Investigation of a Natural pH-Indicator Dye for Nanofibrous Wound Dressings

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Abstract In this study, fast responding and user-friendly biocompatible, halochromic nanofibrous mat was successfully produced for the purpose of wound healing monitoring, including a natural pH-indicator dye which was extracted from Red cabbage (*B. oleracea L.*) inside an alginate (NaAlg) and polyvinyl alcohol (PVA) mixture solution. The morphological characterization of the halochromic nanofibrous mat was examined by scanning electron microscope. Nanofibrous mats exhibited a pink color when exposed to pH 4-6, simulating the wound-milieu salubrious to the wound healing, and a green-blue color when exposed to pH 7-10, simulating the wound-milieu that obstacle wound healing.

***Keywords*— pH-sensitive dyes, polyvinyl alcohol, red cabbage, smart textile, sodium alginate, wound dressing .**

I. Introduction

Color changing textiles are intelligent textile materials which change color upon an external stimulus. These materials can change their colors due to the change of several triggers, such as temperature (thermochromism), light (photochromism) and pH (halochromism)[1]. The usage of halochromic textiles in the determination of acidity of sweat, urine, and coryza is possible. The pH degree of a wound gives information about its healing property and pH of the wound leakage changes according to the healing or worsening of the wound [2]. For this reason, the pursuit of the change of wound pH is of vital importance in wound healing [3].

In this study, natural pH-indicator anthocyanins which are compatible with human health and environment were extracted from red cabbage (RC) plants. Nanofiber wound dressings (mats) were produced by electrospinning method by adding this extract in sodium alginate (NaAlg) and polyvinyl alcohol (PVA) mixture solution. Morphological characteristics of electrospun PVA/NaAlg/RC nanofiber mats were investigated in terms of extract concentrations. Further, PVA/NaAlg/RC nanofiber mats were tested against simulated conditions having different pHs for the purpose of wound healing monitoring.

II. Methods and Procedures

Red cabbage (*B. oleracea L.*) leaves (300 g) were smashed by using a domestic blender and soaked in 500 ml of alcohol solution (80% ethanol+20% pure water) in a magnetic stirrer for 24 h at room temperature. The product solution was filtered through a filter paper and the remaining solid was extracted two times with 500 ml of fresh solvent. The extract was lyophilized (0.188 mBar pressure and 72 h at -45°C). At the end of the process, the solid extract was dissolved in 100 ml of pure water.

The NaAlg polymer $(NaC_6H_7O_6)_n$ which was used for the production of nanofibrous mats had a viscosity of 700-900 cp. It was a commercial product, Cecalgum® S1300, and it was purchased from Cargill. It was dissolved in distilled water by mixing on magnetic stirrer at 60°C for 6 h with a concentration of 1% (w/w). PVA polymer $(CH_2CHOH)_n$ with Mw 85.000-124.000 g/mol (%87-89 hydrolyze) was purchased from Sigma-Aldrich. It was dissolved in distilled water by mixing on magnetic stirrer at 80°C for 12 h with a concentration of 12% (w/w). PVA solution with a concentration of 12% and NaAlg solution with a concentration of 1% were mixed in the volume ratio of 2/1 PVA/NaAlg. After that different extract (RC) concentrations were (2-3%) added the solution.

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The pH, conductivity, surface tension and viscosity properties of the electrospun solutions were measured. Viscosity was measured by using a Brookfield RV-DV II viscometer. Conductivity and pH were determined by HANNA HI-98129 pH-meter and surface tension was determined by using Attension Theta surface tensiometer.

Electrospinning of the prepared solutions was carried out by a set-up consisting of a high-voltage DC power supply. The feed rate of the solution was controlled at about 0.7 ml/h by using a syringe pump and a tip-to collector distance of 12 cm.

In order to stabilize the PVA/NaAlg/RC nanofibers, electrospun mats were cross-linked by immersing them in acetone solution of 39.3 ml including 0.5 ml glutaraldehyde (50%) and 0.2 ml hydrochloric acid (37%) at room temperature for 10 min. The cross-linked nanofibrous mats were neutralized in ethyl alcohol (99.5%) for 5 min. The cross-linked and neutralized mats were washed with phosphate buffer solution (1%) three times and then dried.

The following buffer solutions were used to test the color schemes of the PVA/NaAlg/RC nanofibrous mats: sodium acetate (CH_3COONa) and acetic acid (CH_3COOH) buffer for pH 4–5; sodium phosphate dibasic dehydrate ($Na_2HPO_4.2H_2O$) and sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) buffer for pH 6–9; sodium phosphate dibasic dehydrate ($Na_2HPO4.2H_2O$), sodium dihydrogen orthophosphate ($NaH_2PO4.2H_2O$) and NaOH buffer for pH 9–10.

The morphological appearance of the electrospun PVA/NaAlg/RC mats was observed under CARL ZEISS EVO 40 scanning electron microscope. The mean diameters of the resultant fibers were calculated from the measurements of fiber diameters on the images of X 10.000 magnification by using Image J program. Approximately 100 measurements were carried out on different parts of each sample.

III. RESULTS AND DISCUSSION

Solution properties are very important for electrospinning process and nanofiber surface structure. The properties of the solutions were given in Table I. When the concentration of red cabbage extract added to the PVA/NaAlg solutions increased, the viscosity of the solutions gradually decreased. The reason was the decrease in PVA / NaAlg polymer concentration per unit volume after mixing the aqueous extract solutions with the PVA / NaAlg solution. On the other hand, increases in conductivity of solutions with increasing concentrations of red cabbage extract were observed. It was possible for the conductivity of the solution to increase because of the presence of calcium, iron, and sodium ions in extract solutions [4]. The nanofiber surfaces obtained by electrospinning are affected by the pH of the solutions. When the pH of the solution is basic, smooth and fine structured nanofibers are obtained. When nanofibers are obtained from acidic solutions, beads are observed due to the presence of protons [5]. After the addition of red cabbage extract into PVA/NaAlg solutions, the pH slightly increased. The results of surface tension measurements did not significantly change, however a slight decrease was observed.

TABLE I
PROPERTIES OF ELECTROSPUN SOLUTIONS

Solutions	Viscosity (cp)	Conductivity (µS/cm)	Surface tension (mN/m)	pH
2/1 12 %PVA /1% NaAlg	880	1344	48.48	5.62
2/1 12 %PVA /1% NaAlg with 2% RC	649.60	2564	44.56	5.75
2/1 12 %PVA /1% NaAlg with 3% RC	499.20	3212	45.69	5.86

Figs. 1-3 illustrate the SEM images and diameter distributions of electrospun PVA/NaAlg and PVA/NaAlg/RC mats with different RC concentrations. Based on these images, the calculated mean diameters of fibers, standard deviation, minimum and maximum values were given in Table II.

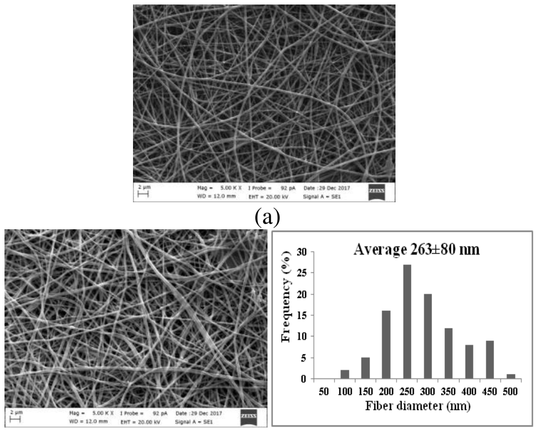

Fig. 1. SEM image of (a) the electrospun 2/1 12 %PVA /1% NaAlg (X 5.000) (b) the cross-linked electrospun 2/1 12 %PVA /1% NaAlg (X 5.000) (c) cross-linked electrospun diameter distribution

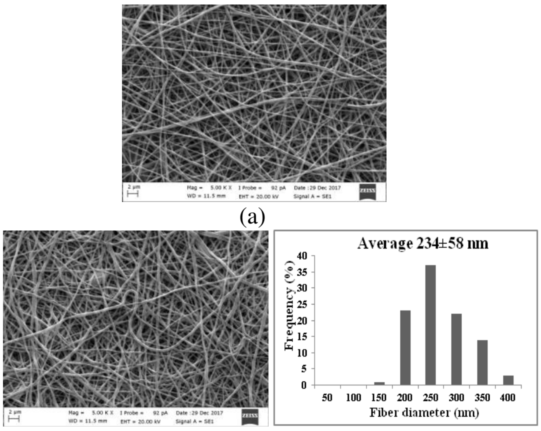

Fig. 2. SEM image of (a) the electrospun 2/1 12 %PVA /1% NaAlg with 2%RC (X 5.000) (b) the cross-linked electrospun 2/1 12 %PVA /1% NaAlg with 2%RC (X 5.000) (c) cross-linked electrospun diameter distribution

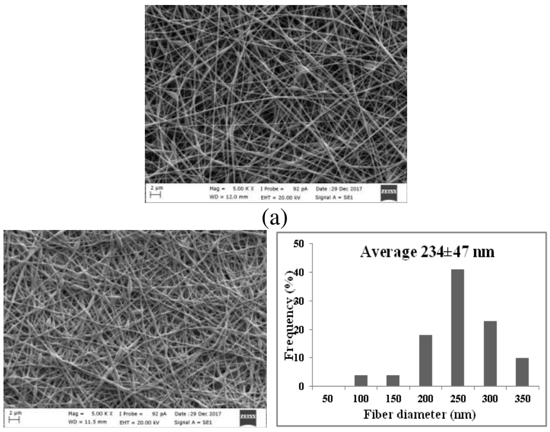

Fig. 3. SEM image of (a) the electrospun 2/1 12 %PVA /1% NaAlg with 3%RC (X 5.000) (b) the cross-linked electrospun 2/1 12 %PVA /1% NaAlg with 3%RC (X 5.000) (c) cross-linked electrospun diameter distribution

SEM images (Fig. 1) showed that smooth, beadless, uniform and continuous fibers were successfully produced from PVA/NaAlg polymer solutions. Fiber diameter average (263±80nm) value was a little bit higher than that of PVA/NaAlg/RC nanofibers mats because of high viscosity and low conductivity properties of PVA/NaAlg. It has been found that the fiber diameter distribution was close to the normal distribution and it was obtained between 200-350 nm.

SEM images of the 2/1 12 %PVA /1% NaAlg with 2-3% of RC extract mats (Figs. 2 and 3) showed that uniform and continuous fibers were produced with fewer beads. This could stem from the decrease in solution viscosity. When there is low viscosity, there is low chain complexity and the influence of surface tension forces on the jet during electrospinning is dominant [4]. After the addition of 2-3% red cabbage extract to the PVA / NaAlg solution, the conductivity values increased as the viscosity values decreased. As a result, fine fibers with lower diameters (234 nm) were produced. SEM images showed that the morphology of electrospun nanofibers progressively changed from a uniform structure to a beaded-like structure as the viscosity of the blended solutions decreased.

It was seen from SEM images (Figs. 1b, 2b and 3b) of cross-linked nanofibrous mats that the fibers appeared flattened, and bundle structures were obtained so that average fiber diameters increased. In the cross-linking process, acetal bonds are formed between the aldehyde groups of glutaraldehyde (which was used as crosslinker) and the hydroxyl groups present in the structure of polyvinyl alcohol and alginate [6]. This flattened structure of nanofibrous mats could be due to formation of new crosslinking bonds which leaded to squeeze the nanofibrous structure [7]. Furthermore, the rapid evaporation of the solvent from the nanofiber surfaces causes the formation of flattened structure. When a quantity of solvent evaporates which is cramped in the fibers, the fibers become flat [8].After cross-linking process, it is possible that the flattening may happen due to the compression of the nanofiber structure which could stem from the formation of new-crosslinking bonds. This could be the result of the separation of water molecules presented in the structure. It was observed that the average diameters of cross-linked electrospun nanofibers increased by the decreasing viscosity of the blended solutions.

TABLE II
MEAN DIAMETER, STANDARD DEVIATION, MINIMUM AND MAXIMUM VALUES OF ELECTROSPUN AND CROSS-LINKED ELECTROSPUN PVA/NAALG AND PVA/NAALG/RC NANOFIBERS

Nanofibrous mats	Mean Diameter (nm)	Standard Deviation (SD)	Minimum (nm)	Maximum (nm)
2/1 12 %PVA /1% NaAlg	242.29	82.74	68.72	416.67
cross-linked electrospun 2/1 12 %PVA /1% NaAlg	263.08	79.99	104.42	479.66
2/1 12 %PVA /1% NaAlg with 2% RC	221.24	61.78	79.94	371.67
cross-linked electrospun 2/1 12 %PVA /1% NaAlg with 2% RC	234.14	57.61	111.17	388.68
2/1 12 %PVA /1% NaAlg with 3% RC	203.12	55.48	59.51	333.49
cross-linked electrospun 2/1 12 %PVA /1% NaAlg with 3 %RC	233.71	46.79	132.64	390.67

The PVA/NaAlg/RC nanofibers took approximately 10 s to develop color in each pH solution (pH 4-10). The PVA/NaAlg/RC nanofiber mats exhibited rather visibly distinguishable colors as seen in Fig. 4, especially at pHs of 4-6 and 7-10. When compared with the PVA/NaAlg /RC solutions, the PVA/NaAlg /RC nanofibrous mats exhibited very similar color patterns. Consequently, the use of the nanofibrous mat as a pH sensor is advantageous; being an alternative and a simple tool for pursuing the wound healing process [9-11].

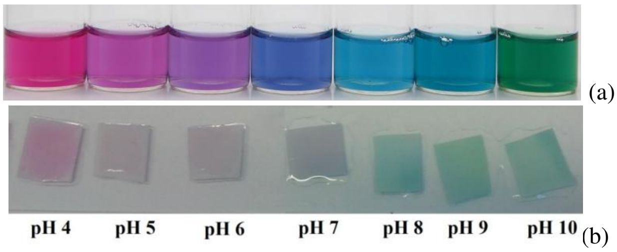

Fig. 4. The color schemes of **(a)** 2/1 12 %PVA /1% NaAlg with 3% RC electrospun solution **(b)** 2/1 12 %PVA /1% NaAlg with 3% RC nanofibers mats at pH 4-10

IV. Conclusion

In this research, the preparation of a nanofibrous mat including a natural halochromic dye (extracted from RC) for detecting the changes in wound environments' pH values was presented. Nanofibrous mat was successfully prepared by electrospinning using sodium alginate (NaAlg) and polyvinyl alcohol (PVA). Uniform and continuous fibers were obtained from PVA/NaAlg/RC solutions, with diameters of 200-250 nm. It is clear that PVA/NaAlg/RC nanofibrous mats exhibit different properties depending on pH of the surrounding environment. It could be concluded that natural pH-indicator dye (RC) was successfully incorporated into nanofibrous mats and the obtained nanofibrous structures could be used as pH-biosensors.

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References

[1] H. Mattila.,P.Talvenmaa, *Intelligent Textile and Clothing*, Woodhead Publishing London:, 2006 , ch 3.
[2] L. V. Schueren, K. , Clerck , "Coloration and Application of pH-Sensitive Dyes on Textile Materials", *Coloration Technology*, vol.128, no.2, pp. 82-90, 2012.
[3] A. Pakolpakçıl "Examination of Wet Fastness Halochromic Dyed Polyamide 6,6 Fabrics for Using Smart Textiles*", MSc. Thesis,* Namık Kemal University, 2013.
[4] S. Ramakrishna, K. Fujihara, W Teo. , T Lim., Z Ma. , *An Introduction to Electrospinning and Nanofibers.* Singapore: World Scientific Publishing Co., 2005, pp.80-81.
[5] W. K. Son, J. H. Youk, T. S. Lee, W. H. Park, "Effect of pH on Electrospinning of Poly(vinyl alcohol)", *Materials Letters*, vol. 59, no. 12, pp.1571-1575, 2005.
[6] M. C. Hassan, N.A. Peppas, "Structure and applications of poly(vinyl alcohol) hydrogels produced by conventional crosslinking or by freezing/thawing methods", *Advances in Polymer Science*, 153, pp.37-65, 2000.
[7] M. K.Sinha, B. R. Das, A. Srivastava, A. K. Saxena , "Needleless Electrospinning and Coating of Poly Vinyl Alcohol with Cross-Linking Agent via in-Situ Technique", *International Journal of Textile and Fashion Technology*, vol.3, no.5, pp. 29-38, 2013.
[8] A. Baji, Y. W. Mai, S. C. Wong, M. Abtahi, P. Chen, "Electrospinning of Polymer Nanofibers: Effects on Oriented Morphology, Structures and Tensile Properties", *Composites Science and Technology*, vol. 70, pp. 703–718, 2010.
[9] K. Devarayan, B. S. Kim, "Reversible and Universal pH Sensing Cellulose Nanofibers for Health Monitor", *Sensors and Actuators B:Chemical*, vol. 209, pp. 281-286, 2015.
[10] A. Agarwal, A. Raheja, T. S Natarajan, T. S.Chandra, "Development of Universal pH Sensing Electrospun Nanofibers", *Sensors and Actuators B: Chemical*, vol. 161, no. 1, pp. 1097-1101, 2012.
[11] L. V. Schueren, T. Mollet, Ö. Ceylan, K. Clerck, "The Development of Polyamide 6.6 Nanofibres with a pH-Sensitive Function by Electrospinning", *European Polymer Journal*, vol. 46, no. 12, pp. 2229-2239, 2010.