TEKSTİL VE KONFEKSİYON

Vol: 33, No: 1
DOI: 10.32710/tekstilvekonfeksiyon. 1030386

Characterization and Antibacterial Activity of Electrospun Polyethylene oxide/Chitosan Nanofibers

Hanan Yunus[1] 0000-0002-1704-956X
Emel Ceyhun Sabır[1] 0000-0002-2385-1524
Halil İbrahim İçoğlu[2] 0000-0003-0687-4721
Behzat Yıldırım[3] 0000-0002-7787-0595
Osman Gülnaz[4] 0000-0003-4714-7651
Mehmet Topalbekiroğlu[3] 0000-0003-4345-8815

[1] Çukurova University / Textile Engineering Deptartment / Adana, Türkiye
[2] Gaziantep University / Metallurgical and Materials Engineering Department / Gaziantep, Türkiye
[3] Gaziantep University / Department of Textile Engineering Department / Gaziantep, Türkiye
[4] Çukurova University / Science and Technology Education Department / Adana, Türkiye

Corresponding Author: Hanan Yunus, hanantex@gmail.com

ABSTRACT

This study aims to characterize and evaluate polyethylene oxide (PEO) and chitosan (CS) nanofibers produced by electrospinning method. Electrospinning solutions were used at three different concentrations (1, 2, 3 wt%) with five different PEO/CS mixing ratios (30/70, 40/60, 50/50, 60/40, 100/0). FESEM, XRD and FTIR tests were applied for characterization of the nanofibers. Antibacterial activity of the nanofibers against Staphylococcus aureus and Klebsiella pneumoniae microorganisms was investigated using disk diffusion method. While 1 wt% of concentration was not suitable to obtain regular nanofibers, the nanofibers were uniform and largely free of beads at the other ones (2, 3 wt%). The average diameters of the nanofibers varied from 59 to 298 nm depending on the concentration and mixing ratio. Strong hydrogen bonds were formed between two polymers, while the crystal structure of PEO did not change significantly when mixed with chitosan. According to the study, whereas chitosan is resistant to a wide range of germs, PEO/CS nanofibers were not. The reason for this is because when chitosan is electrospun with PEO, the characteristics of the chitosan are altered by the concentrations and ratios used.

ARTICLE HISTORY
Received: 23.11.2021
Accepted: 26.05.2022

KEYWORDS
Chitosan, polyethylene oxide, electrospinning, nanofibers, antibacterial activity

1. INTRODUCTION

Nanotechnology plays an important role in human life as it offers advantages in various aspects of life. On the one hand, it is able to provide new physical properties to the polymers produced with nanotechnology; on the other hand, it is characterized by a high surface area compared to its size and its application in various industrial and medical fields [1]. Electrospinning technology is considered the simplest and least expensive technique for obtaining nanofibers. This technology is based on the production of nanofibers from the desired polymer contained in the extrusion needle, using a high electric field generated by applying a positive voltage to the polymer material, at the tip of the needle, and a negative voltage to the collector plate [2].

The need to preserve nature leads mankind to constantly seek natural alternatives and use them in various aspects of their lives. This has led them to use natural polymers such as chitosan (CS), which is obtained by the deacetylation process of chitin found in the shells of marine animals and fungi [3]. Chitosan is characterized by the presence of amine groups in its molecular structure, which are positively charged when chitosan is dissolved in weak or concentrated acids and thus is able to interact with other groups found in other compounds to obtain various mixtures in the form of films, gels, molecules or nanofibers [4]. Chitosan is characterized by its biocompatibility and biodegradability, in addition to its large presence in nature, which allows it to be easily obtained and applied in various

To cite this article: Yunus H, Sabır EC, İçoğlu Hİ, Yıldırım B, Gülnaz O, Topalbekiroğlu M. 2023. Characterization and antibacterial activity of electrospun polyethylene oxide/chitosan nanofibers. *Tekstil ve Konfeksiyon* 33(1), 1-8.

fields [5]. Because of the high viscosity of chitosan, it is very difficult to obtain it in the form of nanofibers by electrospinning method, so it must be mixed with other materials that help reduce its viscosity and facilitate the process of electrostatic spinning. Among the materials, there is polyethylene oxide (PEO), which is a non-toxic, water-soluble, synthetic polymer and stable in acidic media. It is characterized by biocompatibility and it becomes capable of being an electrospun nanofiber [6].

In a study by Singh, PEO was used to reduce the interlocking chitosan chains and obtain nanofibers. In this study, PEO/CS different mixing ratios and different concentrations of acetic acid were used. The study showed that increasing the acetic acid concentration and PEO content helped to obtain nanofibers with a larger diameter and a smaller number of beads [7]. To determine the effect of deacetylation degree of chitosan on its adhesion properties in mucous membranes, nanofibers were formed from the mixture of PEO/CS with different deacetylation degree of chitosan. It was found that the degree of deacetylation plays an important role in changing the physicochemical properties of chitosan. The higher the deacetylation degree of chitosan, the greater the stability of nanofibers in aqueous media and the degree of adhesion to mucous membranes increases [8]. In another study, the anti-inflammatory teicoplanin was coated with PEO/CS nanofibers, and this material showed a higher ability to resist bacteria when coated with PEO/CS nanofibers than when not coated, and the concentration of 4% teicoplanin was the best in resisting bacteria [9]. PEO/CS nanofibers were also used as a supporting membrane to which metal-organic frameworks-5 (MOF-5) nanoparticles were added, and they were used as effective filters to clean the air from PM2.5 (particulate matters≤ 2.5 μm), which are harmful to the lungs and cause cancer [10]. In another study, the PEO/CS nanofibers showed resistance to *S. aureus* but no resistance to e.coli except when phenolic was added to the PEO/CS nanofibers [11]. Pomegranate peel extract solution was added to the PEO/CS mixture and showed antibacterial resistance against e.coli [12].

There have been many studies that have looked at the method of producing PEO/CS nanofibers with good specifications. Some studies discussed the effect of the type of solvent and the addition of sodium chloride on the morphology of PEO/CS nanofibers [13], other studies discussed the different mixing ratios and concentrations of the chitosan and PEO mixture [14, 15], and there were many studies about the applications of PEO/CS nanofibers as filters [16] and antibacterial membranes [17], as well as their role in controlling some diseases caused by bacteria infecting plants [18].

There was limited studies on the effect of the solution concentrations below 4 wt% and the CS ratios below 50 wt% on morphological and structural properties of PEO/CS electrospun nanofibers. In this study, eletrospun PEO/CS nanofibers were produced by using electrospinning solutions at three different concentrations (1, 2, 3 wt%) with five different PEO/CS mixing ratios (30/70, 40/60, 50/50, 60/40, 100/0). Before electrospinning process, viscosity, surface tension and electrical conductivity of the solutions were measured. Morphology, chemical bonds and crystal structure of the nanofibers were investigated by using FESEM, FTIR and XRD, respectively. Lastly, the resistance of the nanofibers to some types of bacteria were tested.

2. MATERIAL AND METHOD

2.1 Material

In this study, two polymers (CS and PEO) were used. Low molecular weight CS (Mw: 50-190 kDa), and medium molecular weight PEO (Mw: 900 kDa) obtained from Sigma-Aldrich, were dissolved with (50 wt%) acetic acid by magnetic stirring for 24 h at room temperature. The electrospinning solutions were obtained by mixing of CS and PEO solutions at three different PEO/CS concentrations with five different mixing ratios by stirring at room temperature for 5 h (Table 1).

Table 1. The PEO/CS electrospinning solutions

Solution concentration (wt%)	PEO/CS ratio (wt/wt)
1	30/70-40/60-50/50-60/40-100/0
2	30/70-40/60-50/50-60/40-100/0
3	30/70-40/60-50/50-60/40-100/0

2.2 Nanofiber Production

Electrospinning method was used to produce PEO/CS nanofibers. The electrospinning setup with two variable DC high voltage power supplies (+50 kV and -50 kV), consisted of a syringe with needle, a syringe pump and a flat plate collector. While the needle was connected to the positive voltage supply, the collector plate was connected to a negative voltage supply. The applied voltage, solution flow rate, tip to collector distance (TCD), and needle inner diameter were kept constant as 25 kV, 15 μL/min, 20 cm, and 0.8 mm, respectively. During the electrospinning process, the ambient temperature and relative humidity were also constant at 30 °C and 50%, respectively.

2.3 Characterization

Viscosity, surface tension and electrical conductivity of PEO/CS electrospinning solutions were determined using Brookfield DV-III Ultra Rheometer, Attention Theta optical tensiometer and Orion 4 Star Plus meter, respectively. These measurements were made under standard laboratory conditions at 23 ± 2 °C of ambient temperature and 45 ± 5% of relative humidity. FTIR spectra were recorded in a frequency range of 4000-500 cm^{-1} with a resolution of 4

cm^{-1}. The Jasco FTIR 6800 was used. QUANTA FEG 650 scanning electron microscope (FESEM), was used to show the morphologies of the nanofibrous mats. The samples were coated with gold at 15 kV for 90 s before observing the fiber morphologies. Depending on the density of the nanofiber sample, between 20 and 40 nanofibers were randomly selected to determine the average diameters of the PEO/CS nanofibers. The Image J program was used to measure diameters of the nanofibers. A PANalytical X-ray diffractometer (model EMPYREAN XRD) was used for XRD measurements, with CuKα radiation (λ: 0.154059 nm) accelerated at a voltage and current of 45 kV and 40 mA, respectively. XRD patterns were recorded from 10° to 90° 2θ with a step size of 0.013°. Statistical analyzes were performed using SPSS statistical program (latest trial version) to investigate the relationship between nanofiber diameters at different mixing ratios and different concentrations. ANOVA tests were used and the results are considered significant at $p \leq 0.05$. The antibacterial activity of the PEO/CS nanofiber samples against the microorganisms *Staphylococcus aureus* ATCC25923 as Gram positive organism and *Klebsiella pneumoniae* ATCC43816 as Gram negative organism was investigated using the disk diffusion method. Two types of bacteria *S. aureus* and *K. pneumoniae* were distributed with sterile cotton swabs on the surface of Petri dishes filled with Mueller Hinton agar (LAB039, A Neogen company). Before spreading the bacteria in the Petri dishes, two types of bacteria were suspended in saline ($0.85\%wv^{-1}$) the day before the test to prepare them for the test. Samples containing only pure PEO nanofibers were used as control sample. After the different PEO/CS nanofiber samples were added to the Petri dishes with two types of bacteria, they were incubated at 37°C for 24h in the bacteriological incubator (Binder). In order to easily apply this assay, PEO/CS nanofibers were collected onto a nylon layer and these nylon layers loaded with PEO/CS nanofibers were cut as samples with the dimensions as 1×1 cm^2.

3. RESULTS AND DISCUSSION

3.1 Solution Characterization

The surface tension, viscosity, and electrical conductivity values of PEO/CS electrospinning solutions at different concentrations and mixing ratios were measured and given in Table 2.

According to Table 2, slight changes in the surface tension values of the solutions can be observed, but these changes did not follow a certain rule. The highest value was reached at sample PEO/CS: 30/70-2%, while the lowest value was at the sample of PEO/CS:50/50-3% with values 37.99, 36.43, respectively. However, significant changes occurred in the viscosity values with the increase in CS ratio, as well as with the increase in concentration of the solutions. The highest value of viscosity was obtained for the sample of PEO/CS:30/70-3%. Similarly, the cationic nature of chitosan causes the electrical conductivity of a PEO/CS mixture to increase by increasing the CS ratio and by increasing solution concentration [19, 20], so that the highest value of conductivity was also obtained for the sample of PEO/CS:30/70-3%. The high viscosity of this sample makes it difficult to increase the solution concentration to higher values, which makes it impossible to obtain nanofibers by electrospinning, so the concentration and CS ratio were not exceeded to higher values than in the sample of PEO/CS: 30/70-3%.

Table 2. The surface tension, viscosity and electrical conductivity of PEO/CS electrospinning solutions

PEO/CS solution concentration (wt%)	PEO/CS ratio (wt/wt)	Surface tension (mN/m)	Viscosity (cP)	Electrical conductivity (µS/cm)
1	30/70	36.84	121	860
	40/60	36.84	115	816
	50/50	37.04	112	783
	60/40	36.91	108	758
	100/0	36.76	54	730
2	30/70	37.99	947	1142
	40/60	36.93	895	1033
	50/50	36.62	713	945
	60/40	36.89	553	854
	100/0	36.62	401	702
3	30/70	37.45	4692	1463
	40/60	36.83	4613	1294
	50/50	36.43	4463	1151
	60/40	36.81	3630	986
	100/0	36.61	1695	675

3.2 FTIR Analysis of PEO/CS Nanofibers

Due to the steady increase in chitosan ratio at each concentration in the PEO/CS polymeric combination, analyzing all samples will reveal minor differences in the test. Thus it was preferable to chose two samples with the same concentration and leave it at that (2 wt percent). One sample containing the highest ratio of CS (70%) while the other containing chitosan free- pure PEO sample. To determine the effect of the incorporation of CS into the polymer blend with PEO. Figure 1 shows the relationship between the wavenumber of the functional groups present in both samples PEO/CS:30/70-2% and PEO 2% with the peak intensities of these groups.

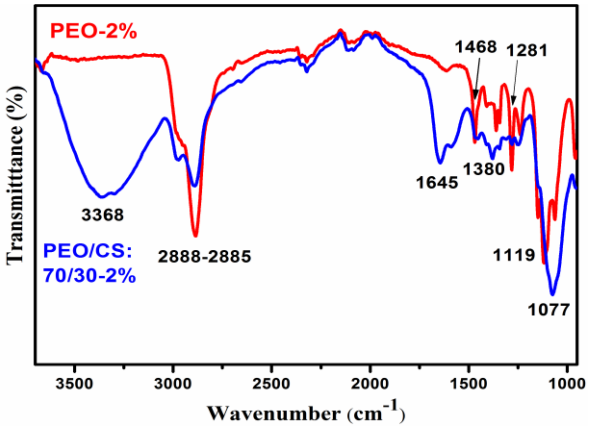

Figure 1. The FTIR spectra of PEO 2% and PEO/CS: 30/70-2%

A broad range from 3100 cm^{-1} to 3600 cm^{-1} can be observed in the sample PEO/CS:30/70-2%, indicating the formation of hydrogen bonds between the CS and PEO. A peak appears at the wavenumber 3368.07 cm^{-1} which is due to the OH stretching vibration of the polysaccharide. While at the same broad range in the pure PEO 2% sample, no peak or extended bond is seen, indicating the presence of hydrogen bonds, confirming that hydrogen bonds were formed only when CS entered the PEO sample [7, 9, 21]. In PEO/CS nanofibers, the peaks at 2887.88, 1453.10, 1281.47, 1077.05 cm^{-1} indicate the stretching of aliphatic C-H, pending of C-H, stretching vibration of C-OH and stretching vibration of C-O groups in glycoside bond of polysaccharide structure of chitosan, respectively. Whereas these groups were observed in PEO sample at wavenumbers of 2884.99, 1468.53, 1281.47, 1063.55 cm^{-1}, respectively [7-9, 21]. In the PEO/CS sample, pending of secondary amine group NH and stretching of CN group can be observed at wave numbers 1645.95 and 1379.82 cm^{-1}, respectively [7, 8]. A peak at 1119.48 cm^{-1} is seen, indicating the presence of the C-O-C group in the PEO sample [9, 21].

3.3 XRD Analysis of PEO/Chitosan Nanofibers

The highest CS ratio (70 wt%) was chosen for XRD analysis to see obvious differences on the crystalline structures according to neat PEO nanofibers at each concentration of 1, 2, 3 wt% (Figure 2). In PEO 1% sample, peaks were observed at angles 78.23°, 64.90°, 44.55°, while in PEO/CS:30/70-1% they were at angles 78.60°, 65.25°, 45.25° (Figure 2a). Similarly, peaks in PEO 2% appear at angles 78.39°, 64.72°, and 44.49°, while in PEO/CS:30/70-2% they were at angles 78.39°, 65.68°, and 45.24° (Figure 2b). Peaks also appear in PEO 3% at angles 77.88°, 64.90°, 44.90°, while in PEO/CS:30/70-3% they were at angles 78.95°, 65.25°, 44.90° (Figure 2c).

From the previous results, besides the fact that the incorporation of chitosan, even at its highest percentage, that there was a large congruence in the angles of refraction. However, as a result of the amorphous chitosan structure there was a decrease in the height of the peaks, so it can be said that chitosan had no effect on the crystal structure of PEO, also increasing the concentration of the polymer mixture from 1 wt% to 3 wt% did not lead to any significant effect on the crystal structure of the samples [7, 11, 22]. According to the results, the introduction of chitosan in the PEO sample did not lead to any significant change in the crystal structure.

3.4 Morphological Analyses

By applying FESEM analysis to each of the samples, the average diameters of PEO/CS nanofibers were calculated according to different mixing ratios and concentrations (Table 3).

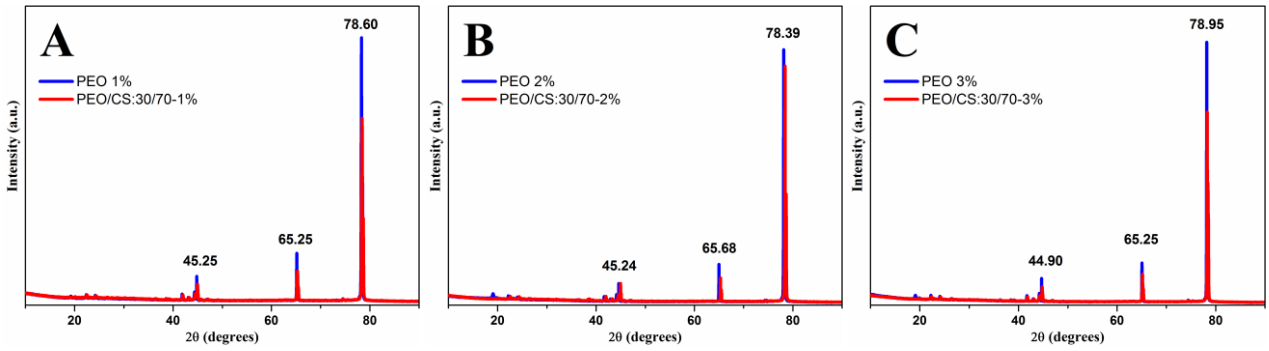

Figure 2. XRD analysis for PEO 1% and PEO/CS:30/70-1% nanofibers (A), PEO 2% and PEO/CS:30/70-2% nanofibers (B), PEO 3% and PEO/CS:30/70-3% nanofibers (C)

Table 3. PEO/CS average nanofiber diameters [nm] according to different mixing ratios and concentrations

PEO/CS concentration (wt%)	PEO/CS (wt/wt)				
	30/70	**40/60**	**50/50**	**60/40**	**100/0**
1	59 ± 14	82 ± 17	89 ± 21	102 ± 18	97 ± 19
2	137 ± 16	164 ± 28	191 ± 49	208 ± 56	225 ± 46
3	138 ± 24	166 ± 26	216 ± 31	222 ± 30	298 ± 48

The average diameters of the nanofibers increase with the increase of the concentration of the solution, which was statistically significant ($p<0.05$). Since the increase in the concentration of the solutions is accompanied by an increase in its viscosity, fibers with a thicker diameter and fewer beads can be obtained.

The chitosan-free PEO sample should theoretically have a larger diameter than any other sample containing chitosan, but the concentration of 1 wt% was very low viscosity, which made it difficult to form nanofibers by electrospinning, so PEO 1% nanofibers appeared with small diameters filled with beads (Figure 3). For all of the concentrations, the diameters of PEO/CS nanofibers decreased with the increase of the ratio of chitosan. Although the presence of chitosan contributes to the high viscosity of the solution, this must be accompanied by an increase in the diameters of the nanofibers. However, the strong hydrogen bonds that chitosan forms with other polymers, in addition to its cationic nature, which increases the electrical conductivity of the solution, makes the diameters of the fibers decrease even if the viscosity of the solution increases [7, 11]. While the relationship between average diameter and mixing ratio is not statistically significant for 1 wt% ($p>0.05$), it is signicant for 2 wt% and 3 wt% ($p<0.05$).

As can be seen in (Figure 3), the 1 wt% of concentration was completely unsuitable for obtaining regular, bead-free nanofibers. The chitosan-free PEO nanofibers showed full of beads. The introduction of chitosan helped to improve the viscosity of the solution, but the beads still occurred, so the fibers resulting from the 1 wt% concentration cannot be considered as regular nanofibers. The PEO/CS:60/40 ratio can be considered the best in terms of small number of beads, but the nanofibers were like flat strips. Figure 4 shows FESEM images of the nanofibers at 2 wt% of concentrations.

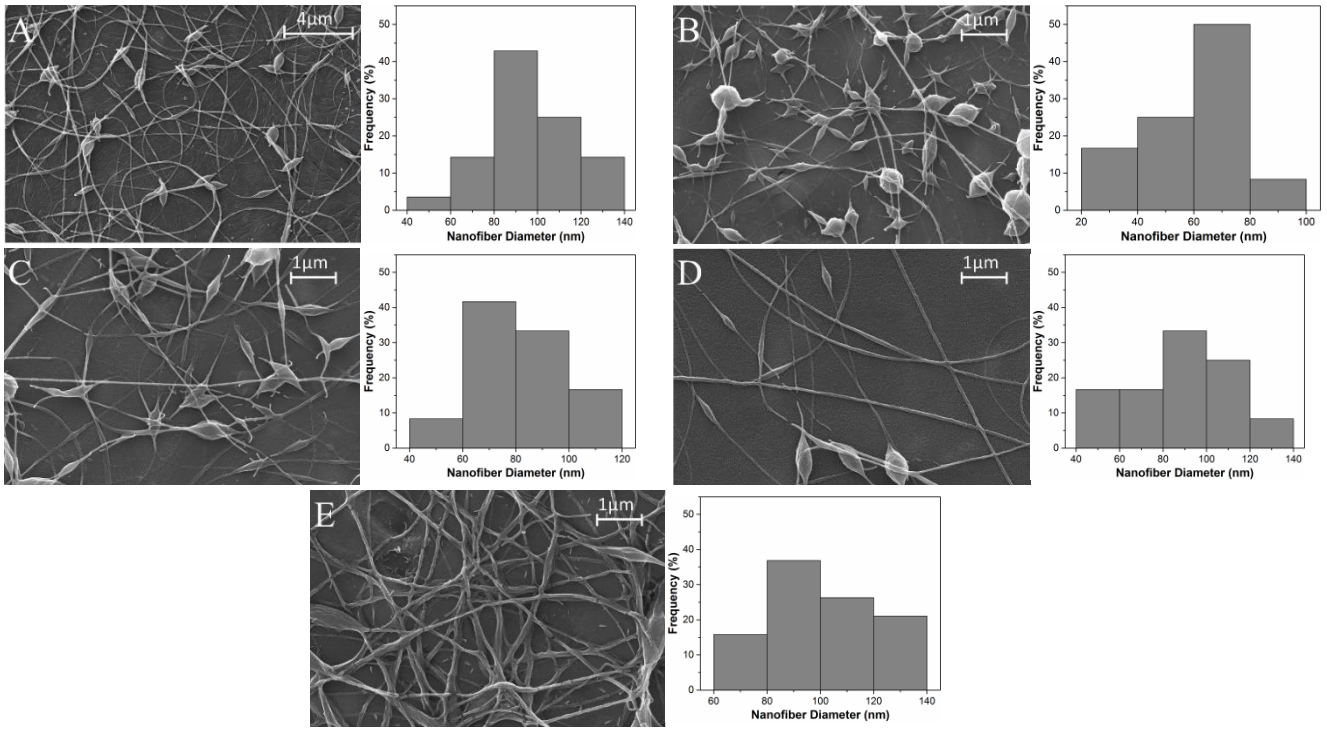

Figure 3. The electrospun nanofibers at concentration of 1 wt%: PEO/CS:100/0 (A), PEO/CS:30/70 (B), PEO/CS:40/60 (C), PEO/CS:50/50 (D), PEO/CS:60/40 (E)

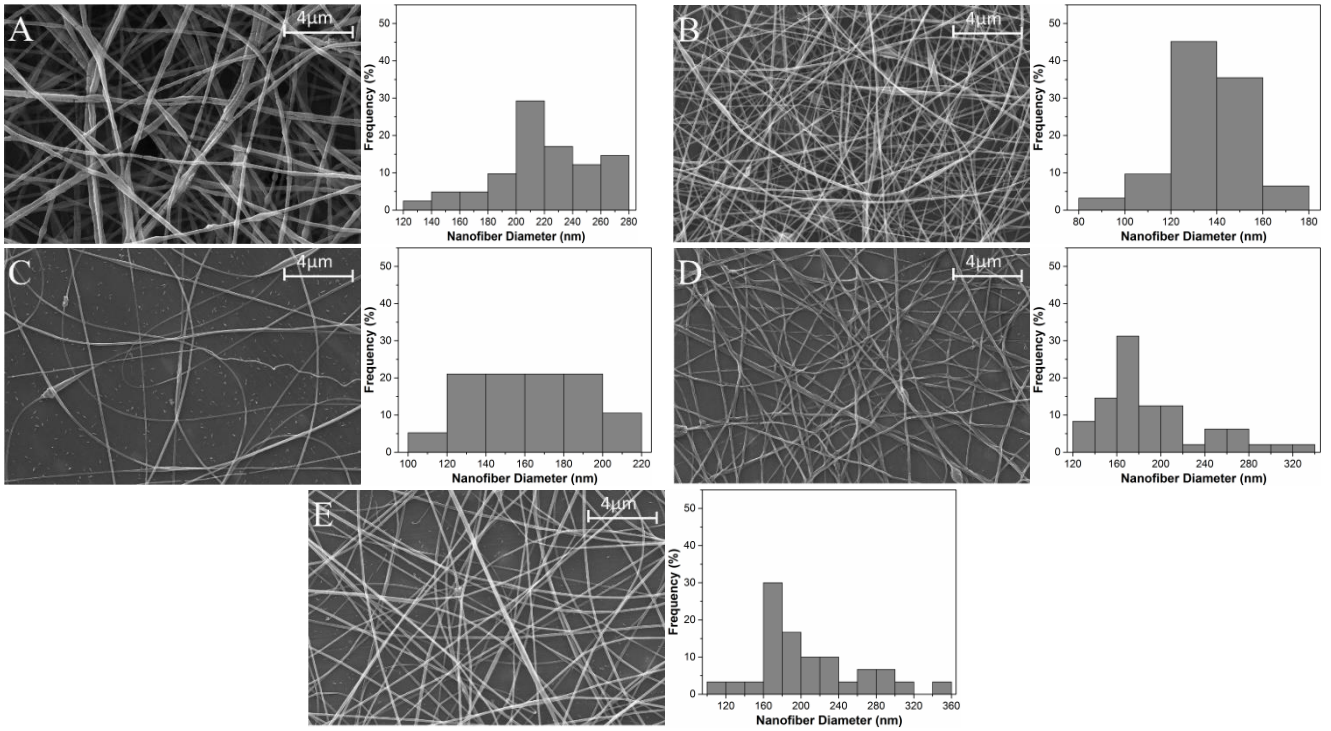

Figure 4. The electrospun nanofibers at concentration of 2 wt%: PEO/CS:100/0 (A), PEO/CS:30/70 (B), PEO/CS:40/60 (C), PEO/CS:50/50 (D), PEO/CS:60/40 (E)

The nanofibers at concentration of 2 wt% were produced as more uniform and regular and bead-free. The nanofibers containing chitosan at different ratios compared to the neat PEO nanofibers have a more regular shape. When the histogram graphs of nanofibers were examined, the nanofiber diameter distribution of the PEO/CS:30/70 was more uniform than the others (Figure 4). FESEM images of PEO/CS nanofibers at 3 wt% of concentration were given in Figure 5.

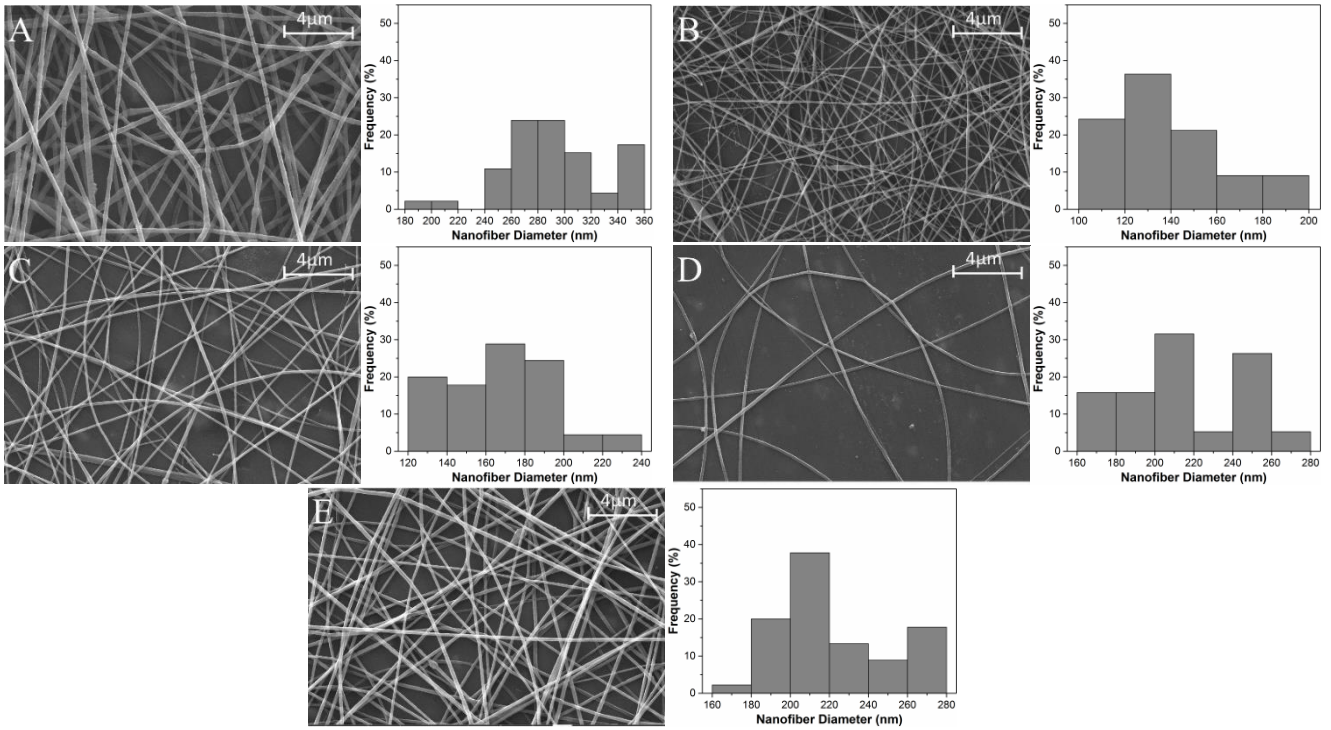

Figure 5. The electrospun nanofibers at concentration of 3 wt%: PEO/CS:100/0 (A), PEO/CS:30/70 (B), PEO/CS:40/60 (C), PEO/CS:50/50 (D), PEO/CS:60/40 (E)

The nanofibers at concentration of 3 wt% were genarally produced more uniformly and regularly than those of 1 wt% and 2 wt%. Similarly, the diameter distributions of the nanofibers at 3 wt% are more regular for all mixing ratios. Neat PEO nanofibers showed a wider range of nanofiber diameter distribution. The nanofibers at the highest CS ratio at 3 wt% of concentration were more regular and uniform.

3.5 Evaluation of Antibacterial Activity

Antibacterial activity of PEO and PEO/CS samples, against *S. aureus* and *K. pneumoniae* was given in Figure 6 and Figure 7, respectively. PEO sample was applied disk diffusion method for the determination of its antibacterial properties. Three disks were placed on agar and PEO prepared in different concentrations (1, 2 and 3 wt%) were poured onto the disk as a solution using a needle. As Figure 6 shows, two types of bacteria completely surrounded the disks and areas of inhibition were not formed [23].

The PEO/CS nanofiber samples with different mixing ratios and concentrations showed no inhibition zone against *S. aureus* or *K. pneumoniae* bacteria in the surrounding area of PEO/CS samples. The nylon layer loaded with PEO/CS nanofibers appeared as an transparent area within the agar, with no space between it and the bacteria. Thus, it can be said that despite the antibacterial properties of chitosan as a cationic polymer, the process of electrospinning with other polymers prevented the occurrence of these properties because the PEO molecules were able to surround the chitosan molecules in the process of electrospinning and prevent the occurrence of the antibacterial properties [12]. At the same time, other studies indicate that the antibacterial property of chitosan does not occur continuously and is closely related to its physical properties, especially its molecular weight [24]. Therefore, this study tested the highest concentrations and the highest proportions of chitosan that can be obtained from this low molecular weight of chitosan in the form of nanofibers via electrospinning technique.

At all the high concentrations and proportions of chitosan, the PEO molecules were able to prevent the occurrence of the antibacterial property of chitosan. Therefore, it is difficult to use these fibers in an application that depends on the antibacterial properties of chitosan, such as wound dressing or medical tissues, without adding other materials with antibacterial properties to support the property of chitosan. However, due to the uniformity of the fibers resulting from the concentrations (2, 3 wt%) and their absence of beads, it is recommended to use them as packaging fibers for different materials such as medicines, fertilizers or in the field of filtration. The identical results were seen in all tested samples, so just antibacterial tests of PEO/CS:30/70-3% sample was given as an example (Figure 7).

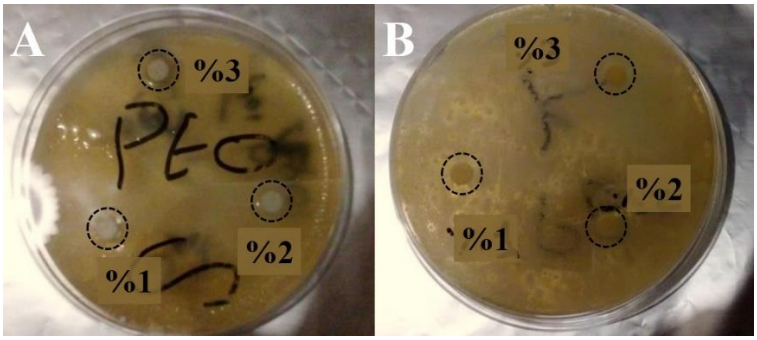

Figure 6. Antibacterial activity for PEO solution against *S. aureus* (A) and *K. pneumoniae* (B)

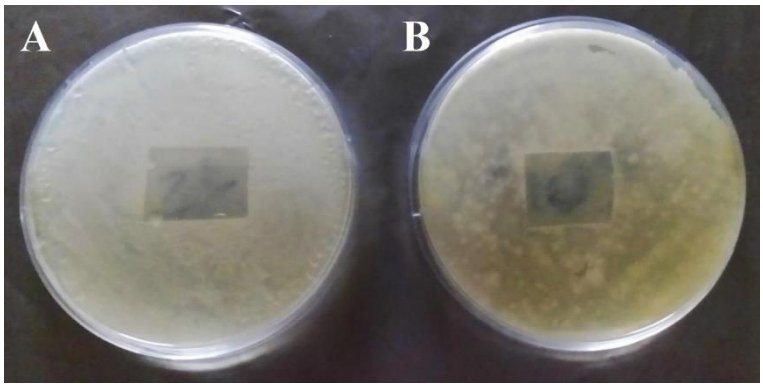

Figure 7. Antibacterial activity of PEO/CS:30/70-3% sample against *S. aureus* (A) and *K. pneumoniae* (B)

4. CONCLUSION

In this study, nanofibers were produced from a mixture of low molecular weight chitosan and medium molecular weight PEO at different concentrations and mixing ratios by electrospinning method. Increasing of CS ratio and soltion concentration caused to increase both viscosity and electrical conductivity of the solutions. While 1 wt% of concentration was not suitable to obtain regular nanofibers, the other concentrations are better to obtain more regular, uniform and bead-free nanofibers. The sample of PEO/CS:60/40 at 3 wt% has the largest diameter and the least amount of beads among CS containing nanofibers. The average diameters of the nanofibers increased with the increase of the concentration of the solution, which was statistically significant ($p<0.05$). The average diameters of PEO/CS nanofibers decreased with the increase of chitosan ratio at all concentrations. CS caused to increase of H bonds intensity of PEO nanofibers. Existance of CS in the PEO nanofibers did not lead to any significant change in the crystal structure. There was no antibacterial activity of the PEO/CS nanofibers to two types of bacteria *S. aureus* and *K. pneumoniae*. PEO/CS nanofibers can be used as packaging fibers for different materials such as medicines, fertilizers or in the field of filtration due to their small diameters, uniform and bead-free nanfiber formation.

Acknowledgement

This study was supported by the Scientific Research Projects Unit of Çukurova University (Project code: FDK-2019-11674).

REFERENCES

1. Cengiz Çallıoğlu F. 2013. The production of nanofiber by roller electrospinning method. *Tekstil ve Mühendis* 20(91), 35-49.
2. Göktepe F, Mülayim BB. 2015. Nanofiber yarn production methods by electrospinning. *Tekstil ve Mühendis* 22(99), 51-67.
3. Antonino RSCMQ, Fook BRDL, Lima EPN, Lima RJS, Fook MVL, Covas CAP, Rached RIF, Lima VAO. 2017. Preparation and characterization of chitosan obtained from shells of shrimp. *Marine Drugs* 15(141), 1-12.
4. Ayodele O, Okoronkwo AE, Oluwasina OO, Abe TO. 2018. Utilization of blue crab shells for the synthesis of chitosan nanoparticles and their characterization, *Songlanakarin Journal of Science and Technology* 40(5), 1043-1047.
5. Sandeep A, Sangameshwar K, Mukesh G, Chandarktan R, Avinash D. 2013. A brief overview on chitosan applications. *Indo American Journal of PHarmaceutical Research* 3(12), 1564-1574.
6. Hung L, Nagapudi K, Apkarian R, Chaikof E, 2001. Engineered collagen–PEO nanofibers and fabrics. *Journal of Biomaterials Science, Polymer Edition* 12(9), 979–993.
7. Singh Y, Dasgupta S, Nayar S, Bhaskar R. 2020. Optimization of electrospinning process & parameters for producing defect-free chitosan/polyethylene oxide nanofibers for bone tissue engineering. *Journal of Biomaterials Science, Polymer Edition* 31(6), 1-23.
8. Stie M, Catke J, Wan F, Chronakis I, Jascobsn J, Nielsen H. 2020. Swelling of mucoadhesive electrospun chitosan/polyethylene oxide nanofibers facilitates adhesion to the sublingual mucosa. *Carbohydrate Polymers* 242, 1-29.
9. Amiri N, Ajami S, Shahroodi A, Jannatabadi N, Darbab S, Bazzaz B, Pishavar E, Kalalinia F, Movaffagh J. 2020. Teicoplanin-loaded chitosan-PEO nanofibers for local antibiotic delivery and wound healing. *International Journal of Biological Macromolecules* 162, 645-656.
10. Pan W, Wang J, Sun X, Wang X, Jian J, Zhang Z, Hao Qu C, Zelong Y, Feng G. 2020. Ultra uniform metal−organic framework-5 loading along electrospun chitosan/polyethylene oxide membrane fibers for efficient PM2.5 removal. *Journal of Cleaner Production* 291, 1-29.
11. Kuntzler S, Costa J, Morais M. 2018. Development of electrospun nanofibers containing chitosan/PEO blend and phenolic compounds with antibacterial activity. *International Journal of Biological Macromolecules* 117, 800-806.
12. Surendhiran D, Li C, Cui H, Lin L. 2020. Fabrication of high stability active nanofibers encapsulated with pomegranate peel extract using chitosan/PEO for meat preservation. *Food Packaging and Shelf Life* 23, 1-9.
13. Martinova L, Lubosova D. 2008. Electrospun chitosan based nano fibers. *Tekstil ve Konfeksiyon* 12(2), 22-79.
14. Lemma SM, Bossard F, Rinaudo M. 2016. Preparation of pure and stable chitosan nanofibers by electrospinning in the presence of poly ethylene oxide. *International Journal of Molecular Sciences* 17, 1-16.
15. Bizarria MTM, Davila MA, Mei LHI. 2014. Non woven nano fiber chitosan/PEO membranes obtained by electro spinning. *Brazilian Journal of Chemical Engineering* 31(1), 57-68.
16. Min LL, Yuan ZH, Zhong LB, Liu Q, Wu RX, Zheng YM. 2014. Preparation of chitosan based electrospun nanofiber membrane and its adsorptive removal of arsenate from aqueous solution. *Chemical Engineering Journal* 267, 132-141.
17. Rieger KA, Schiffman JD. 2014. Electrospinning an essentialoil: cinnamaldehyde enhances the antimicrobial efficacy of chitosan/ Poly(Ethylene Oxide) nanofibers. *Carbohydrat Polymers* 113, 561-568.
18. Noruzi M. 2016. Electrospun nanofibers in agriculture and the food industry: A review. *Society of Chemical Industry* 10, 1-16.
19. Koosha M, Mirzadeh H. 2015. Electrospinning, mechanical properties, and cell behavior study of chitosan/PVA nanofibers, *Society For Biomaterials* 103(9), 3081-3093.
20. Paipitaka K, Pornprac T, Mongkontalangc P, Techitdheera CW, Pecharapaa W. 2011. Characterization of PVA-chitosan nanofibers prepared by electrospinning. *Science Direct* 8, 101-105.
21. Aliabadi M, Irani M, Ismaeili J, Piri H, Parnian MJ. 2013. Electrospun NanofiberMembrane of PEO/Chitosan for the Adsorption of Nickel, Cadmium, Lead And Copper Ions From Aqueous Solution. *Chemical Engineering Journal* 220, 237-243.
22. An J, Zhang H, Zhang J, Zhao Y, Yuan X. 2009. Preparation and antibacterial activity of electrospun chitosan/poly(ethylene oxide) membranes containing silver nanoparticles, Colloid and Polymer Science 287(12), 1425-1434.
23. Gatti J, Smithgall M, Paranjape S, Rolfes R, Parajope M. 2013. Using electrospun poly(ethylene-oxide) nanofibersfor improved retention and efficacy of bacteriolytic antibiotics. Biomedical Microdevices 15(5),887-93.
24. Benhabiles MS, Salah R, Lounici H, Drouiche N, Goosen MFA, Mameri N. 2012. Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. *Food Hydrocolloids* 29, 48-56.