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Schiff Base Synthesis as a Capping Agent for Green Synthesized Silver Nanoparticles

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A SCHIFF base **3** was synthesized and used as a new capping agent for synthesis of silver nanoparticles through preventing overgrowth of silver oxide. Schiff base **3** helps to enhance the structural properties of the resulted silver nanoparticles in a distinguishable style. The structure of Schiff base **3** was confirmed on the basis of spectral data (^{1}H nuclear magnetic resonance, mass, and Fourier-transform infrared spectroscopy) and melting point. The ultraviolet–visible was used to monitor the formation of silver nanoparticles within a surface plasmon band at 400 nm. Scanning electron microscopy image shows the particle size of silver nanoparticles which have a spherical shape (6-45 nm). The resulted silver nanoparticles were screened against four types of bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli.*

Keywords: Antibacterial activity, Capping agent, Schiff base, Silver nanoparticles.

Introduction

Schiff bases or the compounds that bear an azomethine group (-CH=N- group) gain importance in an organic synthetic field [1]. Schiff bases act as intermediates in the synthesis of a number of biological and industrial compounds through cycloaddtion, ring closure and replacement reactions. Schiff bases derivatives are useful compounds for synthesis of bioactive agents such as antiviral [2], anticonvulsant [3, 4], antifungal [5], anti-trypanosoma cruzi [6], antimicrobial [7-9], anti-inflammatory [10], antitumor [11], gastroprotective activity [12] and anticancer [13-15]. In addition, Schiff bases offer diverse applications due to they have been used as excellent chelating agents [16], corrosion inhibitors [17, 18], asymmetric catalytic systems [19-21] and an excellent selectivity toward specific metal ions: Mn(II), Co(II), Cu(II), Ni(II), Zn(II), Gd(III), Hg(II), Pb(II) and Y(III)[22-25].

Many publications have reported that metallic nanoparticles own a wide range of applications [26], particularly in biomedical domains (nano-medicine) as they can be used in gene and drug delivery systems [27, 28]. Among of these nanoparticles, silver nanoparticles (AgNPs) which are considered as an important type of nanoparticles due to a variety of their applications in biological and industrial field [29-31]. The general methods for synthesis of silver nanoparticles include a reduction reaction of silver salts by using organic or inorganic agents such as polyacrylate [32], carboxymethyl chitosan [33], sodium borohydride ($NaBH_4$) [34], Tollen's reagent [35] and ascorbic acid [36]. These agents were used in the reduction reaction of silver ions in an aqueous or non-aqueous solutions to form metallic silver. The resulted metallic silver undergoes an agglomeration process to form oligomeric clusters. Ultimately, these clusters convert to metallic colloidal silver particles. Therefore, the use of capping agents for stabilization of dispersive nanoparticles is highly desirable [37].

In this work, we use maltose sugar (a reducing agent) and synthesized Schiff base of 4-((2-hydroxybenzylidene) amino) benzoic acid **3** (Scheme 1) as a new capping agent for synthesis of silver nanoparticles. In addition, antibacterial activities of these nanoparticles have been evaluated.

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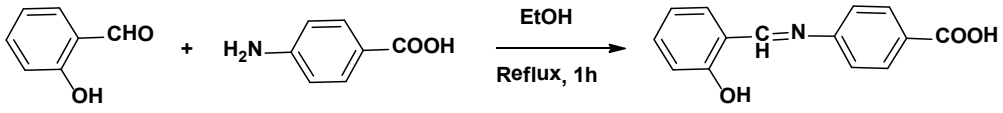

Scheme 1. Synthetic route of Schiff base (3).

Materials and Methods

Solvents and chemicals were purchased from Sigma-Aldrich. Analytical properties of TLC were carried out by using plates precoated with a silica gel 60 ultraviolet (UV) 254 and the UV light was used to visualize the obtained compounds. FTIR spectra were recorded on Shimadzu FTIR-8300 infrared spectrophotometer (Iraq) and the absorbance were taken between 3600-600 cm^{-1}. ^1H NMR spectra were recorded on a Bruker inovo AV-400 spectrometer (Iran) at room temperature in DMSO-d_6 as solvent with a signal peak of ^1H spectra at δ 2.50 ppm. The completed proton of decoupling values (J) are given in Hz. Melting points were obtained from a Gallenkamp melting point apparatus in capillary tubes. Accurate mass was performed by using a Micro Mass LCT operating in Electrospray mode (ES) (Iran). The UV-Vis spectra was performed by using UV-160v, Shimadzu spectrophotometer at the regions (400-700 nm). Scanning electron microscopy (SEM) images were taken by using a Zeiss instrument with an accelerating voltage of 200kv (Iraq). Energy dispersive analysis (EDX) of silver nanoparticles was carried out by using (EDX instrument, Iraq). Antibacterial activity of silver nanoparticles has been performed against *S. aureus, P. aeruginosa, K. pneumonae* and *E. coli* by utilizing an agar-well diffusion method (Iraq).

Synthesis of 4-((2-hydroxybenzylidene) amino) benzoic acid (3)

To a solution of salicylaldehyde (0.5 mL, 5 mmol) and 2 drops of glacial acetic acid in ethanol (15 mL), 4-aminobenzoic acid (0.68 g, 5 mmol) was added. The resulting mixture was refluxed for 2 h. The reaction was cooled to precipitate a yellow product. The product that separated out was filtered, washed with cooled ethanol, recrystallized from ethanol and dried under vacuum desiccators to give compound **3** as yellow powder. The purity of Schiff base **3** was checked by TLC on a silica gel. Yield 90%, m.p. 153-154 º C. FTIR spectrum (KBr, v, cm^{-1}): 2989 (C-H), 2825 (C-H), 1681 (C=O), 1598 (C=O), 1570 (C=C), 1531 (C=C). ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.69 (s, 1H, COOH), 8.99 (s, 1H, CH=N), 8.0 (d, 2H, J = 7.4 Hz, CH of phenyl ring), 7.7 (d, 1H, J = 8.9 Hz, CH of phenyl ring), 7.49-7.42 (m, 3H, CH of phenyl ring), 7.01-6.97 (m, 1H, CH of phenyl ring). Mass (ESI^+) ($M+H^+$) calculated for $C_{14}H_{11}NO_3$: 242.0088, found: 242.0000 (Fig. 1–3).

Synthesis of silver nanoparticles

An eco-friendly method was used for synthesis of silver nanoparticles (AgNPs). To an aqueous solution of silver nitrate (0.1 g / 100 mL) in a beaker (250 mL), D (+) maltose (0.1 g) was added. Schiff base **3** (0.1 g) as a capping agent was added into above mixture in order to avoid sliver oxide precipitation. The resulting mixture was heated and stirred at 60 º C for 10 min. After that the color of mixture changed from light to light brown due to the reduction reaction of silver ions as shown in Fig. 4. In Fig. 4, the leaser pointer was used to confirm the formation of silver nanoparticles. This process follows by Tyndall effect that represents the scattering of light to result a light beam through a suspension solution.

Results and Discussion

UV-Vis spectroscopy of silver nanoparticles

Silver nanoparticles solutions were measured to detect a plasmon spectra by using UV-Vis spectroscopy. The reduction reaction of Ag^+ ions to form Ag^0 was monitored by recording UV-Vis spectra of silver nanoparticles (1.0 mL) after dilution with deionized water (3.0 mL). The absorbance peaks appear at regions (400-700 nm) due to an excitation of localized surface plasmon band in silver nanoparticles [38]. The maximum absorbance at 400 nm attributes a surface plasmon band (SPB) due to the formation of silver nanoparticles as shown in Fig. 5.

Scanning electron microscopy (SEM)

The particle size of silver nanoparticles was determined by using SEM images. The use of Schiff base **3** as a capping agent in the synthesis of silver nanoparticles is very important due to the Schiff base **3** can stabilize dispersive nanoparticles [39]. The image of the silver nanoparticles exhibited a clear separation (Fig. 6) than in the absence of Schiff base **3** [40]. These nanoparticles also show uniform style in the solution. The particle size of silver nanoparticles was found to be 6-45 nm and the shapes of these nanoparticles were a spherical shape.

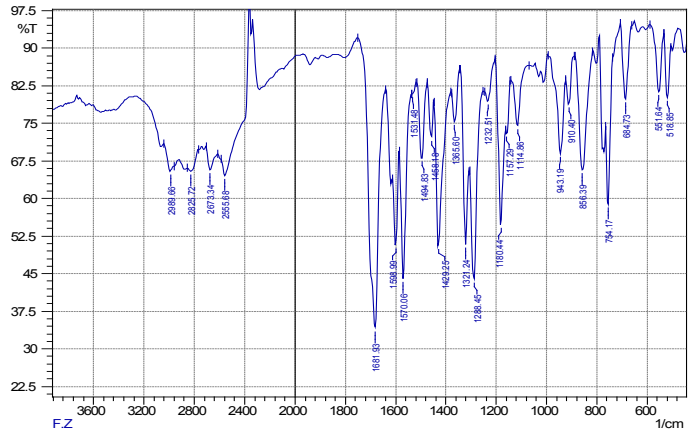

Fig. 1. Fourier-transform infrared spectroscopy spectrum of Schiff base (3).

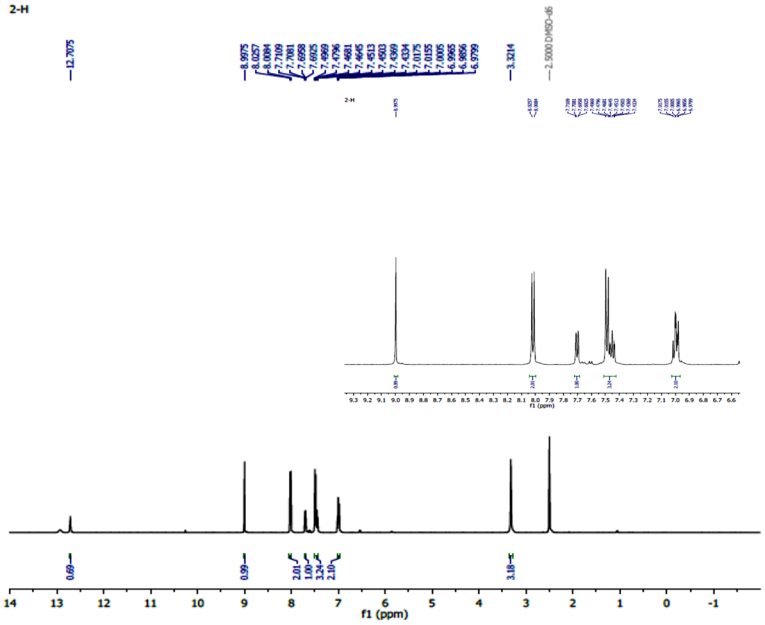

Fig. 2. ^{1}H nuclear magnetic resonance spectrum of Schiff base (3).

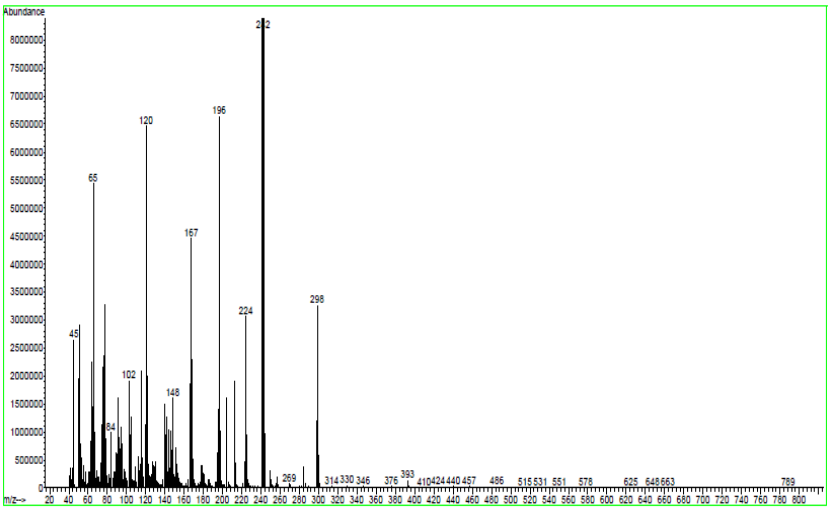

Fig. 3. Mass spectrum of Schiff base (3).

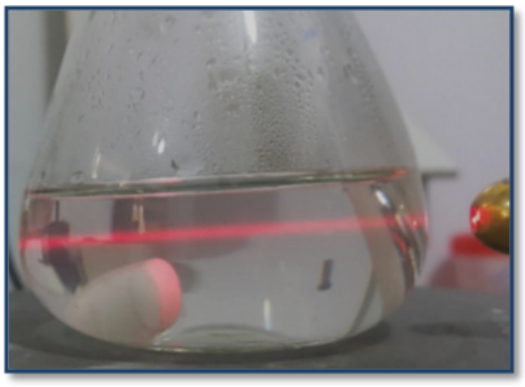

Fig. 4. Formation of silver nanoparticles confirmed by laser beam.

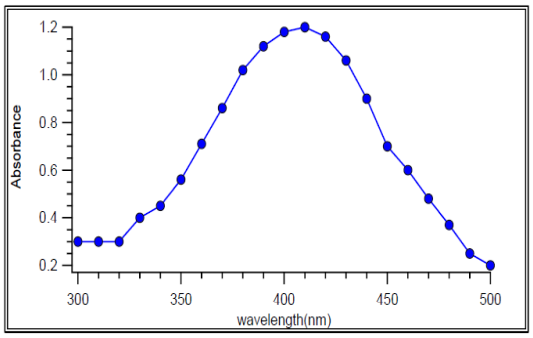

Fig. 5. Ultraviolet–visible absorbance spectra of silver nanoparticles.

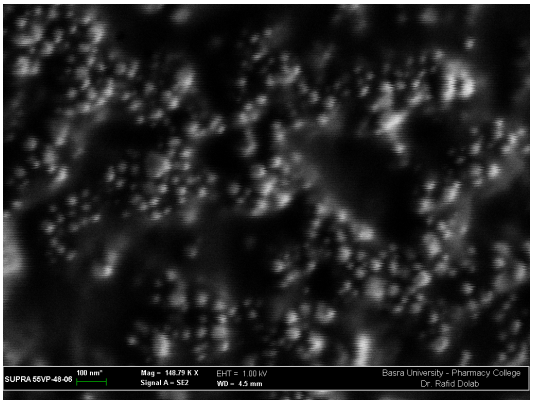

Fig. 6. Scanning electron microscopy image of silver nanoparticles in the presence of Schiff base (3).

Energy dispersive analysis (EDX).

The introduced method for synthesis of silver nanoparticles in the presence Schiff base **3** is a convenient to enhance silver particles toward a narrow size distribution and to make a spherical shape [41]. The morphological analysis for the droplets of silver nanoparticles solution on a layer of aluminum foil offer good image for these nanoparticles in SEM (Fig. 6). Silver possesses a high percentage in the silver nanoparticles solution and the map of an elemental analysis displays the loading of silver on magnetite nanoparticles. Intensity of silver EDX peaks show their adsorption on the surfaces of silver nanoparticles solution (Fig. 7). The silver nanoparticles were found to have a wide distribution of pore size which may offer a significant contributions during the adsorption process.

Zeta sizer and zeta potential calculations

Zeta sizer and zeta potential were measured in order to scan the size of nanoparticles in the resulted solution. The distribution of silver ion due to the addition of Schiff base **3** can be observed in Fig. 8. It was noted that the particle size of nanoparticles was less than 10 nm. Zeta sizer provides a good agreement with SEM results and the best results with regard to a small size of the nanoparticles can be achieved by Zeta sizer because of the measurement of colloidal is simple in the particles with free distribution and migration. The detection of a small size more than 10 nm depends on the type of instruments. Moreover, zeta potential distribution of silver nanoparticles was a negative value (-27 mV) due to the presence of silver ions (Fig. 9a). Zeta potential can be counted by tracking the colloidal particles through a microscope as they transfer in a voltage field. Hence the positive ions collect on the voltage as a negative value (Fig. 9b).

Biological activity of silver nanoparticles

Silver nanoparticles can affect cell membrane and intracellular unites due to these nanoparticles have a high reactivity and large ratio (surface areas per volume) [42]. Silver nanoparticles can produce free silver ions that can pass into the cellular wall to cause lysis and death [43]. Encouraged by above properties of silver nanoparticles, the antibacterial activities of these nanoparticles were performed against four types of bacteria (*S. aureus, P. aeruginosa, K. pneumonae and E. coli*) by using agar-well diffusion method. The solutions of silver nanoparticles (30 µg) in DMF (0.01 mL) were prepared as standard solutions and tested on a paper disc. The susceptible zones (mm) were clearly calculated around the discs. The discs were put in an incubator at 35 ºC for 48 h and carried out on agar plates of the bacterial growth. The agar plates that were used for the growth of bacterial species were prepared from agar (40 g) MacConkey (Merck). These plates were suspended with a fresh water (500 mL), allowed to soak for 20 min and boiled in a water bath till the agar was completely dissolved. The activity of low concentrations of silver nanoparticles was tested against four types of bacteria and found

to be biologically active. These nanoparticles show different degrees of an effective inhibition toward the growth of bacterial species. The results revealed that silver nanoparticles have significant antibacterial activities against Gram-positive bacteria *S. aureus* with inhibition zone (15 mm), *P. aeruginosa* with inhibition zone (15 mm), Gram-negative bacteria *E. coli* with inhibition zone (13 mm) and *K. pneumonae* with inhibition zone (11 mm) as shown in Fig. 10.

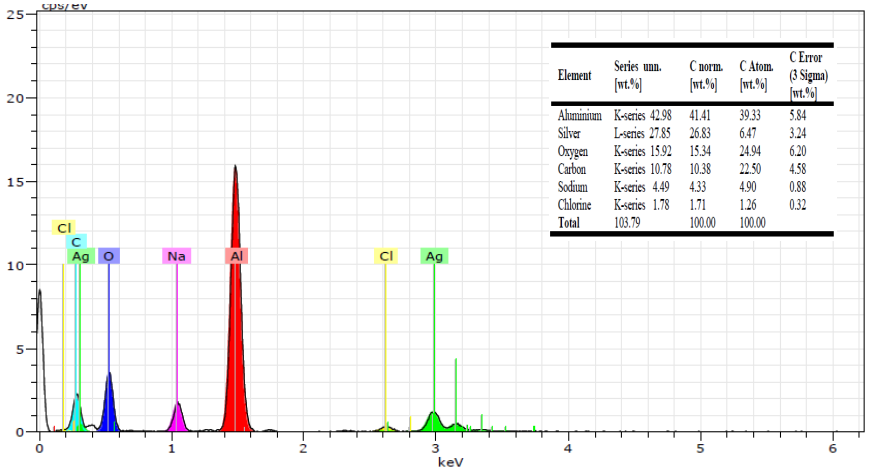

Fig. 7. Energy dispersive analysis spectra of silver nanoparticles.

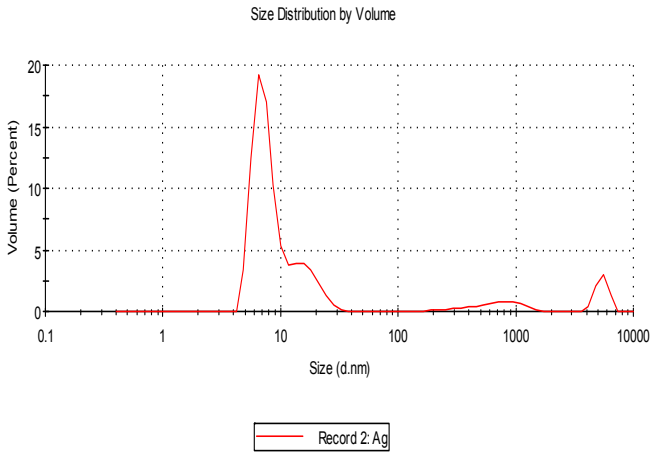

Fig. 8. Size distribution by volume of silver ion in the presence of Schiff base (3).

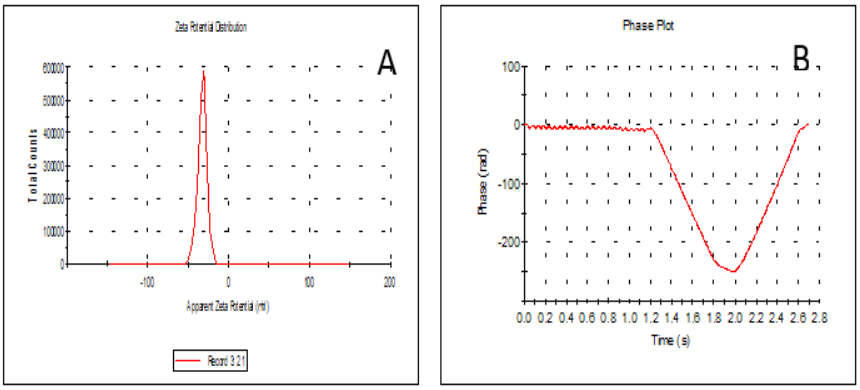

Fig. 9. Zeta potential distribution of silver nanoparticles (a) and the phase plot (b)..

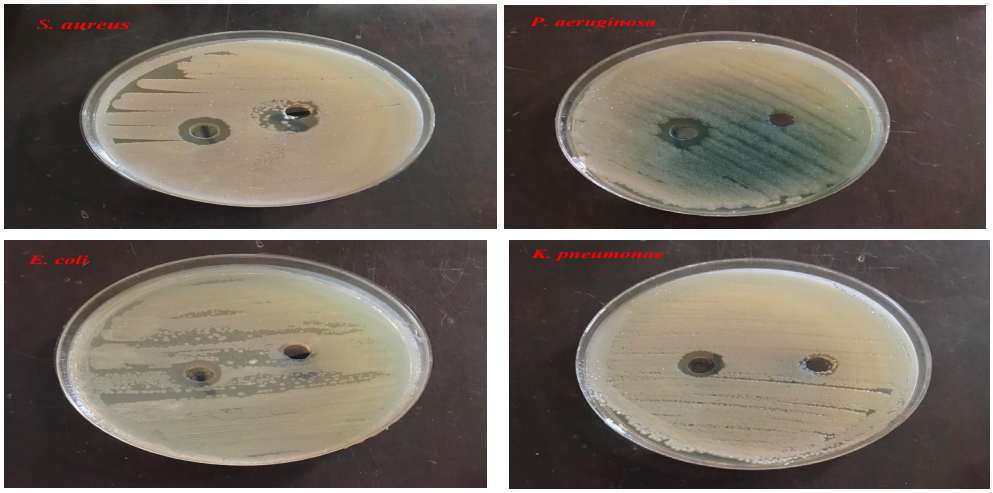

Fig. 10. The antibacterial activity of silver nanoparticles against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli.*

Summary and Conclusion

An economic and green method was used for synthesis of silver nanoparticles. Schiff base 3 was employed as a capping agent and used to enhance an aggregation process of silver nanoparticles. This capping agent helps to create silver nanoparticles in a distinguishable style. The appearance of surface plasmon resonance peak (SPR) at 400 nm provides a convenient spectroscopic signature for the formation of silver nanoparticles. SEM analysis and zeta sizer confirm that silver nanoparticles have a spherical shape. The distribution of silver nanoparticles (zeta sizer and zeta potential) in ethanol media was analyzed. The zeta analysis revealed that the particle size of silver nanoparticles was 6-45 nm and zeta potential was a negative value. Silver nanoparticles were tested against *S. aureus, P. aeruginosa, K. pneumonae* and *E. coli* and found to have a significant inhibitory activity against these bacteria species.

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تحضير قاعدة شف كعامل مثبت في الطريقة الخضراء لتحضير جزيئات الفضة النانوية

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تم تحضير قاعدة شف من تفاعل تكثيف السالسلديهايد مع بارا امينو بنزويك اسد بوجود قطرات قليلة من حامض الخليك الثلجي كعامل مساعد وشخصت قاعدة شف المحضرة باستخدام التحليل الطيفي بالاشعة تحت الحمراء (FT-IR), الرنين النووي المغناطيسي (^{1}HNMR)، طيف الكتلة ودرجات الأنصهار. استخدمت قاعدة شف المحضرة لأول مرة كعامل مثبت في تحضير جزئيات الفضة النانوية وتم معرفة خصائص هذه الجزئيات بواسطة استخدام التحليل الطيفي بالأشعة المرئية وفوق البنفسجية حيت استخدمت تلك الأشعة لمتابعه تكوين جزئيات الفضة النانوية في المحلول عن طريق اختزال Ag^+ إلى Ag^0. كما استخدم المجهر الالكتروني الماسح (SEM) لدراسة خصائص وحجم هذه الجزيئات النانوية والتي تتراوح بين 45-6 نانومتر, كما استخدم التحليل التشتت للطاقة (EDX) لتقدير الكمي لشدة الفضة في محلول الفضة النانوية. تم حساب قيم zeta sizer و zeta potential من اجل تقدير حجم الجزيئات الفضة النانوية واظهرت النتائج ان معامل التوزيع zeta potential هو قيمة سالبة (-27mV) نتيجة لوجود ايونات الفضة.

اثبتت النتائج أن إضافة قاعدة شف تعمل على تثبيت محلول جزيئات الفضة النانوية من خلال تحسين الخصائص التركيبة لتلك الجزيئات واظهارها بأسلوب مميز. وقد تم ايضا دراسة الفعالية البايلوجية لجزيئات الفضة النانوية المحضرة ضد اربعة أنواع من البكتريا حيث اظهرت هذه الجزيئات فعالية جيدة تجاه الأنواع البكتيرية المستخدمة.