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Fabrication of bioactive wound dressing containing carbopol, hyaluronic acid and Na-alginate

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Abstract

Novel wound dressings were prepared by treating non-woven cotton fabric samples with different formulations of carbopol (CP), hyaluronic acid (HA), and sodium alginate (HA) followed by crosslinking with Ca^{2+}, Zn^{2+} or Cu^{2+} ions. The impacts of such formulations on the antibacterial besides, some physico-mechanical properties of such dressings were studied. Loading the crosslinked CP/HA/SA dressing with sodium diclofenac and its in-vitro releasing from that dressing at pH 7 was investigated. To improve the antibacterial activities of such dressing, silver nano-particles were incorporated as a bioactive agent in the dressing formulation. The CP/HA/SA crosslinked film was characterized via FTIR analysis whereas the CP/HA/SA dressing SEM and EDX images were investigated.

Keywords: Wound dressing; Carbopol; Hyaluronic acid; Sodium alginate; Metal ions

1. Introduction

Exposure of the human anatomical tissues to a physical or thermal factor may lead to a disruption in continuity of that tissue causing a wound. Healing of a wound is an energetic process of tissue regeneration as well as growth progress. The wound healing progresses through four phases called haemostasis, inflammation, proliferation, and remodeling [1,2]. Usually, the wound healing needs to a convenient dressing material to protect the wound from the external factors. Many wound dressing materials types are at the hand in the wound care market. Upon the nature of action, the wound dressings are classified into bioactive, interactive, and passive dressings. The former are consisted of bio-materials possessing healing activities. They are prepared commonly from biocompatible, biodegradable, as well as non-toxic polymers, natural or synthetic, such as chitosan, collagen, hyaluronic acid, alginates, polypropylene, and polyester [2,3].

Carbopol® is a polyacrylic acid exhibits a sol-to-gel transition when the pH of its aqueous solution is raised to above pK ca 5.5 [4,5]. Stimuli-responsive hydrogels can be prepared from Carbopol polymers. Such hydrogels exhibit changes in their swelling behavior upon exposure to external stimuli like pH, temperature, light or electric field [6-8]. Currently, carbopol is highly candidate for the controlled drug-delivery systems because of its ability to deliver a drug to a specific area of the body. The greater swelling of carbopol in alkaline pHs renders it to deliver the maximum drug in an alkaline medium [9].

Hyaluronic acid is a linear polysaccharide of high molecular weight and composed of N-acetylglucosamine and glucuronic acid repeats. As a bio-polymer, hyaluronic acid is one of the most hydrophilic polymers in nature. Owing to the hyaluronic acid characteristics such as the viscoelastic nature, biocompatibility as well as the non-immunogenicity, it is widely used in many medical applications like supplementation of joint fluid, tissue repairing and wound healing [10-12].

Alginate is considered as polysaccharide pertaining hydrophilic character and extracted from the marine brown algae, it is composed of linear chains of structure namely α-L-guluronic acid and β-

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D-mannuronic acid linked together by 1,4-glucosidic linkages [10,13,14]. It has non-toxicity, biocompatibility, moisture retention, biodegradability, flexibility, permeability, and excellent film-forming properties, the matter that renders it convenient for the hydrocolloid wound dressings fabrication.

Both the alginate and hyaluronic acid can be crosslinked via divalent cations such as Ca^{2+}, Zn^{2+} and Cu^{2+} forming three-dimensional polymeric networks (hydrogels) [10,13-15]. Their based hydrogels have the ability to retain a moist medium around the wound which practically accelerate the wound healing. Besides, they act as a drug vehicle in the drug delivery systems [10,16-18].

In that work, different wound dressings were prepared via treating non-woven cotton fabric samples with different formulations containing carbopol, sodium alginate, and hyaluronic acid followed by crosslinking by means of the Ca^{2+}, Zn^{2+} or Cu^{2+}ions. The impact of such formulations on some performance and antibacterial characteristics of such dressings was discussed.

2. Experimental

2.1. Materials

Cotton fabric (100%)(Non-woven) (NWC fabric), Hebitex Co., Egypt, was used. Sodium salt of hyaluronic acid (HA) of an average molecular weight $1.48*10^6$ Da, Euromededx,France, was used. Sodium alginate (SA), Sigma-Aldrich, USA, was used. Carbopol 934 (CP), purchased from China. Sodium diclofenac (SD) is of pharmaceutical grade, China. Silver nitrate, copper chloride, tri-sodium citrate, calcium chloride, acetic acid, $ZnCl_2$, and nitric acid were availble in the lab.

2.2. Methods

2.2.1. CP/HA/SA dressings preparation

All solutions of CP, SA or HAwere arranged by dissolving certain polymer masses in distilled water with stirring at 80 °C/45 min [2,10,19,20]. Different formulations of these polymers were prepared by blending different volumes of these polymers' solutions, keeping the net concentration of blends solutions at 2%. Non-woven cotton fabric (NWC fabric) wound dressings were prepared by padding NWC fabric samples in solutions of the previous formulations, squeezing and drying at 80 ^{O}C/5 min. The dried samples were then immersed for interval times (1-7 min) into a particular divalent metal ion aqueous solution of a specific concentration (0.5 – 2%) to crosslink the deposited polymers followed by washing using dis. H_2O to eliminate the excess metal cation and finally dried at 80 ^{O}C/5 min. The treated fabric samples are then stored in a desiccator at 52%humidity for 24 h for analysis.

2.2.2. The preparation of Ag-NPs

Ag-NPs was performed by the method mentioned elsewhere [21].

2.2.3. Production of CP/HA/SA dressings containing sodium diclofenac and/or silver nanoparticles

The NWC fabric-based CP/HA/SA dressings containing sodium diclofenac (SD) and/or Ag-NPs were prepared by adding 1% of sodium diclofenac and/or 2% of the Ag-NPs to the nominated formulation solution followed by padding the NWC fabric in that solution, squeezing and drying in a same procedure mentioned in part 2.2.1.

2.2.4. The *In-Vitro* releasing of sodium diclofenac

Sodium diclofenacis released *in vitro* from the crosslinked CP/HA/SA dressing by mixing of a specific weight of the dressing to a 100 ml glass bottle filled with buffer solution at pH 7.0. Afterwards, 2ml were withdrawn from the above solution every interval of time and the sodium diclofenac released was assessed via"PG-T80", "UV/Visible Spectrophotometer". Equal volumes of buffered solutions were sited into the cells subsequently to each trial to preserve the medium volume constant [22,23].

2.3. Characterization of the fabricated wound dressing

- The percent swelling (% SW) was assessed using the following equation: SW (%) = (Wa – Wi)/Wi×100, (where Wa is the dressing wetted weight and Wi is the dressing initial weight) [19,20,24].

- The percent gel fraction (% GF) was assessed using the subsequent equation:

GF (%) = (Wa/Wi)×100, where Wi is the dressing initial weight and Wa is the dressing dry weight [19,20].

- The tensile strength of the dressing (TS) was assessed conferring to ASTM standard way D882.
- The air permeability of the dressing (AP) was assessed according to ATSM (D 737-96).
- The antibacterial activities of the dressing were assessed by the bacterial count method against Staphylococcus aureus (*SA*) as Gram-positive bacteria and Escherichia coli (*EC*) as Gram-negative bacteria [19,20].
- "SEMas well as EDX images of $CaCl_2$, $CuCl_2$ and $ZnCl_2$ crosslinked samples were inspected via "scanning electron microscope; JEOL, JXA-840A Electron Probe Microanalyzer Japan" armed with an "energy dispersive X-ray system; INCAX-Sight–England" for the elemental investigation.

3. Results and discussion

3.1. Factors affecting CP/HA/SA dressing formation

3.1.1. Na-alginate weight ratio

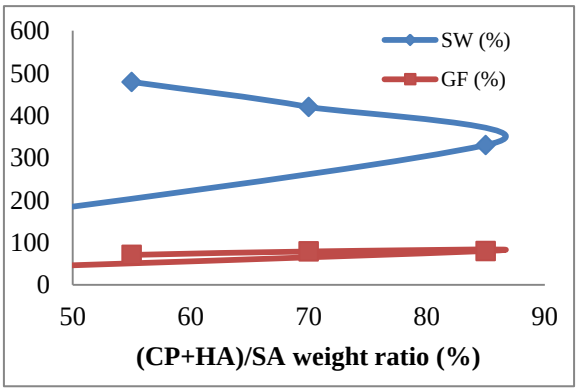

Figure1: Effect of Na-alginate weight ratio on percent swelling and gel fraction of CP/HA/SA dressing. Total polymers concentration, 2%; CP/HA weight ratio, 1; $CaCl_2$ concentration, 2%; immersion time, 5 min.

As far as the changes in percent swelling and gel fraction of CP/HA/SA dressing as a function of SA weight ratio in that dressing, Figure 1 demonstrates that decreasing of SA weight ratio from 55 to 85%, keeping the total polymer concentration at 2%, results in a significant enhancement in gel fraction along with a gradual decreasing in percent swelling of that dressing. The matter that can be considered as a direct consequence for increasing of such dressing crosslinking with the Ca^{2+}cations and the subsequent decreasing in that dressing solubility [25]. However, the alteration in extent of such properties is governed by the ionic interactions among the Ca^{2+} and the carboxylate groups of CP, HA and/or guluronate component of SA [10,26].

3.1.2. Immersion time

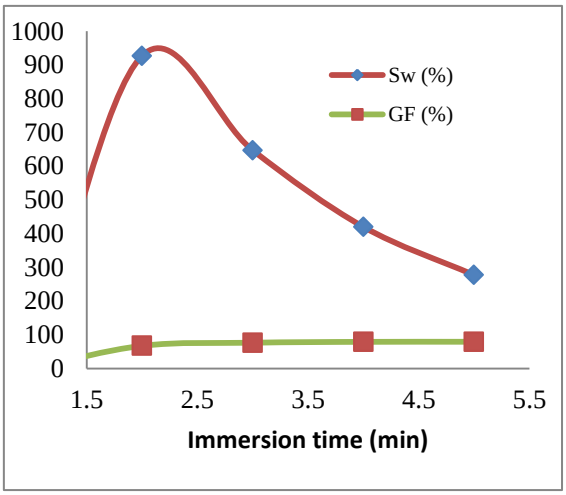

Figure 2: Effect of immersion time on percent swelling and gel fraction of CP/HA/SA dressing. CP/HA/SA weight ratio, 0.3/0.3/1.4; $CaCl_2$ concentration, 2%.

Figure 2 clearly shows the swelling degree as well as gel fraction properties of the prepared CP/HA/SA dressing as a function in the immersion time of that matrix in 2% calcium chloride aqueous solution. It is well seen that increasing of the steeping time gives rise to an enhancement in gel fraction accompanied with a decreasing in swelling degree of that dressing. This outcome can be attributed to increasing of the chemical interaction between Ca^{2+} ions and carboxylic groups of the matrix components by time, and the subsequent enhancement in extent of cross-linking of the CP/HA/SA dressing. Longer immersion time up to 7

min results in a dressing having lower swelling degree and higher gel fraction. It seems that 5 min is the proper immersion time from the percent swelling and gel fraction points of view [19,27-29].

Bakar A A, Ghazali C and Amin K M 2018 Sodium Alginate/Ageratum Conyzoides Extract Film for Wound Dressing Materials IOP Conf. Ser. Mater. Sci. Eng.374 012087
[6] RashidzadehA, Olad A, Salari D and Reyhanitabar A 2014 On the preparation and swelling properties of hydrogel nanocomposite based on sodium alginate-g-poly (acrylic acid-co-acrylamide)/clinoptilolite and its application as slow release fertilizer J. Polym. Res.21 2 344
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3.1.3. Calcium chloride Concentration

Figure 3 illustrates the impact of the $CaCl_2$ solution concentration on percent swelling and gel fraction of the CP/HA/SA dressing. It is obvious that increasing of the $CaCl_2$ concentration form 0.5 to 2% results in a crosslinked CP/HA/SA dressing having progressive increasing in the gel fraction and decreasing in the swelling degree.

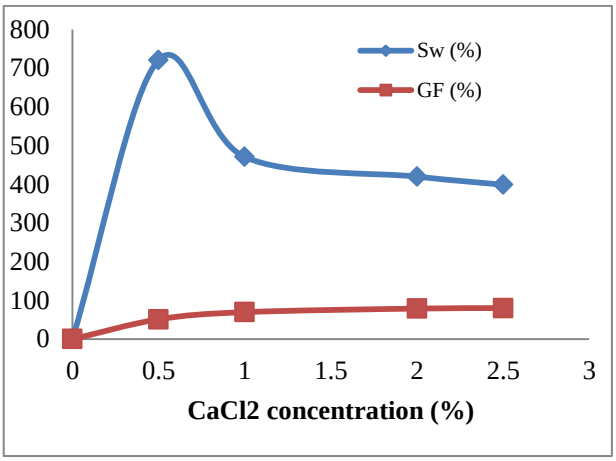

Figure 3: Effect of calcium chloride concentration on percent swelling and gel fraction of CP/HA/SA dressing. CP/HA/SA weight ratio, 0.3/0.3/1.4; immersion time, 5 min.

The matter that may be associated with the enhancement in the interaction probabilities of such matrix's carboxyl groups with Ca^{+} ions which subsequently enhance the extent of crosslinking of such dressing. The further increasing in $CaCl_2$ concentration up to 2.5%, gives rise to higher degrees in the aforementioned properties magnitudes. It seems that the concentration of 2% $CaCl_2$ solution is the appropriate concentration to crosslink the nominated CP/HA/SA dressing [19,27-29].

3.1.4. Metal cation type

Table 1 shows the impact of using $CaCl_2$, $ZnCl_2$ and $CuCl_2$ as ionic crosslinkers on some performance and anti-bacterial properties of the CP/HA/SA dressing. Table 1 clearly reveals that:

- the percent swelling decreases in the order: $Ca^{2+} > Cu^{2+} > Zn^{2+}$
- the gel fraction decreases in the order: $Zn^{2+} > Cu^{2+} > Ca^{2+}$

Table 1: Effect of the metal ion type on the performance and antibacterial characteristics of the CP/HA/SA dressing.

Metal ion type	Sw (%)	GF (%)	TS (Kg)	AP $Cm^3/ Cm^2.S$	% Reduction			
					Without Ag-NPs		With 1% Ag-NPs	
					G+ve	G−ve	G+ve	G−ve
Ca^{2+}	420	78.9	47.2	41	43.1	25.9	95.3	91.4
Zn^{2+}	369	82.3	49.5	38	72.3	53.4	100	99.1
Cu^{2+}	396	79.5	48.1	39	82.4	60.6	100	100

CP/HA/SA weight ratio, 0.3/0.3/1.4; metal ion concentration, 2%; immersion time, 5 min.

- the air permeability decreases in the order:
  Ca^{2+}> Cu^{2+} > Zn^{2+}
- the tensile strength, in the warp direction, decreases in the order:
  Zn^{2+}> Cu^{2+} > Ca^{2+}, and
- The antibacterial activity decreases in the order:
  Cu^{2+}> Zn^{2+} > Ca^{2+}, keeping other parameters constant.

The alteration in extents of nominated properties is a direct consequence for: i) the differences among such cations in their ionic radius which meaningly influence the extent of the ionic interface between that metal cations and the CP, HA, and SA active sites that sequentially, verifies the crosslinking density of the CP/HA/SA matrix, ii) number and accessibility of the CP, HA, and SA active sites which significantly distress the magnitude of crosslinking of the CP/HA/SA dressing, and iii) distribution and location of the metals cations onto and/or within the CP/HA/SA dressing [11,12,15,26,30–32].

Furthermore, the antibacterial characteristics of the CP/HA/SA dressing against the Gram +ve and Gram −ve bacteria is significantly influenced by the difference in antibacterial activities of the nominated metal cations [26] beside the HA antibacterial activity as a component in the CP/HA/SA matrix [2,10,20]. Needless to say, that the higher magnitude of crosslinking leads to lower diffusion of the bio-active ingredients that thereby reduces the antibacterial activity of the dressing [22]. Moreover, incorporation of the Ag-NPs in the CP/HA/SA dressing indeed will enhance the antibacterial activity of the dressing against the bacterial cells via formation of Ag^+ ions, in the presence of moisture, that then attached to the bacterial DNA and causes its inactivation (Eq. 1), and/or generating of oxygen radicals which oxidize the bacterial molecular structure (Eq. 2) [19,33-35].

:

$$O_{2(aq)} + 4H_3O^+ + 4Ag_{(s)} \rightarrow 4Ag^+{}_{(aq)} + 6H_2O \quad (1)$$

$$H_2O + (1/2)O_2 \xrightarrow{Ag^+} H_2O_2 \rightarrow H_2O + (O) \quad (2)$$

1.2. In vitro releasing of Na-diclofenac

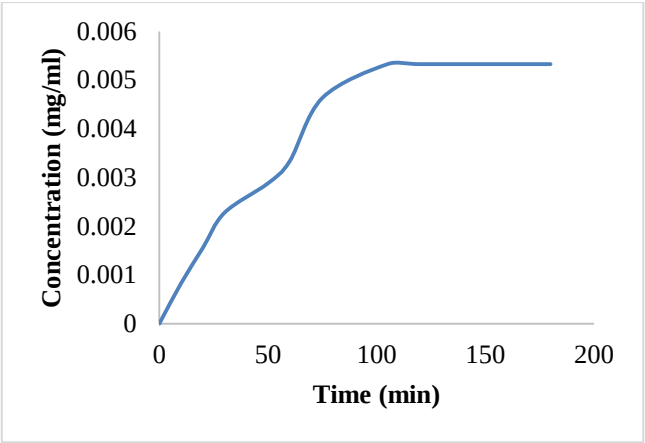

Figure 4: Releasing of Na-diclofenac from the CP/HA/SA dressing.

Figure 4 shows the releasing of Na-diclofenac from the loaded the CP/HA/SA dressing at pH 7. It is well seen that the drug releasing reached a fixed value after about 105 min reflecting the CP/HA/SA matrix opened – structure [22,36].

1.3. Characterization of the crosslinked CP/HA/SA film

1.3.1. FTIR

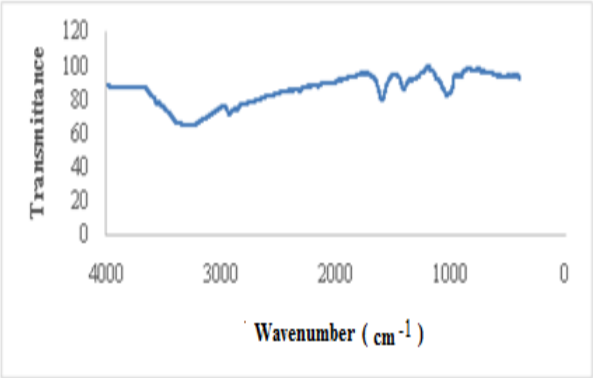

Figure 5: FTIR spectrum of sodium alginate.

Figure 5 shows the sodium alginate FTIR spectrum. It includes a broad band 3255-3425 Cm^{-1} corresponding to H-bonding of -OH groups, a band at 2932 cm^{-1} of corresponding to stretching vibration of -CH, a band at 1055–1084 cm^{-1} of corresponding to the C-O-C (cyclic ether) stretching vibration, two peaks at 1616 cm^{-1} assigned to $-COO^-$ groups stretching vibrations [22,37,38].

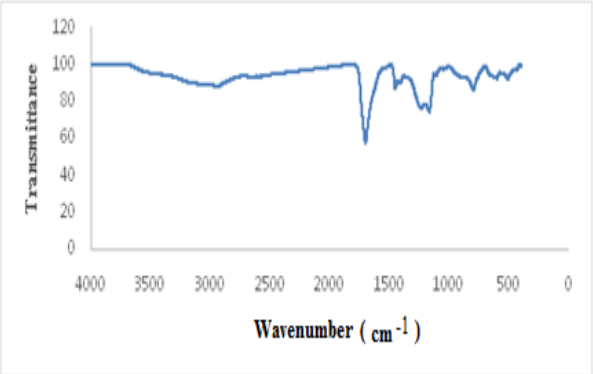

Figure 6: FTIR spectrum of carbopol 934.

Figure 6 illustrates FTIR spectrum of carbopol 934. It is obvious that spectrum includes a peak at 2965 cm^{-1}, corresponding to -OH stretching vibration, a peak at 1695 and cm^{-1}corresponding to -C=O stretching vibration, a peak at 1436 cm^{-1} corresponding to C-O, a band at 1222 cm^{-1}corresponding to C-O-C of acrylates, and a peak at 1101 cm^{-1}assigned to the C-O-C group stretching vibration [39,40].

Figure 7: FTIR spectrum of hyaluronic acid.

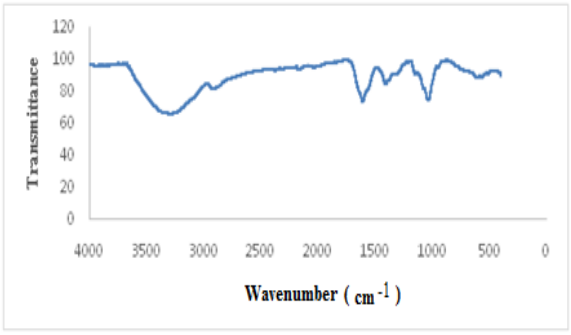

Figure 7 depicts FTIR spectrum of hyaluronic acid sodium salt. It includes a broad stretching band of hydroxyl groups at 3435 Cm^{-1}, a band at 2979 Cm^{-1} assigned to the –CH groups stretching vibration, a band at 1621 Cm^{-1} characteristic to the carbonyl group of carboxyl group and amide I, a band at 1565 Cm^{-1} corresponding to the amide II stretching vibration, a band at 1050 Cm^{-1} corresponding to the C-O-C group [41,42,43].

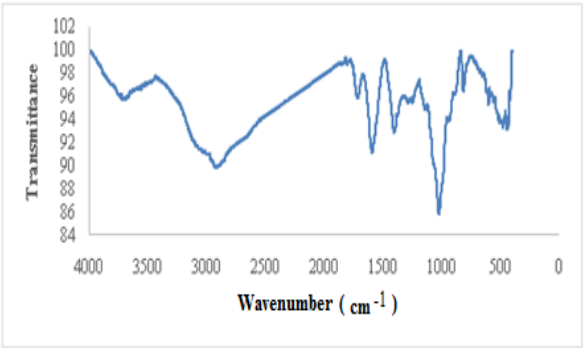

Figure 8: FTIR spectrum of the crosslinked CP/HA/SA film.

Figure 8 illustrates the FTIR spectrum of the crosslinked CP/HA/SA film. It is clear that the spectrum comprises peaks belonging to the aforementioned bio-polymers such as the peak at 3691 Cm^{-1}characteristic to the hydroxyl groups stretching vibration, a band at 2912 Cm^{-1} assigned to –CH groups stretching vibration, a peak at 1700 Cm^{-1} characteristic to the carbonyl group of carboxyl group and amide I, a peak at 1583 Cm^{-1} corresponding to the amide II stretching vibration, a band at 1124 Cm^{-1} characteristic to C-O-C group of the acrylates.

1.3.2. SEM and EDX analysis

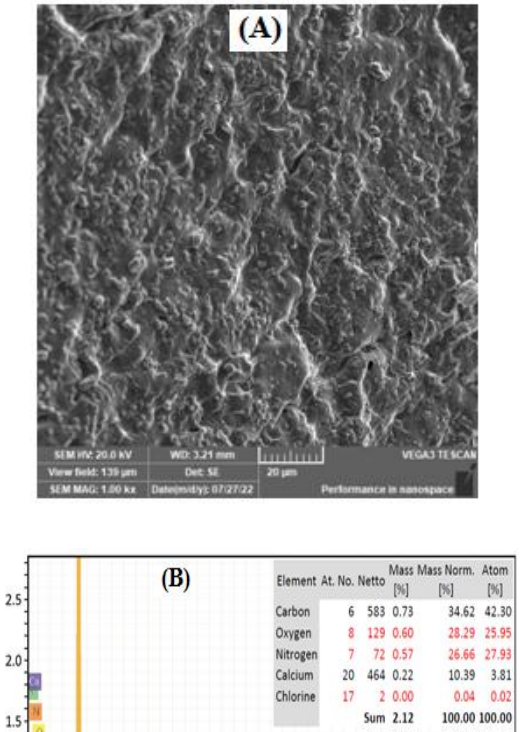

Figure 9: (a) SEM of the CP/HA/SA dressing crosslinked with $CaCl_2$, and (b) EDX spectra of the CP/HA/SA dressing crosslinked with $CaCl_2$.

The SEM and EDX images of the CP/HA/SA dressing are represented by Figure 9 (A and B). Figure 9 (A) depicts that the dressing has homogeneous surface without any cracks whereas Figure 9 (B) confirms loading of such dressing with Ca^{2+} as the dressing was crosslinked with $CaCl_2$.

1.4. Conclusion

- Novel CP/HA/SA wound dressings were prepared by treating non-woven cotton fabric with different formulations of CP, HA, and SA followed by crosslinking by the Ca^{2+}, Zn^{2+}, or Cu^{2+}ions.
- The prepared dressings have significant physico-mechanical as well as antibacterial characteristics.
- Inclusion of Ag-NPs as a bioactive agent into the CP/HA/SA dressing formulation remarkably promoted the antibacterial activities of such dressing.
- The aforementioned dressing can be loaded and release sodium diclofenac at pH 7.
- The SEM image indicted that the prepared CP/HA/SA dressing having homogeneous structure without any cracks whereas the EDX image confirmed crosslinking of the dressing with $CaCl_2$.

Conflict of interest: There is no conflict of interest.

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