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# Antibacterial Activity with Hummer's Method for Nano Graphene Oxide

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## Abstract
The current work describes how Schiff's reaction was used to create unique nanoparticles. Particularly, graphene oxide and hydrazine hydrate combined with astatine and guanine, respectively, to produce Schiff bases (II and III). On the other hand, graphite was added to a solution of sodium nitrate and strong sulfuric acid using Hummer's method, which also involves the use of oxidizing chemicals like hydrogen peroxide and potassium permanganate, to produce nanoparticle graphene oxide (I). A range of positive and negative gram bacteria were used to test the manufactured nanocomposite's biological activity using the Mueller-Hinton Agar well diffusion method. According to the research, the Nano Schiff base II exhibits an interesting inhibitory function, especially when it comes to Staphylococcus aureus germs.

***Keywords*:** Graphene oxide; Schiff base; Hummer’s method; Antibacterial activity

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## 1. Introduction

In the last few years, Schiff reaction response presents the most well-known technique for the preparation of many organic compounds. Schiff base derivatives have exhibited a variety of interesting properties [1, 2]. The general protocol of Schiff bases synthesis is often carried out with acid- catalyzed and generally by refluxing the mixture of amine and ketone (or aldehyde) in the organic medium [3-4]. Schiff bases received considerable attention in different areas because of their fascinating synthetic chemical and physical properties [5-8], these proprieties including intermediates on the organic synthesis [9,10], polymer stabilizers [11], dyes and tinctures [12]. Additionally, Schiff bases were possessed various assorted pharmacological enacts like [13, 14] antifungal [15], anti-malarial [16], antibacterial [17], anti-proliferative [18], anti - viral, anti - inflammatory [19] and anti- pyretic properties [20]. The biological activities such as compounds comes from the existence of the azo-methine group (C=N) [21] that have lone-paired of pi electrons in an Sp2 hybridized orbital of nitrogen particle [22-23].

However, Graphene oxide (GO) is a particle with two-dimensional structure and it may be known as a two-dimensional polymer (2D) made up from the covalently bind of oxygen-derivative carbon atoms. It is an exceptional material with superior properties, this is due to its small size and large surface areas [24]. It possesses multiple wonderful attributes characteristics that are originating from its chemical structures composed of various functional groups such as hydroxyl, epoxy, and carboxyl groups and [25]. Because of the low toxicity of graphene oxide and exceptional physical and chemical properties, it gained a widely antimicrobial property [26] and focused on drug delivery [27].

## 2. MATERIAL AND METHODS

### 2.1.1 Material

All chemicals Graphite, hydrazine hydrate, Isatin, Guanine, Sodium nitrate, potassium permanganate and hydrogen peroxide got from Fluka and Sigma-Aldrich Co., which were used as received.

### 2.1.2 Instruments

Fourier-transform infrared spectroscopy (FT-IR) for title compounds were recorded using Shimadzu Fourier Transform WQF-520 Spectrophotometer (400-4000 $cm^{-1}$).

### 2.1.3 Antibacterial Testing

All equipment’s required and bacteria, *Staphylococcus aureus*, *Bacillus thyuringiensis* and *Pseudomonas aeruginosa*, formamide (solvent), were acquired from the Ministry of Science and Technology, Industrial Microbiology Department.

**2.1.4 Microbiological tests**

The microorganisms used to commence microbiological tests are: (1) Staphylococcus aureus is Satisfactory bacteria and was isolated from burned skin, (2) Bacillus thyuringiensis 13 is Ecologic and was isolate from minced meat (3) Bacillus thyuringiensis 13, (4) Bacillus thyuringiensis esp., (5) Bacillus thyuringiensis 3, and (6) Bacillus thyuringiensis bacteria are Biological Control, (7) Pseudomonas aeruginosa 17 which is Environmental bacteria and was isolated from water, finally (8) Pseudomonas Iutllae 26 that was isolated from the rumen of the camel.

**2.2- Synthesis**

**2.2.1 Synthesis of graphene oxide (GO) Nanoparticle (I)**

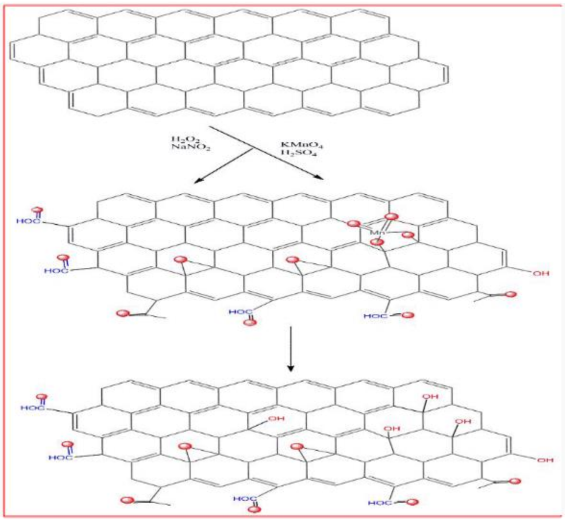
In a suitable 50 ml beaker concentrated sulfuric acid (23 ml) was added and placed in an ice- bath to reduce the homogeneity of the temperature, then, 0.5 g, 0.005 mole of sodium nitrate was added gradually for about 15 minutes with constant stirring. After that, 0.2 g of graphite dust (G) was added step by step for 10 minutes with continuous stirring. A 3 g, 0.021 mole of potassium permanganate which was added for about 15 minutes with stirring. The mixture left in an ice-bath for 5 minutes, then it was stirred at room temperature at 25°C for 60 minutes. After that, 46 ml of distilled water dropped for 20 minutes, warm water (140 ml) added and the mixture left to stir for period 10 minutes, then 9 ml of hydrogen peroxide 30% added with continued stirring for 30 minutes. The mixture was cooled and filtered with special filter paper and washed with distilled water (3x5 ml), dried at a temperature of 60-70°C until the weight is stable (4 hours) (Scheme 1). Percentage of compound was 85 % with Black color.

**2.2.2 Synthesis of Nano Schiff bases II and III**

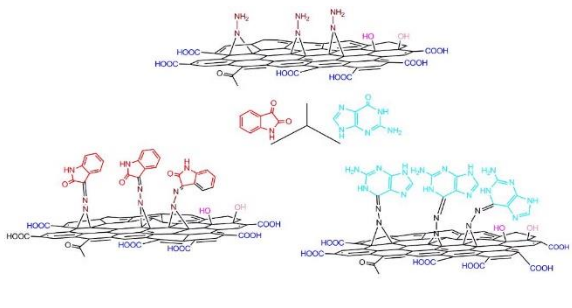
Graphene oxide (0.1 g) mixed with (2mL) of hydrazine hydrated 80% (without solvent), then the mixture was put on soil-bath until the color and the nature of compound changed. After that, (0.1 g) of isatin, guanine was added respectively. The mixture heated in mental until the color and the texture of the synthesized nanoparticle was changed. The nanocomposites (**II** and **III**) were recrystallized from ethanol (Scheme 2) Yield (%) and Colors for the synthesized nanoparticles was 70 and 75 and pale gray.

**2.2.3 Antimicrobial activity of Nano particles**

The antimicrobial activity of nanoparticles was determined by using the agar well diffusion method on Mueller-Hinton agar [28]. Sterilized medium Muller Hinton agar was cooled to about (50± 2 ℃), then it was poured into petri-dish and allowed to gel firmly before inoculating by bacterial species. Bacterial cultures of *Staphylococcus aureus*, *Bacillus thyuringiensis*, and *Pseudomonas aeruginosa*, were used as challenge specimens. Bacterial cultures were crashed out from the nutrient agar plate and suspended in sterilized peptone water. McFarland standard tube number 1 was applied for bacterial turbidity evaluation; it equivalents approximately amounts to (1X108 CFU/mL). A cotton swab was immersed in the bacterial suspension and spread over Muller Hinton agar which let stand for about 10 min to ensure bacterial adherence. Meanwhile, the borer applicator was sterilized by flame, cooled, and pressed on the top of seeded Muller Hinton agar to make a well with a 7 mm radius. Distances were kept away between wells because of the aspect of the plate. Each well was filled with 100, 50, and 25 µl of a solution containing nanoparticles; control wells were filled with 100, 50, and 25 µl of formamide. Plates were left for 10 minutes, in the laboratory and then were incubated for 24 h at 37 °C. Three replicas of each plate were prepared and the diameter of the inhibition zone was recorded from the edge of the well to the end of the halo zone. The inhibition zone was measured in mm




**Scheme (1) Synthesis of graphene oxide (GO) Nanoparticle (I) by Hummer's method from graphite**



II III

**Scheme (2) Synthesis of Nano Schiff particles II and III particles by composite method**

## 3. Results and Discussion

### 3.1 FTIR analysis

FT-IR of the synthesized nanoparticles (**I –III**) were recorded in KBr medium in the spectral range 4000–500 $cm^{-1}$. Graphene oxide (GO) was synthesized by using Hummers' method without any alteration [29], the mechanism of the reaction was shown in (Scheme 1). FT-IR of nanoparticle GO I (Fig. 1) showed a broad absorption band at (3200-3300) cm-1 due to the overlapping of *v* (O-H str.) of carboxylic and alcohol groups. Another absorption band was at 1650-1670 $cm^{-1}$ for the overlapping *v* (C=O str.) of carboxylic and ketones groups, stretching band at 1200 cm-1 due to *v* (C-OH str.) and stretching band at 1060 cm-1 attributed to *v* (C-O str.) of epoxy groups.

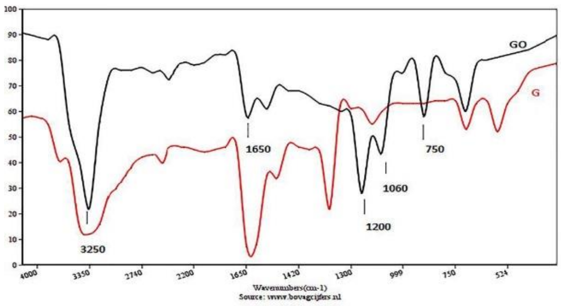



**Fig. (1) The FT-IR spectrum [1] graphite (G) and [2] graphene oxide I (GO)**

FTIR spectrum of nanoparticle compound II (Figure-2) had shown a number of IR absorption bands 1730 for cm-1 for *v* (C=O str.) of five-member isatine ring, 1618 cm-1 for v (C=N str.) group, stretching vibration occurs at (3192-3109) cm-1 assignable to *v* (NH str.), bands at 3060-3041 cm-1 for *v* (C-H str.) of aromatic, sharp band at1462 cm-1 due to *v* (C=C str.). A stretching band was at 3446 cm-1 due to the overlapping of OH carboxylic and hydroxyl groups of GO. The FTIR of nanoparticle III (Figure- 3) showed a new sharp band at 1697cm-1 which was attributed to *v* (C=O str.) of six-member purine ring, stretching band at 1674 cm -1 due to carbonyl groups for carboxylic acid and ketones. Another stretching bands were at 1560 cm -1 for *v* (C=N str.), (1473-1417) cm-1 for *v* (C=C str.) aromatic groups. It was observed that a broad band of (3113-3170) cm-1 was assigned to *v* (NH, NH2 str.). Broadband at 3327 cm-1 was due to the overlapping of O-H stretching vibration for both carboxylic and hydroxyl groups of GO. A bands at (2991- 2906) cm-1 due to the *v* (C-H str.) of aromatic.

### 3.2 Antibacterial activity

The microbial activity of the nanoparticles Schiff bases (II and III) were tested in vitro by using several types of bacteria and using the agar well diffusion method. The tested bacteria were Pathological and Environmental isolates. The nanocomposites exhibited different bioactivity ranging from (high, medium, and low) effect. The biological activity was examined by using different concentrations (25, 50, and 100 µL) and types of bacteria to increase the chance of detecting antibiotic principles in tested materials.

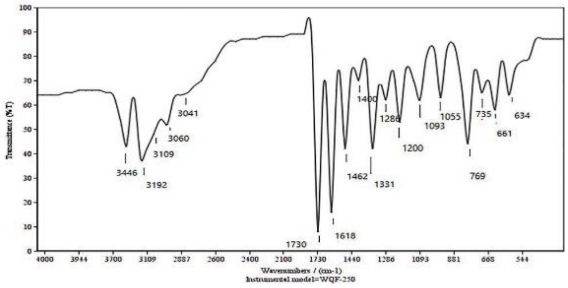



**Fig. (2) The FTIR spectrum of Nano particle (II)**

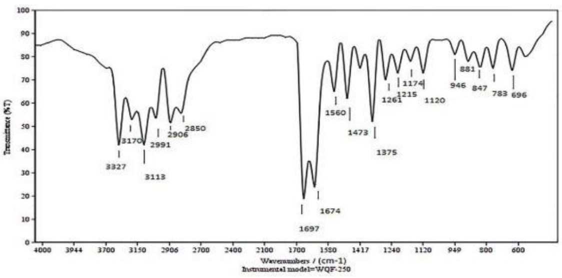



**Fig. (3) The FT-IR spectrum of Nano particle (III)**

The results of the bactericidal examination are listed in Tables 1 and 2. In this paper, we used GO as drug delivery [30]. The effect of the nanoparticle Schiff base II in the anti-bacterial activity against the tested Gram (+ve) and Gram (-ve) organism's exhibits that the synthesized nano Schiff base can be used in treating the bacteria associated with burns as well as its activity against environmental bacteria that used extensively in biological control program against insects. A reason was given for an active presence of imine groups, also due to p-electron delocalization, on the whole, the nanoparticles that affected.

This figure shows the antibacterial activity of Nanoparticle (II) against tested bacteria in 100, 50 and 25 µL.

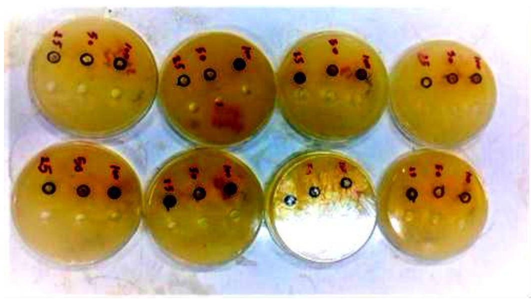


**Fig. (4) Antibacterial activity of Nanoparticle (II) against tested bacteria**

Furthermore, the synthesized nno Schiff base III was found to have moderate activity against Staphylococcus aureus only at high concentration 100µL and low action against Bacillus thyuringiensis at concentration 100µL, which indicated for dose dependent activity of nano Schiff base II as presented in (Fig. 5).

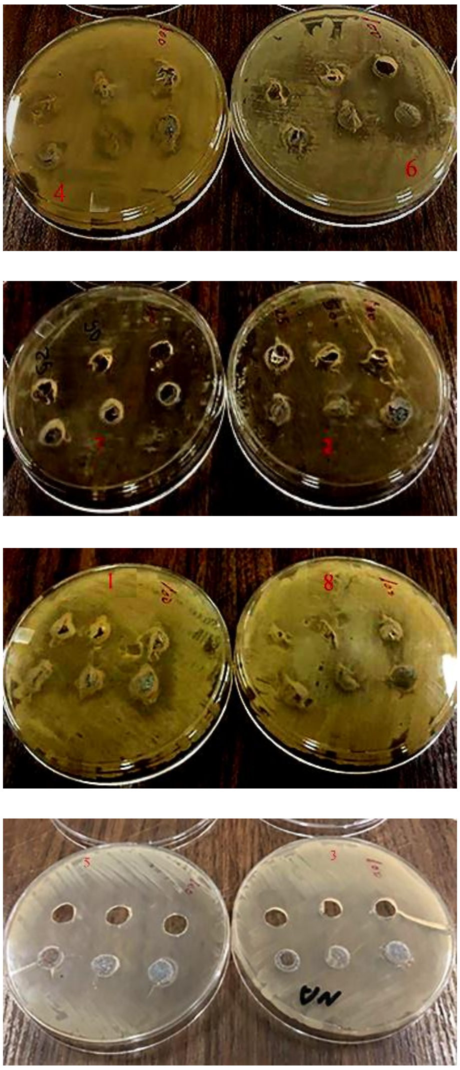


**Fig. (5) Antibacterial activity of Nanoparticle (III) against the tested bacteria**

**Table (1) Anti-bacterial activity of nano Schiff base compound II**

| Tested bacteria | Concentration 100µL | Concentration 50µL | Concentration 25µL |
|---|---|---|---|
| *Staphylococcus aureus* | 4 | 8.4 | 5.4 |
| *Bacillus thyuringiensis 13* | 5.4 | 5.4 | 4 |
| *Bacillus thyuringiensis esp.* | 5.4 | 8 | 4 |
| *Bacillus thyuringiensis 3* | 5.4 | 4 | - |
| *Bacillus thyuringiensis* | 4 | 5.4 | 4 |
| *Pseudomonas aeruginosa* | - | 7 | 6.4 |
| *Staphylococcus aureus* | - | 4 | 5 |
| *Pseudomonas lutellae 26* | 4 | 5.4 | 5 |

**Table (2) The *in vitro* anti–bacterial activity of nano Schiff base compound III, inhibition zone (mm)**

| Tested bacteria | Concentration 100µL | Concentration 50µL | Concentration 25µL |
|---|---|---|---|
| *Staphylococcus aureus* | 5 | 2 | - |
| *Bacillus thyuringiensis 13* | - | - | - |
| *Bacillus thyuringiensis esp.* | 5 | - | - |
| *Bacillus thyuringiensis 3* | - | - | - |
| *Bacillus thyuringiensis* | 5 | - | - |
| *Pseudomonas aeruginosa* | 5 | 5 | - |
| *Staphylococcus aureus* | - | - | - |
| *Pseudomonas lutellae 26* | - | - | - |

*µL = Microliters, (-)= no inhibition*

## 4. Conclusion

In this paper, we reported the preparation of the nanocomposites by using the Schiff's reaction which has been diagnosed by using FT-IR technology. The biological activity was studied for the nanocomposites on different types of bacteria. We concluded that the nanocomposite II that was prepared from the combination reaction of graphene oxide and Isatin has a wide range of inhibition for gram positive bacteria and gram negative bacterial species. Moreover, nanocomposite III has a low bacterial inhibition capacity comparing with the first nanoparticle. In the future, we believe that it is possible to use nanoparticle II as an ointment to treat S. aureus that extensively associated with infections of burn especially with long period of hospitalized patients. Graphene oxide (GO) possesses multi properties that make it more attractive for biomedical applications and it can be used as a therapeutic delivery. Finally, the main reason for the effort is due to the unique effect of the nanocomposites as bacterial inhibitors which could provide usefulness in biomedicine and therapy.

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