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Electrochemical determination of sildenafil citrate as standard, in tablets and spiked with human serum at gold and cystein modified gold electrode

Jelena Lović[a], Nemanja Trišović[b], Jelena Antanasijević[b], Nebojša D. Nikolić[a], Sanja Stevanović[a], Dušan Mijin[b], Dragan Vuković[c], Aleksandar Mladenović[d], Slobodan Petrović[b], Milka Avramov Ivić[a,*]

[a] ICTM–Institute of Electrochemistry, Belgrade University, Njegoševa 12, Belgrade, Serbia
[b] Faculty of Technology and Metallurgy, Belgrade University, Karnegijeva 4, Belgrade, Serbia
[c] Faculty of Medicine, Belgrade University, Dr Subotića 8, Belgrade, Serbia
[d] Hemofarm A.D. Stada Company, Vršac, Serbia

*Corresponding author: milka@tmf.bg.ac.rs, tel/fax: +381 11 337 03 89

Abstract

Nonmedical use of sildenafil citrate (SC) requires new methods for drug determination in human serum and in tablets. SC as standard and in Sildena® tablets is determined by square wave voltammetry (SWV) in 0.1 M H_2SO_4 (harmful for human serum) at gold electrode in a range: (1×10^{-3}, 1×10^{-2}, 0.1, 0.5, 1) μM and on cysteine (Cys) modified gold electrode (Au/Cys) in a range: (1×10^{-3}, 1×10^{-2}, 0.05, 0.1) μM. The presence of Cys causes two times larger peak currents and shifting of the incipient potential of the SC oxidation to 0.1 V in negative direction. A safe procedure for SC determination as standard and in Sildena® tablets spiked with human serum is developed by SWV at gold electrode in 0.05 M $NaHCO_3$ (suitable for human liquids) in a range: (1×10^{-3}, 1×10^{-2}, 0.1, 0.2, 0.3) μM. Microscopic characterization of the surfaces morphology was also performed. The SC concentrations were checked by High Performance Liquid Chromatography-Ultraviolet spectroscopy (HPLC-UV) showing that electrochemical method is more sensitive and can be used for the measurements of very low concentrations of the analyte.

Keywords: sildenafil citrate; Au/Cys electrode; human serum analysis; surface microscopic analysis.

1. Introduction

In a treatment of erectile dysfunction (efficacy for antidepressant associated sexual dysfunction), pulmonary arterial hypertension and altitude sickness [1, 2], oral phosphodiesterase (PDE) inhibitors are the first option. Three main PDE inhibitors are sildenafil, tadalafil, and vardenafil [3]. SC, which is chemically 1-[{3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1-H-pyrazolo [4,3-d] pyrimidin-5-yl)-4-ethoxyphenyl} sulfonyl]-methyl piperazine citrate (Fig. 1) attracts much attention.

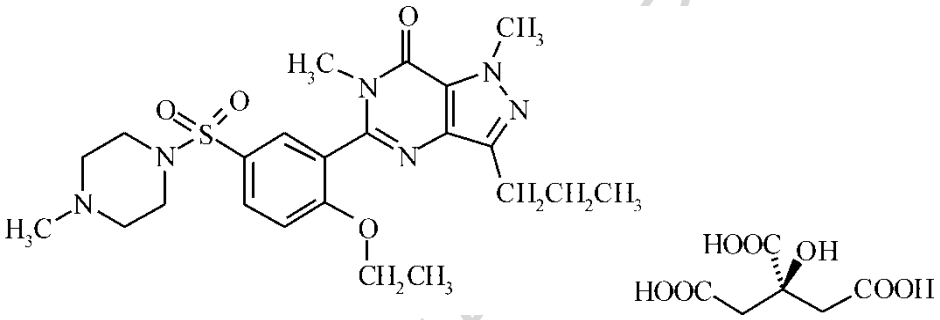


Fig. 1. Structural formula of sildenafil citrate.

Nonmedical use of SC has increased over the years [4, 5]. SC treats and recovery of extreme tiredness after a long flight [6]. The most common adverse effects of sildenafil 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 [7].

Protease inhibitors (treatment for HIV) inhibit metabolism of SC, effectively multiplying SC plasma levels, increasing the incidence of side effects [8]. For patients who are taking nitroglycerin or erythromycin, who have decreased liver or/and renal function, low blood pressure, SC may be quantified in plazma, serum or whole blood to toxic level. That's why development of fast and safe methods for quantifying SC in plasma or serum is of great importance. Its determination in tablets requires also new procedures.

Analytical methods for the determination of SC concentration include spectrometric, chromatographic and electroanalytical methods [3]. Spectrometric methods include UV-VIS spectrophotometric [9] and spectrofluorimetric methods [10], NMR spectroscopy [11] and electrospray tandem mass spectrometry [12]. Examples of HPLC and gas chromatography comprise: liquid chromatography-electrospray-mass spectrometry (MS) [13], ultra-performance liquid chromatography with tandem MS [14-16], HPLC coupled with electrochemical detection [17], HPLC with UV detector [18], rapid resolution reversed-phase HPLC with diode array detector [19] and gas chromatography coupled with MS [20].

In 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 [21]. At glassy carbon electrodes (GC), cyclic voltammetry and SWV were applied in determination of SC in the mixture with paracetamol and carvedilol [22] and in biological and pharmaceutical formulations

[23]. 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 pH [24]. By SWV at Pb film modified GC a new method for determination of SC is developed [25].

Using DPV and SWV at boron-doped diamond and at diamond paste electrodes SC was determined [26, 27]. GC was modified with a chitosan-supported ruthenium film in order to prepare sensor for SC [28].

The aim of this work is to develop a fast and safe SWV method for SC determination at gold electrode. Since acid electrolyte is harmful for human serum, SC was successfully determined as standard and as a content of Sildena® tablets at gold and at Au/Cys electrode in 0.1 M H_2SO_4. Additionally, SC was determined as standard and in Sildena® tablets spiked with human serum at gold electrode in 0.05 M $NaHCO_3$ respecting that the concentrations are in accordance with clinical serum level. Microscopic characterization of the surfaces morphology was performed using optical microscope. The SC concentrations in electrolytes were confirmed by HPLC-UV.

2. Experimental

2.1. Chemicals

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, H_2SO_4 and $NaHCO_3$ were purchased from Sigma Aldrich. Deionized water was obtained from GenPure ultrapure water system (TKA, Niederelbert, Germany).

2.2. Electrochemical measurements

For SWV, PGZ 402 Volta Lab (Radiometer Analytical, Lyon, France) was used. The three electrode electrochemical cell and the preparation of polycrystalline gold electrode (surface area 0.5 cm^2) were described in detail previously [29]. 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.

2.3. Surface characterization

The surface characterization was performed using optical microscope (Olympus CX41) connected to the computer.

2.4. Electrode modification, stock solutions and Sildena® tablets spiked with human serum preparations

A stock solution of SC (2×10^{-5} M) was prepared by a dissolving the Sildena® tablets in deionized water and stored at 4°C in the dark. The pharmaceuticals were prepared according to the reported procedure [25].

The freshly pretreated electrode was immersed into phosphate buffer (pH 5.5) containing 10 mM L-cysteine for 24 h [30]. The modified electrode was rinsed with water, and dried under the nitrogen atmosphere.

The human serum was collected and clinically prepared from ten healthy volunteers and spiked with Sildena® tablets [31, 32].

2.5. HPLC-UV analysis

By Agilent HPLC 1100 system with UV detector the samples were analyzed using Nucleodur Gravity C_{18} column (250 mm × 4.6 mm, 5 μm) in isocratic mode of elution using mobile phase consisting of 50 % acetonitrile and 50 % ammonium acetate solution (0.1 M) with a flow rate of 1 $mLmin^{-1}$. Injection volume was 20 μL with UV detection at 230 nm. The HPLC method was validated for linearity, accuracy, selectivity, precision and robustness.

3. Results and discussion

The physical insight in electrode surfaces morphology using optical microscope is presented first. SC as standard crystallizes on Au in the form of regular crystals (Fig. 2a). However, when SC was used as a content of Sildena® tablet, the regularity of crystals was lost (Fig. 2b). The differences between images shown in Figures 2a and 2b can be primarily ascribed to the effect of excipients, i.e. to their effect on crystal growth from the solution. The difference caused by crystal damage during tablet pressing is excluded. It is observed by a systematic investigation of the surface morphology in the presence of SC standard and Sildena® tablet. The SC standard on Au/Cys keeps some regularity, as shown in Fig. 2c. The irregularity of crystals was kept with Sildena® tablet on Au/Cys (Fig. 2d), but it can be noticed that large crystals are interrupted and a multitude small irregular crystals were obtained. The serum and Cys did not show any crystallization effect on Au, as shown in Fig. 2e. Finally, irregular globules were obtained when Sildena® tablet was spiked with serum (Fig. 2f).

Although the regularity of crystals is kept, the real surface area of the SC standard on Au/Cys (Fig. 2c) was increased in relation to the SC standard on Au (Fig. 2a) due to a coalescence of regular crystals in strip-like forms. The irregularity of crystals was kept with Sildena® tablet on Au/Cys (Fig. 2d), but it can be noticed that large crystals are interrupted and a multitude small irregular crystals were obtained. Due to interruption of the large crystals, the real surface area of this electrode was also increased in relation to the Sildena® tablet on Au (Fig. 2b).The mean size of the irregular globules was about 210 μm (Fig. 2f). It is necessary to note that the real surface area of this electrode was considerably increased by formation of the large irregular globules.

Due to the strong correlation between the electrochemical behavior and the state of electrode surface, the observed differences in morphology of the gold surface obtained with various components suggest their impact on the electrochemical characteristics and ability for SC oxidation as is later presented in Fig. 3.

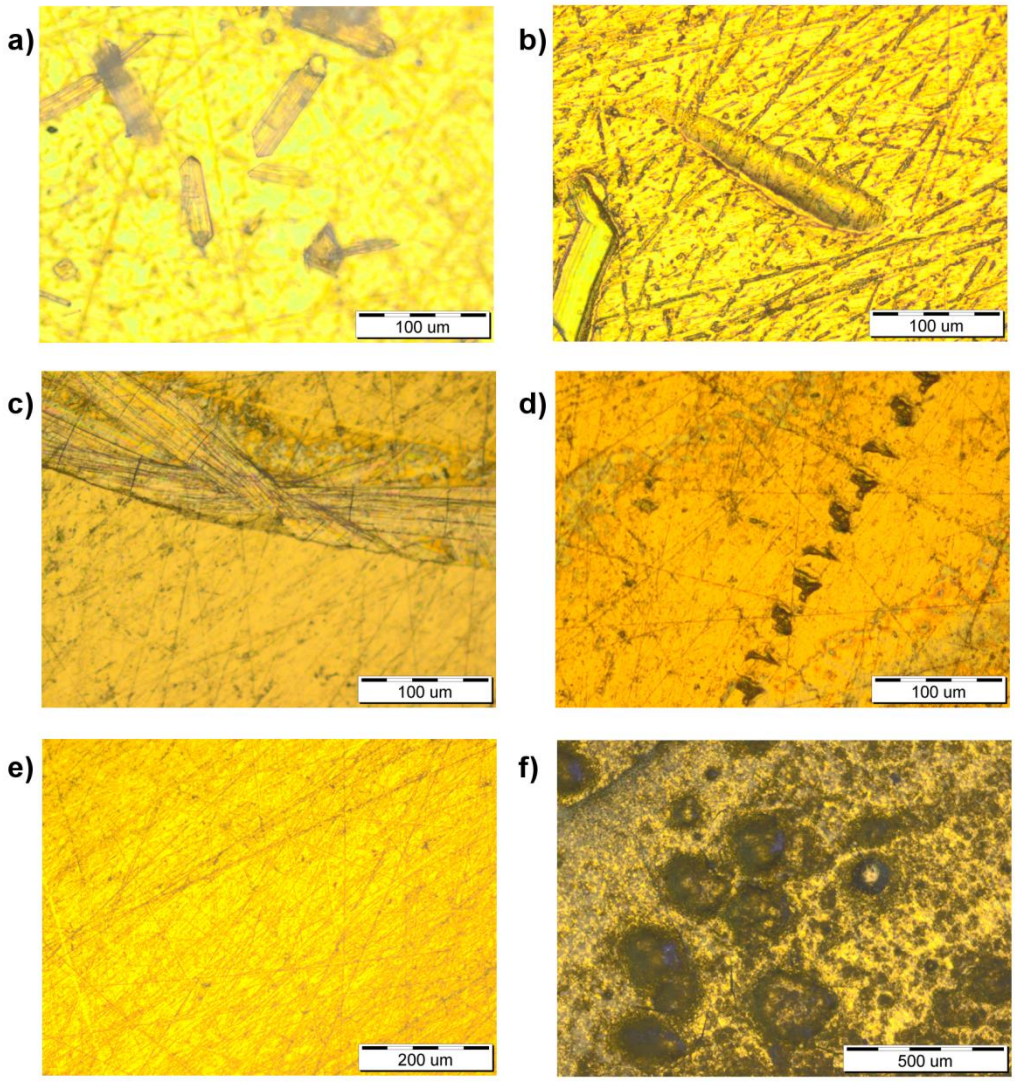
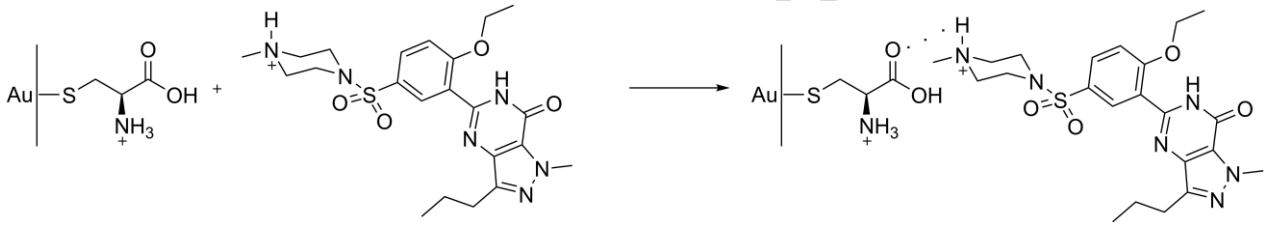


Fig. 2. Images obtained by optical microscope: a) SC standard on Au, b) Sildena® tablet on Au, c) SC standard on Au/Cys, d) Sildena® tablet on Au/Cys, e) serum on Au, f) Sildena® tablet spiked with serum.

SC standard is determined by SWV at Au in 0.1 M H_2SO_4 (red lines) in a range: (1×10^{-3}, 1×10^{-2}, 0.1, 0.5, 1) μM and at Au/Cys (green lines) in a range: (1×10^{-3}, 1×10^{-2}, 0.05, 0.1) μM as is shown in Fig. 3. Comparing to Au at Au/Cys two times larger peak currents and 0.1 V more negative incipient potential are observed for the all tested concentrations. As is presented in Fig. 2c the SC standard on Au/Cys shows a coalescence of regular crystals in strip-like forms compared to regular crystals on Au, additionally increasing the surface area of electrode as already mentioned. It could be one of the origins of the improved activity of the modified electrode in 0.1 M H_2SO_4. The role of Cys can also be explained by the mechanism proposed by Ozkan, Uslu and Zuman for the electrochemical oxidation of SC on a GC electrode in an aqueous solution containing 30 % (v/v) acetonitrile [24]. The authors indicated that an 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 [33], it seems to act as both a mediator of the electrode reaction and an electron-transferring accelerator. Namely, we believe that the interaction between the carboxylic group of Cys and the protonated SC through hydrogen bonding formation facilitate the dissociation of the protonated form of the drug (Scheme 1). This results in the more negative incipient potential and the observed increase in the peak currents.



Scheme 1. A possible mechanism of accumulation of SC on the Au/Cys electrode surface involving hydrogen bonding.

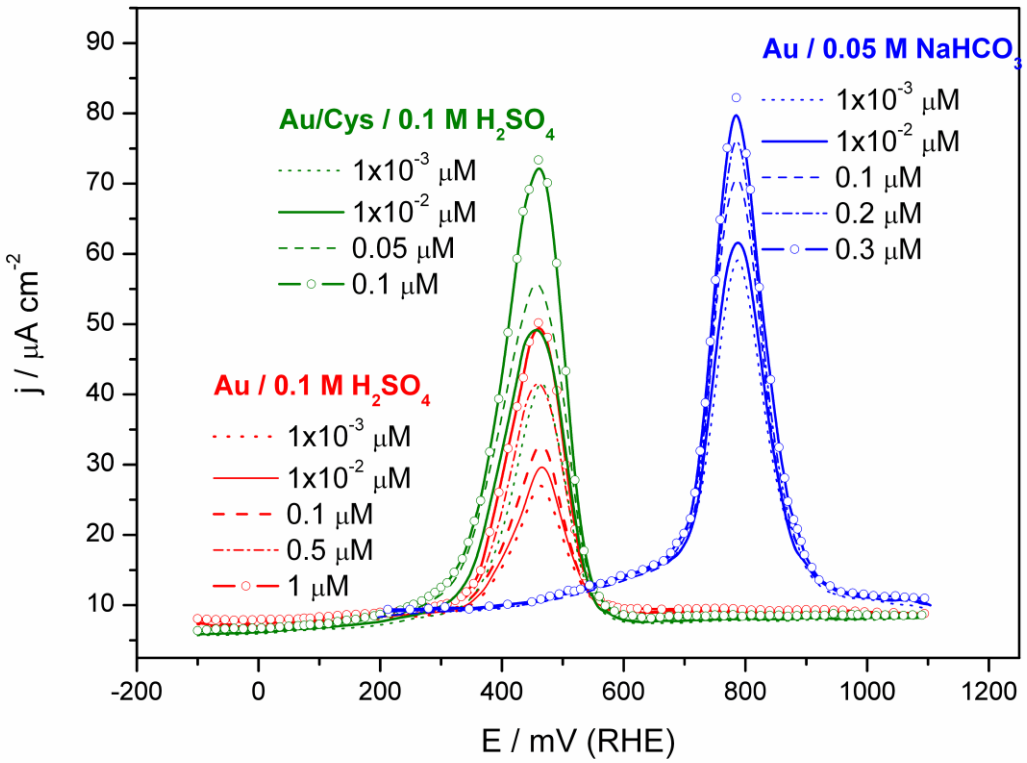


Fig. 3. The SC standard (the concentrations added in electrolyte are presented in legend) determined by SWV at Au (red lines), at Au/Cys (green lines) in 0.1 M H_2SO_4 and at Au in 0.05 M $NaHCO_3$ (blue lines). Step size 5 mV, pulse size 25 mV and scan rate 10 mV s^{-1}, accumulation time 60 s at –400 mV.

In order to determine content of SC in human serum, 0.05 M $NaHCO_3$ is tested and SC standard was analyzed in a range: (1×10^{-3}, 1×10^{-2}, 0.1, 0.2, 0.3) μM. It is obvious from Fig. 3 that without any modification Au electrode itself (blue lines) depicts comparable currents with Au/Cys (green lines) and two times larger than Au (red lines) in acid medium. This is explained by the fact that a conjugate base is always more easily oxidized than the corresponding acid [24]. Hence, the oxidation currents following an acid–base equilibrium increases with increasing pH. The structure of Cys also depends upon pH of the solution. Because its carboxyl group is unprotonated at pH 8.4, the interaction with SC is absent; thus the effect of Cys is not observed in this case.

Using the linear dependency of peak currents vs. SC concentrations (the data from Fig. 3), the calibration curves are constructed for both electrolytes and presented in Fig. 4. Previously the each excipient as a content of pharmaceutical formulation Sildena® was electrochemically tested under the experimental conditions presented in Fig. 3 and no one exhibited electrochemical activity and did not affect the SC electrooxidation. The values of unknown SC concentrations in Sildena® tablets were determined in 0.1 M H_2SO_4 on Au (Fig. 4a), on Au/Cys (Fig. 4b) and in 0.05 M $NaHCO_3$ on Au (Fig. 4c) including and Sildena® tablets spiked with human serum.

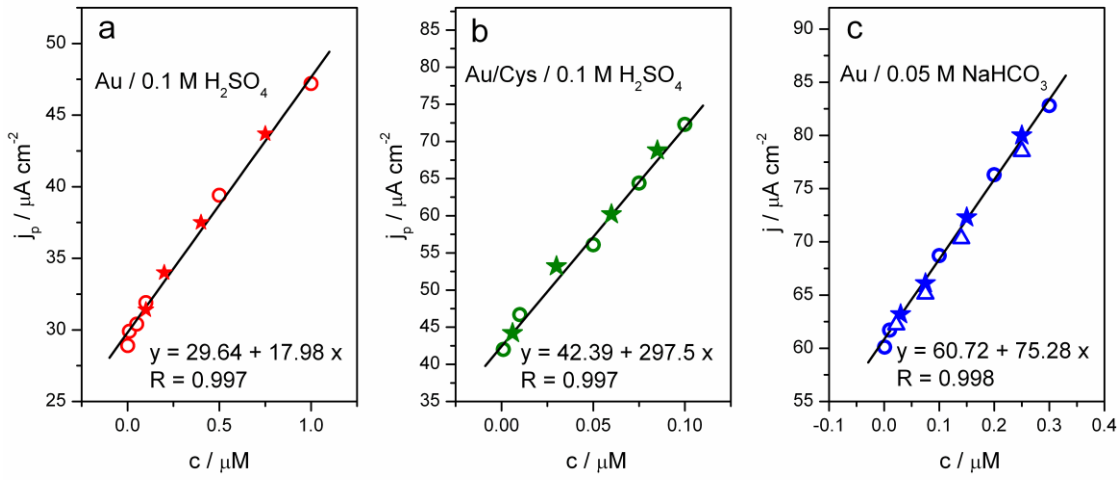


Fig. 4. The SC standard concentrations (assigned by circles) determined on Au (Fig. 4a), on Au/Cys (Fig. 4b) in 0.1 M H_2SO_4 and on Au in 0.05 M $NaHCO_3$ (Fig. 4c), from the data collected from Fig. 3. The unknown SC concentrations determined in Sildena® tablets are assigned by stars and in Sildena® tablets spiked with serum by triangles.

The all SWV determined SC concentrations presented in Fig. 3 were confirmed by HPLC-UV and the comparison with SWV is presented in Table 1.

Table 1. Determination of SC by SWV and HPLC-UV methods

Parameter	SWV	HPLC-UV

Range/μM	1×10^{-3} – 0.3	3.7×10^{-2} – 7.338
Regression equation*		
Slope	75.11/μA cm^{-2} μM^{-1}	53.23/mAU μM^{-1}
S.D. of slope	3.125	0.135
Intercept	60.84	0.433
S.D. of intercept	0.523	0.458
Regression coefficient	0.997	0.999
Recovery/%	99.6	103.8
S.D./%	0.799	1.101
LOD/μM	0.031	0.028
LOQ/μM	0.106	0.086

*Y=a+bC where C is SC concentrations, μM; Y is current per area unit, μA cm^{-2}, peak area: mAUsec, S.D.: standard deviation, LOD is limit of detection, and LOQ is limit of quantification.

The determination of SC by SWV in Sildena® tablets and tablets spiked with human serum showed that the mean recoveries were 100.11 % and 98.82 % respectively, with relative standard deviations: 1.52 % and 1.74 %, respectively.

Both analytical methods have excellent linear regression coefficients in the investigated ranges, with values above 0.99 indicating that SWV can be used for reliable determination of SC. In contrast to HPLC-UV, electrochemical method is more sensitive and can be used for the measurements of very low concentrations of analyte. Comparing to previously published electrode surfaces used for SC determination gold electrode exhibits some advantages. The preparation of gold electrode is very simple regarding to the preparation of amperometric dot-sensors based on zinc porphyrins [34], cathodically pre-treated boron-doped diamond electrode [26], glassy carbon electrode modified with a chitosan-supported ruthenium film [28] and lead film modified glassy carbon electrode [25]. All these electrodes are less toxic than hanging mercury electrode [35]. The proposed methods using glassy carbon electrode [24], electrode based on natural diamond paste [27] and gold electrode are simple and fast for SC analysis in pharmaceutical formulations. Gold electrode exhibited excellent performances for SC determination in human serum and it is to be noticed that for the in vivo assay of SC in biological samples, diamond paste being one of the most biocompatible materials [27]. Nevertheless, extension of the linear concentration range to a very low concentration (up to picomoles per liter magnitude order), and decrease of the limit of detection recorded for pharmaceutical formulations at aforementioned electrodes [25-28, 34, 35] in acid electrolytes is observed. In acid electrolyte gold electrode modified by Cys comparing to bare gold exhibits two times larger peak currents and shifting of the incipient potential 0.1 V in negative direction. Only for gold electrode, the physical insight in electrode surfaces morphology using optical microscope is presented with correlation between the electrochemical behavior and the state of electrode surface.

4. Conclusion

The fast and safe SWV method for SC determination as standard and in Sildena® tablet is developed in 0.1 M H_2SO_4 and for tablet spiked with human serum in 0.05 M $NaHCO_3$. In acid electrolyte bare gold electrode enables wider range of currents vs. concentrations linearity compared to modified electrode but with Au/Cys an enlargement of SC anodic currents and shift of the incipient potential of 0.1V to the negative direction is observed. It is explained by possible oxidation mechanism and by electrode surfaces morphology. In 0.05 M $NaHCO_3$ Au electrode for SC oxidation exhibits the comparable anodic currents as was obtained with Au/Cys in acid.

In investigated ranges SWV and HPLC-UV methods have excellent linear regression coefficient with values above 0.99 and SWV can be used for reliable determination of SC. In contrast to HPLC-UV, electrochemical method is more sensitive method and can be used for the measurements of very low concentration of analyte.

Acknowledgements

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References

[1] J.O. Stream, C.K. Grissom, Update on high-altitude pulmonary edema: pathogenesis, prevention, and treatment, Wild. Environ. Med. 19 (2008) 293–303.

[2] D.J. Webb, S. Freestone, M.J. Allen, G.J. Muirhead, Sildenafil citrate and blood-pressure-lowering drugs: results of drug interaction studies with an organic nitrate and a calcium antagonist, Am. J. Cardiol. 83 (1999) 21C–28C.

[3] A. Muneer, J. Kalsi, I. Nazareth, M. Arya, Erectile dysfunction, BMJ 348 (2014) doi: 10.1136/bmj.g129.

[4] K. Peterson, Young men add Viagra to their drug arsenal, USAToday (2001-03-21).

[5] K.M. Smith, F. Romanelli, Recreational use and misuse of phosphodiesterase 5 inhibitors, J. Am. Pharm. Assoc. 45 (2003) 63–72.

[6] P.V. Agostino, S.A. Plano, D.A. Golombek, Sildenafil accelerates reentrainment of circadian rhythms after advancing light schedules, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 9834–9839.

[7] Viagra U.S. Physician Prescribing Information and U.S. Patient Product Information for Health Care Professionals,(PDF).September 2015, available online : http://www.pfizer.com

[8] Prescribing information for Viagra (PDF).Pfizer. September 2015 available online: http://www.pfizer.com/files/products/uspi_viagra.pdf

[9] A.S. Amin, A.M. El-Beshbeshy, Utility of certain σ and π-acceptors for the spectrophotometric determination of sildenafil citrate (Viagra), Microchim. Acta 137 (2001) 63–69.

[10] C.C. Wang, R.A. Silva, A.N. Masi, L. Fernandez, Sensitive spectroflurimetric determination of sildenafil, Anal. Meth. 2 (2010) 519–524.

[11] I. Wawer, M. Pisklak, Z. Chilmonczyk, ^{1}H, ^{13}C, ^{15}N NMR analysis of sildenafil base and citrate (Viagra) in solution, solid state and pharmaceutical dosage forms, J. Pharm. Biomed. Anal. 38 (2005) 865–870.

[12] M.E. Abdel-Hamid, Determination of Sildenafil, Tadalafil and Varendfil in tablets and adulterated herbal products by ESI-MS-MS, J. Liq. Chromat. Rel. Technol. 29 (2006) 591–603.

[13] K.M. Alkharfy, Simple and sensitive LC-ESI-MS method for the quantitation of sildenafil in plasma samples, J. Sep. Sci. 32 (2009) 3866–3870.

[14] B.C. Witjies, J.M. Ahsman, B.C. van der Nagel, D. Tobboel, R.A. Mathot, Simultaneous assay of sildenafil and desmethylsildenafil in neonatal plasma by ultraperformance liquid chromatography-tandem mass spectrometry, Biomed. Chromat. 24 (2010) 180–185.

[15] S. Magiera, I. Baranowska, A rapid method for determination of 22 selected drugs in human urine by UHPLC/MS/MS for clinical application, J. AOAC Int. 97 (2014) 1526–1537.

[16] K.M. Younes, E.F. El-Kady, A new validated bio-analytical liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method for the quantification of azithromycin in human plasma, Eur. J. Chem. 5 (2014) 241–246.

[17] M. Al-Ghazawi, M. Tutunji, S. Aburuz, Simultaneous determination of sildenafil and N-desmethyl sildenafil in human plasma by high-performance liquid chromatography method using electrochemical detection with application to a pharmacokinetic study, J. Pharm. Biomed. Anal. 43 (2007) 613–618.

[18] H. Al-Hroub, B. Alkhawaja, E. Alkhawaja, T. Arafat, Sensitive and rapid HPLC-UV method with back-extraction step for the determination of sildenafil in human plasma, J. Chromatogr. B 1009–1010 (2016) 1–6.

[19] Y.J. Yang, D.M. Song, W.M. Jiang, B.R. Xiang, Rapid resolution RP-HPLCDAD method for simultaneous determination of sildenafil, vardenafil, and tadalafil in pharmaceutical preparations and counterfeit drugs, Anal. Lett. 43 (2010) 373–380.

[20] S. Strano-Rossi, L. Anzillotti, X. de La Torre, F. Botre, A gas chromatography/mass spectrometry method for the determination of sildenafil, vardenafil and tadalafil and their metabolites in human urine, Rapid Commun. Mass Spectro. 24 (2010) 1697–1706.

[21] J.J. Berzas, J. Rodriguez, G. Castañeda, M.J. Villaseñor, Voltammetric behavior of sildenafil citrate (Viagra) using square wave and adsorptive stripping square wave techniques: Determination in pharmaceutical products, Anal. Chim. Acta 417 (2000) 143–148.

[22] I. Baranowska, M. Koper, P. Markowski, Electrochemical determination of carvedilo, sildenafil and paracetamol using glassy carbon electrode, Chem. Anal. 53 (2008) 967–981.

[23] R.A. Farghali, R.A. Ahmed, A novel electrochemical sensor for determination of sildenafil citrate (Viagra) in pure form and in biological and pharmaceutical formulations, Int. J. Electrochem. Sci. 7 (2012) 13008–13019.

[24] S.A. Ozkan, B. Uslu, P. Zuman, Electrochemical oxidation of sildenafil citrate (Viagra) on carbon electrodes, Anal. Chim. Acta 501 (2004) 227–233.

[25] K. Tyszczuk, M. Korolczuk, Voltammetric method for the determination of sildenafil citrate (Viagra) in pure form and in pharmaceutical formulations, Bioelectrochemistry 78 (2010) 113–117.

[26] E.F. Batista, E.R. Sartori, R.A. Medeiros, R.C. Rocha-Filho, O. Fatibello-Filho, Differential Pulse Voltammetric Determination of Sildenafil Citrate (Viagra) in Pharmaceutical Formulations Using a Boron-Doped Diamond Electrode, Anal. Lett. 43 (2010) 1046–1054.

[27] R.-I. Stefan-van Staden, J.F. van Staden, H.Y. Aboul-Enein, Diamond paste-based electrodes for the determination of sildenafil citrate (Viagra), J. Solid State Electrochem. 14 (2010) 997–1000.

[28] F.G. Delolo, C. Rodrigues, M. Martins da Silva, L.G. Dinelli, F.N. Delling, J. Zukerman-Schpector, A.A. Batista, A new electrochemical sensor containing a film

of chitosan-supported ruthenium: detection and quantification of sildenafil citrate and acetaminophen, J. Brazil. Chem. Soc. 25 (2014) 550–559.

[29] N.P. Trišović, B.Dj. Božic, J.D. Lović, V.D. Vitnik, Ž.J. Vitnik, S.D. Petrović, M.L. Avramov Ivić, Electrochemical characterization of phenytoin and its derivatives on bare gold electrode, Electrochim. Acta 161 (2015) 378–387.

[30] W. Yang, J.J. Gooding, D.B. Hibbert, Characterisation of gold electrodes modified with self-assembled monolayers of L-cysteine for the adsorptive stripping analysis of copper, J. Electroanal. Chem. 516 (2001) 10–16.

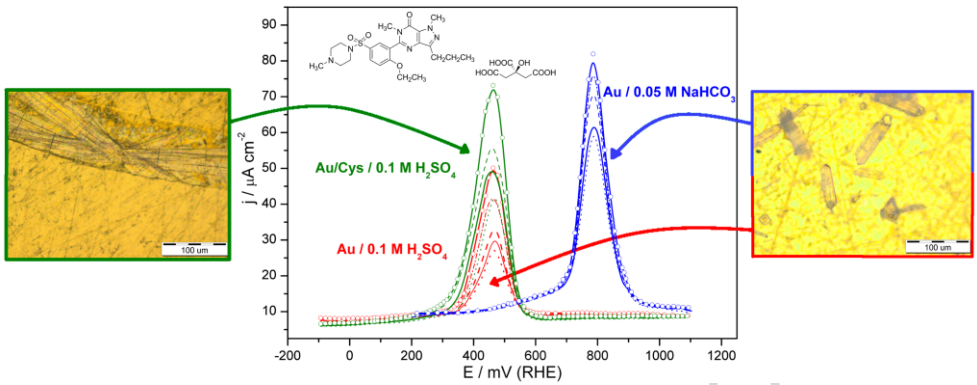
[31] R. Richterich, Clinical Chemistry, Theory and practice, Academic Press, 1969.

[32] K.M. Drljevic-Djurić, V.D. Jović, U.Č. Lacnjevac, M.L. Avramov Ivić, S.D. Petrović, D.Ž. Mijin, S.B. Djordjević, Voltammetric and differential pulse determination of roxithromycin, Electrochim. Acta 56 (2010) 47–52.

[33] S. Wang, D. Du, Studies on the electrochemical behaviour of hydroquinone at L-cysteine self-assembled monolayers modified gold electrode, Sensors 2 (2002) 41–49.

[34] S.-C. Balasoiu, R.-Io. Stefan-van Staden, J. Frederick van Staden, R.-M. Ion, G.-L. Radu, H. Y. Aboul-Enein, Amperometric dot-sensors based on zinc porphyrins for sildenafil citrate determination, Electrochim. Acta 58 (2011) 290–295.

[35] J. Rodríguez, J.J. Berzas, G. Castañeda, N. Rodríguez, Determination of sildenafil citrate (viagra) and its metabolite (UK-103,320) by square-wave and adsorptive stripping square-wave voltammetry. Total determination in biological samples, Talanta 62 (2004) 427–432.



Graphical abstract

Highlights

- Clinical needs caused the new method development for SC determination.
- SWV method for SC standard, tablet and in human serum in 0.05 M $NaHCO_3$ is applied.
- In 0.1 M H_2SO_4 for SC standard and tablet catalytic effect of Au/Cys is observed.
- SWV is more sensitive than HPLC and can be used for very low concentrations of SC.
- Microscopic characterization of the morphology of the used electrode surfaces.