

The Effect of Aloe vera Extract Variation in Electrospun Polyvinyl Alcohol (PVA)-Aloe vera-Based Nanofiber Membrane

by Dyah Hikmawati

Submission date: 02-Nov-2022 06:20PM (UTC+0800)

Submission ID: 1942304479

File name: C15._Prosiding_Karya_Ilmiyah.pdf (1.35M)

Word count: 3185

Character count: 16355

PAPER · OPEN ACCESS

The Effect of *Aloe vera* Extract Variation in Electrospun Polyvinyl Alcohol (PVA)-*Aloe vera*-Based Nanofiber Membrane

To cite this article: D Hikmawati ⁶ *et al* 2018 *J. Phys.: Conf. Ser.* **1120** 012096

View the [article online](#) for updates and enhancements.



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

The Effect of *Aloe vera* Extract Variation in Electrospun Polyvinyl Alcohol (PVA)-*Aloe vera*-Based Nanofiber Membrane

D Hikmawati¹, A R Rohmadanik¹, A P Putra², Siswanto¹, and Aminatun¹

¹Physics Study Program, Department of Physics, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 61110, Indonesia

²Biomedical Engineering Study Program, Department of Physics, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 61110, Indonesia

Corresponding author: dyah.hikmawati@yahoo.co.id

Abstract. Electrospinning technique is a method to produce nanofiber with high potential difference. Several main parameters in the electrospinning process are solution parameter, such as viscosity, surface tension and conductivity. Those parameters affect the morphology of nanofiber membrane produced by the electrospinning process. This study used 10% PVA in aquadest with several *Aloe vera* concentration (0, 2.5, 5, 7.5 and 10 % w/v). The viscosity of the solution increased when the concentration of the *Aloe vera* increased. SEM image of the nanofiber membrane showed that the diameter of the nanofiber increased with the increase of the *Aloe vera* concentration, with the highest diameter of 379.80 nm on the membrane with *Aloe vera* concentration of 10% w/v. The result of DSC test showed that there were shifts on the glass transition (T_g) and melting point (T_m) value along with the addition of *Aloe vera* in the PVA solution. This results was also emphasized with the result of FTIR test which showed the shift on the wavenumber absorbance due to the crosslinking between PVA and *Aloe vera*. The degradation test indicated that the presence of crosslinking could maintain the nanofiber structure, thus the membrane was not easily degraded.

1. Introduction

Electrospinning is an emerging technique to produce polymer-based micro or nano fibres with high quality characteristics in textile industry, especially as wound dressing, tissue engineering materials, drug delivery systems or coating technique in medical field. This technique utilizes electrostatic force to form fibres in micro or nano scale. By using this technique a diameter less than 10 nanometres could be obtained [1]. There are several parameters that affect the quality of nanofiber, such as electrospinning parameters (the high voltage, the distance between the needle and collector, the needle inner diameter, the speed of solution exerting from the needle etc.), polymer solution parameters (molecular weight, viscosity, surface tension, conductivity etc.), and environmental parameters (temperature and humidity) [2]. One of the most affecting parameters is the viscosity of the solution. Viscosity would determine the morphology of nanofiber. The study of Sukigara et al., (2003) mentioned that continuous and smooth fibres could not be obtained by a solution with low viscosity



Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

[3]. On the other hand, high viscosity also produce irregularity in the fibres. Thus, the determination of viscosity of the solution plays an important role in producing nanofiber with high quality.

Several studies were already performed to produce a high quality nanofiber by using polymers such as polyvinyl alcohol (PVA), Poly ϵ -Caprolactone (PCL), Poly Ethylene Oxide (PEO) etc. [2, 4-5]. PVA was known for its hydrophilicity which could easily bond hydrogen molecule in its chain. 10% w/v PVA in water could be electrospun with high voltage of 5-20 kV with the result of nanometre scale fibres. The lack of this result was the presence of beads along the fibres which was undesirable. It was caused by the tendency of the PVA molecule which have strong bonds and is difficult to be pulled by the potential difference and form beads. The addition of *Aloe vera* at the right amount of concentration expected to decrease the presence of the beads. The study conducted by Abdullah (2014) showed that the *Aloe vera* could bind to PVA and have a suitable structure to be applied as a biomaterial due to its biodegradability, biocompatibility, and non-toxicity [1].

Aloe vera has been used in biomaterials as an additive agent to provide wound healing in wound dressing. It also has been utilized for burn wound for many decades. The study of Isfandiary et al., (2017) on using *Aloe vera* in wound dressing scaffold in chitosan-collagen scaffolds showed that the presence of *Aloe vera* could be beneficial in this scaffold to be applied as wound dressing [6]. The review from Venugopal et al., (2014) and Khan et al., (2018) said that the presence of *Aloe vera* was proven in accelerating the wound healing with nanofiber to perform a better treatment to the wound [5, 7]. The *Aloe vera* also has antibacterial properties which was remarkable [7].

This study was focus on the effect of viscosity towards the characteristics of nanofiber membrane from PVA-*Aloe vera*. Several concentration of *Aloe vera* was used in this study. The nanofiber membrane was characterized by using functional group test with Fourier Transform Infrared (FTIR Test), morphology test with Scanning Electron Microscope (SEM Test), thermal analysis test with Differential Scanning Calorimetry (DSC Test), and degradation test.

2. Materials and Methods

2.1. Materials

The Polyvinyl alcohol (PVA) (Mw: 66000 g/mol) was purchased from Duksan Pure Chemicals Co. Ltd. (Korea) The *Aloe vera* extract from Kangcare Bioindustry Co. Ltd., deionized water, Phosphate Buffer Saline (PBS), and ethanol.

2.2. Methods

10% w/v PVA solution was dissolved in water on the magnetic stirrer with temperature of 80°C for three hours. The addition of *Aloe vera* extract was varied from 0, 2.5, 5, 7.5, and 10 % w/v to the PVA solution on the magnetic stirrer with the same temperature for three hours. Then, ethanol was added to the mixed solution of PVA-*Aloe vera* with a ratio of 70:30 using a magnetic stirrer for an hour. The final solutions were tested with Viscotester VT-04F RION Co. Ltd. to obtain its viscosity before entering the electrospinning process [8].

The electrospinning process was started with the insertion of the solution into a syringe and make sure the solution filled the tip of the needle to get a better fibre. An aluminium foil was placed in the collector part of the electrospinning device to catch the resulted nanofiber membrane. The potential difference was 19 kV with 20 cm of distance between the needle and the collector. By using the same process, all variation of PVA-*Aloe vera* solution were electrospun.

The nanofiber membrane sample was characterized by FTIR (Shimadzu) in Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia. KBr was mixed with the sample and formed into a pellet. The FTIR test was performed along the wavenumber of 4000 - 400 cm⁻¹. The morphology test on the electrospun PVA+*Aloe vera* based nanofiber membrane was carried out with Phenom Pro X Desktop SEM. The sample was coated first with Au and Pd. The thermal analysis using DSC test was performed by using a METTLER TOLEDO DSC-3. The test was done by raising the temperature from room temperature (27°C) to 300°C at a rate of 10°C/minute. The degradability test aimed to observe the degradation time and rate of the sample by using PBS. The membrane with area

of 2.25 cm² and mass of 0.01 gr was dropped by the 0.05 mL PBS on the middle of the membrane surface. The time needed for the membrane to degrade completely was recorded.

3. Result and Discussion

The solution was tested with Viscotester to obtain its viscosity. The result was shown in Figure 1. The increase of *Aloe vera* concentrations also increase the viscosity of the solution since the presence of the solute increased too.

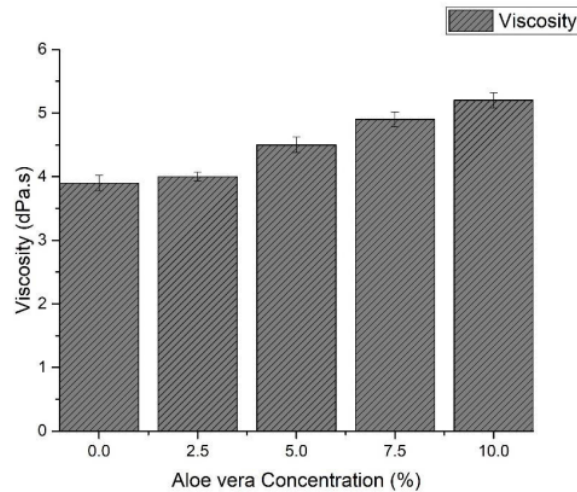


Figure 1. Viscosity of PVA-*Aloe vera* Solution with Several *Aloe vera* Concentrations

3.1. Nanofiber Membrane Electrospinning

The PVA-*Aloe vera* nanofiber membrane making with electrospinning process considers the device, solution and environment parameters. The process used the distance of the needle and the collector at 20 cm because it could form fibres in the collector smoothly. Besides that, the potential difference and flow rate also affected the result. This study used potential difference of 19-21 kV and flow rate of 0.2 mL/hour.

The electrospinning process was four hour to obtain a membrane that had an adequate thickness to remove from the aluminium foil. The result of the electrospinning process of PVA-*Aloe vera*-based nanofiber membrane was shown in Figure 2. The result was then characterized using several characterization as mentioned above.

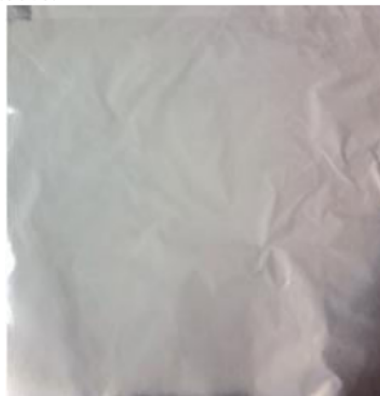


Figure 2. Electrospun PVA-*Aloe vera*-Based Nanofiber Membrane on the Aluminum Foil

3.2. Functional Group Test Using FTIR

The FTIR result of PVA and PVA+Aloe vera was shown in Figure 3. The FTIR result of PVA showed the stretching O-H absorbance at 3309.15 cm⁻¹, the bending O-H at 1425.19 cm⁻¹, 1374.04 cm⁻¹ and 1327.92 cm⁻¹, the stretching C-H at 2939.55 cm⁻¹, and the bending C-H at 944.15 cm⁻¹, 842.33 cm⁻¹, and 604.46 cm⁻¹ [8]. The stretching aldehyde (C=O) group appeared at wavenumber of 1732.47 cm⁻¹ and the stretching C-O group at wavenumber of 1245.67 cm⁻¹ and 1089.79 cm⁻¹.

The FTIR of PVA+Aloe vera indicated the stretching OH group at wavenumber of 3310.70 cm⁻¹. The stretching C-H group appeared at wavenumber of 2915.04 cm⁻¹, while the bending C-H group had absorbance at wavenumber of 1420.39 cm⁻¹, 1374.33 cm⁻¹, 937.54 cm⁻¹, 884.06 cm⁻¹, 575.23 cm⁻¹ and 479.33 cm⁻¹. The stretching and bending aldehyde group was shown at wavenumber of 1734.76 cm⁻¹ and 1245.37 cm⁻¹, respectively [1, 9]. The addition of Aloe vera in the PVA showed a strong sulfoxide functional group (S=O) at wavenumber of 1021.38 cm⁻¹. It also showed a shift on the wavenumber due to the hydrogen bond between PVA and Aloe vera. PVA chains could form the intramolecular and intermolecular hydrogen bonding because of its hydrophilicity. With the presence of Aloe vera among the PVA chains, the hydrogen bonding would also occur between them [1, 10].

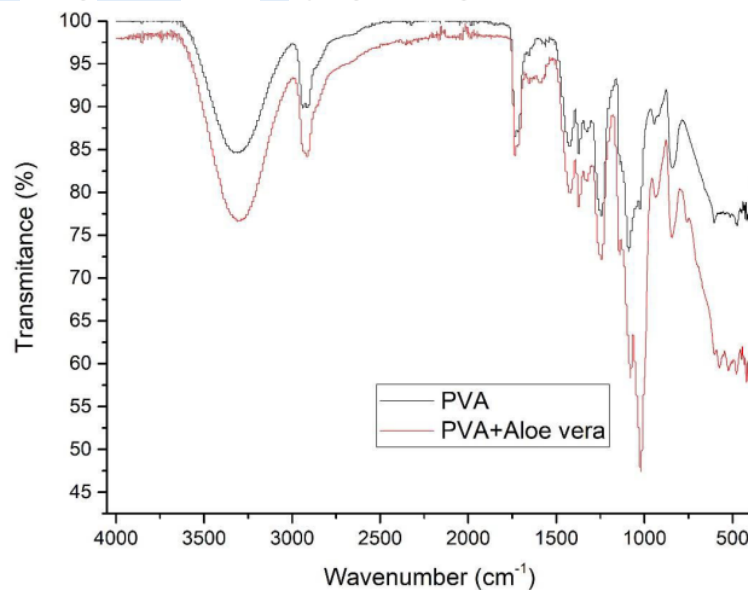


Figure 3. FTIR Spectra of Electrospun PVA and PVA+Aloe vera Based Nanofiber Membrane

3.3. Morphology Test Using SEM

The SEM images of the sample were shown in Figure 4. 10% w/v PVA with viscosity of 3.9 dPa.s formed continuous and homogeneous fibres, even though at some points, the membrane was still wet due to the lack of fast evaporation of the solution before reaching the collector, thus it formed beads. The sample with the addition of 2.5% Aloe vera showed that there was no beads present in the fibres. The presence of Aloe vera in the mixture might decrease the membrane conductivity, thus produces fibres with no beads [1]. The diameter of fibres shown in Figure 4 was measured and the result was shown in Figure 5.

The average diameter of PVA membrane without Aloe vera was 330 nm and with beads in several points. This value was higher than the fibre diameter of PVA+Aloe vera membrane. With the increase of Aloe vera concentration in the mixture, the average diameter of the membrane also increased. With 10% Aloe vera, the average diameter of the membrane was 313.2 nm. This value was smaller than the result of Uslu et al. (2010) study which was 553 nm for membrane from PVA/PEG/PVP/Aloe vera [10]. The study of Abdullah et al. (2014) also showed the same range of fibre diameter of PVA/Aloe

vera. This behaviour might be caused by the presence of hydrogen bonding between the PVA chains and the Aloe vera which also promoted the increase of columbic repulsion and electrostatic force. The molecule would be closer among them [1, 10-11].

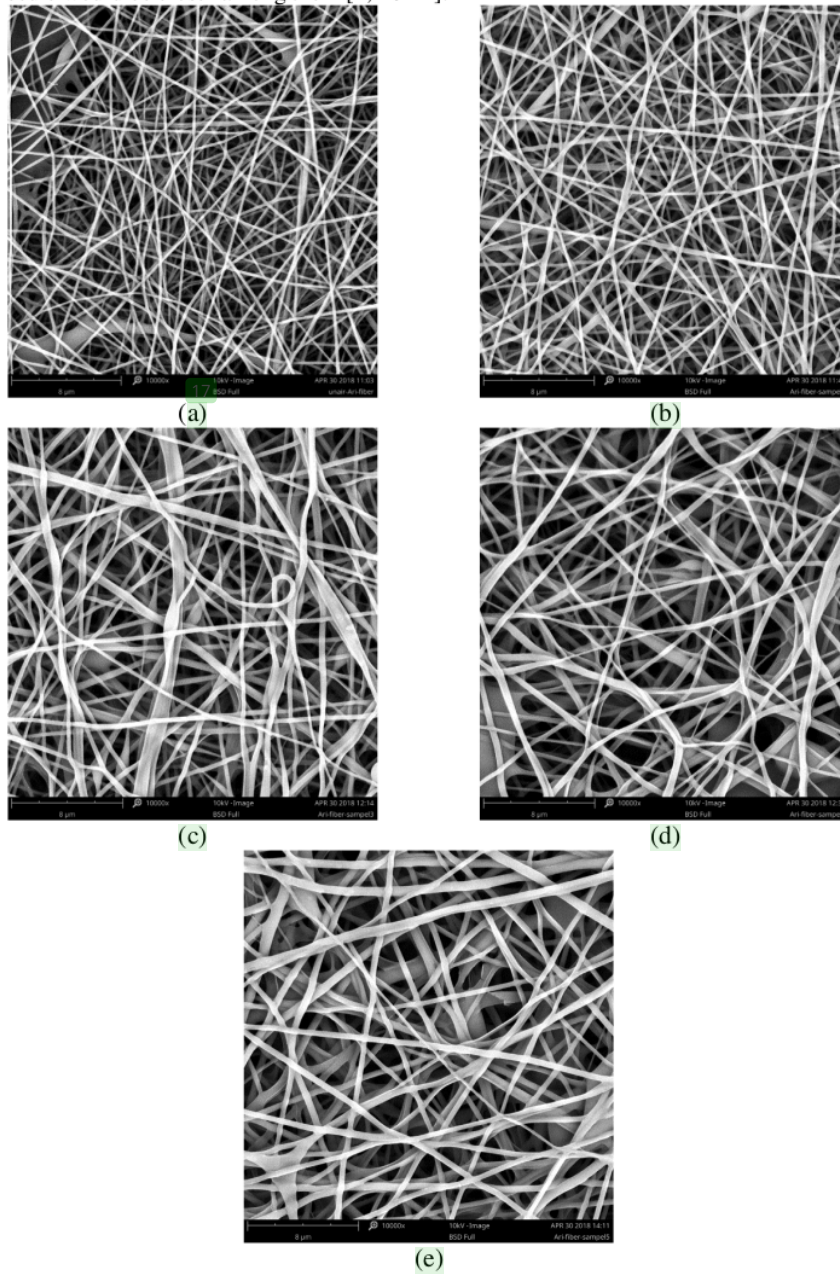


Figure 4. SEM images of Electrospun PVA-Aloe vera-Based Nanofiber Membrane with Several Aloe vera Concentration (a) 0%, (b) 2.5%, (c) 5%, (d) 7.5%, and (e) 10%

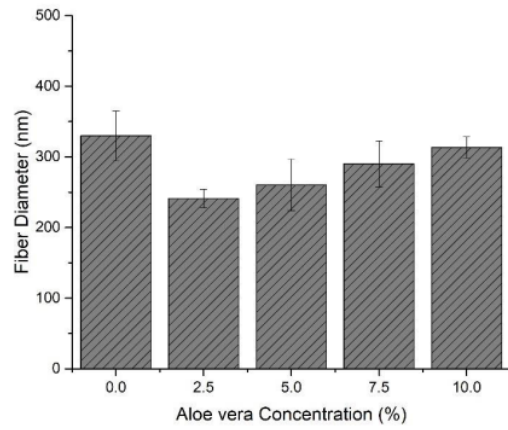
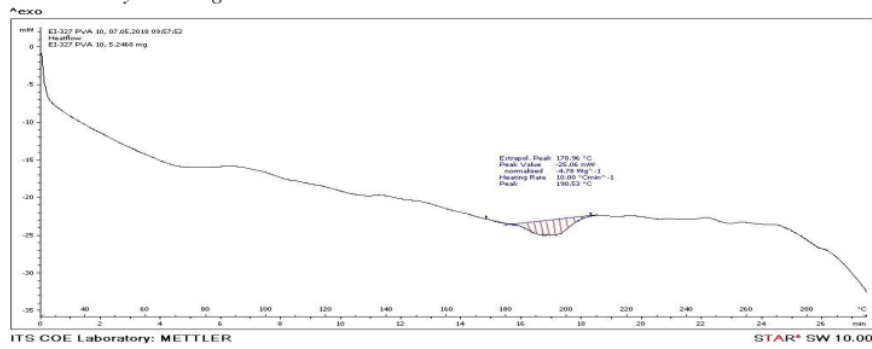
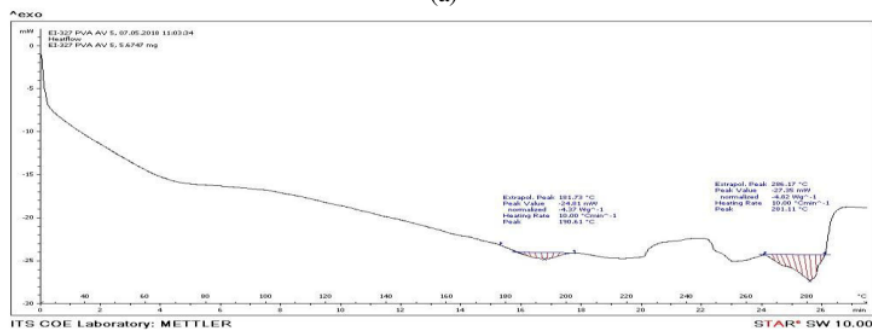


Figure 5. Fiber Diameter of Electrospun PVA-Aloe vera-Based Nanofiber Membrane with Several Aloe vera Concentration

3.4. Thermal Analysis Using DSC



(a)



(b)

Figure 6. DSC Thermogram of Electrospun PVA-Aloe vera-Based Nanofiber Membrane with Several Aloe vera Concentration

28 Thermal analysis of the membrane was performed by using DSC Test. The test was done on two samples, PVA membrane and PVA+Aloe vera membrane to observe the effect of the addition of the Aloe vera in the mixture. The result was shown in Figure 6 that showed an endothermic curve. The PVA membrane showed that the glass transition temperature (T_g) was at 85.08°C and the melting point (T_m) was at 190.53°C. The PVA+Aloe vera membrane indicated the glass transition temperature (T_g) at 90.00°C and the melting point (T_m) at 190.61°C. The glass transition temperature of the membrane slightly changed due to the addition of Aloe vera. The bonds between PVA and Aloe vera was the reason why the T_g shifted to the higher temperature. The Aloe vera in this mixture the crystallinity of the polymer and leads to more amorphous structure [10]. The shift on T_m was caused by the same problem as T_g, but it did not change drastically. This behaviour was emphasized by the research of Uslu et al. (2014) which also had an increase in T_m [10].

3.5. Degradation Test

The degradation test resulted that the Aloe vera decreased the degradation rate, hence increased the degradation time as shown in Figure 7. This behaviour is caused by the smaller diameter of the PVA membrane. It would absorb more liquid compared to the PVA-Aloe vera membrane and leads to more degradable. The increase in degradation time means the increase in mass loss of the sample. The polymer with lower molecular weight, high hydrophilicity and having acetic, amide, or ester bonding could be easily degraded. The lack of crystallinity and crosslinking on the polymer chains without the addition of Aloe vera could also make the membrane easily degraded [12].

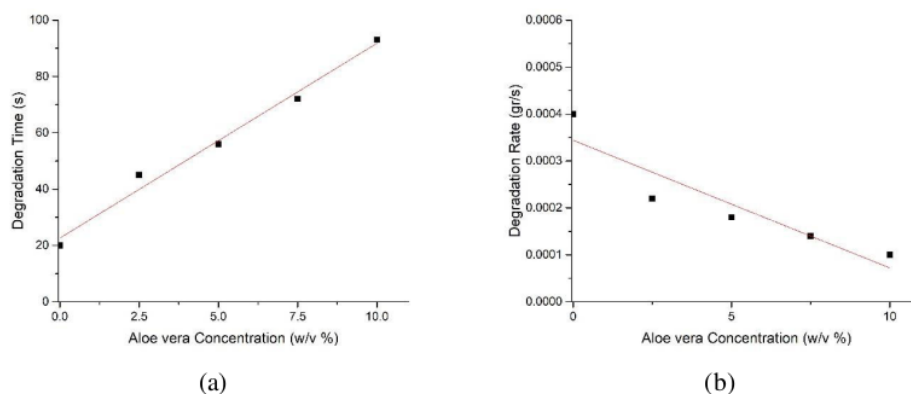


Figure 7. Degradation Time and Rate of Electrospun PVA-Aloe vera-Based Nanofiber Membrane with Several Aloe vera Concentration

4. Conclusion

The Aloe vera concentration affects the viscosity of the solution hence it affects the result of electrospinning process. FTIR Test showed that there was a shift in absorbance due to the presence of the hydrogen bond between PVA and Aloe vera. The diameter of the fibres increases with the increase of the Aloe vera concentration. The DSC test implied that there was a shift in glass transition and melting point temperature which give the information about the durability of the membrane. The presence of Aloe vera decreased the fibre diameter of the membrane and it increased with the increase of the Aloe vera concentration. The degradability of the membrane showed that the crosslink in the membrane prolong the time to degrade of the membrane, hence shorten the degradation rate of the membrane.

Acknowledgments

The authors would like to say thank you to Faculty of Mathematics and Natural Science, State University of Semarang and Energy Laboratorium, Sepuluh Nopember Institute of Technology.

References

- [1] Shukry N A A, Sekak K A, Ahmad M R and Effendi T J B 2014 Characteristics of Electrospun PVA- Aloe vera Nanofibres Produced via Electrospinning in *International Colloquium in Textile Engineering, Fashion, Apparel, and Design 2014 (ICTEFAD 2014)* pp. 139.
- [2] Li Z and Wang C 2013 Effects of Working Parameters on Electrospinning *One-Dimensional Nanostructure* (Springers) pp 15–29.
- [3] Sukigara S, Gandhi M, Ayutsede J, Micklus M and Ko F 2003 *Regeneration of Bombyx mori silk by electrospinning - Part 1: Processing parameters and geometric properties* **44**.
- [4] Isfahani F R, Tavanai H and Morshed M 2017 Release of Aloe vera from Electrospun Aloe vera-PVA Nanofibrous Pad **18** 2 pp 264–271.
- [5] Venugopal J R, Sridhar S and Ramakrishna S 2014 Electrospun plant-derived natural biomaterials for Tissue engineering *Plant Sci. Today* **1** 3 pp. 151–154.
- [6] Isfandiary A, Widiyanti P and Hikmawati D 2017 Composite of Chitosan-Collagen- Aloe vera for Scaffolds Application on Skin Tissue *J. Biomimetics, Biomater. Biomed. Eng.* **32** pp. 82–89.
- [7] Khan R, Xiangyang S, Ahmad A and Mo X 2018 Electrospinning of Crude Plant Extracts for Antibacterial and Wound Healing Applications : A Review *SM J. Biomed. Eng.* **4** 1 pp. 1–8.
- [8] Putra A P, Rahmah A A, Fitriana N, Rohim S A, Jannah M and Hikmawati D 2018 The Effect of Glutaraldehyde on Hydroxyapatite-Gelatin Composite with Addition of Alendronate for Bone Filler Application *J. Biomimetics, Biomater. Biomed. Eng.* **37** pp. 107–116.
- [9] Aytimur A, Koçyigit S, Uslu İ and Gökmeşe F 2015 Preparation and Characterization of Polyvinyl Alcohol Based Copolymers as Wound Dressing Fibers *Int. J. Polym. Mater. Polym. Biomater.* **64** 3 pp. 111–116.
- [10] Uslu İ, Keskin S, Gül A, Karabulut T C and Aksu M L 2010 Preparation and Properties of Electrospun Poly (vinyl alcohol) Blended Hybrid Polymer with Aloe vera and HPMC as Wound Dressing 2010 *Hacettepe J. Biol. Chem.* **38** 1 pp. 19–25.
- [11] Sirima S, Phiriyawirut M and Suttisintong K 2017 Comparison of the Release of Aloe vera Extracts from Poly (Vinyl Alcohol) Electrospun Fibers and Hydrogel Films for Wound Healing Applications *Key Engineering Mater.* **751**, pp. 592–598.
- [12] Hajian M, Mahmoodi M and Imani R 2017 In Vitro Assessment of Poly (Vinyl Alcohol) Film Incorporating Aloe Vera for Potential Application as a Wound Dressing In Vitro Assessment of Poly (Vinyl Alcohol) Film Incorporating *J. Macromol. Sci. Part B Phys.* **56** 7 pp. 435–450.

The Effect of Aloe vera Extract Variation in Electrospun Polyvinyl Alcohol (PVA)-Aloe vera-Based Nanofiber Membrane

ORIGINALITY REPORT

19%

SIMILARITY INDEX

12%

INTERNET SOURCES

17%

PUBLICATIONS

0%

STUDENT PAPERS

PRIMARY SOURCES

1	Katarzyna Bialik-Wąs, Klaudia Pluta, Dagmara Malina, Mateusz Barczewski, Katarzyna Malarz, Anna Mrozek-Wilczkiewicz. "Advanced SA/PVA-based hydrogel matrices with prolonged release of Aloe vera as promising wound dressings", Materials Science and Engineering: C, 2021 Publication	2%
2	autodocbox.com Internet Source	2%
3	businessdocbox.com Internet Source	1%
4	wjst.wu.ac.th Internet Source	1%
5	e-space.mmu.ac.uk Internet Source	1%
6	www.researchgate.net Internet Source	1%

7

Mitra Naeimi, Nika Noohi. "Fabrication and characterisation of antibacterial porous alginate/aloe vera structures containing chitosan nanoparticles for wound dressing applications", *Materials Technology*, 2021

Publication

1 %

8

W Christijanti, A Z Juniarto, L Suromo. "The effectiveness of Aloe vera peel extract on the reproductive status of streptozotocin-induced diabetic rats", *Journal of Physics: Conference Series*, 2021

Publication

1 %

9

Dyah Hikmawati, Hendita N. Maulida, Alfian P. Putra, Aniek S. Budiatin, Ardiyansyah Syahrom. "Synthesis and Characterization of Nanohydroxyapatite-Gelatin Composite with Streptomycin as Antituberculosis Injectable Bone Substitute", *International Journal of Biomaterials*, 2019

Publication

1 %

10

Novita Sari, Alfian Pramudita Putra, Siswanto, Muhammad Fajar Faliasthiunus Pradipta, Dyah Hikmawati. "Hydroxyapatite-gelatin-HPMC composite as injectable bone substitute with alendronate variation for osteoporotic bone", *AIP Publishing*, 2020

Publication

1 %

11 Reyhaneh Fatahian, Mohammad Mirjalili, Ramin Khajavi, Mohammad Karim Rahimi, Navid Nasirizadeh. "A novel hemostat and antibacterial nanofibrous scaffold based on poly(vinyl alcohol)/poly(lactic acid)", Journal of Bioactive and Compatible Polymers, 2020
Publication

12 repository.ntu.edu.sg
Internet Source

13 openaccess.sku.ac.ir
Internet Source

14 Sharjeel Abid, Tanveer Hussain, Ahsan Nazir, Abdul Zahir, Nabyl Khenoussi. "Acetaminophen loaded nanofibers as a potential contact layer for pain management in Burn wounds", Materials Research Express, 2018
Publication

15 Hanyue Zheng, Bhuvana Kannan, Naveen Ashok Chand, Adam Blake, James Chong, Iain Hosie, Pablo Lepe. "ActiVLayr nanofiber technology", Elsevier BV, 2020
Publication

16 Arefe Zargari, Pooria Gill, Golnar Rahimzadeh, Fatemeh Ghadami, Adele Rafati, Pedram Ebrahimnejad. "Aloe vera Nanofibers Contained Pseudomonas Bacteriophages

Fabrication, Characterization, and Biofunction", BioNanoScience, 2022

Publication

17

biointerfaceresearch.com

Internet Source

<1 %

18

"Eco-friendly and Smart Polymer Systems", Springer Science and Business Media LLC, 2020

Publication

<1 %

19

e-journal.unair.ac.id

Internet Source

<1 %

20

e-sciencecentral.org

Internet Source

<1 %

21

ebin.pub

Internet Source

<1 %

22

hdl.handle.net

Internet Source

<1 %

23

link.springer.com

Internet Source

<1 %

24

Abdolmajid Bayandori Moghaddam, Bitas Shirvani, Mohammad Ali Aroon, Tayebe Nazari. "Physico-chemical properties of hybrid electrospun nanofibers containing polyvinylpyrrolidone (PVP), propolis and aloe vera", Materials Research Express, 2018

Publication

<1 %

25

Joon Seok Lee, Kyu Ha Choi, Han Do Ghim, Sam Soo Kim, Du Hwan Chun, Hak Yong Kim, Won Seok Lyoo. "Role of molecular weight of atactic poly(vinyl alcohol) (PVA) in the structure and properties of PVA nanofabric prepared by electrospinning", Journal of Applied Polymer Science, 2004

Publication

<1 %

26

Md. Ariful Islam, Hosne Ara Begum, Md. Abdus Shahid, Ayub Ali. " Antibacterial electrospun nanofibers from poly (vinyl alcohol) and with augmented moisture properties: formation and evaluation ", The Journal of The Textile Institute, 2020

Publication

<1 %

27

Panneerselvam Jithendra, Abraham Merlin Rajam, Thambiran Kalaivani, Asit Baran Mandal, Chellan Rose. "Preparation and Characterization of Aloe Vera Blended Collagen-Chitosan Composite Scaffold for Tissue Engineering Applications", ACS Applied Materials & Interfaces, 2013

Publication

<1 %

28

acikbilim.yok.gov.tr

Internet Source

<1 %

29

addi.ehu.es

Internet Source

<1 %

30	downloads.hindawi.com Internet Source	<1 %
31	polen.itu.edu.tr Internet Source	<1 %
32	pure.hw.ac.uk Internet Source	<1 %
33	stdjns.scienceandtechnology.com.vn Internet Source	<1 %
34	vdoc.pub Internet Source	<1 %
35	Haleema Khanzada, Abdul Salam, Muhammad Bilal Qadir, Duy-Nam Phan et al. "Fabrication of Promising Antimicrobial Aloe Vera/PVA Electrospun Nanofibers for Protective Clothing", Materials, 2020 Publication	<1 %
36	Bakhtawar Ghafoor, Murtaza Najabat Ali, Umar Ansari, Muhammad Faraz Bhatti, Mariam Mir, Hafsa Akhtar, Fatima Darakhshan. "New Biofunctional Loading of Natural Antimicrobial Agent in Biodegradable Polymeric Films for Biomedical Applications", International Journal of Biomaterials, 2016 Publication	<1 %

Exclude quotes Off

Exclude matches Off

Exclude bibliography On

The Effect of Aloe vera Extract Variation in Electrospun Polyvinyl Alcohol (PVA)-Aloe vera-Based Nanofiber Membrane

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9
