

PREPERATION AND ANTIBACTERIAL INVESTIGATION OF POLYCAPROLACTONE/CHITOSAN NANO/MICRO FIBERS BY USING DIFFERENT SOLVENT SYSTEMS

FARKLI SOLVENT SİSTEMLERİ KULLANILARAK POLİKAPROLAKTON/KİTOSAN NANO/MİKRO LİFLERİN ÜRETİMİ VE ANTİBAKTERİYEL ÖZELLİKLERİNİN İNCELENMESİ

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ABSTRACT

Chitosan (CHI) blended polycaprolactone (PCL) nano/micro fibers were prepared with different CHI content via electrospinning procedure. Two different solvents, acetone and formic acid (FA) were used to dissolve and blend the polymers before electrospinning process. Effect of the solvent on the electrospinnability of the blend, final nano/micro fiber morphologies, chemical and thermal properties and antibacterial activities were investigated with SEM, FTIR, DSC, and ASTM 2149 01 Standard Dynamic Contact Conditions. The results revealed that chitosan particles were encapsulated in the as-spun PCL fibers with using acetone as the solvent resulted in reduced antibacterial activities. Contrarily, when FA is used as the solvent, CHI and PCL were dissolved and blended very well, and enhanced antibacterial activities were obtained from as-spun PCL/CHI nano/micro fibers.

Keywords: Antibacterial materials, electrospinning, chitosan, polycaprolactone, nanofiber

ÖZET

Farklı oranlarda karışımı yapılmış kitosan/polikaprolakton (CHI/PCL) nano/mikro lifler elektroçekim metoduyla üretilmiştir. Elektroçekim çözeltileri aseton ve formik asit (FA) olmak üzere iki farklı solvent kullanılarak hazırlanmıştır. Solvent etkisinin üretilen nano/mikro liflerin morfolojileri, kimyasal ve termal özellikleri ve antibakteriyel aktiviteleri üzerindeki etkileri SEM, FTIR, DSC ve ASTM 2149 01 standardına göre antibakteriyellik analizleri yapılarak ölçülmüştür. Elde edilen sonuçlara göre, solvent olarak aseton kullanıldığında kitosanın partikül olarak PCL lifler tarafından kapsüle edildiği ve liflerin antibakteriyellik özelliklerini düşürdüğü gözlemlenmiştir. Diğer taraftan, solvent olarak formik asit kullanıldığında kitosan ve polikaprolaktonun elektroçekim çözeltisi içerisinde tamamen çözüldüğü, kapsülasyonun olmadığı, üretilen ağsı yapı içerisinde hem PCL hemde CHI fibriller yapıda bulunduğu ve elde edilen yapıların daha iyi antibakteriyel özelliklerde oldukları tespit edilmiştir.

Anahtar Kelimeler: Antibakteriyel malzemeler, elektroçekim, kitosan, polikaprolakton, nanolif

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1. Introduction

Development of antibacterial materials has critical importance due to increased number of pandemics and epidemics all over the world [1]. Therefore antibacterial functionalization of the surfaces is necessary in majority of the areas including textile materials, food packing and the surfaces where people always touch there. Various antibacterial agents including *N*-halamines, metal ions, quaternary ammonium compounds, synthetic mimics of antibacterial peptides and chitosan are reported in the literature [2, 3]. Among these, chitosan draws attention due to its biocompatibility and bioactivity [4]. Being one of the most abundant polymer after cellulose, chitosan is a reasonable antibacterial material which suitable to use for

various applications including textiles, coatings and packing [4].

PCL is widely used in biomedical applications due to its unique biocompatible and biodegradable structure. PCL nanofibers have been developed for use as wound dressings [5], biomedical scaffold for tissue engineering [6, 7], drug delivery [8], vascular grafts [9], and water filters [10]. All of these aforementioned applications require antibacterial functionalities as they are mainly used in medical environment. In this regard, chitosan was mainly used to fabricate antibacterial PCL membranes by blending chitosan and PCL in a common solvent [11, 12]. Along with blend electrospinning, grafting and coaxial electrospinning were also used to develop chitosan grafted PCL

nanoparticles [13] and core-shell PCL/CHI nanofibers, respectively. PCL/ CHI nanofibers for liver tissue engineering has been also investigated [14].

Electrospinning is a novel technology that helps to produce continuous nanofibrous structure from variety of precursors to final structured materials including polymers, ceramics and carbon [15-17]. In the procedure, a proper solution is prepared from the precursor and electrospun into nanofibers by applying high voltage electricity. Because of the application of electricity, the solution droplet is elongated until reaching to collector and form nanofiber structure. During the journey of the solution droplet, solvent is evaporated and the dried fibers are collected.

It is well known that the solvents used to dissolve PCL for electrospinning affects the surface morphologies, electrospinnability and biocompatibility. This relationship is well studied in the literature [18]. Various studies have been reported on PCL/CHI nanofibers with antibacterial activities, solvent effect on the morphologies and the biocidal activities of the PCL/CHI nanofibers has not systematically been investigated yet. In this study compares morphological, thermal and biocidal properties of the PCL/CHI nano/micro fibers fabricated with blending in formic acid and acetone.

2. Materials and Methods

2.1. Chemicals

For electrospinning solution, Chitosan (Mw: 100,000-300,000) was obtained from Acros Organics and polycaprolactone (Mn:80,000) was received from Sigma Aldrich. Acetone and Formic Acid obtained from Sigma Aldrich were used as the solvent. For antibacterial tests, the mediums for supporting growth and cultivation of microorganism such as Tryptic soy broth (TSB), Nutrient Broth (NB) and Nutrient Agar (NA) were supplied by Becton Dickinson. For preparing buffer solution, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (sodium dihydrogen phosphate) and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (disodium hydrogen phosphate dodecahydrate) were purchased from Sigma Aldrich.

2.2. Electrospinning method

15 wt% of PCL was dissolved in acetone and FA separately by magnetically stirring. Then, a proper amount of chitosan (PCL/CHI: 95/5, 80/20, 50/50) was added in the as-prepared solution and stirred. Final prepared solution was loaded in a plastic syringe fitted with a stainless steel needle (0.508 mm i.d.). A grounded metal collector was placed in front of the needle where polymer solution was fed. Applied voltage and the distance were around 15 kV and 15 cm. Flow rate were around 4 ml/hr for acetone samples and 1 ml/hr for FA samples. Polymer solution droplet in front of the metal needle was ejected from needle to collector plate as a result of the applied voltage. Finally, the solvent was evaporated until the nano/micro fibers reach to the collector and dried nano/micro fibers were collected on the grounded collector plate.

2.3. Nano/micro fibers characterizations

The morphology of nano/micro fibers was explored using a scanning electron microscope (ZEISS EVO 40 with thermionic electron gun) with an acceleration voltage of 20 kV. In order to reduce charging during SEM imaging and getting clear images, samples were placed on a sample holder and coated with gold-palladium in a proper thickness

using a BAL-TEC SCD005 sputter coater. Nano/micro fibers were also analyzed with an optic microscope. Attenuated total reflection Fourier transform infrared spectra (ATR-FTIR) of nano/micro fibers were recorded using a Thermo Nicolet iS50 in the wavenumber range of 4,000 to 400 cm^{-1} at room temperature. At least 124 scans were collected to minimize noise. DSC data were obtained using a Perkin Elmer DSC 8000. The experiments were conducted with a heating rate of 10°C/min under nitrogen atmosphere.

2.4. Antibacterial activity measurements

The antibacterial efficiency was quantitatively evaluated by the ASTM 2149 01 Standard Dynamic Contact Conditions. The samples were tested against Gram-negative bacteria (*Escherichia coli* ATCC 35218). In this test, a homogenous suspension of bacteria was prepared in NB and diluted with buffer solution at pH 7. The standardized concentration of about 10^5 cfu/ml was applied to samples for the antibacterial testing. All the samples were incubated at 37°C and shaken in a wrist-action shaker for 24 hours. The antibacterial activity was expressed in % reduction of the organisms after contact with the test sample at time '0' compared to the number of bacterial cells surviving after contact with the sample after 24 hours. The percentage reduction (R) of bacteria was calculated using following formula: $R = 100 (B - A) / B$, where, A is the number of bacteria recovered from the inoculated treated test sample in the jar incubated for 24 hours; and B is the number of bacteria recovered from the inoculated treated test sample at '0' contact time. Antibacterial test procedure were adapted to the previous reported papers by Orhan et al. [19, 20].

3. Results and Discussions

As a concept in this study, first CHI/PCL solutions were prepared by using acetone and FA separately. Schematic illustration of the preparation, electrospinning process and nanofiber morphologies of the as-spun CHI/PCL nano/micro fibers by using acetone and FA solvents are demonstrated in Figure 1. As seen from SEM and optical microscopy images, the as-spun nano/micro fibers structures were differentiated. When acetone was used as the solvent, CHI particles were encapsulated by PCL nano/micro fibers (Figure 1, A1, A2). On the other hand, when FA was used, ultrafine fibrillar structures were formed among the blended nano/micro fibers (Figure 1, B1, B2). The details of the phenomenon are discussed as following.

3.1. Morphology analysis of as-spun nano/micro fibers

High magnification morphology analyses of fibers were conducted via SEM and given in Figure 2 and 4. Fiber average diameters were determined from SEM images. Average fiber diameters range for fibers prepared from CHI/PCL solutions by using acetone, 1234 nm for pure PLC and increases upto 1648 nm for PCL/CHI (95/5), 1682 nm for PCL/CHI (80/20) and 2832 nm for PCL/CHI (50/50). As seen from Figure 2, increased fiber diameter observed with addition of chitosan into PCL nano/micro fibers. Chitosan particles were encapsulated in the PCL nano/micro fibers when acetone was used as the solvent. The encapsulation was clearer in high concentration chitosan sample (Figure 2D).

In order to support chitosan encapsulation in the ultrafine nano/micro fibers, optical microscopy analysis was also

conducted. As seen in Figure 3, encapsulated chitosan concentration increased with addition of more chitosan in PCL-acetone electrospinning solution, since chitosan particles were not totally dissolved in the solution. Because

chitosan particles were encapsulated, fiber breakage was also observed at some points as a result of stress formation along the fibers during electrospinning.

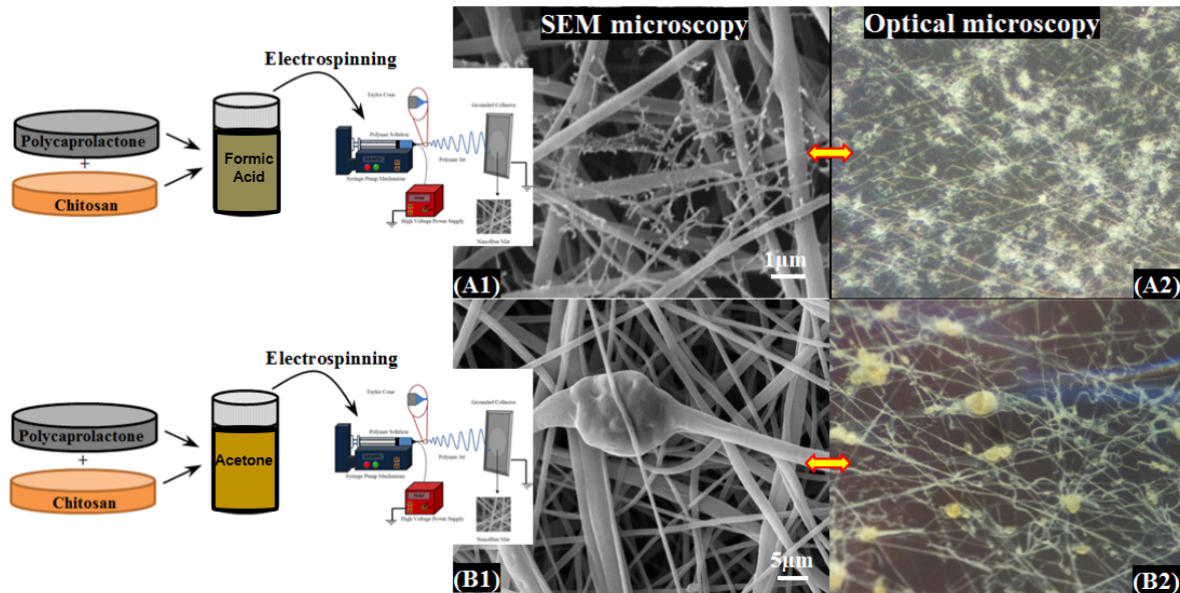


Figure 1. Schematic illustration of solution preparation, electrospinning process and nanofiber morphologies of as-spun CHI/PCL (50/50) nano/micro fibers by using acetone and FA as solvents. The used magnifications for optical microscopy are 600x.

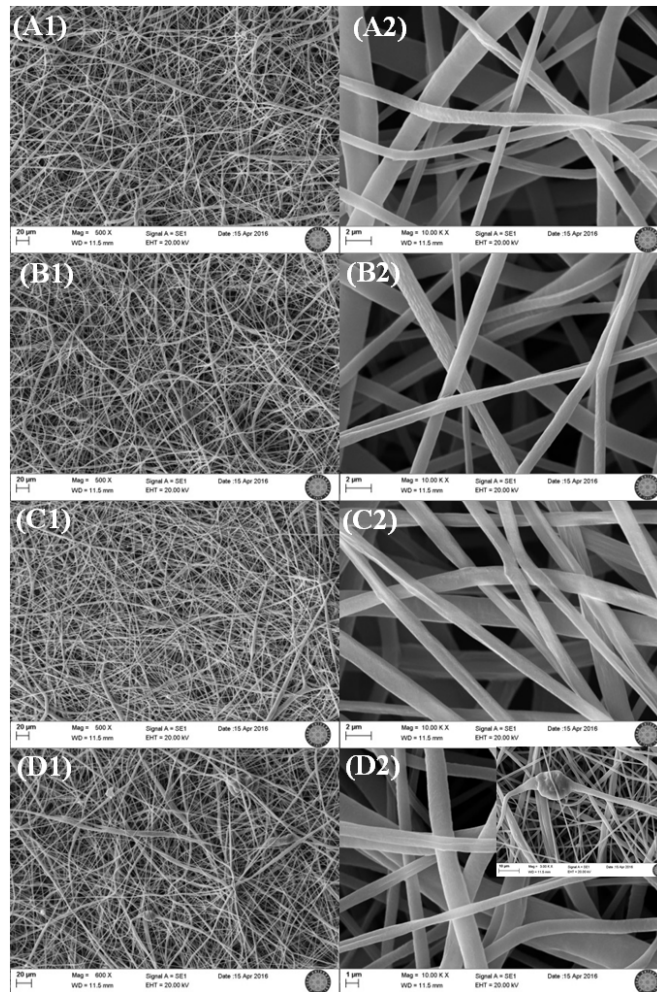


Figure 2. SEM images of acetone solutions of as-electrospun (A1, A2: PCL), (B1, B2: PCL/CHI (95/5)), (C1, C2: PCL/CHI (80/20)) and (D1, D2: PCL/CHI (50/50)) nano/micro fibers.

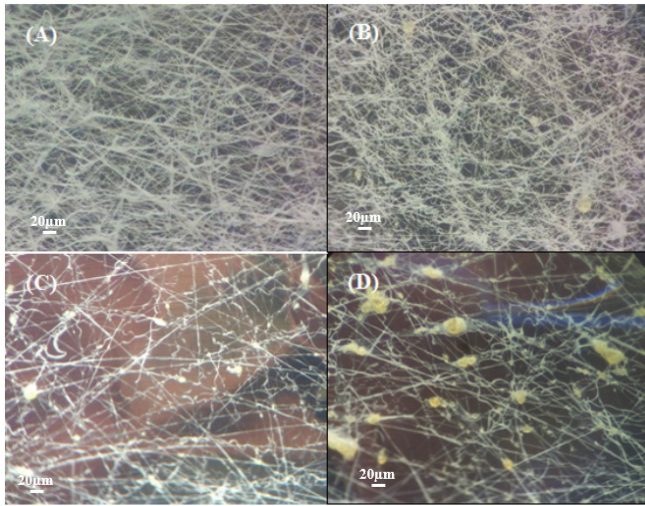


Figure 3. Optic microscope images of acetone solutions of as-electrospun (A: PCL), (B: PCL/CHI (95/5)), (C: PCL/CHI (80/20)) and (D: PCL/CHI (50/50)) nano/micro fibers. These nano/micro fibers are the same as in Figure 2.

Fiber average diameters were determined from SEM images. Except to ultrafine nano fibers, average fiber

diameters in the mat for FA solution prepared nano/micro fibers range from 214 nm for pure PLC and decreases to 171 nm for PCL/CHI (95/5) and increases again by increasing CHI contents in nanofiber upto 187 nm for PCL/CHI (80/20) and 446 nm for PCL/CHI (50/50). Ultrafine nano fibrillation was observed in the nanofiber mat with addition of chitosan and this trend increased with increasing chitosan concentration (Figure 4B1-4B4). Similar trend was also reported by Schueren et al. by electrospinning of PCL/CHI NFs using acetic acid/formic acid mix solution [21].

In order to check if there was encapsulation as in the case of PCL/CHI-fa nano/micro fibers, optical microscopy analyses were also conducted for the PCL/CHI-acetone samples. As seen in Figure 5, no chitosan encapsulation was observed. Instead, blended PCL/CHI nano/micro fibers were obtained. This can be proved by looking at the electrospinning solution, since PCL/FA solution was transparent supporting homogeneous solution formation.

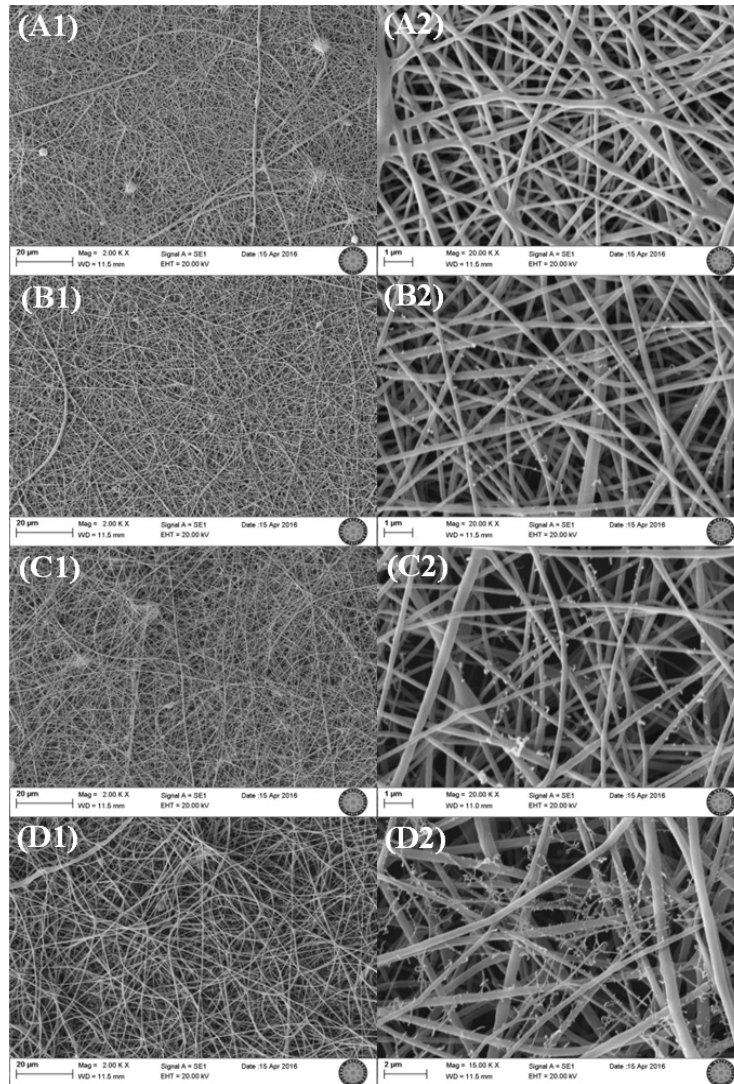


Figure 4. SEM images of FA as-electrospun (A1, A2: PCL), (B1, B2: PCL/CHI (95/5)), (C1, C2: PCL/CHI (80/20)) and (D1, D2: PCL/CHI (50/50)) nano/micro fibers.

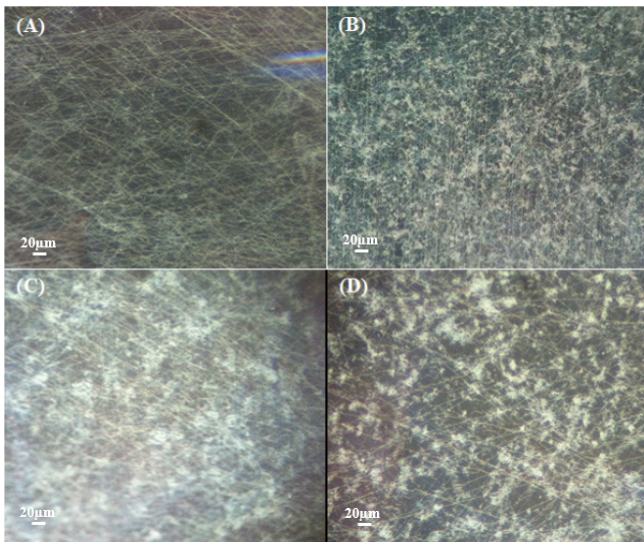


Figure 5. Optic microscope images of FA as-electrospun (A:PCL), (B: PCL/CHI (95/5)), (C: PCL/CHI (80/20)) and (D: PCL/CHI (50/50)) nano/micro fibers. These nano/micro fibers are the same as in Figure 4.

3.2. Chemical analysis via ATR-FTIR

Figure 6A and 6B show FTIR spectra of the produced nano/micro fibers using acetone and FA, respectively.

Along with typical absorption bands of CH_2 stretching at 2943 and 2864 cm^{-1} , the prominent peaks were detected at 1165 , 1467 and 1726 cm^{-1} for the neat PCL nano/micro fibers corresponding to the symmetric stretching of C-O-C, asymmetric deformation of CH_3 groups, and stretching bands of ester carbonyl groups, respectively [22, 23]. There is no distinctive difference obtained between the neat PCL and PCL-chitosan blends for the samples produced with acetone. On the other hand, new

absorption bands were detected at 3356 , 1666 , and 1569 cm^{-1} for the chitosan blended samples produced with FA. Moreover, the intensities of these new bands increased with increasing chitosan amount. These bands are the characteristics absorptions for chitosan, such that the bands at 3356 cm^{-1} correspond to the N-H vibrational stretching, the bands at 1666 cm^{-1} correspond to the C=O vibrational stretching and the bands at 1569 cm^{-1} correspond to the N-H vibrational bending [24]. The reason for not observing any chitosan vibrational bands for the samples produced with acetone is that the chitosan is encapsulated by PCL as evidenced by SEM images. Since only surface functional groups are detected by FTIR with ATR unit, amide group vibrational bands could not be observed for these samples.

3.3. Thermal analysis of as-spun NFs with DSC

As seen from DSC analysis results of the as-spun NFs in Figure 7, all chitosan blended PCL nano/micro fibers showed an endothermic peak around $58\text{ }^\circ\text{C}$ which was associated with the melting temperature of PCL [22]. Even though melting point of the acetone sample did not change with addition of chitosan, a slight left shift was observed for FA samples with chitosan addition up to 50/50. This revealed that miscibility of chitosan and PCL was higher in FA than in acetone [22]. This was also observed at electrospinning solution that even though solution PCL/CHI in acetone was blurry, solution PCL/CHI in FA was totally transparent. On the other hand SEM images of PCL/CHI NFs also support this result that because of partially solubility in acetone some CHI particles were encapsulated by PCL/CHI composite nano/micro fibers.

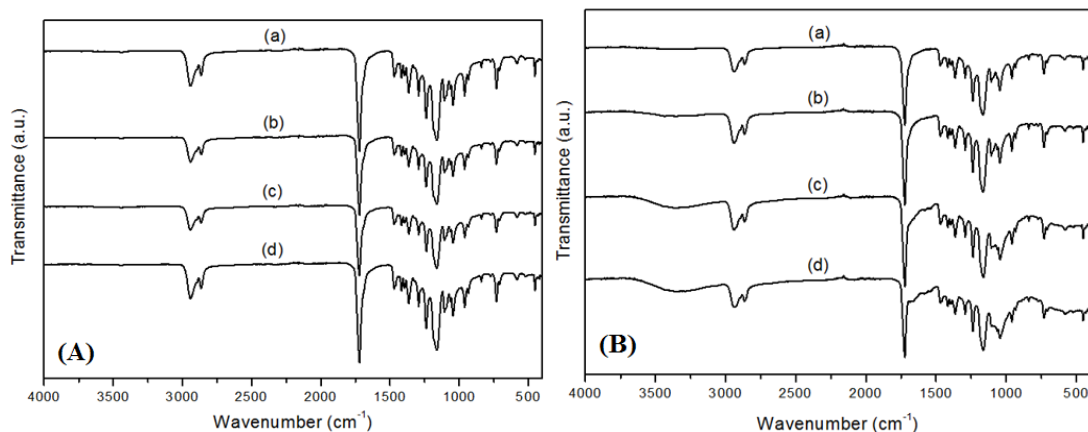


Figure 6. FTIR spectra of PCL/Chitosan nano/micro fibers with different ratio: a) pure PCL nano/micro fibers, b) (PCL/CHI (95/5)), c) (PCL/CHI (80/20)) and d) (PCL/CHI (50/50)) nano/micro fibers. (A): Acetone solution, (B): FA solution.

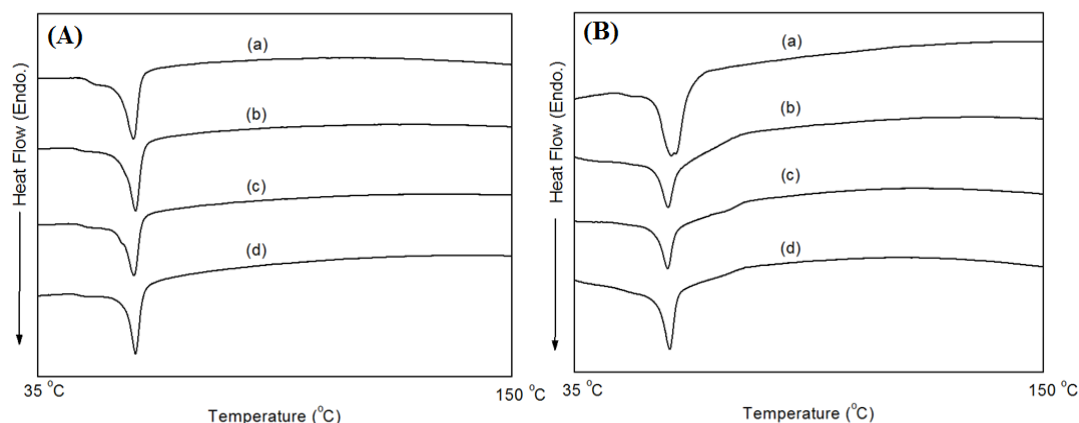


Figure 7. DSC thermo analyses of: a) pure PCL nano/micro fibers, b) (PCL/CHI (95/5)), c) (PCL/CHI (80/20)) and d) (PCL/CHI (50/50)) nano/micro fibers. (A): Acetone solution, (B): FA solution.

3.4. Antibacterial activity measurements

Electrospun PCL/CHI nano/micro fibers produced by using FA and acetone for electrospinning solution preparation results both different nano/micro fibers morphologies and antibacterial activities. In this regards, four different concentration of PCL/CHI nano/micro fibers for both acetone and FA samples were produced and antibacterial activity tests were conducted against *E. coli*, and the results are demonstrated in Table 1.

Chitosan is a biopolymer which represent antibacterial activity [12, 25, 26] It was expected that addition of chitosan into PCL exhibit antibacterial activity. According to test results, it was seen that PCL surfaces obtained using both FA and acetone dissolution solvents exhibited no antibacterial activities (-3.23% and -3.23%, respectively) against *E. coli*. After combining CHI at varying ratios in PCL, the antibacterial activities against *E. coli* were

enhanced by increasing CHI concentrations for FA and acetone solvents (-94.84% and -35.48%, respectively). It has been demonstrated that the antibacterial activities were dramatically increased (from -3.23% to -94.84% for FA) after adding CHI in PCL, which implies that CHI is a good bactericide. These results are compatible with previous studies for the representation of antibacterial property of chitosan [12, 25-28]

Since chitosan particles were encapsulated by PCL in PCL/CHI hybrid nanofiber structure if acetone was used as solvent for electrospinning solution, The acetone solvent system for PCL/CHI showed no sufficient antibacterial activities (from -3.23% to -35.48% for AC) against *E. coli*. PCL/CHI (50-50) surfaces obtained using FA have the best antibacterial activity against *E. coli*. Because CHI encapsulation was not observed at PCL/CHI nano/micro fibers when FA was used as solvent, CHI properly distributed in the mat and helps to promote antibacterial activity.

Table 1. The antibacterial activity results against *E. coli*^a according to test method ASTM E2149

Sample number	Sample name			The antibacterial activity after 24 h
				(Bacteria reduction, %)
1	Control sample			No reduction
	Nanofiber	Solvent	Concentration	
			(PCL/CHI)	
2		FA	(100-0)	3.23
3			(95-5)	35.48
4			(80-20)	79.03
5			(50-50)	94.84
6		AC	(100-0)	3.23
7			(95-5)	6.45
8			(80-20)	19.35
9			(50-50)	35.48

^a The concentration of bacteria was adjusted to $3,10 \times 10^5$ (log 5,49) cfu*/ml for each sample.

* cfu : Colony forming unit

** PCL: Polycaprolactone, CHI: Chitosan, FA: Formic acid, AC: Acetone

Note: The positive values of bacterial reduction (%) demonstrate a decrease in bacterial growth.

CONCLUSIONS

The type of dissolution solvent for electrospun PCL/CHI nano/micro fibers and its effect on nanofiber morphologies and their antibacterial activities were investigated. It was found that when FA was used as a solvent for electrospinning of PCL/CHI polymer blend nano/micro fibers, antibacterial activities considerably increased compared to acetone used as a solvent. This result is believed to be mainly due to more homogenous nanofiber structures were obtained when FA used as solvent. Optical and electron microscope images, and DSC and FTIR analysis also supported that encapsulation of chitosan by PCL

nanofiber matrix when acetone electrospinning solution was used resulting in limited contact of chitosan with bacteria thus weakening the antibacterial properties. In conclusion, the FA system showed more potential for the solution electrospinning of antibacterial PCL/CHI polymer blend nano/micro fibers.

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