EFFECT OF COLLAGEN CONCENTRATION ON MORPHOLOGY OF PVA/CHITOSAN FIBERS MADE BY ELECTROSPINNING METHOD

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ABSTRACT

The purpose of this study was to determine the impact of the addition of collagen concentration on morphology of PVA/chitosan nanofiber. The research was conducted by making 10% PVA solution, 2% chitosan solution, and collagen solutions with various concentrations of 2%, 4%, and 8%. The PVA/chitosan/collagen nanofibers were made with ratios of 8:2:2, 8:2:4, and 8:2:8 and were named P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8. As a control was PVA/chitosan with a ratio of 8:2, which was named P8/Ch2/C0. The nanofiber was made by electrospinning method with the following parameters: a voltage of 10 kV, a distance from the spinneret tip to the collector of 10 cm, and a flow rate of 1.00 µl/h. Nanofiber characterization was carried out using FTIR and SEM. SEM images were analyzed using ImageJ and Origin to measure the diameter and length of the fibers. It obtained that, the best ratio of PVA/chitosan/collagen to make a good nanofiber is 8:2:2, with the characteristics are the least number of beads and solution droplets, the most continuous fiber, and the average diameter is 145.55±8.64 nm. This result showed that, the addition of collagen can improve the morphology of PVA/chitosan nanofibers, where the characteristics of fibers made depending on the concentration of collagen added.

Keywords: Chitosan; collagen; PVA; nanofiber; electrospinning.

Introduction

Nanotechnology is a knowledge that deals with material manipulation on the nanometer scale (in the unit of 10^{-9} m).[1] Indonesia is a country that has a very high biodiversity, so it is called a mega-biodiversity country.[2] With a very high biodiversity, Indonesia has great potential in developing nanotechnology. One of the nano technology applications currently being developed in Indonesia is nanofiber.

Nanofiber is a fiber that has a diameter of less than 500 nm.[3] Nanofiber from a polymer is made and studied by many researchers, generally because of its characteristics, such as a large surface area, small pore size and can be formed in a three-dimensional structure so that it has the potential as a filtration medium, optical fiber, drug delivery system (DDS)), protective clothing/textiles,[4] and as wound dressing.[5] One of the most widely used biopolymer material for biomedical application is chitosan.[6]

Chitosan is a material obtained from deacetylation (the process of removing the acetyl group by adding an alkaline solution) of chitin.[7] Chitosan has been widely used in the medical field. The antibacterial characteristic of chitosan has been widely reported, one of which is used as a wound dressing because of its ability to accelerate wound healing,[8] but chitosan has poor characteristic of water absorption. Absorption of too much water causes the antibacterial characteristic of chitosan to not durable because the absorbed water will become a medium for microbes to

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multiply rapidly. One of the organic material that can improve the water absorption characteristic of chitosan is collagen.[9]

Collagen is one type of protein that has a fiber-shaped structure.[2] In the biomedical field, collagen plays a very important role at every stage of the wound healing process. Chitosan modified with collagen is not only effective as an antibacterial but is also used as a wound dressing. This is because the number of fibroblast formations continues to increase with the addition of collagen so that new tissue in the injured area will be formed more quickly.[9]

Research on chitosan modified with collagen has been carried out. One of the studies conducted by Ratnawati, et al. (2013) has accomplished research on the effect of variations in the composition of collagen/chitosan on the physical and mechanical properties of membranes for the application of wound dressing.[10] It was found that the variation in the composition of collagen/chitosan was able to increase the thickness of the membrane from 0.047 ± 0.03 mm to 0.243 ± 0.24 mm and made the pores of the membrane tighter.

In terms of the application of PVA/chitosan nanofiber as a wound dressing, it is important to conduct research about the improvement of the morphology of PVA/chitosan nanofiber. Based on this problem, this study reported the effect of collagen concentration on the morphology of PVA/chitosan nanofiber so that it is expected to produce better nanofiber with the addition of collagen to be further developed as a wound dressing.

Methods

a. Preparation of Nanofiber Solution

The materials used in this study included chitosan powder derived from Windu shrimp shells, 2% acetic acid, PVA, collagen powder derived from fish, and demineralized water (aqua DM). A 10% PVA solution was prepared by dissolving 10 g of PVA in 100 mL aqua DM. A 2% chitosan solution was prepared by dissolving 2 g of chitosan in 100 mL of 2% acetic acid. A 2% collagen solution was prepared by dissolving 0.2 g of collagen in 10 mL of 0.5 M acetic acid. In the same way, collagen solutions with concentration of 4% and 8% were prepared. Furthermore, it made mixed solutions of PVA:chitosan: collagen with ratio of 8:2:2, 8:2:4, and 8:2:8, which were named P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 respectively. Also, it made a mixed solution of PVA:chitosan with a ratio of 8:2 named P8/Ch2/C0; it was used as a control.

b. Electrospinning of Nanofiber

The nanofibers made by electrospinning method with the following parameters: a voltage of 10 kV, a distance from the spinneret tip to the collector of 10 cm, a relative humidity (RH) of 63.4%, and a flow rate of 1.00 µl/h. The electrospinning process was carried out at room temperature for 3 hours until a layer of nanofiber was formed. In the same way, P8/Ch2/C0, P8/Ch2/C4, and P8/Ch2/C8 nanofibers made.

c. Nanofiber Characterization using FTIR

The nanofibers made were further characterized using FTIR spectrophotometer, that aims to determine the functional groups contained in the nanofiber. Each nanofiber was cut to a size of ± 1 cm^2 and scanning was carried out in the wave numbers region of 400-4000 cm^{-1}.

d. Nanofiber Characterization using SEM

The characterization of nanofibers using SEM aims to determine the diameter and length of the fibers, also to observe the presence of beads and solution droplets in the fibers formed. The obtained nanofibers were cut to a size of ± 1 cm^2. From the SEM characterization obtained morphological images to be analyzed for the presence of beads and solution droplets. Also with the help of ImageJ and Origin applications, it can measured the diameter and length of the fibers.

Result and Discussion

The PVA/chitosan/collagen nanofibers made namely P8/Ch2/C0, P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8, are shown in Figure 1. Figure 1 shows that with the addition

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of collagen, the nanofibers appear more transparent starting from Figure 1a to 1d. This is due to the higher concentration of collagen can cause some of the solution to evaporate before it can finally form fibers, so that the fibers accumulated decreased.[11] This is what causes P8/Ch2/C8 nanofibers to see more transparent than other nanofibers.

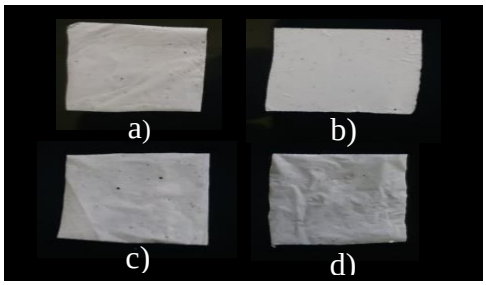


Figure 1. The figures of nanofibers made, a) P8/Ch2/C0, b) P8/Ch2/C2, c) P8/Ch2/C4, and d) P8/Ch2/C8.

Characterization using FTIR aimed to determine the functional groups of the nanofibers have been made. The FTIR spectra of the nanofibers are presented in Figure 2. Figure 2 shows that the spectra of nanofibers have a similar pattern and different percentage of transmittance. For the purpose of labeling, the spectra of P8/Ch2/C0 and P8/Ch2/C2 in Figure 2 are redrawn as shown in Figure 3.

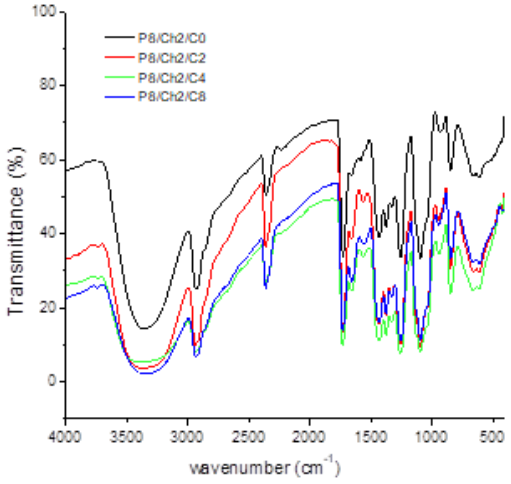
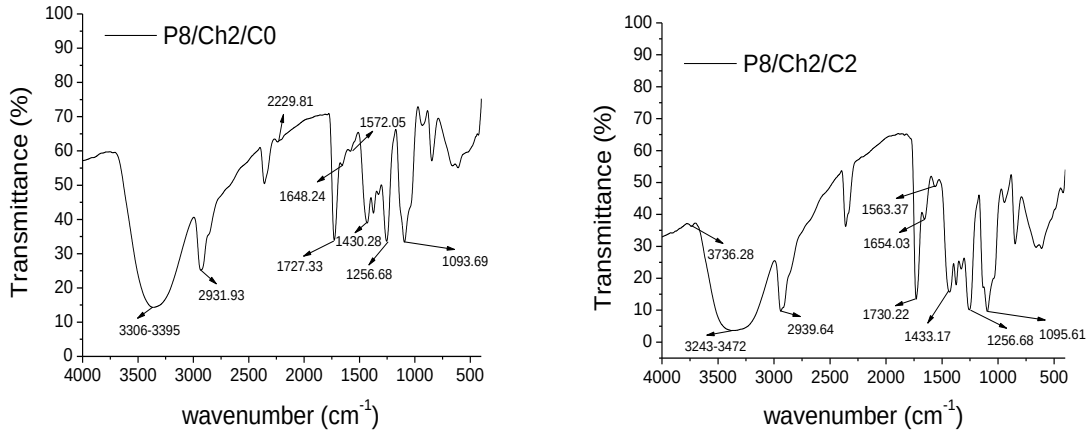


Figure 2. The FTIR spectra of the nanofibers: P8/Ch2/C0, P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8.



a) b)

Figure 3. FTIR Spectrum of nanofibers a) P8/Ch2/C0 and b) P8/Ch2/C2.

Figure 3a shows that there is a broad band at 3306-3395 cm^{-1} which is known as the O-H group of intramolecular and intermolecular hydrogen bonds.[12] Around this area is also observed a band which is known as N-H stretching group. The band is not clearly observed, this is due to the wide -OH region so that the N-H stretch group is covered.[12,13] The C-H stretch group is observed at around 2931.93 cm^{-1} and a small peak at around 2229.81 cm^{-1} which assigned as C≡O bond.[12] Also it observed C=O stretch at around 1727.33 cm^{-1}, C-O group at around 1093.69 cm^{-1},[12] the CH_2 group at around 1430.28 cm^{-1},[14] Amide I, II, and III groups are observed at around 1648.24, 1572.05 and 1256.68 cm^{-1}, respectively.[15]

Figure 3b shows a new peak at around

3736.28 cm^{-1} indicate the N-H stretch group[16] which is shifted due to the addition of collagen.[17] It is also observed the -OH group band widened with the addition of collagen at 3243-3472 cm^{-1},[12] The width of the -OH peak indicates the presence of hydrogen bond forms between PVA, chitosan, and collagen.[18] Meanwhile, the C≡O bond is not observed in the spectrum. The C=O group is observed at around 1730.22 cm^{-1}, and the C-O group at around 1095.61 cm^{-1},[12] The CH_2 functional group is observed at around 1433.17 cm^{-1},[14] Amide I, II, and III groups are observed with sharper peaks with the addition of collagen at around 1654.03, 1563.37, and 1256.68 cm^{-1}, respectively.[15] The results of the analysis for all nanofibers made are written in full in Table 1.

Table 1. The functional groups which observed in P8/Ch2/C0, P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 nanofibers.

Functional Group	*wavenumber (λ^{-1}) from references (cm^{-1})	P8/Ch2/C0		P8/Ch2/C2		P8/Ch2/C4		P8/Ch2/C8	
		λ^{-1} (cm^{-1})	T (%)	λ^{-1} (cm^{-1})	T (%)	λ^{-1} (cm^{-1})	T (%)	λ^{-1} (cm^{-1})	T (%)
-OH, N-H stretch	3000-3750	3306-3395	14.32	3243-3472	3.62	3171-3491	5.22	3233-3450	2.17
C-H stretch	2850-3000	2931.93	25.14	2939.64	9.74	2939.64	7.86	2936.75	6.91
C≡O	2100-2250	2229.81	62.83	-	-	-	-	-	-
C=O	1600-1800	1727.33	33.91	1730.22	13.47	1730.22	9.80	1729.26	13.77
C-O	1000-1300	1093.69	33.38	1095.61	9.61	1095.61	8.02	1093.69	11.38
CH_2	1400-1475	1430.28	38.94	1433.17	15.68	1432.21	11.03	1432.21	16.26
Amide I	1600-1690	1648.24	56.32	1654.03	38.57	1655	24.52	1653.07	27.08
Amide II	1480-1575	1572.05	60.52	1563.37	48.75	1559.51	33.75	1554.69	37.09
Amide III	1229-1301	1256.68	33.61	1256.68	10.14	1255.71	7.75	1252.82	10.92

T = Transmittance.*[12,14,15,16]

From Table 1, it can be seen that the wavenumber of -OH and N-H stretch in the P8/Ch2/C2 and P8/Ch2/C4 nanofibers shifted to larger wavenumber, while in the P8/Ch2/C8 nanofiber shifted to smaller wavenumber. The C-H stretch groups on P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 showed to shift to larger wavenumber. The amide I group on P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 also showed to shift to larger wavenumber. Meanwhile, the wavenumber of Amide III group on P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 showed to shift to smaller region. This shifting indicates an interaction between those functional groups in PVA, chitosan, and collagen.[19] While the wavenumber of C-O, C=O, CH_2, and Amide III groups in P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 nanofibers did not shift. Table 1 also shows that the -OH stretch, N-H stretch, C-H stretch, C-O, C=O, CH_2, Amide I, Amide II, and Amide III groups in P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 decreased in transmittance, which indicates the increasing absorption of these functional groups.[20]

Characterization using SEM aimed to characterize morphology of the fibers formed, including continuity, beads, and the average diameter. The results of the characterization using SEM as shown in Figure 4.

Figure 4 shows that P8/Ch2/C2 nanofiber has the best morphology compared to other nanofibers, where the number of beads and solution droplets observed were small. Beads and solution droplets were mostly observed in P8/Ch2/C0 nanofiber (control). This is due to

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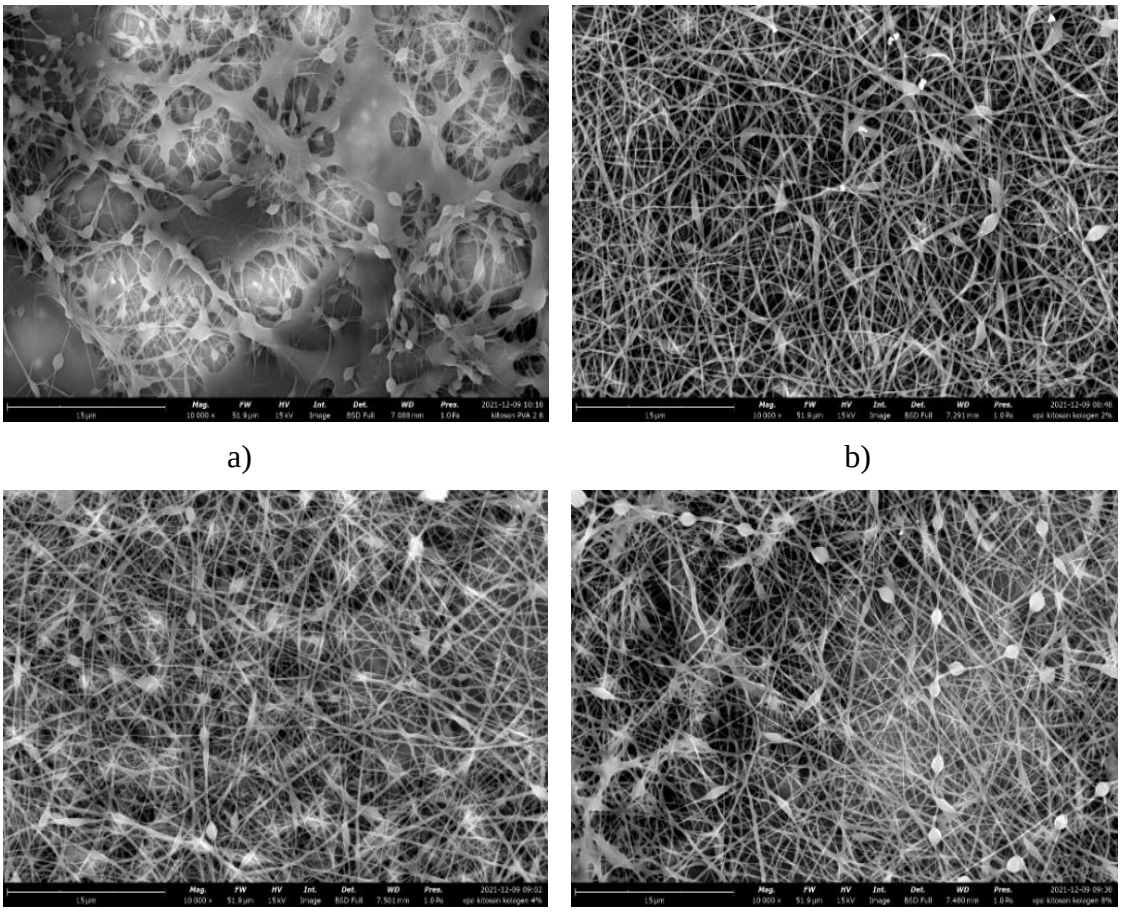
the presence of an electric field and surface tension which causes the polymer chain to break into small fragments before reaching the collector, thus becoming a factor in the formation of beads.[21] Thus, the addition of 2% collagen has been able to improve the morphology of P8/Ch2/C0 nanofiber, where the addition of collagen causes an increase in chain attachment in solution so that can maintain the continuity of the flow of solution during the electrospinning process and can produce nanofiber with a small number of beads.[21]



a) b)

c) d)

Figure 4. The results of the characterization with SEM of a) P8/Ch2/C0, b) P8/Ch2/C2, c) P8/Ch2/C4, and d) P8/Ch2/C8 nanofibers.

In P8/Ch2/C4 and P8/Ch2/C8 nanofibers, more beads were observed than P8/Ch2/C2 nanofiber. In addition, a lot of solution droplets were observed in the two nanofibers. In Figure 4, it can be seen that the higher the concentration of collagen, the bigger number of beads. The addition of 4% and 8% collagen caused the concentration of the solution to approach the maximum limit, thus affecting the results of the nanofibers formed.[21]

Figure 4 measured the average diameter of the fibers using the ImageJ application. Then the fiber diameter data obtained were plotted into a distribution graph using the Origin application, as shown in Figure 5. The average diameter (dc) of each nanofiber is presented in Table 2.

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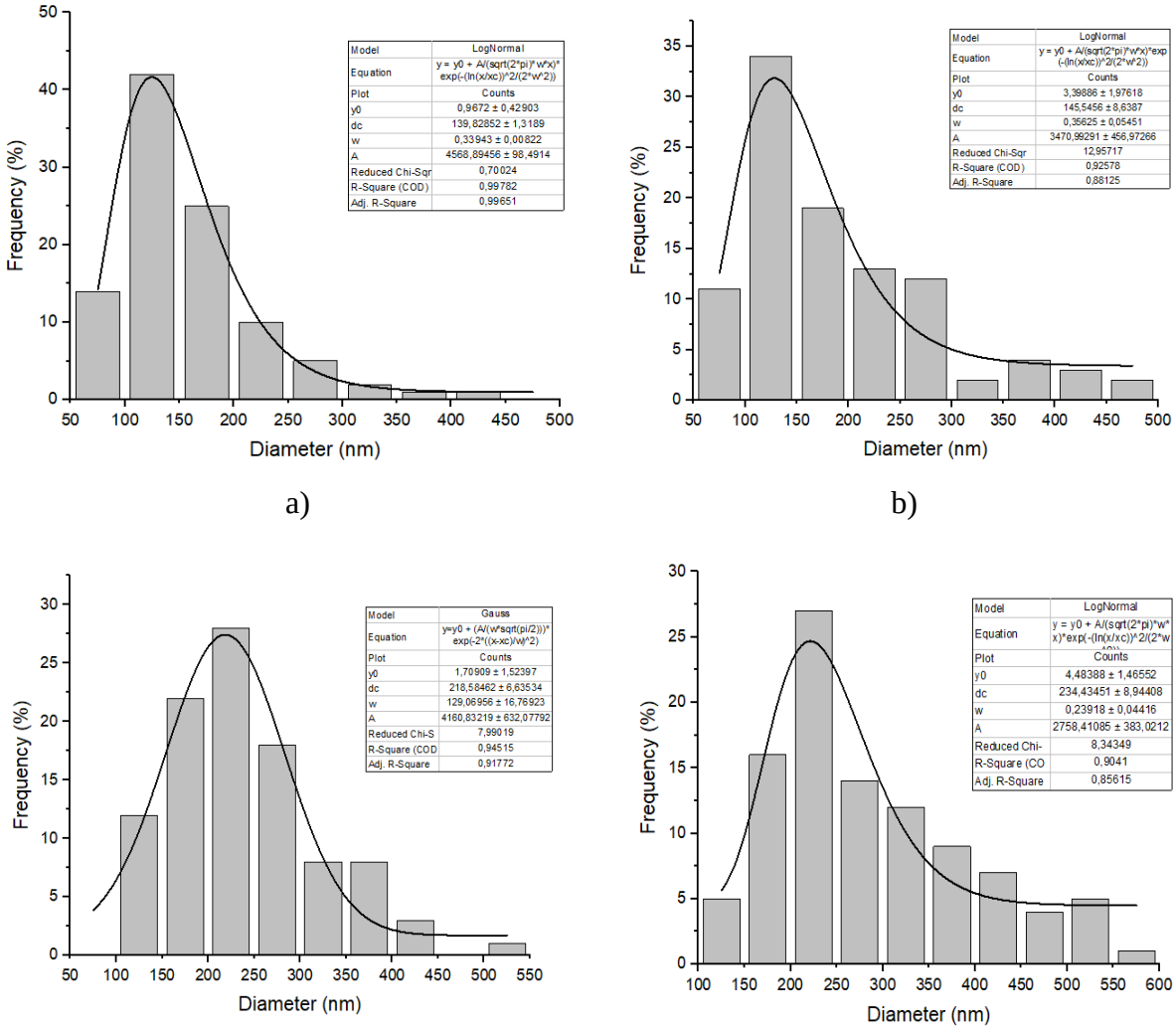

Figure 5. The distribution graph of diameter of the nanofibers a) P8/Ch2/C0, b) P8/Ch2/C2, c) P8/Ch2/C4, and d) P8/Ch2/C8 (Frequency shows the display number of diameter observed within a given range of diameter)[22]

Table 2. The average diameter of P8/Ch2/C0, P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 nanofibers.

No.	Nanofiber	The average of diameter (nm)
1	P8/Ch2/C0	139.83±1.32
2	P8/Ch2/C2	145.55±8.64
3	P8/Ch2/C4	218.58±6.64
4	P8/Ch2/C8	234.43±8.94

Table 2 shows that the addition of collagen causes an increase in diameter of the P8/Ch2/C0 nanofiber. However, the diameter of the nanofibers made were still in the range of diameter of the nanofibers made for wound dressing, which are in the range of 50 to 300 nm.[23] It also appeared that P8/Ch2/C2 nanofiber has the smallest average diameter compared to 2 other nanofibers PVA/chitosan/collagen.

From Figure 4, it has also measured the continuity of the nanofibers. The measurement of the continuity has done by measuring the length of each nanofiber using the ImageJ application. The data obtained is graphed with a distribution graph using the Origin application as shown in Figure 6. The average length (lc) of each nanofiber were presented in Table 3.

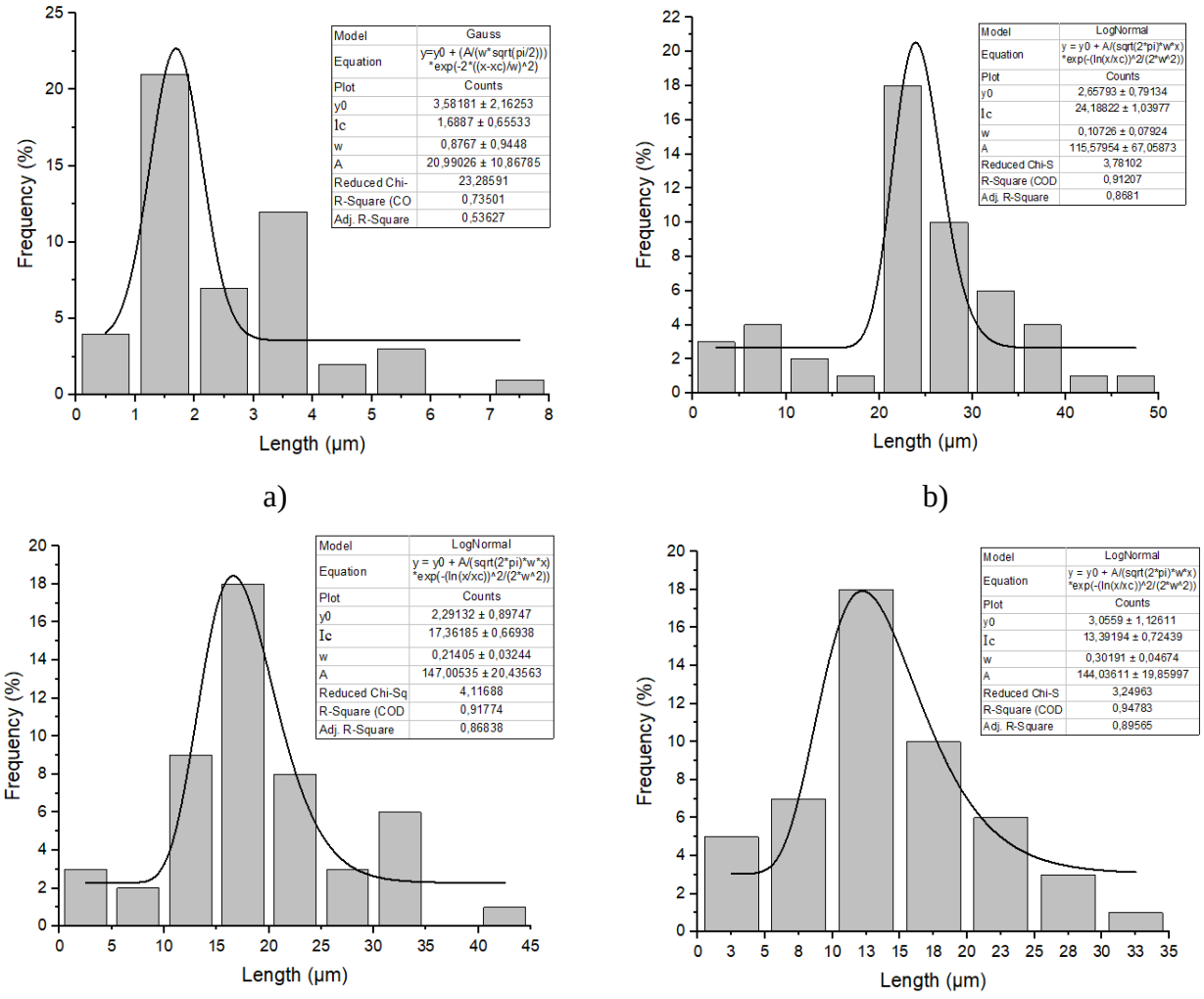

Figure 6. The distribution graph of the length of the nanofibers of a) P8/Ch/2/C0, b) P8/Ch/2/C2, c) P8/Ch2/C4, and d) P8/Ch/2/C8 (Frequency shows the display number of length observed within a given range of length)[22]

Table 3. The average length of P8/Ch2/C0, P8/Ch2/C2, P8/Ch2/C4, and P8/Ch2/C8 nanofibers.

No.	Nanofibers	The average of length (μm)
1	P8/Ch2/C0	1.69±0.66
2	P8/Ch2/C2	24.19±1.03
3	P8/Ch2/C4	17.36±0.67
4	P8/Ch2/C8	13.39±0.72

Table 3 shows that the addition of collagen causes an increase in the length of the nanofibers. This indicates that collagen is able to increase the continuity of P8/Ch2/C0 nanofiber. In addition, from Table 3 it is found that P8/Ch2/C2 nanofiber has the highest average length of the nanofiber made compared to other nanofibers, which means it has the highest continuity.

Conclusion

In this study, the effect of the concentration of collagen on the morphology of PVA/chitosan nanofiber has been accomplished. The best ratio of PVA/chitosan/collagen to make a good nanofiber is 8:2:2, with the nanofiber morphology are the least number of beads and solution droplets,

the most continuous fiber, and the average diameter of 145.55±8.64 nm. This result showed that, the addition of collagen can improve the morphology of PVA/chitosan nanofibers, where the characteristics of fibers made depending on the concentration of collagen added.

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References

1. Sirajudin A, Rahmanisa S. Nanopartikel Perak sebagai Penatalaksanaan Penyakit Infeksi Saluran Kemih. Majority. 2016 Oct; 5(4):1-5.
2. Suryawan A, Kinho J. Diversity Species of Eboni (Diospyros Spp.) Tulabolo-Pinoguresort, Boganinani Wartabone National Park, North Sulawesi. Widyariset. 2012 Aug; 15 (2): 117-126.
3. Hulupi M, Haryadi. Sintesis dan Karakterisasi Serat Nano Polivinil Alkohol yang Diikat Silang dengan Glutaraldehid untuk Aplikasi Pembalut Luka. Chimica et Natura Acta. 2018 Dec; 6 (3): 101-105.
4. Herdiawan H, Juliandri, Nasir M, editors. Pembuatan Dan Karakterisasi Co-PVDF Nanofiber Komposit Menggunakan Metode Elektrospinning. Prosiding Seminar Nasional Sains dan Teknologi Nuklir PTNBR – BATAN Bandung; 2013 July 4; Bandung, Indonesia. P.110-116.
5. Supriyanto A, Murni M.L, Marlina F, Pangga D. Uji Biokompatibilitas Nanofiber Komposit Kitosan/PVA Sebagai Pembalut Luka. Jurnal Kependidikan Fisika. 2018 June; 6 (1): 37-42.
6. Yulina R, Winiati W, Bonadies I, Laurienzo P, Dalpogetto G. Optimasi Kondisi Larutan dan Parameter Proses Pemintalan Elektrik pada Sintesis Serat Nano Kitosan-PEO. Arena Tekstil. 2018 July; 33 (1): 1-10.
7. Nuryantini A.Y, Munir M.M, Suciati T, Khairurrijal, editors. Pembuatan Serat Nano PVA/Kitosan dengan Penambahan Sodium Tripolifosfat (STTP) sebagai Agen Ikatan Sambung Silang. Seminar Nasional Material 2013; 2013 Feb 16; Bandung, Indonesia. P. 70-73.
8. Hartomo B.T, Firdaus, F.G. Pemanfaatan Biomaterial Kitosan dalam Bidang Bedah Mulut. Jurnal Kedokteran Gigi Universitas Baiturrahmah. 2019 June; 6 (1): 63-70.
9. Octavian A. Kajian Sifat Fisik-Mekanik dan Antibakteri Plastik Kitosan Termodifikasi Kolagen Limbah Sisik Ikan Kakap Merah [Thesis]. Semarang: Universitas Negeri Semarang; 2015.
10. Ratnawati A, Izak D.R, Supardi A. Sintesis dan Karakterisasi Kolagen dari Teripang-Kitosan sebagai Aplikasi Pembalut Luka. Journal of Physics and Application. 2013 Apr; 1 (2): 12-24.
11. Sudirman, Karo A.K, Sukaryo S.G, Adistiana K.D, Dahlan K. Synthesis of Nanofiber from Poly Vinyl Alcohol (PVA)-Collagen using Electrospinning Methods. Jurnal Kimia Terapan Indonesia. 2020 Apr; 21 (2): 55-65.
12. Pavia D.L, Lampman G.M, Kriz G.S, Vyvyan, J.R. Introduction To Spectroscopy. United States: Brooks/Cole, Cengage Learning; 2001.
13. Windari, N.N.P., Rupiasih, N.N., Sumadiyasa, M. Studi Membran Kitosan dari Kulit Lobster Bambu Sebagai Membran Filtrasi. Buletin Fisika. 2016 Feb; 17 (1): 1-6.
14. Alchaddad, M., Siadi, K., Supartono. Transesterifikasi Etil P-Metoksisinamat Hasil Isolasi Rimpang Kencur dengan Vitamin C Terkatalis Lipase. Indonesian Journal of Chemical Science. 2015 Aug; 4 (2): 84-88.

15. Suptijah, P., Indriani, D., Wardoyo, S.E. Isolasi dan Karakterisasi Kolagen Dari Kulit Ikan Patin (Pangasius sp.). Jurnal Sains Natural Universitas Nusa Bangsa. 2018 Jan; 8 (1): 8-23.
16. Dachriyanus. Analisis Struktur Senyawa Organik Secara Spektroskopi. Padang: Lembaga Pengembangan Teknologi Informasi dan Komunikasi (LPTIK) Universitas Andalas; 2012.
17. Wivanius, N. and Budianto, E. Sintesis dan Karakterisasi Hidrogel Super absorben Kitosan Poli(N-Vinil kaprolaktam) (PNVCL) Dengan Metode Full IPN (Interpenetrating Polymer Network). Pharm Sci Res. 2015 Dec; 2 (3): 152-168.
18. Gonggo, S.T., Diah, A.W.M., Lateene, R. Pengaruh Kaolin Terhadap Membran Blend Kitosan Poli Vinil Alkohol-Litium Sebagai Membran Elektrolit Untuk Aplikasi Baterai Ion Litium. Jurnal Akademi Kimia. 2017 Feb; 6 (1): 55-64.
19. Suryati, Azhari, Pasaribu, D.L. Pembuatan Biokomposit Kitosan/ Alginat/Kolagen untuk Aplikasi Pembalut Luka [Thesis]. Medan: Universitas Sumatera Utara; 2021.
20. Sari, I.W., Nita, W.O., Rupiasih, N.N., Sumadiyasa, M. Pengaruh Paparan Radiasi UVC Terhadap Struktur dan Swelling Membran Kitosan Yang Terpapar Medan Magnet. Buletin Fisika. 2019 July; 20 (2): 35-40.
21. Hidayati, A., and Cahyono, E. Matrix Composition Effect on the Characteristics of Isopulegol Nanofibers Fabrication by Electrospinning Method. Indonesian Journal of Chemical Science. 2016 May; 10 (1): 49-58.
22. Zubair, M. To Increase Data Analysing Power You Must Know Frequency Distribution (Stat-04) [Internet]. 2021 [updated 2021 Aug 10; cited 2022 Oct 30]. Available from: https://towardsdatascience.com/to-increase-data-analysing-power-you-must-know-frequency-distribution-afa438c3e7a4.
23. Darmawan, M., Syamdidi, Yennie, Y., Wibowo, S. Karakteristik Serat Nano Komposit Kitosan-Polivinil Alkohol (PVA) dari Cangkang Rajungan melalui Proses Electrospinning. JPB Kelautan dan Perikanan. 2016 Nov; 11 (2): 213-222.