

## Electrochemical determination of acetaminophen in pharmaceutical formulations and human urine using Ag-Au bimetallic nanoparticles modified electrode

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### Abstract

In the present paper, the Ag-Au bimetallic nanoparticles (Ag-AuNPs) was prepared by the simultaneous reduction of silver and gold with dextran solution. A novel glassy carbon electrode (GCE) modified by Ag-AuNPs was employed for the voltammetric determination of acetaminophen (ACT). The nanoparticles were characterized by UV-visible spectroscopy method which confirmed the homogeneous formation of the bimetallic alloy nanoparticles. Transmission electron microscopy (TEM) revealed that the synthesized bimetallic nanoparticles' size was in the range of 20-50 nm. The electrochemical behavior of ACT at the Ag-AuNPs/GCE exhibited a higher electrocatalytic effect compared to that observed when GCE was modified with each constituent of the composite and bare GCE. A linear relationship between anodic current of ACT concentration was obtained over the range of 0.4 to 10.2  $\mu\text{M}$  with a detection limit of 0.54  $\mu\text{M}$ . The proposed method was simple, less time consuming and showed a high sensitivity. The application of this electrode to determine ACT in tablets and urine samples was proposed. The application of this electrode to determine ACT in tablets and urine samples was proposed. The results analyzed by the proposed method was not significantly different from that analyzed by standard HPLC method.

**Keywords.** Acetaminophen, paracetamol, voltammetry determination, Ag-Au bimetallic nanoparticles.

### 1. INTRODUCTION

Acetaminophen (ACT), ( $\text{C}_8\text{H}_9\text{NO}_2$ , N-(4-hydroxyphenyl)ethanamide, N-(4-hydroxyphenyl)acetamide) also known as paracetamol is an analgesic drug which is extensively used to alleviate muscular aches, chronic pain, pain and fever.<sup>[1]</sup> Using high doses of ACT may come out with adverse effects in the body, although in proper doses it does not exhibit any side effects. Recently, ACT is widely employed for its remarkable therapeutic characteristics thus precise determination and control of its quality is critical. Therefore, several techniques have been used to determine the ACT content in tablet formulations or biological samples comprising spectrophotometry gas,<sup>[2,3]</sup> chromatography with

mass spectrometry (GC-MS),<sup>[4]</sup> high-performance liquid chromatography (HPLC)<sup>[5]</sup> liquid chromatography with mass spectrometry (LC-MS).<sup>[6,7]</sup> However, these techniques are time-consuming, expensive, and involve a complicated pre-treatment samples along with tedious operation. Hence, the simple, sensitive and accurate approaches to determine the active ingredients in drugs have been developed. Electroanalytical methods are more preferred mainly due to their accurate, simplicity, low cost, and applicability.<sup>[8]</sup> Glassy carbon electrodes (GCEs) have been widely used in voltammetric approaches for trace level detection of organic compounds because they offers high sensitivity, negligible porosity, and good mechanical stability. GCEs have been modified by means of

various nanomaterials, porous materials, hybrid inorganic-organic compounds to develop the new analytical approaches. Novel nanometals (Ag, Au or its alloy) have been successfully modified on the electrodes to determine several organic compounds.<sup>[9-11]</sup> Attractive electrochemical and electrocatalytic properties were exhibited for novel metal nanoparticles modified electrode, which should be attributed to the acceleration of interface electrons transfer due to high electric conductivity. Silver nanoparticles modified carbon nanotube paste electrode was employed to determine simultaneously of dopamine and ascorbic acid.<sup>[9]</sup> Chang *et al.* reported gold nanoparticle modified electrode for non-enzyme detection of glucose.<sup>[11]</sup> Pal *et al.* reported silver nanoparticles modified electrode for determination of nitrite.<sup>[10]</sup> Voltammetric determination of anthracene on Ag-Au alloy nanoparticles/overoxidized-polypyrrole composite modified glassy carbon electrodes was reported by Mailu *et al.*<sup>[12]</sup> To our best knowledge, a few articles has been reported in voltammetric determination of ACT using Ag-Au bimetallic nanoparticles modified electrode.

In the present work, we prepared Ag-Au bimetallic nanoparticles modified glassy carbon electrodes (Ag-Au NPs/GCE). The electrochemical behaviors of the Ag-AuNPs/GCE were studied in comparison to those of AgNPs/GCE and AuNPs/GCE by the electrochemical measurements. Cyclic and different pulse voltammograms were applied to evaluate the oxidation of ACT on the Ag-AuNPs/GCE. The Ag-AuNPs/GCE presents excellent catalytic activity and selectivity for the ACT oxidation, which is promising for the application in electrochemical sensors.

## 2. MATERIALS AND METHODS

### 2.1. Reagents and Materials

Analytical grade dextran ( $(\text{H}(\text{C}_6\text{H}_{10}\text{O}_5)_x\text{OH}$ , 99%), hydrogen tetrachloroaurate(III) trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ , 99 %), silver sulfate pentahydrate ( $\text{Ag}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ , 98 %), ammonium hydrate ( $\text{NH}_3 \cdot \text{H}_2\text{O}$ , 25-28 %), ethanol ( $\text{C}_2\text{H}_5\text{OH}$ , 98 %) were obtained from Sigma-Aldrich. Potassium hydroxide (KOH), phosphoric acid ( $\text{H}_3\text{PO}_4$ , 85 %), and boric acid ( $\text{H}_3\text{BO}_3$ , 99 %) were obtained from Daejung, Korea and used to prepare the buffer solution. The Britton–Robinson buffer solutions (B–R BS) were prepared from 0.5 M  $\text{H}_3\text{BO}_3$ , 0.5 M  $\text{H}_3\text{PO}_4$ , and 0.5 M  $\text{CH}_3\text{COOH}$  solutions. The desired pH of the B–R buffer was adjusted using 1 M KOH or 1 M  $\text{H}_3\text{PO}_4$  solutions. ACT ( $\text{C}_8\text{H}_9\text{NO}_2$ , 99 %) were purchased

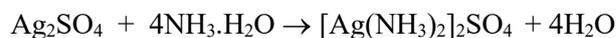
from Sigma-Aldrich. The stock solution of  $10^{-2}$  M ACT was prepared daily. Standard solution was prepared by diluting the stock solution with the B–R–BS.

### 2.2. Measurement and Instrumentation

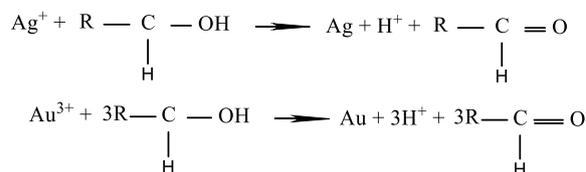
UV-Vis spectra measurements were recorded with the UV-Vis Jasco-V630, Japan. X-ray diffraction patterns were measured on a D8 ADVANCE (Bruker, Germany). Transmission electron microscopy (TEM) images were acquired using a JEOL JEM-2100F. EDX elemental mapping is measured by using a FESEM HITACHI S-4800 instrument. All electrochemical measurements were conducted with a CPA-HH5 Computerized Polarography Analyzer, Vietnam) in a conventional three-electrode cell.

### 2.3. Preparation of Ag monometallic nanoparticles (Ag NPs), Au monometallic nanoparticles (AuNPs) and Ag-Au bimetallic nanoparticles (Ag-Au NPs)

The AgNPs/dextran was prepared by the reduction of  $[\text{Ag}(\text{NH}_3)_2]_2\text{SO}_4$  with the dextran according a previous report.<sup>[13]</sup> Briefly, the solution of 1 mM  $[\text{Ag}(\text{NH}_3)_4]\text{SO}_4$  was prepared by mixing 5 mL of 20 mM  $\text{Ag}_2\text{SO}_4$ , 2 mL of 5 %  $\text{NH}_3$  and 78 mL of distilled water. The reaction is expressed as follows:



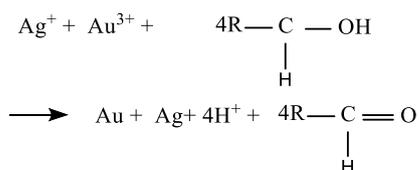
To this solution 1 mL of 5 % dextran was added dropwise and was heating to  $90^\circ$  and then add 7 mL of 5 %  $\text{NH}_3$  solution to this solution to obtain the colloidal silver solution. 50 mL of  $\text{C}_2\text{H}_5\text{OH}$  (96 %) was added to the colloidal silver solution. During this process, the solution was stirred using a magnetic stirrer. The solid was collected by centrifugation and dried at  $60^\circ\text{C}$  for 24 hours to obtain the Ag-NPs/dextran. The reaction is expressed as follows:



Au nanoparticles/dextran was prepared through the reduction of  $\text{HAuCl}_4$  using dextran solution as a reduction agent according to Refs. [14], [15]. 1 mL of 5 % dextran was added into 1 mM  $\text{HAuCl}_4$  solution using a magnetic stirrer. The colloidal gold solution was obtained by adding 7 mL 5 %  $\text{NH}_3$  to the mixture of  $\text{HAuCl}_4$  and dextran. 50 mL of 96 %  $\text{C}_2\text{H}_5\text{OH}$  was then added into the colloidal gold solution. The solid was collected by centrifugation and dried at  $60^\circ\text{C}$  for

24 h to obtain AuNPs/dextran. The reaction is expressed as follows:

The Ag-Au NPs/dextran was prepared via a previous paper.<sup>[15]</sup> The colloidal Ag-Au solution with the Ag:Au molar ratio of 5:5 was prepared by mixing 5 mL  $[\text{Ag}(\text{NH}_3)_2]\text{SO}_4$  and 5 mL  $\text{HAuCl}_4$ , 1 mL of 5 % dextran and 7 mL of 5 %  $\text{NH}_3$ . 50 mL of 96 % ethanol was added into the Ag-Au colloidal solution. The solid was collected by centrifugation and dried at 60 °C for 24 hours to obtain Ag-Au NPs/dextran. The reaction was expressed as follows:



The metallic nanoparticles/dextran (Ag, Au or Ag-Au/dextran) were calcined at 400 °C for 4 hours to obtain the nanoparticles of Ag NPs, Au NPs and Ag-Au NPs.

#### 2.4. The preparation of electrodes

The glassy carbon electrode was firstly polished to mirror-like by using polishing paper and 0.2  $\mu\text{m}$  alumina powders. Then they were washed in ethanol and distilled water with ultrasonication each for 15 min and dried naturally. The metal nanoparticles were dispersed in distilled water containing 10 mL with ultrasonication for 10 hours to get a homogenous suspension ( $1 \text{ mg mL}^{-1}$ ). Then, 5  $\mu\text{L}$  of the suspension was dropped onto the surface of cleaned glassy carbon electrode (GCE) and dried at room temperature, resulting in Ag NPs modified GCE (AgNPs/GCE), Au NPs modified GCE (AuNPs/GCE) and Ag-AuNPs modified electrode (Ag-AuNPs/GCE).

#### 2.5. Prepare urine and pharmaceutical samples

The ACT determination was performed in humane urine samples and pharmaceutical preparations. Urine samples were collected from the clinical laboratory and filtered through membrane filters (0.45  $\mu\text{m}$ ), all samples were stored in freeze condition. The samples were diluted five times by distilled water two times and 0.2 M B-R-BS pH 7 before measurement. The spiked samples were used to evaluate the content of ACT in samples. The tablets containing ACT were purchased from the local area. The tablets were ground to powder and dissolved in 0.2 M B-R-BS pH 7 then ultrasonic for five minutes, the solution was filtered out the insoluble solid. The developed method was employed for the analysis of a

kind of ACT commercial tablet by the standard addition method.

### 3. RESULTLS AND DISCUSSION

#### 3.1. Charactrization of materials

Figure 1 shows the UV-Visible spectra for colloidal silver, gold, and silver-gold solution. The absorption peaks at 556 and 426 nm are assigned to surface plasmon absorption of Au and Ag nanoparticles, respectively. If gold and silver ions are reduced simultaneously by dextran, it is possible that gold-silver nanoparticle alloy or homogeneous mixture of gold nanoparticles and silver nanoparticles are formed. The optical absorption spectrum exhibits single and broad plasmon band at which maximum absorbance appeared to between absorption peaks of AgNPs and AuNPs indicating the possibility that Ag-Au bimetallic alloy nanoparticles, the separated Ag- and Au-nanoparticles were coexisted.

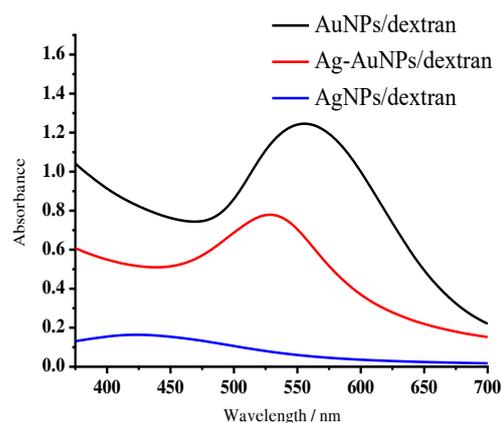


Figure 1: UV-Vis spectra of (a) colloidal AgNPs/dextran solution; (b) colloidal AuNPs/dextran solution and (c) colloidal Ag-AuNPs/dextran solution

The XRD patterns of Ag, Au and Ag-Au nanoparticles are presented in figure 2. The characteristic diffractions of Ag and Au according JCPDS file No. 04-0783 and JCPDS file No.04-0784, respectively were observed in figure 2 a and b indicating the formation silver and gold. In the case of Ag-Au bimetallic nanoparticles, all diffractions are similar to the monometallic NPs of Au and Ag. Since the Ag and Au possess the similar space group of  $Fm-3m$  and the similar lattice constant of 0.408 and 0.409 nm for silver and gold respectively the formation of Ag-Au solid solution and two separated Ag and Au nanoparticles should be coexisted. The mean size of these Ag-Au NPs was calculated to be

25-30 nm using the Scherrer equation.

The morphology of nanoparticles is observed by TEM images (figure 3). TEM observation consists of almost uniformly sized nearly spherical nanoparticles of average size  $\sim 100$  nm for Ag NPs and  $\sim 15$  nm for Au NPs (figures 3 a and b). The average size of Au-Ag NPs is around  $\sim 10$  nm but tends to be agglomerated (figure 3c). The average particle size of Ag-Au NPs seems to be smaller than those of individual particles. It may be due to suppressing the growth of crystals in Ag-Au NPs.

The average composition of synthesized Ag-Au NPs was performed using the EDX analysis of Ag-Au alloy by films loaded on carbon disks (figure 4). The figure 4b contains the values of the Au/Ag molar ratios obtained by EDX analysis in comparison with the ratios calculated from the colloid preparation. A good agreement between obtain and predicted values indicates that almost no metal (within the experimental error of EDX analysis) was dissolved in

solution. Energy dispersive X-ray elemental mapping (EDX-elemental mapping) of Ag-Au NPs are shown in figure 4c,d, which further conformed the composition of Au-Ag NPs. The full coverage of both Au and Ag indicates the high dispersion of Ag-Au nanoparticles.

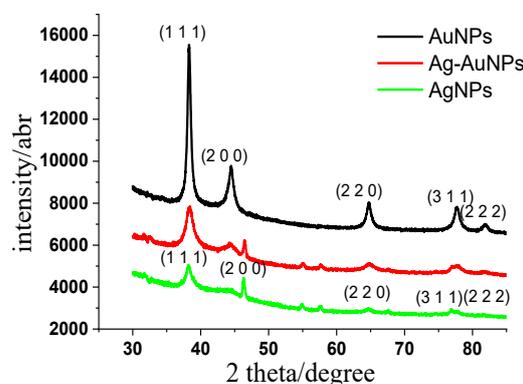


Figure 2: XRD patterns of AgNPs, AuNPs and Ag-AuNPs

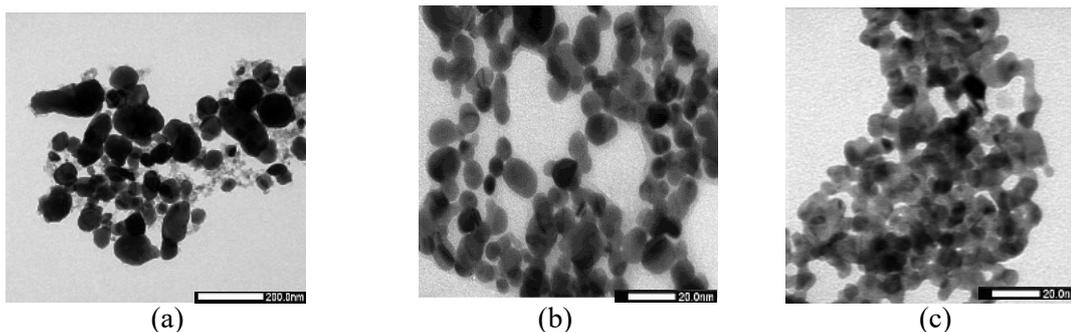


Figure 3: TEM images of AgNPs, AuNPs and Ag-Au NPs

### 3.2. Voltammetric determination of acetaminophen (ACT) using the Ag-Au NPs modified electrode (Ag-AuNPs/GCE)

#### 3.2.1. Electrochemical behaviors of ACT at different electrodes

Figure 5 presents CVs of ACT at the bare GCE; AgNPs/GCE; Au NPs-GCE and Au-AgNPs/GCE. The bare GCE provides the broad peak and the peak current is not observed. Whereas, the modified GCE exhibits the clear peak currents. It is worth noting that the peak current at Au-AgNPs/GCE presents clear anodic and cathodic currents. The anodic peak current at Ag-AuNPs/GCE is 1.4 times higher than that for Ag NPs-GCE and 1.3 times higher than that for AuNPs-GCE.

#### 3.2.2. The electroactive surface area of the modified-GCE

Electrochemical properties of the bare and modified

electrodes were characterized by  $K_3[Fe(CN)_6]$  as a probe by CVs. Figure 6 a and b shows the CVs of bare GCE and Ag-AuNPs/GCE in 1.0 mM  $[Fe(CN)_6]^{3-/4-}$  containing 0.1 M KCl. For a reversible process, relation of the anodic peak current ( $I_{pa}$ ) with the square root of scan rates ( $v^{1/2}$ ) is expressed by the Randles-Sevcik formula

$$I_{ap} = 2.69 \times 10^5 n^{3/2} A D^{1/2} C_0 v^{1/2} \quad (1)$$

where  $I_{ap}$  is the peak current (anodic peak current),  $n$  is the number of electrons transferred ( $n = 1$  in the  $[Fe(CN)_6]^{3-/4-}$  redox system),  $A$  is electroactive surface area of electrode ( $cm^2$ ),  $C_0$  is the concentration of  $[Fe(CN)_6]^{3-/4-}$ ,  $D_0$  is the diffusion coefficient of the  $[Fe(CN)_6]^{3-/4-}$  ( $C_0 = 1$  mM;  $6.7 \times 10^{-6} cm^2 \cdot s^{-1}$  [16]) and  $v$  is the scan rate ( $V \cdot s^{-1}$ ).

Hence, from the linear plot of the  $I_{pa}$  versus  $v^{1/2}$  (the insets of figures 6 a and b), the value of  $A$  for Ag-AuNPs/GCE ( $0.121 cm^2$ ) was found to be 1.6 times higher than that for the bare GCE ( $0.074 cm^2$ ) infesting that the effective electroactive surface of the

modified electrode was enhanced significantly in the presence of Ag-Au NPs.

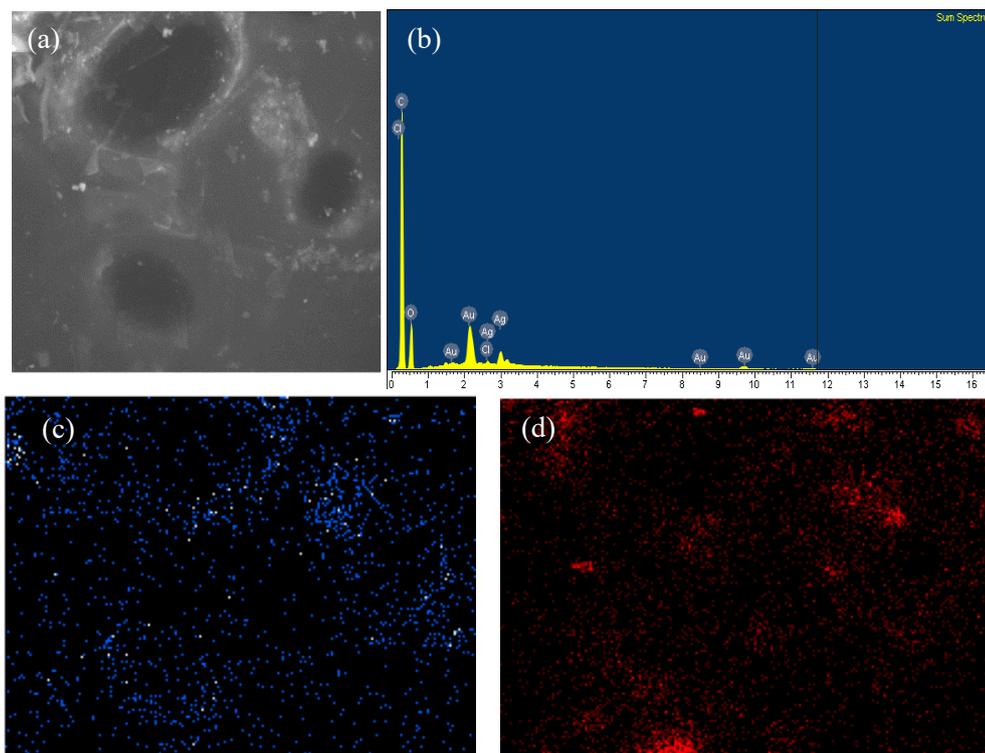


Figure 4: (a) SEM observation for area analyzed EDX, (b) EDX spectrum, elemental mapping image of (c) Au and (d) Ag

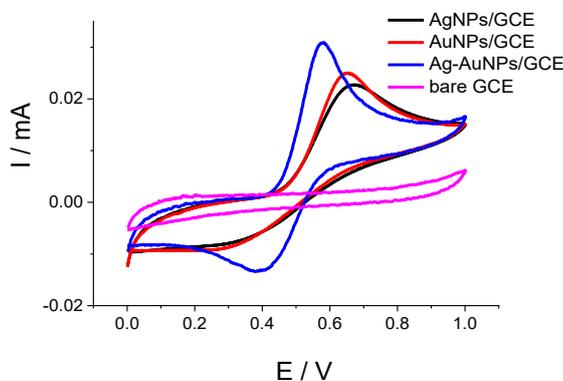


Figure 5: Cyclic voltammograms (CVs) of ACT at different electrodes (Sweeping range of potential: 0-1 V,  $10^{-4}$  M ACT in 0.2 M B-RBS pH 3)

### 3.2.3. Effect of pH

The pH effect on the electrocatalytic oxidation of ACT at the Ag-AuNPs/GCE was performed by CV technique using Britton-Robinson buffers solution (B-R-BS) within the pH range of 2-9 (figure 7a). It was found that anodic current ( $I_{ap}$ ) tends to increase with increasing pH and given their highest anodic current responses at pH 6-7 values and reduced in further increasing pH. The pH value of 7 was selected for further experiments (figure 7b). Also the pH of the

solution has a significant effect on the peak potential of the catalytic oxidation of ACT, *i.e.* the anodic peak potentials ( $E_{ap}$ ) shifted negatively with an increase in the solution pH (figure 7c), which indicates the protons have taken part in electrocatalytic oxidation at the Ag-AuNPs/GCE. Also, the peak potential for ACT oxidation varies linearly with pH.

The linear regression equation is as follows:

$$E_{ap} = (0.79 \pm 0.01) + (-0.046 \pm 0.002)\text{pH}, \quad r = 0.993 \quad (2)$$

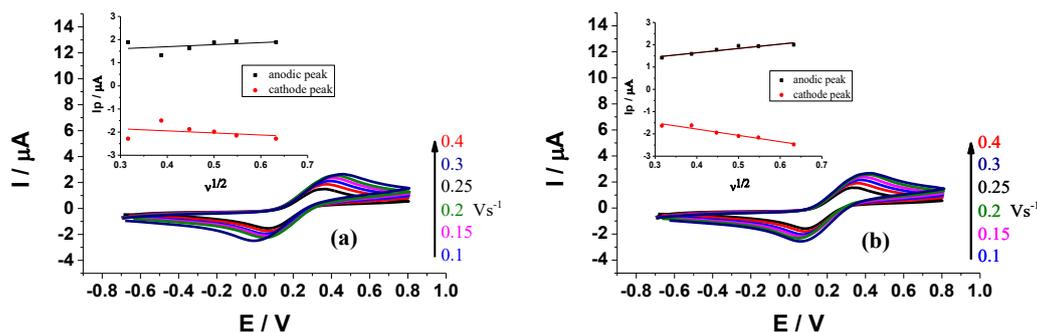


Figure 6: The CVs in  $\text{Fe}(\text{CN})_3/\text{Fe}(\text{CN})_4$  at (a) the bare GCE; (b) the Ag-Au NPs/GCE; the insets present the plots of peak current vs square root of scan rate

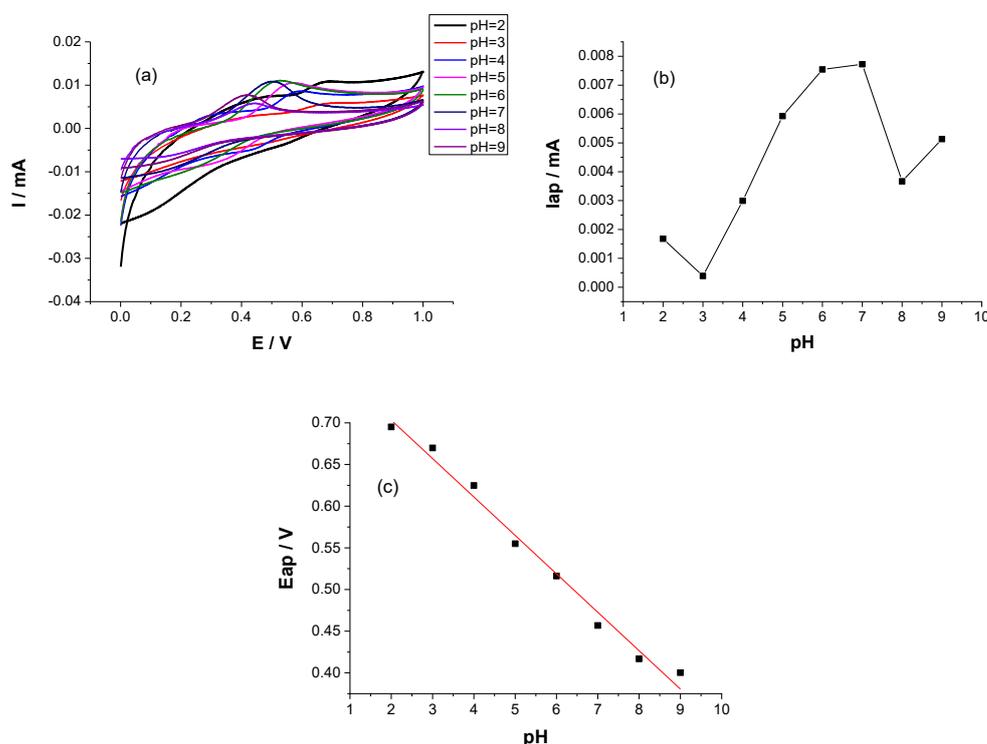


Figure 7: (a) CV of ACT at Ag/Au-GCE at different pH values; (b) the dependence of peak current on pH; (c) the linear plot of anodic peak potential ( $I_p$ ) of ACT vs. pH. Sweeping range of potential: 0 to 1 V,  $10^{-4}$  M ACT in 0.2 M B-R-B-S

The slope of 0.046 is close to the theoretical value of 0.0599 at 298 K indicating the reaction of ACT oxidation is involved in the equal number of protons and electrons transferred.

### 3.2.4. Effect of scan rate

The effect of varying scan rates ( $\nu$ ) on the anodic peak currents of 0.1 mM ACT at the Ag/Au-GCE in 0.2 M B-RBS pH 7.0 was studied by cyclic voltammetry (CVs) (figure 8). Figure 8a presents the CVs of ACT obtained in the scan rate range of 0.04 to 0.1  $\text{V}\cdot\text{s}^{-1}$ . The  $I_{ap}$  is observed to increase with scan rate (Figure 8b). In order to examine whether or not the oxidative behaviors of ACT were controlled by diffusion or

adsorption process, the plot of natural logarithm of peak current vs. natural logarithm of scan rate was performed in the above given range. A straight line was obtained for ACT with a slope of 0.46 (Fig. 8c), which is comparable with theoretical slope of 0.5 for diffusion controlled process.

For irreversible system, the relation of anodic peak potential *versus* scan rate is expressed by Laviron's equation:<sup>[17]</sup>

$$E_{ap} = E^0 - \frac{RT}{(1-\alpha)nF} \ln \frac{RTk_s}{(1-\alpha)nF} - \frac{RT}{(1-\alpha)nF} \ln \nu \quad (3)$$

in which  $E^0$  is the formal redox potential,  $k_s$  is apparent electron-transfer rate constant,  $\alpha$  is the charge transfer coefficient,  $n$  is the number of

electrons exchanged in oxidation,  $\nu$  is the scan rate ( $\text{V}\cdot\text{s}^{-1}$ ) and,  $T = 298 \text{ K}$ ,  $R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$  and  $F = 96480 \text{ C mol}^{-1}$ .

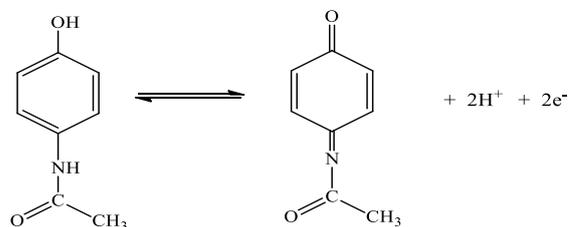
The linear regression equation of  $E_{\text{ap}}$  vs.  $\ln \nu$  is as follows (figure 8d):

$$E_{\text{ap}} = (0.611 \pm 0.005) + (0.023 \pm 0.02) \ln \nu$$

$$r = 0.987 \quad (4)$$

The value of  $n\alpha$  obtained from the linear slope of the linear regression line of eqs. (6) was found as 1.12. The value of  $\alpha$  is assumed to be 0.5, which is commonly used for a totally irreversible system.<sup>[18]</sup> Therefore, the number of electrons transferred ( $n$ ) in the electrooxidation of ACT was found to be 2.24.

Consequently, assuming  $n = 2$ , the oxidation mechanisms for ACT can involve two electrons and two protons. The oxidation mechanism of ACT is illustrated in the scheme 1.



Scheme 1: The oxidation mechanism of ACT

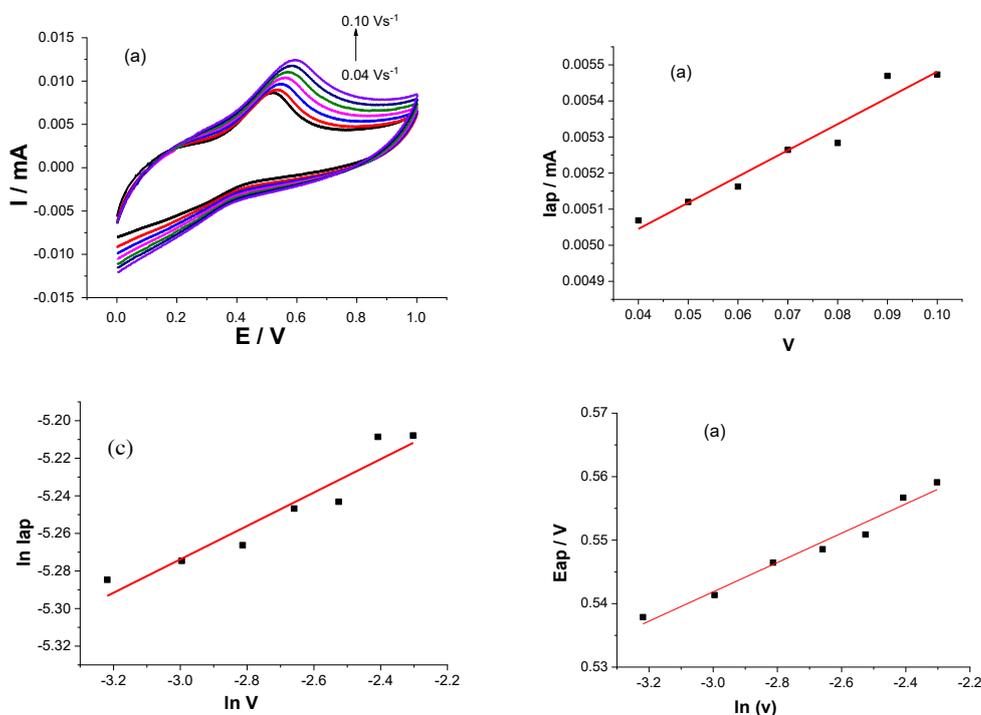


Figure 8: (a) CVs of ACT on Ag/Au-GCE at different scan rates; (b) the plot of anodic peak current ( $I_{\text{ap}}$ ) vs square root of scan rate ( $\nu$ ); (c) the linear plot of  $\ln I_{\text{ap}}$  vs.  $\ln \nu$  (d) anodic peak potential ( $E_{\text{ap}}$ ) vs  $\ln \nu$

### 3.2.5. Interferents, reproducibility and stability, linear range and limit of detection

Relative Error (RE) ( $(I_0 - I)/I_0$  where  $I_0$  is peak current measured without and with interference) is used to examine the effect some potential interfering compounds on the detection of ACT. The tolerance limit is considered as the maximum concentration of the interferent that leads to a RE excess  $\pm 5\%$ . This value of some possible inorganic and organic substances in the use of  $1.0 \mu\text{M}$  ACT are given in table 1.

For inorganic salts, except for  $\text{Na}_2\text{SO}_4$ , tested at 50-fold excess, all other inorganic substances at 100-

fold excess show no interference effect on the detection of ACT. Similarly, saccharine (at 100-fold excess), glucose (60-fold excess), sodium benzoate (50-fold excess) do not interfere the signal of ACT.

The proposed electrode reproducibility was conducted by means of pulse voltammetric techniques (DPV) method over nine electrodes prepared by the same procedure. A relative standard deviation (RSD) of the  $I_{\text{ap}}$  is 6.1% which manifests good reproducibility. In addition, stability tests for the modified GCE were demonstrated by measuring the current response of  $2.0 \mu\text{M}$  of ACT over a period of one week, and it is found that the Ag-Au/GCE maintains 7.3% of its initial anodic peak current

under proper storage.

Table 1: Tolerance limit of interfering species at 2.0  $\mu\text{M}$  ACT

Interferent	Concentration Interferent ( $\mu\text{M}$ )	Concentration ratio of Interferent / ACT	RE (%)
Saccharine	200	100	4.2
Glucose	120	60	5
Sodium benzoate	100	50	4.9
$\text{K}_2\text{CO}_3$	280	>100	< 5
$\text{Na}_2\text{SO}_4$	100	50	4.3
$\text{Ca}(\text{H}_2\text{PO}_4)_2$	230	>100	< 5
$\text{NaCl}$	290	>100	< 5

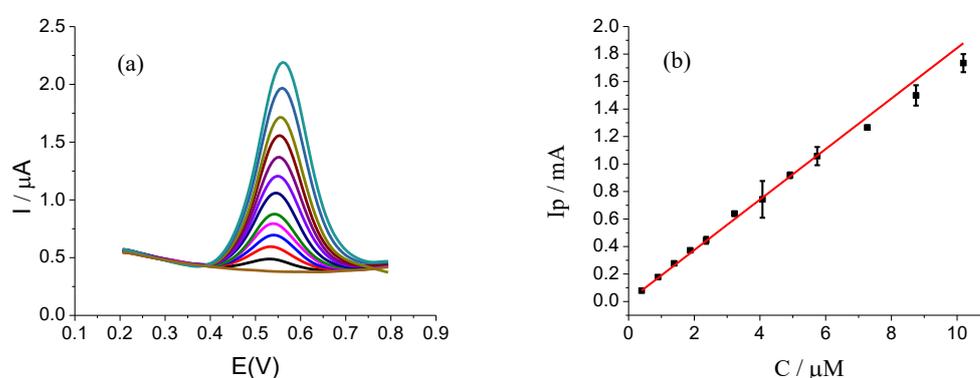


Figure 9: (a) Voltammetric current responses of successive additions of PRA using Ag/Au-GCE in 0.2 M B-R-BS pH 7; (b) The linear plot of anodic peak current vs concentration of ACT

Pulse voltammetric techniques method was employed to determine the ACT concentration. The plot of anodic peak current vs. ACT concentration in 0.2 M BR-BS pH 7 (figure 9) presents a linear range of 0.4 to 10.2  $\mu\text{M}$  with the regression equation of  $I_{ap} = (0.006 \pm 0.002) + (0.183 \pm 0.004) \cdot C$  with  $r = 0.995$ . The limit of detection (LOD) is found as 0.54  $\mu\text{M}$ .

Table 2 presents a comparison of LOD for the Ag-AuNPs/GCE with the reported methods for the determination of ACT. As seen in the table the present modified electrode exhibits the good LOD compared with literature<sup>[19-24]</sup> reported previously.

### 3.2.6. Determination of ACT in tablets and human urine

ACT tablets containing 200 mg or 500 mg of ACT were in commercial name as Efferalgan Vitamin C, Effe Paracetamol, Ameflu C and Ameflu. The tablets were weighed and finely pulverized. The appropriate amount of this powder was dissolved in double distilled water. The content of the tablet was diluted to get the concentration of ACT in the working range and then the differential pulse voltammetry (DPV)

was measured using Ag-AuNPs/GCE. The concentration of ACT in the tablet was determined from the calibration curve (Table 3). The results are compared by using paired samples *t*-test. With  $\alpha = 0.05$ , It was found that the results analyzed by the proposed DPV method agree well with the labelled contents ( $t(3) = -1.08, p = 0.36$ ) and consistent with the results of standard HPLC analysis ( $t(3) = 1.15, p = 0.33$ ).

The prepared urine samples with spike of 100  $\text{mg} \cdot \text{L}^{-1}$  were measured by the proposed DPV method. The results are listed in table 4. It was found that the results analyzed by the proposed DPV method were consistent with the results of standard HPLC analysis. Recovery tests were also conducted and ranged from 99.13 to 109.85 % revealing that the results obtained by Ag-AuNPs/GCE are reliable and feasible. There is no statistical difference between the results analyzed by the proposed method and by the HPLC ( $t(2) = 2.59$  and  $p = 0.122 > 0.05$ ). These results present the potential applicability of Ag-Au electrode for the determination of ACT in pharmaceutical formulation and human urine samples.

Table 2: Comparison of the Ag/Au-GCE with the papers reported previously for the determination of ACT

Electrode	Linear range (mM)	LOD (mM)	References
C60-modified glassy carbon electrode	50-1500	50	[19]
Electropolymerized-molecularly imprinted polypyrrole modified pencil graphite electrode	5-500	0.79	[20]
Poly(3,4-ethylenedioxythiophene)-modified screen-printed electrodes	4-400	1.39	[25]
Graphene/GCE	0.1-20	0.03	[21]
GC/Cu(II)-complex	20-5000	5.00	[22]
GC/nano-TiO <sub>2</sub> /PAY	12-120	2.00	[23]
Ag/Au-GCE	0.4-10.2	0.54	This work

Table 3: The results of ACT in pharmaceutical preparations analyzed by the proposed method and by the HPLC

Samples	Labeled (mg/tablet)	Methods	
		DPV	HPLC
Efferalgan Vitamin C	500	495±12	491.8±1.5
Effe Paracetamol	200	196±5	197.5±0.9
Ameflu C	500	491±7	491.0±0.7
Ameflu	500	496±6	489.6±1.1

Table 4: Results analyzed by the proposed method and HPLC, and recovery data obtained by standard addition method for ACT in human urine samples

Samples	Proposed DPV			Rev (%)	HPLC
	Original Conc. (mg.L <sup>-1</sup> )	Spike (mg.L <sup>-1</sup> )	Found (mg.L <sup>-1</sup> )		Found (mg.L <sup>-1</sup> )
Sample 1	0	100	109.85	109.85	101.2±3.9
Sample 2	0	100	101.08	101.08	99.7±2.5
Sample 3	0	100	99.13	99.13	91.4±1.7

#### 4. CONCLUSIONS

In the present work, a DPV method based on GCE modified with nanoparticles of Ag-Au alloy was used for electrochemical determination of ACT. The advantages of the Ag-Au nanoparticles improved the sensitivity of the modified electrode significantly.

The results showed that the method was simple and sensitive enough for determination of ACT in human urine and in commercial tablet with good precision and accuracy. Compared with other modified electrodes for the assay of ACT, the Ag-AuNPs/GCE exhibits a good current response and stability with low detection limit. The results are satisfactory with the results of standard HPLC analysis.

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