Int. J. Electrochem. Sci., 10 **(2015)** 1494 - 1505

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Gold Nanoparticles-Modified Screen-Printed Carbon Electrode for Voltammetric Determination of Sildenafil Citrate (Viagra) in Pure Form, Biological and Pharmaceutical Formulations

R. A. Farghali[1,2], *Rasha A. Ahmed*[1,3,*]

[1]Taif University, Faculty of Science, Chemistry Department, Taif, Hawiya 888, Saudi Arabia
[2]Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt.
[3]Forensic Chemistry Laboratories, Medico Legal Department, Ministry of Justice, Cairo, Egypt
[*]E-mail: rashaauf@yahoo.com

Received: 16 November 2014 / *Accepted*: 21 December 2014 / *Published*: 30 December 2014

In this work, a highly sensitive electrochemical sensor for the determination of sildenafil citrate (SILC) drug (the active component of Viagra) was fabricated by electrodeposition of gold nanoparticles (AuNPs) onto a screen-printed glassy carbon electrode (SPGCE). Cyclic voltammetry (CV) and Square wave voltammetry (SWV) were used to characterize the redox behavior of SILC in absence and presence of AuNPs. The response of the SPGCE electrode for SILC determination was observed to be enhanced in presence of AuNPs. The peak currents for SILC at AuNPs/SPGCE show a linear response in the concentration range from 1.8×10^{-6} to 3.3×10^{-5} mol L^{-1} with good reproducibility. The limit of detection was found to be 5.2×10^{-10} mol L^{-1}.An interference study was also carried out in presence of high concentration of ascorbic acid (AA), and uric acid (UA) to estimate the high selectivity of the electrode. The modified sensor was successfully applied for the determination of SILC in simulated human urine samples and pharmaceutical formulation with good agreement between the added and recovery values.

Keywords: Sildenafil citrate, gold nanoparticles, cyclic voltammetry, biological samples.

1. INTRODUCTION

Viagra is a citrate salt of Sildenafil and is commonly used as oral therapy for erectile dysfunction. Sildenafil is a potent and a selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5) [1-4]. Sildenafil citrate (SILC) is known chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1-H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]-

sulphonyl]-4-methyl piperazine citrate[5-7]. Its formula is $C_{28}H_{38}N_6O_{11}S$. It has the structure given in Fig. 1. Sildenafil citrate (Viagra) induces in vitro apoptosis of B-chronic lymphocytic leukemia (CLL) cells, suggesting that Viagra has anticancer activity [8]. This drug can also be efficient as therapy for a range of cardiovascular diseases [9–10]. The mode of action of Sildenafil in the erection of the penis involves the release of nitric oxide (NO) which activates the enzyme guanylatecyclase, results in increased levels of cGMP, producing smooth muscle relaxation which improve penile erectile function by allowing inflow of blood [3,11].

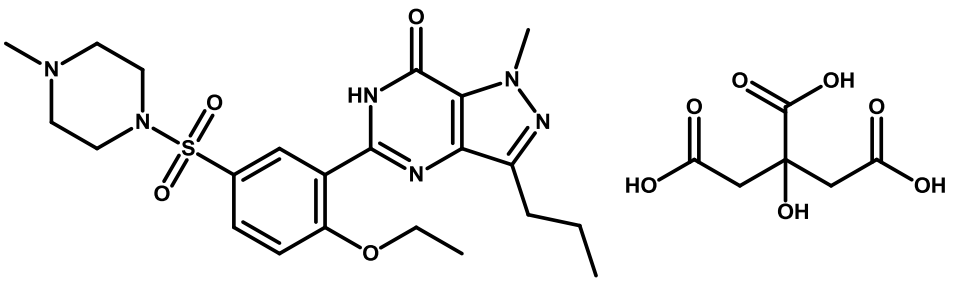

Figure 1. Structure of sildenafil citrate

NO may also participate in disease processes such as hypertension, and diabetes [12]. Oral administration of SILC with other drugs like nitrates or nitroglycerine can induce headaches and low blood pressure [13]. Thus, the residual presence of SILC even in trace (nanomolar) concentration levels could result in adverse side effects. A sensitive method for selective determination of SILC in nanomolar level is warranted. In the last decade, several works have been published reporting the identification of SILC in pure form or in pharmaceutical formulations by spectrophotometric and chromatographic techniques [14–16]. Most of these methods are expensive, suffer from lack of selectivity, and require careful control of conditions and considerable time for routine control analysis [17, 18]. Recently, application of potentiometric sensors in the field of pharmaceutical and biomedical analysis have been developed [19]. Although, these approach provides simple, fast, and selective technique for determination of various drugs [19–25] but very little research is known about the use of this technique for Viagra quantification [26,27]. In previous work, our group has developed and validated a simple and fast electrochemical method for the determination of SILC in pharmaceutical formulations [28].

In recent years, nanotechnology, including nanoparticles, nanotubes, nano-quantum dots and nanowires, has been used in various applications. This is owed to the essential properties of high chemical and thermal stability, surface to volume ratio, elasticity, and tensile strength. These properties along with their metallic conductivity allow for their use as functional components in the fabrication of medical sensing devices [29, 30].

In the present work, the determination of Sildenafil citrate in biological and pharmaceutical assays has been investigated by using a novel sensor of screen-printed glassy carbon electrode modified with gold nanoparticles (SPGCE/AuNPs). Using AuNPs works on increasing the electroactive surface area which enhances the electron-transfer between the electrode and the analyte. This electrode provides

fast, accurate, and reproducible determination of SILC with low detection limit and high sensitivity and selectivity.

2. EXPERIMENTAL

2.1. Materials and reagents

All chemicals were of analytical reagent grade unless otherwise specified. Doubly distilled water was used for the preparation of stock solutions of sildenafil citrate. $HAuCl_4.3H_2O$, uric acid, and ascorbic acid were supplied by sigma Aldrich Company and pure sildenafil citrate was supplied from Pfizer. Britton-Robinson buffer (B-R) (pH 2.3–9) was prepared from 0.12 M CH_3COOH, 0.12 M H_3BO_3 and 0.12 M H_3PO_4, and adjusted with 0.5 M NaOH. The pharmaceutical formulation of sildenafil citrate (Viagra tablet, 50 mg) was purchased from the local market.

2.2. Apparatus and measurements

Voltammetric measurements were carried out with a mini Autolab PGSTAT 910 potentiostat connected to a personal computer. The measurements were performed in a conventional electrochemical cell.

Screen-printed glassy carbon electrode (SPGCE) strips purchased from Metrohm were used. The electrode is based on an alumina ceramic base(s) 35 mm long, 10 mm wide and 0.45 mm thick.

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used for the determination of SILC using SPGCE electrode in the potential window of +1.5 to −2.0 V, with a scan rate of 100 mV s^{-1}.

Scanning electron microscopy (SEM) (Philips, XI 30) was used for characterization of the homogeneity of AuNPs deposited over the electrode.

2.3. Fabrication of the metal nanoparticle-modified SPGCE

Gold solution, 6.0 mM $HAuCl_4.3H_2O$ was prepared in 0.1 M HNO_3. The electrodeposition of Au was performed in 10 mL of the solution that totally cover the screen printed electrode while applying a constant potential of −0.4 V (vs Ag/AgCl within SPGCE) for 600 sec. The metal nanoparticle-modified SPGCE was rinsed using ultra-pure water, and blot-dried.

2.4. Analysis of Sildenafil in pharmaceutical preparations

Five tablets of the pharmaceutical product (Viagra, 50 mg) were weighed and then the average mass per tablet was determined. The tablets were carefully grounded to a fine powder, and then a quantity of homogeneous powder equivalent to 50 mg of SILC was dissolved in 100 mL of water by sonication for 15 min. The desired concentration of SILC was obtained by accurate dilution with B-R

buffer. The sample solution so prepared was added to the supporting electrolyte in the voltammetric cell and SWV of the solution was recorded and the redox peak current was evaluated.

2.5. Analysis of Sildenafil citrate in human urine

For this purpose, the urine samples were diluted 10 times in B-R buffer (pH 7.3) to minimize any matrix effect. In 10 mL measuring flasks, three different amounts of 0.5 mmol L^{-1} SILC solutions were spiked to 2.0 mL of urine sample, diluted with B-R buffer, poured into the electrolytic cell, and the corresponding SWVs were recorded.

3. RESULTS AND DISCUSSION

3.1. Characterization of Au nanoparticles-modified SPGCE

The SEM images of the working electrode for SPGCE and SPGCE/ AuNPs are shown in Fig. 2A–B. The surface of a bare SPGCE is shown in Fig. 2A. After electrodeposition, AuNPs were observed to be distributed uniformly on the working electrode in which it possesses more sites for binding with SILC, extending the interaction area and enhancing the electron transfer rate on the surface of the electrode. The AuNPs layer could be observed clearly in Fig. 2B.

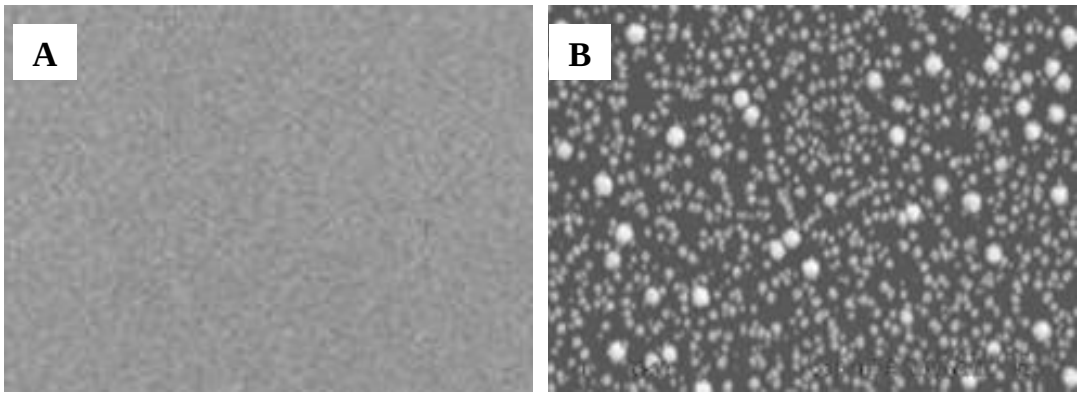

Figure 2. SEM images of the working electrode at: (A) bare SPGCE, and (B) AuNPs electrodeposited SPGCE prepared from 0.1M HNO_3 solution (pH 1.2) containing 6.0 mM $HAuCl_4.3H_2O$.

3.2. Electrochemical behavior of SILC

3.2.1. At pH 2.3

Using cyclic voltammetry (CV), the electrochemical behavior of 0.5 mmol L^{-1} SILC in 0.12 M B-R buffer, pH 2.3, on a bare SPGCE in the potential range of 1.5 to –2.0 V was investigated. It is well known that sildenafil contains basic functional groups with a weak acidic moiety. In the substituted and fused rings of pyrimidine and pyrazol, protonation is highly difficult due to resonance and steric effects.

Therefore, the only site in sildenafil avaliable for protonation is the nitrogen bonded to electron-donating methyl group in the piperazine ring [32-33]. Figure 3A (curve a) shows the cyclic voltammogram for SILC at bare SPGCE which give rise to an irreversible oxidation peak at 0.08 V, due to the oxidation of piperazine ring. In Fig.3 (curve b), two peaks were observed at 0.42 V and 0.12 V representing the oxidation-reduction of Au at AuNPs/SPGCE in buffer solution. However, by using AuNPs/SPGCE in SILC solution, Fig. 3 (curve c), a high response for anodic current of 186 µA at 0.30 V, and a small reduction peak were observed, indicating that the homogenous film formed from AuNPs enhances the peak current of SILC by increasing the active sites responsible for the redox reaction.

3.2.2. At pH 7.3

As pH value changes, a great change in SILC behavior occurs. It is well known that sildenafil has two ionization constants; the first constant (pK_{a1}) at 5.5 is a characteristic for the acidic group while the second constant (pK_{a2}) at 8.7 was attributed to its basic group [25]. As pH increases above the ionization constant the drug ionized differently. Figure 3B (curve a) shows the cyclic voltammogram for SILC at bare SPGCE which give rise to a reversible oxidation peak at 0.5 V and a reduction peak at –0.96 V. The appearance of reduction peak at pH 7.3 attributed to the completely reduction of the drug at pH above its ionization potential value. One oxidation peak and two broad reduction peaks were observed for Au in buffer solution at this pH, (Fig.3B (curve b)). In Fig. 3B (curve c) at AuNPs/SPGCE in SILC solution, a broad oxidation peak current of 179 µA was obtained at 0.11 V, and two small reduction peaks were noticed clearly at 0.05 V and –0.23 V, this can be attributed to the deposition of AuNPs on SPGCE which contributes in stabilizing the redox reaction of SILC and enhancing the current values at pH 7.3.

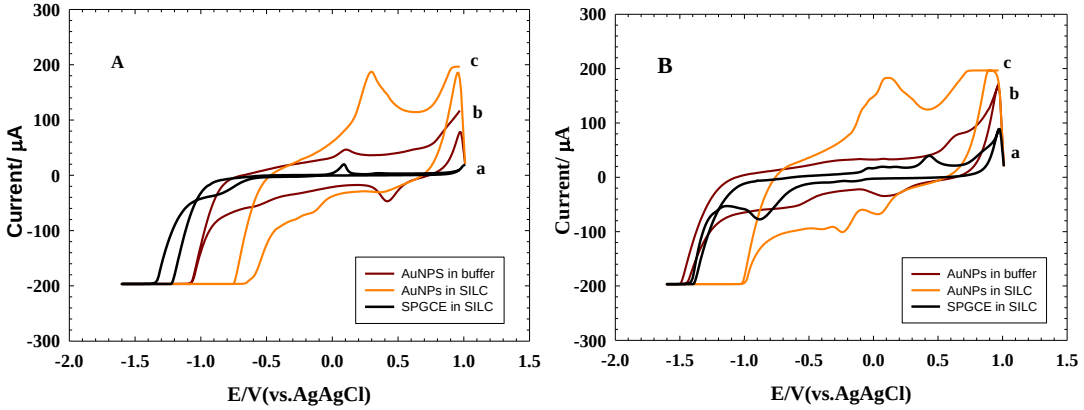

Figure 3. CVs of bare SPGCE in 0.5 mmol L^{-1} SILC/0.1 mol L^{-1} B-R buffer (a), AuNPs/SPGCE in B-R buffer (b) and AuNPs/SPGCE in 0.5 mmol L^{-1} SILC (c), at pH 2.3 (A), and pH 7.4 (B), with scan rate of 100 mVs^{-1}.

The dependence of the oxidation peak currents and the potential response of AuNPs/SPGCE of 0.5 mmol L^{-1} solutions of SILC over various pH (2.3-9.0) was critically investigated. In general, as the pH value increases the oxidation potential peaks is shifted to more negative values either in absence or presence of AuNPs (Fig.4A) [34]. In case of AuNPs/SPGCE, at pH>7.3, the potential returned to be shifted to more positive values, Fig.4A (curve b). The data in Fig.4B revealed that in the presence of AuNPs, oxidation current peaks for SILC enhanced greatly as pH values increases. Throughout the pH range studied, the maximum current intensity (I_p) at AuNPs/SPGCE was observed at pH 7.3. Thus, B-R buffer of pH 7.3 was employed for further studies.

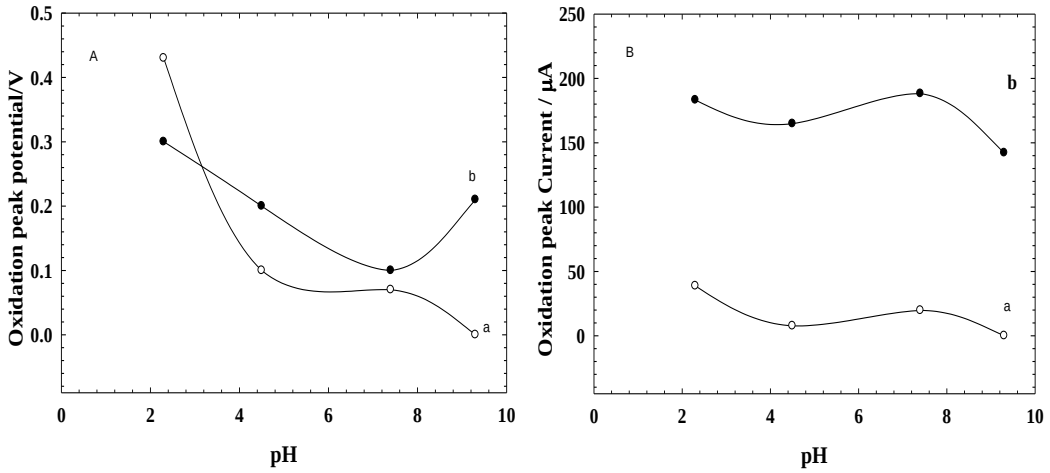

Figure 4. (A) Dependence of the oxidation peak potential, and (B) oxidation peak Current on pH in absence (a), and in presence (b) of AuNPs. Scan rate: 100 mVs^{-1}

3.3. Effect of scan rate

The oxidation peak current (I_p) of SILC at SPGCE electrode in 0.5 mmol L^{-1} SILC solution (pH 7.3) was varied with change of scan rate (v), as shown in Figure 5. The logarithm of anodic peak current was proportional to the logarithm of scan rate in the range of 20–160 mVs^{-1} with the regression equation:

$\log I_p$ (μA) = 1.31 + 0.55 log v (mV s^{-1})

(R^2=0.923, where v is in mVs^{-1} and I_p is in μA)

This linear relation indicates that the electrode process for electrooxidation of SILC was controlled *mainly* by diffusion. That is confirmed by the slope of 0.55, which is very close to the theoretically expected 0.5 value for a diffusion-controlled process [33].

The dependence of the anodic peak current on the scan rate has been used for the estimation of the "apparent" diffusion coefficient, D_{app}, for the compounds studied. D_{app} values were calculated from Randles–Sevcik equation [35, 36]:

$I_p = 2.69 \times 10^5 n^{3/2} A C_0 D^{1/2} v^{1/2}$

Where I_p is the peak current density (Acm^{-2}), n is the number of electrons transferred at T=298 K, A is the geometrical electrode area (0.0176 cm^2), C_0 is the analyte concentration (5×10^{-6} mol cm^{-3}), and D is the diffusion coefficient of the electroactive species (cm^2 s^{-1}). D_{app} value at AuNPs/SPGCE for SILC is found to be 8.0×10^{-9} cm^2 s^{-1}, which is larger than its corresponding value at bare SPGCE 6.7×10^{-7} cm^2 s^{-1}. It is clear that the AuNPs affect remarkably the diffusion component of the charge transfer at the electrode surface as indicated by the D_{app} values [37].

The size of the diffusion layer at the electrode surface proximity changes with the voltage scan rate used. At relatively slow voltage scans, the diffusion layer grows much further towards the solution side and further from the electrode surface. Therefore, as the scan rate increases the flux to the electrode surface increases considerably. At relatively higher scan rates, and in presence of AuNPs, more active sites were provided with large specific surface area ready for oxidation of SILC. The values indicated for D_{app} show that the diffusion is enhanced in presence of AuNPs than in absence of it.

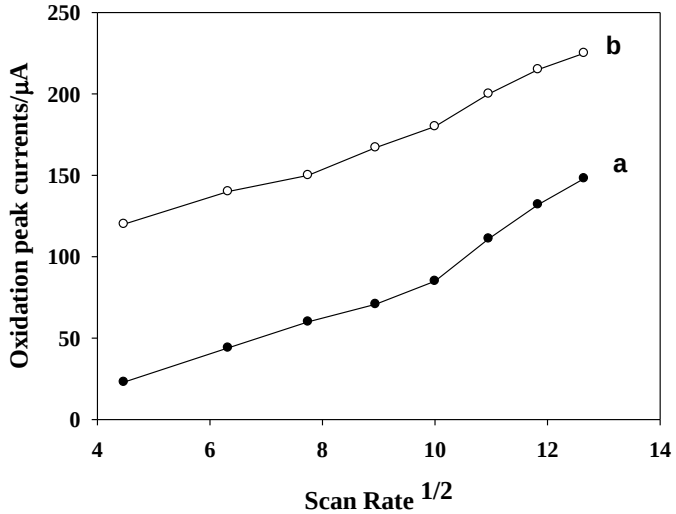

Figure 5. Dependence of the oxidation peak current on square root of scan rate of 0.5 mmol L^{-1} SILC/0.1 mol L^{-1} B-R buffer solution in absence (a) and presence (b) of AuNPs.

3.4. Response Stability of the Electrode

The stability of the modified electrode is very important for practical applications, so the stability of AuNPs/SPGCE was tested by cyclic voltammetry in two ways. Firstly, the operational stability was examined by recording 40 successive CVs at AuNPs/SPGCE in 0.5 mmol L^{-1} SILC in 0.1 mol L^{-1} B-R (pH 7.3) solution, and a decrease by just ≈30% of the initial anodic and cathodic peak currents was observed (Fig. 6A) after 15 cycles, afterwards remaining constant.

Secondly, AuNPs/SPGCE was tested during 30 days, recording 10 successive CVs in 0.5 mmol L^{-1} SILC in 0.1 mol L^{-1} B-R (pH 7.3) once every 5 days. When not in use, the modified electrode was stored at room temperature, away from direct contact with UV light. Results are shown in Fig. 6B, where anodic peak currents of AuNPs/SPGCE are plotted versus days and it can be seen that there is a sharp decrease of anodic current after 7 days, but then reach a plateau, and remaining almost constant. Thus, AuNPs/SPGCE electrode shows high stability for determination of SILC.

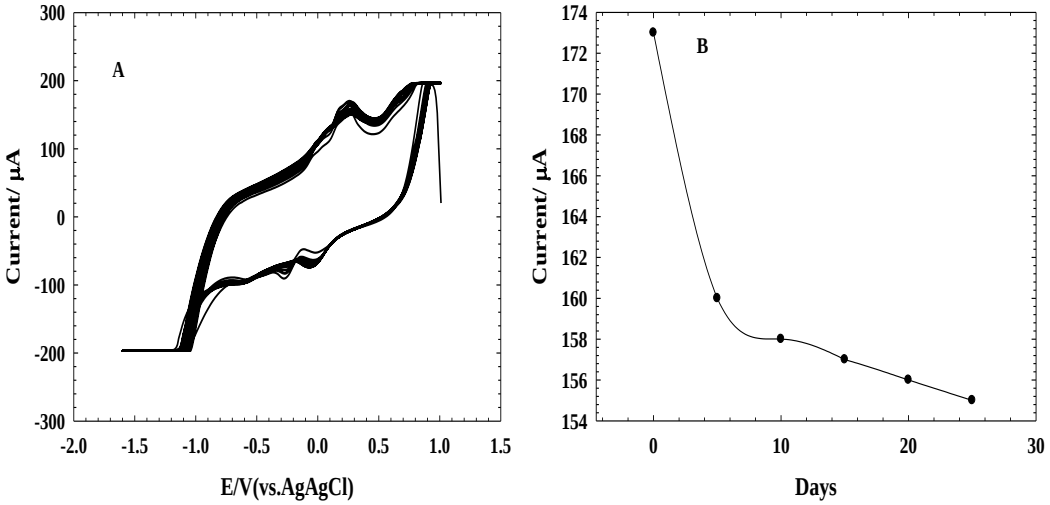

Figure 6. (A) Repeated cyclic voltammograms (40 runs) of 0.5 mmol L^{-1} SILC/0.1 mol L^{-1} B-R buffer at AuNPs/SPGCE at pH 7.3, scan rate 100 mV s^{-1}. (B) Relation between days and anodic peak currents (within day variation).

3.5. Calibration graph, limit of detection

In Fig. 7, The calibration graph for oxidation peak was linear from 1.8×10^{-6} to 3.3×10^{-5} mol L^{-1} and obeyed the equation **y=3.355x+0.779**, where y and x are the peak current (µA) and SILC concentration (µM), respectively. The correlation coefficient (r) was 0.995. The detection limit for oxidation peak is estimated as 5.2×10^{-10} mol L^{-1}.

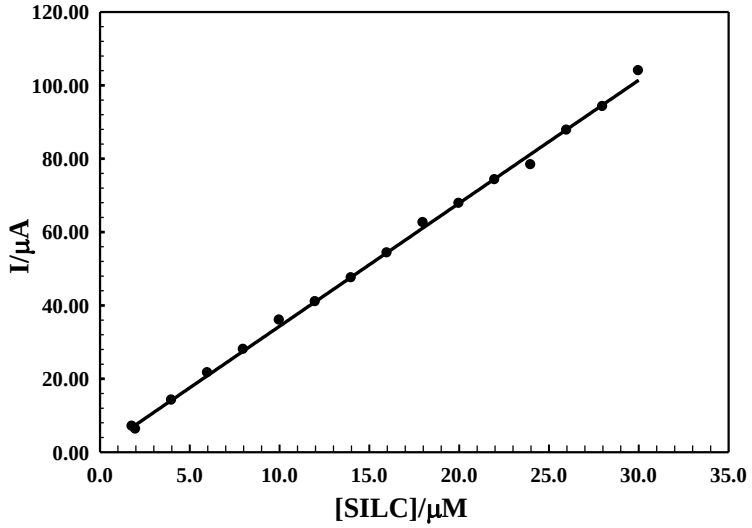

Figure 7. Calibration curve of SILC of concentrations from (1.8×10^{-6} mol L^{-1} to 3.3×10^{-5} mol L^{-1}) in 0.1 molL^{-1} B-R pH 7.3 at AuNPs/SPGCE electrode.

3.6. Selective Determination of SILC in Presence of Uric acid and Ascorbic acid

Uric acid (UA) and ascorbic acid (AA) co-exist in the extracellular fluid of the central nervous system, serum and exerted in urine with high concentration. AA and UA can cause a matrix effect in drug determination. The ability to determine SILC in the presence of these species has been a major goal of electroanalysis research. Therefore, the electrochemical behaviors of SILC, UA and AA in a mixture solution were studied. Cyclic voltammograms were recorded at SPGCE for the mixture solution of sildenafil, ascorbic acid and uric acid with concentrations 5×10^{-4} mol L^{-1}, 5×10^{-2} mol L^{-1}, 5×10^{-3} mol L^{-1}, respectively. Figure 8 (curve a) Shows the cyclic voltammogram for AuNPs/SPGE in buffer solution. It has been noticed that at B-R solution pH 7.3, (curve b) ascorbic acid has no oxidation current peak at AuNPs/SPGE, while uric acid has an oxidation current peak at 0.25 V. By addition of SILC solution to the AA and UA mixture solution a sharp oxidation peak was noticed with high current value. The results indicated that, although the concentration of ascorbic acid and uric acid is much higher than that of SILC by 1000 and 100 times, respectively, AuNPs/SPGCE electrode still can determine traces concentration of SILC. The good selectivity of AuNPs/SPGCE electrode towards SILC can be attributed to that pK_a of ascorbic acid is 4.1 and for uric acid is 5.4 at pH 7.3, while that of SILC is 8.7 so the nitrogen sites in piperidine ring is expected to be protonated and give rise to an oxidation current peak. These results confirm that ascorbic acid and uric acid have no matrix effect on the adsorption of SILC at AuNPs/SPGCE.

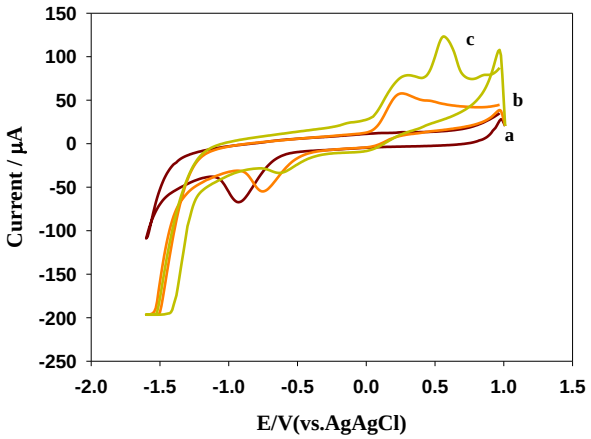

Figure 8. SWVs for SPGCE in B-R (a), SPGCE in a mixture of 5×10^{-4} mol L^{-1} ascorbic acid and 5×10^{-2} mol L^{-1} uric acid (b) and in addition of 5×10^{-3} mol L^{-1} SILC in the same mixture (c) with scan rate 100 mVs^{-1}, pH 7.3.

3.7. Analytical Applications

3.7.1. Determination of SILC in human urine

The proposed method was used to detect SILC in urine samples, which obtained from healthy volunteer. No signal was observed for SILC in urine samples; therefore, the urine samples were spiked by different concentrations of SILC standard solution, and then used for further determination. The

voltammograms were recorded using the SWVs, and the corresponding values were recorded. The results obtained are given in Table 1. As can be seen for the determination of SILC, good recoveries were obtained ranging from 99.3-102.2 %.

Table 1. Recoveries in spiked human urine samples

Urine sample	Spike (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)	R.S.D. (%)[a]
1	5.0	5.11	102.2	2.2
2	15.0	14.90	99.3	2.6
3	25.0	24.97	99.9	2.8

[a] average of 3 times repetition

3.7.2. Determination of SILC in Viagra Tablets

In order to verify the reliability of AuNPs/SPGCE electrode for analysis of SILC in a pharmaceutical product, the modified electrode was used to determine SILC in Viagra tablets (50 mg Sildenafil citrate per tablet).

The modified electrode was applied for the recovery assessment of SILC in tablets using standard addition method by adding different standard concentrations of SILC to the dissolved tablet sample. The results in Table 2 indicate that the amounts obtained by the proposed modified electrode are in good concurrence with the declared specifications on the pharmaceutical samples with recoveries values between 95.7 and 102.6 % for four measurements.

Table 2. Tablet results and recoveries obtained for four determinations of SILC in spiked Viagra tablets

Sample	content (μmol L^{-1})	SILC added (μmol L^{-1})	SILC found (μmol L^{-1})	Recovery (%)
1	7.00	3.00	10.26	102.6
2	7.00	7.00	13.84	98.6
3	7.00	10.00	16.48	96.9
4	7.00	12.00	18.19	95.7

4. CONCLUSION

This work shows that SILC can be determined using Voltammetric techniques on the basis of its oxidation process over AuNPs/SPGCE electrode. Enhancement the oxidation peak current indicates the high efficiency of AuNPs in modifying SPGCE using its high specific surface area and increasing the active sites for SILC determination.

The modified sensor offers simple, rapid, low cost, accurate and selective method for determination of SILC in pure form, human urine and in pharmaceutical preparations without sample pretreatment or separation. The limit of detection (5.2×10^{-10} mol L^{-1}) of the proposed sensor for SILC compared favorably with some of the reported methods for SILC determination [14, 16]. This method is sensitive, selective and there is no interference in the analysis from the other components present in tablets and biological fluids.

The good results obtained in the analysis of commercial formulations suggest that the proposed sensor is suitable for the determination of SILC and can be used in the routine control analysis with high stability and repeatability.

ACKNOWLEDGMENT
We gratefully acknowledge chemistry department (University of Taif, kingdom of Saudi Arabia) for financial support to carry out the above investigations.

References

1. A.T. Chuang, J.D. Strauss, R.A. Murphy, W.D. Steers, *J. Urol.* 160 (1998) 257.
2. A. Morales, C. Gingell, M. Collins, P.A. Wicker, I.H. Osterloh, *Int. J. Impot. Res*. 10 (1998) 69.
3. S.A. Ballard, C.J. Gingeli, K. Tang, L.A. Turner, M.E. Price, A.M. Naylor, *J. Urol.* 159 (1998) 2164.
4. F. Montorsi, T.E.D. McDermott, R. Morgan, A. Olsson, A. Schultz, H.J. Kirkeby, I.H. Osterloh, *Urology* 53 (1999) 1011.
5. J.J. Berzas, J. Rodrıguez, G. Castaneda, M.J. Villasenor, *Anal. Chim. Acta* 41, (2000) 143.
6. S.A. Ozkan, B. Uslu, P. Zuman, *Anal. Chim. Acta* 501, (2004) 227.
7. J. Rodrıguez, J.J. Berzas, G. Castaneda, N. Rodrıguez, *Talanta*, 62, (2004) 427.
8. M. Sarfati, V. Mateo, S. Baudet, M. Rubio, C. Fernandez, F. Davi, J. L. Binet, J. Delic, *Blood* 101(2003) 265.
9. K.F. Croom, M.P. Curran, *Drugs* 68 (2008) 383.
10. P. Sandner, J. Hutter, H. Tinel, K. Ziegelbauer, E. Bischoff, *Int. J. Impot. Res*. 19 (2007) 533.
11. F. Montorsi, T.E.D. McDermott, R. Morgan, A. Olsson, A. Schuitz, H.J. Kirkeby, I.H. Osterloh, *Urology* 53 (1999) 1011.
12. L. Bosca, S. Hortelano, *Cell. Signal*. 11 (1999) 239.
13. B. Tunctan, B. Korkmaz, H. Yıldırım, L. Tamer, U. Atik, C. K. Buharalıolu, *Am. J. Infect. Dis*. 1 (2005) 111.
14. J.D.H. Cooper, D.C. Muirhead, J.E. Taylor, P.R. Baker, *J. Chromatogr. B* 701 (1997) 87.
15. Y.M. Liu, H.C. Yang, J.R. Miao, *Yaowu Fenxi Zazhi.* 20 (2000) 61.
16. X. Zhu, S. Xiao, B. Chen, F. Zhang, S. Yao, Z. Wan, D. Yang, H. Han, *J. Chromatogr*. A 1066 (2005) 89.
17. N.D. Dinesh, P. Nagaraja, N.M. Made Gowda, K.S. Ranappa, *Talanta* 57 (2002) 757.
18. A.S. Amin, A. El-Beshbeshy, *Micro. chim. Acta* 137 (2001) 63.
19. V.V. Cosofret, R.P. Buck, *CRC Press, Boca Raton, Florida* (1992) 448.
20. K. Vytras, *J. Pharm. Biomed. Anal.* 7 (1989) 789.
21. S.S.M. Hassan, M.M. Abou-Sekkina, M.A. El-Ries, A.A. Wassel, *J. Pharm. Biomed. Anal.* 32 (2003)175.
22. S.S.M. Hassan, N.M.H. Rizk, *Analyst* 122 (1997) 815.
23. S.S.M. Hassan, M.M. Amer, S.A. Abd El-Fatah, A.M. El-Kosasy, *Anal. Chim. Acta* 363(1998) 81.

24. S.S.M. Hassan, M.M. Amer, S.A. Abd El-Fatah, A.M. El-Kosasy, *Talanta* 46 (1998) 1395.
25. A.M. Othman, N.M.H. Rizk, M.S. El-Shahawi, *Anal. Chim. Acta* 515 (2004) 303.
26. S.S.M. Hassan, E. M. Elnemma, W. H. Mahmoud, A.H.K. Mohammed, J. Appl. *Electrochem*. 36 (2006)139.
27. E.F. Batista, E.R. Sartori, R.A. Medeiros, R.C. Filho, O.F. Filho, *Anal. Lett*. 43 (2010)1046.
28. R. A. Farghali, R. A. Ahmed, *Int. J. Electrochem. Sci*., 7 (2012) 13008.
29. T.Vo-Dinh, B. M. Cullum, D. L. Stokes, *Sens. Actuat*. B 74 (1-3) (2001) 2.
30. N. F. Atta, R. A. Ahmed, H. Amin, A.Galal, *Electroanal*. 24(2012) 2135.
31. S.P. Liu, L. Fan, X.L. Hu, Z.F. Liu, S. Li,.*Anal. Sci*. 22(2006) 819.
32. N.D. Dinesh, P. Nagaraja, N.M. Made Gowda, K.S. Rangappa, *Talanta,* 57 (2002) 757.
33. H. S. Wang, A. M. Zhang, H. Cui, D. J. Liu, R. M. Liu, *Microchem. J.* 64(2000)67.
34. S.S.M. Hassan, S.A. Marzouk, *Talanta* 41 (1994) 891.
35. J. Wang, *Anal. Electrochem., 3rd ed., Wiley, Hoboken, NJ* 2006, p. 32.
36. N. F. Atta, A. Galal, R. A. Ahmed, *Electroanalysis*, 23(2011) 737.
37. P. Masawat, J. M. Slater, *Sens. Actu.s B* 124 (2007) 127.