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Synthesis of Gold Nanoparticles Conjugates with Ceftriaxone Sodium and Two Schiff base Derivatives Using Sodium Borohydride as a Reducing Agent.

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ABSTRACT

Conjugates of gold nanoparticles with ceftriaxone sodium (CR) and two Schiff base derivatives of CR with isatin (L_1) and N-acetyl isatin (L_2) was studied by the reduction of sodium tetrachloroaurate (III) ($NaAuCl_4$) to AuNPs with sodium borohydride in presence and absence of trisodium citrate (TSC)with concentration ratios of CR: $NaBH_4$ and Schiff base ligand: $NaBH_4$ 0.0055-0.0066 and 0.014-0.0148 respectively. The addition of high concentrations of CR protected Au(III) ions against reduction with $NaBH_4$ and TSC. The synthesized AuNPs were characterized by uv- visible spectroscopy, scanning electron microscope (SEM) and atomic force microscope (AFM) analysis. The presence of citrate affected the sizes and shapes of AuNPs conjugates

Keywords: Ceftriaxone, Schiff base, sodium borohydride, AuNPs, trisodium citrate.

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INTRODUCTION

The synthesis of AuNPs by wet chemical methods involves the reduction of Au(III) ion precursors such as hexachloroauric (III) acid ($HAuCl_4$), sodium tetrachloroaurate (III)($NaAuCl_4$) and gold(III) chloride ($AuCl_3$) in solution with different reducing agents such as trisodium citrate (TSC) ($C_6H_5O_7Na_3$) [1-4], sodium borohydride ($NaBH_4$)[5-9], hydrazine (N_2H_4)[10] and ascorbic acid($C_6H_8O_6$) [10-12] …etc., to convert gold ions into Au^0 nanoparticles in aqueous solutions. The reduction process using $NaBH_4$ as a reducing agent in aqueous solutions is reported to proceeds more successfully at low temperature and in presence of stabilizers[9]. The reduction mechanism was suggested as follows [9]

Oxidation reaction: $BH_4^- + 3H_2O \rightarrow B(OH)_3 + 7H^+ + 8e^-$

Reduction reaction: $AuCl_4^- + 3e^- \rightarrow Au\ (0) + 4Cl^-$

In this work we are studying the synthesis of AuNPs in presence of the antibiotic ceftriaxone sodium(CR) and two of its Schiff base derivatives with isatin (L_1) and N-acetyl isatin (L_2) (Figure-1) using sodium borohydride as a reducing agent in presence and absence of TSC as reducing and stablising agent.

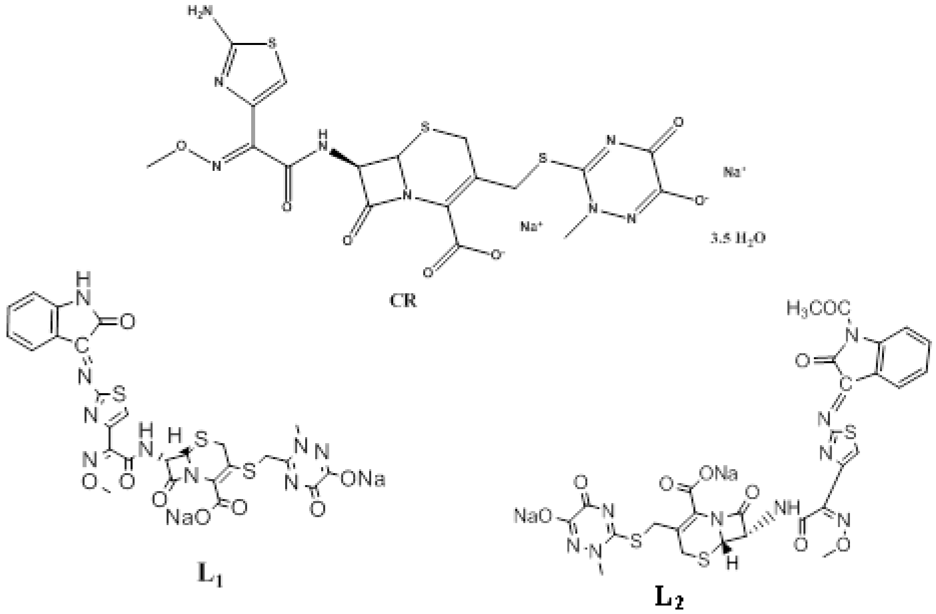

Figure – 1: Chemical structures of Ceftriaxone sodium(CR)and its two Schiff basederivatives

EXPERIMENTAL

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 ($NaAuCl_4.2H_2O$) (BDH), Trisodium citrate dihydrate ($C_6H_5O_7Na_3.2H_2O$ 99% (Fluka), Sodium borohydride ($NaBH_4$, 99%) (Fluka). The preparation and characterization of the two Schiff base ligands has been reported earlier [13].

Instuments

Electronic spectra for prepared solutions in the (UV-Visible) region (200-1100 nm) were recorded on SHIMADZU 1800 Double Beam UV-Visible spectrophotometer. SEM images were acquired using KYKY-EM3200. AFM images were acquired using AFM model AA 3000 SPM 220 (V-Angstrom Advanced INC. USA). Samples were prepared by applying few drops of metal nanoparticles solutions on a glass slide followed by vacuum drying.

Preparation of solutions:-

A stock aqueous solution of the gold salt $NaAuCl_4.2H_2O$ (2.778x10^{-3} M) was prepared by dissolving 0.1105 g of $NaAuCl_4$.$2H_2O$ in 100 mL distilled deionized water (DDW) in 100 mL volumetric flask. A standard solution of $AuCl_4^-$ ions, (2.778x 10^{-4} M) was prepared by diluting 10 mL of the stock solution to 100 mL with (DDW). An aqueous solution of the antibiotic CR (1.51×10^{-3} M) was prepared by dissolving 0.1 g of ceftriaxone sodium (CR) in 100 mL DDW. A standard solution of ceftriaxone sodium salt (1.51×10^{-4} M) was prepared by diluting 10 mL of the stock solution to 100 mL with (DDW). Stock solutions of the Schiff base ligands L_1 and L_2 (2.53×10^{-3} M and 2.4×10^{-3} M respectively) were prepared by dissolving the ligand (0.2053 and 0.2015 g respectively) in 100 mL DDW. Then 10 mL of each stock solution was diluted to 100 mL to prepare the standard solutions (2.53×10^{-4} and 2.4×10^{-4} M respectively). A stock solution of TSC ($C_6H_5O_7Na_3.2H_2O$) (0.01 M) was prepared by dissolving 0.2941 g of the salt in 100 mL distilled deionized water (DDW. A standard solution of TSC (2.5×10^{-3} M) was prepared by diluting 2.5 mL of the stock solution to 10 mL with (DDW). Sodium borohydride solution (0.1 M) was prepared by dissolving 0.3783 g of the compound in 100 mL (DDW) in 100 mL volumetric flask .

Preparation of AuNPs - CR conjugates

I. To a stirred aqueous solution of $AuCl_4^-$ (2.778×10^{-4} M, 18 mL) was added, an aqueous solution of CR (1.51×10^{-4} M, 3.5 mL) and the mixture was stirred for 3 min at (10°C). Then an aqueous solution of $NaBH_4$ (0.1 M, 0.6 mL) cooled to 10°C was added slowly to the mixture. The color of solution changed to pink. After continuous stirring for 40 min, the absorbance of the solution was measured after 1hour, 24h, 1week and 2weeks.

II. To an aqueous solution of $AuCl_4^-$(2.778×10^{-4} M, 18 mL), was added an aqueous solution of TSC (2.5×10^{-3} M, 2 mL), followed by the addition CR solution (1.51×10^{-4} M, 3.5 mL). The mixture was stirred vigorously for 4-5 min at (10°C). An aqueous solution of $NaBH_4$ (0.1 M, 0.6 mL) cooled to 10°C was added slowly to the mixture. The color of solution changed to pink. The same color was obtained when the volume of $NaBH_4$ solution (0.1 M) was increased to 0.8 mL,

III. To a cold aqueous solution (10°C) of $AuCl_4^-$(2.778×10^{-4} M, 18 mL) was added an aqueous solution of TSC (2.5×10^{-3} M, 2mL), the solution mixture was stirred vigorously for 4-5 min at 10°C. Then a cold solution (10°C) of $NaBH_4$ (0.1 M, 0.6 mL) was added slowly with continuous stirring. A pink color was developed indicating the formation of AuNPs. Then an aqueous solution of CR (1.51×10^{-3} M, 3.5 mL) was added to the mixture and stirring was continued for 40 min. Adding 3.5 and 2mL of CR solution (1.51×10^{-3} M) to $AuCl_4^-$ solution (2.778×10^{-4} M, 18 mL) before the addition of $NaBH_4$ (0.1 M, 0.6 mL) changed the color of solution to brown and the SPB was only observed after leaving the resulting solutions for two weeks. In two parallel experiments the CR solution (1.51×10^{-3} M, 3.5 mL) was added to a mixture of TSC (2.5×10^{-3} M, 2 mL) and $AuCl_4^-$ (2.778×10^{-4} M, 18 mL) with continuous stirring for 5 min. A brown color was observed. The two mixtures were cooled to (10°C) followed by the addition of two volumes of cold $NaBH_4$ solution (0.1 M, 0.6 and 0.8 mL). The brown color remained unchanged and no SPB was observed.

IV. The CR solution (1.51×10^{-3} M, 3.5 mL) was added to a mixture of TSC (2.5×10^{-3} M, 2 mL) and $AuCl_4^-$ (2.778×10^{-4} M, 18 mL) with continuous stirring for 5 min. The mixture was then heated to boiling point with stirring for 30 min. The color of solution was changed to brown. The resulted solution was cooled to10°C in ice bath. Then a cold solution of $NaBH_4$ (0.1 M, 0.6 mL) was added with continuous stirring. No color change was observed for more than one month.

Preparation of AuNPs Schiff base conjugates

I. To a stirred aqueous solution of $AuCl_4^-$ (2.778×10^{-4} M) in DDW (18 mL) was added a cold aqueous solution of the Schiff base ligand L_1 or L_2 (2.53×10^{-4} and 2.4×10^{-4} M respectively , 3.5 mL each), and the mixture was vigorously stirred for 3 min. Then an aqueous solution of $NaBH_4$ (0.1 M, 0.6 mL) cooled to 10°C, was added slowly to the reaction mixture with continuous stirring. The color of solution was changed to wine red. Stirring was continuous for 40 min.

II. To an aqueous solution of $AuCl_4^-$ (2.778×10^{-4} M, 18 mL) cooled to 10°C in an ice bath was added, a cold solution of TSC (2.5×10^{-3} M, 2 mL), and the mixture was stirred for 3 min at10°C when a cold solution of

$NaBH_4$ (0.1 M, 0.6 mL) was added slowly to the reaction mixture. The color of solution was changed to wine red. Then an aqueous solution of each ligand was added to the colored solution with continuous stirring. The color of the two solutions remained unchanged.

III. The same solution mixture was prepared as in II, except that the solution of L_1 or L_2 (2.53×10^{-4} M, and 2.4×10^{-4} M respectively, 3.5 mL) was added to the ($AuCl_4^-$, TSC) mixture before the addition of $NaBH_4$ (0.1 M, 0.6 mL) at 10°C. The color of the solution changed to wine red, indicating the formation of AuNPs.

RESULTS AND DISCUSSION

Synthesis of AuNPs using different concentration ratios of CR: $NaBH_4$

Figure-2 shows the variation of intensity and position of SPR of AuNPs solutions that have been formed by the addition of sodium borohydride (0.1M) to a solution of gold salt (2.778×10^{-4} M) in presence of CR(1.51×10^{-4} M). The final concentrations of $AuCl_4^-$, CR and $NaBH_4$ in the mixture were (2.263×10^{-4}, 2.391×10^{-5} and 2.715×10^{-3} M respectively and the concentration ratio of CR: $NaBH_4$ is (0.0088). The color of solution was changed immediately to pink and SPB of AuNPs appeared as a single peak at 512 nm indicating the formation of spherical AuNPs[6,14-18] with estimated particle diameter between 10-20 nm [12, 19].The solution remained stable for more than two weeks. The same reduction process was repeated, by adding $NaBH_4$ solution (0.1 M) to an aqueous solution of TSC (2.5×10^{-3} M), CR (1.51×10^{-4} M) and $AuCl_4^-$ (2.778×10^{-4} M). The final concentrations of reactants were (2.489×10^{-3}, 2.075×10^{-4}, 2.193×10^{-5} and 2.075×10^{-4} M respectively) and the concentration ratio of CR: $NaBH_4$ is (0.008). The color of solution was changed to pink and SPR of AuNPs appeared as a single peak at 501 nm (Figure -3). The AuNPs remained stable with increased intensity and preservation of the SPB positions for more than two weeks. Similar results were obtained on increasing the volume of $NaBH_4$ to give the final concentrations of reactants [3.29×10^{-3}, 2.058×10^{-4}, 2.058×10^{-4} and 2.175×10^{-5} M respectively] where the ratio of CR: $NaBH_4$ is (0.0066). The band of SPR of AuNPs appeared as a single peak at 505 nm (Figure -4).

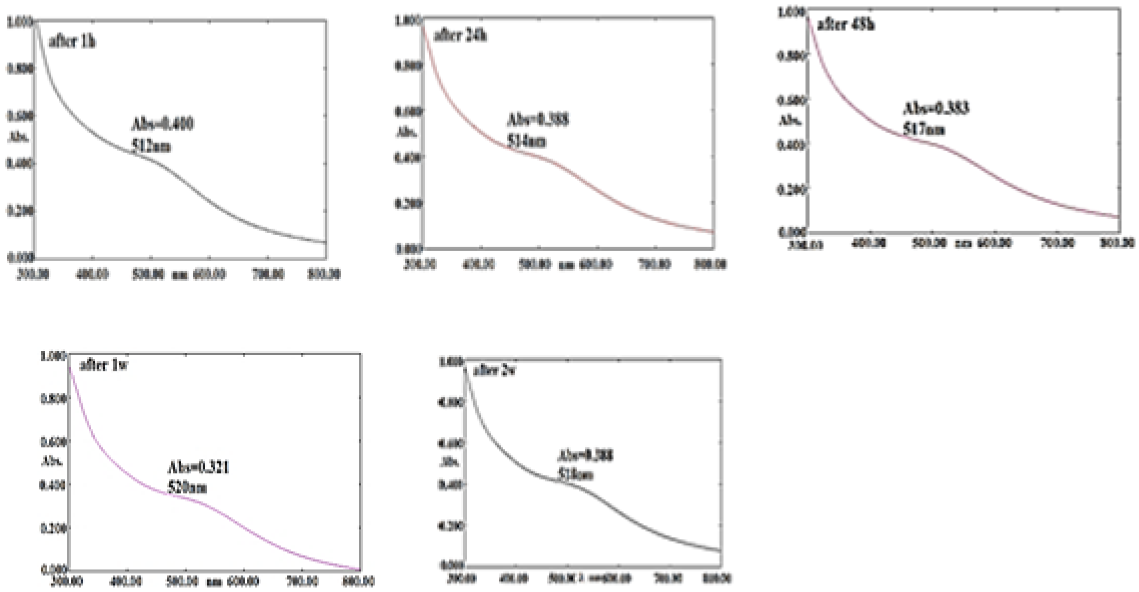

Figure -2: Absorption spectra of AuNPs prepared from the reduction of $AuCl_4^-$ with $NaBH_4$ in presences of CR (the concentration ratio of CR: $NaBH_4$ 0.0088).

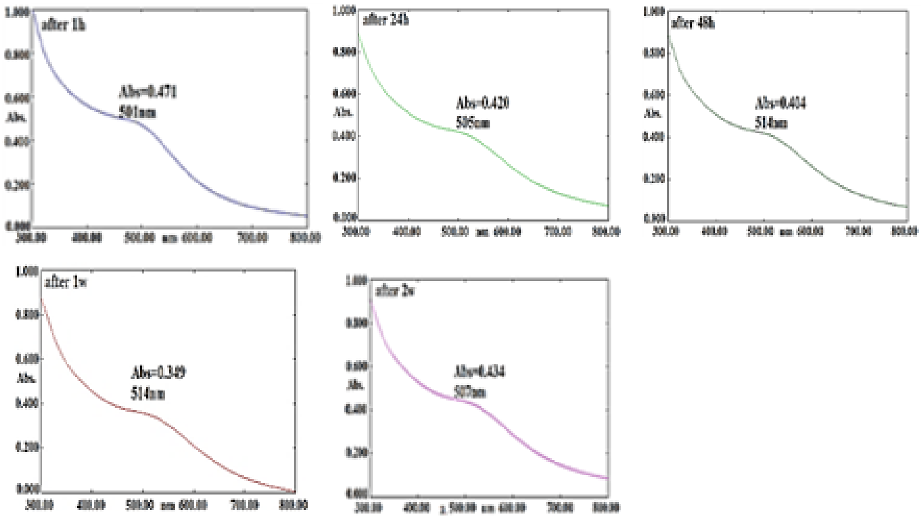

Figure- 3: Absorption spectra of AuNPs in presences of TSC and CR (concentration ratio of CR: $NaBH_4$ 0.008).

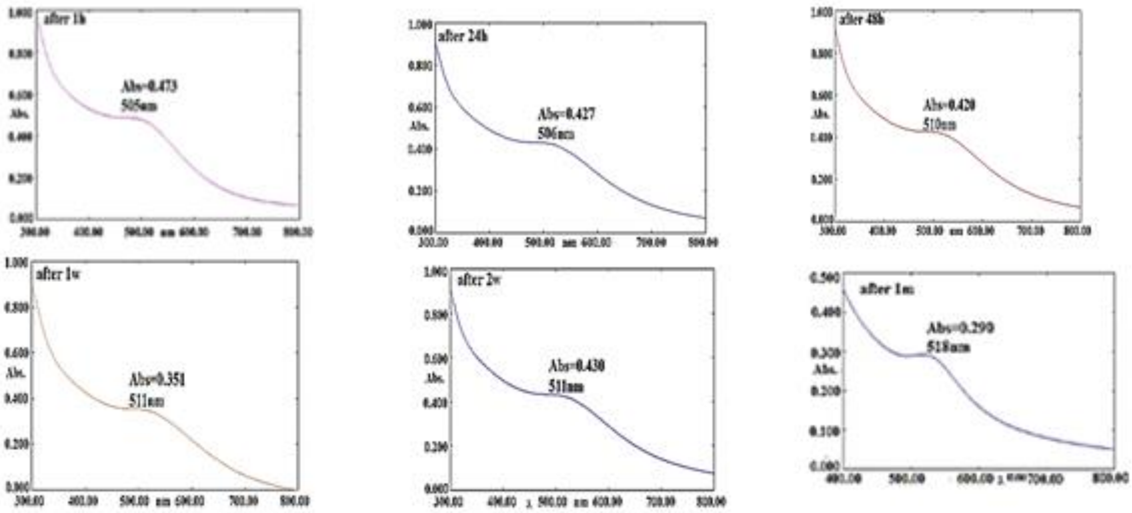

Figure 4: Absorption spectra of AuNPs in presences of TSC and CR (concentration ratio of CR:$NaBH_4$ 0.0066).

The AuNPs have been synthesized by the addition of TSC (2.5×10^{-3} M) to gold salt solution (2.778×10^{-4} M), followed by the addition of sodium borohydride (0.1 M) at 10 ° C. The color of solution was changed to wine red. Then a solution of CR (1.51×10^{-3} M) was added. The final concentrations of reactants were (2.075×10^{-4}, 2.075×10^{-4},2.489×10^{-3} and 2.193×10^{-4} M) for $AuCl_4^-$,TSC, $NaBH_4$and antibiotic respectively) and the concentration ratio of CR:$NaBH_4$ is (0.088) . The color remained unchanged and the SPB was observed as a single peak at λ 521 nm which corresponds to spherical AuNPs [6, 14-18] (Figure -5), with estimated size about 10-23 nm [12]. The solution remained stable for 1 month within the wavelength range 520-529 nm which refers to the stabilization of AuNPs with CR molecules. When the CR solution ($1.51\text{x}10^{-3}$) was added to the $AuCl_4^-$ solution before $NaBH_4$ in absence of TSC, using the reactant concentrations (2.263×10^{-4}, 2.391×10^{-4} and 2.715×10^{-3} M for $AuCl_4^-$, CR and $NaBH_4$ respectively) and the concentration ratio of CR:$NaBH_4$ (0.088), the solution turned brown and no SPB for AuNPs was detected . After two weeks, the SPB was observed at λ 514 nm as is shown in (Figure -6) which refers to the formation of spherical AuNPs with estimated particle size about10-20 nm[12,19]. The same result was obtained when the volume of CR was reduced so that the final concentrations of $AuCl_4^-$, CR and $NaBH_4$ were (2.427×10^{-4}, 1.466×10^{-4} and 2.913×10^{-3} M respectively) and the concentration ratio of CR: $NaBH_4$ is (0.0503). The peak of SPR appeared at λ 526 nm (Figure -7).

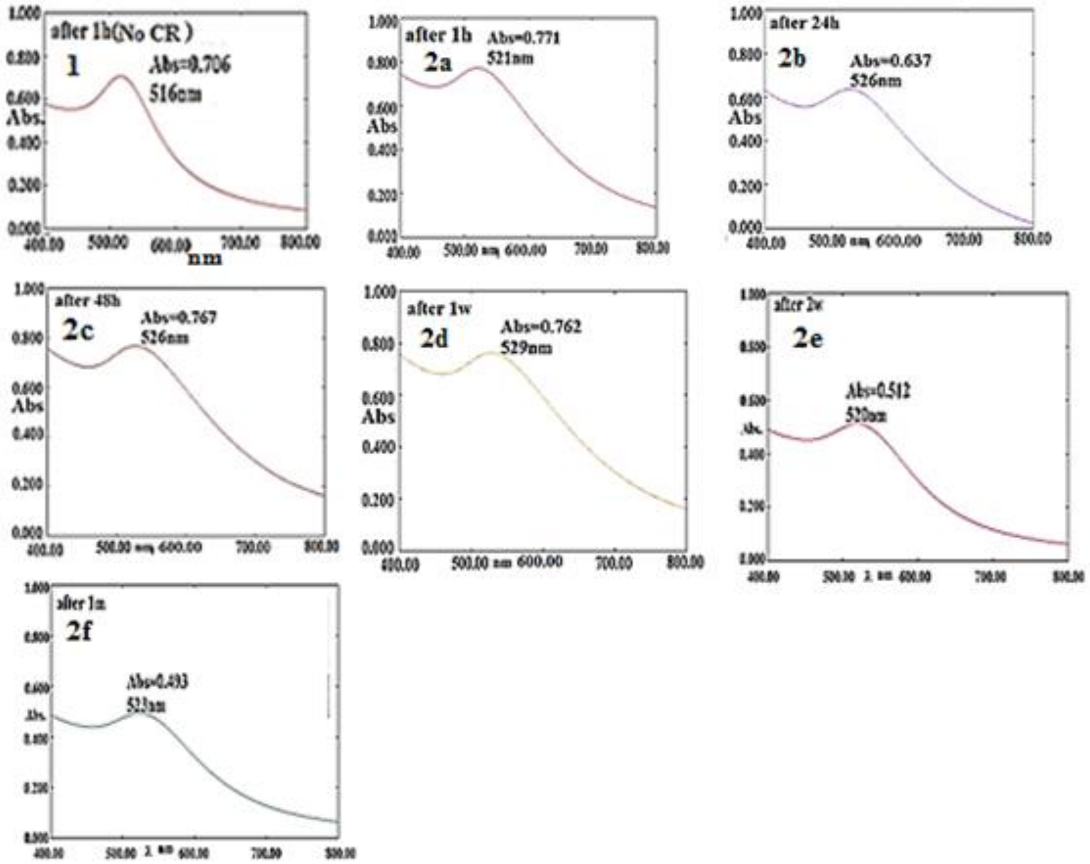

Figure -5: Absorption spectra of AuNPs1- in absence of CR and TSC and 2(a-f)-before the addition of CRin presence of TSC (CR:NaBH$_4$ 0.088).

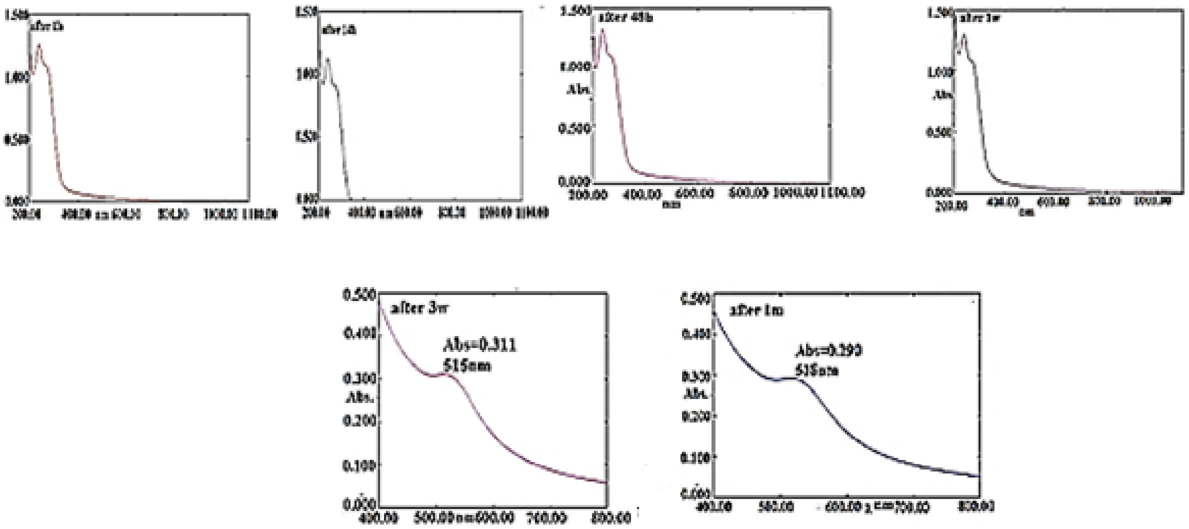

Figure -6: Absorption spectra of AuNPs synthesized from the reduction of AuCl$_4^-$ with NaBH$_4$after the addition of CR (CR: NaBH$_4$ 0.088).

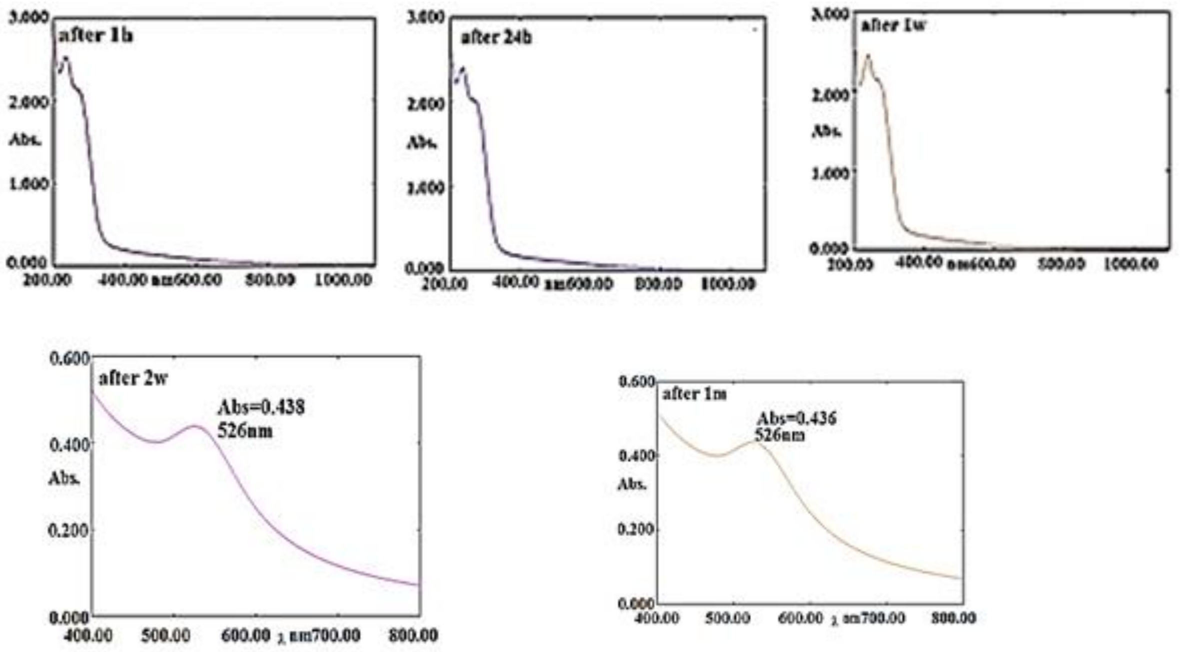

Figure -7: Absorption spectra with time of AuNPs synthesized from the reduction of $AuCl_4^-$ with $NaBH_4$ after the addition of CR (CR:$NaBH_4$ 0.0503).

No reduction took place and no SPB was detected on adding CR ($1.51x10^{-3}$) to a solution mixture of $AuCl_4^-$ and TSC before $NaBH_4$even on increasing the concentration of the latter (Figures -8a and -8b) and the colors of resulted solutions remained brown. Heating the solution mixture of $AuCl_4^-$, TSC and CR to boiling point did not show any sign of reduction or formation of AuNPs (Figure- 9a). Cooling the solution to 10 °C followed by the addition of sodium borohydride showed no sign of AuNPs either (Figure -9 b). These observations indicate that, at high concentrations (1.466×10^{-4} - 2.391×10^{-4} M) , the antibiotic CR forms stable Au(III) complexes against reduction of Au(III) ions with $NaBH_4$ to AuNPs . Although TSC is reported as a good reducing agent for AuNP synthesis at boiling point [20-24], the CR molecules acted as a masking agent against the reduction process. However, the addition of high concentrations of CR, after reduction of Au(III) ions with $NaBH_4$, was found to stabilize the AuNPs compared with low concentrations ($2.193\text{-}2.39\times10^{-5}$ M).

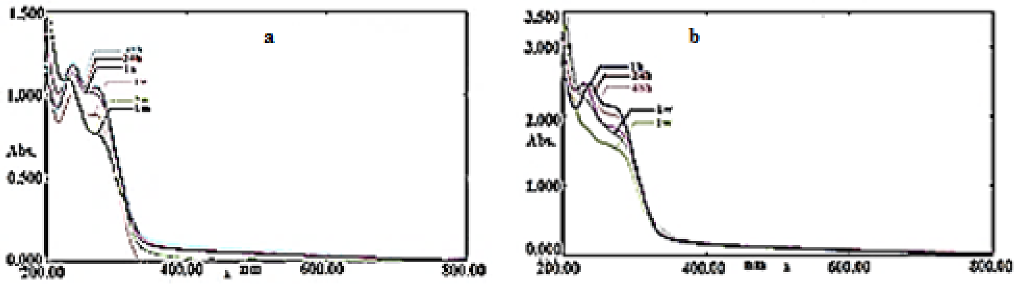

Figure -8: Absorption spectra of a solution mixture prepared from addition of $NaBH_4$, to a solution of $AuCl_4^-$, TSC and CR a- CR:$NaBH_4$ 0.088; b- CR:$NaBH_4$ 0.066)).

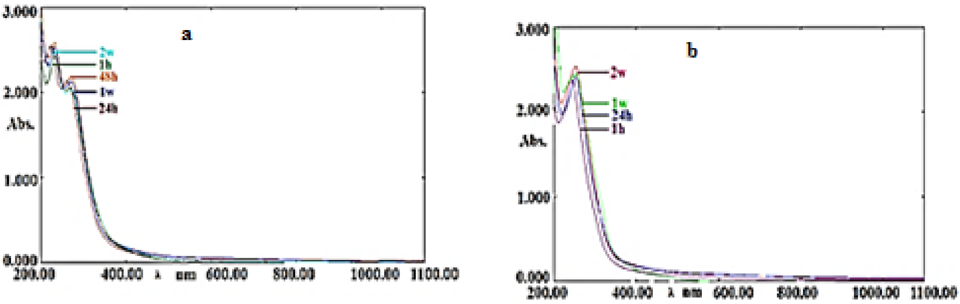

Figure -9: Absorption spectra of a solution prepared from a- a mixture of $AuCl_4^-$, TSC and CR) b-The same mixture in (a) after the addition of $NaBH_4$ solutionat 10°C (CR:$NaBH_4$ (0.088)) .

Synthesis of AuNPs using $NaBH_4$ in presence of Schiff base ligands

Figure -10 shows the variations of intensity and position of SPR of AuNPs solutions that have been synthesized by the addition of sodium borohydride (0.1 M) to a solution of TSC (2.5×10^{-3} M) and $AuCl_4^-$ (2.778×10^{-4} M), followed by the addition of an aqueous solution of L_1 (2.53×10^{-4} M). The final concentrations of $AuCl_4^-$, TSC, $NaBH_4$ and L_1 (2.075×10^{-4}, 2.075×10^{-4}, 2.489×10^{-3} and 3.674×10^{-5}M respectively) and the concentration ratio of L_1:$NaBH_4$0.0148. The color of the solution turned to pink and SPB of AuNPs appeared as a single peak at 513 nm . The same reduction process was repeated with the $NaBH_4$ solution being added after the addition of L_1. The color of solution was changed immediately to wine red and SPB of AuNPs appeared as a single peak at 515 nm (Figure -11). The same result was obtained in absence of TSC(Figure-12) when a solution of L_1(2.53×10^{-4} M) was mixed with $AuCl_4^-$ solution (2.778×10^{-4} M) for 4 min followed by the addition of $NaBH_4$ solution (0.1M) to give the final concentrations (2.263×10^{-4}, 4 $\times10^{-5}$, 2.715×10^{-3} M) for $AuCl_4^-$, L_1 and $NaBH_4$ respectively and the concentration ratio of L_1:$NaBH_4$ 0.0147. The color of solution was changed immediately to wine red and SPB of AuNPs appeared as a single peak at 515 nm. The AuNPs prepared in presence of L_1 remained stable for three weeks. Figure -13 shows the variation of intensity and position of SPB of AuNPs solutions that have been formed from the addition of $NaBH_4$ (0.1 M) to a solution mixture of TSC (2.5×10^{-3} M) and $AuCl_4^-$(2.778×10^{-4} M), followed by the addition of solution of L_2 (2.4×10^{-4} M). The final concentrations of $AuCl_4^-$, TCS, $NaBH_4$ and L_2 in the mixture are (2.075×10^{-4}, 2.075×10^{-4}, 2.489×10^{-3} and 3.485×10^{-5} M respectively) and the concentration ratio of L_2:$NaBH_4$ 0.014 . The color of solution was changed immediately to wine red and SPR of AuNPs appeared as a single peak at 512 nm. The color of solution was also changed immediately to wine red when $NaBH_4$ was added after the addition of L_2 and SPR of AuNPs appeared as a single peak at 504 nm (Figure -14). Figure-15 shows the spectra and images of AuNPs solutions prepared in absence of TSC, by the addition of $NaBH_4$ to mixture of L_2 and $AuCl_4^-$ to give the final concentration (2.263×10^{-4}, 3.80×10^{-5} and 2.715×10^{-3} M) for $AuCl_4^-$, L_2 and $NaBH_4$ respectively where the concentration ratio of L_2:$NaBH_4$ 0.014. The color of solution was changed immediately to wine red and SPR of AuNPs appeared as a single peak at 508 nm. The AuNPs in presence of L_2 remained stable with increased intensity and preservation of SPB positions for more than two weeks. These results show that the two Schiff base complexes with $AuCl_4^-$ ions were unstable toward reduction with $NaBH_4$ in presence and absence of TSC, compared with CR complexes. However AuNps were stabilized by the two Schiff base ligands.

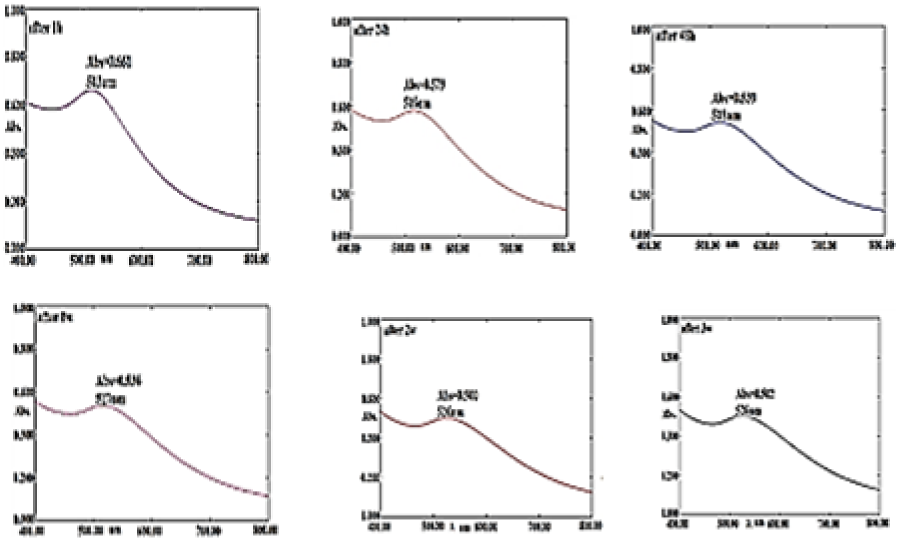

Figure -10: Absorption spectra of AuNPs solution synthesized in presence of TSC followed by the addition of L_1 (concentration ratio of L_1:$NaBH_4$ 0.0148)·

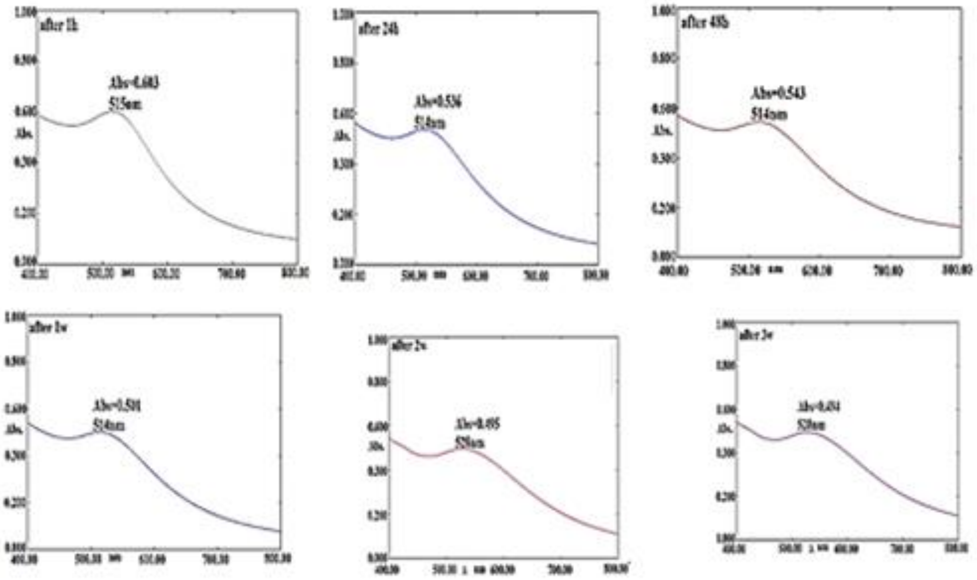

Figure- 11: Absorption spectra of AuNPs solution prepared in presence of TSC and L_1 (L_1:$NaBH_4$ 0.0148)

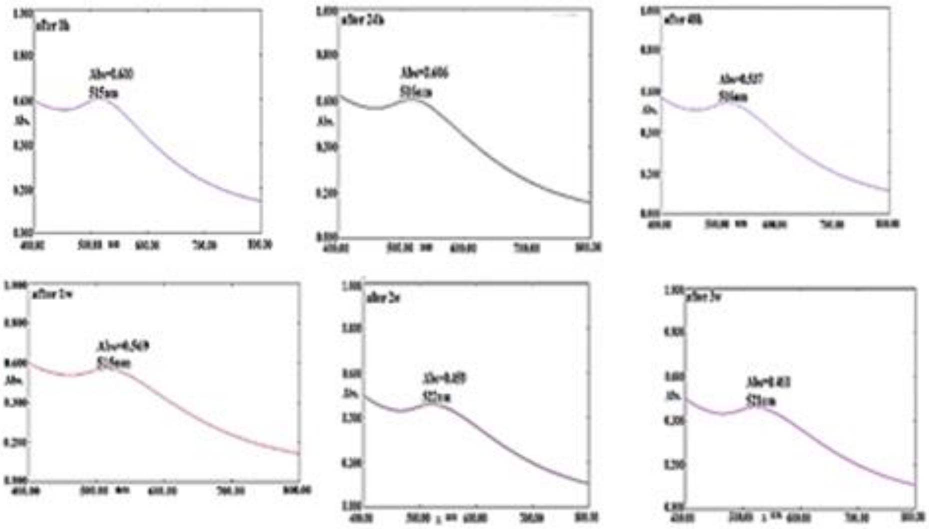

Figure -12: Absorption spectra of AuNPs prepared in presences of L_1 and in absence of TSC. (Concentration ratio of L_1:$NaBH_4$ 0.0147)

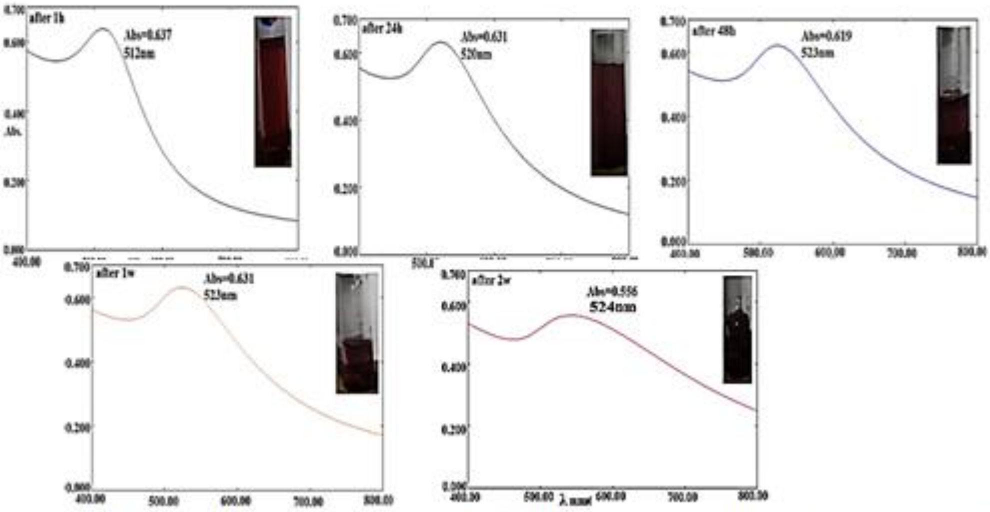

Figure -13: Absorption spectra and images of AuNPs after the addition of L_2 in presence TSC (concentration ratio of L_2:$NaBH_4$ 0.014)

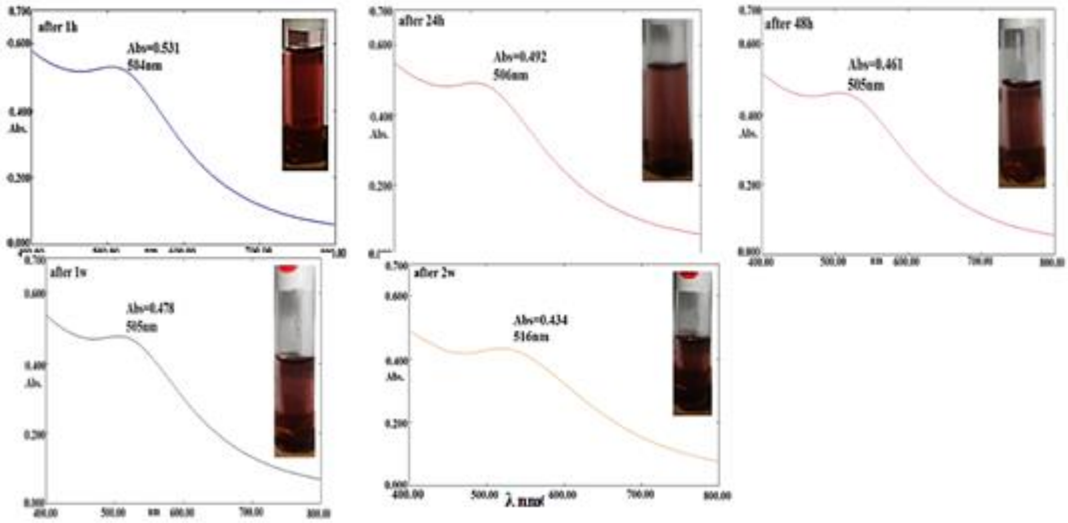

Figure -14: Absorption spectra of AuNPs synthesized from $AuCl_4^-$ and $NaBH_4$ in presences of L_2 and TSC (L_2:$NaBH_4$ 0.014)

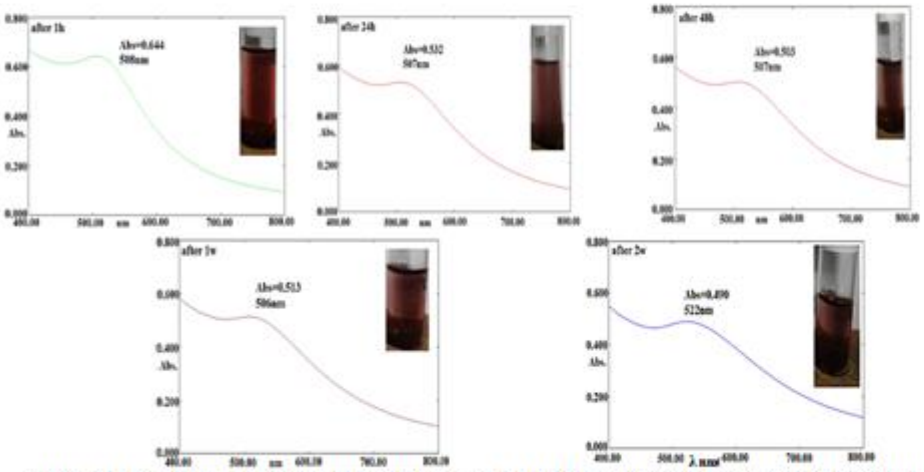

Figure -15: Absorption spectra and images of AuNPs prepared from $AuCl_4^-$ and $NaBH_4$ in presences of L_2 in absence of TSC (L_2:$NaBH_4$ 0.014).

Scanning Electron Microscopy (SEM):-

Figure-16 shows the SEM image of AuNPs prepared from the addition of $NaBH_4$ to the solution of $AuCl_4^-$ and TSC followed by addition of CR (2.193×10^{-4} M). The resulting nanoparticles had cubic shapes with a wide size distribution (48.9-100 nm) and average particle size 72.9 nm. In absence of TSC (Figure -17), the particles appeared spherical in shapes with an average diameter 52.8 nm**.** The SEM image of AuNPs prepared from the addition $NaBH_4$ to the solution of $AuCl_4^-$ and L_1 in presence of TSC is shown in Figures 18. They have cubic shapes with a wide size distribution and average diameter 46.6 nm. In absence of TSC (Figure -19), highly dispersed spherical and cubic shapes of AuNPs are observed with average size of 49.2 nm. SEM micrographs of AuNPs conjugates with L_2 are shown in Figures -20 and -21. In presence of TSC (Figure -20), the AuNPs have irregular and spherical shapes, with an average size 50.446 nm. In absence of TSC (Figure -21), the particles were of regular spherical shapes with average diameter about 71.856 nm. The shapes and size distribution of AuNPs were affected by type of ligand as well as the presence and absence of TSC. In absence of TSC, the morphology of conjugated AuNPs were of more regular shapes and better dispersion compared with those in presence of TSC**.**

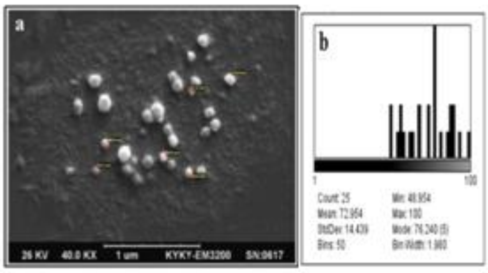

Figure- 16: a-SEM and b-particle size distribution of CR- AuNPs conjugates prepared in presence of TSC (CR: $NaBH_4$ 0.088)

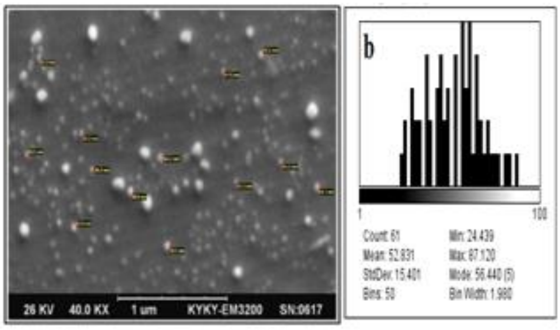

Figure -17: a-SEM and b-particle size distribution of CR- AuNPs conjugates prepared in absence of TSC (CR: $NaBH_4$ 0.088)

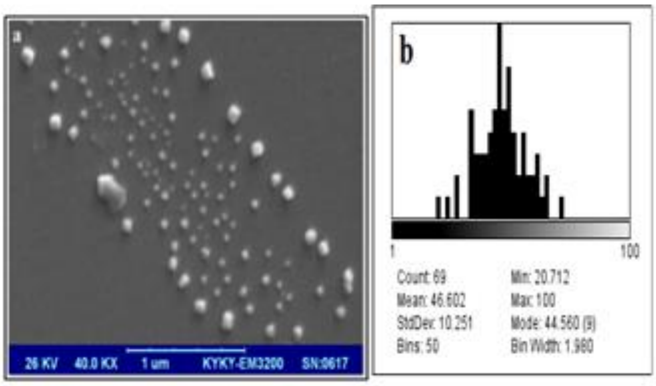

Figure- 18: a-SEM and b-particle size distribution of L_1-s AuNPs conjugates prepared in presence of TSC. (L_1/ $NaBH_4$ 0.0148)

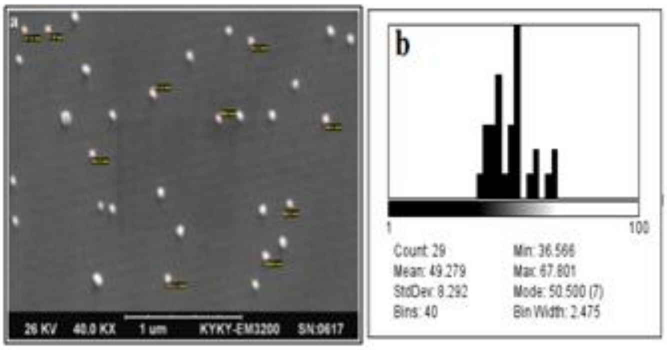

Figure- 19: a-SEM and b-particle size distribution of AuNPs- L_1 conjugates in absence of TSC). (L_1/ $NaBH_4$ 0.0147)

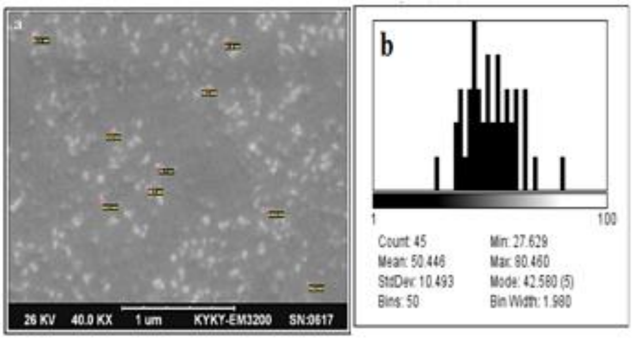

Figure -20: a-SEM and b-particle size distribution of L_2- AuNPs conjugates prepared in presence of TSC and L_{2-}(L_2/ $NaBH_4$ 0.014)

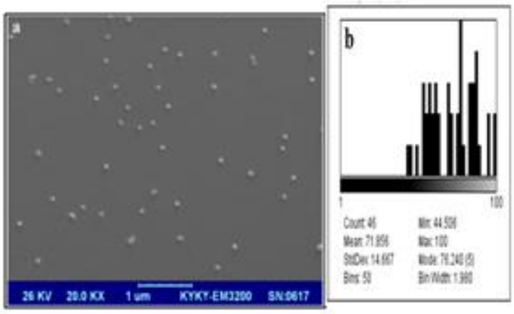

Figure- 21: a-SEM and b-particle size distribution of L_2-AuNPs conjugates prepared in absence of TSC. (L_2/ $NaBH_4$ 0.014)

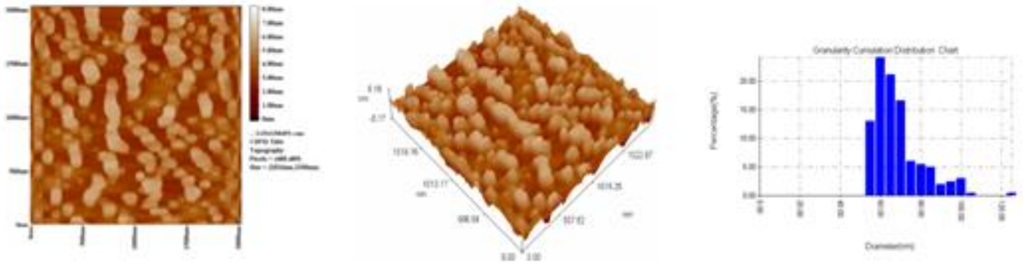

Figure- 22: AFM images and size distribution CR- AuNPs conjugates prepared TSC in presence of TSC (CR: $NaBH_4$ 0.088). Average diameter around 66.08 nm).

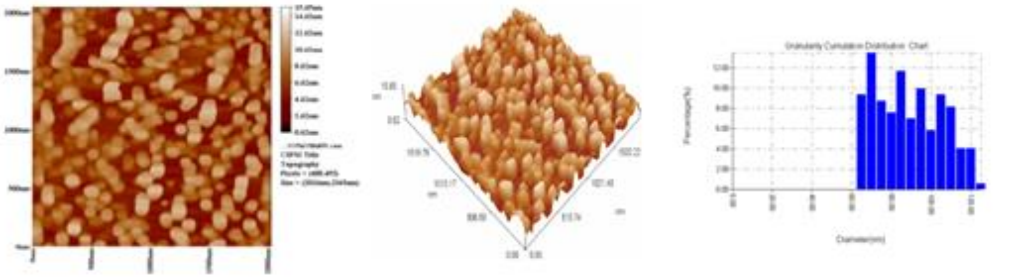

Figure -23: AFM images and size distribution CR- AuNPs conjugates prepared in absence of TSC (CR: $NaBH_4$ 0.088). Average diameter 86.33 nm.

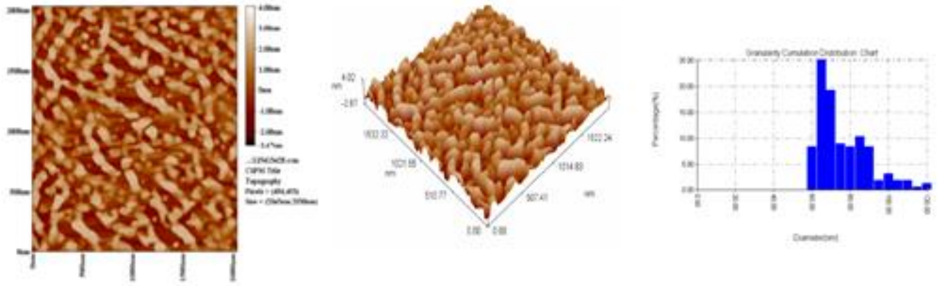

Figure -24: AFM images and size distribution of L_1-AuNPs conjugates prepared in presence of TSC (L_1/ $NaBH_4$0.0148)). Average diameter 73.83 nm.

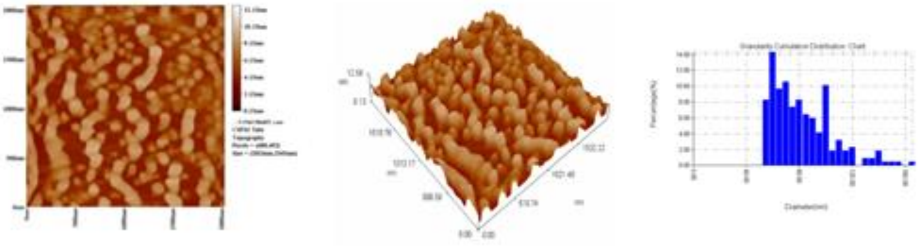

Figure -25: AFM images and size distribution ofL_1-AuNPs conjugates prepared in absence of TSC (L_1/ $NaBH_4$0.0147). Average diameter 79.62 nm.

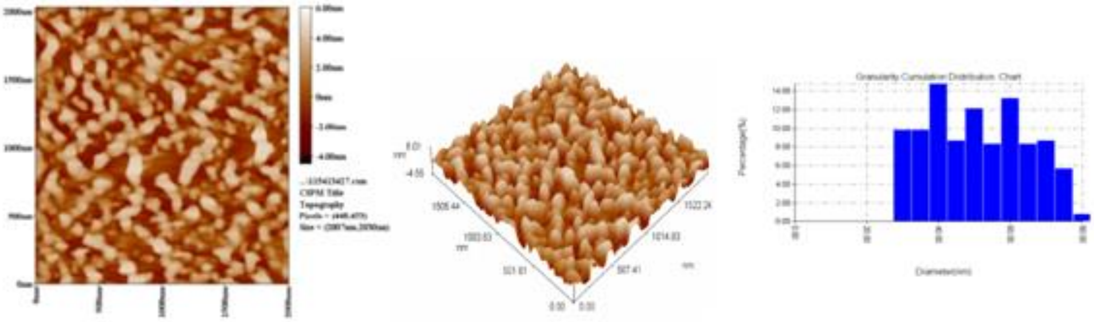

Figure -26 AFM images and size distribution of the L_2- AuNPs conjugates prepared in presences of TSC (L_2/ $NaBH_4$0.014). Average diameter 48.57 nm.

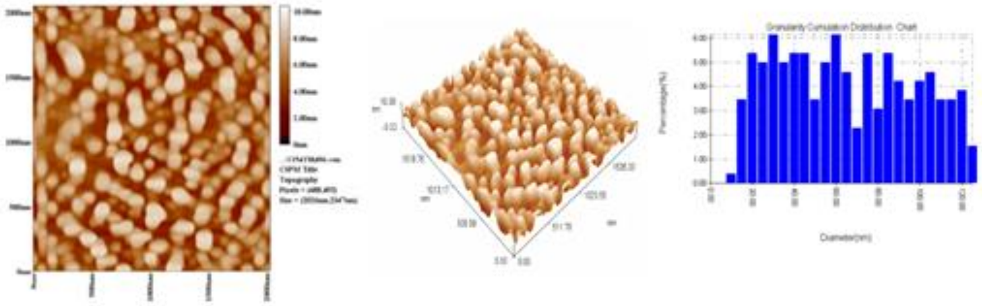

Figure- 27: AFM images and size distribution of the L_2-AuNPs conjugates prepared in absence of TSC (L_2/ $NaBH_4$0.014). Average diameter 62.56 nm.

Atomic Force Microscopy (AFM) analysis

The AFM images showed that morphology of gold nanoparticles conjugated with CR, L_1 and L_2 were agreeable with those observed in the SEM micrographs. In presence of TSC(Figure- 22) the particles had nearly cubic and spherical shapes with average diameter 66.08 nm. In absence of TSC (Figure -23), the particles had spherical shapes with average diameter 86.33 nm. The AFM images and histograms of AuNPs prepared from the addition of $NaBH_4$ to a mixture of Au(III) salt and the Schiff base ligands L_1 and L_2 in presence and absence of trisodium citrate showed that the AuNPs conjugates had average particle sizes 73.83 and 79.62 nm respectively in presence of L_1 (Figures -24 and -25) respectively) and 48.57 and 62.56 nm respectively in presence of L_2 (Figures -26 and -27 respectively).

CONCLUSION

The synthesis of AuNPs conjugated with the antibiotic CR and two of its Schiff base ligands as capping and stabilizing agents was investigated using $NaBH_4$ as a reducing agent in presence and absence of TSC. The AuNPs were formed when the concentration ratios of the ligands :$NaBH_4$ were 0.0066- 0.0088 and 0.014-0.0148 for CR and the two Schiff base ligands respectively. The Uv-visible spectra showed that no reduction of Au(III) ions to AuNPs by $NaBH_4$ at the concentration ratio of CR:$NaBH_4$ 0.066-0.088 when CR was added before $NaBH_4$ especially in presence of trisodium citrate compared with the two Schiff base derivatives. This leads to the conclusion that CR acted as a masking agent by forming stable chelate complexes with Au (III) ions and hence prevented the reduction of Au (III) ions by $NaBH_4$ or TSC. On the other hand, AuNPs were more stabilized when CR solution was added after reduction of $AuCl_4^-$ with $NaBH_4$, in the same concentration ratio 0.066-0.088. The size and morphology of the AuNPs were affected by the type of ligand and were more dispersed and regular in shapes in absence of citrate.

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