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# Synthesis of Gold Nanoparticles Using a Schiff Base Derivative of Ceftriaxone in Absence of Reducing Agents

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## ABSTRACT

Gold nanoparticles AuNPs were prepared by the reduction of sodium tetrachloroaurate (III) (NaAuCl4) with Schiff base derivative of Nacetylisatin with ceftriaxone antibiotic: (sodium(6S)-7-((Z)-2-(2-((E)-1-acetyl-2-oxoindolin-3-ylideneamino)thiazol-4-yl)-2ethoxyimino)acetamido)-3-((2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2carboxylate) (SCR). The size, morphology and crystal structure of the synthesized AuNPs were characterized by UV- visible spectroscopy, Xray Diffraction (XRD), Scanning Electron Microscope (SEM), and Atomic Force Microscope (AFM) analysis. The FTIR spectra supported the conjugation of AuNPs with the ligand molecules.

Keywords: N-acetyl isatin, Schiff base, AuNPs, Surface plasmon resonance, Scanning Electron Microscope (SEM)

## INTRODUCTION

The synthesis of gold nanoparticles by Schiff base derivatives of  $\beta$ -lactam antibiotics was reported to be enhanced and stabilized by groups other than the free amino group that has been involved in condensation reaction with different carbonyl compounds [1,2]. In this work the synthesis of AuNPs in aqueous solutions is investigated in absence of free amino group by condensation of ceftriaxone antibiotic with N-acetylisatin to form Schiff base derivative: (sodium(6S)-7-((Z)-2-(2-((E)-1-acetyl-2-oxoindolin-3-ylideneamino)thiazol-4-yl)-2-ethoxy-imino)acetamido)-3-(2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylate) (SCR) [3] (Figure 1) as a reducing and capping agent using different concentration ratios of SCR/AuCl<sub>4</sub><sup>-</sup>, temperature and pH media.



Figure 1: Structure of Schiff base ligand (SCR) [3]

#### MATERIALS AND METHODS

#### Chemicals

The following chemicals were used as received from suppliers: Ceftriaxone Sodium ( $C_{18}H_{16}N_8O_7S_3Na_2.3.5H_2O$ ) (LDP), Sodium Tetrachloroaurate (III) dihydrate (NaAuCl<sub>4</sub>.2H<sub>2</sub>O) (BDH). The synthesis and characterization of the Schiff base ligand SCR has been reported earlier.

#### Instruments

Electronic spectra for prepared solutions in the (UV-Visible) region (200-1100 nm) were recorded on SHIMADZU 1800 Double Beam UV-Visible spectrophotometer.

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The Fourier Transform Infrared Spectroscopy (FTIR) spectra were performed by using SHIMADZU FT-IR 8400S Fourier transforms. Separation of nanoparticles by centrifugation was performed by using Centrifuge C41 7800 14000 r.p.m., Jouan, (France). SEM images were obtained by using (KYKY-SEM3200). AFM images were taken using AFM model AA 3000 SPM 220 V-Angstrom Advanced INC. USA. XRD analysis was carried out on a SHIMADZU XRD-6000 x-ray diffractometer (XRD).

#### **Preparation of solutions**

Each of the two reactants NaAuCl<sub>4</sub>.2H<sub>2</sub>O,  $(2.514 \times 10^{-4} \text{ M})$ , and the Schiff base ligand SCR  $(1.2 \times 10^{-4} \text{ M})$  (100 ml each) were prepared by dissolving 0.1000 and 0.1007 g respectively in 100 ml Deionized Water (DDW) in two separate 100 ml volumetric flasks.

#### Synthesis of GNPs

To study the Effect of the concentration ratios of SCR/AuCl<sub>4</sub><sup>-</sup> on the synthesis rate of GNPs, aliquots of 1.5 mL of ligand aqueous solution (1.20  $\times 10^{-4}$  M) were added to different volumes of AuCl<sub>4</sub><sup>-</sup> solutions (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0 and 3.5 ml, 2.514  $\times 10^{-3}$  M) in ten volumetric flasks, and the volumes were completed to 5 ml by DDW. The resultant concentrations of ligand was  $3.6 \times 10^{-5}$  M, while the concentrations of AuCl<sub>4</sub><sup>-</sup> were: (1.257  $\times 10^{-5}$ , 2.514  $\times 10^{-5}$ ,  $3.771 \times 10^{-5}$ ,  $5.28 \times 10^{-5}$ ,  $6.285 \times 10^{-5}$ ,  $7.542 \times 10^{-5}$ ,  $1.006 \times 10^{-4}$ ,  $1.257 \times 10^{-4}$ ,  $1.504 \times 10^{-4}$  and  $1.76 \times 10^{-4}$  M) respectively. The concentration ratios of SCR/AuCl<sub>4</sub><sup>-</sup>=2.864, 1.432, 0.955, 0.682, 0.573, 0.477, 0.358, 0.286, 0.239 and 0.205 respectively (solutions 1-10 respectively). The absorbance of each solution mixture was measured at room temperature after 1 h, 24 h, 48 h, 1 w, 2 w and 3w to select the optimum concentration ratio. Temperature effect was studied by heating ten (5 ml) solutions containing the selected ligand/Au (III) concentration ratio, at the selected temperature for different heating times (5, 10, 15, 20, 25, 30, 35, 40, 45 and 60 min respectively). The absorbance of each solution mixture in the UV-Visible region was measured after being cooled to room temperature for 1 h. The synthesis of AuNPs of solutions containing the selected SCR/AuCl<sub>4</sub><sup>-</sup> concentration ratio was studied at different pH values (2.60, 3.7, 4.24, 5.47, 5.89, 7.29, 8.42 and 8.87).

#### **RESULTS AND DISCUSSION**

#### **UV-Vis spectrophotometry**

#### **Concentration effect**

Figure 2 shows the UV-Visible spectra of the Schiff base ligand SCR (a),  $AuCl_4^-$  (b) and AuNP solution prepared from mixing 1.5 ml of Schiff base ligand SCR  $(1.2 \times 10^{-4} \text{ M})$  with 2 ml of  $AuCl_4^-$  (2.514 × 10<sup>-4</sup> M) diluted to 5 ml at room temperature. The spectrum of SCR displayed two bands at  $\lambda$  230 and 290 nm which were attributed to  $\pi \rightarrow \pi^*$ [3], while the spectrum of  $AuCl_4^-$  solution showed a high intensity band at 212 nm and a shoulder at 290 nm which are characteristic of the square planar geometry of the complex [4,5]. The mixture of both reactants developed a pink color solution with an absorption band at 535 nm assigned to the SPR of spherical AuNPs [6-11] with estimated size range of about 10-23 nm [12]. This indicates that  $AuCl_4^-$  has been reduced by Schiff base ligand SCR to form AuNPs. However the color was observed after 24 h of mixing which refers to low rate of reduction.



Figure 2: The UV-Visible spectra of a-Schiff base ligand SCR, b- AuCl<sub>4</sub><sup>-</sup> and c-synthesized AuNPs in aqueous solutions at concentration ratio of SCR/Au(III) (0.358) at room temperature, after 24 h

Figure 3 exhibits the variation of intensity and position of SPB of AuNPs solutions prepared from a constant concentration of SCR ( $3.6 \times 10^{-5}$  M) and different concentrations of AuCl<sub>4</sub><sup>-</sup>. After 1 h no color change and no SPB were detected in all solutions. After 24 h the solutions (4-10) gave single absorption bands at  $\lambda$ =536, 536, 538, 535, 536, 548 and 551 nm respectively, corresponding to SPR of spherical AuNPs [6-11]. After 48 h solution 3 exhibited a weak absorption band at 517 nm. The spectra of solutions (4-10) displayed increased intensity of SPB which appeared at  $\lambda$ =535, 535, 534, 538, 565 and 570 nm respectively with bathochromic shift in the solutions 7-10 with estimated size range 38-60 nm [8-10]. After one week solutions (3, 5, 8 and 10) exhibited decreased intensity of SPB, while the solutions (4, 6, 7 and 9) exhibited increased intensity of SPB observed at  $\lambda$ =(531, 540, 539, 539, 541, 548, 548 and 551 nm respectively. After two weeks absorption bands of solutions 3-10 were observed at  $\lambda$ =531, 541, 541, 541, 544, 550, 550 and 549 nm respectively. The solutions 1 and 2 remained colorless for or more than two weeks then solution 2 exhibited a weak absorption band at  $\lambda$ =519 nm.



Figure 3: Absorption spectra of AuNPs solution prepared from different concentration ratios of SCR/AuCla

The above mentioned results indicate that the rate of synthesis of AuNPs increased with decreased concentration ratio of SCR/AuCl<sub>4</sub><sup>-</sup> in the range (0.955-0.286). The highest performance was exhibited by solution 7 (SCR/AuCl<sub>4</sub><sup>-</sup> 0.358) which showed higher intensity of SPB and better stability of AuNPs compared with the other solutions.

## **Temperature effect**

Figure 4 shows variations of spectra of ten AuNPs solutions prepared at different temperatures (35-80°C) using concentration ratio of SCR/AuCl<sub>4</sub> (III) (0.358). The first five solutions exhibited color change and their spectra showed weak SPB after more than 24 h. Color change and SPBs were observed only in the last five solutions at temperature range 60-80°C, the spectra of which exhibited increased intensity with temperature and showed SPBs of spherical AuNPs [6-11] which appeared at wavelength range (535, 530, 530, 534 and 531 nm respectively) with maximum absorption at 80°C. The spectra of ten solutions heated at 80°C for 5-60 min, are shown in Figure 5. The color of all solutions were changed and their spectra after 1 h exhibited absorption bands appeared at  $\lambda$ =528, 537, 528, 528, 526, 521, 525, 520, 527 and 528 nm respectively, assigned to the SPB of spherical AuNPs with size range about 10-23 nm [12]. After 24 h all solutions exhibited increased intensity of absorption bands. The solutions heated for 5, 15, 25, 30, 35, 45 and 60 min were shifted to higher wavelength and appeared at (531, 532, 530, 528, 533, 534 and 534 nm respectively. The solutions remained stable for more than one week when the intensity of SPBs decreased and their positions were shifted to higher wavelength at  $\lambda$ =544, 542, 536, 544, 536, 542, 543, 542, 542 and 544 nm respectively, referring to increased size of AuNPs. The best heating time to give good particle size and higher stability of AuNPs was for 30 min.



Figure 4: Absorption spectra with time for AuNPs synthesized from a solution of SCR/AuCl<sub>4</sub>(III) (0.358), heated at 35°C-80°C





Figure 5: Absorption spectra of AuNPs solutions of SCR/AuCl<sub>4</sub> (III) (0.358), heated at 80°C for different time intervals (5-60 min)

#### pH effect

Figure 6 shows the absorption spectra of SCR-synthesized AuNPs of SCR/AuCl<sub>4</sub><sup>-</sup> ratio (0.358), studied at different pH media 2.60, 3.7, 4.24, 5.47, 5.89, 7.29, 8.42 and 8.87, solutions 1-8 respectively. Solutions of higher pH values 10.12 and 11.09, 9 and 10 were excluded as they remained colorless for more than one week. No colour change and no SPB were detected in all eight solutions on day of preparation. After 24 h the spectra of solutions at pH 2.60-5.89, solutions 1-5 respectively exhibited single absorption bands at  $\lambda$  539,533, 534, 528 and 529 nm respectively related to spherical AuNPs [6-11].



Figure 6: Absorption spectra of AuNPs synthesized by SCR at concentration ratio SCR/AuCl<sub>4</sub>(III) (0.358), at different pH values

After 48 h the spectra of solutions (2-5) (3.7-5.89) exhibited increased intensity of SPB which were shifted to higher wavelength and appeared at 542, 544, 535 and 538 nm respectively. After one week the spectrum of solution 6 (pH=7.29) exhibited a weak absorption band appeared at 540 nm, while the solutions 7 and 8 (pH 8.42 and 8.87) remained colorless for more than two weeks. The optimum pH values for a higher rate of AuNPs synthesis by SCR with smaller particle size and higher stability, were at pH=5.47 (solution 4) and to less extent at pH 5.89 (solution 5) which were stable for more than two weeks. These results indicate that the synthesis of GNPs by SCR is inhibited in highly acidic and in basic media.

#### X-ray diffraction (XRD)

Figure 7 shows the XRD pattern of AuNPs synthesized at concentration ratio of SCR/AuCl<sub>4</sub><sup>-</sup> (0.358). Four diffraction peaks were observed at  $2\theta$ =38.1181, 44.3110, 64.5129 and 77.3573, corresponding to the planes (111), (200), (220) and (311) of face centered cubic crystal lattice structure of AuNPs [13].

The average particle size was found 45.42 nm corresponding to the planes (111) and (200) and 55.8 nm according to the planes (111), (200), (220) and (311) using Scherrer's equation [14,15].



Figure 7: XRD of SCR-capped AuNPs

## FT-IR spectra

The infrared spectrum of SCR, (Figure 8a), was reported in the previous work [3]. The bands appeared at (1739.63, 1654.81, 1639.83, (1550.09 and 1340.98) and  $1249.79 \text{ cm}^{-1}$ ).



Figure 8: FT-IR spectrum of a- SCR and b-SCR-capped AuNPs

Which were attributed to v C=O of lactam stretching vibration, azomethine (-HC=N, v C=O overlapped amide and ester,  $v_{asy}(COO^{-})$  and  $v_{sy}(COO^{-})$  and v C-COCH<sub>3</sub> respectively were shifted to lower or higher wavenumbers in the spectrum of AuNPs (Figure 8b) and appeared at (1743.53, 1650.10, 1625.10 and (1525.08 and 1317.29) cm<sup>-1</sup> respectively. These results indicate that the AuNPs have been conjugated with SCR through the mentioned functional groups.

#### Scanning Electron Microscopy (SEM)

The SEM micrographs of AuNPs prepared at room temperature in Figure 9 shows that the particles had different sizes and shapes ranging between spherical (diameter 56.79 nm), nanorods (diameter and length 45.138 and 277.491 nm respectively) and irregular shapes. Figure 10 shows the same morphology when the SCR-conjugated AuNPs were prepared at heating temperature 80°C for 30 min, with the average diameter of spherical shapes 23.96 nm and average diameter and length of Nano rod 43.504 and 178.087 nm respectively. Similarly, the SEM image of AuNPs at pH=5.47 (Figure 11) showed nanospheres of average diameter 40.486 and nanorods with average diameter and length about 57.86 and 215.164 nm respectively.



Figure 9: a-SEM image and particle size distribution of b-spherical and (c and d)-rod like SCR- synthesized AuNPs at room temperature



Figure 10: a-SEM micrograph and particle size distribution of b- spherical and (c and d)-nanorods of SCR-synthesized AuNPs heated at 80°C for 30 min



Figure 11: a-SEM image and particle size distribution of b-spherical and (c and d) nanorod of SCR-synthesized AuNPs at pH=5.47

## Atomic Force Microscopy (AFM)

The AFM images in Figures 12-14, showed aggregates of different shapes and sizes of AuNPs at concentration ratio of SCR/AuCl<sub>4</sub> (III) (0.358) at room temperature, 80°C and at pH=5.47 respectively. Results came in agreement with those of SEM.





Figure 12: AFM picture and size distribution of the SCR synthesized AuNPs at room temperature (SCR/AuCl<sub>4</sub> (III) (0.358). Average diameter 99.12 nm



Diameter(nm)





Figure 14: AFM picture and size distribution of the SCR- synthesized AuNPs at pH=5.47 (SCR/AuCl<sub>4</sub> 0.358). Average diameter 90.15 nm

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#### Antibacterial activity

Inhibition zone exhibited by SCR and SCR-conjugated AuNPs against bacterial cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumonia* are described in Table 1. Results showed that bacterial cultures were weakly affected by both materials. However, the growth inhibition zones caused by SCR-AuNPs were twice higher than those exhibited by the free ligand except *Staphylococcus aureus* which gave the same response against both materials.

#### Table 1: Inhibition zone exhibited by SCR and SCR-conjugated AuNPs against some pathogenic bacteria

Type of ligand	Inhibition zone (mm)			
	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus	Streptococcus pneumonia
SCR	5	2	5	3
SCR-capped AuNPs	10	5	5	7.5

## CONCLUSION

The synthesis of AuNP conjugates was achieved by using Schiff base derivative of ceftriaxone with N-acetyl isatin in absence of reducing agent. The sizes and stability of the synthesized AuNPs were highly dependent on  $SCR/AuCl_4$  (III) ratio, temperature, heating time and pH. The synthesis of AuNPs were enhanced in moderately acidic medium and highly inhibited in basic medium. SEM and AFM analyses showed that sizes and morphology of the synthesized AuNPs were highly affected by the Schiff base ligand. The antibacterial activity of SCR-AuNP conjugates against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pneumonia* were twice higher than the activity of the free ligand.

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