RELEASE CHARACTERISTICS OF NAPROXEN LOADED POLY(VINYL ALCOHOL) NANOFIBERS CROSSLINKED WITH POLYCARBOXYLIC ACIDS

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ABSTRACT

Poly(vinyl alcohol) (PVA) hydrogel nanofibers are believed to be a potential candidate for drug release applications. Polycarboxylic acids; 1,2,3,4 butanetetracarboxylic acid (BTCA) and citric acid (CA) are low cost, non-toxic alternatives that have been reported to crosslink electrospun PVA hydrogel. They could directly be added into the spinning solutions. Naproxen which is one of the most efficient NSAIDs was selected as a model drug for this study. The release mechanisms of drug-loaded electrospun PVA nanofibers are based on the diffusion of the drugs through the swollen PVA fibrous matrix and the release due to partial dissolution of the matrix. Control over the release characteristics of the drugs can be provided through partial crosslinking of the PVA fibrous matrix.

Key Words: Electrospinning, PVA, Crosslinking, Polycarboxylic acids, Naproxen, Drug Release

1. INTRODUCTION

Electrospinning is a promising and emerging method for the production of medical textile materials with controlled release properties. One of the main advantages of the electrospinning process over the conventional film-casting technique is the highly porous structure of electrospun fiber mats which exhibit greater surface area that assumingly could allow drug molecules to diffuse out from the matrix much more conveniently [1]. In terms of material selection, electrospun poly(vinyl alcohol) (PVA) hydrogel nanofibers are believed to be a potential candidate for drug release applications. Poly(vinyl alcohol) (PVA) is a hydrophilic, semi-crystalline polymer with good chemical and thermal stability [2-3]. Electrospun PVA nanofibers have been one of the most extensively studied topics due to its biocompatibility, nontoxicity, hydrophilicity and ease of processability [4-5].

Many researchers have been studied drug release from PVA fibers in recent years [1-3, 6-7] The release mechanisms of drug-loaded electrospun PVA nanofibers are based on the diffusion of the drugs through the swollen PVA fibrous matrix and the release due to partial dissolution of the matrix [2-3,6]. Control over the release characteristics of the drugs can be provided through partial crosslinking of the PVA fibrous matrix [6]. Methanol treatment and chemical crosslinking with glutaraldehyde are found to be most applied treatments within the literature, but they display toxicity problems and thus their potential applications as biomaterials are limited [8-9]. Also, during methanol treatment there is a loss of the drug entrapped in the fibers [3]. Electrospun PVA nanofibres containing model drugs could be also crosslinked with the vapor from the aqueous solution of either glutaraldehyde or glyoxal, followed by heat treatment. It is believed that crosslinking by this method could minimize the toxicity [6-7]. But vapor induced crosslinking needs a post-treatment and it is difficult to achieve reproducible results. Therefore in this study, polycarboxylic acids; 1,2,3,4 butanetetracarboxylic acid (BTCA) and citric acid (CA) were selected as crosslinking agents

(Figure 1.). BTCA and citric acid are low cost, non-toxic alternatives that have been reported to crosslink electrospun PVA hydrogels [8-9]. Another advantage is the miscibility of them with PVA electrospinning solutions, thus they can directly be added into the spinning solutions.

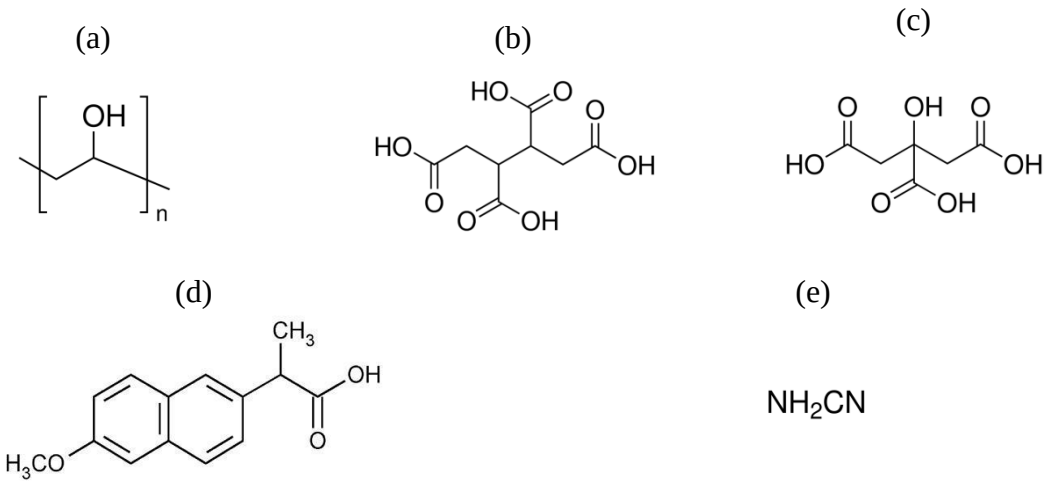

Figure 1. Poly(vinyl alcohol) (PVA) (a), 1,2,3,4 butanetetracarboxylic acid (BTCA) (b), citric acid (CA) (c), naproxen (d) and cyanamide (e) [10]

The use of electrospun fibers as drug carriers will be promising in the future of medical textile applications especially, for pain related topical administrations. Non-steroidal anti-inflammatory drugs (NSAIDs) are used for controlling pain and inflammation in rheumatic diseases. Naproxen which is one of the most efficient NSAIDs [11-12] was selected as a model drug for this study.

In this study, the crosslinking of PVA nanofibers with polycarboxylic acids and its effect on the resultant nanofiber morphology, swelling behavior and naproxen release characteristics were investigated.

2. EXPERIMENTAL

2.1. Materials

Poly(vinyl alcohol) (PVA) (Figure 1) with average molecular weight of ~125,000 g/mol, naproxen, 1,2,3,4 butanetetracarboxylic acid (BTCA), citric acid (CA), cyanamide were purchased from Sigma Aldrich Chemical Company. Chemical structures are given in Figure 1 [10].

2.2. Electrospinning

A weighed amount of PVA was dissolved in distilled water at 100 °C to prepare an aqueous PVA stock solution at a fixed concentration of 10% w/w. It was stirred for 4 hours and cooled down to room temperature while it was stirring. After allowing the solution to cool down to room temperature, BTCA or CA, as crosslinking agents, was directly added into the spinning solution with cyanamide as a catalyst in ratio of 2:1 (w/w) followed by stirring for 15 min.

The concentration of the BTCA and CA were fixed at 20% ($w/w_{polymer}$). 10% ($w/w_{polymer}$) naproxen was dissolved in the PVA solution followed by stirring for 4 h prior to electrospinning.

Electrospinning of the polymer solutions was carried out by a set-up consisting of a syringe (10 mL) and a stainless steel needle (1.5-cm long, 22 gauges, and flat tip), a ground electrode and a high voltage supply (Simco, MP Series CM5 30 P, Charging Generator Output 30 kV DC). Polymer solutions were electrospun at a voltage of 18 kV, a tip-to-collector distance of 15 cm with a feeding rate of 0.7 ml/h. A grounded stationary rectangular metal collector covered by a piece of aluminum foil was used for the nanofiber deposition. Electrospinning of each sample were carried out for 10 h.

After electrospinning, PVA/BTCA and PVA/CA nanofibrous mats were heat set at 110^{o}C for 20 min in an oven to enhance the esterification reaction.

2.3. Characterization

The morphology of drug-loaded electrospun mats was observed by a scanning electron microscope (SEM; FEI Quanta250 FEG scanning electron microscope). The electrospun mats were sputtered by EMITECH K550X ion sputtering device with a thin layer of gold prior to SEM observation. The mean diameter of the resultant fibers was calculated from measurements on SEM images of 5000× magnification by using Image J program. Approximately 100 measurements were carried out from the different parts of each sample.

The thickness of the nanofibrous mats were measured by Mitutoyo digital micrometer at 0,001 mm accuracy.

Swelling and weight loss of the Naproxen-loaded electrospun non-crosslinked and BTCA and CA crosslinked PVA fiber mats were characterized in phosphate buffer at pH 7.4 (37 ^{o}C) (PBS) for 24 h according to the following equations:

$$\text{Degree of swelling}(\%) = \frac{M - M_d}{M_d} \text{ x } 100 \quad (1)$$

and

$$\text{Weight loss } (\%) = \frac{M_i - M_d}{M_i} \text{ x } 100 \quad (2)$$

where M is the weight of each fiber mat sample after submersion in PBS for 24 h and samples were carefully blotted with tissue paper to remove excess water from the surface. Md is the weight of the sample after drying at 37 ^{o}C, Mi is the initial weight of the samples.

2.5. Release characteristics

Total immersion method was used to study the release characteristics of the drug from the drug-loaded electrospun PVA fiber mats. The samples (in dimension of 3x3 cm) were accurately weighed and immersed in a glass bottle containing phosphate buffer pH 7.4 USP and the bottles were incubated in a horizontal incubation shaker (Thermo-Scientific MaxQ6000) at of 37± 0.5°C, 60 rpm. At a specified immersion period ranging between 0 and 4 h (240 min), 0.5 ml of the sample was taken out at selected times and an equal volume of medium was

returned to the system after withdrawal. The samples were then assayed spectrophotometrically at 263 nm. 3 repetitions were carried out for each type of samples. The release of the drug from the samples was reported as the cumulative release of the drug as a function of the immersion period.

3. RESULTS AND DISCUSSION

Crosslinking of PVA nanofibers with polycarboxylic acids and its effects on the resultant nanofiber morphology and naproxen release properties were investigated in this study. The SEM micrographs and mean diameter of the nanofibres and the mean thickness of the membranes are given in Figure 2 and Table 1, respectively. It was observed that BTCA or CA incorporated, naproxen loaded PVA nanofibres were successfully electrospun. No naproxen crystals were detected by SEM either on the surface of the fibers or outside the fibers as shown in Figure 2. This indicated that, naproxen was totally embedded within the fibers.

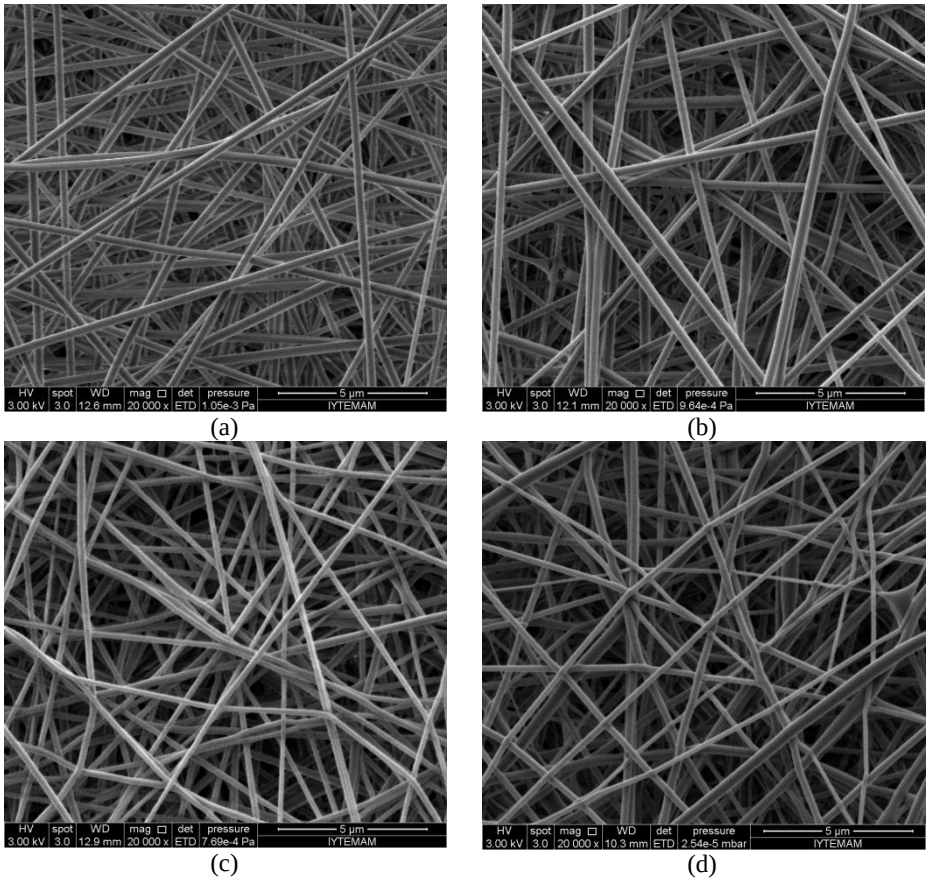

Figure 2. SEM Images of the neat (a), non-crosslinked NAP loaded (b), BTCA (c) and CA (d) crosslinked electrospun PVA mats

Differences in fiber diameters and thickness were analyzed by one-way analysis of variance (ANOVA) followed by a Tukey test for pairwise comparison. It was observed that, there is no significant effect on the fiber diameter by the incorporation of naproxen. Considering polycarboxylic acid crosslinking, the resultant fiber diameters decreased by the addition of BTCA and CA compared to non-crosslinked samples. This difference at $P<0.05$ was considered statistically significant.

In case of mean thickness of the nanofibrous membranes, naproxen loading did not cause a significant difference in the thickness whereas, BTCA and CA addition caused statistically significant increase in thickness values. It is possibly related to the diameter of the deposition area. BTCA and CA addition reduced the jet path and the bending instability spreads over a smaller area which increases the thickness of the mats.

Table.1 Mean diameter of electrospun PVA nanofibers, and mean thickness of electrospun PVA mats

	Mean Diameter ± S.D.	**Mean Thickness ± S.D.**	**Diameter of the deposition area**
10%PVA	*291.60 ±38.70*	*0.1271 ± 0.03*[a]	*~20 cm*
10%PVA-10% NAP non-crosslinked	*274.26 ±47.88*	*0.1829 ± 0.03*[a]	*~20 cm*
10%PVA-10% NAP-BTCA crosslinked	*228.80 ±46.66***	*0.3271 ± 0.07*[b]	*~17 cm*
10%PVA-10% NAP-CA crosslinked	*246.99± 66.09***	*0.2417 ± 0.12*[c]	*~17 cm*

[a,b,c,] ** ***P<0.05.*** *One Way ANOVA, TukeyHSD post hoc test*

Swelling and weight loss of the naproxen-loaded non-crosslinked electrospun PVA nanofibres and BTCA and CA crosslinked electrospun PVA nanofibres are given in Figure 3. Without crosslinking, the degree of swelling of naproxen loaded electrospun PVA nanofibers was ~631%, while the percentage of weight loss was as much as ~44%. BTCA and CA crosslinking led to an increase in the degree of swelling, which were tested to be ~1110% and ~1580 %, respectively. On the other hand, the weight loss decreased to a great extent by BTCA crosslinking (~3% and ~42% for BTCA and CA, respectively).

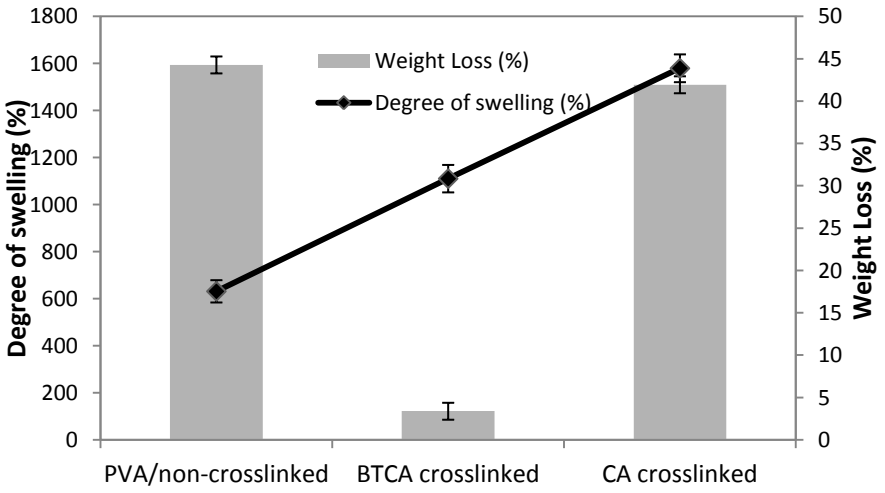

Figure 3. Swelling and weight loss of the NAP-loaded non-crosslinked, BTCA and CA crosslinked e-spun PVA fiber mats

Figure 4 shows the scanning electron micrographs of the samples after immersion into PBS for 24 h and a further drying. When neat and non-crosslinked naproxen loaded PVA nanofiber membranes were immersed in PBS, they shrunk immediately and became almost transparent. On the other hand, BTCA crosslinked mats became bulky and stay opaque whereas CA crosslinked samples became bulky but gel like transparent appearance (Figure 5). The nanofibrous structure of non-crosslinked PVA nanofibers was totally destroyed due to high

solubility [Figure 4(a) and (b)]. The nanofibrous structure of the BTCA crosslinked membranes was observed to be preserved however CA crosslinked membranes almost lost their fibrous structure with a swollen and conjoint appearance. This is probably due to the highest swelling capacity of the CA crosslinked nanofibers. The results point out that the stabilization of the structure against water in terms of weight loss was successfully achieved by the use of BTCA as crosslinker, and it is categorically clear that the use of BTCA is more appropriate when the preservation of nanofibrous structure is an issue.

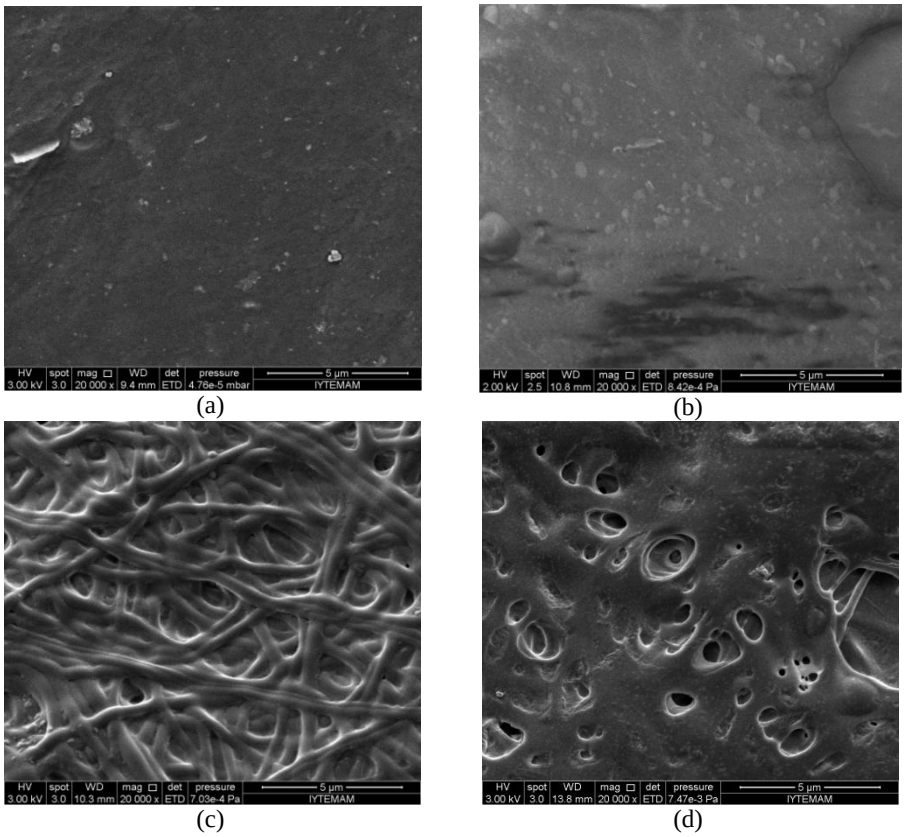

Figure 4. SEM images of nanofibers after immersion of PBS for 24 h (a) neat PVA nanofibers, NAP loaded non-crosslinked PVA (b) BTCA (c) and CA crosslinked PVA nanofibers

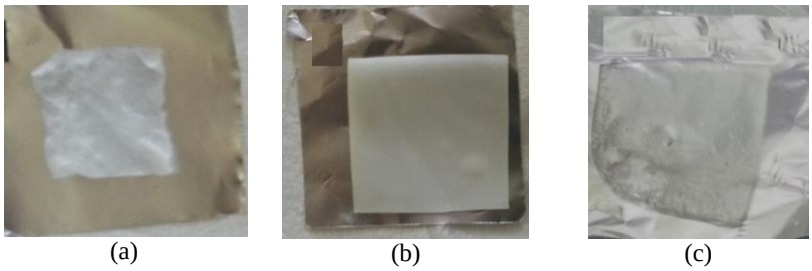

Figure 5. Swelling images of the non-crosslinked (a), BTCA (b) and CA (c) crosslinked PVA electrospun mats after immersion in dissolution medium for 24 hours

The release characteristics of naproxen from the naproxen-loaded electrospun fiber mats are shown in Figure 6. Evidently, naproxen release from non-crosslinked PVA mats showed burst effect during 15-30 min due to high solubility of PVA, which lost its all drug content immediately. It is known that, for a drug delivery system, one of the factors controlling the release of a drug is the swelling behavior of the hydrogel carrier [2]. As soon as the PVA

matrix began to swell; molecules of naproxen drug were leached out from the matrix very rapidly. Another contributing factor was the dissolution of the PVA matrix within the medium. This indicated that the mechanism for the burst release of naproxen included the swelling and the partial dissolution of the PVA matrix.

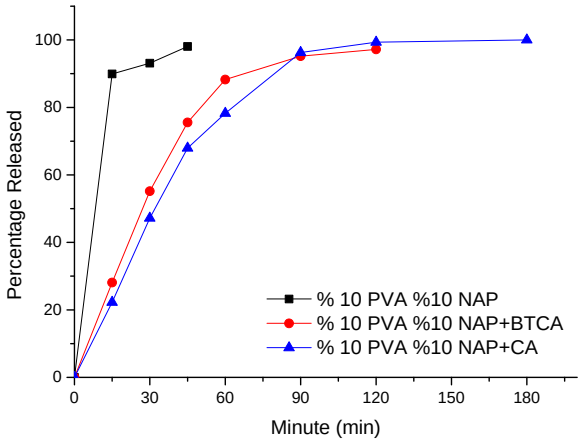

Figure 6. Percentage release of non-crosslinked NAP loaded (■), BTCA (●), CA (▲) crosslinked 10% PVA mats

Obviously, crosslinking enhance the stability of the mats against dissolution in water and slowed down the release of naproxen from the drug-loaded nanofibers. It was observed that higher swelling (see figure 3) leads to lower release rate. Thus CA crosslinking led to slower release compared to those of BTCA crosslinked. The release of drugs from hydrogels involves the absorption of water into polymer matrix and simultaneous desorption of the drug via diffusion [13, 14]. So, it is thought that due to the higher swelling, longer diffusion path of BTCA and CA crosslinked PVA nanofibers resulted lower drug release rates. The total amount of the drug released from the BTCA and CA crosslinked naproxen loaded electrospun PVA fiber mats were 88% and 78% respectively within 60 min whereas non-crosslinked naproxen loaded PVA mats released ~98% of drug within 45 min. Crosslinking did not decrease the total released drug amount.

3. CONCLUSIONS

Electrospun non-crosslinked, BTCA and CA crosslinked PVA nanofibers containing naproxen was produced. The effect of BTCA and CA crosslinking were investigated in terms of resultant fiber diameters, membrane thicknesses, swelling and weight loss of the structure and their naproxen release characteristics were compared. It was seen that, stabilization of the PVA mats against dissolution in water could be significantly enhanced by crosslinking with polycarboxylic acids even at fixation of 110 ^{o}C. BTCA crosslinking is more advantageous in terms of the preservation of the nanofibrous structure. On the other hand, CA crosslinked PVA nanofibres showed higher swelling and lower naproxen release rate.

4. REFERENCES

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