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Original Research

Immobilization of glucose oxidase by starch-based nanofibers using plasma surface modification

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ABSTRACT:

In this research in order to produce blood sugar biosensor, an appropriate membrane for glucose oxidase immobilization by using nanofibers created from polymers of polyacrylic acid and starch are studied. They are biocompatible and biodegradable respectively and were prepared by electro-spinning method for nanofiber fabrication. Dimethylformamide and distilled water were used as solvent for PAA and starch respectively to get a homogeneous solution. Because nanofibers made of polyacrylic acid-starch face with enzymes, due to its extremely high hydrophilic 'OH' groups may lose their cohesion, crosslinking as chemical surface modification and for better enzyme immobilization, non-thermal plasma surface modification using atmospheric pressure Dielectric Barrier Discharge (DBD) were used. Crosslinking was carried out by APTMS and Glutaraldehyde (GA). The effect of electro-spinning process variables on morphology of nanofibers was examined by Scanning Electron Microscopy (SEM). Nanofibers structure and chemical composition to demonstrate the successful linking and immobilization of enzymes in the composite membrane was obtained by Fourier Transform Infrared spectroscopy (FTIR) and improved thermal stability of nanofibers in presence of enzyme and surface modifications was determined by Thermal Gravimetric Analysis (TGA).

Keywords:
Polyacrylic acid biosensor, Glucose oxidase, Enzyme stabilization.

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INTRDUCTION

Enzymes are considered as natural catalysts which exist in all organisms and they accelerate all processes (Reach and Wilson, 1992). In the recent decades, following the appearance of new technologies, speed accuracy and selectivity of sensors have increased, and hereon nanotechnology has played more roles by presenting nanostructures. One of these nanostructures which have been useful in many aspects especially in biosensors is polymeric nanofiber. Nanofiber layers due to high specific surface and highly porous structure are the best option in order for stabilizing the enzyme in biosensors (Yarin *et al.,* 2001). Also, in order for stabilize the enzyme against unsuitable environmental condition, electrospinning approach with a composite of polymer alloys is used and it is possible to take advantage of a polymeric material which is more compatible with natural polymers. Accordingly, the end of the present research is the amendment of surface properties of alloy nanofiber based on starch and by using plasma for stabilization of glucose oxidase enzyme

MATERIAL AND METHODS

Materials

Polyacrilic acid was purchased from the Sigma-Aldrich and Co with the medium molecular weight of 130000 gr/Mol. Solvent such as starches in water, Dimethylformamide (DMF), the glutaraldehyde solvent 25% in water, with molecular weight of 100.1 gr/Mol, Aminopropyl Trimethoxysilane (APTS) with purification of 99% and sodium acetate with molecular weight of 82.03 gr/Mol were purchased from the Merck company. Acetic acid with molecular weight of 60.05 gr/Mol, beta D glucose, ABTS, and HRP were available in the Nano laboratory of Shahid Beheshti University, and all the chemical compounds with laboratory grade were purchased from the Merck company.

Methods

Creating nanofiber of polyacrilic acid/starch

In order to create the pure polymer solvent of polyacrilic acid, the DMF solvent was used. To form nano- fiber polymer on the inverting framework, weight is different percentages were created, and among them, only the polymer solvent with 5% weight found to be was suitable. To create this solvent, 0.25g of poly acrylic acid powder was dissolved in 5 CC DMF solvent and the solvent was put on the mixer for 2 hours in atmospheric temperature and with 700 rpm to gain a homogeneous and wholly transparent polymer solvent. 5 % of 5 ml starch was added to the mixer and kept for 2 hours in 80ºC. After preparing these two solvents, at different percentage the starch and polyacrylic acid were mixed together, and 30:70 relevancy was chosen as the weight efficient relation. According to the efficient parameters, electro spinning was conducted (Yao *et al.,* 2013).

Electro spinning of nano composite fibers

In order to collect polymer of nanofiber, the aluminum foil was used. The aluminum frame was first fully washed with water and alcohol, and then was put on the inverting framework. The hypodermic needle with the outside diameter of 12 mm was attached to the syringe, and the distance from the hypodermic needle to the inverting frame was set on 15 cm, and the voltage difference of 16 kV was applied to the feed source. Discharge was set on 0.5 mm per hour. The electro spinning time for reaching all the solvents was considered as seven hours. The humidity in the system was 27ºC and the environmental temperature was 30ºC. The mentioned conditions using the trial and guess method resulted in a desired procedure followed by us (Theron *et al.,* 2001).

Surface modification and fiber cross- linking

After creating the fiber, it is required that the produced nanofiber maintain its sustainability so that at the time of being put in water environment, it does not melt (solvent). So, to stabilize the enzyme on the

nanofiber, it is required first to modify the nanofiber surface. For this purpose, different physical and chemical methods were used. First of all, the thermal modification on the nanofiber was used. With regard to the SEM results of the nanofiber surface, the cross linking was not applied. Then, the chemical modification on the nanofiber surface using aminopropyl trimethoxysilane and Glutaraldehyde (GA) was applied. In order to create cross linking, first, different percentages of (GA) were used. Regarding the structure of glutaraldehyde it causes the link from one side to the nanofiber polymer and from the other side to the enzyme and maintains the nanfiber's structure. The utilized percentages were 1, 2, 5 and 10 per weight, which, according to the results, the cross linking was not applied here as well, and as soon as putting it inside the water, the electrospinning fibers were dissolved. In the next level, the APTMS was used along with the GA. APTMS due to the silane linking, caused the prevention of dissolving of nanofiber inside water. (Lu *et al.,* 2008)

Plasma modification

The other way of physical modification used in this survey for creating surface modification on the produced nanofiber was using plasma. For so doing, the samples were exposed to air plasma in time durations of 2, 6, and 10 min, and using the Fourier Transform Infrared Spectroscopy (FTIR), the chemical changes gained from plasma modification was analyzed. Also, the samples were analyzed by the Scanning Electron Microscope (SEM) (Lue *et al.,* 2006).

Enzyme immobilization

After preparing the proper substrate for fixing and then creating cross linking on the substrate and modification of plasma surface, the enzyme should be immobilized. For this purpose, the commercial glucose oxidase enzyme with special activity of 118 units in return for each MM, in a given amount being 1*1cm for each laboratory sample was used (Song *et al.,* 2013).

RESULTS AND DISCUSSION

Scanning Electron Microscope (SEM) analysis

Polyacrylic acid / starch nanofibers

In Figure 1, the SEM pictures of the nanofiber has been displayed. Figure 1a. relates to the pure polyacrylic acid and Figure 1b relates to the polyacrylic acid/ starch (70:30). The diagonal of the fibers in electro spinning procedure depends on viscosity of the solution, resistance and conductance, surface tension, molecular weight, molecular weight distribution, and polymer topology (Fong *et al.,* 1999). The diagonal measurement of the fiber has a direct relationship with viscosity (ŋ) '0' and an inverted relationship with the conductance of solution (Sheldon, 2007). The increase in starch increases the diagonal of the fiber, which is due to the reduction of electro spinning conductance of the solution. But by exceedingly increasing starch, the diagonal is again reduced; that is because the viscosity of the starch solution is less compared to the polyacrylic acid solution, and the diagonal of fibers is against these two factors.

Modified nanofibers

In order to immobilize the glucose oxidase enzyme on the nanofiber, first, the polymer surface is modified by plasma atmospheric pressure of dielectric barrier discharge, and the enzyme was immobilized according to the mentioned way. In Figure 2, the gained SEM pictures of immobilization on these fibers are seen. Regarding the picture, it can be seen that the plasma modification, in addition to maintaining the shape of the fibers, causes more stability of the enzyme on the

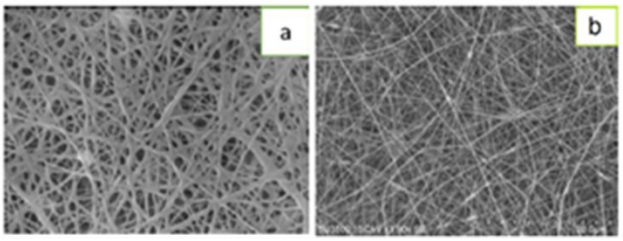

Figure 1. The composite nanofiber with fixed parameter conditions of electro spinning procedure (a). Pure PAA (b). PAA/ starch (70:30)

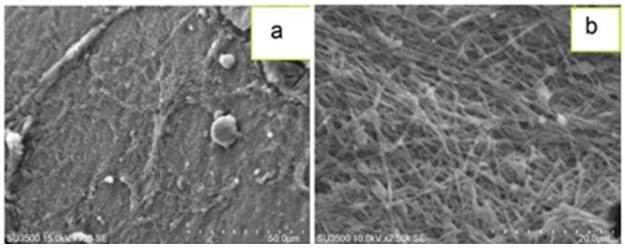

Figure 2. Enzyme immobilization on the nanofiber polymer with stable conditions of parameters of electro spinning (a) Without plasma modification (b) with plasma modification

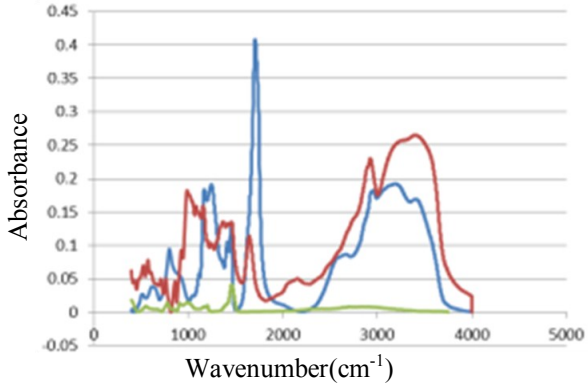

Figure 3.The ATR spectrum of nanofiber of pure polyacrylic acid (blue), pure starch (red) and nanofiber of polyacrylic acid/starch (green) (70:30)

nanofiber surface. The morphology pictures showed that the chemical modification had an important role in forming cross linking and plasma modification caused the improvement of the enzyme fixing on the nanofiber (Matsuoka *et al.,* 2003).

Attenuated Total Reflectance (ATR) analysis

Polyacrylics acid/ Starch nanofibers

In Figure 3, the related spectrum of the pure polyacrylic acid and polyacrylic acid/ starch (70:30) is shown. The feature peak of nanofiber of polyacrylic acid as reported by Li and Wu (2009) is 1780 cm and 2936 cm, belonging to the carboxylic acid group, existing in the whole synthesized nanofiber. In the spectrum related to starch, the peak is 3418 cm and the peak 1646 cm relates to the free hydroxyl groups (OH). The peaks 1156 cm and 1080 cm relates to the stretching shake of carbonyl groups (C-O) in hydroxyl groups (C-O-H) and the peak 986 cm is related to the stretching vibration of carbonyl groups in ether linking(C-O-C]) (Namazi *et al.,* 2011).

In composite fibers, i.e. polyacrylic acid/ starch observing the C=O stretching acid linking in 1730 cm, it could be concluded that the polyacrylic acid and starch are well combined and have formed a linking. Also, the weak peaks of tensional C-O and bending O-H in 974 and 1369 cm, respectively, shows the establishment of composite linking of polyacrylic acid and starch.

Nanofiber with plasma modification

Figure 4 shows FTIR spectrums of the nanofiber with plasma fiber at different time durations. According to the results of this analysis, the severity of the peak of features of polyacrylic acid and starch in time duration of six min is more and stronger than two and ten min, which is itself an evidence of more absorption of enzyme on the nanofiber being under plasma for six min. By modifying plasma, new links on the surface of the nanofiber has been created. These peaks, viewed in 700, 1000, 1600, 2800, 3300 cm, respectively, have showed different severity regarding the amount of applying plasma, which, regarding the results, and the efficient time of six min, the severity of peaks in the related curve to this time is stronger.

Thermal Gravimetric Analysis (TGA)

Polyacrylic acid/ Starch nano- fiber

The analyzing chart of TGA for the provided nanofibers has been illustrated. For pure polyacrylic acid at 235.49ºC the first mass reduction, approximately 30% is observed which is related to carboxylic acid groups and at 479.31ºC, the substance is totally destructed which matches with the previous literature. The TGA chart relating to nanofiber of poly acrylic acid/starch without cross link is also illustrated. In the first mass reduction at 77.28ºC and at 398.07ºC the polymer is completely destructed which indicates that on the basis of the reported numbers in 73 and 42, starch has destruction degree close to 300ºC which is higher than destruction degree of polyacrylic acid. Consequently, by composing these two polymers, destruction degree is increased.

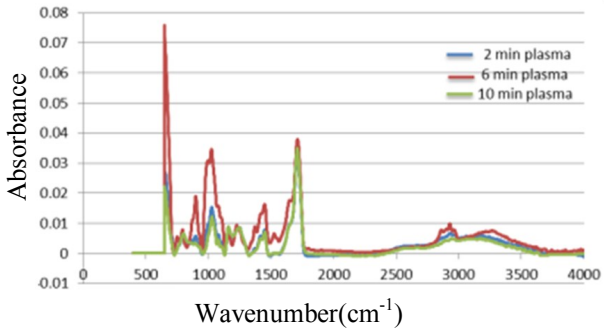

Figure 4. FTIR spectrum of nanofiber with plasma modification

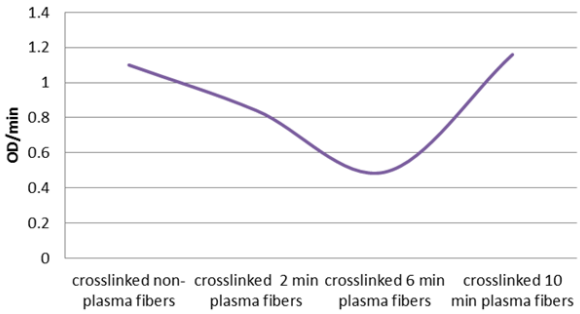

Figure 5. Absorbed enzyme by nanofiber surfaces

Furthermore, due to the crystallinity in nanofiber and its conversion into net state, the warmth stability of cross linking condition is more than pure polyacrilic acid condition. In cross-linking condition, polymer at 470.04ºC is totally destructed which compared to non-cross linking condition, warmth stability has increased. The reason for the increase in warmth stability after cross linking is that oxygenized links are involved in the reaction. Therefore, the number of them decreases and warmth stability increases. The red curve indicates the warmth stability of nanofiber after enzyme fixing in which a destruction peak at 42.36ºC is observed. Furthermore, a total destruction peak at 387.12ºC occurs which has been reduced compared to the polymer with cross linking of warmth stability in which case, due to the hydrolization of links and 24 hours exposure to enzyme solution, part of the link is destructed. The destruction degree of this sample does not differ much from the sample without cross link and plasma modification and is about 399.58ºC. On the basis of these findings, the plasma does not have much effect on increasing the stability of polymer fibers and that is due to the fact that plasma modification is related to the surface characteristics of polymers and leads to the creation of oxygen links. The warmth analyzes is also relevant to the mass features and the destruction of the structures forming the nanofiber (Chronakis *et al.,* 2006). Consequently, the warmth stability of samples not being exposed to plasma modification after vertical link is more than samples with plasma modification.

Analyzing enzyme activity

In order to analyze the immobilized enzyme on the fiber, the sewage of enzyme solution for samples with chemical modification and plasma modification, plasma was put in the samples for 24 hours for immobilizing enzyme and using the optical method (Liu and Liu, 2001) the absorption within 1 minute was measured. Furthermore, from washing buffers in which nanofibers has been put for 2 hours, the absorption was measured.

Based on the results of the absorbed enzyme in enzyme waste waters, the absorbed enzyme on nano-fiber having surface modification of plasma is more than the nanofiber not having this modification. Regarding these results, oxygen links resulted from plasma modification within six minutes on the surface links to enzyme glucose oxidase and has increased enzyme absorption. Based on the Figure 5, plasma modification within ten minutes leads to the destruction of surface links, thus destroying the improvement effect of surface and leads to a reduction in enzyme absorption and the lost enzyme on the nanofiber is even more than the condition in which fibers do not have plasma modification which is due to the destruction of the required links with enzyme. As can be seen, plasma modification for two minutes also leads to more absorption of enzyme compared to the condition without plasma modification. However, regarding the fact that

the intended links have been created within six minutes, enzyme absorption compared to plasma modification for six minutes reduces and the created links under this condition have not been enough for enzyme absorption. Enzyme activity and density on fibers having plasma modification for six minutes have been more than the others which is in harmony with the expressed analyses and formed links within efficient modification of plasma absorbed enzymes (Doshi and Reneker, 1993).

CONCLUSION

The analysis showed that, the nanofiber of polyacrylic acid/starch in efficient combination is created by adding starch to the polyacrylics acid. Due to the reduction of solution conductance, the diagonal of the fiber increases, and by exceedingly increasing starch due to the reduction of viscosity of solution, the diagonal of the fibers decreases. Using the Aminopropyl Trimethoxysilane (APTS) and glutaraldehyde, the chemical modification for use in watery environments like enzyme solutions was done for a long time and using the dielectric barrier, the physical modification was done on the surface of the nanofiber. Then, the glucose oxidase enzyme was fixed on the nanofiber, and physical modification of plasma had a significant effect on the amount of the fixed enzyme on the nanofiber polymer and increased the amount of the enzyme absorption. The related FTIR spectrums to the fibers show that the polymer components formed hydrogen linking well. The thermal stability of the rolling nanofiber produced was available through networking them, and weighing was done by the heat experiments. It was also shown that the surface modification of plasma did not create any difference in establishing thermal stability of the nanofiber.

REFERENCES

Chronakis IS, Grapenson S and Jakob A. (2006). Conductive polypyrrole nanofibers via electrospinning: electrical and morphological properties. *Polymer,* 47 (5):1597-1603.

Doshi J and Reneker DH. (1993). Electrospinning process and applications of electrospun fibers. In Industry Applications Society Annual Meeting, Conference Record of the 1993 IEEE. 1698-1703 p.

Fong H, Chun I and Reneker DH. (1999). Beaded nanofibers formed during electrospinning. *Polymer,* 40(16): 4585-4592.

Li SF and Wu WT. (2009). Lipase-immobilized electrospun PAN nanofibrous membranes for soybean oil hydrolysis. *Biochemical Engineering Journal,* 45(1):48-53.

Liu F and Liu G. (2001). Poly (solketal methacrylate)-b lock-poly (2-cinnamoyloxyethyl methacrylate)-b lock-poly (allyl methacrylate): Synthesis and Micelle Formation. *Macromolecules,* 34(5):1302-1307.

Lu J, Askeland P and Drzal LT. (2008). Surface modification of microfibrillated cellulose for epoxy composite applications. *Polymer,* 49: 1285–1296.

Lue SJ, Shih TS and Wei TC. (2006). Plasma modification on a Nafion membrane for direct methanol fuel cell applications. *Korean Journal of Chemical Engineering,* 23:441–446.

Matsuoka H, Matsutani M, Mouri E and Matsumoto K. (2003). Polymer micelle formation without Gibbs monolayer formation: Synthesis and characteristic behavior of an amphiphilic diblock copolymer having strong acid groups. *Macromolecules*, 36(14):5321-5330.

Namazi H, Fathi F and Dadkhah A. (2011). Hydrophobically modified starch using long-chain fatty acids for preparation of nanosized starch particles. *Scientia Iranica,*18(3): 439-445.

Reach G and Wilson GS. (1992). Can continuous glucose monitoring be used for the treatment of diabetes? *Analytical Chemistry*, 64(6): 381A-386A.

Sheldon RA. (2007). Enzyme immobilization: the quest for optimum performance. *Advanced Synthesis and Catalysis,* 349(8-9): 1289-1307.

Song C, Sheng L and Zhang X. (2013). Immobilization and characterization of a thermostable lipase. *Marine Biotechnology,* 15(6): 659-67.

Theron A, Zussman E and Yarin AL. (2001). Electrostatic field-assisted alignment of electrospun nanofibres. *Nanotechnology,* 12: 384–390

Yao S, Wang X, Liu X, Wang R, Deng C and Cui F. (2013). Effects of ambient relative humidity and solvent properties on the electrospinning of pure hyaluronic acid nanofibers, *Journal of Nanoscience and Nanotechnology,* 13(7): 4752–4758

Yarin AL, Koombhongse S and Renker DH. (2001). Taylor cone and jetting from liquid droplets in electro spinning of nanofibers. *Journal of Applied Physics*, 90 (9): 4836-4846