Research Progress in Mechanical and Manufacturing Engineering Vol. 2 No. 2 (2021) 354-361
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RPMME

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/rpmme
e-ISSN : 2773-4765

Physiochemical, Mechanical and Antibacterial Properties of Sodium Alginate/Copper Oxide BioComposite Film for Wound Healing Applications

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DOI: https://doi.org/10.30880/rpmme.2021.02.02.040
Received 02 Aug. 2021; Accepted 27 Nov. 2021; Available online 25 December 2021

Abstract: The objectives of this study are to fabricate alginate and copper oxide biocomposite film via solution casting method. At the earlier stage, the alginate solution with 1wt% was prepared and later mix with the 0.5, 1.0 and 1.5 gram of copper oxide. The combination of alginate and copper oxide was stirred for 3 minutes before proceeding to casting process and left for 24 hours. When the films are ready, it was crosslinked with calcium chloride. The biocomposite film was dried in the room temperature for 24 hours. Then, the fabricated film was characterised and analysed by using Fourier Transform Infrared Spectroscopy (FTIR) and contact measurement, thickness analysis and viscosity analysis also being conducted in order to study the characterization of the film. Also the antibacterial assessment was carried out by using disc diffusion method (Kirby-Bauer test).

Keywords: Alginate, Copper Oxide, Physiochemical, Antibacterial Properties

1. Introduction

Wound healing is a characteristic helpful reaction to tissue damage. Despite tremendous advancements in wound dressing development, wound healing remains a major difficulty that places a huge burden on patients and the healthcare system. Wound healing is a complex process in which the skin, and the tissues under it, repair themselves after injury. Biomaterial can be used as wound healing material in any injury at tissue organ. Layer of tissue in human body is important. The skin is considered the largest organ of the body and has many different functions [1]. In general, a good wound dressing should maintain a moist wound environment, guard against secondary infections, remove wound exudate, and promote tissue regeneration.

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Among various natural polymers, Na-alginate has gained remarkable recognition due to its unique features including protection of wounds from bacterial burden, providing moist microenvironment, promoting re-epithelialization and granulation tissue formation for faster wound closure, non-immunogenicity, biocompatibility, biodegradability, and controlled release properties [2]. Besides, Copper plays a key role in angiogenesis and in the synthesis and stabilization of extracellular matrix skin proteins, which are critical processes of skin formation [3]. By performing few testing and data analysis on sodium alginate with copper oxides film, properties of the film can be investigated. These are the list of analysis and testing that being done on the samples:

1. Thickness analysis
2. Viscosity analysis
3. Contact angle estimation (Goniometer)
4. Fourier-transform infrared spectroscopy (FTIR)
5. Antibacterial testing

The use of Alginate is an anionic polymer derived naturally from brown seaweed that has been investigated and used in a variety of therapeutic applications. The physicochemical properties of the alginate gels are dependent on the type of crosslinking, crosslinking density, and molecular weight and composition of the alginate [4]. The use of alginate can provide several advantages including ease of preparation, biocompatibility, biodegradability, and nontoxicity [5] Alginate has carboxyl groups which are charged at pH values higher than 3-4, making alginate soluble at neutral and alkaline conditions to promote the widespread use of alginates [5]. As a biomaterial, alginate has a number of advantageous features including biocompatibility and non-immunogenicity and they are likely related to its hydrophilicity [6]. Alginate is suitable in pharmaceutical industry and food industry at which acts as emulsion stabilizers, thickeners, suspension and importantly as viscosity increasing agents.

Alginates as a chosen polymer in several delivery systems can be adapted to the limitations that drugs or proteins, or the system itself, must overcome. They promote wound healing, permeability to water vapour, carbon dioxide, and oxygen, and protect the wound from bacterial infections. Alginate dressings absorb wound fluid resulting in gels that maintain a physiologically moist environment and minimize bacterial infections at the wound site [7].

Copper ions play an important role in augmenting wound healing [8]. With respect to nanoparticulate metals, the antimicrobial properties of silver and copper have received the most attention [9]. It has been revealed that CuO nanoparticles have high affinity to carboxyl and amine groups on the surface of B. subtilis cells, this affinity being higher than the interaction of silver nanoparticles with these bacteria cells [10].

The potential of metallic copper as an intrinsically antibacterial material is gaining increasing attention in the face of growing antibiotics resistance of bacteria [11]. Recent findings suggest that the direct contact between bacteria and metallic copper is an important factor in contact killing [11]. Copper ions destroy bacterial cell membranes, or "envelopes," and can destroy the microbe's DNA or RNA as well.

2. Materials and Methods

2.1 Materials

In preparation this film, material that had been used as shown in Table 1. The composition of the film showed in Table 2 and Table 3.

Table 1: Lists of material used

No	Item	Brand
1.	Sodium Alginate	SIGMA-ALDRICH
2.	Copper	SIGMA-ALDRICH
3.	Calcium Chloride Dihydrate	SIGMA
4.	Distilled water	-

Table 2: Composition of sodium alginate solution

Alginate (g)	Distilled water (ml)
1	100

Table 3: Composition of sodium alginate with copper oxide

No.	Alginate (ml)	Copper (gram)	Label
1.	100	-	SA
2.	100	0.5	SC0.5
3.	100	1.0	SC1.0
4.	100	1.5	SC1.5

Sodium alginate solution is prepared by preparing 1g sodium alginate and added to the beaker. Then, pour 500 ml of distilled water. By using stirring hotplate, setting the speed to 300 rpm in 30 minutes at room temperature. In the fume hood, 100 ml of Alginate in the small beaker been mix with 0.5g of copper oxide. Stir it well for about 3 minutes. Next, measure 5 ml (alginate + copper oxide) using a measuring cylinder. Pour the solution into the 12 mm of the petri dish. Label the petri dish based on Table 3. Same method is repeated for concentration with 1.0g and 1.5g of copper oxide. Next, for the casting process. The biocomposites dry at room temperature for 24 hours in the fume hood. When the biocomposites film is ready next step is to cross-linked process, calcium chloride, $CaCl_2$was used to harden the film. $CaCl_2$ was mixed with 20 ml of distilled water by using stirring hotplate. Dip the petri dish into $CaCl_2$ for one second. After 30 minutes, the petri dish dip under the tap water and let it dry for 1 hour in the fume hood.

3. Results and Discussion

3.1 Thickness analysis

Sodium alginate film with 1.5g copper oxide has the thinnest film compared to others which is the thickness is 0.127mm in average. Based on the graph in Figure 1, it can be seen that the average thickness of sodium alginate films is decreasing as the copper oxide content increased as well as the value of standard deviation of the sample.

Table 4: Thickness analysis of the films

	Thickness 1 (mm)	Thickness 2 (mm)	Thickness 3 (mm)	Average (mm)	Standard deviation (mm)
SA	0.140	0.136	0.159	0.145	0.381
SC0.5	0.144	0.149	0.142	0.145	0.381
SC1.0	0.148	0.144	0.139	0.144	0.380
SC1.5	0.130	0.126	0.125	0.127	0.356

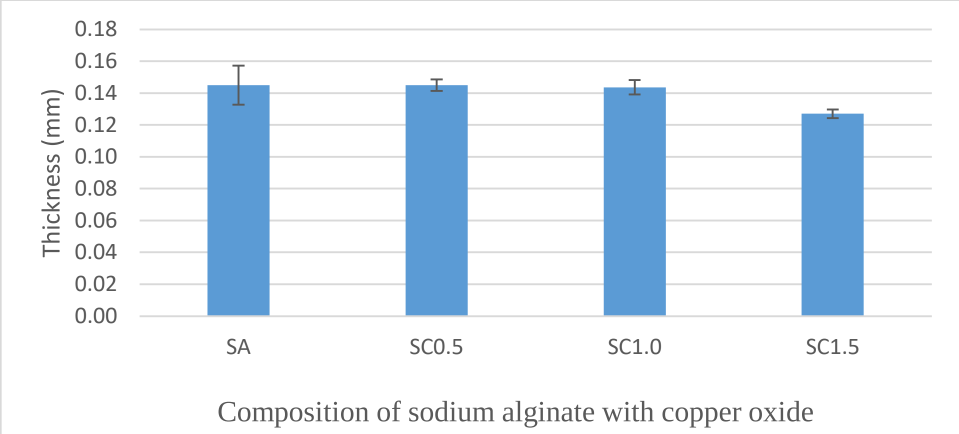

Figure 1: Thickness analysis graph

3.2 Viscosity analysis

Based on Figure 2, it clearly shows that the viscosity of pure sodium alginate is 6.8 mPa.s which is the lowest viscosity compared with others. The viscosity of pure sodium alginate is the lowest. The viscosity of sodium alginate with copper oxide is increasing as the concentration increase. Theoretically, a larger molecular weight should, result in more binding between chains. A studies have stated that the molecular weight and polymer content distribution is affecting the viscosity of an alginate solution [6]. The highest viscosity among these samples is sample SC1.5 which is 11.7mPa.s. This shows that SC1.5 has the stronger intermolecular forces since previous studies stated that high viscosity is characteristic of liquids with strong intermolecular forces [12].

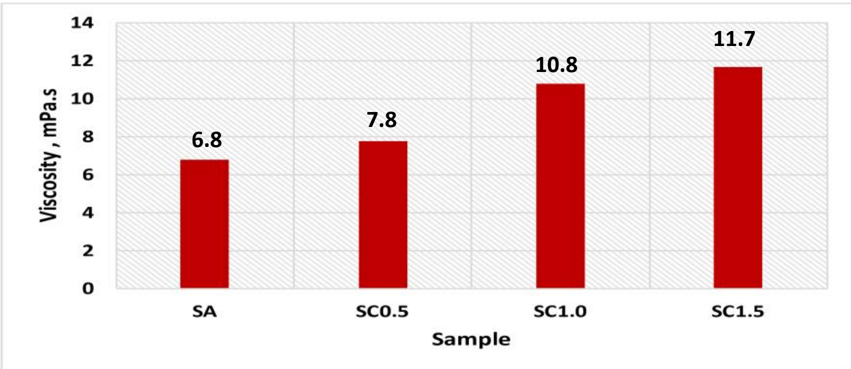

Figure 2: Viscosity analysis

3.3 FTIR analysis

Hydrogen bonding and other interactions between composite polymer components were investigated using the FT-IR method. Based on the results obtained, in Figure 3, there have main broad absorption band detected at 3250cm^{-1} due to O-H stretching vibration of sodium alginate in all spectrums of samples. Next, there has a high peak detected at 1590cm^{-1} and 1420cm^{-1} is symmetric and asymmetric stretching vibrations of the carboxylate group. Peak at 1022.39cm^{-1} is attributed for vibrations in esters which is Acetates and lastly peak at 980cm^{-1} can be ascribed as stretching vibration of C-O-C group. The similar pattern of FTIR wavelength was observed either with or without copper oxide. It may be the copper oxide wavelength was overlapped with alginate. As it was reported that from previous work that the wavelength for copper oxide was in the range of 1376cm^{-1} and 1420cm^{-1} at which it detect the presence of CuO by C-H bending vibrations [13].

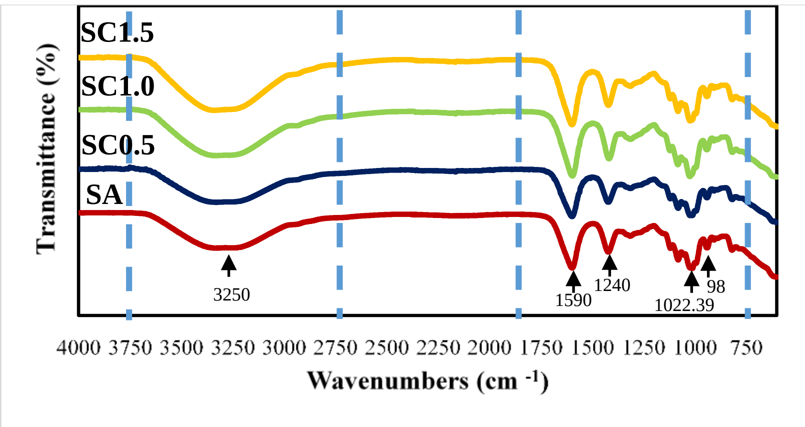

Figure 3: FTIR analysis on SA, SC0.5, SC1.0 and SC1.5

3.4 Contact angle measurement

In Figure 4 it shows the surface morphology of surface and static angle of sodium alginate film and also film of mixture between sodium alginate and copper oxide with different compositions.

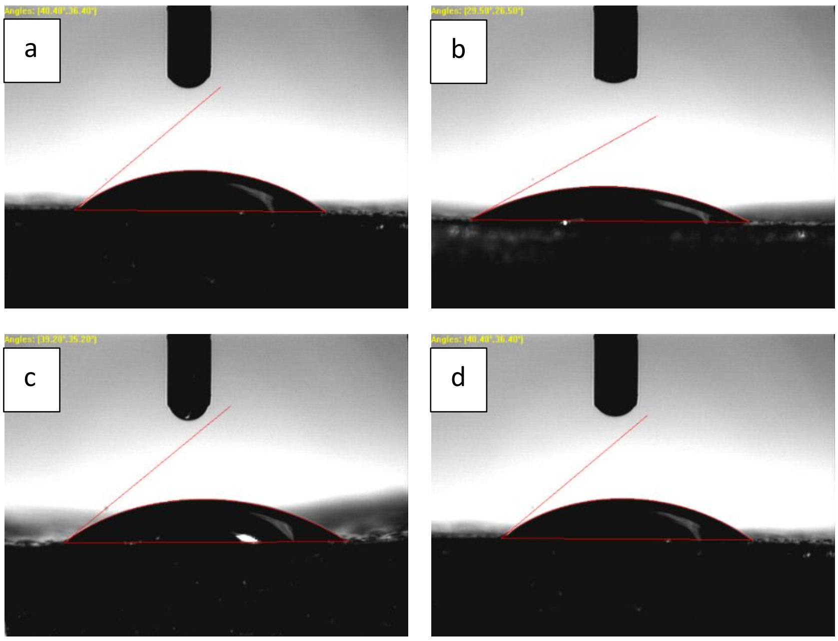

Figure 4: Contact angle measurement

The sample result's shows an increasing pattern as the concentration of film of sodium alginate with copper oxide increasing. Refer from Table 5, although the angle of the sample is increasing, it still does not exceed 90˚ which shows that all samples have a low contact angle and practically it indicates that it has high wetting surface. Sample SC0.5 with concentration 0.5g of copper has the lowest contact angle which is 29.50˚ followed by sample SC1.0 with 39.20˚ and sample of SC1.5 is the highest contact angle with 40.40˚. In sodium alginate sample with absence of copper oxide in the film, it has the highest contact angle compared to others which is 61.40˚.

Table 5: Summary of contact angle

Sample	Contact Angle (˚)	Hydrophilic / Hydrophobic
SA	61.40	
SC0.5	29.50	Hydrophilic
SC1.0	39.20	(< 90˚)
SC1.5	40.40	

3.5 Antibacterial properties

Antimicrobial activity was analysed against *Staphylococcus aureus*. Figure 5 shows the activity of antibacterial properties in sodium alginate and sodium alginate with 1.0g of copper oxide in bacteria in a well diffusion assay. From the results, it can be seen that sodium alginate with 1.0g copper oxide exhibited antibacterial efficacy against S. aureus. The size of the inhibition zone reflects the microbes' susceptibility. For sodium alginate sample, it can be seen that there has no antibacterial activity showed against S. aureus bacteria.

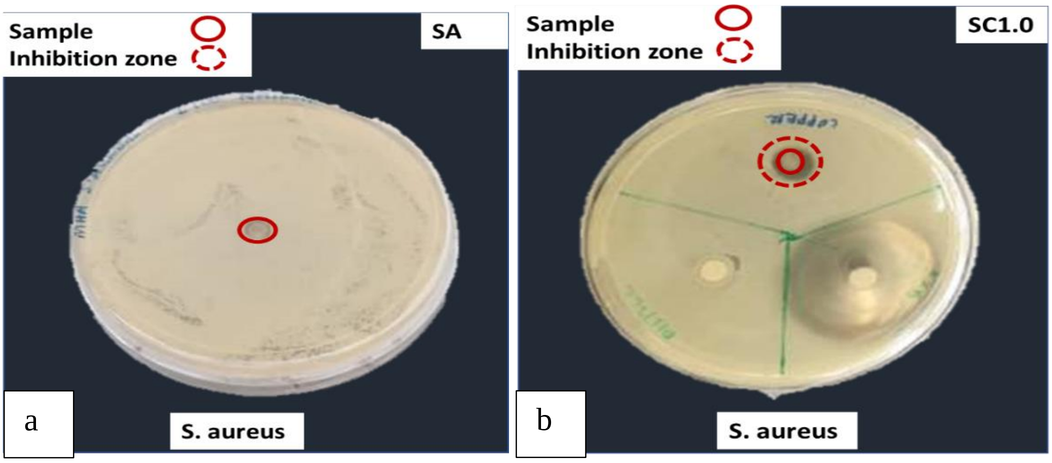

Figure 5: Result of activity of antibacterial of a) sodium alginate film, b) sodium alginate with 1.0g copper oxide film by using Saureus

4. Conclusion

The film of sodium alginate with copper oxides was fabricated successfully by using solution casting method. The testing that was successfully done were thickness of the film, viscosity, FTIR and also antibacterial testing. In thickness analysis, it can be seen that as the concentration on copper increase, the film produced was thin. The viscosity of sodium alginate mix with copper oxide is increasing as the concentration of mixture increasing. This shows that the intermolecular of sodium alginate with copper oxide is stronger as the concentration increase. When there has increasing in molecular weight, the viscosity of the solution is increasing and the physical properties is improved. FTIR analysis, the interaction of any bonding between sodium alginate and copper oxide. Between pure sodium alginate and with copper oxide, there has no significant comparisons that can be detected. In contact angle measurement, the results shows that the films are hydrophilic which also can be concede that this film is capable to absorb exudates. Lastly, results from antibacterial testing, it can be seen that with presence of copper oxide, it helps in enhancing antibacterial activity as copper oxide nanoparticles can pass through microbial membranes and induce cell death by disrupting the cell membrane and destroying DNA and protein.

Acknowledgement

The authors wish to thank to the Faculty of Mechanical and Manufacturing Engineering, Universiti Tun Hussein Onn Malaysia that has supported on the accomplishment of research activity.

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