Halochromic composite nanofibrous mat for wound healing monitoring

Ayben Pakolpakçıl, Bilgen Osman, Elif Tümay Özer, Yasemin Şahan, Behçet Becerir, Gökhan Göktalay and Esra Karaca

1. Introduction

Chromic textiles, a subclass of smart textiles, change color depending on environmental changes. The number of studies focusing on the fabrication of chromic textiles has rapidly increased in recent years [1, 2]. Numerous external stimuli such as temperature, light, pressure, and pH can lead to changes in color. Halochromism is defined as a reversible color change arising from a pH change [3]. Although halochromic textiles are lesser known than other chromic textiles [4], they present an increasing usage potential, especially in the future of medical textiles.

Halochromic properties have been added to textile materials either by conventional methods [5–9] or by using nanotechnological applications. The number of publications on immobilization of many synthetic halochromic dyes using electrospinning has increased in recent years. Examples of the polymers used in these reports could be polyanime 6,6 [7, 10], polycyrolonitrile [11, 12], polycaprolactone and polycaprolactone/chitosan [13, 14] and cellulose acetate [15].

Electrospinning is a straightforward and affordable method used to generate ultrathin polymer fibers via an electric field. Electrospun nanofibrous mats have distinctive characteristics, such as high specific surface area, high porosity, small pore sizes, permeability to moisture and air, and resistance to microorganisms [16, 17]. They present a wide range of application possibilities, especially in biomedical applications such as medical.
prosthesis [18], tissue engineering scaffold [19], drug delivery [20], adhesion barrier [21], and wound dressing [22].

One of the most interesting and promising areas of biomedical use of nanofibers is wound dressing. The advantages of electrospun nanofibrous mats as wound dressing have been indicated in studies focused on natural and synthetic polymers such as collagen [23], chitosan [24], fibroin [25], sericin [26], polyurethane [27], polycaprolactone [28], gelatin [29], alginate [30], poly (lactic-co-glycolic acid) [31], hyaluronic acid [32], cellulose acetate [33], polyglycerol [34]. Moreover, in many previous studies, a myriad of therapeutic additives such as drugs [35], antibiotics [36], proteins [37], growth factors [38], cells [39], antimicrobial agents [40] and essential oils [41] were able to be incorporated into the electrospun fibrous mats produced as wound dressing materials.

Sodium alginate (SA) is a natural biopolymer derived and isolated from brown algae. SA has specific properties that facilitate wound healing, which make it a unique raw material in the production of highly absorbent wound dressings [42, 43]. However, SA cannot be used for electrospinning due to its polyelectrolyte characteristics and inadequate chain entanglement. Therefore, synthetic polymers such as polyvinyl alcohol (PVA) and polyethylene oxide have been added to aqueous solutions of SA in the electrospinning process [44, 45]. PVA is a semi-crystalline and water-soluble polymer that is of special interest for application in wound dressings due to its biocompatibility and ability to absorb water [46].

Halochromic textiles can be used to provide information about the surrounding medium because the processes in nature are generally affected by pH. For instance, the pH of the skin varies during the wound healing process [47]. The pH of chronic wounds has been measured within the range of 7.15–8.9. As the wound progresses toward healing, the pH moves to neutral and then becomes acidic. The normal skin surface exhibits a slightly acidic pH, ranging from 4.0 to 6.0 [48–50]. Thus, it is possible to indicate the progression of the healing process by a pH-sensitive wound dressing without removal of the dressing.

The textiles prepared by synthetic halochromic indicator dyes can be harmful, especially in medical applications, due to the toxic effects of these synthetic compounds [51, 52]. Anthocyanins are natural counterparts of the synthetic halochromic pigments, which are derived from red-colored fruits or vegetable sources [53]. The concentration, ratio, and chemical structure of these natural halochromic pigments vary by plant. The pH change from low to high results in a bathochromatic shift from red to purple and red to blue, respectively [54, 55]. The anthocyanins extracted from plants can be used to prepare halochromic nanofibers. However, several papers have been published in which only red cabbage anthocyanins were used as a natural halochromic indicator in the preparation of electrospun nanofibrous pH sensors with cellulose acetate [51], zein [52], and PVA [56].

Black carrot (BC) also contains anthocyanins and is applicable as a natural food colorant due to the color properties of its extracts under various pH conditions [57]. In addition, BC anthocyanins have superior characteristics such as good water solubility, exceptional color resistance at higher temperatures, and light stability [58]. However, to the best of our knowledge, no research has exploited superior characteristics of BC anthocyanins for pH sensors.

The objective of this study was to develop a halochromic nanofibrous mat including anthocyanins extracted from BC plants. The pH-sensing nanofibrous mat was produced via the electrospinning method. The electrospinning solution was prepared by mixing BC extract, sodium PVA, and SA. The color stability of BC anthocyanins in the PVA/SA electrospinning mixture was investigated at different pHs using UV–vis spectrophotometry. The functionalized electrospun PVA/SA/BC mat was crosslinked by glutaraldehyde (GA) to obtain water-insoluble crosslinked nanofibers. The characterization studies were conducted using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). The pH-sensing ability of the PVA/SA/BC nanofibrous mat in aqueous solution was tested to determine the applicability of the nanofibrous mat to wound healing monitoring. The colors of the nanofibrous mat in the pH range of 4.0–10.0 were monitored via photographs taken with a digital camera. The storage stability of the pH-sensing PVA/SA/BC nanofibrous mat was also studied. The prepared pH-sensing mat has a short response time and a high sensing ability, demonstrating the applicability for pH sensing in wound healing.

2. Experimental

2.1. Materials

BCs (*Daucus carota* ssp. *sativus var. atrorubens*) were purchased from the local market in Bursa, Turkey. SA (viscosity range: 700–900 cP) was purchased from Cargill (Cecalgum® S1300). PVA (CH2CHOH)n with a molecular weight of 85,000–124,000 g mol−1 (87%–89% hydrolyzed) was obtained from Sigma-Aldrich. Ethyl alcohol (99%), sodium acetate trihydrate, acetic acid, ammonium chloride, sodium hydrogen phosphate dihydrate, and sodium dihydrogen orthophosphate were supplied by Merck. Hydrochloric acid (37%,
1.18 g ml\(^{-1}\) and GA (50%) were supplied by Sigma-Aldrich, and acetone purchased from Merck was used in the crosslinking process. All other chemicals were of analytical grade. Elga Flex3 (Veolia Water Solutions & Technologies, France) was used for water purification.

2.2. Extraction of BC pigment anthocyanins

Fresh BCs were milled into a uniform sample using a blender. After that, ultrasonic-assisted extraction was performed in a temperature-controlled ultrasonic bath (Wiseclean Ultrasonic Cleaner). In the procedure, approximately 350 g of the homogenized sample was placed into a glass Erlenmeyer flask containing 500 ml of the ethanol/water (80:20) mixture. Then, the sample was exposed to ultrasonic treatment at 35 kHz for 60 min. The obtained mixture was filtered through a filter paper. The ethanol/water mixture containing BC anthocyanins was kept in a refrigerator at 4°C. A second portion of ethanol/water mixture (500 ml) was replenished to the filtered solid, and ultrasonic-assisted extraction was performed using the same procedure. After the completion of the extraction procedure, the two portions were put together. The total extract was lyophilized at 0.188 mBar pressure at −45°C for 72 h using a freeze drier (Labconco Freezone Freeze Dry Systems). Finally, the residual solid was dissolved in pure water (100 ml) and stored at −24°C until use.

The amount of anthocyanins extracted from BCs was determined via the pH differential method described in the AOAC Official Method 2005.02 [39] and expressed as milligrams of cyanidin-3-glucoside equivalent per 1 g dried weight.

2.3. Preparation of the pH-sensing PVA/SA/BC nanofibrous mat

The electrospinning solution of the PVA/SA/BC nanofibrous mat was prepared by mixing SA, PVA, and BC extract. A magnetic stirrer (Cleaver Scientific LTD) was used to prepare solutions. PVA and SA solutions were separately prepared under a reflux condenser to prevent water from boiling away. Firstly, the SA solution (1%, w/w) was prepared by stirring the aqueous solution for 6 h at a 400 rpm stirring rate at 60°C. PVA (12%, w/w) was dissolved in distilled water by stirring for 12 h at a 400 rpm stirring rate at 80°C. After that, a PVA/SA mixture was prepared by mixing the PVA and SA solutions at a volume ratio of 2/1 (v/v). BC extract was added to the PVA/SA mixture at a concentration of 1% (w/v). The obtained PVA/SA/BC mixture was gently stirred for 3 h at room temperature to obtain a homogeneous mixture. The PVA/SA mixture was also prepared using the same procedure without addition of BC extract.

The pH, surface tension, electrical conductivity, and viscosity of the electrospun solutions were rigorously determined before the electrospinning process. The pH and electrical conductivity measurements were performed using HANNA pH and Conductivity Meter (HI-98129). A Brookfield viscometer (RV-DV II) was used to perform viscosity measurements. An Attension Theta KSV instrument was used to determine the surface tension. All measurements were repeated three times.

The electrospinning of the PVA/SA and PVA/SA/BC solutions (20 ml for each) was conducted using an electrospinning system (Inovenso Technology Inc.) including a high-voltage DC power supply. Electrospinning parameters for PVA/SA and PVA/SA/BC solutions were the same, with the exception of applied voltage. In the electrospinning process, the feed rate and tip-to-collector distance were 0.7 ml h\(^{-1}\) and 12 cm, respectively. The electrospinning procedure was carried out by applying a voltage of 18 kV and 14 kV for PVA/SA and PVA/SA/BC solutions, respectively. The PVA/SA and PVA/SA/BC electrospun nanofibrous mats were collected on aluminum foil wrapped around a cylindrical drum rotating at 200 rpm.

The PVA/SA/BC and PVA/SA electrospun nanofibrous mats were crosslinked by GA to obtain water-insoluble crosslinked nanofibers. The crosslinking was achieved by immersing the electrospun nanofibrous mats in acetone solution containing 0.5 ml of GA and 0.2 ml of concentrated HCl at room temperature for 10 min. After that, the crosslinked nanofibrous mat was washed with ethyl alcohol and phosphate buffer (pH 7.0) and dried at room temperature for 24 h.

2.4. Characterization studies

2.4.1. UV–vis spectrophotometry analyses

The halochromic behavior of the PVA/SA/BC electrospinning solution at different pHs was investigated using a UV–vis spectrophotometer (Shimadzu, UV-1700). For this purpose, a small amount of BC extract (40 µl) was added to 3 ml of PVA/SA mixtures and 3 ml of a set of buffer solutions, prepared at pH values ranging from pH 4.0 to 10.0. Acetate buffers (pH 3.0–4.0), phosphate buffers (pH 6.0–9.0), or ammonium chloride/ammonia buffers (pH 10.0) were used to adjust the pH of the medium. The absorption spectra were recorded in the wavelength range of 200–800 nm.
investigated by soaking the samples in the buffer solutions PVA electrospinning solutions such as viscosity, conductivity, pH, and surface tension. The electrospinning process and nano

3.1. Solution properties

3. Results and discussion

2.4.2. Morphological, chemical, and thermal analysis of the nanofibrous mats

The morphology of the PVA/SA, PVA/SA/BC, crosslinked PVA/SA, and PVA/SA/BC electrospun nanofibrous mats were examined using a scanning electron microscope (CARL ZEISS AG-EVO 40 KVP). The mean nanofiber diameter and its distribution were determined from 100 random measurements using ImageJ software (National Institute of Health, USA) on SEM images of each sample. The thickness of the electrospun mats was measured using a digital micrometer (INSIZE Thickness Gauge) with 0.005 mm accuracy at five points. The tests were conducted at 25 ± 2 °C. The average values and standard deviations were calculated from the obtained data.

An FTIR spectrometer (Perkin Elmer, Spectrum100) equipped with an attenuated total reflectance (ATR) apparatus was used to study the chemistry of the PVA/SA, PVA/SA/BC, and crosslinked PVA/SA/BC nanofibrous mats within the range of 650–4000 cm⁻¹.

The TGA curves of the PVA/SA, PVA/SA/BC and crosslinked PVA/SA/BC nanofibrous mats were obtained using a thermogravimeter (Perkin Elmer, STA 6000). TGA was performed by heating the sample (10 mg) in the temperature range of 30 °C to 850 °C under a nitrogen flow of 20 ml min⁻¹ and at a heating rate of 20 °C min⁻¹.

2.4.3. Color measurements

The color schemes of the PVA/SA/BC electrospinning solutions and the crosslinked PVA/SA/BC nanofibrous mats at different pHs were tested by color measurements. The PVA/SA/BC electrospinning solutions were diluted with prepared buffer solutions in the ratio of 1:6. The PVA/SA/BC nanofibrous mats (cut into 2 × 2 cm squares) were soaked in the buffer solutions for 1 min. The color schemes of the PVA/SA/BC electrospinning solutions and the PVA/SA/BC nanofibrous mats were compared through the CIELAB (International Commission on Illumination) color space coordinates. The colors were recorded as digital photographs using a camera (CANON EOS 5D Mark III camera). RGB and CIE L’a*b* values of the PVA/SA/BC solutions and the PVA/SA/BC nanofibrous mats were determined using the Adobe Photoshop CS5 program. Color analyses were carried out by using the photographs obtained under the same illumination conditions. An average of ten readouts each comprising an average of 100 pixels were considered for analysis. The color of each electrospinning solution and nanofibrous mat at different pHs was compared through the total color difference (ΔEab*) using equation (1):

\[
\Delta E_{ab}^* = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)}
\] (1)

where Δ is the difference between the numeric value of the sample and the numeric value of the standard; ΔEab* is the distance between the two colors in the CIELAB color space [37].

2.5. Stability of the pH-sensing PVA/SA/BC nanofibrous mat

The stability of the pH-sensing PVA/SA/BC nanofibrous mat was evaluated under different conditions with respect to temperature. The nanofibrous mat samples were incubated at two temperatures of −20 °C (storage temperature) and 37 °C (body temperature) for 24 h. Subsequently, the responses to pH changes were investigated by soaking the samples in the buffer solutions (pH 4.0, 7.0 and 9.0). RGB values were calculated from the photographs of nanofibrous mats. The measurements were repeated three times.

3. Results and discussion

3.1. Solution properties

The electrospinning process and nanofibrous surface morphology are significantly affected by the properties of electrospinning solutions such as viscosity, conductivity, pH, and surface tension [60, 61]. The properties of the PVA/SA and PVA/SA/BC electrospinning solutions are summarized in table 1.

| Solutions | Viscosity (cP) | Conductivity (μS cm⁻¹) | Surface tension (mN m⁻¹) | pH |
|-----------|---------------|------------------------|--------------------------|----|
| PVA/SA   | 880.00 ± 57.45 | 1344 ± 40.83           | 48.48 ± 0.50             | 5.62 ± 0.12 |
| PVA/SA/BC| 521.60 ± 24.18 | 1609 ± 27.66           | 47.77 ± 0.41             | 5.42 ± 0.03 |

An FTIR spectrometer (Perkin Elmer, Spectrum100) equipped with an attenuated total reflectance (ATR) apparatus was used to study the chemistry of the PVA/SA, PVA/SA/BC, and crosslinked PVA/SA/BC nanofibrous mats within the range of 650–4000 cm⁻¹. The TGA curves of the PVA/SA, PVA/SA/BC and crosslinked PVA/SA/BC nanofibrous mats were obtained using a thermogravimeter (Perkin Elmer, STA 6000). TGA was performed by heating the sample (10 mg) in the temperature range of 30 °C to 850 °C under a nitrogen flow of 20 ml min⁻¹ and at a heating rate of 20 °C min⁻¹.

The TGA curves of the PVA/SA, PVA/SA/BC and crosslinked PVA/SA/BC nanofibrous mats were obtained using a thermogravimeter (Perkin Elmer, STA 6000). TGA was performed by heating the sample (10 mg) in the temperature range of 30 °C to 850 °C under a nitrogen flow of 20 ml min⁻¹ and at a heating rate of 20 °C min⁻¹.

The TGA curves of the PVA/SA, PVA/SA/BC and crosslinked PVA/SA/BC nanofibrous mats were obtained using a thermogravimeter (Perkin Elmer, STA 6000). TGA was performed by heating the sample (10 mg) in the temperature range of 30 °C to 850 °C under a nitrogen flow of 20 ml min⁻¹ and at a heating rate of 20 °C min⁻¹.
calcium, iron, and sodium in the BC extract solution, besides anthocyanins [62]. The pH and surface tension values of the PVA/SA solution did not significantly change, but an insconsiderable decrease was detected with the addition of BC extract.

3.2. Halochromic behavior of the electrospinning solution

The color of BC is mainly due to a group of natural halochromic pigments called anthocyanins. The major anthocyanins of BC are derived from cyanidin being essentially acylated [63]. BC contains cyanidin 3-xylosylgalactoside and cyanidin 3-xylosyl(glucosyl)galactoside as nonacylated anthocyanins (figure A.1 is available online at stacks.iop.org/MRX/6/1250c3/mmedia). Additionally, nuclear magnetic resonance (NMR) spectroscopy studies showed that cyanidin 3-xylosyl(coumaroylglucosyl)galactoside, cyanidin 3-xylosyl(feruloylglucosyl)galactoside and cyanidin 3-xylosyl(sinapoylglucosyl)galactoside are monoacylated anthocyanins of BC [58].

The halochromic behavior of electrospinning solution including BC anthocyanins is indicative of the color properties of the PVA/SA/BC nanofibrous mat. Therefore, the UV–vis absorption characteristics of the PVA/SA/BC electrospinning solutions at different pHs (4.0–10.0) were investigated in the wavelength range of 200–800 nm. Additionally, UV–vis spectra of the PVA/SA/BC solutions were compared with those of the buffer solutions including BC extract prepared at pH values ranging from pH 4.0 to 10.0.

The UV–vis spectra of the PVA/SA/BC solutions in the pH range of 4.0–10.0 are depicted in figure 1. The absorption maximums in the visible region were observed in the range of 539–565 nm for pH 4.0–7.0 and 603–605 nm for pH 8.0–10.0. A bathochromic shift was clearly observed from low to high pH. The absorption maximum was determined to be 605 nm at pH 10.0, whereas it was measured as 539 nm at pH 4.0. The UV–vis spectra of the buffer solutions including BC extract in the same pH range are also shown in figure A.2. The results showed that the halochromic behavior of the BC extract in the PVA/SA/BC electrospinning solutions was exactly the same as that of the BC extract in the buffer solutions in the pH range of 4.0–10.0. The halochromic behavior of the PVA/SA/BC electrospinning solution demonstrated that the PVA/SA/BC nanofibrous mat can sense the pH changes in the environment with a short response time and a high sensing ability.

3.3. Characterization of the nanofibrous mats

3.3.1. SEM analysis

Before and after the crosslinking process, the morphology of the PVA/SA and PVA/SA/BC nanofibrous mats was determined via SEM analysis (figure 2). The SEM image of the PVA/SA electrospun nanofibrous mat (figure 2(a)) clearly demonstrated that beadless, uniform, and ultrafine fibers with an average diameter of 242 ± 83 nm were obtained. However, the SEM image of the PVA/SA/BC nanofibrous mat (figure 2(b)) proved that continuous fibers with some spindle-shaped beads were produced because of the lower viscosity of the PVA/SA/BC electrospinning solution than that of the PVA/SA solution.

During the production of the PVA/SA and PVA/SA/BC nanofibrous mats, the process parameters other than applied voltage were kept completely equal. Therefore, the solution properties played a determinant role in the morphology of the electrospun mats. The addition of aqueous BC extract into the PVA/SA solution caused a
decrease in viscosity because the polymer concentration decreased. The effect of the concentration/viscosity on the morphology of the nanofibers was reported in many previous studies \[64–66\]. When the viscosity is low, weak chain entanglement among the polymer chains is obtained and the effect of surface tension force on the jet becomes dominating during electrospinning. Therefore, continuous jet formation may not be obtained, and the formation of beads or beaded nanofibers might be observed.

The conductivity of electrospinning solution also plays a significant role in the electrospinning process. Usually, an increase in conductivity of the solution causes the formation of fine fibers because the polymer solution is exposed to more stretching under the high electrical field \[67–69\]. The addition of BC extract to the PVA/SA polymer solution caused an increase in conductivity due to the existence of ions in the BC extract, which results in an increase in the surface charge density of the polymer jet. Therefore, the PVA/SA/BC nanofibrous mat had approximately a 20% lower fiber diameter \((211 \pm 39 \text{ nm})\) and lower fiber diameter variation \((18\%)\) than the PVA/SA nanofibrous mat. In addition, sticking was observed at the junction points of the nanofibers in the nanofibrous mat containing BC extract. When the concentration of free solvent molecules in the solution is high, solvent molecules have a tendency to accumulate because of surface tension, and they cannot evaporate \[70\]. This partial dissolving on the collector could lead to the fibers sticking to each other.

The PVA/SA or PVA/SA/BC nanofibrous mats were crosslinked using water-soluble GA. When the SEM photographs (figures 2(c),(d)) obtained after crosslinking were examined, it was observed that the integrity of the fibrous structure was kept but the fibers were flattened. In previous studies, it has been reported that the newly formed crosslinks lead to squeezing of the fibrous structure, and some amount of solvent remaining in the fibrous structure evaporates, causing the formation of flat fibers \[70, 71\]. The morphologies obtained from SEM analyses of the crosslinked PVA/SA and PVA/SA/BC electrospun mats supported the results reported in previous studies. As a result of the flattening after the crosslinking process, the average diameter values of the crosslinked PVA/SA nanofibers and the crosslinked PVA/SA/BC nanofibers increased to \(263 \pm 80 \text{ nm}\) and \(213 \pm 37 \text{ nm}\), respectively. Moreover, the porosity on the crosslinked mats decreased visibly. In addition to flattening, fusions at nanofiber junction points increased after crosslinking by GA. It has been reported that the nanofibrous structures crosslinked by GA in aqueous solution are immediately changed into a dense membrane \[72\]. In fact, vapor crosslinking by GA is generally preferred to avoid the collapse of the nanofibrous matrix in the aqueous system \[73\]. However, in this study, PVA/SA nanofibers and PVA/SA/BC nanofibers were
successfully crosslinked by GA in aqueous medium without a significant morphological change, although fusions at nanofiber junction points slightly increased after crosslinking. It can be concluded that the crosslinking medium consisting of a small amount of water (0.5%) and a short crosslinking time (10 min) enabled the formation of a morphologically functional crosslinked PVA/SA/BC electrospun mat.

3.3.2. Thickness measurements

Thickness is an important characteristic of electrospun mats because the thickness of the mats directly affects performance properties such as permeability. Because the crosslinked PVA/SA/BC electrospun mat is expected to respond to different pHs with a color change, the thickness value becomes even more important. The thicknesses of the nanofibrous PVA/SA, PVA/SA/BC, crosslinked PVA/SA, and crosslinked PVA/SA/BC mats were measured as 82.4 ± 3.4 μm, 84.0 ± 4.8 μm, 78.4 ± 2.4 μm, and 81.6 ± 4.2 μm, respectively. The thickness was controlled through the volume of the electrospinning solutions. Although both PVA/SA and PVA/SA/BC nanofibrous mats were produced under the same conditions, the slightly higher thickness value of the PVA/SA/BC electrospun mat (84.0 ± 4.8 μm) could be attributed to the existence of beaded nanofibers with thicker diameters in structure. In addition, the crosslinking process did not significantly affect the thicknesses of the PVA/SA and PVA/SA/BC nanofibrous mats, but a insignificant decrease was observed (3%–5%), probably associated with the flattening of the nanofibers after crosslinking. As a result, the crosslinked PVA/SA/BC electrospun mat with a convenient thickness was prepared for monitoring pH changes.

3.3.3. FTIR analysis

FTIR analysis was conducted to examine the effect of BC extract addition and the crosslinking procedure. FTIR spectra of PVA/SA, PVA/SA/BC, and crosslinked PVA/SA/BC nanofibrous mats are shown in figure 3.

In the FTIR spectrum of the PVA/SA nanofibrous mat, the broad adsorption band at 3399 cm⁻¹ mainly resulted from the stretching of −OH groups of PVA [74]. The absorption bands at 2841 cm⁻¹ and 2916 cm⁻¹ are due to the symmetric and antisymmetric stretching vibrations of C−H from alkyl groups, respectively [75]. The absorption band at 1734 cm⁻¹ is related to the stretching vibration band of the ester carbonyl (C=O) group of PVA because it is a semi-crystalline polymer prepared by hydrolysis of the poly(vinyl acetate) [76]. The characteristic band belonging to stretching vibrations of the carboxylic acid carbonyl group of SA appeared at 1713 cm⁻¹ [45]. The absorption bands resulting from the C−O stretching of PVA and SA were also observed at 1091 cm⁻¹ [77].

The adsorption band at 3306 cm⁻¹ resulting from the stretching of −OH groups more intensely appeared in the FTIR spectrum of the PVA/SA/BC nanofibrous mat because the anthocyanins in BC extract also have −OH groups and are present as glycosides [78]. Additionally, the characteristic adsorption band at 1046 cm⁻¹ corresponding to aromatic ring C−H deformation of anthocyanins supported [79] that BC anthocyanins were successfully incorporated into the nanofibrous mat.

The GA crosslinking agent used in the crosslinking process activates the hydroxyl groups in the polymer molecules generating the nanofibrous structure and ensures the formation of acetal bonds between them [80]. In
the FTIR spectrum of the crosslinked PVA/SA/BC mat, the absorption bands at 1130 cm\(^{-1}\) belonging to C–O–C stretching of acetal groups showed that the crosslinking with GA was successfully performed [81]. Moreover, the intensity of the absorption band at 3398 cm\(^{-1}\) considering –OH groups considerably reduced after crosslinking because the crosslinking reaction occurs via free hydroxyl groups. The aldehyde peak observed at 2867 cm\(^{-1}\) in the FTIR spectrum of the crosslinked PVA/SA/BC mat probably resulted from the participation of only one aldehyde group of some GA molecules in the crosslinking reaction, whereas the other remained unreacted. As a result, the crosslinking was successfully performed in the presence of BC extract.

The anthocyanin content of the nanofibers was also calculated as 1.37 ± 0.06 mg anthocyanin/g for the PVA/SA/BC nanofibrous mat.

### 3.3.4. Thermal properties

Thermal degradation profiles of PVA/SA, PVA/SA/BC, and crosslinked PVA/SA/BC nanofibrous mats were examined by TGA to determine the thermal stability of their components (figure 4).

TGA curves of the PVA/SA, PVA/SA/BC, and crosslinked PVA/SA/BC mats showed similar patterns. The degradation of the mats occurred in three stages of mass loss [74]. The first was between approximately 50 and 100 °C due to loss of adsorbed water. The mass losses of PVA/SA and PVA/SA/BC nanofibrous mats were 10% and 4%, respectively. The mass loss of the PVA/SA/BC nanofibrous mat slightly decreased as compared to the PVA/SA. This was probably because the amount of water adsorbed onto the PVA/SA/BC mat comparatively decreased due to the existence of BC extract in the nanofibrous structure. The second mass losses, about 90%, occurred between 220.69 °C and 251.07 °C (figure A.3). The second stage of thermal degradation includes both melting point and the degradation temperature of PVA. The result was considered a chemical degradation resulting from the breaking of carbon-carbon bonds in the polymeric backbone [82]. In the second stage, the mass losses of PVA/SA and PVA/SA/BC nanofibrous mats started at 251.07 °C and 220.69 °C, respectively. The addition of BC extract caused a slight decrease (~30 °C) in thermal stability, indicating that the extract has a low thermal stability. On the other hand, the mass loss of the crosslinked PVA/SA/BC started at 243.85 °C. When compared to PVA/SA/BC, the thermal stability of the crosslinked PVA/SA/BC nanofibrous mat showed a slight increase (~20 °C), which suggests that the reaction between PVA and GA caused a decrease in the amount of hydroxyl groups involved in the polyene formation, leading to a decrease in weight loss [83]. In the third stage, the mass losses around 510 °C~540 °C were attributed to the fragmentation of the macromolecular structures of the mats. As a result, TGA clearly showed that the crosslinked PVA/SA/BC nanofibrous mat was thermally stable until the temperature of 50 °C, thus having thermal stability to be applied in wound healing monitoring at body temperature.

### 3.4. RGB and CIELAB values

The visual color change of PVA/SA/BC electrospinning solutions and crosslinked PVA/SA/BC nanofibrous mats are presented in figure 5. The crosslinked PVA/SA/BC nanofibrous mats had a short response time (5–10 s) for each solution at different pHs (4.0–10.0). This result was consistent with the one reported by Devaray and Kim [31], who investigated immobilization of natural pH indicator dyes extracted from red cabbage into a cellulose acetate nanofibrous mat. The visual color changes of electrospinning solutions and nanofibrous mats demonstrated that the color-changing property of BC extract in both cases resulted from the pH changes in the medium.

![Figure 4. TGA curves of PVA/SA, PVA/SA/BC and crosslinked PVA/SA/BC nanofibrous mats.](image-url)
The RGB values calculated for PVA/SA/BC electrospinning solutions and crosslinked PVA/SA/BC nanofibrous mats at each pH are presented in figure 6. RGB values and their corresponding changes in the pH range of 4.0–10.0 clearly showed the red color of the acidic samples at pH 4.0–6.0, the blue color of the samples at pH 7.0–9.0, and the black-green color at pH 10.0. The differences in RGB values were more visible in electrospinning solutions than nanofibrous mats, probably because of the light transmittance property of the solutions. In both cases, the R value showed the most remarkable changes between pH 4.0 and 10.0, especially for the electrospinning solutions. It could be concluded that the change in R value primarily determined the color change for the two cases. This could be because of the natural color of BC extract, which is pure red.

CIELAB color coordinates were calculated to evaluate the colors in the CIELAB color space. The CIELAB model is similar to human vision and is not dependent on the device on which the colors are displayed or the method of creation. The observable colors and the colors that are out of range for human vision are included in the CIELAB color space [84].

CIELAB L*a*b* coordinates were determined on the digital photographs of the electrospinning solutions and nanofibrous mats using Adobe Photoshop CS5. Total color differences (ΔE*) of the standard PVA/SA/BC electrospinning solutions and crosslinked PVA/SA/BC nanofibrous mats were calculated and presented in table 2. It can be clearly seen that the ΔE* values for the PVA/SA/BC solutions were significantly higher than those of the crosslinked PVA/SA/BC mats between each pH. It is generally reported that the color differences are visibly distinguished when the ΔE* value is greater than 5. Furthermore, the colors generally belong to different color hues if ΔE* values are higher than 12 [51]. ΔE* values calculated for the crosslinked PVA/SA/BC nanofibrous mats were higher than 5 at all studied pHs. These results demonstrated that the colors obtained at different pHs belonged to different color hues. Moreover, they were easily detected by the unaided eye.
3.5. Stability of the pH-sensing nanofibrous mat

Long life, reusability, and photostability are the main requirements for halochromic materials. The crosslinked PVA/SA/BC electrospun mats were examined at two temperature conditions of -20 °C (storage conditions) and 37 °C (body temperature) for 24 h. The electrospun mats developed different colors for pH 4.0, 7.0, and 9.0 (figure 7). Interestingly, there were no significant changes in color at -20 °C and 37 °C, demonstrating that the halochromic properties of the electrospun mats were not adversely influenced by temperature. The RGB values of the stored mats were also similar to those of the freshly prepared PVA/SA/BC electrospun mat (figure 6(b)).

4. Conclusion

In this study, a PVA/SA-based halochromic nanofibrous mat for wound healing monitoring was prepared using the electrospinning process. The halochromic characteristic was added to the nanofibrous mat with the addition of natural BC anthocyanins into the electrospinning solution. The SEM images showed that electrospinning of the PVA/SA/BC solution produced rarely beaded continuous nanofibers. The PVA/SA/BC electrospun mat was successfully crosslinked by GA. The morphological structure of the electrospun mat did not change significantly after crosslinking processing. However, it was seen from SEM images that flattening and fusions of the nanofibers occurred, and the average diameter of the nanofibers increased to 213 ± 37 nm. The thickness of the halochromic PVA/SA/BC nanofibrous mat was 81.6 ± 4.2 μm and applicable for color changes at different pHs. The PVA/SA/BC nanofibrous mats exhibited halochromic behaviors exactly same to PVA/SA/BC electrospinning solutions, demonstrating that natural pH-indicator anthocyanins (extracted from BC) were successfully integrated into the nanofibrous mats. TGA demonstrated that the crosslinking of the PVA/SA/BC mat increased the thermal stability despite the fact that thermal stability was weakened by the addition of BC extract. The halochromic PVA/SA/BC nanofibrous mat with a short response time presented visibly
distinguishable colors, especially at pHs of 4.0–6.0 and 8.0–10.0, which are consistent with the pH changes in the wound healing process. As a result, the crosslinked PVA/SA/BC nanofibrous mat can be used as a pH sensitive wound dressing for monitoring the healing progress.

Acknowledgments

This study was financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK) and was assigned project number 116M540.

ORCID iDs

Ayben Pakolpakçıl  https://orcid.org/0000-0002-6981-4980
Bilgen Osman  https://orcid.org/0000-0001-8406-149X
Elif Tümay Özer  https://orcid.org/0000-0002-5225-0146
Esra Karaca  https://orcid.org/0000-0003-1777-3977

References

[1] Coyle S and Diamond D 2015 Medical Applications of Smart Textiles: Advances In Smart Medical Textiles (Cambridge: Woodhead Publishing)
[2] Koncar V 2016 Smart Textiles and Their Applications (Cambridge: Woodhead Publishing)
[3] Talvenmaa P 2016 Intelligent Textile and Clothing: Chronic and Conductive Materials (London: Woodhead Publishing)
[4] Van der Schueren I and De Clerck K 2012 Coloration and application of pH-sensitive dyes on textile materials Color Technol. 128 82–90
[5] Van Der Schueren I and De Clerck K 2010 The use of pH-indicator dyes for pH-sensitive textile materials Text. Res. J. 80 590–603
[6] Schaudle C, Fröhlich E, Meindli C, Attard J, Binder B and Mohr GJ 2017 The development of indicator cotton swabs for the detection of pH in wounds Sensors 17 1365–77
[7] Van Der Schueren I, Hemelsote K, Van Spybroeck V and De Clerck K 2012 The influence of a polyamide matrix on the halochromic behaviour of the pH-sensitive azo dye nitrazine yellow Dyes. Pigments. 94 415–51
[8] De Meyer T, Steyaert I, Hemelsote K, Hoogenboom R, Van Spybroeck V and De Clerck K 2016 Halochromic properties of sulphonphthaleine dyes in a textile environment: the influence of substituents Dyes. Pigments. 124 249–57
[9] Sun X, Branford-White C, Yu Z and Zhu L 2015 Development of universal pH sensors based on textiles J. Sol-Gel Sci. Technol. 74 641–9
[10] Steyaert I, Vancocilde G, Hoogenboom R and De Clerck K 2015 Dye immobilization in halochromic nanofibers through blend electrospinning of a dye-containing copolymer and polylamid-6 Polym. Chem. UK 6 2685–94
[11] Zhang C, Li Y, Wang W, Zhan N, Xiao N, Wang S, Li Y and Yang Q 2011 A novel two-nozzle electrospinning process for preparing microfiber reinforced pH-sensitive membrane with enhanced mechanical property Eur. Polym. J. 47 2228–33
[12] Shanifábad A N and Bahrami S H 2016 Halochromic chemosensor from poly (acrylonitrile)/ phenolphthalein nanofibers as pH sensor IEEE Sens. J. 16 873–80
[13] Van Der Schueren I, De Meyer T, Steyaert I, Ceylan O, Hemelsote K, Van Spybroeck V and De Clerck K 2013 Polycaprolactone and polycaprolactone/chitosan nanofibers functionalised with the pH-sensitive dye nitrazine yellow Carboryd. Polym. 91 284–93
[14] Schoelch E, Steyaert I, Vancocilde G, Geltmeyer J, Lava K, Hoogenboom R and De Clerck K 2016 Blend electrospinning of dye-functionalized chitosan and poly(e-caprolactone) towards biocompatible pH-sensors J. Mater. Chem. C. 4 4507–16
[15] Kurecci M, Hribernik S, Virant N, Ojstred A, Smole M S and Kleinschek K S 2016 Polysaccharide based nanofibers with pH-sensitive function Tekstil: čopit za tekstilno in odjevno tehnologijo 65 165–70
[16] Bhadarwaj N and Kundu S C 2010 Electrospinning: a fascinating fiber fabrication technique Biotechnol. Adv. 28 325–47
[17] Supaphol P, Suwantong O, Srinivasan S, Jayakumar R and Nair S V 2012 Electrospinning of biocompatible polymers and their potentials in biomedical applications Adv. Polym. Sci. 246 213–40
[18] Subramanian R, Mani M P and Jaganathan S K 2018 Fabrication and testing of electrospin polyurethane blended with chitosan nanofibers for vascular graft applications Cardiovasc. Eng Techn. 9 903–13
[19] Sadeghi A, Pezeshki-Modares M and Zandi M 2018 Electrospun polyvinyl alcohol/gelatin/chondroitin sulfate nanofibrous scaffold: fabrication and in vitro evaluation Int. J. Biol. Macromol. 114 1248–56
[20] Mendes A C, Gortzzeny C, Halter N, Schneider SW and Chronakis IS 2016 Hybrid electrospin chitosan-phospholipid nanofibers for transdermal drug delivery Int. J. Pharm. 510 68–56
[21] Shi R, Xue J, Wang H, Wang R, Gong M, Chen D and Tian W 2015 Fabrication and evaluation of a homogeneous electrospin PCL-gelatin hybrid membrane as an anti-adhesion barrier for craniofacial Journal of Materials Chemistry B 3 4063–71
[22] Liu M, Duan X, Li Y, Yang D and Long Y 2017 Electrospin nanofibers for wound healing Mat Sci Eng C-Mater. 76 1413–23
[23] Chen J P, Chang G Y and Chen J K 2008 Electrospin collagen/chitosan nanofibrous membrane as wound dressing Colloid Surface A 313 183–8
[24] Zhou Y, Yang H, Liu X, Mao J, Gu S and Xu W 2013 Electrospinning of carboxyethyl chitosan/poly(vinylalcohol)/silk fibroin nanofibers for wound dressings Int. J. Biol. Macromol. 53 88–92
[25] Calamak S, Erdogdu C, Ozalp M and Ulubayram K 2014 Silk fibroin based antibacterial bionanotextiles as wound dressing materials Mat. Sci. Eng. C-Mater. 43 11–20
[26] Zhao R, Li X, Sun B, Zhang Y, Zhang D, Tang Z, Chen X and Wang C 2014 Electrospin chitosan/sericin composite nanofibers with antibacterial property as potential wound dressings Int. J. Biol. Macromol. 68 92–9
[27] Hacker C, Karahaliloglu Z, Seide G, Denkbas E B and Gries T 2013 Functionally modified, melt-electrospin thermoplastic polyurethane mats for wound-dressing applications J. Appl. Polym. Sci. 131 40132
[28] Saeed S M, Mirzadeh H, Zandi M and Barzin J 2017 Designing and fabrication of curcumin loaded PCL/PVA multi-layer nanofibrous electrospin structures as active wound dressing Prog. Biomater. 6 39–48
[29] Shirazaki P, Varshosaz J and Khraazi A Z 2017 Electrospun gelatin/poly(glycerol sebacate) membrane with controlled release of antibiotics for wound dressing Adv. Biomed. Res. 6 105–11
[30] Ustundag C G, Karaca E, Ozayrutlu M, Ozbek S, Yermazler A and Cavusoglu I 2014 Histological evaluation of wound healing performance of electrospun gelatin/poly(vinyl alcohol)/sodium alginate as wound dressing in vivo Bio-Med. Mater. Eng. 24 1527–36
[31] Shahverdi S, Hajimiri M, Esfandiar M A, Larjani B, Atyabi F, Rajabiani A, Dephour A R, Gharehaghi A F and Dinardarv B 2014 Fabrication and structure analysis of poly(lactide-co-glycolic acid)/silk fibroin hybrid scaffold for wound dressing applications Int. J. Pharm. 473 345–55
[32] Uppal R, Ramaswamy G N, Arnold C, Goodhand R and Wang Y 2011 Hyaluronic acid nanofiber wound dressing: production, characterization, and in vivo behavior J. Biomed. Mater. Res. B 89 20–9
[33] Tsekov P B, Spasova M G, Manolova N E, Markova N D and Rashkov I B 2017 Electrospun curcumin-loaded cellulose acetate/polyvinylpyrrolidone fibrous materials with complex architecture and antibacterial activity Mat. Sci. Eng. C-Mater. 73 206–14
[34] Perumal G, Pappuru S, Chakraborty D, Nandakumar A, Chand D K and Doble M 2017 Synthesis and characterization of curcumin loaded PLA-hyperbranched polyglycerol electrospun blend for wound dressing applications Mat. Sci. Eng. C-Mater. 76 1196–204
[35] Ignatova M, Manolova N and Rashkov I 2007 Electrospinning of poly(vinyl pyrrolidone)—iodine complex and poly(ethylene oxide)/poly(vinyl pyrrolidone)—iodine complex—a prospective route to antimicrobial wound dressing materials Eur. Polym. J. 43 1609–23
[36] Nagaraja R, Soussan L, Bechelany M, Teyssier C, Cavalles Y, Pochat-Botarier C, Miele P, Kalkura N, Imano J and Balme S 2016 Novel biocompatible electrospun gelatin fiber mats with antibiotic drug delivery properties J. Mater. Chem. B 4 1134–41
[37] Sun X, Li K, Chen S, Yao B, Zhou Y, Cui S, Hu J and Liu Y 2016 Rationally designed particle preloading method to improve protein delivery performance of electrospun polyester nanofibers Int. J. Pharm. 512 204–12
[38] Garcia–Orue I, Gainza G, Gutierrez F B, J. Aguirre J J, Evora C, Pedraz J L, Hernandez R M, Delgado A and Igartua M 2017 Novel nanofibrous dressings containing rhEFG and Aloe vera for wound healing applications Int. J. Pharm. 523 556–66
[39] Van Aalst J A, Reed C R, Han L, Andrady T, Hromadka M, Bemacki S, Kolappa K, Collins J B and Loboa E G 2008 Cellular incorporation into electrospun nanofibrous retainer: proliferation, retention and function in fibroblasts Ann. Plast. Surg. 60 577–83
[40] Kohsari I, Shiratinta Z and Pourmortazavi S M 2016 Antibacterial electrospun chitosan-polycrylonitrile nanocomposite mats containing bioactive silver nanoparticles Carbohyd. Polym. 140 267–98
[41] Manikandan A, Mani M P, Jagathan S K, Rajasekar R and Jagathan M 2017 Formation of functional nanofibrous electrospun polyurethane and muriavena oil with improved haemocytometric activity for wound healing Polym. Test. 61 106–13
[42] Paul W and Sharma C P 2004 Chitosan and alginate wound dressings: a short review Trends Biomater. Artif. Organs. 18 18–23
[43] Lee J Y, Shin D S, Kwon O W, Park W H, Choi H G, Lee Y R, Han S S, Noh S K and Lyoo W S 2007 Preparation of acetic poly(vinyl alcohol)/sodium alginate blend nanowebs by electrospinning J. Appl. Polym. Sci. 106 1357–42
[44] Ustundag G C, Karaca E, Ozbek S and Cavusoglu I 2010 In vivo evaluation of electrospun poly (vinyl alcohol)/sodium alginate nanofibers as wound dressing Tekst. Konfekcijom. 20 290–8
[45] Safi S, Morshed M, Ravandi S H and Ghiaci M 2007 Study of electrospinning of sodium alginate, blended solutions of sodium alginate/poly(vinyl alcohol) and sodium alginate/poly(ethylene oxide) J. Appl. Polym. Sci. 104 3245–35
[46] Fu R, Li C, Yu C, Xie H, Shi S, Li Z, Wang Q and Lu L 2016 A novel electrospun membrane based on mioxiloxacin hydrochloride/polylvinyl alcohol/sodium alginate for antibacterial wound dressings in practical application Drug Deliv. 23 818–29
[47] Dargaville T R, Farrugia B L, Broadbelt J A and Pace S 2013 Sensors and imaging for wound healing: a review Biosens. Bioelectron. 41 30–42
[48] Tsukada K, Tokumaga K, Iwama T and Mishima Y 2002 The pH changes of pressure ulcers related to the healing process of wounds Wounds 14 16–20
[49] Geethin 2007 The significance of surface pH in chronic wounds Wounds 3 52–5
[50] Khan M A, Ansari U and Ali N M 2015 Real-time wound management through integrated pH sensors: a review Sensor. Rev. 35 183–9
[51] Devarayan K and Kim B S 2015 Versatile and universal pH sensing cellulose nanofibers for health monitor Sensors Actuat. B Chem. 209 281–6
[52] Pietro L, Pinto Z, Mello L, Halal E, De Moraes M G, Vieira A, Lim L, Renato A, Dias G and Zavareze R 2018 Ultrafine fibers of zein and anthocyanins as natural pH indicator J. Sci. Food. Agric. 98 2735–41
[53] Bondre S, Patil P, Kulkarni A and Pillai M M 2017 Study on isolation and purification of anthocyanins and its applications as pH indicator Int. J. Adv. Biotechnol. Res. 3 698–702
[54] Khoj E H, Azlan A, Tang S T and Lim S M 2017 Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and potential health benefits Food Nutr. Biol. 61 1361779
[55] Jami R and Babalou F 2017 Stability of blueberry (Cornus mas—Yuluyuk) anthocyanin nanofiber under pH conditions and co-pigment treatments Int. J. Food Prop. 20 2128–33
[56] Maftoonazad N and Ramaswamy H 2019 Design and testing of an electrospun nanofiber nanofiber as a pH biosensor and monitoring the pH associated quality in fresh date fruit (Rutab) Polym. Test. 75 76–84
[57] Ersus S and Yurdagel U 2007 Microencapsulation of anthocyanin pigments of black carrot (Daucus carota L.) by spray drier J. Food Eng. 80 805–12
[58] Montilla E, Arzaba M R, Hillebrandt S and Winterhalter P 2011 Anthocyanin composition of black carrot (daucus carota ssp. sativus var. bratonea var.)cultivars antonina, beta sweet, deep purple, and purple haze J. Agric. Food Chem. 59 3385–90
[59] Lee J, Durst W R and Wrolstad R E 2005 Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study J. Amoc. Int. 88 1269–78
[60] Li Z and Wang C 2008 Effects of Working Parameters on Electrospinning: One-Dimensional Nanostructures. (New York: Springer) pp 15
[61] Mit-Upphanth C, Nithitanakul M and Supaphol P 2004 Ultrathin electrospun polyamide-6 fibers: effect of solution conditions on morphology and average fiber diameter Macrom. Chem. Phys. 205 2327–38
[62] Ramakrishna S, Fujihara K, Teo W, Lim T and Ma Z 2005 An Introduction To Electrospinning and Nanofibers (Singapore: World Scientific Publishing Co.)
[63] Algarra M, Fernandes A, Matues N, Freitas V, Esteves da Silva J C G and Casado J 2014 Anthocyanin profile and antioxidant capacity of black carrots (Daucus carota L. ssp. sativus var. atrobrunet Atfen) from Cuevas Bajas, Spain J. Food Compos. Anal. 33 71–6
[64] Doshi J and Reneker D H 1995 Electrospinning process and applications of electrospun fibers J. Electrostat. 35 151–60
[65] Shamim Z, Saeed B, Amir T, Abo saleed R and Rogheih D 2012 The effect of flow rate on morphology and deposition area of electrospun nylon 6 nanofiber J. Eng. Fabrics Fibers 7 42–9
[66] Haider S, Al-Zeghayer Y, Ahmed A L, Haider A, Mahmood A, Al-Masyr W, Imran M and Aijaz M 2013 Highly aligned narrow diameter chitosan electrospun nanofibers Polym. J. 20 11–1
[67] Uyar T and Besenbacher F 2008 Electrospinning of uniform polystyrene fibers: the effect of solvent conductivity Polymer 49 5336–43
[68] Moghe A K, Hufenus R, Hudson S M and Gupta B S 2009 Effect of the addition of a fugitive salt on electrospinnability of poly(ε-caprolactone) Polymer 50 3311–8

[69] Angammana C J and Jayaram S H 2011 Analysis of the effects of solution conductivity on electrospinning process and fiber morphology IEEE T. Ind. Appl. 47 1109–17

[70] Baji A, Mai Y W, Wong S C, Abtahi M and Chen P 2010 Electrospinning of polymer nanofibers: effects on oriented morphology, structures and tensile properties Compos. Sci. Technol. 70 703–18

[71] Sinha M K, Das B R, Srivastava A and Saxena A K 2013 Needleless electrosprining and coating of poly vinyl alcohol with cross-linking agent via in situ technique Int. J. Text. Fashion Technol. 3 29–38

[72] Rhoo K S, Jeong L, Leea G, Seod B M, Parka Y J, Honge S D, Roha S, Choa J J, Park W H and Mina B M 2006 Electrospinning of collagen nanofibers: effects on the behavior of normal human keratinocytes and early-stage wound healing Biomaterials 27 1452–61

[73] Taepaiboon P, Rungsardthong U and Supaphol P 2007 Effect of cross-linking on properties and release characteristics of sodium salicylate-loaded electrospun poly(vinyl alcohol) fibre mats Nanotechnology 18 175102

[74] Islam M S and Karim M R 2010 Fabrication and characterization of poly(vinyl alcohol)/alginate blend nanofibers by electrospinning method Colloids. Surfaces. A. 366 135–40

[75] Awada H and Daneault C 2015 Chemical modification of poly(vinyl alcohol) in water Appl. Sci. 5 840–50

[76] Wang T, Turhan M and Gunasekaran S 2004 Selected properties of pH-sensitive, biodegradable chitosan—poly (vinyl alcohol) hydrogel Polym. Int. 91 911–8

[77] Pereira V A, Arruda I N Q and Stefani R 2015 Active chitosan/PVA films with anthocyanins from brassica oleraceae (red cabbage) as time-temperature indicators for application in intelligent food packaging Food Hydrocolloids. 43 180–8

[78] Ramamoorthy R, Radha N and Maheswari G 2016 Betalain and anthocyanin dye-sensitized solar cells J. Appl. Electrochem. 46 929–41

[79] Zhai X, Shi J, Zou X, Wang S, Jiang C, Zhang J, Huang X, Zhang W and Holmes M 2017 Novel colorimetric films based on starch/polyvinyl alcohol incorporated with roseelle anthocyanins for fish freshness Monitoring Food Hydrocolloids. 69 308–17

[80] Fonseca dos Reis E, Campos F S, Lage A P, Leite R C, Heneine L G, Vasconcelos W L, Lobato Z I P and Mansur H S 2006 Synthesis and characterization of poly (vinyl alcohol) hydrogels and hybrids for rMPB70 protein adsorption Mater. Res. 9 185–91

[81] Mansur H S, Sadahira C M, Souza A N and Mansur A A P 2008 FTIR spectroscopy characterization of poly (vinyl alcohol) hydrogel with different hydrolysis degree and chemically crosslinked with glutaraldehyde Mat. Sci. Eng. C-Mater. 28 539–48

[82] Pawar R P, Nagar N and Road C 2016 Study of thermal decomposition and instrumental analysis of synthesized polyvinyl alcohol J. Ultra Chem. 11 1–6

[83] Figueiredo K C S, Alves T L M and Borges C P 2014 Myoglobin entrapment in poly(vinyl alcohol) dense membranes Braz. J. Chem. Eng. 31 747–56

[84] Mery D, Leo K, Pedreschi F and Leo J 2006 Color measurement in L’ a’ b’ units from RGB digital images Food Res. Int. 39 1084–91