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Original scientific paper

Electrochemical behaviour of sildenafil citrate at gold and cystein modified gold electrode in acid solution

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Abstract

Electrochemical behavior of sildenafil citrate (SC) at gold and cystein (Cys) modified gold electrode (Au/Cys) in 0.1 M H_2SO_4 was investigated by cyclic voltammetry (CV) and by square wave voltammetry (SWV). Effect of scan rate on the CVs of SC standard was performed in order to examine the mode of transport and irreversibility of process. SC as standard is determined by SWV in acid solution at Au electrode in a range: 10^{-3}, 10^{-2}, 0.1, 0.5, 1 µM and on Au/Cys in a range: 10^{-3}, 10^{-2}, 0.05, 0.1 µM. The presence of Cys causes two-time larger peak currents and shifting of the incipient potential of the SC oxidation to 0.1 V in negative direction. In investigated range of concentration of SC standard in acid solution on modified and unmodified Au electrodes SWV method have excellent linear regression coefficient with value 0.997 promoting SWV for reliable determination of SC.

Keywords

Sildenafil citrate standard; cystein modified electrode; sensitivity; quantitative analysis.

Introduction

Sildenafil citrate (SC) is chemically designated as 1-[[3-(6,7- dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylopiperazine citrate. SC is the active ingredient of one of the widely used pharmaceutical formulations of Viagra. Studied initially for its use in treatment of hypertension and angina, Viagra was very efficient for the treatment of erectile dysfunction. Nonmedical use of SC has increased over the years [1,2]. SC treats and recovery of extreme tiredness after a long flight [3]. The most common adverse effects of SC use included nasal congestion, impaired vision (blurriness, loss of peripheral vision, photophobia) and headache. Serious adverse effects include severe low blood pressure, heart attack, stroke, increased intraocular pressure, sudden hearing loss [4].

Many various methods for determination of sildenafil citrate in pharmaceutical preparations and biological samples have been described. The most frequently used method for determination of SC is high performance liquid chromatography [5-7]. Other reported methods for confirmation of SC in pharmaceutical preparations or biological fluids are optical methods such as UV-VIS spectrophotometric methods [8], NMR spectroscopy [9] or Electrospray tandem mass spectrometry (ESI-MS-Ms) spectrophotometry [10] and electroanalytical methods [11-15]. Among electrochemical methods different electrodes and voltammetric techniques were used. The SC determination at pH 2.0 was performed by SWV and adsorptive stripping techniques at hanging mercury drop electrode [16]. At glassy carbon electrodes (GC), CV and SWV were applied in determination of SC in the mixture with paracetamol and carvedilol [17] and in biological and pharmaceutical formulations [14]. The cyclic and linear sweep voltammetric methods as well as differential pulse voltammetry (DPV) and SWV at GC were applied for oxidative determination of SC in solutions containing 30 % (v/v) acetonitrile at different pHs [18]. By SWV at Pb film modified GC a new method for determination of SC is developed [19].

Different electrodes were used for electrochemical examination of SC. For example, electroanalytical methods were applied at boron-doped diamond and at diamond paste electrodes [12,20]. Delolo *et al.* used GC modified with a chitosan-supported ruthenium film in order to prepare sensor for SC [13].

The aim of this work is to examine the electrochemical behavior of SC at Au and Au/Cys in 0.1 M H_2SO_4 by CV and SWV. Scan rate studies were carried out to assess whether the process on Au and Au/Cys was under diffusion or adsorption-controlled. Linear dependency of peak currents from concentrations is established using SWV in order to investigate the sensitivity of the electrodes. Also, the linear dependency was constructed as a calibration curve and tested for quantitative determination of SC.

Experimental

Sildenafil citrate, provided by Hemofarm A.D. Stada, was used as SC standard and as content of Sildena® tablets (pharmaceutical formulation) with excipiences: lactose monohydrate, cellulose, microcrystalline, hydroxypropylcellulose, croscarmellose sodium, sodium stearyl fumarate, silica colloidal anhydrous, Opadry II Blue, Opadry fx silver. L-cysteine and H_2SO_4 were purchased from Sigma Aldrich.

For CV and SWV measurements, PGZ 402 Volta Lab (Radiometer Analytical, Lyon, France) was used and the three-electrode electrochemical cell. Polycrystalline gold (surface area 0.5 cm^2) which served as the working electrode, was polished with diamond paste, cleaned with a mixture of 18 MΩ cm deionized water and sulfuric acid and further cleaned with 18 MΩ cm deionized water in an ultrasonic bath. A gold wire was used as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. All potentials are given *vs.* SCE. The electrolytes were deoxygenated by purging with nitrogen.

Electrode modification and stock solutions were prepared according to [21]. All the solutions used were prepared with high purity water (Millipore, 18 MΩ cm resistivity).

Results and discussion

The electrochemical behavior of SC on Au and Au/Cys electrode was studied by cyclic voltammetry (CV) and its determination in pharmaceutical formulations was carried out by square wave voltammetry (SWV). The CV of SC standard on gold electrode in 0.1 M H_2SO_4 alongside the

voltammetric response of Au electrode in blank solution (dot line) is presented in Fig. 1. In the presence of SC, the CV was changed so that current increase occurs in the region of oxide formation. It can be said that highly developed gold oxide on surface is necessary as a catalyst for the beginning of the SC electrooxidation reaction.

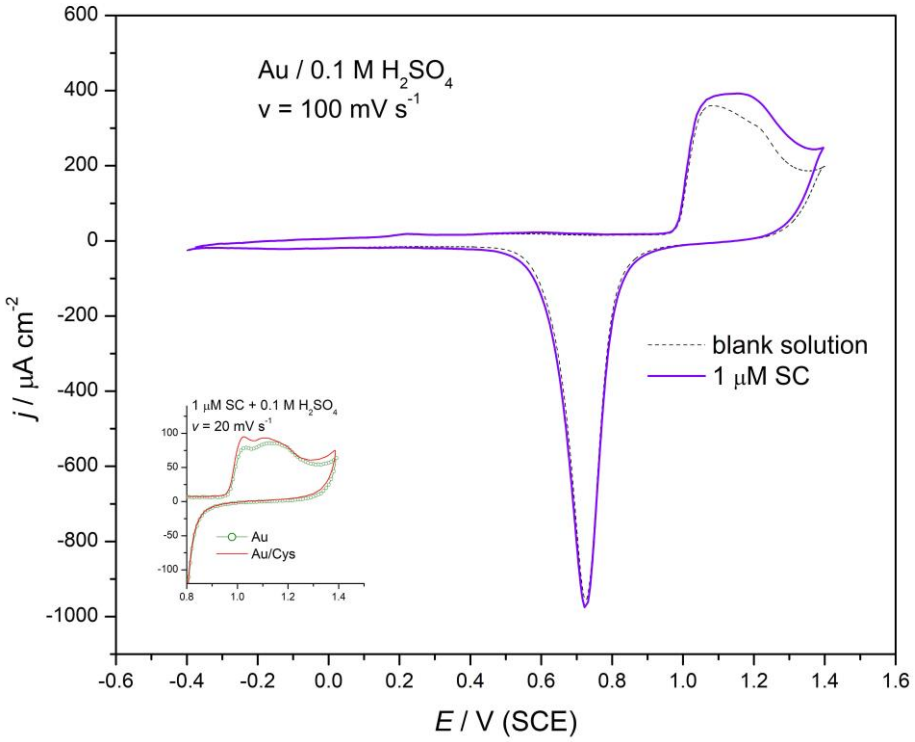

Figure 1. *CV of Au electrode using 0.1 M H_2SO_4 (dot line) and after the addition of 1 μM SC standard (full line), scan rate: 100 mV s^{-1}. Inset: CV of 1 μM SC standard on Au (dash line) and Au/Cys (full line) electrode, scan rate: 20 mV s^{-1}.*

In order to improve the activity, the electrode was modified with cysteine as was previously described [21]. In the inset of Fig. 1 it is show that in the presence of Cys the electrode becomes more active for the electrochemical oxidation of SC. The improved activity of the modified electrode in 0.1 M H_2SO_4 could be due to the increase of the electrode surface area according to the observed differences in morphology of the gold surface modified with Cys as was confirmed by optical microscope [21]. The role of Cys can also be explained by the mechanism proposed by Ozkan *et al.* for the electrochemical oxidation of SC on a GC electrode in an aqueous solution containing 30 % (v/v) acetonitrile [18]. The authors indicated that acid–base equilibrium precedes the electrooxidation of the piperazine moiety at pH 5.5. Cys is chemisorbed on an Au surface through the thiol group, while its amino and carboxylic groups remain available to interact with SC. As suggested by Wang and Du [22], it seems that Cys act as a mediator of the electrode reaction and as an electron-transferring accelerator. Possible mechanism of interaction between SC and Au/Cys is presented in detailed in [21], explaining the observed reactivity for SC electrooxidation.

Sildenafil citrate was used as SC standard and as content of Sildena® tablets (pharmaceutical formulation) containing excipients. Figure 2 show CVs of SC standard and SC in pharmaceutical formulation on modified and unmodified Au electrode. Overlap of CVs confirm that excipients are electrochemically inactive and did not affect the SC electrooxidation.

Furthermore, effect of the scan rate on the CVs of SC standard was studied on Au/Cys electrode as is presented in Fig. 3. Scan rate studies were carried out to assess whether the process on modified gold electrode was under diffusion or adsorption-controlled.

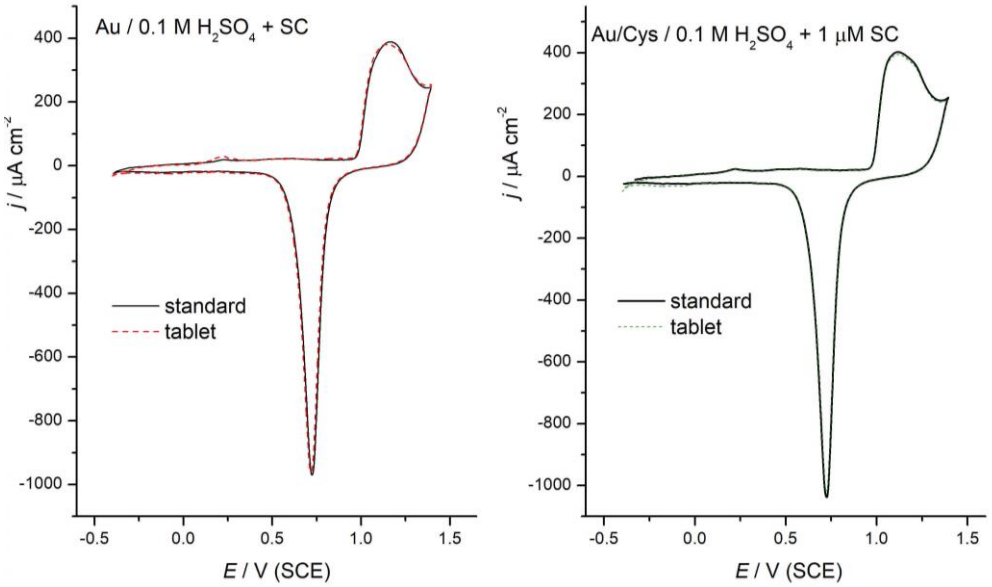

Figure 2. *CV of SC standard (full line) and SC tablet (dot line) on Au electrode (a), CV of SC standard (full line) and SC tablet (dot line) on Au/Cys electrode (b); 1 µM SC + 0.1 M H_2SO_4 scan rate: 100 mV s^{-1}.*

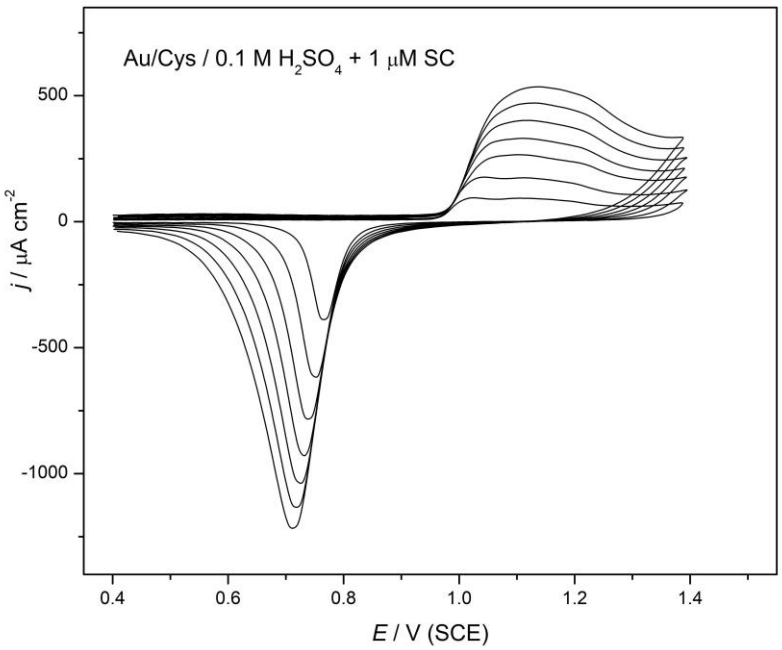

Figure 3. *CVs of SC standard on Au/Cys electrode for different scan rates 20-140 mV s^{-1}.*

Figure 4 demonstrates the dependency of peak current and peak potential from scan rates recorded in the range 20-140 mV s^{-1} (data are taken from Fig. 3). The peak current densities are linearly proportional to the scan rates suggesting adsorption-controlled electrode process (Fig. 4a). Furthermore, plot of logarithm of anodic peak current *vs*. logarithm of scan rate gave a straight line with a slope of 0.89 close to the theoretical value of 1.0, which is expected for an ideal reaction for the adsorption-controlled electrode process [23]. The peak potential was also dependent on scan rate. The plot of E_p versus log *v* was linear suggesting that it is irreversible electrode process (Fig. 4b). Nevertheless, close inspection of the dependency of peak potential from scan rates in backward direction indicates partially deviation from linearity for slower scan rates suggesting the interference of some reaction intermediates on the electrode process. The same experiments were performed on Au electrode exhibiting the same conclusion about the mode of

transport and irreversibility of process. Effect of scan rate on SC at GC [14,18] and at the hanging mercury dropping electrode[24] demonstrate also the adsorption-controlled process.

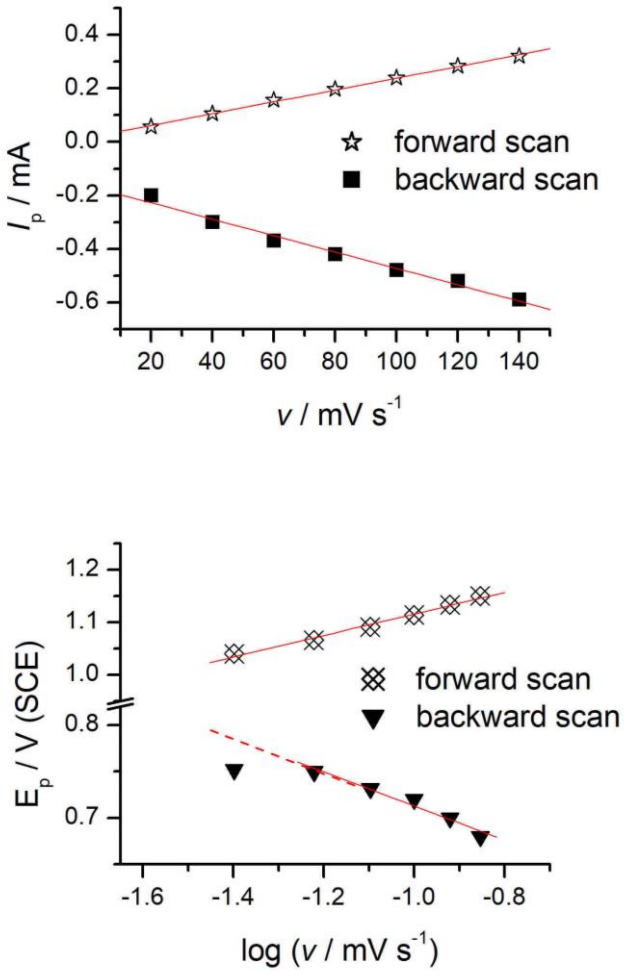

Figure 4. *Electrochemical behavior of SC standard on Au/Cys electrode for scan rates 20-140 mV s^{-1}, presented as dependence of peak current* vs. v *(a) and peak potential vs. log of scan rates (b).*

Apart from apparent increase in the voltammetric response caused using higher scan rates, the significant improvement in sensitivity can be achieved by employing SWV method. In order to establish the optimal parameters in SWV pulse size, scan rate and accumulation time were varied. The peak current appeared with maximum values for an accumulation potential of -400 mV while the accumulation time of 60 s enables electrode surface saturation. With increasing scan rate, the peak current increases also but the peak becomes less sharp and poorly defined. Higher peak current was observed by increasing the pulse size, but the background current also increases. The peak current increases linearly with step size up to 5 mV. Thus, the best defined voltammetric signal for the determination of SC was recorded under the conditions: step size 5 mV, pulse size 25 mV, scan rate 10 mV s^{-1}, accumulation time 60 s at - 400 mV.

SC standard is determined by SWV in 0.1 M H_2SO_4 at Au in a range: 10^{-3}, 10^{-2}, 0.1, 0.5, 1 µM as is shown in Fig. 5. It can be notice that the selected concentrations of SC standard start to oxidize with the beginning of oxide formation on gold electrode. SW voltammograms recorded at Au electrode for increasing amount of SC standard concentrations showed a linear increase of anodic peak currents with the concentration (inset of Fig. 5) with an excellent correlation coefficient (R = 0.997). Obviously, the linear dependency can be used as a calibration curve for quantitative determination, providing a convenient way for quantitative analysis.

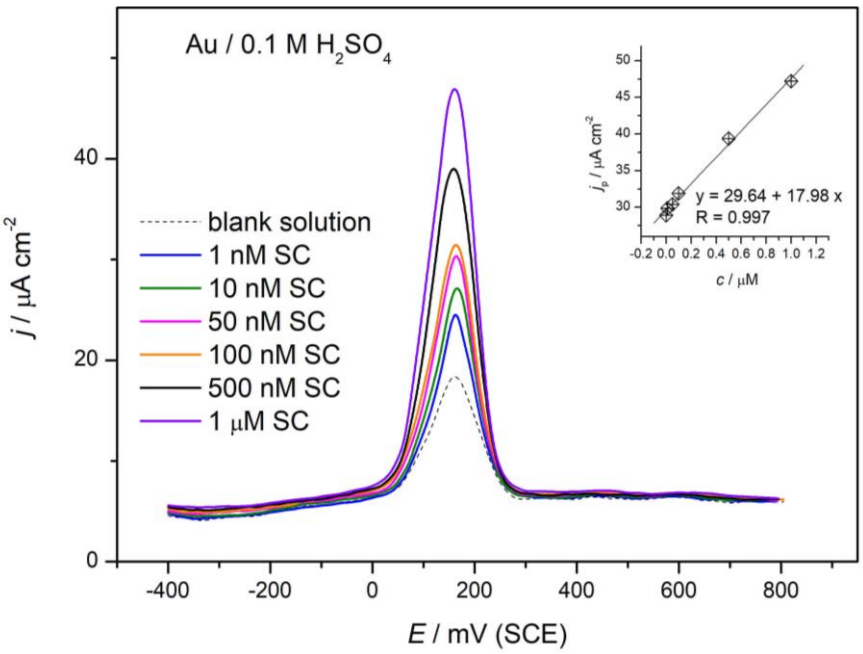

Figure 5. *SW voltammograms of SC standard (10^{-3}, 10^{-2}, 5×10^{-2}, 10^{-1}, 5×10^{-1}, 1.0 μM) on Au electrode using 0.1 M H_2SO_4. Step size 5 mV, pulse size 25 mV and scan rate 10 mV s^{-1}, accumulation time 60 s at -400 mV. Inset: the linear dependency of anodic peak currents vs. concentration of SC standard.*

SC standard is determined by SWV at Au/Cys electrode in a range: 10^{-3}, 10^{-2}, 0.05, 0.1 μM as is shown in Fig. 6. The currents increase with increasing the concentration of SC standard, shifting the peak current towards less positive value. A linear increase of anodic peak currents with the concentration with an excellent correlation coefficient (R = 0.997) is observed and presented in inset of Fig. 6. Comparing the equations for linear dependency of peak currents from concentrations it can be notice that higher slope of calibration curve is obtained on Au/Cys electrode indicating the higher sensitivity of SW voltammetry for concentration detection. Nevertheless, linear dependency can be obtained in extensive concentration range on Au electrode regarding Au/Cys.

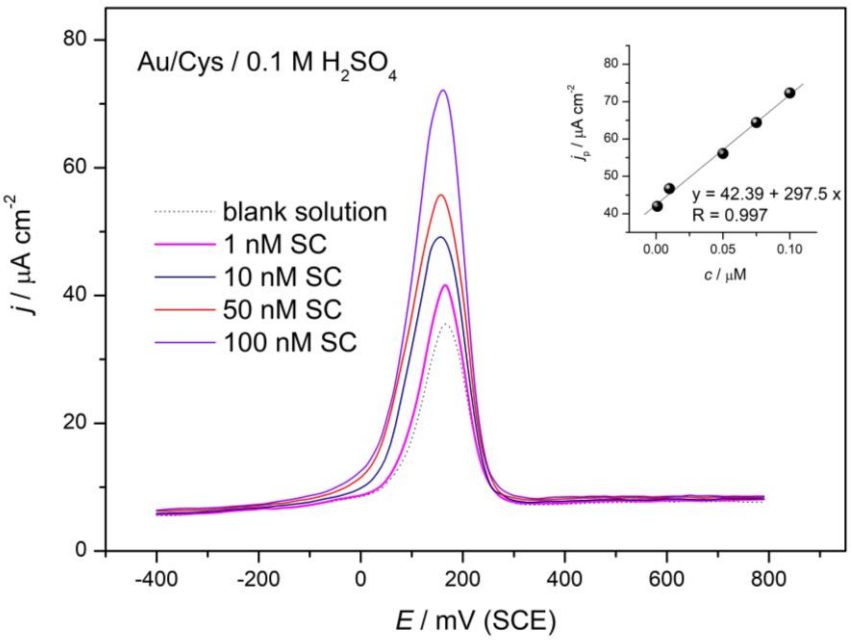

Figure 6. *SW voltammograms of SC standard (10^{-3}, 10^{-2}, 5×10^{-2}, 10^{-1}, 5×10^{-1}, 1.0 μM) on Au electrode using 0.1 M H_2SO_4. Step size 5 mV, pulse size 25 mV and scan rate 10 mV s^{-1}, accumulation time 60 s at -400 mV. Inset: the linear dependency of anodic peak currents* vs. *concentration of SC standard.*

Better insight in the obtained activity of Au and Au/Cys electrode in SC standard electrooxidation can be observed by overlapping of SW voltammograms for a chosen concentration, as is presented in Fig. 7. Comparing the results of SW voltammograms two times larger peak currents and 100 mV more negative incipient potential obtained on Au/Cys in regard to Au electrode can be noticed. It could be explained by possible oxidation mechanism and by different electrode surfaces morphology [21]. Concerning SW voltammogram of SC tablet on Au/Cys electrode, the same shape and peak position was obtained. Examination of the electrochemical behavior of various concentrations of SC tablet by SWV reveal excellent fitting in the calibration curve, as is presented in [**Error! Bookmark not defined.**]. Besides, unknown concentrations of SC tablet are confirmed by HPLC-UV method. It was highlighted that in contrast to HPLC-UV, electrochemical method is more sensitive and can be used for the measurements of very low concentrations of analyte.

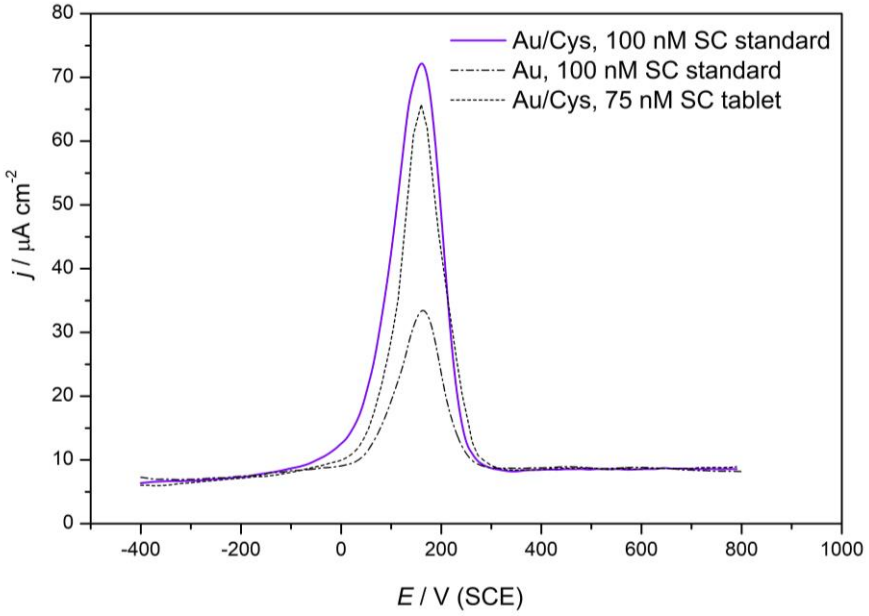

Figure 7. *SW voltammograms of 0.1 µM SC standard on Au and Au/Cys electrode and of 0.75[1] µM SC tablet using 0.1 M H_2SO_4. Step size 5 mV, pulse size 25 mV and scan rate 10 mV s^{-1}, accumulation time 60 s at -400 mV.*

Table 1 presents comparative characteristics of the quantitative determination of SC obtained with different electrochemical methods. The calibration curves provided reliable linear responses on a suitable range although with Au/Cys electrode linear concentration range was extent to a very low concentration (up to nanomoles per liter magnitude order).

Table 1. *Comparison of electrochemical methods for SC determination*

method	electrode	Linear range, M	LOD, M	reference
AdCSV	Bismuth film/GC	10^{-7}-10^{-6}	$1.8x10^{-8}$	[25]
potentiometry	Ppy/Cit/Graphite	$3.4x10^{-5}$-$1.7x10^{-3}$	$3x10^{-5}$	[26]
SWV	screen-printed GC	10^{-6}-$1.4x10^{-5}$	$5.5x10^{-8}$	[14]
SWV	Au/Cys	10^{-9}-10^{-7}	$1.04x10^{-8}$	Present work

Conclusions

Investigation of electrochemical behavior of SC at Au electrode in acid solution reveal that highly developed gold oxide on surface is necessary as a catalyst for the beginning of the SC electrooxidation reaction. Effect of the scan rate on the CVs of SC standard was studied on Au/Cys and Au electrode suggesting adsorption-controlled electrode process since the peak current

densities are linearly proportional to the scan rates. Also, the peak potential was linear dependent on logarithm of scan rate suggesting that it is irreversible electrode process. SWV measurements indicate wider range of currents *vs.* concentrations linearity on Au electrode compared to modified electrode. Nevertheless, with Au/Cys an enlargement of SC anodic currents and shift of the incipient potential of 0.1 V to the negative direction is noticed. In investigated ranges of SC standard concentration in acid solution on modified and unmodified Au electrodes SWV method have excellent linear regression coefficient with value 0.997 and SWV can be used for reliable determination of SC.

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