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Fabrication of bio-active wound dressings containing carbopol blends and chitosan

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Abstract

The wound dressing is an essential material tailored for protecting a wound from the environmental conditions as well as healing of that wound. Actually, many dressings are present in the medical market. This research work aims to formulate different wound dressings via treating non-woven cotton fabric samples with different blends of carbopol® (CP) with arabic gum (AG), sodium alginate (ALG), or carboxymethyl cellulose (CMC) as a polyanionic layer followed by crosslinking with chitosan (CS) as a polycationic layer. Factors affecting building up of the CP/AG/CS dressings such as AG/CP weight ratio, CS concentration, coating layers number, and dressing fomulation type were investigated. The results revealed that the best performance properties, i.e. percent swelling, air permeability, stiffness, and tensile strength, of the prepared CP/AG/CS dressing were achieved at weight ratio 25/75 of CP/AG respectively and 2% of CS. Moreover, increasing number of the coating layers positively affects swellability as well as tensile strength but negatively reduces the air permeability of such dressing. Among the CP/AG, CP/ALG, and CP/CMC blends, the later was the best blend from the performance properties point of view. Furthermore, to enhance the antibacterial properties of the prepared CP/CMC/CS dressing, silver nano-particles were added during its preparation. The CP/CMC/CS dressing was characterized via SEM and EDX analysis.

Keywords: Wound dressing; Carbopol; Chitosan; Arabic gum; Sodium alginate; CMC.

1. Introduction

A wound can be defined as disintegration of the skin epithelial lining due to a thermal or physical reason. Wound dressings materials are applied for healing and protecting wounds from infection as well as preventing the further injury. They are obtainable in several types and forms; each having its own benefits and purposes. There are diverse types of dressing materials in the wound care market. The suitable dressing for a wound is important to realize faster healing. The dressings are classified due to their action to passive products, interactive products and bioactive products. The latter, are prepared from natural or synthetic bio-polymers that have healing properties like chitosan, alginate and hyaluronic acid. Sometimes growth factors and antimicrobials are incorporated to the dressing to enhance the wound healing process [1-10]. The prim function of wound dressings is to impart moist medium for wound which facilitates the healthy cells growth that in turn accelerates the wound healing [1-10].

Carbopol® (CP) is a water soluble poly acrylic acid, used as a suspending, stabilising, thickening and gelling agent in many products. Carbopol® is available in different grades, which renders it to be used in preparation of cosmetics, lotions and creams, detergents, as well as air fresheners [11-16]. Fahmy et al. prepared new wound dressings using non-woven cotton fabrics treated with formulations containing carbopol, hyaluronic acid, and sodium alginate then crosslinked with Ca^{2+}, Cu^{2+} or Zn^{2+} ions [17].

Chitosan (CS) is a natural occurring biopolymer derived from chitin which is a main component of outer skeletons of the crustacean. Chitosan is well known in the wound management domain by its haemostatic properties. It possesses antimicrobial activities and helps in fasting of wounds healing. In addition, it has the adequacy to stimulate the cell proliferation as well as the histoarchitectural tissue organization [18-20].

Arabic gum (AG) is the natural exudate obtained from the Acacia trees, Family: Fabaceae. Arabic gum is considered as a dietary fiber with a high percentage of carbohydrates and low protein content. It has pharmacological and medical effects like antihypertensive, anti-hyperlipidemic, anti-diabetic, nephroprotective, anti-inflammatory, anti-coagulant, and many other effects [21]. Singh et al reported the

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importance of gums for wound care by using AG in designing hydrogel wound dressing containing AG [22]. Ngece et al prepared a wound dressing sponges containing alginate/arabic gum/carbopol® crossinked with $CaCl_2$. The sponges prepared were loaded with a mixture of norfloxacin and ampicillin [23].

Sodium alginate (ALG) is a hydrophilic polysaccharide has anionic properties and used for fabrication of hydrocolloid wound dressings because of its biocompatibility, biodegradation as well as excellent film forming properties[7]. The hydrogels based on alginates promote the wounds healing due to their retaining of a moist environment throughout the wounds. In addition, ALG can be exploited for loading and releasing drugs in the drug delivery systems [5].

Carboxymethyl cellulose (CMC) is a promising material for preparing wound dressingsbecause of their noble characteristics like the biodegradability, biocompatibility, hydrophilicity, non-toxicity, tissue resembling, and low cost [24,25].

This research work aims to formulate different wound dressings via treating of non-woven cotton fabric samples with different polymers blends containing CP, AG, ALG, and CMC followed by crosslinking the treated samples with CS as a polycations crosslinker. Moreover, silver nano-particles, as antibacterial agent, will be added to any of the above mentioned blends to enhance the antibacterial properties of the prepared dressings. The prepared dressings will be characterized via SEM and EDX analysis and some of their performance as well as antibacterial properties will be also studied.

1. Experimental

2.1. Materials

Non woven fabric of cotton (NWC fabric) of Hebitex Company, Egypt, was used. Chitosan (CS) of high molecular weight (Fluca Co.), sodium alginate (ALG) (Sigma-Aldrich), carboxymethyl cellulose (CMC) (Fluca Co.), arabic gum (AG) (Alpha chemiKa Co.), and carbopol® 934 (CP), purchased from China, were used. Silver nitrate and sdium hydroxide are used chemicals of a laboratory grade.

2.2. Methods

2.2.1. The CP/AG/CS dressing preparation

The preparation of the CP, AG, CMC, ALG, and CS solutions were performed by dissolving specific weights of such polymers in distilled water at 80 °C/45 min with stirring to prepare (2%) of each of such bio-polymers, except chitosan (0.5-2%). Formulations of different ratios of such polymers solutions were prepared with keeping in mind that the net concentration of such polymers in their blends solutions is 2%. Moreover, the CP/AG/CS dressing was prepared through padding NWC fabric in the CP/AG blend solution then squeezed, and dried at 80 °C/5 min. The dried fabric was then crosslinked by padding in CS solution followed by squeezing and then drying at 80 °C/5 min.

2.2.2. The preparation of silver nano-particles (Ag-NPs)

The silver nano-particles (Ag-NPs) were synthesized using CMC as reducing agent and silver nitrate as a precursor [26].

2.2.3. Preparation of CP/AG/CS dressing containing Ag-NPs

The CB/AG/CS dressing containing Ag-NPs was prepared by padding NWC fabric in the above mentioned CP/AG solution formulations containing 1% Ag-NPs followed by the procedure that mentioned in section 2.2.1.

2.3. Characterization of the fabricated wound dressing

- The percent swelling (SW %) was evaluated through the following equation:
  SW (%) = (Wa –Wi)/Wi×100, (where Wa is the wetted dressing weight and Wi is the dressing initial weight) [4,7,8,10].
- The tensile strength of the dressing (TS) was evaluated conferring to ASTM standard way D882.
- The air permeability of the dressing (AP) was assessed according to ATSM (D 737-96).
- The stiffness (S) was assessed due to ASTM Test Method D 1388-96.
- The antibacterial activities was assessed by the bacterial count method against Gram-positive bacteria (*Staphylococcus aureus, SA*) and Gram-negative bacteria (*Escherichia coli, EC*) [25,26].
- SEM as well as EDX images of untreated and the prepared dressing 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.

2. Results and Discussion

3.1. Factors affecting CP/AG/CS dressing formation

3.1.1. Carbapole to arabic gum weight ratio

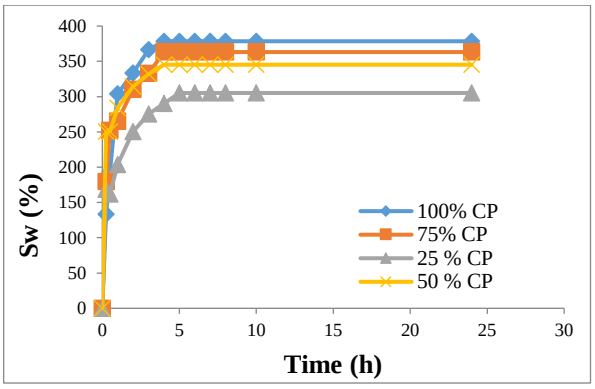

Figure 1: Effect of carbapole to arabic gum weight ratio on percent swelling of the CP/AG/CS dressing. [CS], 2%; [CP], 2%; [AG], 2%; No. of layers, 2.

Table 1: Effect of carbopole to arabic gum weightratio on some performance properties of the CP/AG/CSdressing.[CS], 1%; [CP], 2%; [AG], 2%; No. of layers, 2

CB/AG ratio	TS (Kg)	S (mg)	AP $cm^3/cm^2.sec$
100/0	14	4575	183.7
75/25	16	3924	171.8
50/50	17	4350	166.4
25/75	19	4470	157.2

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Figure 1 shows the impact of CP/AG weight ratio on percent swelling of the CP/AG/CS dressing. It is well seen that decreasing of CP ratio as a component in building up of such dressing from 100 to 25% brings about in a decreasing in the percent swelling of the dressing reflecting of the increasing in the intermolecular interactions between CS as a polycationic layer and the CP/AG blend as a polyanionic layer [4,17,27-31]. However, the percent swelling of the aforementioned CP/AG ratios can be arranged as follows: 100/0 > 75/25 >50/50 >25/75, respectively.

On the other hand, Table 1 shows the effect of CP to AG ratio on some performance properties such as tensile strength, stiffness, as well as air permeability, of the prepared CP/AG/CS dressing. It is clear that decreasing of CP ratio in the CP/AG/CS dressing formulation gives rise to: i) an enhancement in tensile strength of such dressing, ii) a decreasing of stiffness of the prepared dressing, and iii) a reduction in the air permeability at the ratio of 75/25 that gradually increases again but still lower than that of the CP without blending, compared to the CP without blending. The matter that reflects the differences among such formulations in the CP content which indeed affects the intermolecular interactions between CS as a polycationic and such blend as a polyanionic layers [4,17,28-31].

3.1.2. Chitosan concentration

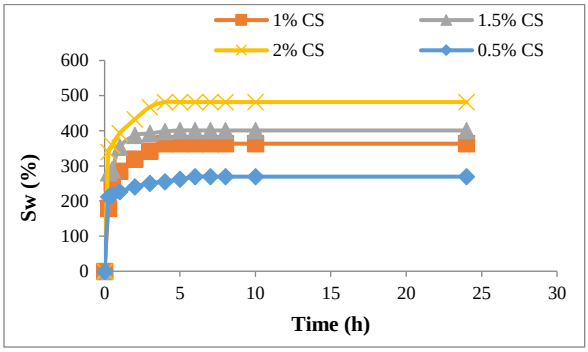

Figure 2: Effect of chitosan concentration on percent swelling of the CP/AG/CS dressing. CB/AG ratio, 75/25; [CP], 2%; [AG], 2%; No. of layers, 2.

Table 2: Effect of chitosan concentration on some performance properties of the CP/AG/CS dressing.CB/AG ratio, 75/25; [CP], 2%; [AG], 2%; No. of layers, 2.

CS Conc. (%)	TS (Kg)	S (mg)	AP $cm^3/cm^2.sec$
0.5	13	2741	197.8
1	16	3924	171.8
1.5	18	4628	147.2
2.0	19	5732	138.3

In that research paper chitosan was used to crosslink the CB/AG blend containing dressing due to its cationic and antibacterial properties. Figure 2 shows the impact of chitosan concentration on swellability of the CB/AG/CS dressing. It is well seen that increasing of the chitosan concentration from 0.5 to 2% gives rise to a progressive enhancement in percent swelling of the CB/AG/CS dressing suggesting the chitosan high swelling properties[4,17,28-310].Moreover, Table 2 reveals the impact of chitosan concentration on stiffness, tensile strength, and air permeability of theCB/AG/CS dressing. It is clear that increasing of chitosan concentration from 0.5 to 2.0% is accompanied by a progressive reduction in air permeability along with an increasing in tensile strength and stiffness of the CB/AG/CS dressing which may be attributed to the gradual increasing in the electrostatic attraction between the chitosancationic groups, i.e. $–NH_2$ groups, and the anionic groupsof the CB/AG blend, i.e. –COOH groups, which indeed enhances subsequently the crosslinking extent withinsuch dressing structure [4,17,28-31].

3.1.3. Number of coating layers

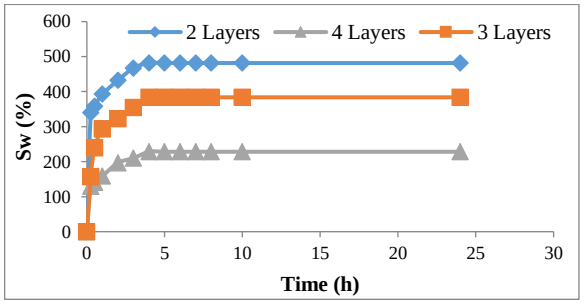

Figure 3: Effect of coating layers number on percent swelling of the CP/AG/CS dressing. CB/AG ratio, 75/25; [CP], 2%; [AG], 2%; [CS], 2%.

Table 3: Effect of layers number of the CP/AG/CS dressing on its performance properties.CB/AG ratio, 75/25; [CP], 2%; [AG], 2%; [CS], 2%.

No. of coating layers	TS (Kg)	S (mg)	AP $cm^3/cm^2.sec$
2	19	5732	138.3
3	20	6550	133.2
4	21	7405	127.9

The impact of dressing coating layers on swellability of CB/AG/CS dressing is clearly seen by Figure 3.It is obvious that increasing of suchdressing coating layers results in a significant enhancement swellability of such dressing. Moreover, Table 3 reveals the effect of that dressing coating layers number on its performance properties. It is clearly seen that increasing of the coating layers brings about a reduction in air permeability along with an enhancement in tensile strength as well as stiffness of such dressing reflectingincreasingof deposition magnitude of the used biopolymers ingredients withinsuch dressing structure with increasing of number of coating layers number [4,17,28-31].

3.1.4. Dressing formulation

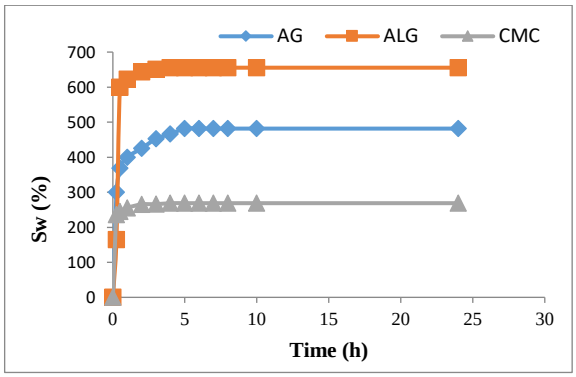

Figure 4: Effect of the dressing formulation on percent swelling of the CP/polymer/CS dressing. CP/polymer ratio, 75/25; [CP], 2%; [AG], 2%; [CS], 2%; No. of layers, 2.

Table 4: Effect of dressing formulation on its performance properties. CP/polymer ratio, 75/25; [CP], 2%; [polymer], 2%; [CS], 2%; No. of layers, 2.

No. of coating layers	TS (Kg)	S (mg)	AP $cm^3/cm^2.sec$
AG	19	5732	138.3
CMC	17	5680	161.4
ALG	16	4340	168.1

Figure 4 illustrates the percent swelling of CP/AG/CS, CP/CMC/CS, and CP/Alg/CS dressings. It is obvious that the swellability of such dressings can be arranged in the following order: CP/Alg/CS >CP/AG/CS >CP/CMC/CS. Moreover, Table 4shows the impact of the dressing formulation on its performance properties. It is obviously seen that magnitudes of the performance properties of such prepared dressings can be arranged as follows:

- tensile strength: CP/AG/CS > CP/CMC/CS > CP/Alg/CS,
- stiffness: CP/AG/CS > CP/CMC/CS > CP/Alg/CS, and
- air permeability: CP/AG/CS < CP/CMC/CS < CP/Alg/CS

This could be associated with the differences among such anionic polymers with respect to the chemical structure, molecular weight, degree of solubility, orientation and configuration of the molecules, rheological characteristics, polarity, film-forming characteristics, chain branching, location and extent of penetration, ionic interaction among each other, cohesive and adhesive characteristics, in addition to the polymer degree of substitution [32-34].

3.2. Antibacterial properties of the CP/CMC/CS dressing

Table 5: Effect of type of Ag-NPs on the antibacterial properties of the CP/CMC/CS dressing.

Treatment type	Reduction(%)	
	S. aureus	*E. coli*
No bio-additive	89.21	85.54
Ag-NPs (2%)	96.66	93.78

According to the performance properties of the CP/CMC/CS dressing, with respect to the other dressings, Ag-NPs asa bio-additive were added during building up of such dressing. Table 5 illustrates the impact of the Ag-NPs on antibacterial properties of the CP/CMC/CS dressing. It is obvious that introducing of the Ag-NPs in such dressing outer layer, gives rise in an enhancement in the antibacterial properties of that dressing because of:

a) generation of Ag^+ that inactivates the bacterial DNA as shown from the following equation:

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

and/or

b) oxidizing of the bacterial molecular structure because of the generated oxygen free radicals as shown from the following equation [4-7, 35-40]:

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

3.3. Characterization of the CP/CMC/CSdressing

3.3.1. SEM and EDX images

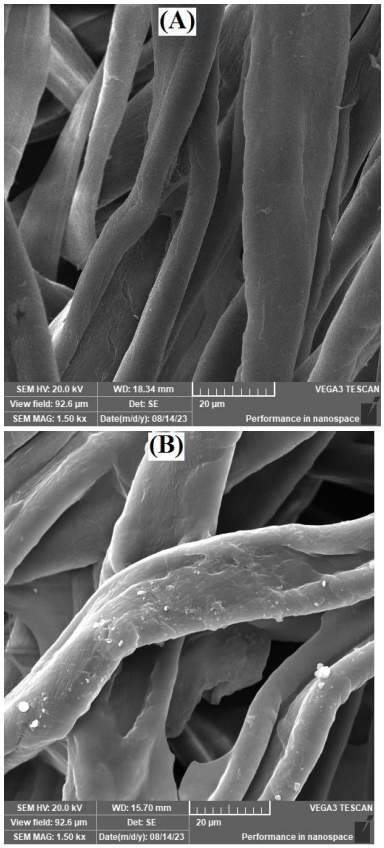

Figure 5: SEM of (A) untreated NWC fabric and (B) the CP/CMC/CS dressing having Ag-NPs in its structure.

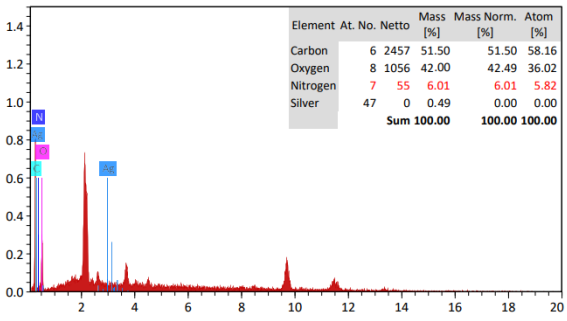

Figure 6: EDX of the CP/CMC/CS dressing having Ag-NPs in its structure.

Figure 5 shows the SEM images of an untreated NWC cotton fabric sample (A) and the CP/CMC/CS dressing (B). It is clearly seen that the dressing fabric sample comprises a NWC fabric covered with a layer of the aforementioned bio-polymers with respect to the untreated fabric. Moreover, Figure 6, the EDX image of the CP/CMC/CS dressing, confirms the presence of elements of nitrogen, belonging to chitosan, and silver onto the nominated dressing structure.

3.4. Conclusions

The best performance properties of the CP/AG/CS dressing were achieved at weight ratio 25/75 of CP/AG and 2% of CS. Increasing coating layers of such dressing brings about an increasing in swellability and tensile strength along with a reduction in air permeability of such dressing. The CP/CMC (75/25, repetivelly) is the best blendfrom the performance properties point of view. Ag-NPs were added during the CP/CMC/CS dressing preparation to enhance the antibacterial properties of such dressing. The CP/CMC/CS dressing was characterized via SEM and EDX analysis.

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