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**ELECTROCHEMICAL DETECTION OF THE *L*-  
CYSTEINE USING A CARBON PASTE ELECTRODE  
MODIFIED WITH A CONJUGATE  
OF TETRAAMINO COBALT (II)  
PHTHALOCYANINE AND GOLD  
NANOPARTICLES**

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## SUMMARY

Gold nanoparticles protected with a mixture of hexanethiol and MUA (11-mercaptoundecanoic acid) (AuNP1) were reacted with Co(II) tetraaminophthalocyanine (CoTAPc). The resulting nanoparticles formed *via* covalent bonds, AuNP2, were characterized by absorption spectra, infrared spectroscopy and transmission electron microscopy. Modified carbon paste electrodes were prepared with AuNP1, CoTAPc and AuNP2 and evaluated for electrochemical detection of *L*-cysteine by cyclic voltammetry. Sensing of *L*-cysteine was uniquely observed with the electrode modified with AuNP2.





## KEY WORDS

Gold, nanoparticles, phthalocyanine, carbon electrodes, sensing, *L*-Cysteine.

## INTRODUCTION

*L*-cysteine (Cys) is an amino acid present in natural proteins. Abnormal concentrations of Cys in urine are indicators of hepatic cystinuria. Consequently, the development of efficient strategies for Cys detection is today an exciting challenge. In that respect, a number of electroanalytical methods have been successfully employed owing their high sensitivity and selectivity. In particular, the use of chemically modified electrodes have permitted to overcome the large overvoltage required for the naked electrode surfaces as well as the surface fouling problem due to slow electrochemical oxidation of Cys or other analites. Among the numerous methods described for electrode modification (Salimi and Pourbeyram, 2003; de Taconi *et al.*, 2003) have emerged those using conjugates of metal nanoparticles (MNPs) and other components, such as metallophthalocyanines (MPcs) (Wang *et al.*, 2005; Lokesh *et al.*, 2009) or Prussian blue (Pandey *et al.*, 2012; Pandey and Chauhan, 2012).

In this paper report the synthesis of conjugates of cobalt(II) phthalocyanine and gold nanoparticles through covalent bonds. We aimed to use this system to modify the carbon paste electrode (CPE) for electrochemical detection of *L*-cysteine.

## EXPERIMENTAL SECTION

*Chemicals and equipment:* All solvents and reagents were purchased from Aldrich. The carbon paste oil base and carbon paste electrode OD: 6.0 mm ID: 3.0 mm were obtained from BAS Inc. CoTAPc (Achar and Lokesh, 2004) and AuNPO (Brust *et al.*, 1994) were synthesized according to the literature.

All electrochemical measurements were performed in a Bio-Logic SAS potentiostat Model SP-150 s/n: 0220. A three electrochemical cell comprising of modified carbon paste electrode (CPE) as the working electrode, platinum wire (Pt) as a counter electrode, and an Ag/AgCl/KCl 3 M reference electrode was used. All solutions for voltammetric study were prepared in 0.1 M phthalate buffer pH 4.0 containing 0.5 M M KCl.





The modified carbon paste electrode (CPE) was prepared using carbon paste oil base and CoTAPc/AuNP1/AuNP2. The composition of the typical active paste was found to be CoTAPc/AuNP1 = 2.0 % (w/w), AuNP2 = 8.0 % (w/w), carbon paste oil base = 98.0 or 92.0 % (w/w). The active paste was filled into well of electrode body and the surface was manually smoothed on a clean butter paper.  $^1\text{H}$  NMR spectra were performed on Bruker DXR 250 and Varian Mercury 400 instruments. Chemical shifts are reported in parts per million relative external standard  $\text{SiMe}_4$ . Infrared spectra were carried out in a Nicolet 520 FT-IR. High resolution transmission electron microscopy (HRTEM) measurements were performed at 200 kV with a JEOL 2100 microscope.

#### *Synthesis of AuNP1*

A sample of 0.151 g of gold NPs capped with hexanethiol was reacted with MUA (11-mercaptoundecanoic acid) (13.9 mg,  $6.36 \times 10^{-5}$  mol) in about 50 mL of  $\text{CH}_2\text{Cl}_2$ . The solution was stirred at room temperature until the nanoparticles containing the mixture of ligands precipitated in the medium. AuNP1 nanoparticles were centrifuged and washed with hexane and  $\text{CH}_2\text{Cl}_2$  and dried under vacuum.

$^1\text{H}$  NMR (300 MHz, acetone- $d_6$ ):  $\delta$  2.73 (t,  $^3J_{\text{HH}} = 6.0$  Hz, SCH<sub>2</sub>); 2.28 (t,  $^3J_{\text{HH}} = 6.0$  Hz, CH<sub>2</sub>COOH); 1.76-1.22 (br, CH<sub>2</sub>); 0.89 (t,  $^3J_{\text{HH}} = 6.0$  Hz, CH<sub>3</sub>). IR (KBr):  $\nu_{\text{max}}/\text{cm}^{-1}$  3400 (O-H), 2953w (CH<sub>3</sub>), 2918sw and 2849m (CH<sub>2</sub>), 1708m (C-O). UV-Vis (CH<sub>3</sub>OH):  $\lambda_{\text{max}}$  525 and 750 nm. HRTEM:  $2.2 \pm 0.6$  nm. TGA (%): Au, 81.2; organic, 18.8. Ligands ratio: 1 mol 1-hexanethiol: 0.6 mol MUA.

#### *Synthesis of AuNP2*

A mixture of AuNP1 (265 mg,  $\sim 0.16$  mmol MUA), EDC·HCl (31 mg, 0.16 mmol) and NHS (23 mg, 0.20 mmol) was dissolved in 20 mL of degassed DMF and kept stirring under nitrogen for 2 hours. Then Co(II)TAPc (26.9 mg, 0.04 mmol) was added dissolved in a mixture of 5 mL of degassed DMF and 5 mL of an aqueous solution of  $\text{NaHCO}_3$  previously deoxygenated. The mixture was kept stirring for 12 h. Upon completion of the reaction, the nanoparticles were centrifuged, washed with DMF and water and dried under vacuum.

$^1\text{H}$  NMR (300 MHz, CH<sub>3</sub>OH- $d_4$ ):  $\delta$  2.69 (t,  $^3J_{\text{HH}} = 6.0$  Hz, SCH<sub>2</sub>); 2.28 (t,  $^3J_{\text{HH}} = 6.0$  Hz, CH<sub>2</sub>CONR); 1.77-1.22 (br, CH<sub>2</sub>); 0.92 (t,  $^3J_{\text{HH}} = 6.0$  Hz, CH<sub>3</sub>). IR (KBr):  $\nu_{\text{max}}/\text{cm}^{-1}$  2953w (CH<sub>3</sub>), 2919 and 2849m (CH<sub>2</sub>), 1608m (NHCO). UV-Vis (CH<sub>3</sub>OH):  $\lambda_{\text{max}}$  525 and 736 nm. ICPOes Analysis (%): Au, 74.25 and Co, 0.15.

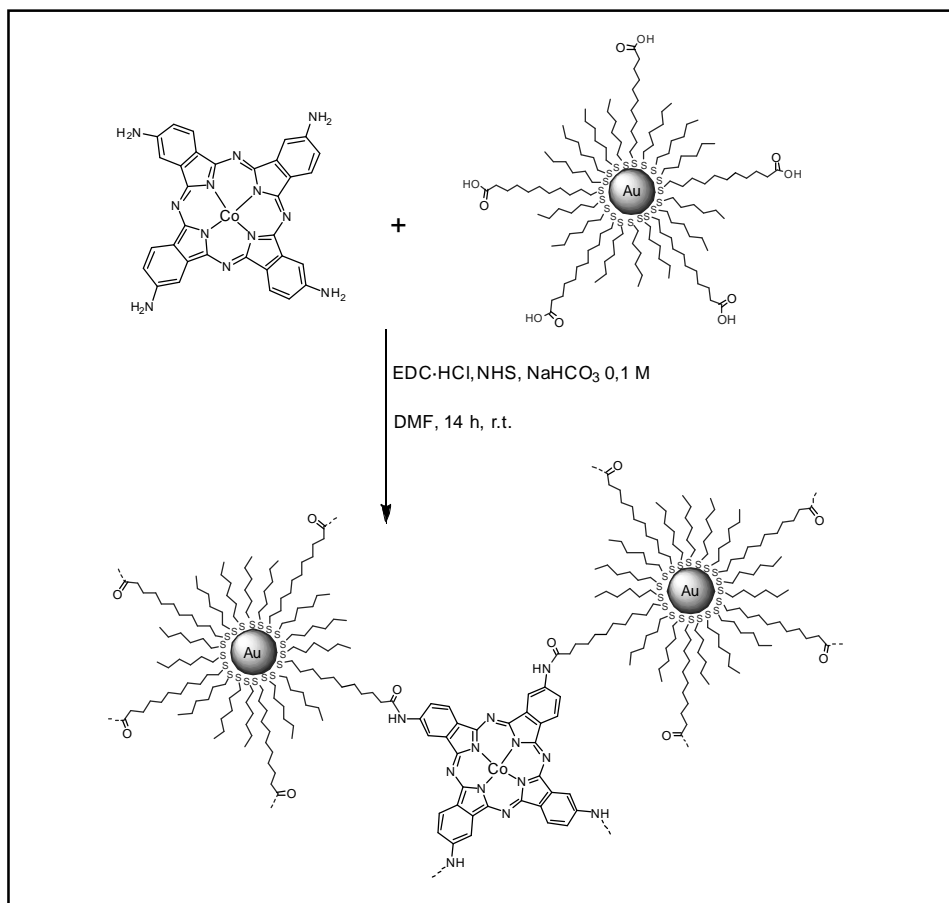




Elemental Analysis (%): C, 11.72; H, 1.80; N, 0.39; S, 3.18. HRTEM:  $1.9 \pm 0.4$  nm. TGA (%): metal, 75.7%; organic, 24.3. Raman:  $\nu_{\max}/\text{cm}^{-1}$  1568 (CNC isoindol).

## RESULTS AND DISCUSSION

The first step for the formation of the conjugates of tetraamino cobalt (II) phthalocyanine and gold nanoparticles consisted on treating Au nanoparticles stabilized with 1-hexanethiol (AuNP0) with 11-mercaptoundecanoic acid (MUA) in dichloromethane. The reaction proceeded until complete precipitation of the gold nanoparticles AuNP1 in the reaction medium. The second step involved the cross-linking between AuNP1 and CoTAPc *via* formation of the amide function (Scheme 1).



**Scheme 1.** Aggregate formation of Au nanoparticles (AuNP2) through cross-linking amide bond between AuNP1 and CoTAPc.





The presence of hexanethiolate ligands provides solubility to AuNP1 in DMF in order to carry out the cross linking process.

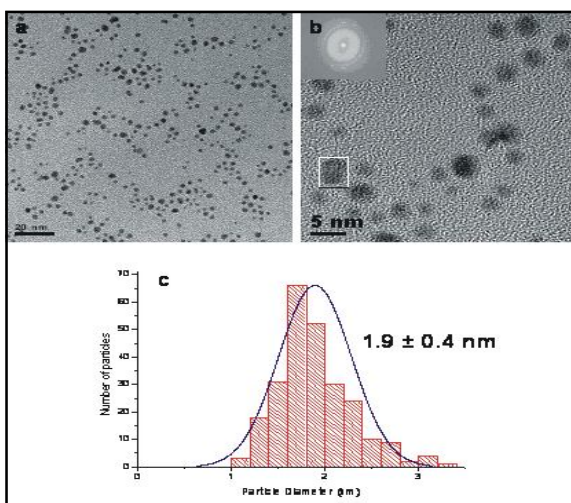
The new material AuNP2 was characterized by IR, Raman, UV-Vis,  $^1\text{H}$  NMR spectroscopies and TEM.

The FTIR spectrum showed a band at  $1609\text{ cm}^{-1}$  due to the vibration of the amide function (Fig. S1 Supplementary material). The presence of CoTAPc was evidenced by the broad band in the range  $1500\text{--}1570\text{ cm}^{-1}$  in the Raman spectrum due to the vibration of the isoindole group of the phthalocyanine (Dent and Farrell, 1997; Tackley *et al.*, 2001) (Fig. S2).

The UV-Vis spectra in methanol showed the surface plasmon band centered at approximately  $525\text{ nm}$  (Fig. S3) due to the excitation of the surface plasmon vibrations of the gold nanoparticles.

The  $^1\text{H}$  NMR spectrum (Fig. S4(a)) revealed the presence of wide signals poorly defined, from which it was not possible to estimate the relative ratio between the two different thiolate ligands on the gold surface. To overcome this drawback, we decomposed AuNP2 with  $\text{I}_2$  in deuterated methanol. This reaction released the thiolate ligands as disulfide species and the integration of the methyl protons of 1-hexanethiol and the methylene protons to the amide function is in accordance with a 1:0.6 (1-hexanethiol:thiol-amide) molar ratio (Fig. S4(b)).

Fig 1 shows the TEM image and size distribution histograms of AuNP2. The size of nanoparticles is around  $2.0\text{ nm}$ , and they show spherical shape. The inset in Fig. 1(b) shows the spots due to electron diffraction with crystallographic planes [111] of Au nanoparticles with a fcc structure. TEM images at a scale of  $20\text{ nm}$  (Figure 1(a)) shows partial aggregation of nanoparticles due to the cross-linking via amide linkage between the CoTAPc and AuNP1.



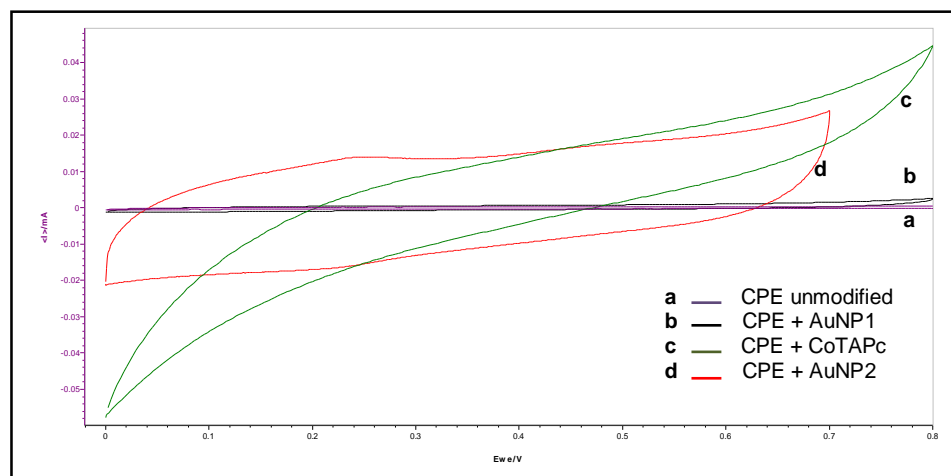
**Figure 1.** (a) and (b) HRTEM of AuNP2 at scales of  $20$  and  $5\text{ nm}$  respectively, and (c) size distribution histogram.





The potential application of the conjugate nanoparticles AuNP2 was tested in the electrocatalytic oxidation of *L*-cysteine. For this, a carbon paste electrode (CPE) was modified with AuNP2 and for comparative purposes we also prepared CPEs modified with tetraaminophthalocyanine of Co(II) (CoTAPc) or AuNP1.

The behavior of the carbon paste electrodes (CPE) was evaluated by cyclic voltammetry in a range of potential of 0.0 to 1.0 V versus Ag/AgCl/KCl 3 M. Before exploring the electroactivity of the conjugate AuNP2, we tested separately the potential electroactivity of their precursors, CoTAPc and AuNP1. The Fig. 2 shows the cyclic voltammograms for a solution of *L*-cysteine at pH = 4.0 using CPE(s) unmodified and modified with AuNP1 or CoTAPc. As can be seen from Fig. 2, no electrocatalytic activity for the direct electrooxidation of *L*-cysteine was observed in any case.



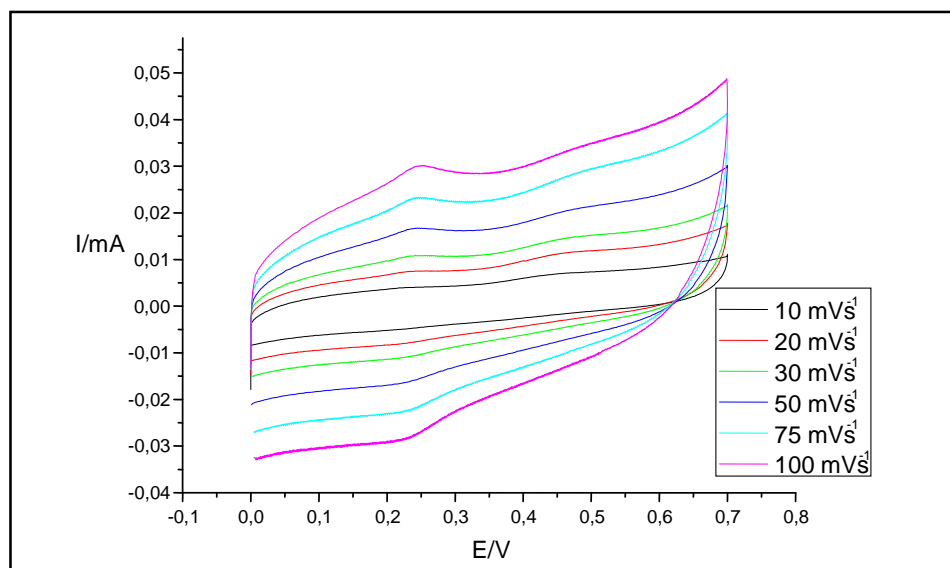
**Fig. 2.** Cyclic voltammograms of a solution of *L*-cysteine 1.0 mM in an aqueous solution of potassium hydrogen phthalate 0.1 M pH=4.0 + KCl 0.5 M at a scan rate of 50 mVs<sup>-1</sup> in: (a) CPE unmodified; (b) CPE + AuNP1 2 %; (c) CPE + CoTAPc 2 %; and (d) CPE + AuNP2 8 %.

On the other hand, due the low content of Co (II) (0.15 w% according the ICPoes analysis) in AuNP2 we had to increase the amount required for the modification of CPE up to 8.0 w%. In these conditions two electrochemical signals were observed: one quasireversible centered at 0.25 V (A1) and another irreversible at 0.45 V (A2). These peaks are assigned to the oxidation of Co (II) phtalocyanine species to Co (III) (A1/C1) and to the chemical oxidation of *L*-cysteine, respectively (A2). The last one serves to regenerate the Co(II) oxidation state of the cobalt bonded to the phtalocyanine molecule (Halber and Baldwin, 1985). Both peaks are not well defined, since the capacitive current due to the double layer of the electrode





surface is high. Probably, the adsorption of the AuNP2-L-cysteine to the electrode surface is the cause of this high capacitance current. The Fig. 3 shows the cyclic voltammograms obtained at different scan rates. A quasi symmetric redox couple on surface A1-C1, which shows a linear relationship of peak current vs. scan rate, is clearly visible.



**Fig. 3.** CPE modified with AuNP2 8 % : Cyclic voltammograms at different rates of a solution of *L*-cysteine 1.0 mM in an aqueous solution of potassium hydrogen phthalate 0.1 M pH= 4.0 + KCl 0.5M.

The electrochemical sensing of *L*-cysteine by using modified carbon paste electrodes with the conjugate species AuNP2 contrasts with the results obtained simply using their precursors AuNP1 or CoTAPc. The important role of the conjugate in the electron transfer between *L*-cysteine and electrode surface can be explained on the basis of the synergetic effect between cobalt centers and Au nanoparticles. That is, the nanoparticles provide a suitable microenvironment facilitating the direct electron transfer between the electroactive species and the electrode surface.

## CONCLUSION

We propose a new method for the detection of *L*-Cysteine based on the nanoparticles AuNP2 formed by gold nanoparticles covalently bonded to Co(II) tetraaminophthalocyanine. Modified carbon paste electrodes with conjugates AuNP2 evidenced electrochemical activity of *L*-Cysteine in cyclic voltammetry.







Interestingly, carbon paste electrodes modified with the precursors of the conjugate AuNP2 did not show electrochemical activity in any case.

## RESUMEN

### **DETECCIÓN ELECTROQUÍMICA DE LA *L*-CISTEÍNA UTILIZANDO UN ELECTRODO DE PASTA DE CARBONO MODIFICADO CON UN CONJUGADO DE TETRAAMINOFTALOCIANINA DE COBALTO(II) Y NANOPARTÍCULAS DE ORO.**

Nanopartículas de oro protegidas con una mezcla de hexanotiol y MUA (ácido 11-mercaptoundecanoico) (AuNP1) se hizo reaccionar con tetraaminofalocianina de cobalto(II) (CoTAPc). Las nanopartículas resultantes formadas vía enlaces covalentes, AuNP2, fueron caracterizadas por espectroscopía UV-Vis, IR y microscopía de transmisión electrónica. Electrodo de pasta de carbono modificados fueron preparados con AuNP1, CoTAPc y AuNP2 y se evaluó su actividad electrocatalítica mediante la detección electroquímica de *L*-cisteína por voltametría cíclica. La detección de *L*-cisteína sólo se observó con el electrodo modificado con AuNP2.

#### **PALABRAS CLAVES:**

Oro, nanopartículas, ftalocianina, electrodos de pasta de carbono, sensor, *L*-cisteína.

#### **ACKNOWLEDGEMENTS**

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APPENDIX. Supplementary material

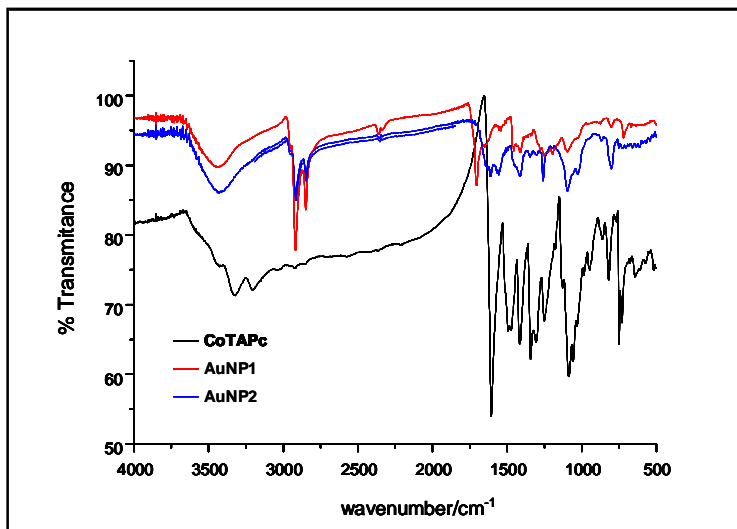


Figure S1: FTIR spectra of CoTAPc, AuNP1 and AuNP2

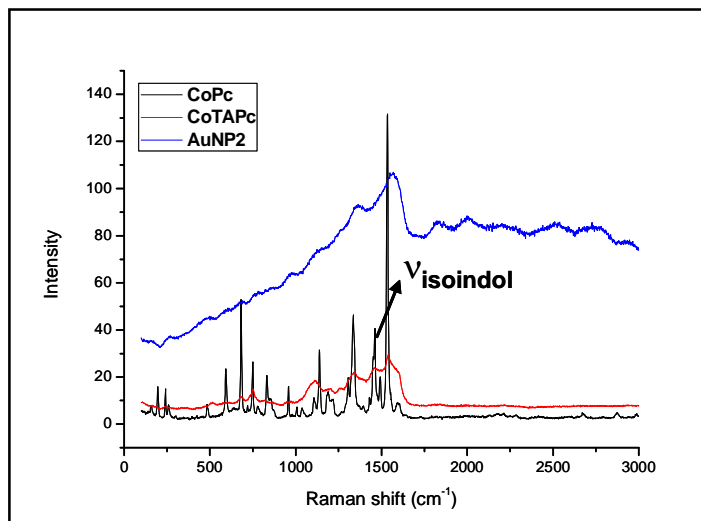
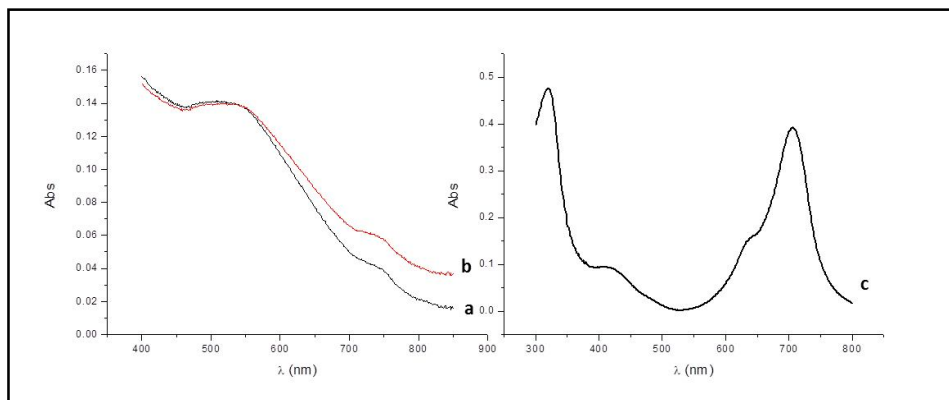
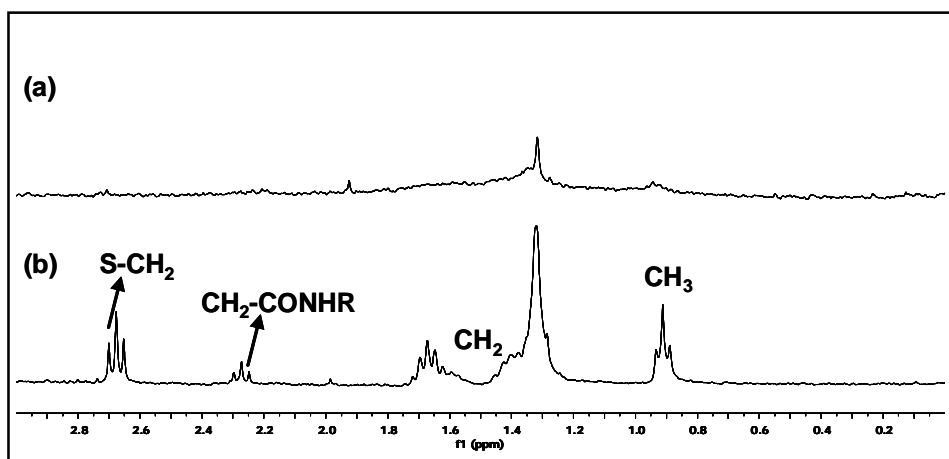


Figure S2: Raman spectra of CoPc, CoTAPc and AuNP2.



**Figure S3:** UV-Vis spectra of (a) AuNP1, (b) AuNP2 and (c) CoTAPc.



**Figure S4:**  $^1\text{H}$  NMR spectrum of AuNP2 (a) before and (b) after oxidative decomposition with  $\text{I}_2$  in  $\text{MeOH-d}_4$ .

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