45th and 60th minutes. It can be said that lavender oil containing membranes reached to maximum swelling capacity value (~177%) at 45th minute and can uptake water more than 1.5 fold of its weight. Water uptake capacities of the electrospun nanofibrous polymeric membranes reported in the literature changes between ~20% and ~220% due to the high surface area to volume ratio of the nanofibers [28]. It can be concluded that the water uptake capacity of the membranes was satisfactory.

3.6. Mass Loss in time

Membranes were kept in buffer solution (PBS at ~pH 7,4; 37°C) for defined periods (2,4,6,8,10,12,14 days). Initial and final dry samples were weighed and remaining mass (%) graph was sketched (Figure 4). A rapid mass loss was observed within first two days (mass remaining ~53% in 2 days). Half of the membrane mass was lost at the end of 2nd day because of fast dissolution of water soluble hydrophilic PEG polymer that exist in membrane structure and consequently fast release of lavender oil encapsulated with PEG polymer. A slower rate of mass loss was observed between days 2nd and 14th. Total mass loss was 77% at the end of 14th day. Mass remaining (approximately 33%) came from PCL polymer that has a low degradation rate compared to PEG dissolution rate. Considering mass remaining graph (Figure 4), the great portion of the lavender oil was released within two weeks by the fast dissolution of PEG polymer existed in membrane structure, and PCL polymer kept maintaining the membrane integrity.

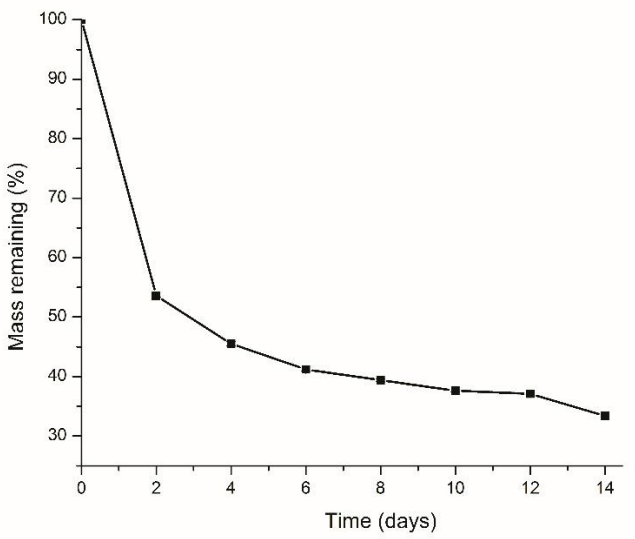

Figure 4. Mass remaining (%) of lavender oil containing membranes.

3.7. Controlled release of lavender oil

Circular membrane sections with a diameter of 7.5mm that contain lavender oil were kept in PBS (pH 7,4; 37°C) for 14 days. Released amount of oil was determined using spectrophotometric measurements of samples that were taken from the PBS medium every 12 hours during 14 days lavender oil release graph was sketched (Figure 5). Oil release was fast within first two days. Oil release was slowed down after second day because of the reduction of the oil exist in the membrane section. After the 9th day, a significant increase in oil release was observed. Oil droplets came out by dissolution of PEG fibers in the membrane may merge because of the hydrophobic character of the oil, and form larger droplets. In such case, diffusion of oil out of the membrane from the pores of the membrane becomes harder. Release of oil accumulated within the membrane pores might have been eased because of the erosion on the membrane surface caused by polymer dissolution. Although small amount of increase and decrease in oil release rates were observed after the 2nd

and 9th days respectively within 14 days period, a controlled and linear oil release from membrane sections can be seen when the release graph is broadly considered.

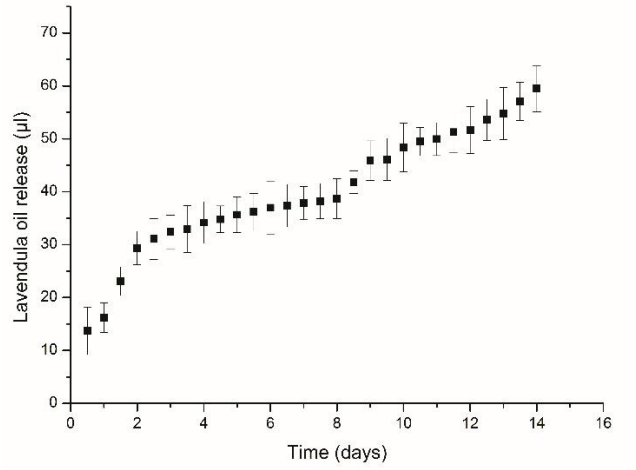

Figure 5. Controlled release of lavender oil containing membrane with time.

3.8. Antibacterial activity

Antibacterial activity of lavender oil loaded sterile disks, lavender oil loaded and plain membranes were tested against *E. coli* and *S. aureus* which are gram negative and gram positive bacteria respectively, by disc diffusion test. Effect of lavender oil release by diffusion from membrane and sterile disk on microorganism growth was evaluated by measuring the inhibition zone measurements (Table 3). No inhibition zone was observed for plain membranes (Figure 6). Plain membranes did not exhibit any antibacterial effect on both *E. coli* (-) and *S. aureus* (+). Inhibition zones around lavender oil loaded sterile disks were measured as 14 and 11 mm for *E. coli* (-) and *S. aureus* (+) media respectively. Inhibition zones around oil containing membranes were measured as 12 mm for *E. coli* (-) medium and 9 mm for *S. aureus* (+) medium. Since *S. aureus* (+) is a cell walled bacterium, penetration of lavender oil that diffused from disk and membrane to the medium into the cell was harder. It's thought that relatively lower inhibition zone diameter at *S. aureus* (+) medium to *E. coli* (-) was observed because of this reason. Lavender oil loaded sterile disks and oil loaded membranes exhibited antibacterial effect on both *E. coli* (-) and *S. aureus* (+). It can be concluded that antibacterial effect can be given in to plain membranes that have no antibacterial effect by including lavender oil in its structure as antibacterial agent.

Figure 6. Antibacterial effects of lavender oil containing membranes on *E. coli* and *S. aurues.*

Table 3. Inhibition zone diameters of oil containing membranes and oil loaded steril disks.

	Inhibition Zone Diameters (mm)	
	E.coli (-)	*S.aureus* (+)
Oil loaded steril disk	14	11
Oil containing membranes	12	9
Plain membrane	-	-

4. Conclusion

In this study, lavender oil used as antibacterial agent was entrapped in PEG fibers and these fibers were included in polymeric membranes. Thus the membranes were given antibacterial property. However, porosity, morphology, mechanical strength, hydrophilicity and wettability of the membranes were characterized. Water uptake capacity was determined to be high by means of porous membrane structure formed by the gaps between the electrospun fibers. It was determined by standard characterization tests that membrane surfaces were hydrophilic and the mechanical strength values of the membranes were high. Mass loss in time and controlled lavender oil release from the membrane due to dissolution of hydrophilic PEG polymer in aqueous media was observed by in vitro studies. It was concluded that oil release and mass loss occurred in controlled manner. It was observed that the oil included in the membranes exhibited antibacterial effect on *S. aureus* and *E. coli* species by diffusion from membrane into the medium. Finally, it is concluded that hydrophilic, durable, swellable and antibacterial membranes produced from biodegradable polymers can be used in the medical field as wound dressing, hernia mesh, cardiac patch etc.

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