

EFFECTS OF CROSSLINKING TEMPERATURE AND TIME ON THE PROPERTIES OF POLY- (ACRYLIC ACID-CO-MALEIC ACID) (PAMA) /POLY (VINYL ALCOHOL) (PVA) ELECTROSPUN NANOFIBER MATS**Thapakorn Charoenying, Tanasait Ngawhirunpat, Theerasak Rojanarata, Prasopchai Patrojanasophon, Prasert Akkaramongkolporn, Praneet Opanasopit***Pharmaceutical Development of Green Innovations Group (PDGIG) Department of Pharmaceutical Technology,
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Abstract: The advantage of thermal crosslinking of nanofiber mats is that the nanofibers will not be easily dissolved and be able to adsorb drug substance when they are soaked in drug solution. Due to the simplicity and environmental friendliness of thermal crosslinking method, previous research prepared crosslinked electrospun nanofiber mats of PAMA/PVA using the thermal crosslinking process to obtain the mats with a negative charge, which can be used to immobilize a positive charge substance by adsorption method. However, the optimal temperature and time for the crosslinking process have not been reported. This study aimed to investigate the thermal crosslinking of electrospun PAMA/PVA fibers. Electrospinning of 50% PAMA/10% PVA solution at weight ratio of 45:55 was completed. The PAMA/PVA nanofibers were thermally crosslinked by placing them in a hot air oven at 100-140 °C for 0.5-5 h. The morphology of the nanofiber mats was investigated by scanning electron microscopy (SEM) and the diameter was measured by J-micro vision® software. The water insolubilization and FT-IR were employed to evaluate the success of the crosslinking process. The smooth bead-free uniform fibers were obtained. The average fiber diameter of the electrospun nanofibers was 376.56 ± 72 nm. Increasing crosslinking time and temperature caused an increment in the degree of crosslinking resulting in a decrease in water solubilization. The desirable temperature and time for crosslinked PAMA/PVA nanofibers was 130 °C for 5 h.

Keywords: Thermally Crosslink, Nanofibers, PAMA, PVA**INTRODUCTION**

Electrospinning is a technique that has attracted much interest in recent years due to its versatility and potential to be applied in diverse fields. Various materials including synthetic, natural, and hybrid polymers have been successfully used for producing nanofibers (Zhang *et al.*, 2016).

Nanofibers are important materials that have been employed in tissue engineering. This is because nanofibers mimic the extracellular matrix structure, having high surface area, high porosity with small pore size, and are able to adsorb biomolecules. However, the biomolecules cannot be directly loaded into nanofibers due to the loss of activity during nanofiber preparation. In addition, the nanofibers readily dissolve when they make contact with a solution. Therefore, biomolecules also cannot be loaded into the nanofibers using an adsorption method. In order to improve the properties of nanofiber mats, crosslinking of the nanofibers is performed to improve the stability of nanofibers when contacted with an aqueous medium. One of the most popular crosslinking methods is thermal crosslinking for its

simplicity and environmental friendliness. The factors related to thermal crosslinking are temperature and time (Tonglairoum *et al.*, 2014a).

Many synthetic and natural polymers have been used to produce nanofiber scaffolds because of their biocompatible and biodegradable properties (Zulkifli *et al.*, 2013). PVA displays good film forming property, biodegradability, hydrophilicity, biocompatibility and chemical resistance (Rezaee and Moghbeli, 2014). PAMA has a negative charge which can be used to immobilize positive charge substances by an adsorption method. In the previous study, the PAMA/PVA crosslinked nanofiber mats were produced and loaded with lysozyme for tissue engineering (Tonglairoum *et al.*, 2014a). However, the optimal temperature and time for the crosslinking process has not been reported. Therefore, this study aimed to investigate the thermal crosslinking parameters of the PAMA/PVA fibers by varying the temperature and time used for the thermal crosslinking process. The success of crosslinking was confirmed by FT-IR and water insolubilization test.

MATERIALS AND METHODS

Materials

50% Poly-(acrylic acid-co-maleic acid) in water, M.W. = 3 kDa) (PAMA) and poly (vinyl alcohol) (PVA, M.W. = 85-124 kDa, degree of hydrolysis 87-89%) were purchased from Sigma Aldrich, MO, USA.

Preparation of PAMA/PVA electrospun nanofiber mats

PVA solution (10% w/v) was prepared by dissolving PVA in distilled water at 80°C until a clear solution was obtained. PAMA solution (50% w/v) was mixed with the 10% PVA solution at the weight ratio of 45:55. The electrospinning solution was contained in a 5-mL glass syringe and underwent the electrospinning process. The feeding rate was 0.25 mL/h and the distance between the end of needle and collector was 15 cm. The electric potential was fixed at 15 kV. The experimental setup for the electrospinning process is illustrated in Fig. 1.

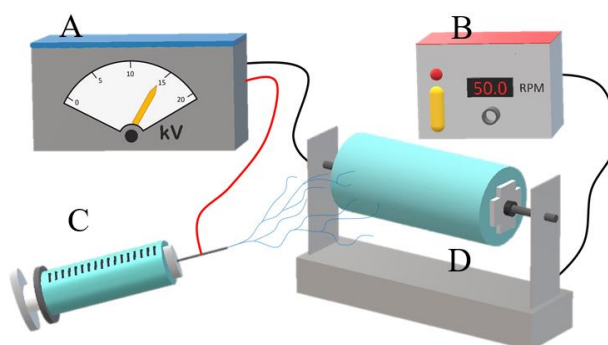


Figure 1. The Electrospinning process. (A) Power supply. (B) Rotation speed controller. (C) Syringe with polymer solution. (D) Collector.

Crosslinking process

The PAMA/PVA nanofiber mats were thermally crosslinked by placing them in a hot air oven at 100, 120, 130, and 140°C for 0.5, 1, 3, and 5 h.

Characterizations of PAMA/PVA electrospun nanofiber mats

The diameter and morphology of the PAMA/PVA electrospun mats were examined using a scanning electron microscope (SEM; S-3400N, Hitachi, Japan). In brief, a small piece of each nanofiber mat covering the aluminium foil was sputtered with a thin layer of gold before the SEM observation. Image analysis software (J-micro vision[®]) was used to measure

50 randomly chosen nanofibers to determine the average diameter. The chemical properties were characterized by Fourier-transform infrared spectroscopy (FT-IR).

Water insolubilization

The crosslinked PAMA/PVA nanofiber mats were cut into small pieces and weighed (W_1). The nanofiber mats were immersed in water and shaken at 150 rpm for 24 h. Then, the fibers were dried at 30°C until a constant weight was achieved (W_2). The water insolubilization of crosslinked nanofiber mats was calculated according to equation (1).

$$\text{insolubilization (\%)} = (W_2/W_1) \times 100 \quad (1)$$

Data analysis

Each experiment was measured in triplicate. The results were reported as mean $\pm$ standard deviation (SD).

RESULTS AND DISCUSSION

Fabrication of PAMA/PVA nanofiber mats

The nanofiber mats of PAMA/PVA were prepared using an electrospinning process from a polymer mixture of PAMA/PVA at the weight ratio of 0.8:1. Smooth fibers without beads were obtained with an average diameter of 376.56 ± 72 nm (Table 1).

Table 1. SEM images (10,000X) of the crosslinked PAMA/PVA nanofiber mats at various crosslinking times and temperatures

Time (h)	Temperature (°C)			
	100	120	130	140
0.5				
1				
3				
5				

Crosslinked PAMA/PVA nanofiber mats

The SEM images of the crosslinked PAMA/PVA nanofiber mats showed similar fiber morphology compared with the non-crosslink fibers. This is concordant with the previous

study which reported that the morphology of nanofibers was not changed after the crosslinking process (Zulkifli *et al.*, 2013).

FT-IR spectroscopy

The FT-IR spectra of the non-crosslinked PAMA/PVA and the crosslinked PAMA/PVA nanofibers are shown in Fig. 2. The spectra of the non-crosslinked PAMA/PVA fibers displayed a strong absorption peak at 1720 cm^{-1} which indicates the C=O stretching of COOH group. The crosslinked PAMA/PVA showed a slightly different FT-IR spectrum compared with that of the non-crosslinked PAMA/PVA nanofibers. The intensity of C=O stretching at 1181 cm^{-1} representing ester bond was increased in spectrum of the crosslinked PAMA/PVA. This is because the crosslink process enables the formation of ester bonds between the COOH group of PAMA and OH group of PVA. These results confirmed that the PAMA/PVA nanofiber mats were successfully crosslinked by the thermal process. According to the literature, the FT-IR spectrum of crosslinked PAMA/PVA had a higher absorption peak intensity at 1181 cm^{-1} than that of the non-crosslinked one (Tonglairoum *et al.*, 2014a).

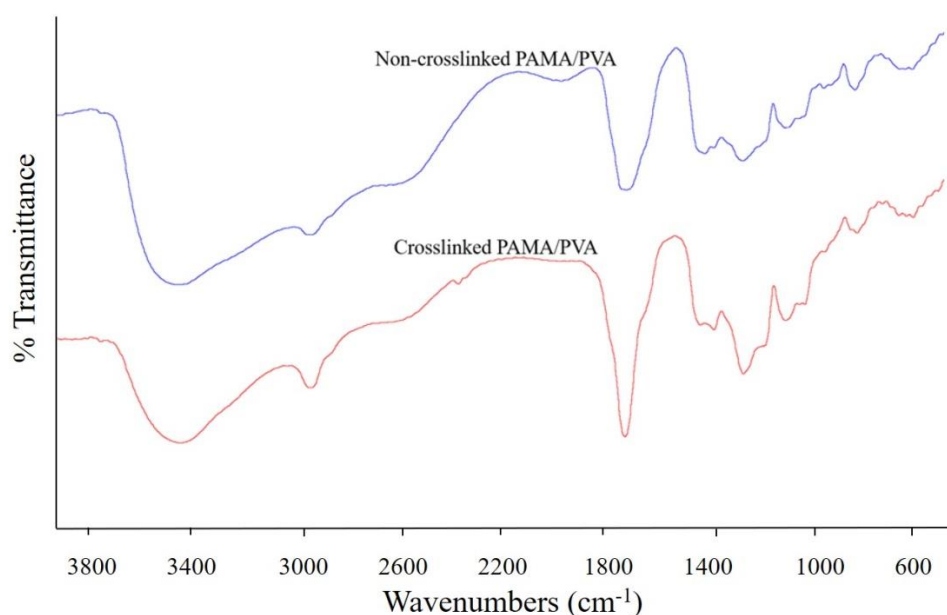


Figure 2. The FT-IR spectra of the non-crosslinked PAMA/PVA and the crosslinked PAMA/PVA nanofiber at 130°C for 5 h.

Water insolubilization

The results of water insolubilization of the crosslinked PAMA/PVA nanofiber mats are presented in Fig. 3. The crosslinking time and crosslinking temperature significantly affect the water insolubilization of the nanofibers. The remaining fibers' percentages were increased as the crosslinking temperature and the crosslinking time increased. This result is in agreement with the previous study (Tonglairoum *et al.*, 2014b). The percentages of remaining fibers crosslinked at 130°C for 5 h was $97.93 \pm 1.88\%$. The fibers crosslinked at 140°C for 5 h turned to dark brown color and became brittle which were unusable. Thus, the PAMA/PVA nanofibers cannot be crosslinked at these thermal crosslinking parameters.

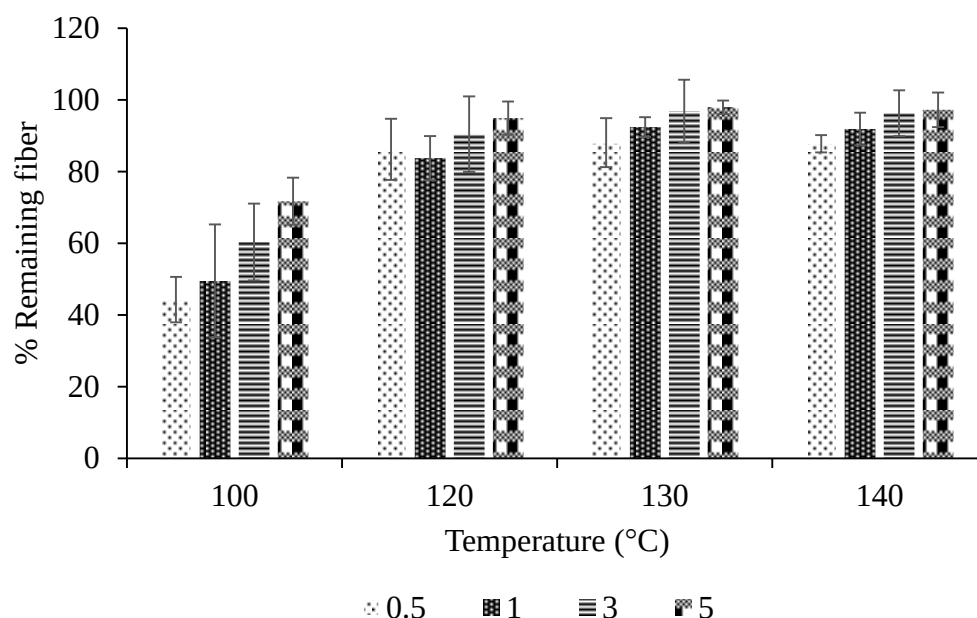


Figure 3. Water insolubilization of crosslinked PAMA/PVA nanofiber mats at different crosslinking times (0.5, 1, 3, 5 h) and temperatures (100, 120, 130, 140°C).

CONCLUSION

In this study, the thermal crosslinking conditions for the crosslink of PAMA/PVA nanofibers were proposed. The appropriate crosslinking of temperature and time for the PAMA/PVA fibers was 130°C for 5 h. This study features the easy approach to crosslink the PAMA/PVA fibers for biomedical application.

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REFERENCES

- Rezaee S, Moghbeli M.R. 2014. Crosslinked Electrospun Poly (Vinyl Alcohol) Nanofibers Coated by Antibacterial Copper Nanoparticles. *Iranian Journal of Chemical Engineering*. 11(3): 45-58. (Accessed on Feb. 19, 2019, at http://www.ijche.com/article_10196.html)
- Tonglairoom P, Ngawhirunpat T, Rojanarata T, Opanasopit P. 2014a. Lysozyme-immobilized electrospun PAMA/PVA and PSSA-MA/PVA ion-exchange nanofiber for wound healing. *Pharm Dev Technol*: 1-8. doi: 10.3109/10837450.2014.954726
- Tonglairoom P, Sutananta W, Rojanarata T, Ngawhirunpat T, Opanasopit P. 2014b. Thermally Crosslinked Chitosan-EDTA/PVA Electrospun Nanofiber Mats: Crosslinking Conditions. *Advanced Materials Research*. 1060:192-5. doi: 10.4028/www.scientific.net/AMR.1060.192
- Zhang X, Tang K, Zheng X. 2016. Electrospinning and crosslinking of COL/PVA Nanofiber-microsphere Containing Salicylic Acid for Drug Delivery. *J Bionic Eng*. 13(1):143-149. doi: [https://doi.org/10.1016/S1672-6529\(14\)60168-2](https://doi.org/10.1016/S1672-6529(14)60168-2)
- Zulkifli FH, Shahitha F, Yusuff MM, Hamidon NN, Chahal S. 2013. Cross-Linking Effect on Electrospun Hydroxyethyl Cellulose/Poly (Vinyl Alcohol) Nanofibrous Scaffolds. *Procedia Engineering*. 53: 689-695. doi: <https://doi.org/10.1016/j.proeng.2013.02.089>