



Formulation of a developed wound dressing containing chitosan, poly (N-vinyl-2-pyrrolidone), and carbopol

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Abstract

A wound dressing is an important element tailored for wound care. The wound dressing should protect the wound from the environmental conditions, absorb wound fluids, as well as provide the moisture for healing the wound. Recently, many wound dressings are available in the medical products market. Despite the substantial advancements in the wound dressings manufacturing, no single material can produce ideal dressing for all wounds and for all the wound healing process stages. In that research work chitosan (CS)/ poly (vinylpyrrolidone) (PVP)/ carbopol® (CP) wound dressing was prepared through treating non-woven cotton fabric samples with different CS/PVP blends as polycations followed by crosslinking with CP as polyanions. Factors affecting the prepared CS/PVP/CP dressing formation such as CS/PVP weight ratio, CP concentration, and number of coating layers were studied. The results indicated that the best CS/PVP weight ratio is 50/50 from the percent swelling, tensile strength, stiffness, and air permeability points of view. Moreover, increasing of the CP concentration results in a gradual increasing in percent swelling, tensile strength, and stiffness in addition to a lowering in air permeability of the CS/PVP/CP dressing. Furthermore, increasing of the coating layers of the CS/PVP/CP dressing brings about a significant increasing the percent swelling, tensile strength, and stiffness accompanied but reduces the air permeability of such dressing. Moreover, incorporation of either clove oil or silver nano-particles (Ag-NPs) in formulation of such dressing leads to an enhancement in the antibacterial properties of such dressing. Furthermore, combining of clove oil with Ag-NPs in the CS/PVP/CP dressing formulation upgrades the antibacterial properties of such dressing. The CS/PVP/CP dressing containing the clove oil and Ag-NPs was characterized via SEM and EDX analysis.

Key words: Wound dressing; Chitosan; Poly (vinylpyrrolidone); Carbopol, Clove oil.

1. Introduction

A wound is the disintegration of mucosa or skin epithelial lining or because of a thermal or physical damage. A dressing material is necessary to be applied to a wound to heal and protect such wound from the external conditions. The dressing material should provide the wound tissue with the right amount of moisture in order to heal the wound as soon as possible. Selecting the correct dressing will reduce the wound healing time, produce a cost-effective care, as well as enhance the life quality of the patient [1-4].

The wound dressing materials are available in the wound care market in many forms suitable for all the wounds types. Wound dressings can be categorized based on their action nature to passive, interactive and bioactive products [5,6]. The

bioactive dressings are commonly produced from biomaterials possess healing properties. They are biocompatible, biodegradable, as well as non-toxic products and produced from natural or synthetic polymers like alginates, hyaluronic acid, chitosan, and collagen or polyester and polypropylene. Sometimes such dressings are combined with growth factors and the antimicrobials to enhance the healing process of a wound [5,6].

Poly (vinylpyrrolidone) (PVP) is a synthetic polymer having unique chemical and physical properties including the biocompatibility, non-toxicity, solubility in water and several organic solvents, chemical stability, and affinity to complex the hydrophobic as well as hydrophilic materials. The matter that renders it convenient as a biomaterial in many medical and nonmedical applications including the medicine and pharmaceutical industry,

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membranes, optical and electrical applications, ceramics, adhesives, coatings and inks, paper, household, fibers and textiles, photography, environmental applications [7]. PVP films are glossy transparent and in the dry state they are hard and clear [15]. Different plasticizers can be added to reduce the hygroscopicity or control the tack and/or brittleness, without affecting the luster or clarity of that films [8]. In aqueous solutions, PVP acts as a polybase and forms interpolymer complexes with polyacids such as the poly (acrylic acid) [9].

Chitosan (CS) is a natural polysaccharide comprises of D-glucosamine and N-acetyl-D-glucosamine and prepared by the deacetylation of chitin, which is extracted from the crustaceans shells and many fungi cell wall [10]. It is commonly used in the pharmaceutical and medicine fields, the agriculture and food industry, cosmetics and waste water treatments. Chitosan is considered as one of the most important biopolymers in the pharmaceutical as well as biomedical fields due to its porous structure, antibacterial activity, water binding capacity, haemostatic properties, biodegradability, low immunogenicity, non-toxicity, anti-tumor, and biocompatibility properties. It has the adequacy to stimulate the cell proliferation as well as histoarchitectural tissue organization [11–14]. Because of the strong inter- and intra-molecular H-bonding between the chitosan chains, it forms a stable film [15]. The chitosan/PVP films containing silver nano-particles possess fast and long-acting anti-bacterial properties against the *Escherichia coli* and *Staphylococcus aureus* bacteria [15]. Li et al. reported [16] that blending of PVP with chitosan reduces the chitosan-based films yellowish color with rendering such films to be easier to tear. Moreover, the chitosan/PVP films containing a PVP ratio 75% are of higher water vapor permeability than the 100% chitosan films. In addition, the antimicrobial activity was reduced when 50% PVP was used in the film. In general, inclusion of PVP in chitosan films significantly reduces production price of chitosan films without affecting the films functionality.

Carbopol (CP) is a poly acrylic acid appears a sol-to-gel transition upon raising its aqueous solution pH above pK ca 5.5 [17,18]. The temperature, pH, light or electric fields affect the swelling behavior of the carbopol hydrogels [19-21]. Carbopol can deliver drugs to a specific area into the body. The carbopol high swellability in the alkaline solutions affords it to deliver a maximum extent of a drug in the alkaline medium [22].

The present work is undertaken to with a view to prepare CS/PVP/CP wound dressings through treating non-woven cotton fabric samples with different CS/PVP blends as polycations followed by crosslinking with CP as polyanions. Some

performance and antibacterial properties of such prepared dressings will be studied.

2. Experimental

2.1. Materials

Non-woven cotton fabric (NWC fabric), Hebitex Co., Egypt, was used. High molecular weight chitosan, Fluca Co., poly (N-vinyl-2-pyrrolidone) (PVP) of molecular weights 40,000 and 1300000 Dalton, Alpha chemiKa Co., and carbopol 934 (CP), purchased from China, were used. Silver nitrate, trisodium citrate, and sodium hydroxide are laboratory grade chemicals were used.

2.2. Methods

2.2.1. The CS/PVP/CP dressing preparation

All PVP, CS, and CP solutions were prepared by dissolving a certain weight of that polymer in distilled water with stirring at 80 °C/45 min. Different formulations of such polymers were prepared by blending of these polymers solutions, keeping the net concentration of such blends solutions at 2%. The dressings were prepared by padding NWC fabric samples in the mentioned above formulations solutions followed by squeezing and drying at 80 °C/5 min. The dried samples were then crosslinked by their padding in CP solution, squeezing and drying at 80 °C/5 min.

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

Ag-NPs were synthesized using the method mentioned elsewhere.

2.2.3. Production of CS/PVP/CP dressings containing Ag-NPs

The NWC fabric-based PVP/CS/CP dressings containing Ag-NPs were prepared by adding 1% of the Ag-NPs to the prepared formulations solutions followed by padding the NWC fabric in such solutions in a procedure similar to that mentioned in section 2.2.1.

2.2.4. Loading of CS/PVP/CP dressings with clove oil

Clove oil (1.5 g) was dissolved in ethanol (20% w/w) and then slowly added to the carbopol solution (2%) with stirring for 1h at 50 °C [24].

2.3. Characterization of the fabricated wound dressing

- The percent swelling (% SW) was assessed using the following equation: $SW (\%) = (W_a - W_i) / W_i \times 100$, (where W_a is the dressing wetted weight and W_i is the dressing initial weight) [14,25-28].
- 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).

- Stiffness (S) was determined in the warp direction according to ASTM Test Method D 1388-96 using Jika (Toyaseiki) apparatus.
- The antibacterial activities of the dressing was assessed by the bacterial count method against Staphylococcus aureus (SA) as Gram-positive bacteria and Escherichia coli (EC) as Gram-negative bacteria [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.

3. Results and discussion.

3.1. Factors affecting CS/PVP/CP dressing formation

3.1.1. Chitosan to PVP weight ratio

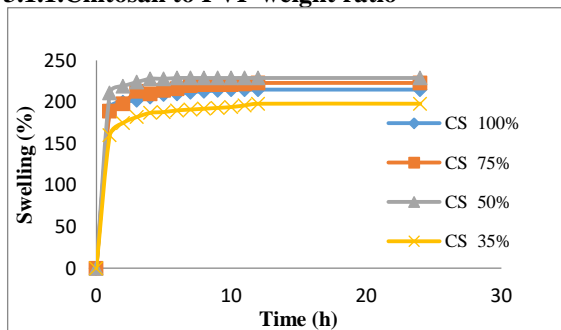


Figure 1: Effect of chitosan to PVP weight ratio on percent swelling of the CS/PVP/CP dressing. [CS], 2%; [CP], 1%; [PVP], 2%; PVP M.W., 40000 D; No. of layers, 2.

Table 1: Effect of chitosan to PVP weight ratio on some performance properties of the CS/PVP/CP dressing. [CS], 2%; [CP], 1%; [PVP], 2%; PVP M.W., 40000 D; No. of layers, 2.

CS:PVP ratio	TS (Kg)	S (mg)	AP cm ³ /cm ² .sec
100/0	11	6230	95
75/25	13	3561	81
50/50	17	3133	83
35/65	15	2741	74

Figure 1 shows the effect of CS/PVP ratio on percent swelling of the prepared CS/PVP/CP dressing as a function in time. It is shown that increasing of the PVP ratio from 0 to 50% gives rise to a gradual increasing in swelling degree of the dressing which may be associated with the reduction in intermolecular interactions among the chitosan chains and/or decreasing of the chitosan solution viscosity by blending it with PVP solution [16]. Moreover, behind 50 and up 65% ratio of PVP, the nominated dressing

swellability slightly decreases reflecting the higher molecular weight of chitosan compared to PVP. Furthermore, according to CS/PVP ratio the percent swelling of the CS/PVP/CP dressing can be arranged descendingly as follows: 50/50 > 75/25 > 100/0 > 35/65.

Table 1 shows the effect of chitosan to poly (N-vinyl-2-pyrrolidone) ratio on some performance properties, expressed in tensile strength, stiffness, and air permeability, of the PVP/CS/CP dressing. It is clear that increasing of the PVP ratio in the PVP/CS/CP dressing formulation results in: i) an improvement in tensile strength of the dressing till the ratio of 50/50 followed by a decreasing in extent of that property, ii) a progressive decreasing in the stiffness of the prepared dressings, and iii) a significant decreasing in the dressing air permeability till the ratio 75/25 of the CS/PVP blend followed by a slight increasing at the ratio of 50/50 and then the air permeability decreases again at the ratio of 35/65. These findings reflect the differences between PVP and chitosan in their chemical structure, degree of polymerization, and film-forming properties [29-34]. Moreover, a synergistic effect has been occurred at 50/50 blending ratio rendering the tensile strength to be the highest value. However, it seems that among all the aforementioned PVP/CS ratios, the 50/50 blending ratio is the appropriate ratio to continue studying of other factors affecting PVP/CS/CP dressing formation.

3.1.2. Carbopol concentration

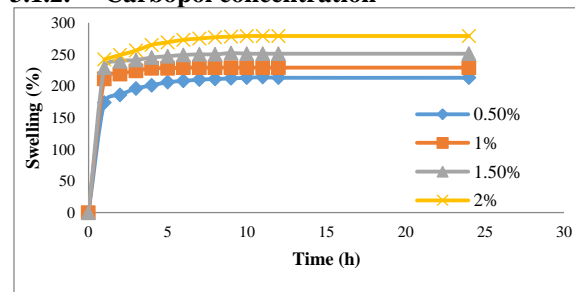


Figure 2: Effect of carbopol concentration on percent swelling of the CS/PVP/CP dressing. CS/PVP ratio, 50/50; [CS], 2%; [PVP], 2%; PVP M.W., 40000 D; No. of layers, 2.

Table 2: Effect of carbopol concentration on some performance properties of the CS/PVP/CP dressing. CS/PVP ratio, 50/50; [CS], 2%; [PVP], 2%; PVP M.W., 40000 D; No. of layers, 2.

CP Conc. (%)	TS (Kg)	S (mg)	AP cm ³ /cm ² .sec
0.5	14	2314	86
1	15	3133	83
1.5	17	4592	81
2.0	18	5340	80

The driving force behind using the carbopol® as a polyanions to build the CS/PVP/CP dressing is its high swellability as well as its ability to crosslink the CS/PVP blend as a polycations layer. Figure 2 shows the effect of carbopol concentration on percent swelling of the CS/PVP/CP dressing. It is clear that increasing of the carbopol concentration from 0.5 to 2% results in a gradual increasing in percent swelling of the CS/PVP/CP dressing reflecting the high swelling properties of carbopol. On the other hand, Table 2 signifies the impact of carbopol concentration on tensile strength, stiffness, and air permeability of the CS/PVP/CP dressing. It is obvious that increasing of the carbopol concentration from 0.5 to 2.5% is accompanied by a progressive enhancement in tensile strength and stiffness along with a reduction in air permeability of the CS/PVP/CP dressing. This may be a direct consequence for increasing of the H-bonding as well as the electrostatic attraction between the CP charged species from one side and the oppositely charged CS/CS species from the other side that indeed enhances the crosslinking extent inside the dressing structure [9,14,17,18,22].

3.1.3. Number of coating layers

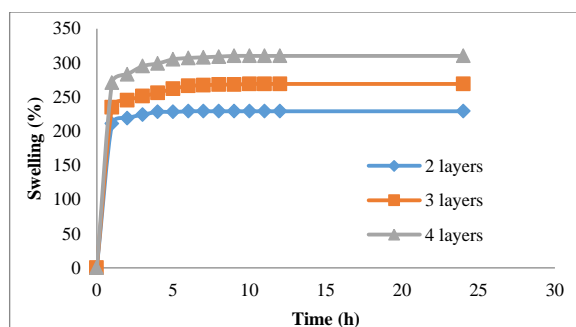


Figure 3: Effect of layers number on percent swelling of the CS/PVP/CP dressing. CS:PVP ratio, 50/50; [CS], 2%; [CP], 2%; [PVP], 2%; PVP M.W., 40000 D.

Table 3: Effect of number of coating layers on some performance properties of the CS/PVP/CP dressing. CS:PVP ratio, 50/50; [CS], 2%; [CP], 2%; [PVP], 2%; PVP M.W., 40000 D.

No. of coating layers	TS (Kg)	S (mg)	AP
2	15	2670	83
3	16	4630	71
4	18	7120	58

The effect of coating layers number on the percent swelling of the aforementioned dressing is well seen by Figure 3. It is clear that increasing of the coating layers of the NWC fabric brings about a significant increasing the percent swelling of such treated fabric samples. On the other hand, Table 2 illustrates the impact of coating layers number on the performance properties of the prepared CS/PVP/CP dressing. It is obviously seen that increasing of the above mentioned dressing coating layers gives rise to an increasing in tensile strength and stiffness along with a reduction in air permeability of such dressing. The matter that can be associated with increasing of deposition extent of the hydrophilic biopolymers constituting such dressing ingredients inside the dressing structure by increasing of coating layers number which subsequently alters extents of the aforementioned properties of that dressing [14,26,27].

3.2. Antibacterial of the CS/PVP/CP dressing

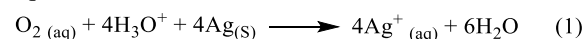
Table 4: Effect of the additive type on antibacterial properties of the CS/PVP/CP dressing. CS:PVP ratio, 50/50; [CS], 2%; [CP], 2%; [PVP], 2%; PVP M.W., 40000 D. No. of layers, 2.

Additive type	Reduction (%)	
	<i>S. aureus</i>	<i>E. coli</i>
Clove oil (1.5%)	24.90	47.49
Ag-NPs (2%)	70.89	74.93
Ag-NPs plus clove oil	94.94	94.69

Table 4 shows the effect of additive type on antibacterial properties of the CS/PVP/CP dressing. It is clear that inclusion of clove oil in the last layer, i.e. the carbopol layer, of the CS/PVP/CP dressing results in an enhancement in the antibacterial properties of the dressing reflecting the antibacterial properties of clove oil [35].

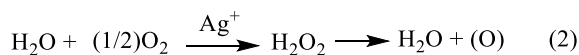
Moreover, incorporation of Ag-NPs in such dressing formulation instead of the clove oil increases the extent of the antibacterial properties of that dressing through:

a) formation of Ag^+ , in the presence of medium of moisture, that combine with o the bacterial DNA resulting in its inactivation according to the following equation:



and/or

b) generation of oxygen free radicals that would oxidize the molecular structure of bacteria according to the following equation [14,25-27, 36,37]:



Furthermore, combining of clove oil with Ag-NPs in the CS/PVP/CP dressing structure leads to a significant enhancement in the antibacterial properties of the dressing suggesting a synergistic effect occurrence as a result of combining of such bio-additives in that dressing structure.

3.3. Characterization of the CS/PVP/ CP dressing

3.3.1. SEM and EDX images

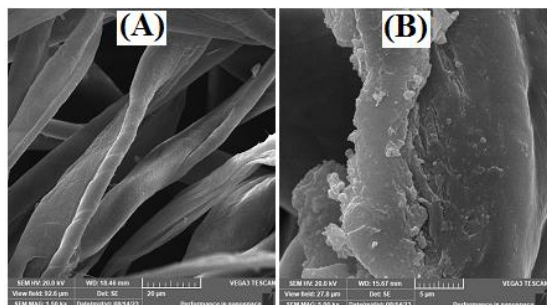


Figure 5: SEM of (A) untreated NWC fabric and (B) the CS/PVP/ CP dressing loaded with Ag-NPs and clove oil.

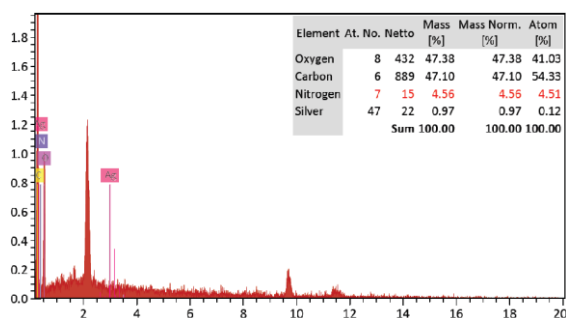


Figure 6: EDX of the CS/PVP/ CP dressing loaded with Ag-NPs and clove oil.

Figure 5 shows the SEM images of an untreated NWC fabric sample (sample A) and the CS/PVP/CP dressing (sample B). It is obviously seen that the dressing structure contains NWC fabric coated with a layer of the nominated bio-polymers compared with the untreated sample. On the other hand, Figure 6 depicts the EDX image of the CS/PVP/CP dressing. This image confirms the presence of silver as well as nitrogen elements onto that dressing structure. The nitrogen element appeared in the EDX image due to the clove oil containing the nitrogen element in its chemical structure [38-48].

Conclusions

The best CS/PVP weight ratio to build CS/PVP/CP dressing having considerable performance properties is 50/50. As the CP concentration increases, the percent swelling, tensile

strength, and stiffness extents increase but the air permeability of the CS/PVP/CP dressing decreases. Increasing of the coating layers of the CS/PVP/CP dressing leads to a significant increasing in percent swelling, tensile strength, and stiffness accompanied with a reduction in air permeability properties of such dressing. Incorporation of either the clove oil or Ag-NPs in formulation of such dressing leads to an enhancement in the antibacterial properties of the CS/PVP/CP dressing. Combining of clove oil with Ag-NPs in the CS/PVP/CP dressing formulation upgrades the antibacterial properties of such dressing. The CS/PVP/CP dressing containing the clove oil and Ag-NPs was characterized via SEM and EDX analysis.

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