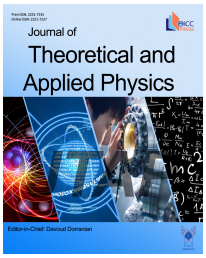

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Preparing and synthesizing modified electrospun nanofibers via online UV method from poly acrylonitrile and poly vinyl alcohol for enzyme (glucose oxidase) immobilization

Sepideh Asadi[1], Mehdi Jahanfar[1,*], Saeed Javadi Anaghizi[2], Dariush Minai-Tehrani[1]

[1]*Faculty of Life Sciences and Biotechnology, Shahid Beheshti University (G.C), Tehran,Iran.*
[2]*Central Laboratory of Shahid Beheshti University (G.C), Tehran, Iran.*

[*]**Corresponding author:** jahanfarmehdi170@gmail.com

Original Research

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Abstract:

The study explores the use of nanofibers for enzyme immobilization, leveraging their high contact area and controlled cavities. By employing ultraviolet (UV) irradiation on a polymeric blend of polyvinyl alcohol (PVA) and poly acrylonitrile (PAN) during electrospinning, nanofibers were fabricated for glucose oxidase (GOD) immobilization. UV treatment enhanced carboxyl group presence on nanofiber surfaces, as confirmed by Fourier transform infrared (FTIR) analysis. Enzyme activity assessments demonstrated a 33% increase in immobilized enzyme activity when PVA content in the nanofibers was raised from 1% to 3%. Scanning electron microscopy (SEM) images revealed that UV treatment helped maintain nano filament structures post-immobilization, aiding enzyme retention and controlled release. Atomic force microscopy (AFM) images displayed increased roughness in UV-treated nanofibers. The study showcased GOD reusability up to 12 cycles in 3% PVA-containing samples.

Keywords: Enzyme immobilization; Nanofibers; Glucose oxidase; PAN; PVA; UV

1. Introduction

Over the recent decades, nanofibers have received particular attention due to their special properties and interesting applications such as having light weight, huge surface-area-to-volume ratio vis-à-vis other known forms, porous texture, and outstanding mechanical features [1]. Fibers are uni-dimensional nanomaterials. They can be synthesized a sundry of approaches; electrospinning is deemed the simplest among others. This boils down to the fact that this method can provide nanofiber fabrications that are extremely elongated in length, have uniform diameter and have a diversified composition [2].

Nanofibers can be adapted in different products, including templates, scaffold of tissue engineering, filtration [3, 4], sensors, pharmaceutical and biomedical applications to, composite materials as well as optical and electronic devices. Specifically, regarding the biosensors, the electrospun nanofibers offer particular properties and advantages. The features of enzyme immobilized electrospun nanofibers applicable futuristically are in the advancement of biofuel cells, biocatalysts and biosensors [5]. Immobilization means the enzyme molecules' fixation on a firm scaffold for constant catalytic activity. It is possible to reuse the immobilized enzymes, better in the control reaction [6]. Evidently, a vast specific surface area and the electrospun nanofiber's fine porous structure can significantly augment the catalytic activity of the immobilized enzymes [3].

The most thoroughly scrutinized method for immobilizing enzymes can be implemented by physical adsorption, covalent bonds to polymers which are water-insolubly functionalized (known as organic or nonorganic polymers) or

entrapment/encapsulation [4]. Adsorption process offers a greater commercial potential amongst other immobilization methods, as this process is simpler and more economical with a high catalytic activity. Also, its most significant advantage is that the support could be reused repeatedly after the immobilized enzyme inactivation [7]. Hence, reversible immobilization of enzyme through adsorption needs a cogent interaction such as a hydrophobic or ionic interaction between the support and enzyme [8].

A quasi-crystalline hydrophilic polymer, PVA, has high chemical and thermal stability. Besides being nontoxic, it has high water-permeability and high biocompatibility, with extensive hygroscopic features making PVA a suitable candidate for various applications. It can also be processed easily and have interaction with other organic and inorganic materials [9]. Because of its functional groups as well as easy preparation as a bulk material and in films and fibers, PVA may be useful in practical studies of functional polymers [10]. Nevertheless, PVA polymer solubility in aqueous media restricts its applications. For rendering electrospun fibers of PVA water insoluble, various approaches have been utilized including heat treatment [11], methanol treatment [12], chemical crosslinking (largely through) glutaraldehyde [13], and irradiation [14, 15]. From these, crosslinking induced by UV has demonstrated various advantages including easy manipulation, posing low hazard for researchers, being highly effective and having controllable reaction. Further, crosslinking and sterilization could be performed simultaneously during the UV irradiation, thus significantly facilitating the adoption of crosslinking induced by UV techniques for biomaterial preparations [16]. PAN is described as an environmentally friendly polymer which has fine stability and mechanical features which can be easily electrospun into nanofibers. Although these polymers have these properties, for immobilization of macromolecules, they do not have enough functional groups. Furthermore, the surface of PAN fibers is modified by grafting with functional polymers for modifying the final surface features. In recent years, extensive research has targeted the modification of PAN surface for adsorption of bio-macromolecules [17]. The research indicated that, with their own separate advantages in the application, PVA and PAN are wildly applied for producing organic fibers through electrospinning. Recently, a myriad of studies has aimed to synthesize the blending of polymers fibers during the electrospinning process through employing various approaches such as thermal treatments for the composite membrane [18, 19]. Among these, a safe and efficient approach for surface modification and blending polymers is irradiation especially UV which is used after electrospinning process called UV cured or during the process called on-line UV [20]. Polymers that are highly cross-linked can be fabricated at room temperature with the UV cure system. In this system, an initiating species is produced by the UV irradiation, such as cations or free radicals, through the photolysis of cationic-type photo initiators or radical type [21]. Ester bond is reported to break by the UV with wavelength around 250 nm; though the break was constrained due to its short etch depth [22] being reported to have the impact of UV-ozone sterilization on surface morphology of polyurethane nanofibers, it has been demonstrated that the UV-ozone significantly morphed the morphology of nanofiber [23].

In this study, PVA and PAN electrospun into nanofibers were exposed to UV irradiation during the process. Then, GOD solution was immobilized on them and the immobilized enzyme's activity was gauged through the colorimetric assay in order to find the best conditions for the support and examining the effect of UV irradiation on nanofibers.

2. Materials and methods

2.1 Materials

PVA with molecular weight of 72,000 was obtained from Merck Chemical Co. (USA). Beta-D-glucose and malonic acid (MA) were purchase from Sigma-Aldrich (St Louis, MO, USA). O-dianisidine $[(CH_3O)(H_2N)C_6H_3]_2$ with molecular weight of 244.29 g/mol was obtained from Fluka. Glucose oxidase (GOD) was bought from Aspergillus Niger. (Sigma Prod. Nos. G-6766). AppliChem Co provided horseradish peroxidase (HRP). Dimethylformamide (DMF), which was used as a solvent for polymeric solution, was obtained from Merck Chemical Co. (USA). Potassium phosphate (0.1 M, at pH 6), prepared from sterilized water and potassium acetate solution, was utilized as the buffer.

2.2 Nanofibers electrospinning

PAN solution (1, 3 and 5% Wt.) and PVA were gleaned by being dissolved in dimethyl formamide $[(CH_3)_2NC(O)H]$, and MA was used as a cross linker in the polymeric solution; the weight ratio for blending PVA was chosen to be 1:1 which was added to the solution simultaneously. Next, through the magnetic stirrer it was stirred for 3 hours at 80 °C until transparent solutions were obtained. The solution was placed at room temperature under stationary conditions for 12 hours for gleaning a uniform solution, devoid of bubbles. The polymer solution was loaded to a syringe and then was fixed into a syringe pump with a flow rate of 0.5 mL/h, with 22 kV of positive voltage applied to the needle through a supply of high voltage power. The distance between nozzle to the aluminum ground collection plate foil was 15 cm. through the colorimetric assay in order to find the best conditions for the support and examination of the effect of UV irradiation on nanofibers.

2.3 Modification of electrospun nanofibers via UV

Figure 1 displays the schematic representation of the electrospinning device with UV lamp for the treatment. As the solution reservoir, a syringe with metal needle (2 cm of length and 0.6 mm of diameter) was utilized. Cylindrical grounded rotating drum collector was installed in front of the nozzle. The distance between the nozzle and the counter electrode (distance from tip-to-collector) was 15 cm, and the applied voltage was 22 kV. The drum had a rotation of 600 rpm. A distance of 5 cm was provided between the collector and the UV lamp. The UV had a continuous irradiation during the electrospinning. To avoid the UV irradiating the solution reservoir, the electrospun fibers were garnered on the top of the drum collector on the bottom

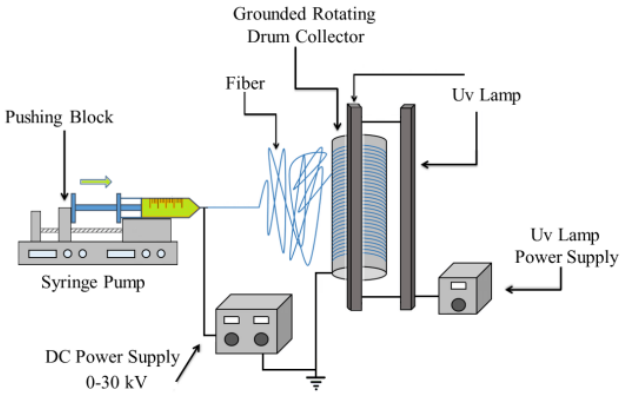

Figure 1. Schematic representation of the electrospinning device with UV lamp.

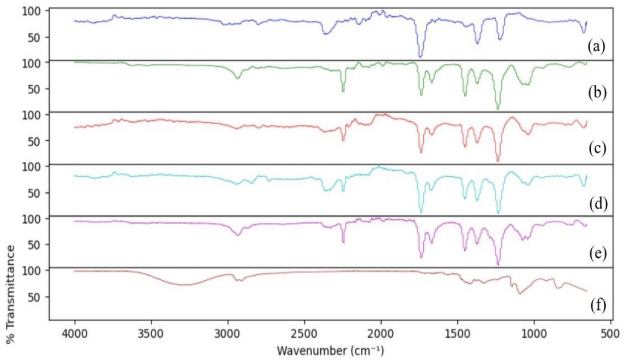

Figure 2. ATR-FTIR spectra of (a) PVA, (b) PAN + PVA (PVA/PAN 3%) UV treated, (c) PAN + PVA (PVA/PAN 3%) non-treated, (d) PAN + PVA (PVA/PAN 1%) UV treated, (e) PAN + PVA (PVA/PAN 1%) non-treated, (f) PAN.

sides of the drum, and two UV lamps (285 nm, 20W-philips Germany) were irradiated [24].

2.4 Characterization

After immobilization and soaking in the buffer solution, the morphology of UV treated and non-treated nanofibers was observed through a SEM (Hitachi SU3500), after 10 nm gold sputter-coating. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) was employed for investigating the surface chemical structure of UV treated and non-treated nanofibers. The nanofibers' ATR-FTIR spectra (ATR-FTIR-NEXUS 470, THERMO NICOLET CO, and USA) were detected within the 4000 to 500 cm^{-1} wavenumbers, via 60 scans with resolution of 4 cm^{-1}. An atomic force microscopy (AFM) (Ara Pajohesh) and a SEM (Hitachi SU3500) delineated the morphology of non-treated and UV treated nanofibers, after 10 nm gold sputter-coating.

2.5 Enzyme immobilization and colorimetric assay

GOD solutions (with activity 37.4 Unit in phosphate buffer pH 7, 0.1 M) were used to spray treated nanofibers for three times. The nanofibers were then cut into tiny pieces (1×1 cm^2). The activity of immobilized enzymes was set by transferring a small piece of wet nanofiber in the test tube which contained 2 mL phosphate buffer pH 7, 0.1 M, 100 μL of 0.02 mg/mL O-dianisidine and 100 μL 18% glucose solution plus 10 μL horseradish peroxidase enzyme (1 mg/mL). Absorbance increase was recorded at 640 nm every 30 seconds. In addition to the above-mentioned method referred in this research as wet assay, the small pieces of nanofibers were incubated at room temperature for 24 h to be dried. The activity of immobilized glucose in the dried nanofibers was monitored according to the above-mentioned assay which is referred to as the dry assay in this research [25].

3. Results and discussion

3.1 ATR-FTIR

The treatments were all applied at atmospheric pressure of 14kV–6kHz. As displayed in the Figure 2, in comparison with the main scaffold PAN + PVA non-treated, the scaffold ATR-FTIR spectra pertained to PAN + PVA UV treated scaffolds indicated some alterations in special bands. As indicated in the figure, a band is seen within the range of 1050 cm^{-1} which is related to the stretching C−O group. The occurrence of this band can be justified due to the reaction of PAN and PVA. For all scaffolds, the absorption band at 2244 cm^{-1} is related to the stretching vibration of the nitrile group whose intensity is diminished in UV treated scaffolds due to the oxidation reaction. C−H asymmetric stretching is deemed as the principal reason of sharp intense absorption at 2932 cm^{-1} and 1448 cm^{-1}.

3.2 Activity of GOD immobilized on the nanofibers

According to the following diagrams in Figure 3 (a) and (b), it can be concluded that as the PVA percentage in nanofibers increased, so did the amount of enzyme activity. The rise in the activity can be the result of the increase in the number of enzymes immobilized on nanofibers. This trait can be explicated through the realization of augmentation in the hydroxyl group of PVA. As observed in Fig. 3 (c) and d, the activity of enzymes immobilized on PAN + PVA nanofibers, after UV irradiation, monitored in dry condition incubating immobilized nanofibers at room temperature for 24 hours; it showed higher activity than the non-treated nanofibers. According to some documentations, the impact of porous matrices in retaining the activity of the enzyme is discernable. That is related to furnishing microenvironment for the enzyme structure for protecting it against deactivation [26]. Thus, it can be concluded that UV irradiation boosts the storage of enzymes in treated nanofibers by making an appropriate microenvironment for the immobilized enzymes through creating more carbonyl group gleaned from the spectrum of ATR-FTIR.

As exhibited in Figure 4 (a), the enzymes immobilized on the UV-irradiated polymer in the first utilization after the activation were less active compared to the enzymes exposed to UV irradiation. Nevertheless, after drying, they have greater activity in reusing the enzyme. This result reveals the ability to store more enzymes in a UV-exposed scaffold. These results can be pertinent to the configuration of more cross-linked polymer chains. To confirm this, the results of the activity of enzymes immobilized on both polymeric scaffolds after 1 hour of continuous activity in the substrate

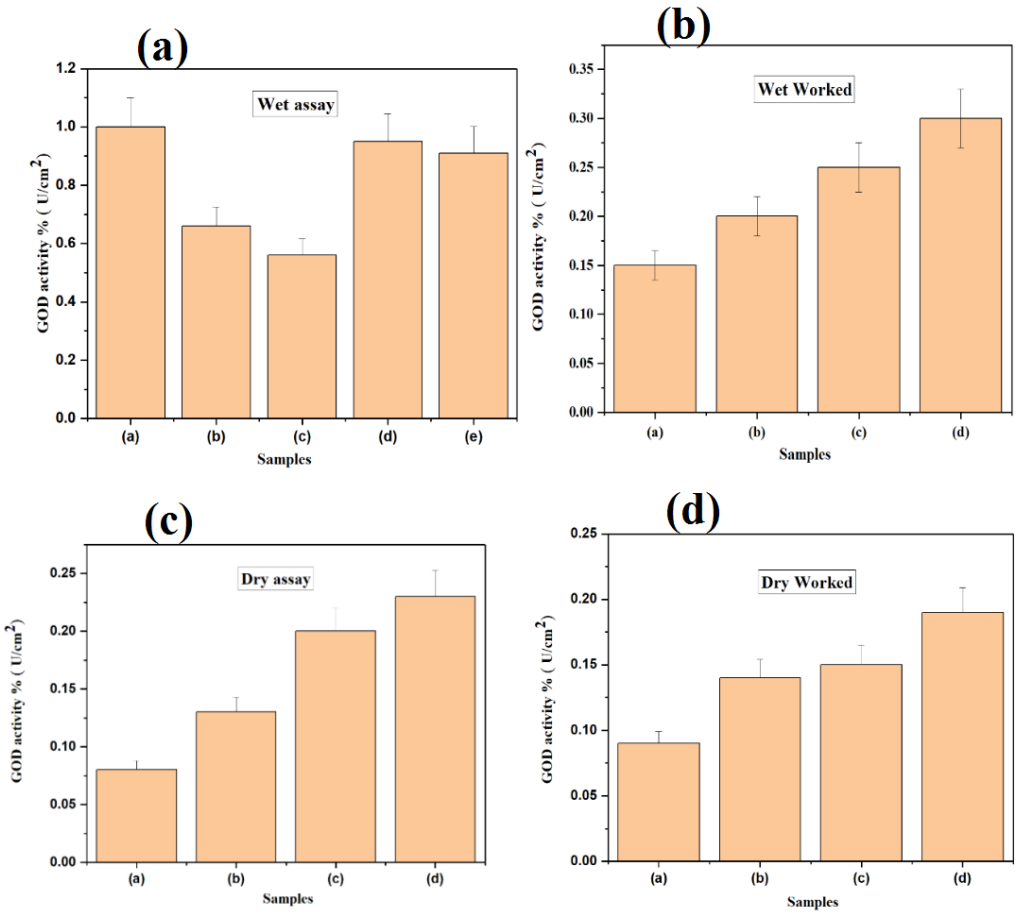

Figure 3. Diagrams of GOD activity immobilized on the surface of (a) PVA/PAN 1%, (b) PVA/PAN 1% UV treated, (c) PVA/PAN 3%, (d) PVA/PAN 3% UV treated nanofibers under different conditions assay (A: wet assay, B: wet worked, dry assay, and D: dry worked).

solution and 1 day of drying were investigated, whereby the same trend was observed again (Figure 4 (b)).

3.3 Nanofibers morphology

Figure 5 presents the electron microscope images of nanofibers synthesized under UV irradiation and nanofibers synthesized without UV irradiation before enzyme immobilization. As represented in the figure, nanofibers synthesized under UV irradiation and nanofibers synthesized without UV irradiation have the same nanofiber structure and do not evince much difference. It can be elicited from Figure 6, that the diameter of UV treated nanofibers measured by image J, was expanded. According to the diagram in Fig. 6, the diameter range of electrospun fibers in this study has demonstrated to be between 650 and 1200 nm. It might be related to the establishment of more cross-links between the polymer chains synthetized under UV irradiation. Figure 7 depicts the electron microscopy images of nanofibers

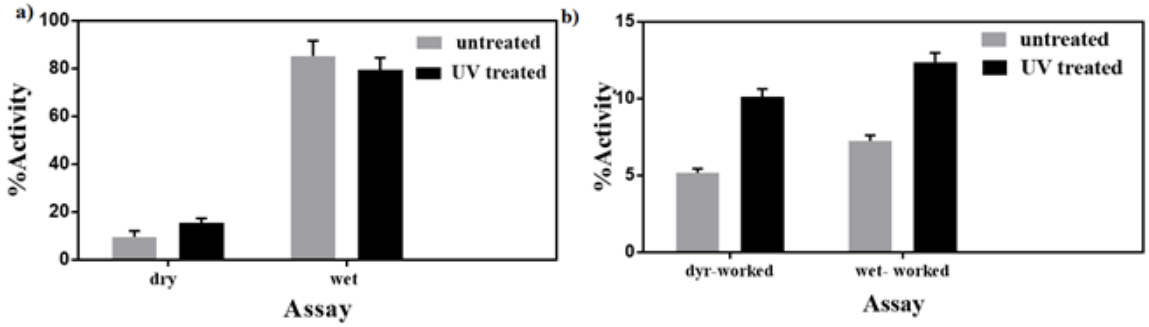

Figure 4. Enzyme activity measurement chart based on the effect of UV irradiation on the enzyme immobilization under different condition of assays, (a) immediately after immobilization (wet) and were incubated in room temperature for 24 h to be dried (dry), (b) previous condition in (a) under condition that being after one hour of continuous activity in the substrate solution and one day of drying.

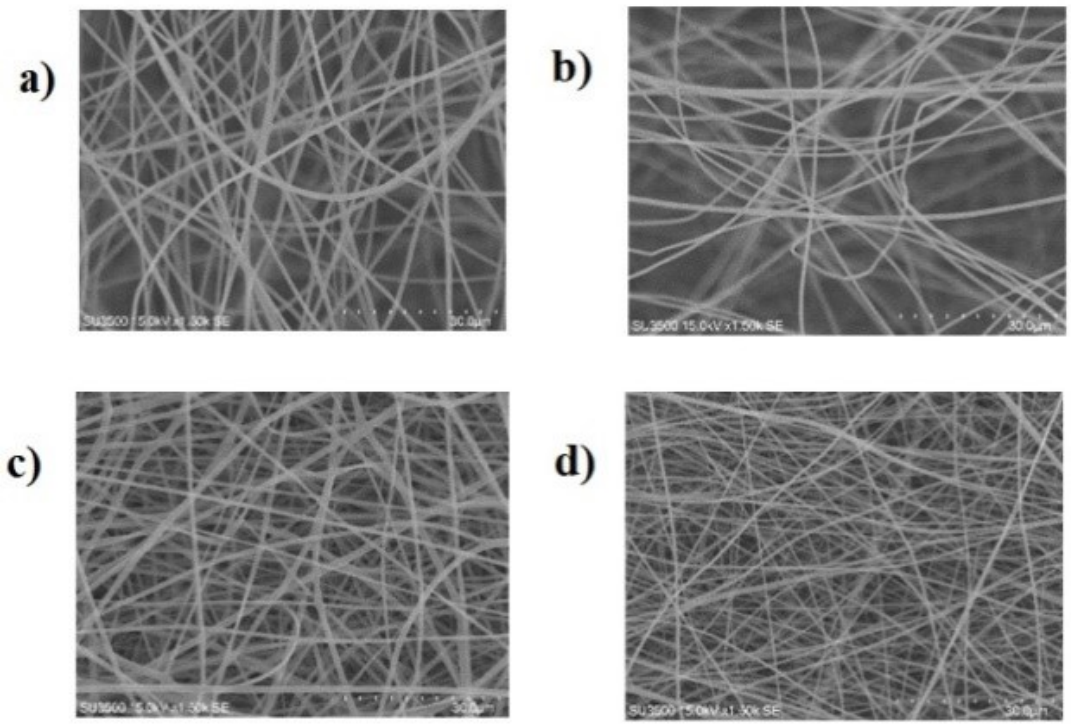

Figure 5. The nanofiber's morphology of membrane using SEM, (a) electrospun nanofibers PVA/PAN 1%, (b) PVA/PAN 1% UV treated, (c) PVA/PAN 3% , (d) PVA/PAN 3% UV treated Nano fibers.

treated with UV and non-treated nanofibers after soaking in enzyme solution for 60 minutes. The images clearly show that the nanofibers which were not treated have lost their nanofiber structure after soaking; however, Figure 8 describes the UV treated samples have retained their fiber-form and little damage was observed in the fiber structure. The UV irradiation has retained nanofiber structure even after 1-hour continuous activation on the substrate solution. It can be related to the generation of more crosslinking bonds between the polymer chains as presented in the spectra of ATR-FTIR (Figure 2).

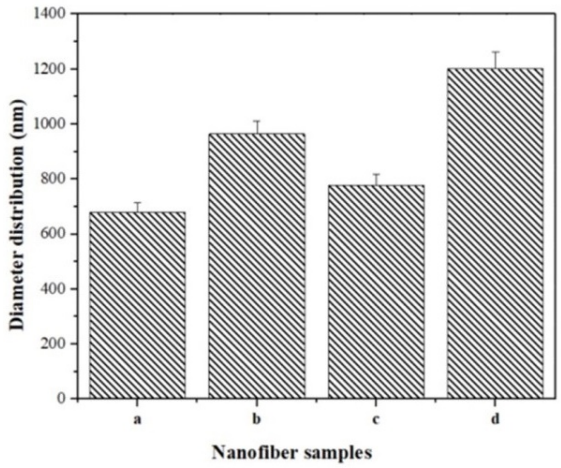

Figure 6. Diameter distributions of (a) electrospun nanofibers PVA/PAN 1%, (b) PVA/PAN 1% UV treated, (c) PVA/PAN 3% , (d) PVA/PAN 3% UV treated.

Figure 9 (a) indicates the AFM image nanofibers which were not treated and Figure 9 (b) shows the nanofibers which were treated with the UV irradiation. As anticipated and the figure clearly presents, once nanofibers of PVA/PAN are exposed to the UV irradiation, rougher surface is observed. The close scrutiny of the AFM images demonstrated an average roughness (Ra) of 88.8 nm for the unmodified membranes and the value of 140 nm was recorded for the membranes treated via UV irradiation. For the nanofibers which were not treated and the ones which were treated by UV, the values of 108 nm and 174 nm were assessed respectively for the root mean square roughness (Sq).

3.4 Reusability of immobilized enzymes

According to the results, PAN + PVA (PVA/PAN 3%) nanofibers without UV irradiation demonstrated the highest relative activity after repeated uses. This process of reuse was as such that after every usage of immobilized enzymes on the nanofibers, it was dried at room temperature for 1 hour and then used again. Figure 10 depicts a graph of reusability of the immobilized enzymes on this nanofiber during a dozen consecutive measurements of the catalytic activity of this nanofiber. The enzyme adsorption (of only physically adsorbed enzyme molecules) lost during the measurement process might be an explanation for the loss of catalytic activity. Further, the nanofiber-likeness of the fibers became damaged and the surface area became smaller after several assays. As presented in Figure 8, after twelve times of usage, while the enzymes immobilized on UV treated scaffolds have kept more than fifty percent

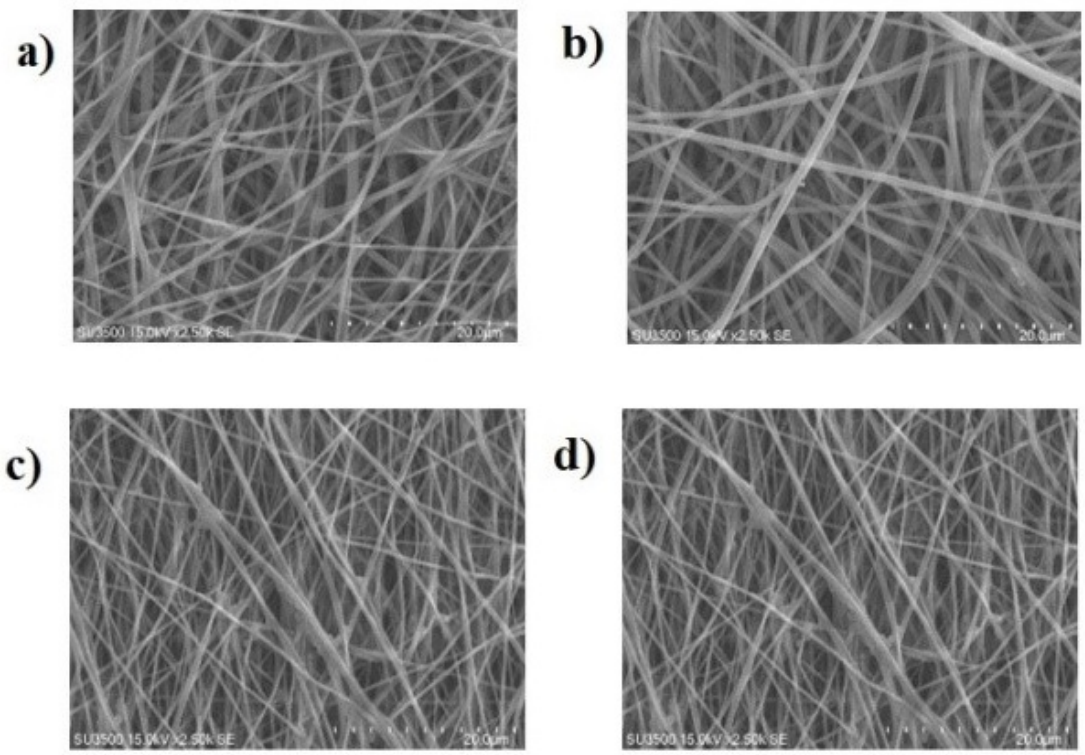

Figure 7. The nanofiber's morphology of membrane using SEM after soaking in enzyme buffer for 60 minutes, (a) electrospun nanofibers PVA/PAN 1%, (b) PVA/PAN 1% UV treated, (c) PVA/PAN 3% , (d) PVA/PAN 3% UV treated Nano fibers.

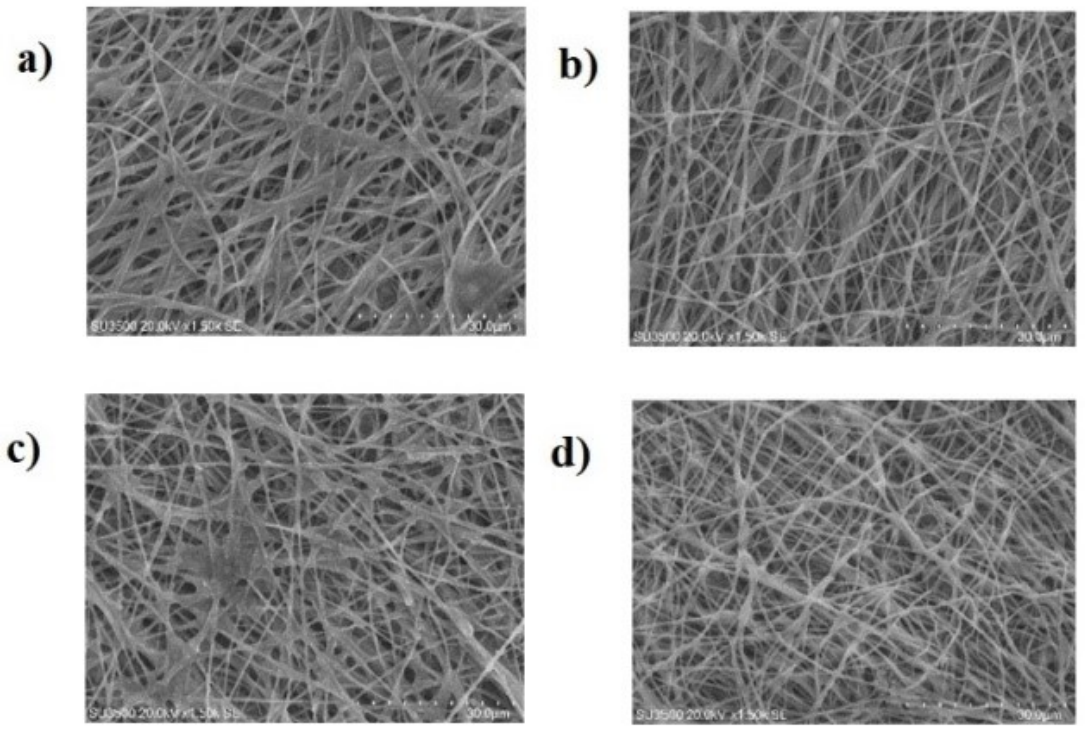

Figure 8. The nanofiber's morphology of membrane using SEM after an hour of continuous activity in substrate solution, electrospun nanofibers (a) PVA/PAN 1%, (b) PVA/PAN 1% UV treated, (c) PVA/PAN 3%, (d) PVA/PAN 3% UV treated Nano fibers.

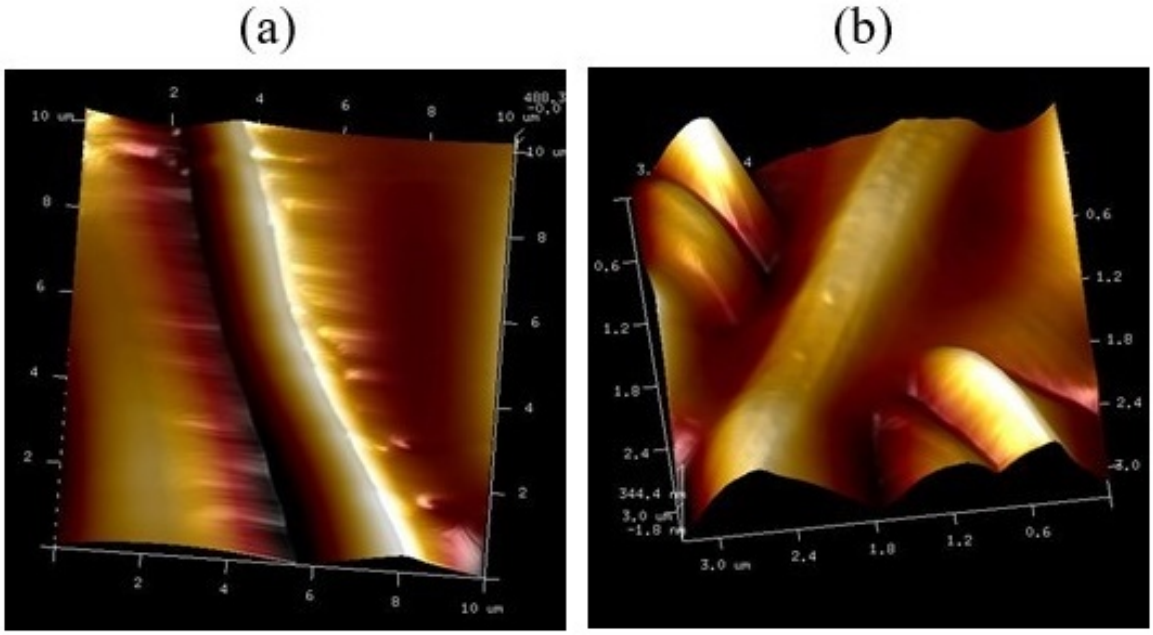

Figure 9. AFM images of PVA/PAN 3% nanofibers (a) non-treated, (b) UV treated .

(52%) of their activity, the non-treated scaffolds have kept only 22%. For the industrial applications, such reusability in the continuous use of this enzyme is valuable.

4. Conclusion

The use of ultraviolet, gamma irradiation or plasma to modify the surface and improve the biocompatibility and mechanical properties of nanofibers has been common among scientists in recent years [27, 28]. But it is worth mentioning that in all previous studies, the polymer solution was either treated before electrospinning, or the polymer nanofibers were treated with plasma or ultraviolet or gamma rays after being fabricated [29, 30].
But in the present study, for the first time, UV irradiation have been used during polymer fabrication, and as a result, every fiber of the polymer layer by layer has been exposed to UV radiation online, and this has enhanced the impact of radiation and a significant improvement in the characteristics of biocompatibility and mechanical stability of the polymeric scaffold, therefore the efficiency of enzyme immobilization and the stability of the immobilized enzymes on the polymer surface. The present research explored the impact of UV irradiation on the PVA/PAN nanofibers. It was demonstrated that the UV irradiation can have a significant influence on the crosslinking between polymer chains and increase in the hydrophilicity of fibers. It was observed that the enzyme storage in the nanofibers increased. The activity degree was enhanced in the non-treated samples vis-à-vis the sample which was modified in wet assay. This is related to releasing the unbounded enzymes to the substrate solution. Nonetheless, the storage stability and reusability showed improvement in the UV treated nanofibers. It was due to the retaining of more maintaining immobilized enzymes.

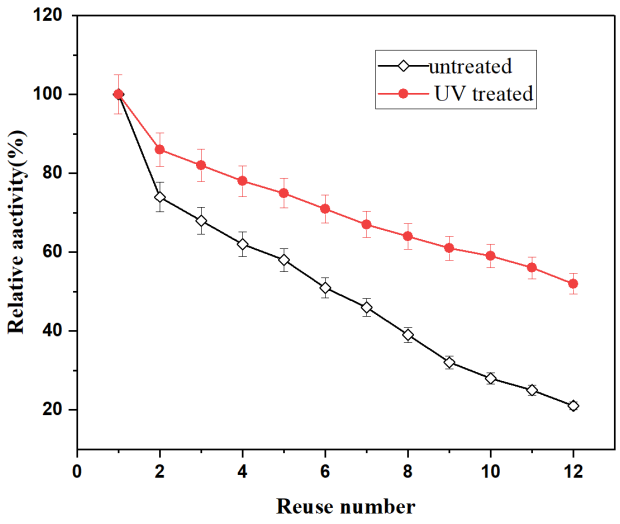

Figure 10. Graph of reusability of immobilized enzymes on, PVA/PAN 3% nanofibers without UV irradiation.

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Ethical Approval
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval is not applicable.

Authors Contributions
All the authors have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript.

Availability of Data and Materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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