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Effect of Silver Complex on Graphene Oxide Alginate (GOAlgAg) for Antibacterial Studies

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ABSTRACT

The effect of the silver complex on graphene oxide and its antibacterial studies was reported in this work. Graphene oxide (GO) has a high specific surface area and is well-known for being an ideal substrate material for growing silver nanoparticles. The simplified Hummer's method was chosen to prepare GO and Silver nanoparticles (AgNPs), which have been decorated on the surface of GO during the reduction of silver complex solution $[Ag(NH_3)_2OH]$ in GO aqueous solution. The obtained nanocomposite was analyzed by using Ultraviolet-visible spectrophotometry (UV-Vis) and X-ray diffraction (XRD). The antibacterial performance against Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*B. subtilis)* was investigated using the disk agar diffusion method. The diameter of the inhibition zone indicates the effectiveness of GOAlgAg in preventing these bacteria from growing while displaying the nanocomposite as a promising material for antibacterial-related applications. The best antibacterial properties were found in a sample of 5.0:2.5ml GOAlgAg, which shows the highest inhibition zone, 0.85cm toward *Escherichia coli* and 1.03cm toward *B. subtilis*.

Keywords: Silver complex; Hummer's method; inhibition zone

1. Introduction

In recent years, researchers from a wide range of fields have become attracted by graphene oxide, a material formed from universal and flexible graphene. The unique properties of graphene, such as its extensive surface area, superior electrical conductivity, and remarkable mechanical strength, make it an excellent choice as a supporting material for hosting and dispersing metal nanoparticles [1]. Graphene oxide has special properties, including its huge surface area, chemical reactivity, and potential for a wide range of applications. The flexibility and versatility of graphene oxide are highlights of its uniqueness and significance. Graphene oxide is easily functionalized and adapted to various uses, including enhanced energy storage solutions and sample preparations in analytical chemistry [2].

Silver nanocomposites and graphene oxide have drawn a lot of attention from researchers due to their strong antimicrobial characteristics. Graphene oxide (GO) can damage the bacterial cell membranes and cause cell death due to the antibacterial effect. Silver is a well-known antibacterial agent and has been demonstrated to have a comprehensive approach to preventing bacterial growth and survival. Combination of graphene oxide based with silver complexes resulting in GOAg composite that has better antibacterial qualities compared to the material alone. Therefore, it is crucial to create a straightforward and effective technique for creating silver-graphene oxide nanocomposites [3].

A common technique for synthesizing AgNPs is a reduction of silver nitrate under microwave irradiation; the formation of AgNPs is not homogenous and ineffective. In this work, we utilize the silver complex to develop a new approach for synthesizing silver nanoparticles with improved homogeneity and efficiency. Additionally, microwave-assisted synthesis can be conducted in aqueous media without hazardous organic solvents or harsh reaction conditions, resulting in a more environmentally friendly approach than chemical synthesis methods [4]. The growing interest in the preparation and study of silver nanoparticles is largely due to their discovered antibacterial activities. Various synthetic methods have been explored for producing nanosized metallic silver particles, and one well-developed approach involves the reduction of silver salts by microwave irradiation [5]. In addition to their antibacterial properties, silver nanoparticles' unique optical, photothermal, and surface chemical characteristics have been extensively investigated, revealing their great potential for various biomedical applications, including diagnostics, therapeutics, and other clinical uses [6].

Therefore, this work aims to investigate the optimal ratio at the different ratios of silver complexes for reducing silver nanoparticles on the graphene oxide layers. To the best of our knowledge, the mixture is the best aqueous solution in the reduction of AgNPs on GO layers. Furthermore, the antibacterial properties of graphene-oxide-based nanocomposite are also investigated by gram-positive and gram-negative bacteria.

2. Materials and Methods

2.1 Graphene Oxide (GO) Production via Improved Hummer's Method

Graphene oxide was generated using Hummer's method. First, 320 mL of sulfuric acid (H_2SO_4), 80 mL of phosphoric acid (H_3PO_4), and 18 g of potassium permanganate ($KMnO_4$) were mixed with 3 g of graphite powder. The mixture was gently stirred with a magnetic stirrer on a hot plate for three days to achieve maximum graphite oxidation. Next, a beaker combined 27 mL of hydrogen peroxide (H_2O_2) with 500 mL of ice cube or frozen distilled water. Subsequently, the combination was treated with an ice cube and hydrogen peroxide solution to stop oxidation until the mixture's color became yellow. The mixture was divided equally among four centrifuge bottles to complete the washing process. The solution was repeatedly rinsed with distilled water and 1 M hydrochloric acid (HCl) at 10,000 rpm for 15 minutes on a centrifuge machine until pH 5 was reached. Finally, graphene oxide was ready for usage as its finished product.

2.2 Formation of Silver Graphene Oxide (GOAg) and GOAlgAg

A silver-ammonia complex solution was prepared by adding five different volumes of 10mM silver nitrate ($AgNO_3$) solution to GO solution (2.0:1.0, 3.0:1.5, 4.0:2.0, 5.0:2.5, 6.0:3.0) and combining it with 100µl of ammonia solution while stirring constantly on a hotplate. The mixture was stirred continuously until a yellowish-brown solution was formed. Further, the mixture was synthesized using a microwave for 5 min. After that, the solutions were centrifuged for 15 minutes at 6,000 rpm. The excessive organic substances were eliminated after three washing cycles during the washing procedure. A sodium alginate solution of 5% w/v was added to the samples with a 1:1 sodium alginate solution ratio to the GOAg solution until the solution became homogeneous. The solution was transferred to the petri dish to form GOAlgAg film.

2.3 Characterization

One of the essential steps in identifying the samples' structures is characterization. The absorbance characteristics of the nanocomposites are measured by using (Thermo Fisher Scientific Evolution 350) UV-Vis Spectrophotometer scan at 420 nm and X-ray diffraction (Bruker D2 Phaser) with Kα source (λ=1.5406 Å).

2.3 Antibacterial Studies

The antibacterial performance against gram-positive and gram-negative bacteria was done by growing *B. subtilis* and *Escherichia coli.* A single bacterial colony will be grown in luria bertani broth (LB broth), and subsequently placed at incubator shakers for the reading of optical density (OD) at 600 nm. After that, when OD is achieved at 0.8-1.0, that bacteria will proceed for the swabbing into new nutrient agar. The disk diffusion method was used to measure the inhibition zone of the bacteria by dipping the paper disk into GOAg solutions and GO/Alg solutions and continuing incubation in a 30 °C incubator. The diameter of the inhibition zone of the bacteria was measured for 0, 8, 16, 20, and 24h.

3. Results and Discussion

Fig. 1 shows the absorption peak of GO, GOAlgAg, and GOAlg, which are recorded at 420 nm. GO peak shows an absorption peak at 230 nm corresponding to the π-π* transitions of aromatic C-C bonds, while GOAlgAg peak shows the absorption peak at 420 nm. The highest peak indicates the formation of silver nanoparticles on the GO layer. As shown in the Fig. 1, the plot absorbance was proportional to the concentration (Fig. 1(a) - 1(c) and the highest absorbance peak at 5.0:2.5 ml (Fig 1(d)) indicates the maximum formation of AgNPs, suggesting the optimum concentration ratio for the reduction of AgNPs in GO. Meanwhile, at the ratios 6.0:3.0 ml (Fig. 1(e)), the absorbance peak starts to decline, suggesting that the Surface Plasmon Resonance (SPR) of AgNPs is disturbed. This is due to the excessive concentration still not being reduced, which affected the surface area of AgNPs [7]. The optical properties affect the SPR of AgNPs since the proton energy will be converted into thermal energy [8]. The size, shape, and surface chemistry of AgNPs are influenced by their optical properties, distorting the SPR properties. These findings are similar to the report by Lou-Franco et al. [9].

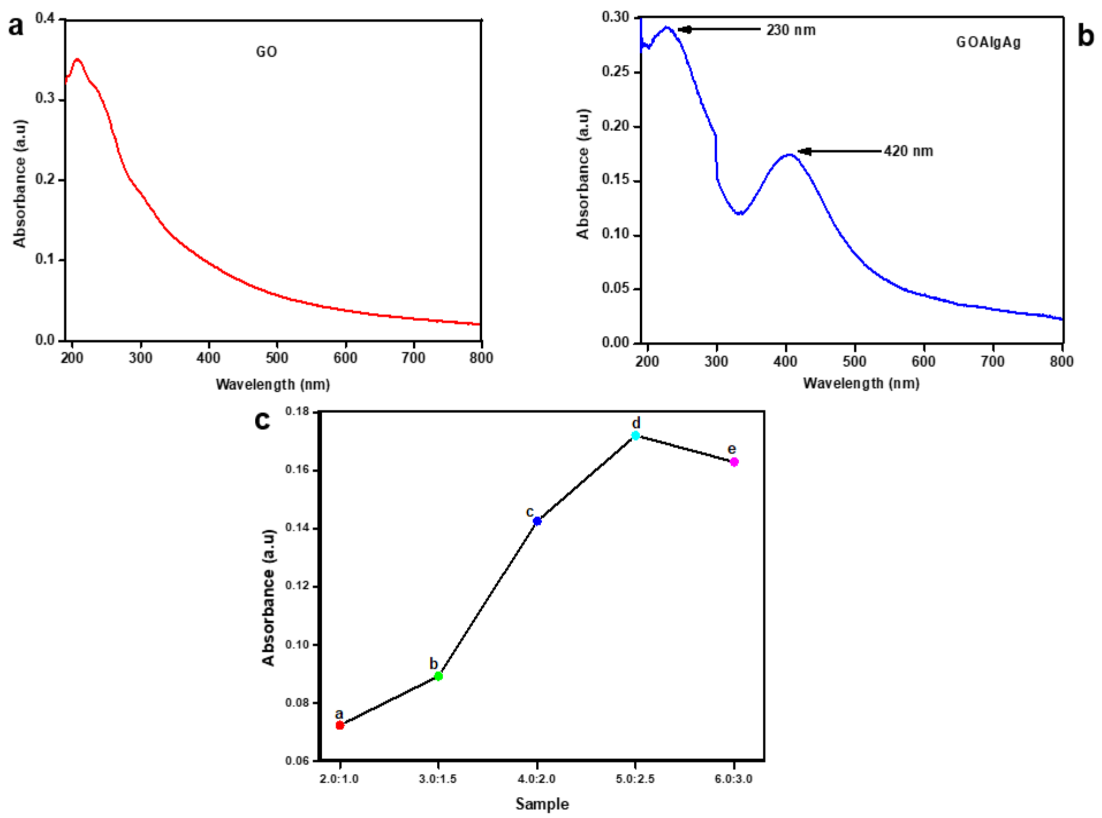

Fig. 1: UV-Vis for a) GO, b) GOAlgAg, and c) Highest absorption peak of GO/Alg at 420 nm for a ratio of (a) 2.0:1.0 ml, (b) 3.0:1.5 ml, (c) 4.0:2.0 ml, (d) 5.0:2.5 ml and (e) 6.0:3.0 ml

Fig. 2 shows an XRD pattern of GO/Alg and the percent crystallinity of GO/Alg at five different concentration ratios of silver complexes to the GO solution. Figure 2 indicated that the GO peak appearing at 13º corresponds to the (0 0 1) after the synthesis process of GO/Alg (Fig. 2A). After the microwave irradiation process, the diffraction peaks of Ag also appeared to correspond to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) crystal planes to face-centered cubic (fcc). In the GO/Alg nanocomposite synthesis, the percent crystallinity of the Ag diffraction peaks increased as the concentration of silver complexes increased (Fig 2B). These findings verify that AgNPs are fully formed on GO layers under microwave irradiation and have a greater Ag content. However, the optimal ratio of 5.0:2.5 ml resulted in the highest crystallinity and most well-defined Ag diffraction peaks, indicating the highest level of AgNP formation on the GO sheets. The peak decreased at 6.0:3.0 ml after an optimal ratio that shows exceeding the optimal Ag concentration leads to aggregation of silver nanoparticles on the GO surface [10].

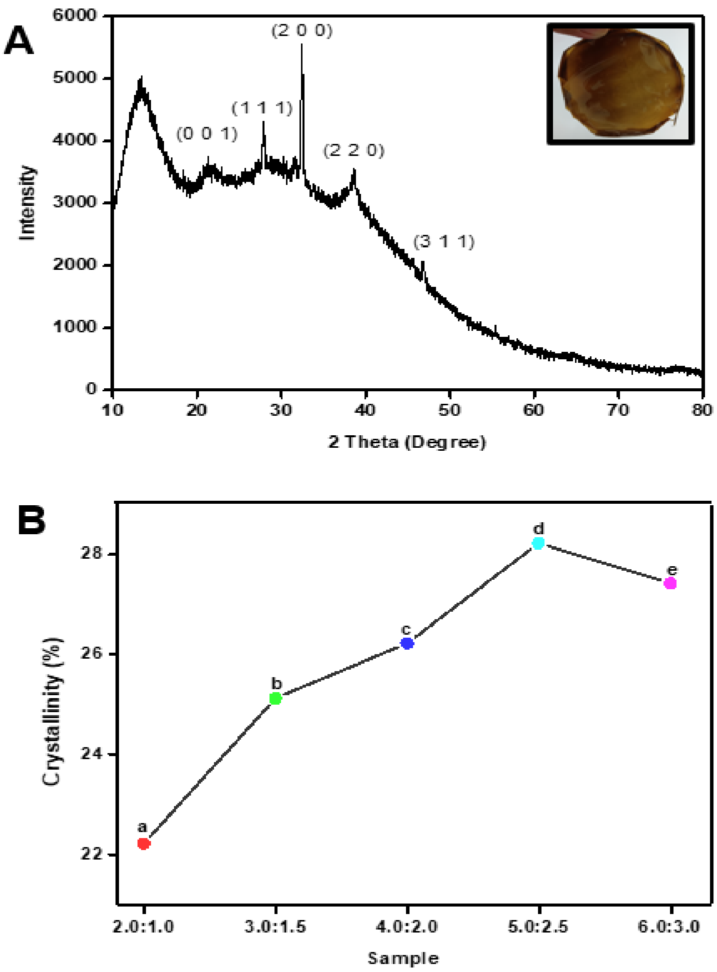

Fig. 2: XRD pattern of A) GO/Alg and B) percent crystallinity of GO/Alg at different concentration ratios of (a) 2.0:1.0 ml, (b) 3.0:1.5 ml, (c) 4.0:2.0 ml, (d) 5.0:2.5 ml and (e) 6.0:3.0 ml. (Inset:GOAlgAg film at the preparation of 5.0:2.5 ml)

Antibacterial Studies of GO/Alg samples were tested against two types of bacteria, *Escherichia coli* as Gram-negative bacteria and *B. subtilis* as Gram-positive bacteria. Tables 1 and 2 shows the GO/Alg samples were more efficient against *B. subtilis* than *E.coli* because *B. subtilis* has greater antibacterial activity than *E.coli* since the maximum diameter of the inhibition zone for *B. subtilis* is 1.03 cm compared to the maximum diameter of *E.coli* is 0.85 cm. The cell wall structure of the gram-positive bacteria is more susceptible to the antibacterial effects and most effective in inhibiting bacterial growth compared to gram-negative bacteria [11].

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Table 1: Inhibition zone of GO/Alg toward *E.coli* after 8h, 16h, 20h and 24h.

Time (h)	Inhibition zone (cm)				
	2.0:1.0	**3.0:1.5**	**4.0:2.0**	**5.0:2.5**	**6.0:3.0**
0	0	0	0	0	0
8	0.53	0.54	0.57	0.60	0.58
16	0.62	0.63	0.65	0.70	0.67
20	0.70	0.72	0.75	0.79	0.75
24	0.78	0.80	0.82	0.85	0.83

Additionally, the interaction of both bacteria reported the highest diameter of the inhibition zone in a concentration ratio of 5.0:2.5 ml due to the optimal antibacterial activity and dispersion. This 5.0:2.5 ml ratio provided the right balance between the amount of silver ions available for antibacterial effects and the stabilizing influence of the graphene oxide, which helps prevent the agglomeration of the nanoparticles. This allows the silver nanoparticles to effectively interact with and disrupt the cell membranes of both bacteria to enhance the overall antibacterial performance [12].

Table 2: Inhibition zone of GO/Alg toward *B. subtilis* after 8h, 16h, 20h and 24h.

Time (h)	Inhibition zone (cm)				
	2.0:1.0	**3.0:1.5**	**4.0:2.0**	**5.0:2.5**	**6.0:3.0**
0	0	0	0	0	0
8	0.67	0.72	0.75	0.81	0.77
16	0.76	0.80	0.83	0.87	0.85
20	0.81	0.83	0.86	0.93	0.88
24	0.85	0.87	0.91	1.03	0.96

4. Conclusion

In summary, this work has successfully synthesized the different concentration ratios of silver complexes to the GO and AlAg solution. It was found that the concentration ratio of 5.0:2.5 ml is the best and optimal concentration ratio of silver complexes to graphene oxide for antibacterial studies. The antibacterial studies of GOAlgAg composite were tested against *E.coli* and *B. subtilis* and showed positive results for antibacterial performance with a value of 0.85cm and 1.03cm, respectively.

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