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Synthesis of Sodium Alginate Graphene Oxide-Silver Film for Antibacterial Activity

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Abstract. A facile preparation of graphene oxide silver alginate film (GO-Ag) has been constructed for the antibacterial activity. Graphene oxide (GO) was used as a substrate towards arranging of unagglomerated silver nanoparticle (AgNPs) by a simple microwave irradiation on combination of GO and silver complexes in the presence of alginate. The obtained nanocomposite were characterized by using Ultraviolet-visible spectroscopy (UV-Vis) and Xray Diffraction to confirm the formation of film composite. AgNPs with spherical-shape deposited on the GO Alginate layer which displayed a good antibacterial towards *E.Coli* and *Staphylococcus aureus*.

1. Introduction

Antibacterial agents are very crucial in most of the industries in worldwide such as food packaging, water disinfection, medicine, and textile industry. These days, one of the most challenging world concerns is caused by pathogenic microorganisms such as fungi, bacteria and viruses that could lead infectious and dangerous diseases [1]. Although most of these infections have decreased over the years with commercialization and the use of conventional method such as penicillin, however this has also led to the increment of the number of drug resistant pathogens. Some of the pathogens include Escherichia coli, E coli and MRSA. According to Vi and Lue (2016), medicines that have been used to treat these infections are having a decline in effectiveness and are creating side effects [2]. This and other infectious related concerns trigger current researchers, scientists and analysts to focus on the development of novel, economical and effective antibacterial treatment strategies to fight pathogenic infections.

Recently, graphene has evolved as a promising material for the future and a highly oxidized graphene layer, graphene oxide (GO) has become one of the favorable materials to be used for antibacterial activity. Besides, it has been discovered that bacteria are less likely to evolve counteraction against silver nanoparticles (AgNPs) [2]. This shows that the tendency of bacteria to develop resistance towards silver (Ag) is low as it exhibits strong cytotoxicity against many microorganisms. A lot of methods have been performed to prepare Ag decorated GO such as chemical reduction [3], hydrothermal [4], microwave [5] and sonochemistry [6]. However, these methods require an expensive instrument and special skills and expertise. In this work, we demonstrate an easy synthesis of AgNPs decorated GO by using a microwave irradiation.

Meanwhile, alginate is one of the natural polysaccharides that, is used in various sector especially in healthcare focusing in drug delivery system, wound dressing and tissue regeneration. Alginate commonly used in sodium alginate form possessed several interesting physicochemical properties such as gelling properties, large surface area and also a good moisture barrier. This properties of alginate can be easily altered by arranging the composition M-blocks and G blocks of the alginate monomer. Hence, the cooperation of sodium alginate into GO-Ag could possibly enhance the antibacterial properties.

Therefore, this project proposes to study the current attempts in the formulation of sodium alginate graphene oxide-silver (GO-Ag) thin film on antibacterial activity as an advanced mechanism to overcome the current treats in confronting against bacterial menaces. Furthermore, the project describes the features of graphene oxide–bacterial interactions to evaluate the antibacterial properties of graphene oxide-based nanocomposite.

2. Experimental

The GO was produced from simplified Hummer's method by using graphite powder.

2.1. Synthesis of Graphene Oxide

Graphene oxide was prepared by using simplified Hummer's method [7]. A coalition 3g of graphite flakes and 18 g of KMnO4 were added into 400 ml of mixture of H_2SO_4 and H_3PO_4 with a ratio of (320 ml: 80 ml), stirred and left for 3 days. The color of the mixture changes from purplish to dark brown indicates the completion of oxidation process. The mixture then poured into the mixture of 500 ml of ice cube containing 27 ml of H_2O_2 to stop the oxidation process, the solution then changes to bright yellow. The graphite oxide formed then washed 3 times with HCl and distilled water for several times with 10 000 rpm until pH 5 is achieved. During the washing process, graphite experience exfoliation which causes the thickening of graphene oxide solution and formation of GO gel.

2.2. Synthesis of Sodium Alginate Graphene Oxide-Silver Film

A complex solution of silver-ammonia was prepared by adding 100 µL of ammonia solution into 10mL of 0.1M silver nitrate (AgNO3) solution and the mixture will be stirred dynamically. Then, the mixture was added with 3 mL of 0.5 mg/mL of GO solution and sonicated for 5 min in 60˚C water bath. Further, the mixture was irradiate under microwave for two minutes. The microwave method was used as a catalyst to assists the attachment between Ag nanoparticles on GO surface [5]. The solution was then centrifuged with 10000 rpm centrifugation force for 15 minutes before being washed thrice by using deionized water to remove excess chemicals [8]. The GO-Ag nanocomposite was synthesized with low concentration of ammonia solution to optimize the synthesis. After that, the composite formed was sonicated again for five minutes.

The dispersion of sodium alginate (5% w/v) was prepared by adding 5 g of sodium alginate powder in 100 ml of distilled water. The dispersion were varies for five different amount ratio of sodium alginate to GOAg solution (5:1, 5:2, 5:3, 5:4 and 1:1). The solutions were stirred at a constant rpm to ensure the solution were mixed homogenously and then transferred into petri dishes. The petri dish is left to dry in oven for 12 hours at 60°C to allow the mixture to form films. The dried solution were peeled of and

were soaked in a 5% w/v aqueous solution of calcium chloride ($CaCl_2$) for 6 hours in order to protect the gelling condition of sodium alginate. The film then left at room temperature to form the novel sodium alginate GO-Ag thin films.

2.3. Antibacterial Application

The antibacterial studies were conducted against gram-negative and gram-positive bacteria, which are *Escherichia coli* and *Staphylococcus aureus.* The inhibition zone of the bacteria ware recorded by using disk diffusion method. The bacterial strains from single bacterial colony will be grown in nutrient broth and swabbed into nutrient agar. The sodium alginate (GO-Ag) film were cut into 6 mm sized disk and were placed into the agar and incubated in 30°C incubator. The diameter of inhibition zone for the bacteria is monitored and recorded for 0h, 8h, 16h, 18h, 20h and 24h using a ruler and a the image were captured using ordinary camera.

3. Result and Discussion

3.1. UV-Visible Spectroscopy of GO and GOAg

Figure 1(a) demonstrates the spectrum signal of GO at 232 nm and a shoulder at 300 nm. The exhibited absorption peak correlated to π–π* transition attributed to C-C bond in aromatic sp2 clusters while at 300 nm, GO is derived from n-π* transitions of the C=O bond [8]. After being irradiated in the microwave and centrifuged, new peaks were formed at 420 nm as shown in Figure 1 (b), (c) and (d). These peaks indicate the formation and distribution of AgNPs on the GO and attributed to the surface plasmon resonance (SPR) absorption feature of the AgNPs. Besides, as the absorbance peak at 230 nm disappears and SPR peak exists, this has further confirmed that after 2 min of the microwave irradiation, the reduction of GO to rGO and Ag^+ ions to AgNPs had successfully formed the GO-Ag composite. Figure 1 (c) shows a very narrow and sharp peak of AgNPs compared to those in Figure 1 (b) and (d), which have broad and wide peaks. The peak that at 400 nm confirms that AgNPs is formed is in spherical shape. The high and sharp peak showed a high homogen sphere nanoparticle produced agree with report elsewhere [5][6].

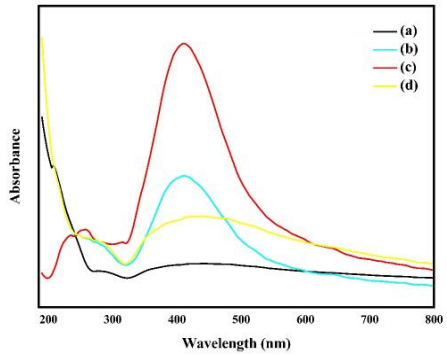

Figure 1. UV-Vis spectrum of (a) bare GO; (b) GO-Ag nanocomposite using 1 ml GO; (c) GO-Ag nanocomposite using 3 ml GO and (d) GO-Ag nanocomposite using 5 ml GO.

3.2. X-ray diffraction of GOAg

The structural characterization of the nanocomposites was determined using the X-ray diffraction (XRD) and the results were presented in Figure 2. The XRD patterns illustrate the spectra obtained from GO and GOAg Alginate film. From the figure, a sharp characteristic peak can be observed at 10.0°, which was attributed to the presence of functional groups such as hydroxyl, carbonyl and carboxyl [12,

13]. After rapid heat treatment with microwave, the peak at 10.0° slightly reduced and shifted to 13.0° suggested the partially reduction of GO [14]. The diffraction peaks observed at 38.1, 44.1 64.2 and 77.2° correspond to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) crystal planes of face-centered cubic (fcc) Ag NPs, respectively. The position of 2θ values matched with the standard JCPDS file no. 89-3722. The results of the XRD analysis reveal that the presence of Ag in the alginate film and assumed GOAg nanocomposite on alginate layer have been successfully synthesized.

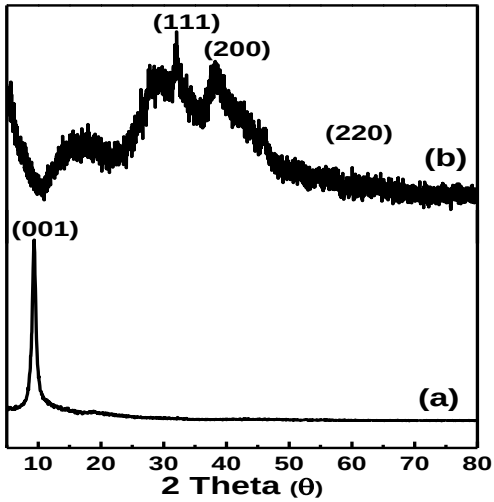

Figure 2. XRD pattern for (a) Graphene Oxide (b) Sodium alginate-GOAg films

3.3. Scanning electron microscopy of GOAg

Figure 3 shows the scanning electron microscopy image of sodium alginate-GOAg films. From the SEM image of sodium alginate-GOAg, the interlayer features were observed due to the folding of graphene oxide sheets and alginate. This interlayer might be formed due to the hydrogen bonding induced at GO layer and alginate surface. Besides, the white dots observed in the images correspond to the AgNPs decorated on GO and alginate sheets which, the AgNP were properly dispersed with less agglomeration thanks to the large surface area provided by GO and alginate.

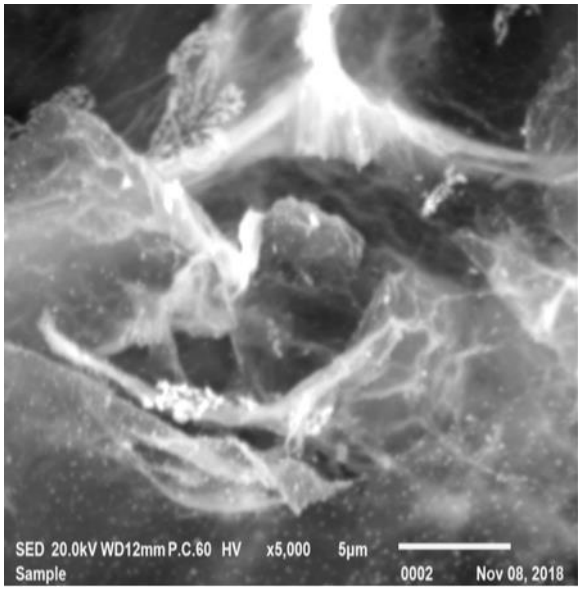

Figure 3. SEM image of sodium alginate GO-Ag film.

3.4. Antibacterial Studies

Antibacterial activity of prepared sodium alginate GO-Ag thin film were tested against two type of bacteria, the Gram-negative and Gram-positive bacteria which are E coli and S. aureus, respectively by applying the disk diffusion method. The zone of inhibition is recorded and measured using a ruler. The outcome show that all the different formulations of sodium alginate GO-Ag thin films are more viable in destroying E. coli than S. aureus. From the observation, S. aureus demonstrate greater resistance towards the sodium alginate GO-Ag thin films than E. coli since the maximum diameter of the inhibition zone for S.aureus is 6.7 mm compared to the maximum inhibition zone of E.coli which is 7.5 mm. This additionally in reliable with the past outcome expressed by Naeem et al. [9]. An increase in the inhibition zone with the increase in volume of GO-Ag nanocomposite is obvious due to the presence of greater antimicrobial agents. The effectiveness of GO-Ag for E. coli than S. aureus bacteria strains is also clear from Table 1 and 2. Sodium alginate GO-Ag thin films demonstrated clear and noteworthy inhibition zones against S. aureus bacterial strain indicating that increasing the concentration of GO-Ag nanocomposite builds its viability against various pathogens.

Table 1. Measurement of inhibition zone in cm after 8, 16, 18, 20 and 24 hours of different ratios of formulations of sodium alginate GO-Ag thin film towards Escherichia coli.

Time (h)	**Inhibition Zone (mm)**				
	5:1	**5:2**	**5:3**	**5:4**	**1:1**
0	0	0	0	0	0
8	6	6	6	6	6
16	6.3	6.5	6.5	6.6	6.7
18	6.3	6.6	6.6	6.7	7.0
20	6.3	6.6	6.7	6.9	7.3
24	6.3	6.7	6.9	7.0	7.5

Similarly, sodium alginate GO-Ag thin films also showed clear and noteworthy inhibition zones against S. aureus bacterial strain indicating that increasing the concentration of GO-Ag nanocomposite increases its viability against different pathogens.

Table 2. Measurement of inhibition zone in cm after 8, 16, 18, 20 and 24 hours of different formulations of sodium alginate GO-Ag thin film towards Staphylococcus aureus.

Time (h)/	Inhibition Zone (mm)				
	5:1	**5:2**	**5:3**	**5:4**	**1:1**
0	0	0	0	0	0
8	6	6	6	6	6
16	6	6	6	6	6
18	6.2	6.2	6.2	6.2	6.3
20	6.2	6.2	6.3	6.3	6.5
24	6.3	6.3	6.4	6.4	6.7

4. Conclusion

In summary, this project has successfully synthesized different formulations sodium alginate GO-Ag thin film. The synthesis of sodium alginate GO-Ag thin films with different ratio of sodium alginate to GO-Ag composite was demonstrated in this research. For the next stage of this research, 1:1 ratio of sodium alginate to GO-Ag composite will be used due to its excellent performance towards both gram-positive and gram-negative bacteria. The antibacterial activity was tested against E. coli and S. aureus demonstrates that the higher the volume of GOAg composite used, the higher the performance of antibacterial activity. All in all, although it was confirmed that the use of GO-Ag composite shows positive results on the antibacterial performance towards E. coli and S. aureus, further investigation must be performed to investigate whether the composite would be suitable for further practical application or not.

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