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Chemical method of preparation reduce graphine oxide_Ag nanoparticles and its applications

A Graduation Project Submitted to the College of Pharmacy – University of Babylon Partial Fulfillment of the Requirement for the B.S. In Pharmacy

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(مَنْ عَمِلَ صَالِحًا مِنْ ذَكَرٍ أَوْ أُنْثَى وَهُوَ مُؤْمِنٌ فَلَنُحْيِيَنَّهُ حَيَاةً طَيِّبَةً)

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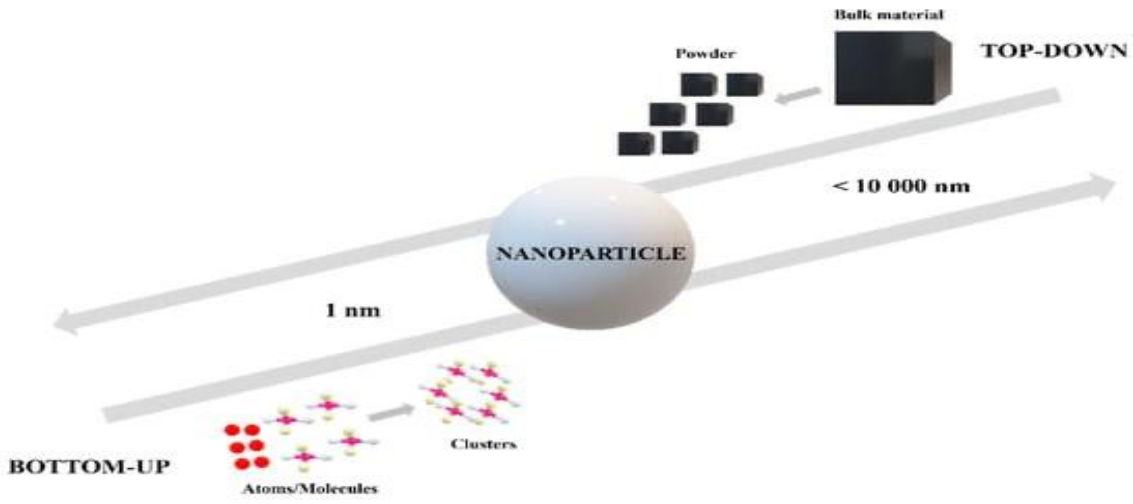
Abstract

This research document focuses on the preparation and applications of reduced graphene oxide- silver nanomaterial. Graphene and its derivatives, such as graphene oxide, a monolayer sheet of graphite, serves as a precursor for reduced graphene oxide (rGO) synthesis. GO exhibits hydrophilic properties, making it water-soluble, and its surface functionalization has led to numerous opportunities for the development of nanocomposite materials. The incorporation of silver nanoparticles (AgNPs) onto GO surfaces its properties, as Ag is highly conductive and reactive. The research addresses the reduction of GO to rGO through chemical methods, which often involve toxic and environmentally harmful. These methods are described as convenient, inexpensive, and capable of strict control over nanoparticle size and morphology.The compound was examined byUV-Visible Spectophotometer wich showen tow peaks (250nm) indicated for partial reduction graphene oxide NPs and (410nm) indicated for Ag NPs ,and (SEM) techniques Which showed the diameters of the nanoparticles about (18.61, 32.61, 47.01) nm .This technique show Required characteristics To be applied In various fields such as antibacterial, antifungal, anticancer,as drug dilvery system and so on .

KEYWORDS :graphene oxide- silver; chemical reduction ; silver nanoparticles; sacannig electron microscopy ; UV spectrophotometer ; antibacterial ; antifungal, anticancer; drug dilvery system

Introduction

Over the last few decades with the advent of nanotechnology, scientists have drawn extreme research interest regarding nanomaterial development. Among the nanomaterials, Nanoparticles are complex molecules with nanometric dimensions less than 100 nanometers and structures composed of three layers. The outside layer allows for the functionalization with other molecules, such as metal ions or polymers. The middle layer, chemically different from the core, assures the bond between the inner of the particle and the outside part. The core of the nanoparticle represents the nanoparticle's material itself [1].
Nanoparticles can be synthesized through different approaches. In general, there are two directions: "top-down" and "bottom-up" methods. Depending on the precursors and materials used, the two approaches are based on different technologies. The "top to bottom" method is based on a bulk material transformation into small particles, while the "bottom to top" method involves the production of nanoparticles using chemical reagents able to assemble atoms to "seed" nuclei which will grow further into clusters and particles of nanometric dimensions, as can be observed in Figure 1 [2,3,4]. Organic particles are usually obtained through bottom-up methods such as chemical reduction, sol-gel, emulsification, or self-assembly processes. These methods lead, in most cases, to nanoparticle fabrication in a spherical form and in a polydisperse size distribution due to the inherent surface tension that makes itself manifest. Besides organic particles, inorganic nanoparticles in different shapes can be obtained through bottom-up techniques, as well, for example, by a nucleation process [5].



(2)

Figure 1. Top-down methods versus bottom-up methods from a nanoparticles perspective.[44}

graphene and related compounds have emerged as a distinctive class of materials because of their unique structure and functionalities. Graphene oxide (GO), a monolayer sheet of graphite, serves as a precursor for the synthesis of reduced graphene oxide (rGO)[6]. Like graphene, GO has a similar hexagonal carbon structure with hydroxyl (−OH), alkoxy (C–O–C), carbonyl (C–O), carboxylic acid (−COOH), and other oxygen-based functional groups [7,8]. Due to the presence of these functional groups, GO exhibits hydrophilic character that makes it a water-soluble nanomaterial[9]. Moreover, the surface functionalization of GO has presented many opportunities regarding its application in the development of nanocomposite materials. GO has high conductivity and shows diverse applications in the field such as sensors, anticancer properties, electronics, biomedicine antibacterial coatings, photocatalytic activity, water decontamination, solar desalination, and drug delivery [10−26]‘

However, the nanocomposite enhances GO properties. Among all transition elements, silver (Ag) is the most conductive and reactive material and has also been used recently in fabricating silver-doped graphene oxide with favorable properties of low resistance, good dispersion, and enhanced mechanical strength[27]. Recently, due to its bactericidal nature, AgNPs are getting much attention in antibacterial applications[28].AgNPs are dispersed on the surface of GO and intercalated in between the layers of GO to study the electrochemical properties and photocatalytic dye degradation rate of the samples. GO reduction by chemi- cal methods results in the formation of limited solubility or even irreversible agglomerates of rGO during preparation in water and most organic solvents . The most commonly used chemical reducing agents are hydrous hydrazine,[30] hydrazine monohydrate, sodium borohydride, and hydrogen sulfide, which are highly toxic to living organisms and the environment[29,31]. Several laboratories have developed biological reducing and stabilizing agents, such as ascorbic acid[32] amino acids,[33] melatonin‘[34] glucose ,[29] humanin,[35] microorganisms,[36-37] and plant extracts. There are many techniques available to synthesize graphene which include physical techniques like mi- cro-mechanical cleavage of highly oriented pyrolytic graphite, thermal decomposition of SiC substrate and various chemical va- pour deposition methods. Although these techniques produce good quality of graphene, having good quantity of graphene along with good quality is possible through chemical route.

The toxic, explosive and corrosive properties of the reductants made us to look for non-toxic and environmentally benign methods for rGO synthesis ، The drawbacks of chemical methods can be overcome thru eco-friendly reducers such as extracts of plants, microbes & other naturally available resources (biological methods)[38][39][40].
Chemical methods for the synthesis of metallic nanoparticles are the most widespread, the most numerous, and at the same time, the most efficient ones. These methods are described as easy, convenient, inexpensive (for large-scale production), and quick to carry out while not requiring the use of complex apparatus. Moreover, the final nanoparticles can be stored for long periods of time without significant loss in stability [41]. An important aspect of the synthesis of nanoparticles by the chemical reduction method is that their size can be strictly controlled, allowing the synthesis of nanoparticles with different morphologies. Further, the method can be easily scaled up for large-scale preparation without the need for high pressure, energy, and temperature [42].
Because of its simplicity, the main chemical method for the synthesis of metal nanoparticles is the reduction in metal ions in solution (chemical reduction method) [43].

Chemical method

Chemical synthesis methods aim to achieve precise control over the size, shape, composition, and structure of nanomaterials. By manipulating these parameters, researchers can tailor the properties of nanomaterials to meet specific requirements.

Materials :
Silver nitrate ($AgNO_3$), Sodium Borohydride ($NaBH_4$) and Graphene oxide were purchased from market commercial, distilled water.

Method of synthesis of silver nanoparticles with graphene oxide :
The preparation of rGO/Ag nanocomposite was done by immediately chemical reduction of GO and $AgNO_3$ as a source of produce a composite in nano-scale with sodium borohydride ($NaBH_4$) as an agent. Practically, Concentration of GO (1 mg mL-1) dissolved in distilled water and sonicated for 20 min to become homogeneous. Plus, $AgNO_3$ (3.4 mg) was added to 5 mL of GO (1 mg mL-1) then the mixture was sonicated for 30 min.

The aqueous solution of 5 mL of NaBH4 (0.1 M) added to the mixture at room temperature.
Spontaneously,the color of the mixture turns from light yellow to dark yellow, then purple, and then dark brown. Then the rGO-Ag NPs nanocomposites were obtained by centrifugation at 8000 rpm, washed with distilled water to get rid of impurities and reduce toxicity. Finally, the rGO-Ag NPs is ready for analysis.
On the other hand, silver nanoparticles were prepared without graphene oxide, mix 5 mL from 0.3 mM of AgNO3 and 5 mL from 0.1M of NaBH4, in the same way.

Result and discussion

Scanning Electron Microscopy (SEM): SEM allows for the visualization of the morphology and distribution of silver nanoparticles on the rGO sheets
• UV-Vis Spectroscopy: UV-Vis spectroscopy can be used to analyze the optical properties and absorbance characteristics of the nanocomposites , so To study the optical properties of rGO-Ag NPs and Ag NPs are checked used UV-Visible Spectrophotometer, the results are shown in figure (2)

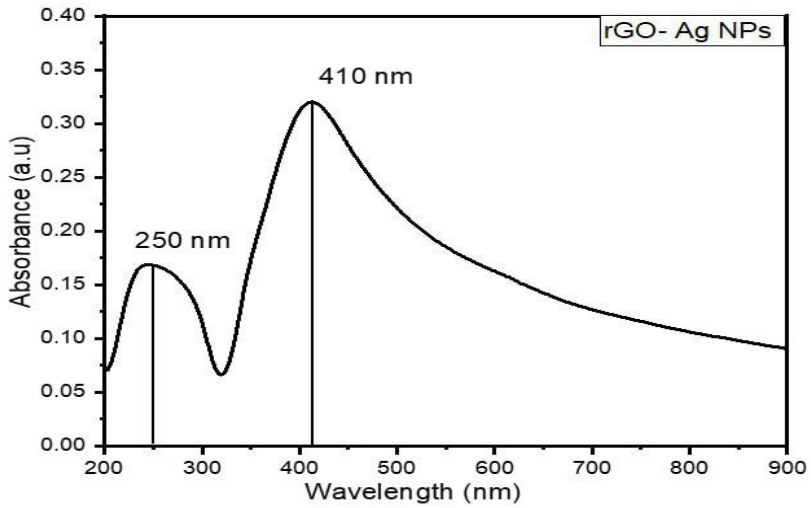


Fig.2 UV-VIS of rGO-Ag NPs

Figure (2) Show the Uv-vis absorption for rGO-Ag NPs. a curve for rGO -Ag NPs shown two peaks ,

, one at(410 nm) refers to Ag NPs and second at (250nm) refers to partial reduced graphene oxide represented the transitions between $\pi = \pi^*$ in C = C bound, the two peaks have redshift that indicates the nanoparticles of silver seemed to be getting smaller and the graphene oxide has been reduced, this indicates the removal of the oxygen groups and the close proximity to graphene.

The curve in figure(2)indicated that the silver nanoparticles have the anchor on the reduced graphene sheets. This result compatible with other reports[45]

The morphology of rGO-Ag NPs shown in figure (3), the images show that the reduced graphene oxide was wrinkled and the silver nanoparticles have homogeneous distribution over rGO sheets. Moreover, all the silver nanoparticles Ag NPs are spherical, and from the figure (3)the aggregation of Ag NPs is absent, approximately. The size for silver nanoparticles on average diameters , the diameters shown in SEM about (18.61,32.61,47.01)nm and they in range .

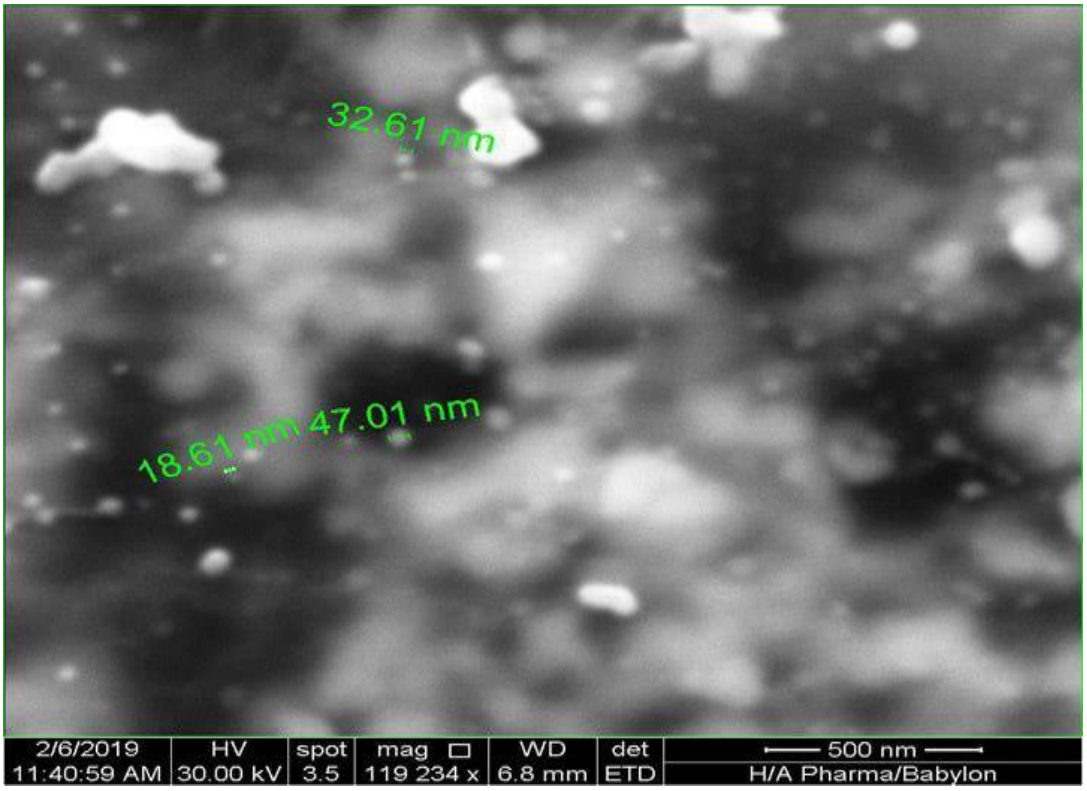


fig (3)SEM for rGO-Ag nanoparticles

Application

applications of reduced graphene oxide-silver nanomaterials:

Reduced graphene oxide-silver nanomaterials have shown great potential in various applications due to their unique properties and synergistic effects. Here are some notable applications of reduced graphene oxide-silver nanomaterials:

Antibacterial activity

In terms of biology, both rGO and AgNPs have antibacterial activities. rGO, however, is not highly effective due to rGO sheet stacking via π-π interac- tions [46,47]. AgNPs do not exert lasting effects because of self-conglomerate. To solve these pro- blems, rGO and AgNPs are combined to form Ag/ rGO nanocomposite. In the structure of Ag/rGO nanocomposite, AgNPs are fixed to the rGO sheets to limit the agglomeration of AgNPs. Meanwhile, AgNPs increase the distance between the rGO layers, resulting in the new material with higher antibacterial activity than the precursor materials [48].The antibacterial activities of Ag/rGO nanocompo- sites were tested against P. aeruginosa and S. aureus by optical density and plate colony-counting methods [49,50,51].
The antibacterial activities of rGO and AgNPs were also compared with the nanocomposites.
Previously, exposure of S. aureus cells to rGO–nAg nanocomposite has been shown to result in cell wrinkling and damage, with some cells being completely covered by the rGO–nAg, whereas exposure of E. coli to the same concentrations of rGO–nAg led to complete cell fragmentation[52]. In other words, for Gram-negative E. coli, the primary mechanism of rGO–nAg bactericidal activity is through disruption of bacterial cell wall integrity, whereas for Gram-positive S. aureus, the effect is bacteriostatic and is associated with dramatic hindering of cell growth[52].They showed that the ratio of nano-silver and GO is important for the antibacterial activity of Ag/GO. They also showed that the bactericidal effect of nano-silver coated onto GO is due to the destruction of the cell membrane of E. coli and the inhibition of S. aureus cell division.

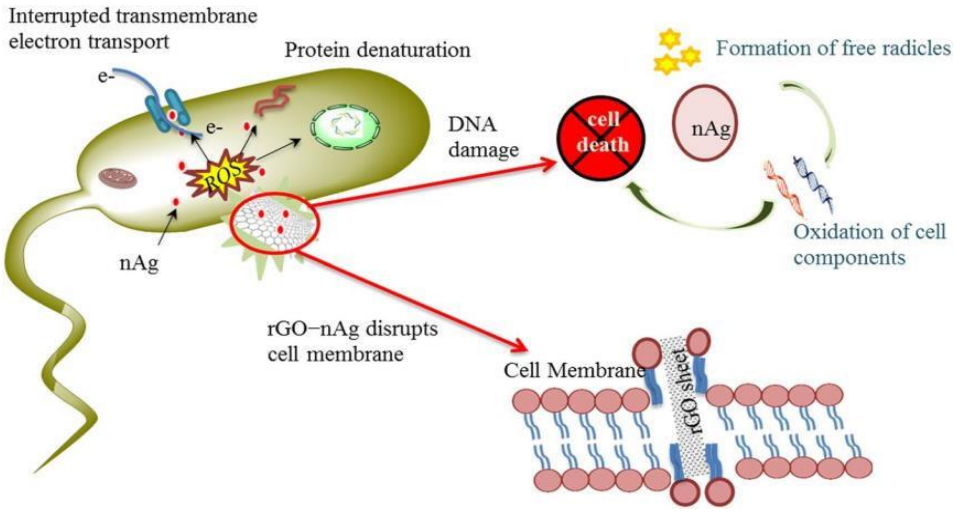


Figure 4. A symbolic representation of the mechanism by which the rGO–nAg nanoparticles kill the bacteria.
$e rGO punctures cell wall and enter the cytoplasm. Silver nanoparticles directly enter into the cell, induces oxidative stress and damage the cell contents.[53]

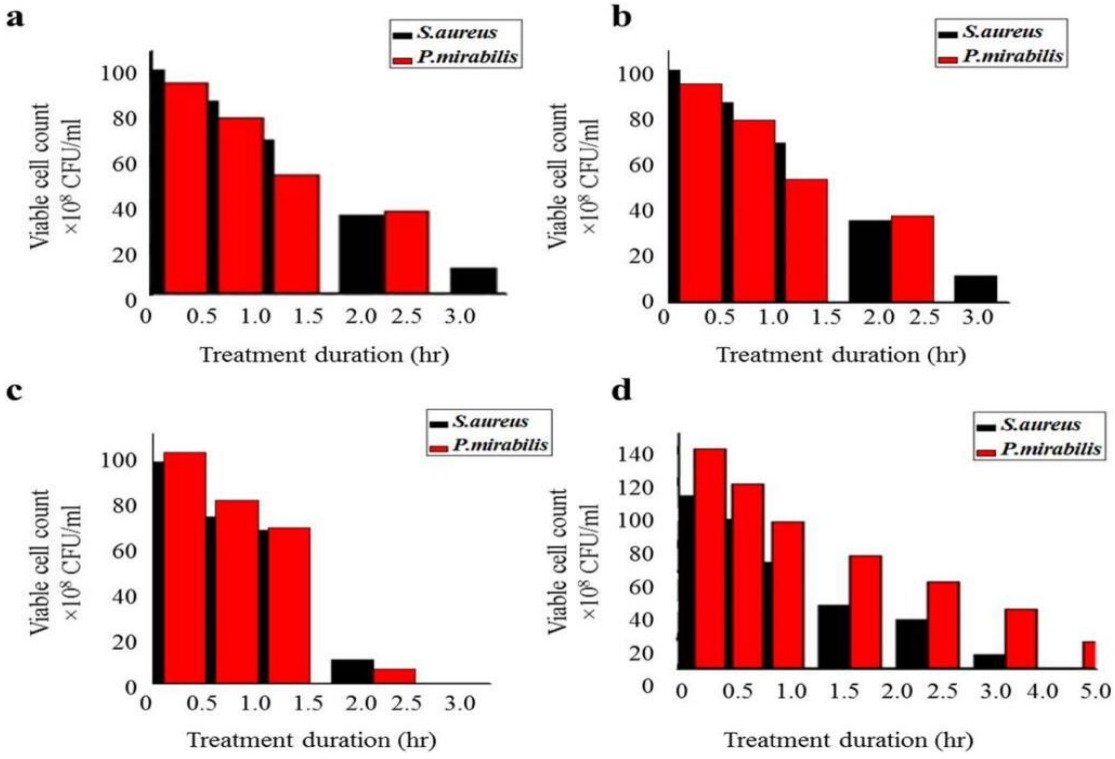


Figure 5. Viable count of bacteria a(er exposure to (a) rGO, (b) nAg, (c) rGO–nAg composite, and (d)standard antibiotic nitrofurantoin.[53]

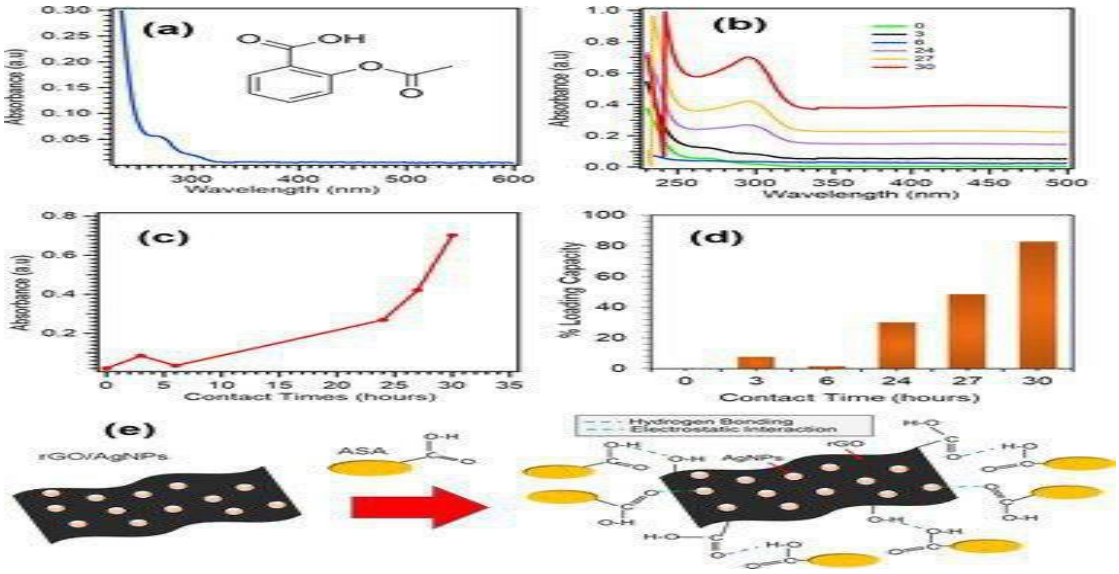
antifungal

The high cost of treatment, toxicity, and the emergence of resistant infectious agents justifies research into new drugs. This work evaluates the fungicidal activity of nanocomposites (NCs) based on reduced graphene oxide (rGO) loaded with silver (Ag) nanoparticles (rGO/Ag) against clinical isolates of dermatophytes and Candida species. This is an unprecedented study in which, for the first time, hybrid nanocompounds based on Ag/rGO were tested against Epidermophytom, Microsporum, and Trichophyton species (dermatophytes agents). In this paper, we synthesize rGO using different concentrations of Ag by hydrolysis of metal salt AgNO3 and follow the growth of nanocrystals on sheets of rGO provided by the NaBH4.Time-kill kinetics was conducted to monitor the effect of the composite to inhibit fungal cells or promote structural changes, avoiding germination. The toxicological evaluation of the NCs was born in an in vivo model based on Galleria mellonella (G. mellonella). Minimum inhibitory concentration (MIC) values of the rGO/Ag NCs ranged from 1.9 to 125 μg/mL. The best inhibitory activity was obtained for rGO/Ag12%, mainly against Candida spp. and Epidermophyton floccosum. In the presence of sorbitol, MIC values of rGO/Ag NCs were higher (ranging from 15.6 to 250 μg/mL), indicating the action mechanism on the cell wall. Both yeast and dermatophytes clinical isolates were inhibited at a minimum of 6 and 24 h, respectively, but after 2 and 12 h, they had initial antifungal interference. All hybrid formulations of rGO/Ag NCs were not toxic for G. mellonella.[54]

rGO_ AgNPS as drug dilvery system:

Acetylsalicylic acid (ASA; Aspirin) is a common anti-inflammatory drug widely prescribed as an anti-platelet to prevent cardiovascular events [55]. It is categorized as Class II drugs according to biopharmaceutics classification systems (BCS) which have poor solubility and bioavailability [56]. The prolonged use of ASA is associated with gastrointestinal mucosa ulcers and gastrointestinal hemorrhaging in severe cases [55]. Here in the present work, the reduced Graphene Oxide/Silver Nanoparticles (rGO/AgNPs) nanocomposite applied as drug loading for ASA. Hybrid materials optimized with nanotechnology synthesized via in-situ method from rGO by decorating AgNPs for a drug delivery system are quite efficient labor and cost. Besides that, the nanocomposites can be applied in the biomedical application with several benefits,

including biocompatible carbon materials as a drug delivery system (DDS); delivery of drug molecules in nanoscale sizes which provide targeted delivery of optimal dose with reduced side effects and toxicity [57]. Moreover, nanocarriers can solve drug solubility and bioavailability problems, thus increasing drug loading on the surface [56].



(Figure6)[59]

The peak of ASA standard shows the absorbance at the wavelength ~270 nm (Figure 6(a)). It can be seen that after stirring time variations are applied, the peak is shifted slightly to a higher wavelength due to interaction rGO/AgNPs in the loading process of ASA (Figure 6(b)). The longer loading process until 30 hrs, the ASA drug being optimally loaded into the rGO/AgNPs nanocomposite (Figure 6(c)), which indicates that the ASA is effectively loaded in the nanocomposite by sticking to the surface of the rGO, which contains AgNPs active ions.

The percentage of drug loading for ASA by rGO/AgNPs is shown in Figure 6(d). The result reveals that rGO/AgNPs nanocomposites are effective as DDS of ASA with the highest loading capacity achieve ~83% at 30 hrs. Compared to the previous study of drug loading for ASA, the result of drug loading capacity of the current method is much higher

The proposed drug loading mechanism of ASA by rGO/AgNPs is shown in Figure 6(e). The ASA molecules are bound to the surface of rGO/AgNPs nanoparticles via intramolecular hydrogen bonding with residual oxygen functional groups of rGO sheet. In addition, the drugs interact by electrostatic interaction with silver nanoparticles as well [58]. The hydrogen bonding and electrostatic interaction give rise to high drug loading and stability of rGO/AgNPs as DDS for ASA.

Anti-cancer

Conventional drug delivery systems and treatment approaches have several limitations, including low aqueous solubility of small molecules, rapid metabolism and elimination of drugs, failure to attain the desired target site concentration, multi-drug resistance, and non-specific cytotoxicity. To address the above limitations, the use of nanomaterials in cancer therapy has led to some cutting-edge research during the last few years [60-65].

The size of nanoparticles impacts on the binding and activation of membrane receptors and consequent protein expression in cancer cells[66] .Silver nanoparticles induced cell death and decreased cell viability of different types of cell lines by inducing apoptosis through the mitochondrial pathway and generation of reactive oxygen species (ROS).[67,68]

Kavinkumar et al. [69] synthesized GO/rGO–AgNP nanocomposites and explored the anticancer effect of this conjugate system against the human lung cancer A549 cell line.. The cytotoxicity of GO/AgNPs, rGO/AgNPs, and GO were evaluated against A549 cells by MTT assay. For GO only, even at a very high concentration (200 µg/mL) after 24 h, the cell viability was higher than 40%. This low cytotoxicity of GO on lung cancer cells can be due to oxygen-containing functional groups, e.g., OH, -COOH, and epoxy groups on the GO surface [70]. Cytotoxicity of rGO was slightly higher than GO, with cell viability IC50 values of 160 µg/mL and 180 µg/mL, respectively, after 24 h. Further, the rGO–AgNP nanohybrid system demonstrated better anticancer activity (IC50 of 30 µg/mL) than the GO–AgNP composite (IC50 of 100 µg/mL) against the A549 cell line. The authors suggested that the improved anticancer activity of the rGO–AgNP composite was a result of the synergistic effect of rGO and AgNPs and enhanced intracellular delivery of rGO.

Gurunathan et al. fabricated an rGO–AgNP composite using Tilia amurensis plant extract and explored its anticancer potential in ovarian cancer cells (A2780) [71]. The synthesized rGO–AgNP nanocomposites were highly stable and water-soluble and did not aggregate for 3 months. The rGO–AgNP composite exhibited a dose-dependent inhibition of viability with an IC50 value of ≈12.5 µg/mL. The composite resulted in the loss of cell membrane integrity, as evidenced by enhanced lactate dehydrogenase leakage. Further, the rGO–AgNP system increased ROS generation and DNA fragmentation in A2780 cells, demonstrating its potential in cancer treatment [71].

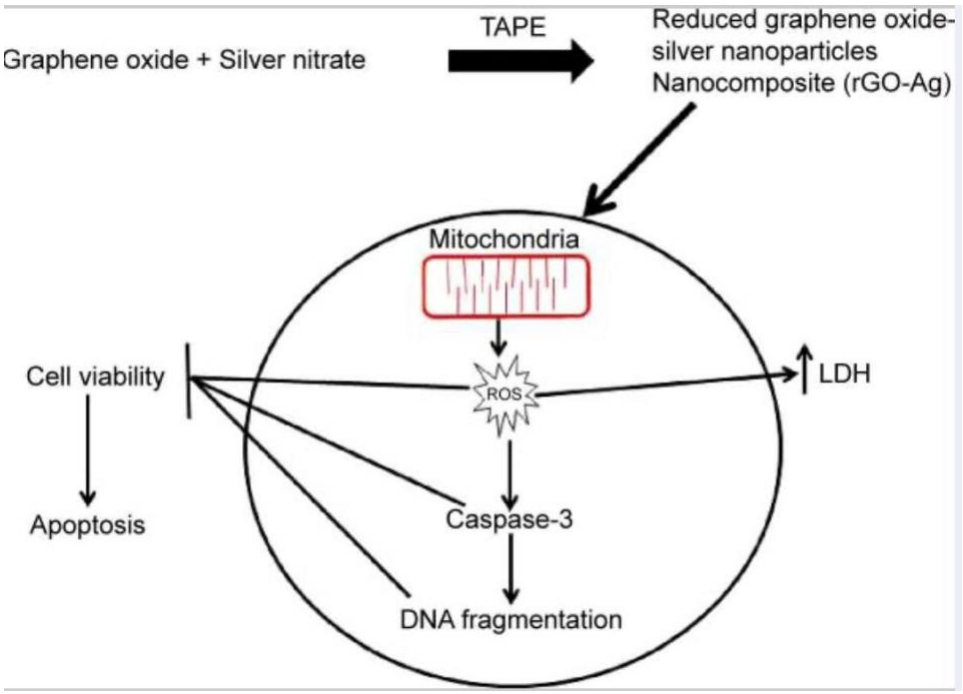


Figure (7) The hypothetical model for synthesis of rGO–Ag and its mechanisms in inducing cell death in human ovarian cancer cells.[72]

rGO–Ag nanocomposite-induced apoptosis in ovarian cancer cells
The data from the previous experiments, such as the increased production of ROS and MDA and the reduced activity of the antioxidant enzymes and GSH, suggested that the rGO–Ag nanocomposite caused induced oxidative stress, which may result in cell damage and apoptosis via DNA damage. Therefore, this experiment was designed to address whether the rGO–Ag nanocomposite could induce DNA fragmentation by oxidative stress. The DNA fragmentation assay enables the assessment of cell death, which is a hallmark of apoptosis. To confirm the induction of apoptosis in A2780 cells, the cells were treated with GO, rGO, and rGO–Ag nanocomposite for 24 hours; the TUNEL analysis was performed on the treated cells. The results indicate that treatment with GO, rGO, and rGO–Ag nanocomposite causes the appearance of a significant number of TUNEL-positive A2780 cells, whereas no apoptotic cells are observed in the control (Figure 8A–C). Interestingly, the rGO–Ag nanocomposite-treated cells show an advanced degree of fragmentation when compared with the cells treated with other tested nanomaterials. This indicates that the cytotoxicity of the rGO–Ag nanocomposite is associated with the induction of apoptosis.[72]

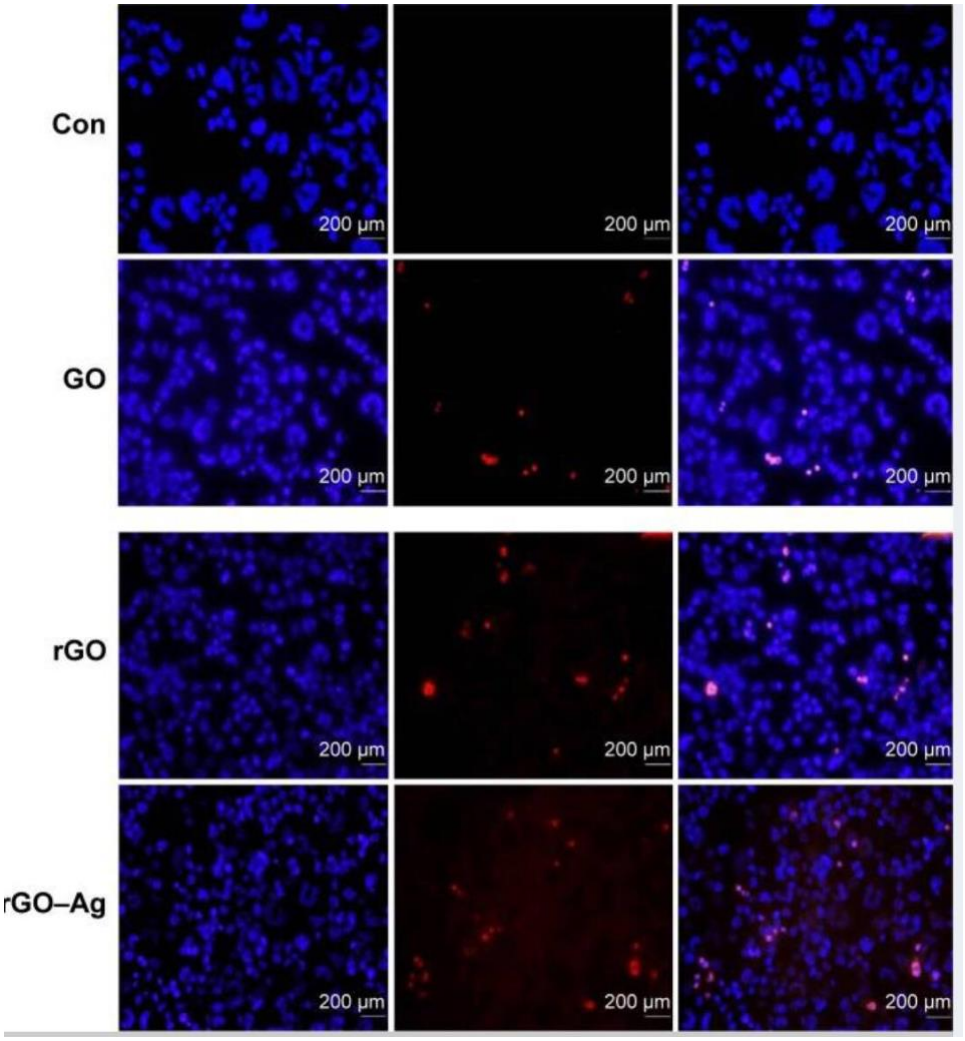


Figure (8)rGO–Ag nanocomposite-induced apoptosis in human ovarian cancer cells.[72]

Notes:

The cells were treated with respective IC50 concentrations of GO, rGO, rGO–Ag nanocomposite, and AgNPs for 24 hours. Apoptosis of human ovarian cancer cells after 24-hour treatment was assessed by the TUNEL assay; the nuclei were counterstained with DAPI. Representative images show apoptotic (fragmented) DNA (red staining) and the corresponding cell nuclei (blue staining).

Conclusion

The study proved that the proposed method was easy, one-step, inexpensive, and a short Time to synthesize RGO-Ag NPs. Additionally, the results, shown that the graphene oxide has Been reduced and at the same time successfully, the silver nanoparticles anchored on the Reduced graphene oxide sheets. Moreover, the grain and crystalline size of the silver Nanoparticles are in the nanoscale, as well, the shape of the AgNPs is spherical, and the Nanoparticles are dispersed evenly distributing on the reduced graphene oxide sheet and optical / morphology properties of rGO-Ag NPs and Ag NPs are study by UV-Visible Spectrophotometer and (SEM) techniques respectively. This Nanocomposite with these good specifications can be applied in several different applications In all areas of life.

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