



## $\kappa$ -carrageenan/poly(ethylene glycol) Based Non-woven Cotton Wound Dressings

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### Abstract

Kappa carrageenan (KC) is sulfated polysaccharides and can be crosslinked by  $K^+$  ions to obtain biohydrogel having a biodegradable nature as well as excellent cytocompatibility. Crosslinking of KC with  $K^+$  ions converts it to strong and brittle gel. In that study, non-woven cotton fabric samples (NWCF) were quaternized to achieve QNWCF samples which were then re-treated with different polyethylene glycol/Kappa carrageenan (PEG/KC) blending ratios and crosslinked with  $K^+$  ions to achieve KC/PEG/ $K^+$ /QNWCF wound dressings. The results depict that the appropriate conditions to achieve that dressing is: PEG/KC weight ratio, 0.25%;  $K^+$  padding bath concentration, 1%; and immersion time, 10 min. The chemical structure of such dressing was confirmed via FTIR while its morphology was analyzed using scan electron microscope. The sulfadiazine in-vitro releasing from the loaded dressing at pH 7 was investigated. The results confirm that the prepared sulfadiazine loaded dressing has significant antibacterial activities that were remarkably enhanced upon addition of silver nano-particle during the dressing preparation.

Key words:  $\kappa$ -carrageenan, Poly (ethylene glycol), Potassium ions, Non-woven fabrics, Wound dressings.

### 1. Introduction

A wound is a disruption in the skin tissue anatomy that can be occurred at any accident or during surgery. A dressing substance is usually used to maintain the wound from the surrounding conditions as well as to promote healing of that wound [1-3].

Wound management is an essential factor for healthcare systems since the wound healing is still a challenge for the medical domain. Saying the fact that no single dressing is convenient for treating all wounds is the driving force for producing a wide range of dressings. In fact, the primary wound dressing function is extracting the wound exudates with maintaining a moist medium for the healing process in addition to ensuring gas permeability, a thermal insulation and an antibacterial medium for the wound.

Besides, the dressing must be also of low-cost, biocompatible, nontoxic, and easy to apply [4-8]. Wound healing is a dynamic process to regenerate and grow the biological tissues. The wound healing process includes hemostasis, inflammation, proliferation, and maturation phases. Dressings can be categorized according to the interaction with the biological tissue into passive, interactive, and bioactive dressings [1,6].

The traditional products that are textile -based products such as gauze and pads are passive dressings. Their combination with topical antibiotic formulations leads to a drug leakage as well as wound dryness and consequently a poor wound healing. The interactive dressings are composed of antimicrobial polymeric films, casted from polymers, permeable to oxygen and water vapor. They are used for the little exuding wounds. The bioactive dressings function is

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delivering the bioactive compounds for wounds curing. These materials comprise natural polymers like alginate and chitosan as well as synthetic polymers like poly ethylene glycol and poly vinyl alcohol in addition to the blends of these types of polymers, in forms of films, hydrogels, sponges, and hydrocolloids [6,9,10]. Films are the simple, effective, flexible and easy applied dressings that serve a moist wound medium to accelerate wound curing [11,12].

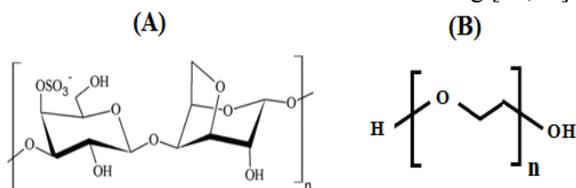


Figure 1: Chemical structures of (A)  $\kappa$ -carrageenan and (B) polyethylene glycol.

Carrageenans are a sulfated polysaccharides family extracted from red seaweeds. They are present in three basic forms: kappa (Figure 1), iota, and lambda. The potassium ions convert kappa carrageenan (KC) into strong and brittle gels; calcium ions transform iota carrageenan into soft and elastic gels; lambda carrageenan cannot form gels. Carrageenans show some limitations in their reactivity as well as processability that may be overcome by blending with other polymers [13-17]. Mohamadnia et al [15] prepared KC beads as interpenetrating polymer networks (IPN) by employing the sodium alginate as a polymer network,  $\text{Ca}^{2+}$  or  $\text{K}^+$  ions as crosslinkers as well as betamethasone acetate as a model drug. IPN matrix tablets of KC and sodium alginate had been prepared by Kulkarni et al [16] for propranolol hydrochloride releasing.

Poly ethylene glycol (PEG) (Figure 1) is a water-soluble synthetic polymer having low intrinsic toxicity and so that it is suitable for the biological applications. The hydrophilic nature of PEG improves the solubility of the hydrophobic drugs upon blending with them. PEG can be chemically crosslinked to form hydrogel. PEG-drug conjugates are widely studied for several molecules and drugs including insulin, peptides and lipids. These conjugates offer reduced protein immunogenicity and enzymatic degradation and increased

residence time in the body. Consequently, most of the conjugated drugs in addition to micellar and liposomal formulations in the market are PEG-containing products [18,19].

Since crosslinking of KC with  $\text{K}^+$  converts KC to a brittle gel, thus, the current study was undertaken with a view to blend PEG with KC to achieve KC/PEG/ $\text{K}^+$ /QNWCF wound dressing having good film properties.

## 2. Experimental

### 2.1. Materials

Non-woven cotton fabric (100%) (NWCF), provided by Hebitex Co., and Kappa carrageenan (KC), provided by Acros Organics, were used. Polyethylene glycols (PEG) with molecular weights 400, 600, 1000, 2000, and 4000 Dalton were used. 3-chloro-2-hydroxypropyl trimethyl ammonium chloride solution (65% w/w) (Quat), Fluca, was used as cationizing agent. Sodium salt of sulfadiazine (SD), Aldrich, was used. Laboratory grade chemicals of acetic acid, sodium hydroxide and potassium chloride were used.

### 2.2. Methods

#### 2.2.1. Fabric quaternization

The quaternization of the non-woven cotton fabric (QNWCF) was prepared by the method that elsewhere described [20]. Two sets of non-woven cotton samples were quaternized using different concentrations of Quat as well as NaOH. Typically, each set was padded in its corresponding solution, squeezed at 100% wet pick up, stored for 24 h in closed plastic bags at room temperature, washed with distilled water, and finally neutralized with acetic acid aqueous solution. Table 1 provides the of Quat and NaOH concentrations that are used in the quaternization process in addition to the percent nitrogen (%N) values of the quaternized NWCF.

Table 1: The concentrations of Quat and NaOH used for quaternization of NWCF samples and % N of that samples.

Quat conc. (g/l)	NaOH conc. (g/l)	% N
30	14	0.1937
50	24	0.3201

### 2.2.2. KC/PEG/K<sup>+</sup> nonwoven dressing preparation

The KC/PEG/K<sup>+</sup> wound dressing was prepared by padding either of the NWCF or QNWCF samples in any of the KC/PEG blends, then the treated samples are squeezed and dried at 85 °C/10 min. The dried samples were then immersed in KCl aqueous solution of specific concentration for a specific time, washed thoroughly with distilled water, dried at 85 °C for 10 min to obtain KC/PEG/K<sup>+</sup> non-woven dressing, and then stored at 60% relative humidity for 24h.

### 2.2.3 Loading of the KC/PEG/K<sup>+</sup>/NWCF dressing with SD and silver nano-particles

Sulfadiazine (SD) and silver nano-particles (Ag-NPs) was loaded into the crosslinked KC/PEG/K<sup>+</sup>/NWC dressing before the dressing crosslinking. Typically, 1% of SD and/or 2% Ag-NPs were added to the KC/PEG blend solution and then stirred to obtain homogenous KC/PEG/SD solution. Nonwoven cotton fabric was then padded into the KC/PEG/SD/Ag-NPs solution followed by squeezing, drying at ambient conditions and crosslinking with KCl solution as mentioned above.

### 2.2.4 In vitro release of sulfadiazine

The sulfadiazine in-vitro releasing from the KC/PEG/SD/K<sup>+</sup>/NWCF dressing was performed by steeping 2.5 × 2.5 cm of the dressing in stoppered glass bottles filled with buffer solution of a pH 7. A Volume of 2 ml was then taken from the aforementioned solution at every 1h, and the sulfadiazine releasing was evaluated at 278 nm by means of PG-T80, UV/Visible Spectrophotometer.

### 2.3. Testing methods

%N was evaluated according to Kjeldahl method [21-28].

The percent swelling was evaluated by steeping the samples in pH 7 distilled water at 37 °C for 24 hours followed by gentle wiping with a filter paper followed by weighing.

$$\text{Swelling (\%)} = (\text{Wh} - \text{Wd}) / \text{Wd} \times 100,$$

where Wd and Wh are the dry weight and hydrated weight respectively of the dressing.

The percent Gel fraction of the dressing was determined as:

$$\text{Gel fraction (\%)} = (\text{Wa} / \text{Wi}) \times 100$$

where Wi is the initial weight and Wa is the dry weight of the hydrated dressing.

Antimicrobial activities of the untreated the NWCF and the nominated dressing were evaluated, as inhibition zone (IZ) per millimeters, according to AATCC Test Method 147-2004 using the following bacteria [29, 30]:

G +ve bacteria: Staphylococcus aureus (SA).

G-ve bacteria: Escherichia coli (EC).

The IR spectra were performed using FT/IR-4700 FTIR Spectrometer.

The Scanning Electron Microscope (SEM) images were obtained using Quanta FEG250 equipped with energy dispersive spectrophotometer.

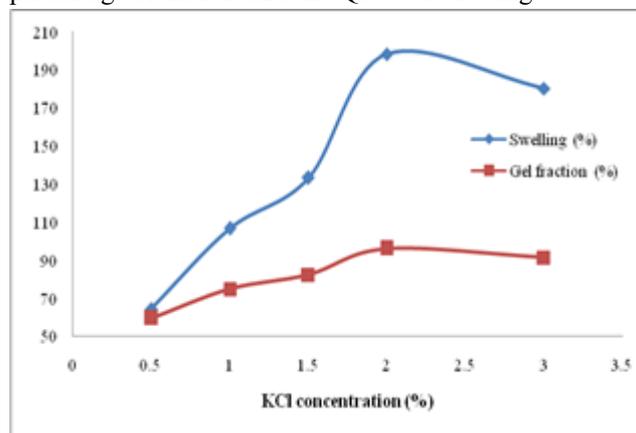
## 3. Results and Discussion

### 3.1. Factors controlling of KC/PEG/K<sup>+</sup>/QNWCF wound dressing preparation.

As reported before, K<sup>+</sup> can crosslink KC [10-13] converting it to strong and brittle gel. Thus, PEG was blended with KC to achieve KC/PEG/K<sup>+</sup>/QNWCF wound dressing with good film properties. Factors affecting such dressing preparation are given below with appropriate discussion.

#### 3.1.1. Potassium chloride concentration

Figure 2: Effect of potassium chloride concentration on swelling and gel fraction percentage of the KC/PEG/K<sup>+</sup>/QNWCF dressing.



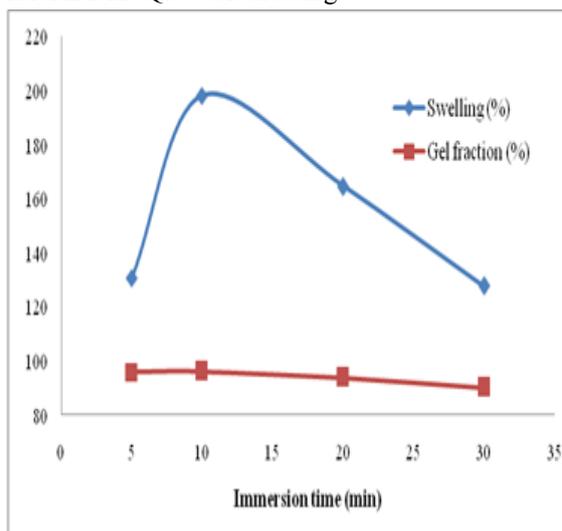
[KC], 1%; PEG/ KC (w/w %), 0.25; immersion time, 10 min; % N of the QNWCF, 0.1937.

Figure 2 depicts the effect of potassium chloride concentration, as a gelling salt, on percent swelling as well as gel fraction of the KC/PEG/K<sup>+</sup>/QNWCF dressing. It is obvious that immersion of KC/PEG/K<sup>+</sup>/QNWCF dressings in potassium chloride solutions of 0.5 to 2% results in a progressive increasing in percent swelling

and gel fraction of the formed dressings suggesting a gradual increasing in crosslinking magnitude of such dressings as a result of increasing of the potassium ions capable to interact with the of KC sulfate groups to form coherent gels [16, 17]. Beyond the potassium concentration of 2 and up to 3%, both the percent swelling as well as gel fraction extents were decreased which can be attributed to the charge screening effect that reduces the ionic bonds by potassium ions and consequently solubilizes the KC/PEG/K<sup>+</sup> matrix, in addition to a breakage of some H-bonds within KC/PEG/K<sup>+</sup>/QNWCF matrix due to the potassium ions concentration increasing [16, 17].

### 3.1.2. Immersion time in potassium chloride aqueous solution

Figure 3: Effect of immersion time on percent swelling and gel fraction of the prepared KC/PEG/K<sup>+</sup>/QNWCF dressing.



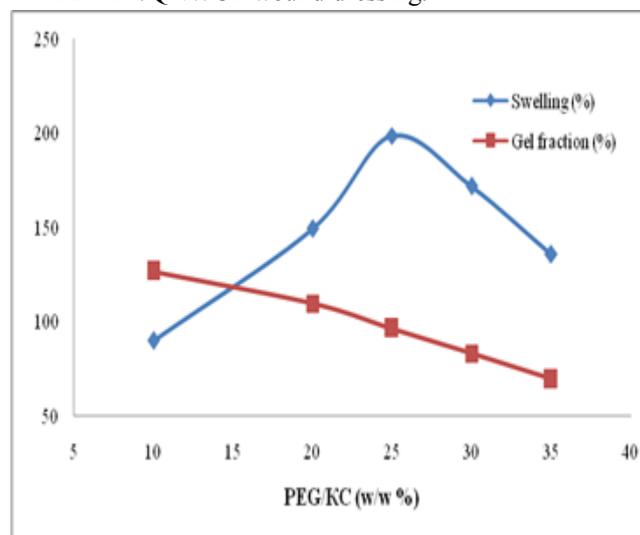
[KC], 1%; PEG/KC (w/w %), 0.25; [KCl], 2%; % N of the NWC,0.1937.

Figure 3 illustrates the percent swelling as well as gel fraction properties of KC/PEG/K<sup>+</sup>/QNWCF dressing as a function in the immersion time. It is obvious that increasing the immersion time of the nominated dressing in 2% KCl solution to 10 min is accompanied by an increasing in both the swelling as well as gel fraction properties extents of the dressing, the matter that can be associated with increasing of the chemical interaction between K<sup>+</sup> ions and KC

sulfate groups and the subsequent increasing in the crosslinking magnitude of such dressing. Further increasing in the immersion time up to 30 min gives rise a progressive decreasing in aforementioned properties which can be explained by a disruption in the H-bonds magnitude inside the dressing structure as a result of the interference with K<sup>+</sup> ions that by time leads to a gradual solubilization of the KC/PEG/K<sup>+</sup> matrix [17].

### 3.1.3. PEG/KC weight ratio

Figure 4: Effect of PEG/KC weight ratio on swelling and gel fraction properties of KC/PEG/K<sup>+</sup>/QNWCF wound dressing.



[KC], 1%; [KCl], 2%; immersion time, 10 min; % N of the NWC,0.1937.

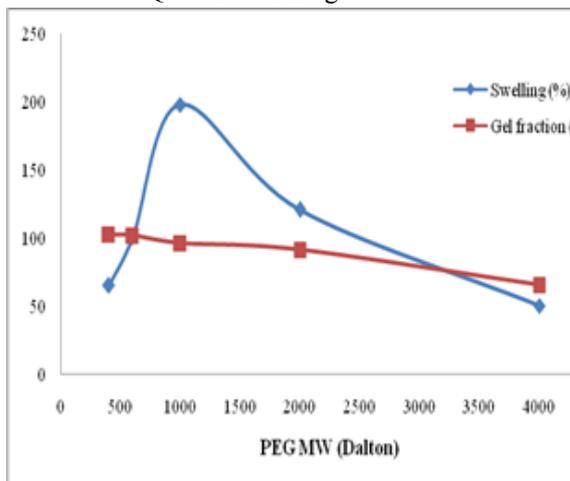
Figure 4 shows the effect of PEG/KC weight ratio on swelling and gel fraction extents of KC/PEG/K<sup>+</sup>/QNWCF dressing. It is clear that increasing of PEG/KC ratio to 25% is accompanied by an increasing in swelling along with a decreasing in gel fraction properties of the prepared dressing reflecting the PEG plasticizing effect that disrupt H-bonds within dressing structure giving rise to higher extent of swelling and reduces the intermolecular interactions resulting in a decreasing in the dressing firmness [31].

Higher weight ratios up to 40% results in a reduction in both the swelling as well as gel

fraction of the dressing suggesting the decreasing in crosslinking extent within the dressing structure that in turn leads to a partial solubilization for the dressing matrix [31-33].

### 3.1.4. PEG molecular weight

Figure 5: Effect of PEG molecular weight on percent swelling and gel fraction of the prepared KC/PEG/K<sup>+</sup>/QNWCF dressing.



[KC], 1%; [KCl], PEG/KC (w/w %), 0.25; 2%; immersion time, 10 min; % N of the NWC, 0.1937.

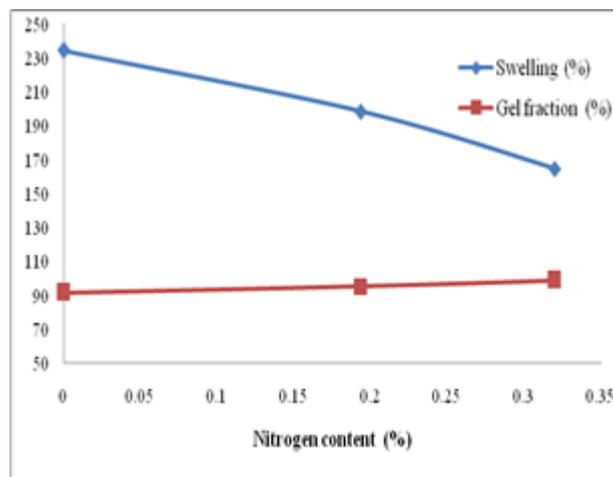
Figure 5 shows the effect of PEG molecular weight on percent swelling and gel fraction extents of KC/PEG/K<sup>+</sup>/QNWCF dressing. The results depicts that increasing of PEG molecular weight from 400 to 1000 Da significantly enhances percent swelling but marginally reduces gel fraction of the formed dressing reflecting the plasticizing effect of such PEG molecular weights [31, 33, 34]. The higher molecular weights up to 4000 Da have a negative impact on the percent swelling and gel fraction of the produced dressing suggesting the reduction in the crosslinking extent inside the dressing matrix and a partial solubility of the dressing matrix [31].

### 3.1.5. Fabric nitrogen content

Figure 6: Effect of fabric nitrogen content on swelling and gel fraction properties of KC/PEG/K<sup>+</sup>/QNWCF dressing.

Figure 6 clarifies the percent nitrogen and gel fraction of KC/PEG/K<sup>+</sup>/QNWCF dressing as a function in the fabric nitrogen content. It is clear that increasing of the percent nitrogen content of

treated fabric gives rise to a reduction in percent swelling accompanied with a little improvement in gel fraction of treated fabric.

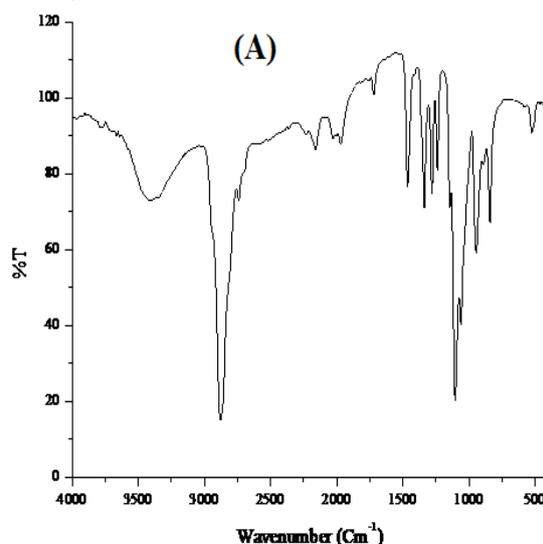


[KC], 2%; PEG/KC (W/W), 0.25; [KCl], 2%; immersion time, 10 min.

The matter that can be interpreted in terms of a formation of ionic bonds between the positively charged quaternized fabric amino groups and the negatively charged KC sulfate groups on a hand and on the other hand, crosslinking of KC with K<sup>+</sup> that in net leads to a closer dressing structure and the subsequent reduction in swellability as well as firmness improving, i.e. increasing of the gel fraction, of the dressing [1].

## 3.2. Characterization of the prepared KC/PEG/K<sup>+</sup>/QNWCF dressing

### 3.2.1. FTIR



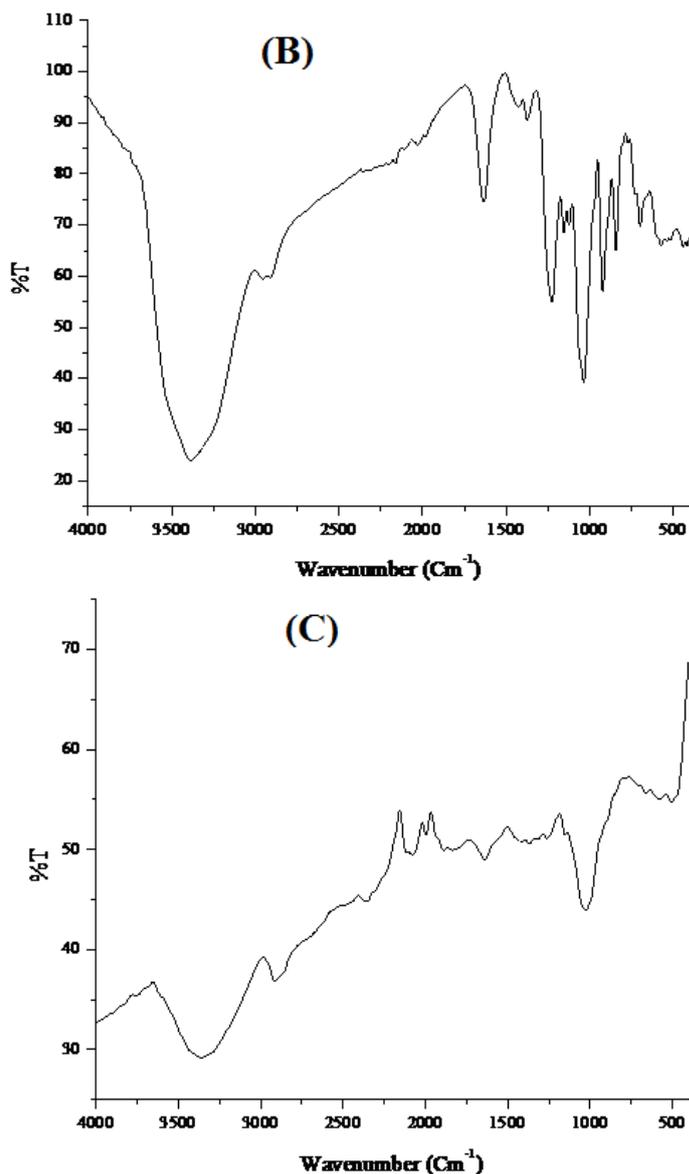


Figure 7: FTIR spectrum of (A) PEG, (B) KC, and (C) KC/PEG/K<sup>+</sup>/QNWCF dressing.

The FTIR spectra of PEG, KC, and KC/PEG/K<sup>+</sup>/QNWCF dressing are represented by Figure 7 (A-C) respectively.

peaks resemble to that of PEG (Figure 7 (A)) that are a broad peak at 3372 cm<sup>-1</sup> assigned to OH group, a peak around 2848 cm<sup>-1</sup> corresponding to methylene group, a peak at 1443 cm<sup>-1</sup> due to binding vibration of -CH<sub>2</sub>, and a peak at 1110 cm<sup>-1</sup> for C-O-C stretching.

peaks resemble to that of KC (Figure 7 (B)) that are a broad peak at 3335cm<sup>-1</sup> due to OH stretching vibrations as well as three peaks at 835, 911 and 1232 cm<sup>-1</sup> corresponding to a d-

galactose-4-sulfate, 3,6-anhydridegalactose and an ester sulfate stretching vibrations respectively. It is clear that the KC/PEG/K<sup>+</sup>/QNWCF dressing spectrum (Figure 7 (C)) has the characteristics peaks of PEG and KC with some shifts due to the interaction between PEG and KC in presence of K<sup>+</sup> ions.

### 3.2.2. SEM image of KC/PEG/K<sup>+</sup>/QNWCF wound dressing

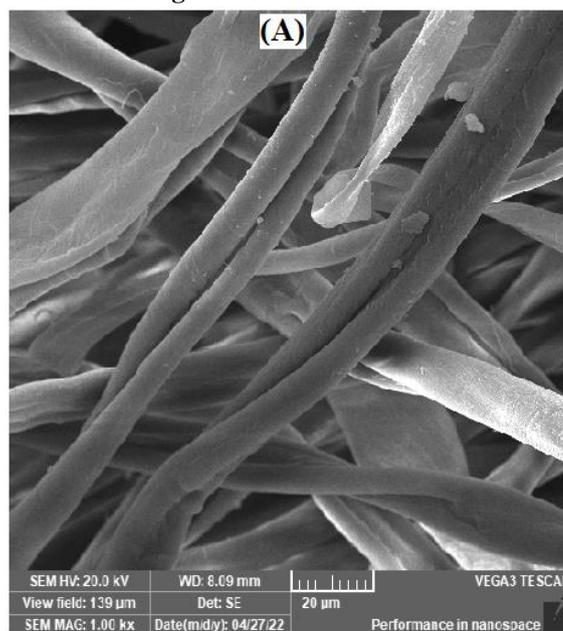
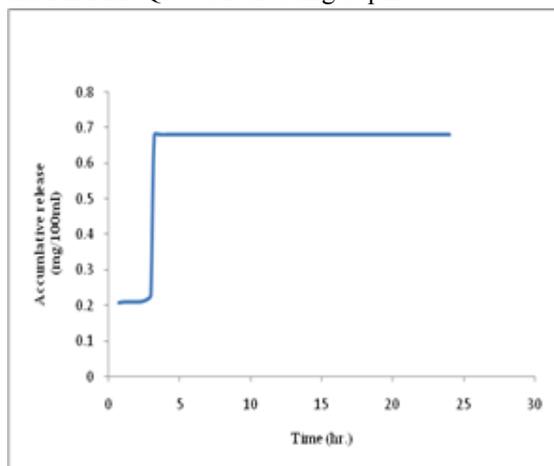


Figure 8: SEM images of (A) an untreated NWCF sample and (B) KC/PEG/K<sup>+</sup>/QNWCF dressing.

The SEM images of (A) an untreated NWCF sample and (B) KC/PEG/K<sup>+</sup>/QNWCF dressing are shown in Figure 8. It is well seen that there is a coating layer of the crosslinked KC/PEG blend on the KC/PEG/K<sup>+</sup>/QNWCF dressing surface compared to the untreated NWCF sample which confirms clearly the KC/PEG/K<sup>+</sup>/QNWCF dressing structure.

### 3.2.3. In vitro Releasing of sulfadiazine

Figure 9: Releasing of SD from KC/PEG/K<sup>+</sup>/QNWCF dressing at pH 7



[KC], 1%; PEG/KC (W/W), 0.25; [KCl], 2%; immersion time, 10 min.

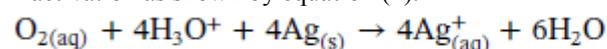
Figure 9 shows the releasing of sulfadiazine from the loaded KC/PEG/K<sup>+</sup>/QNWCF dressing at pH 7. It is clear that the KC/PEG/K<sup>+</sup>/QNWCF dressing chemical structure has the ability to bind as well as effectively release sulfadiazine and such releasing reaches a constant amount after approximately 3 hours [2].

### 3.2.4. Antibacterial properties of the KC/PEG/K<sup>+</sup>/QNWCF dressing

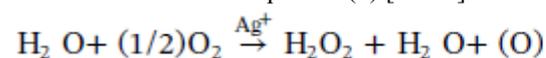
Table 2: Antibacterial properties of the KC/PEG/K<sup>+</sup>/QNWCF dressing.

Treatment type	ZI (mm)	
	G+ve	G-ve
Untreated	0	0
KC/PEG/K <sup>+</sup> /QNWCF dressing loaded with 1% SD	8	5
KC/PEG/K <sup>+</sup> /QNWCF dressing loaded with 1% SD and 2% Ag-NPs	18	17

The antibacterial properties of the KC/PEG/K<sup>+</sup>/QNWCF dressing loaded with SD alone or in combination with Ag-NPs is shown in Table 2. It is clear that introducing of SD into the dressing matrix results in a significant improvement in the antibacterial properties of such dressing while combining of Ag-NPs with SD in that dressing matrix remarkably enhances the dressing antibacterial properties reflecting: i) the SD harmful effect for bacteria through inhibition of the bacterial folic acid synthesis [2,34], and ii) Ag-NPs destroying effect on bacteria the bacterial membrane through formation of Ag ions, in presence of moisture, that in turn bind to the bacterial DNA causing its inactivation as shown by equation (1):



and/or formation of oxygen radicals that consequently oxidize the molecular structure of bacteria as clear from equation (2) [35-43]:



### Conclusions

The proper conditions to prepare KC/PEG/K<sup>+</sup>/QNWCF dressing are: PEG/KC weight ratio, 0.25%; K<sup>+</sup> padding bath solution concentration, 1%; and immersion time, 10 min.

The chemical structure of the prepared dressing was confirmed via FTIR.

The nominated dressing morphology was investigated with SEM.

The in vitro releasing of SD from the prepared loaded dressing at pH 7 reached a constant value after 3 hours.

The SD loaded dressing exhibits antibacterial properties against both Gram-positive and Gram-negative bacteria.

Loading such dressing with SD in combination of Ag-NPs imparts the dressing with remarkable antibacterial properties.

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