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قسم الكيمياء

بحث مقدم لاستكمال درجة البكالوريوس بعنوان:

Comparison between Green Synthesis and Chemical Synthesis of Silver Nanoparticles: Characterization and their Antimicrobial Activities

مقارنة بين التخليق الأخضر والتوليف الكيميائي لجسيمات الفضة النانوية: التوصيف وأنشطتها المضادة للميكروبات

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بسم الله الرحمن الرحيم

قَالَ إِنَّمَا الْعِلْمُ عِنْدَ اللَّهِ وَأُبَلِّغُكُمْ مَا أُرْسِلْتُ بِهِ ﴿٢٣ الأحقاف﴾

الاهداء

اهدي ثمرة علمي هدا الي والدي العزيز الدي غرس في حب العلم والفضيلة...

والدتي التي تعجز الكلمات عن الوفاء ولو ببعض حقها...

الي اخواني واخواتي... الي جميع اقاربي...

والي طلاب العلم والمعرفة ,وكل غيور عن المجتمع

والي كل من قدم لي الدعم والتشجيع بكلمة صادقة كانت حافزا لي

او دعاء صالح بظهر الغيب انار دربي

كلمة الشكر والتقدير

الشكر لله اولا واخيرا والحمد لله الدي وفقنا للإتمام هدا العمل راجيين من الله ان يجعل هدا العمل منفعي لطلبة العلم.

نتوجه بخالص شكرنا وتقديرنا وعظيم امتنانا الي الدكتورة الفاضلة "ونيسة ابراهيم السبيعي" علي كل التوجيهات والتوصيات , فجزاها الله عنا كل خير ويجعله في ميزان حساناتها.

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ولا انسي ان اشكر كل من ساعدنا على اتمام هدا العمل من قريب او بعيد

فهرس المحتويات

Abstract

This study was conducted on the preparation of silver nanoparticles (AgNPs) using chemical ligands, namely sodium benzoate, ascorbic acid, and aspirin. Sodium borohydride ($NaBH_4$) was used as a reducing agent. AgNPs were stable in room condition up to 4 months. The results were confirmed by UV and IR analyzes. In addition, this study confirms the ability of medicinal plants to produce silver nanoparticles with high stability during green synthesis and used $NaBH_4$ as reducing agent. The results of their activities against Pseudomonas aeruginosa bacteria were done as well. The stability of AgNPs were monitored at different pH. The comparison between AgNPs synthesised by two options were done in this work.

1. Introduction

Nanotechnology is an emerging area of research which has a potential in replacement of conventional micron technologies and gives size dependent on the properties of the functional materials. For example, when the dimension of a material is reduced from a large size, the properties remain the same at first, then small changes occur, until finally, when the size drops below 100 nm, dramatic changes in their properties. The interest in synthesis of nanomaterials has grown because of their distinct optical, magnetic, electronic, mechanical, and chemical properties compared with those of the bulk materials [1].

Nanoscience breakthroughs in almost every field of science and nanotechnologies make life easier in this era. Nanoscience and nanotechnology represent an expanding research area, which involves structures, devices, and systems with novel properties and functions due to the arrangement of their atoms on the 1–100 nm scale. The field was subject to a growing public awareness and controversy in the early 2000s, and in turn, the beginnings of commercial applications of nanotechnology. Furthermore, nanotechnologies contribute to almost every field of science, including physics, materials science, chemistry, biology, computer science, and engineering. Notably, in recent years nanotechnologies have been applied to human health with promising results, especially in the field of cancer treatment [2].

1.1. Methods of Synthesis of Nanomaterials.

Nanostructure materials have attracted a great deal of attention because their physical, chemical, electronic and magnetic properties show dramatic change from higher dimensional counterparts and depends on their shape and size. Many techniques have been developed to synthesize and fabricate nanostructure materials with controlled shape, size, and structure. The performance of materials depends on their properties and their properties in tern depend on the atomic structure, composition, microstructure, defects, and interfaces which are controlled by thermodynamics and kinetics of the synthesis [3].

Classification of Techniques for synthesis of Nanomaterials are two general approaches for the synthesis of nanomaterials as shown below including (Top- down) approach and (Bottom–up) approach.

a) Top-down

Top-down approach involves the breaking down of the bulk material into Nano sized structures or particles. Where Top-down synthesis techniques are extension have been used for producing micron sized particles and they are inherently simpler. It is also depending on removal or division of bulk material or on reduction of bulk fabrication processes in order to produce the desired structure with appropriate properties. The biggest problem with the top-down approach is the imperfection of surface structure. For instance, nanowires made by lithography are not smooth and may contain a lot of impurities and structural defects on its surface. Examples of such techniques are high-energy wet ball milling, electron beam lithography, atomic force manipulation, gas-phase condensation, aerosol spray, etc. [3].

b). Bottom-up

The alternative approach, which has the potential of creating less waste and hence the more economical, is the 'bottom- up'. Bottom-up approach refers to the buildup of a material from the bottom: atom-by-atom, molecule-by-molecule, or cluster-by cluster. Many of these techniques are still under development or are just beginning to be used for commercial production of nano-powders. Oregano-metallic chemical route, revere-micelle route, sol-gel synthesis, colloidal precipitation, hydrothermal synthesis, template assisted sol-gel, electrodeposition etc, are some of the well- known bottom–up techniques reported for the preparation of luminescent nanoparticles [4]. (See Figure1)

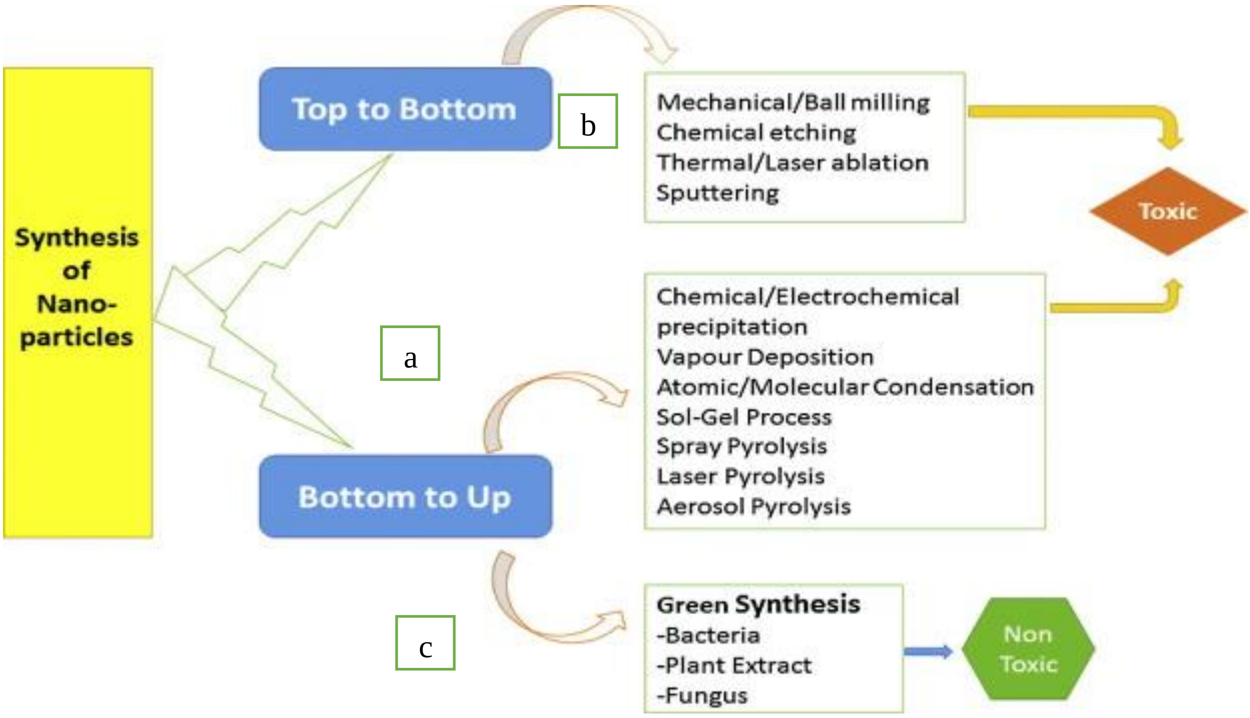

Figure 1: Different approaches of synthesis of silver nanoparticles. 2Protocols employed for synthesis of nanoparticles (a) bottom to top approach and (b) top to bottom approach. (c) Protocol for synthesis of silver nanoparticles using plant extract mediated synthesis of silver nanoparticles.[5].

2. Experiments Section

2.1. Chemical Synthesis

Silver nanoparticles were synthesized by using chemical ligands, where silver colloidal nanoparticles were prepared according to the chemical reduction method in which the $NaBH_4$ was used as a reducing agent in this research and vitamin C, O-acetyl salicylic acid, and sodium benzoate as stabilizing agents.

2.2 . Synthesis Silver Nanoparticles with Plant Extraction.

The huge potential of Nano biotechnology makes it an intensely researched field in modern medicine. Green nanomaterial synthesis techniques for medicinal applications are desired because of their biocompatibility and lack of toxic byproducts. For example, it is reported that the toxic byproducts free Phyto-synthesis of stable silver nanoparticles (AgNPs) using the bark extract of the traditional medicinal plant*Acacia leucophloea* (Fabaceae). Where the visible yellow-brown color formation and surface plasmon resonance at 440 nm indicates the biosynthesis of AgNP [12]. The in vitro agar well diffusion method confirmed the potential antibacterial activity of AgNP functionalized by plant extract and against the common bacteria. This research combines the inherent antimicrobial activity of silver metals with the Teacrium barbeyanum extract, yielding antibacterial activity-enhanced AgNPs. This new biomimetic approach using traditional medicinal plant (Teacrium barbeyanum) to synthesize biocompatible antibacterial AgNPs could easily be scaled up for additional biomedical applications. These stable AgNPs green-synthesized via plant extract can readily be used in many applications not requiring high uniformity in particle size or shape.

2.2.1. Synthesis Silver Nanoparticles Functionalized by Extract of Teacrium barbeyanum

Teucrium species are distinguished by the content of valuable secondary metabolites and long-standing traditional ethno-metallic use. Bearing in mind that the therapeutic benefit of medicinal plants often depends on their antioxidant activity, this chapter is a comprehensive review of the antioxidant activity of potential medicinal species and drugs of the genus Teucrium, as well as the content and diversity of secondary metabolites including methods for determining antioxidant activity. Analysis of the current literature indicates that the antioxidant activity of more than 30 species of this genus has been tested using different systematic approaches [13].

The genus Teucrium is a large and polymorphous genus of the family Lamiaceae distributed in temperate climatic zones, mainly in the Mediterranean basin and Central Asia. Studies of the non-volatile constituents of trichrome species have shown that it is a rich source of neoclirodian diterpenoids, which are chemical markers of the genus. In addition to non-volatile metabolites, there has been great interest in the essential oils of this genus. It is noted that, a complete survey of the chemical composition and biological properties of essential oils isolated from Teucrium cultivars is provided [14].

Extraction method of Teacrium barbeyaumn:

The aerial part of the cleaned plant was ground into a powder, and then the fat was removed by extraction with petroleum ether and dichloromethane, respectively, using a continuous extraction device (Soxhlet). Then the defatted plant was extracted with a solution of 70% aqueous methanol. The alcoholic extract was concentrated under sieved pressure and then dissolved in distilled water. Then the aqueous solution of the extract was fractionated successively with chloroform, then ethyl acetate, and finally normal butanol to obtain the butanol extract used in the study.

2.3. Study of the stability of AgNPs:

According to previous literature, it was found that the addition of acid and base to the AuNPs can be changing the ionic strength of the dispersion. For instance, it is noted that AuNPs were not stable in dispersion with a high concentration (above 0.1M) of a NaCl added and instantly lead to the aggregation [16].

Silver nanoparticles with pH:

The role of pH in the green synthesis of AgNPs is investigated. For the synthesis of AgNPs we use silver nitrate which reduced by $NaBH_4$, extract plants were used as stabilizer. The effect of NaOH addition on the nature of AgNPs is systematically studied. Two reaction pathways are proposed to explain the formation of AgNPs, keeping in view the pH changes that occur on addition of different amounts of NaOH. The aqueous sol of AgNPs prepared at different pH values display different surface plasmon resonance (SPR) behavior. This is explained in terms of size and size distribution of AgNP as mentioned in the literature [17].See Figure 9.

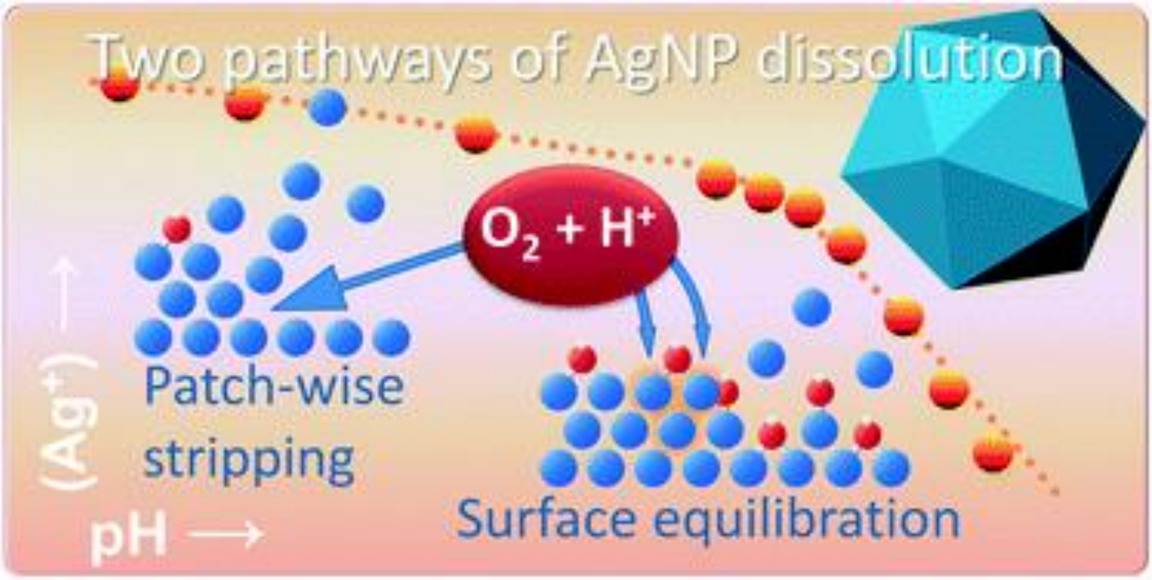

Figure9: Oxidative dissolution has large implications for the environmental fate and toxicity of silver nanoparticles (AgNPs). In this study, we quantify the kinetics, pH, and size dependency of silver ion (Ag^+) release from AgNPs and explain our results in a consistent manner with a mechanistic view [18].

2.3.1. Study of the Stability of Functionalised AgNPs- Teacrium barbeyanum in acid and basic conditions of 1M HCl and 1M NaOH.

According to previous literature, it was found that the addition of acid and base to the AgNPs can be changing the ionic strength of the dispersion. For instance, it is noted that AuNPs were not stable in dispersion with a high concentration (above 0.1M) of a NaCl added and instantly lead to the aggregation [16].

2.4. Antibacterial activities of AgNPs.

Nanoparticles have been widely used today, especially in the latest developed science materials. Silver nanoparticles, one of the most popular antimicrobial materials had been generally utilized in the textile industry and medical engineering. Different silver nanoparticles fabricating methods will make different physical or chemical property. Besides from silver nanofibers and plasma nanoparticles, most textile processing methods involve the dipping method. The entire industrial silver nanoparticles containing waste still has not any proper method to grip them out from the waste before it was drained into the environment. A novel Klebsiella sp. mutant was isolated from the ordinary laboratory analysis, and was revealed and identified as Klebsiella pneumonia by optical, biochemical and 16S rRNA sequence analysis, followed by NCBI genebank sequence blast result. This mutant strain (K pneumonia B50) could degrade 5 different trade mark silver nanoparticles, directly. Under the textile nanoparticles operation concentration (2%~10%), which exhibit a complete antimicrobial activity, all these five different kinds of commercial silver nanoparticles will be degraded into none antimicrobial activity by this mutant within 4 hours. Atomic absorbance analysis observed that the silver nanoparticles were absorbed or uptake by the cell directly. It means that, K pneumonia B50 could be served as a powerful bioremediation tool for industrial silver nanoparticles containing waste managing [19]. In this research, antimicrobial activity was applied on the kind of bacteria and showed excellent results as illustrated in Figures 16,-21.

3. Discussion:

The AgNPs in this work were stable based on the ligands used to functionalize them. For example, we compared between AgNPs synthesised by chemical ligands and plant extraction. The AgNPs prepared by chemical ligands stayed stable for nearly 4 months, while AgNPs synthesised by green methods stayed for nearly 3 months. In addition, AgNPs functionalized by plant extraction were highly biocompatible in comparison with AgNPs functionalized by chemical ligand.

Furthermore, the green synthesis of Ag nanoparticles was characterized by using Fourier transform infrared spectroscopy (FT-IR) as showed in Figure 22 below, which revealed that hydroxy (OH) group at 3300 cm^{-1}, aldehyde/ketone (C=O) at 1650 cm^{-1}, aromatic (2900-3100 $cm^{-1)}$. These function groups may increase the affinity of the extraction plant toward the surface of nanoparticles as mentioned in the research [21]. The Grewia tenax extract may participate in the bio-reduction and capping of the formed AgNPs.

Conclusion

AgNPs in this work prepared with high stability without showing any signs of decomposition, such as particle growth or loss of stability. The effect of the pH on the formation of Ag nanoparticles by reduction of Ag (I) to Ag (0) using $NaBH_4$ and functionalized by compounds containing carboxylate groups in their structures have been described. Also, comparison with green synthesis were taken in consideration in this research. These ligands and plant extraction are indeed provided the AgNPs with excellent stability, which is evidenced by using UV-Vis. The stability observation for 8 weeks at normal laboratory conditions displayed that the AgNPs were highly stable with these ligands and no remarkable shift of the maximum peak was noted. These AgNPs show a promising application in the development of antibacterial products which predicts to be an appropriate choice in medical fields in the future. For this reason, synthesis of monodispersed and size controlled of AgNPs is consequently required.

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