*Int. J. Electrochem. Sci.*, 17 **(2022)** Article Number: 220928, doi: 10.20964/2022.09.36

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

# Ticagrelor Determination via Its Electrooxidation as the Standard, in Tablets and the Spiked Human Serum at Au Solid Electrode

*Jelena Lović*[1,*], *Aleksandra Bogdanović*[2], *Dušan Mijin*[2], *Dragan Vuković*[3], *Slobodan Petrović*[2], *Milka Avramov Ivić*[1]

[1] University of Belgrade-Institute of Chemistry, Technology and Metallurgy, Department of electrochemistry, Njegoševa 12, Belgrade, Serbia
[2] Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade, Serbia
[3] Faculty of Medicine, University of Belgrade, Dr. Subotića 8, Belgrade, Serbia
[*]E-mail: jelena.lovic@ihtm.bg.ac.rs

*Received:* 23 May 2022 / *Accepted:* 8 July 2022 / *Published:* 7 August 2022

Ticagrelor hydrochloride (TCG) is effective in platelet inhibition and useful for preventing cardiovascular death, myocardial infarction, or ischaemic stroke. The drug standard was investigated by cyclic voltammetry (CV) and quantitatively determined using differential pulse voltammetry (DPV) via its electrooxidation at Au electrode in 0.05 M $NaHCO_3$. DPV showed a linear dependency of the anodic peak currents vs. TCG standard concentrations in the range from $3 \times 10^{-7}$ mol $l^{-1}$ to $10^{-5}$ mol $l^{-1}$ with the values of the limit of detection (LOD) and the limit of quantification: 0.288 µmol $l^{-1}$ and 0.96 µmol $l^{-1}$, respectively. Using the constructed and validated calibration curve, the values of unknown ticagrelor concentrations in Ticagrex® tablets and in human serum spiked with the standard were determined. Mechanistic study suggests that the oxidation proceeds at sulfur atom by removal of one electron as the rate determining step. In the next, fast step, formed radical cation loose another electron and sulfoxide is formed (with water molecule as nucleophile). The study of TCG degradation showed that at the Au, after 4.5 h of potential cycling, degradation occurs, which was confirmed by UV spectroscopy.

**Keywords:** ticagrelor hydrochloride, Ticagrex® tablet, human serum, reaction mechanism

## 1. INTRODUCTION

In the prevention and treatment of thrombosis antithrombotic drugs are widely used [1]. These drugs include anticoagulant, fibrinolytic and antiplatelet drugs. Among antiplatelet drugs besides aspirin and thienopyridines (clopidogrel and prasugrel), ticagrelor has gained much attention. It was approved for use in EU and USA more than 10 years ago. TCG reversibly interacts with the platelet

$P2Y_{12}$ ADP-receptor inducing inhibition of adenosine phosphate thus preventing platelet activation [2]. TCG belongs to the cyclopentyl-triazolopyrimidine class of $P2Y_{12}$ inhibitors [3]. Chemically it is (1S,2S,3R,5S)-3-[7-[(1R,2S)-2-(3,4-difluorophenyl)cyclopropylamino]-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol (Fig. 1).

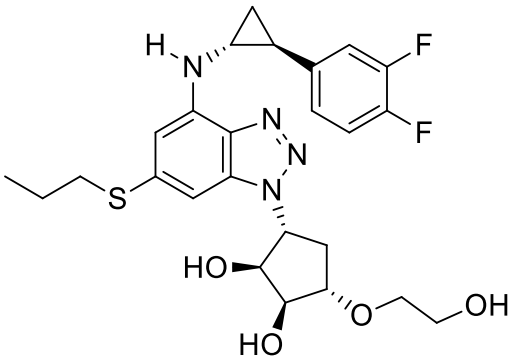


**Figure 1.** Structure of the ticagrelor

TCG can be used to prevent thrombotic events (for example, after a stroke or heart attack) in patients with acute coronary syndrome or ST-elevation myocardial infarction, alone or in combination with acetylsalicylic acid (if not contraindicated). It was concluded that administration of ticagrelor plus aspirin was superior to administration of aspirin alone in reducing the composite of stroke or death. It is taken twice a day orally and side effects of the drug can be shortness of breath, dizziness, headache, nausea, nosebleed [4-7].

Various analytical methods were developed and applied for qualitative and quantitative determination of TCG and its metabolites [8-10]. These methods include UV spectrophotometry, high-performance liquid chromatography (HPLC), reverse phase - high-performance liquid chromatography (RP-HPLC), liquid chromatography coupled with mass spectrometry (LC-MS), bio-analytical techniques. UV spectrophotometric methods were developed and applied for the estimation of ticagrelor in pharmaceutical dosage form, as well in bulk drug (active pharmaceutical ingredient) and formulations [11-13]. Methods for determination of TCG in coated tablets were validated and applied by RP-HPLC [9, 14,15]. Bio-analytical techniques and UV spectrophotometry provide the qualitative and quantitative analysis of ticagrelor with the impurities in the pharmaceutical tablets and in human plasma and urine [10]. LC method was optimized and validated for the determination of ticagrelor and its impurities [16] while LC-MS method was used for simultaneous determination of ticagrelor and its active metabolite in human plasma [17-19]. In addition, HPLC–MS/MS method was used and validated for the determination of ticagrelor and its active metabolite in human plasma [20]. LC-MS/MS methods [21-24] showed very low limits of quantitation ranged from 0.25 to 5.0 ng $ml^{-1}$ ($4.78 \times 10^{-10}$ to $9.57 \times 10^{-9}$ M) and because of that those methods are appropriate for pharmacokinetic studies. Since the plasma concentration range reported in the literature was in the range $2.10 \times 10^{-8}$ to

$1.02 \times 10^{-6}$ M, [25, 26], the reported HPLC methods [27-32] are not efficient enough for some pharmacokinetic studies.

The electrochemical methods as analytical techniques provide feasible, economic, simple and not time consuming highly sensitive analysis of pharmaceutical compounds. However, square-wave voltammetry method at MWCNTs/$TiO_2$NPs/CPE was reported for determination of TCG in human plasma. So, it gives rise to a linearity range of ($1.0 \times 10^{-6}$ to $1.6 \times 10^{-4}$ M) with very high LOQ of $1.0 \times 10^{-6}$ M with the insufficient sensitive limits (LOQ or LOD) for its pharmacokinetic studies [33]. El-Desoky et al. constructed electrochemical sensor using nano-sized manganese ferrite modified graphite powder which exhibited good electrochemical response in determination of TCG in real pharmaceutical formulations in biological sample such as human blood [34].

The aim of this work is to present the electrochemical behavior of TCG at the Au solid electrode combined with the newly developed electroanalytical DPV method for its quantitative determination as the standard, in tablets and the spiked human serum in 0.05 M $NaHCO_3$. The mechanism of TCG oxidation was evaluated and possibility of its electrodegradation at Au solid electrode was tested.

## 2. EXPERIMENTAL

### *2.1 Materials and methods*

Referent standard of ticagrelor hydrochloride and commercial drug consisting ticagrelor - Ticagrex®, were acquired by Hemofarm Stada A.D. (Vršac, Serbia). All other used chemicals were obtained commercially and were p.a. grade. The deionized water was obtained by a Millipore Waters Milli-Q purification unit and used in all experiments.

The electrolyte solution applied in experiments was bicarbonate buffer, 0.05 M $NaHCO_3$ (pH 8.4) and a set of phosphate buffer solutions in the range of pH values 5.8-8. Buffer solutions were prepared according to literature [35] and pH values of the solutions were checked using the pH meter Hanna instruments HI 22 ID.

Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV) measurements performed in the electroanalytical study of TCG were carried out using a Bio-Logic SP-200 potentiostat. The three electrode electrochemical cell with the Au (Gamry, surface area, 0.07) as the working electrode, a gold wire as the counter electrode and a Ag/AgCl / 3.5 M KCl as the reference one, were used. The manner of preparation of the Au electrode was that it was mechanically (on diamond paste) polished, chemically treated (in concentrated sulphuric acid) and washed with deionized water. All the potentials are given vs. Ag/AgCl / 3.5 M KCl.

The potential was scanned from -0.4 to 1.1 V in 0.05 M $NaHCO_3$ and the optimum DPV parameters were determined using pulses height = 25 mV, pulses width = 50 ms and step height = 5 mV and $v$ = 15 mV $s^{-1}$. The accumulation time was 60 s at $E$ = - 0.4 V giving the maximal peak current values. It was necessary to clean the surface of the gold electrode after each DPV scan.

*2.2 Preparation of TCG stock and working solutions*

A standard solution of $10^{-2}$ mol $l^{-1}$ TCG was prepared by dilution in methanol and stored at a low temperature (4 °C). Working solutions in the concentrations: $3 \times 10^{-7}$ mol $l^{-1}$ (0.157 μg $ml^{-1}$); $5 \times 10^{-7}$ mol $l^{-1}$ (0.261 μg $ml^{-1}$); $1 \times 10^{-6}$ mol $l^{-1}$ (0. 522 μg $ml^{-1}$); $3 \times 10^{-6}$ mol $l^{-1}$ (1. 57 μg $ml^{-1}$); $5 \times 10^{-6}$ mol $l^{-1}$ (2. 61 μg $ml^{-1}$); $7 \times 10^{-6}$ mol $l^{-1}$ (3. 66 μg $ml^{-1}$) and $1 \times 10^{-5}$ mol $l^{-1}$ (5. 22 mg $ml^{-1}$) were prepared daily, previous to performed experiments, by the addition of adequate volumes of the previously prepared stock solution to the electrolyte solution. Working solutions of appropriate concentrations were obtained by exact dissolution of adequate volume of the stock solution to the electrolyte solution.

*2.3 Preparation of TCG drug product solutions*

Ten tablets of the commercial drug (Ticagrex®) were weighed and the average mass per tablet was determined. The tablets were then ground to a fine powder. An accurate portion of the grounded powder equivalent to the weight of the two tablets (60 mg) was transferred to a 50 ml volume calibrated flask and dissolved in about 30 ml of methanol, sonicated for 20 min and then filled up to 50 ml with methanol, forming a concentration of $10^{-2}$ mol $l^{-1}$. The solution was then filtered through a 0.45 μm filter. The desired concentrations of TCG for the analysis were obtained by the accurate dilution of the appropriate volume of this solution to the electrolyte solution of 120 ml.

*2.4 Preparation of human blood serum samples*

Human blood was collected from ten healthy volunteers and the serum was clinically prepared in laboratories of the Faculty of Medicine, University of Belgrade and spiked with TCG following the procedure described in [36].

*2.5 UV-VIS samples analysis*

UV-vis analysis was performed on Shimadzu UV-1700 spectrophotometer. Samples were taken from electrochemical cell before and after 4.5 h of electrochemical oxidation and as such analyzed.

## 3. RESULTS AND DISCUSSION

*3.1.1 Electrochemical behavior of TCG at gold electrode*

The electrochemical behavior of TCG was defined at first, with the CV on the Au solid electrode in a 0.05 M $NaHCO_3$ as electrolyte, comparing to the voltammetric response of the Au electrode in a blank solution (dash line) as shown in Fig. 2. The apparent increase of the anodic currents (black line) in the whole region of the oxide formation is observed. The electrocatalytic effect

of gold electrode in the region of oxide formation could be associated to the oxidation of thiol-containing compounds followed by the formation of sulfoxide species as was proposed for the electrochemical oxidation of the sulphur-containing amino acids [37] and it will be discussed further in section 3.2. Consequently, the decreased Au oxide reduction currents are attributed to the reduction of the sulfoxide species formed in anodic direction during TCG electrooxidation at gold electrode surface covered with oxide. The same CV behavior of TCG is observed in a set of phosphate buffers used as electrolyte.

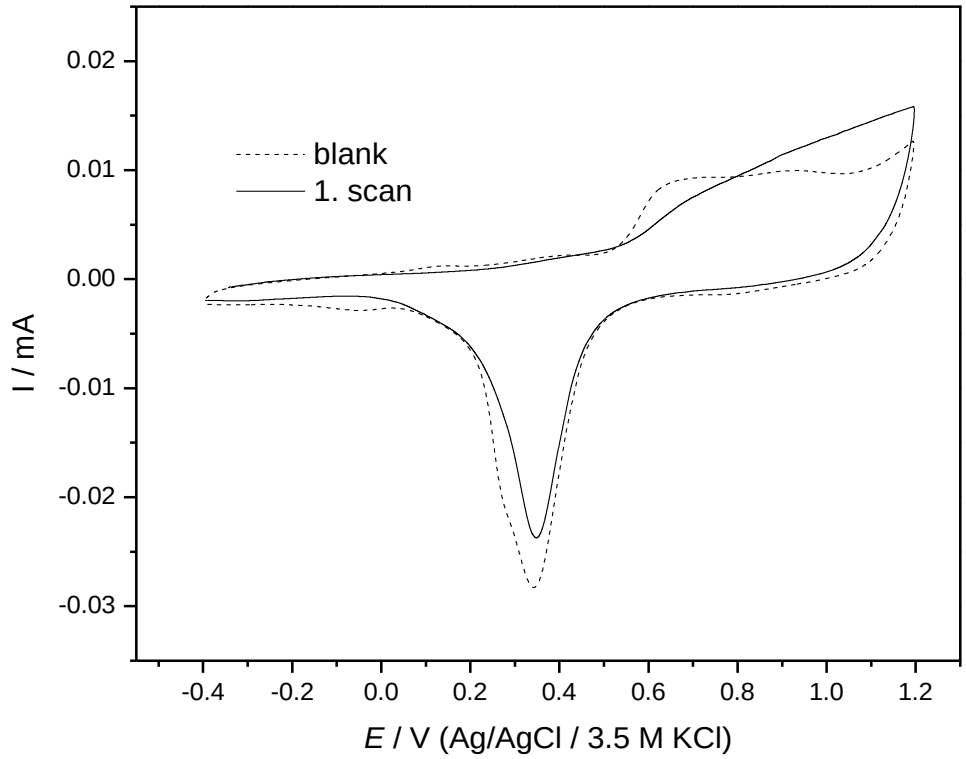


**Figure 2.** CV of the Au electrode (dot line) and in the solution of TCG standard $c = 1 \times 10^{-5}$ mol $l^{-1}$ (solid line) in 0.05 M $NaHCO_3$, $v = 50$ mV $s^{-1}$

*3.1.2. Quantitative TCG detection at gold electrode*

The official analytical procedures for analysis (impurities detection) of TCG standard and tablets for human use are proposed in European Pharmacopoeia 10.7. The most detailed overview of existing methods is given in [10] as the outline of chromatographic, bio-analytical, and spectroscopic methods developed and validated for its estimation with the obvious requirement for the new ones.

According to [21] after the administration of a single oral dose of either (30 or 100 mg), ticagrelor was rapidly absorbed to reach maximum plasma concentration ($c_{max}$) of 138 ng $ml^{-1}$ ($2.63 \times 10^{-7}$ M) and 510 ng $ml^{-1}$ ($9.74 \times 10^{-7}$ M), respectively. Minimum plasma concentration ($c_{min}$) of 15 ng $ml^{-1}$ ($2.86 \times 10^{-8}$ M) and 35 ng $ml^{-1}$ ($6.68 \times 10^{-8}$ M) were achieved with mean elimination half-life of

6.3 and 8.3 h and, respectively. Besides, $c_{max}$ and $c_{min}$ values of ticagrelor after an oral dose of 90 mg ticagrelor were 536.0 ng ml$^{-1}$ (1.02 × 10$^{-6}$ M) and 11 ng ml$^{-1}$ (2.10 × 10$^{-8}$ M), respectively.

It was challenging to test the analytical ability of the non-modified, solid Au electrode for the quantitative determination of the TCG and to compare the results with the electrode covered with nanocomposite [34].

DPV technique was successfully applied and optimized at first for the standard detection. The various concentrations of TCG standard are presented with the obtained their anodic current peaks in Fig. 3. Between two consecutive concentrations, the gold electrode was cleaned as described in the Experimental part. The addition of TCG leads to a well-resolved increase of the peak currents at about 0.55 V. The relevant calibration curve obtained under DPV conditions is presented in Fig. 4. A linear fit calibration shows a correlation of 0.998 in the concentration range from 3 × 10$^{-7}$ mol l$^{-1}$ to 10$^{-5}$ mol l$^{-1}$ TCG (with each data point generated from six experiments). The validation of the DPV method was carried out by the determination of the limit of detection (LOD), limit of quantitation (LOQ), and recovery. The LOD and LOQ were calculated from the calibration curves as k × SD/b where k = 3 for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve [38, 39]. The obtained values of LOD and LOQ were 0.288 µ mol l$^{-1}$ and 0.96 µmol l$^{-1}$, respectively. Lower detection limits were found in regard to the values obtained using MWCNTs/TiNPs/CPE [33] but higher compared to the LOD and LOQ determined with $Mn_{0.2}Fe_{2.8}O_4$ nanoparticles [34]. Recovery studies were also performed to establish the accuracy of the DPV method by performing six measurements at low, intermediate and high TCG concentrations.

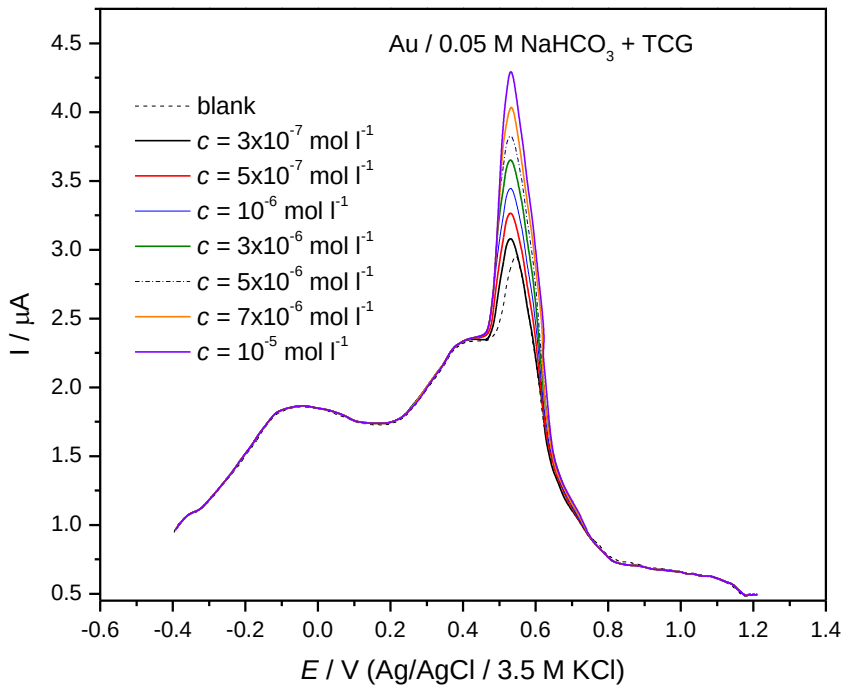


**Figure 3.** DPV of the Au electrode obtained in 0.05 M $NaHCO_3$ (dot line) and containing TCG standard in presented concentrations. The accumulation time was 60 s at $E$ = -0.4 V, pulses height = 25 mV, pulses width = 50 ms, step height = 5 mV and $v$ = 15 mV s$^{-1}$.

From Fig. 3 it is clear that with DPV method using solid Au electrode the levels of the typical plasma concentration range reported in literature are covered, except the minimal one: $2.1 \times 10^{-8}$ mol $l^{-1}$. This is an advantage of the electrode covered with nanocomposite [34]. The preparation of solid gold electrode for the TCG determination is fast and simple and this is important advantage for its use. The choice of the electrode surface depends of the clinical need and existing experimental possibilities and conditions with the two promising methods for the effective TCG electroanalysis.

The constructed calibration curve was used for the evaluation of an unknown concentration of TCG in commercial tablet Ticagrex® and in human serum spiked with TCG standard (Fig. 4). The all excipients as a content of pharmaceutical formulation Ticagrex® were tested under the experimental conditions presented in Figs. 2 and 3, and none exhibited electrochemical activity and consequently, did not affect the TCG electrooxidation. The determination of TCG by DPV in Ticagrex® tablets and in human serum spiked with standard showed that the mean recoveries were 100.12% and 98.84%, respectively, with relative standard deviations: 1.43% and 1.68%, respectively. The selected analytical method has an excellent linear regression coefficient in the investigated range, with values above 0.99 indicating that DPV can be used for the reliable determination of TCG.

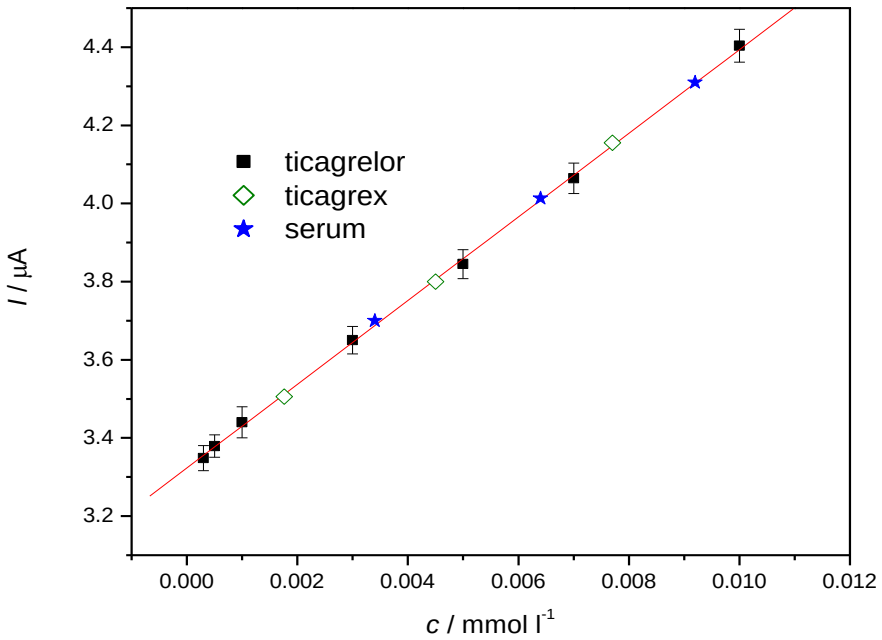


**Figure 4.** The linear dependency of anodic peak currents on concentrations of TCG standard, Ticagrex® tablets and human serum spiked with standard, marked with certain symbols. The data are obtained from Fig. 3.

*3.2 The mechanistic study of TCG electrooxidation in phosphate buffer solutions*

The evaluation of the kinetic parameters and mechanism of TCG electrooxidation was investigated in a phosphate buffer solution of pH from 5.8 to 8. The voltammetric profiles of TCG

were recorded at a different pH (Fig. 5) and it can be noticed that the peak potential was shifted to more negative values with increasing pH. The linear dependence of $E_p$ vs. pH was obtained following the equation: $E_p$ = 1.36 V - 0.062 V × pH, and the slope of 0.063 V (Inset in Fig. 5) suggests the same number of electrons and protons involved in the oxidation process [39].

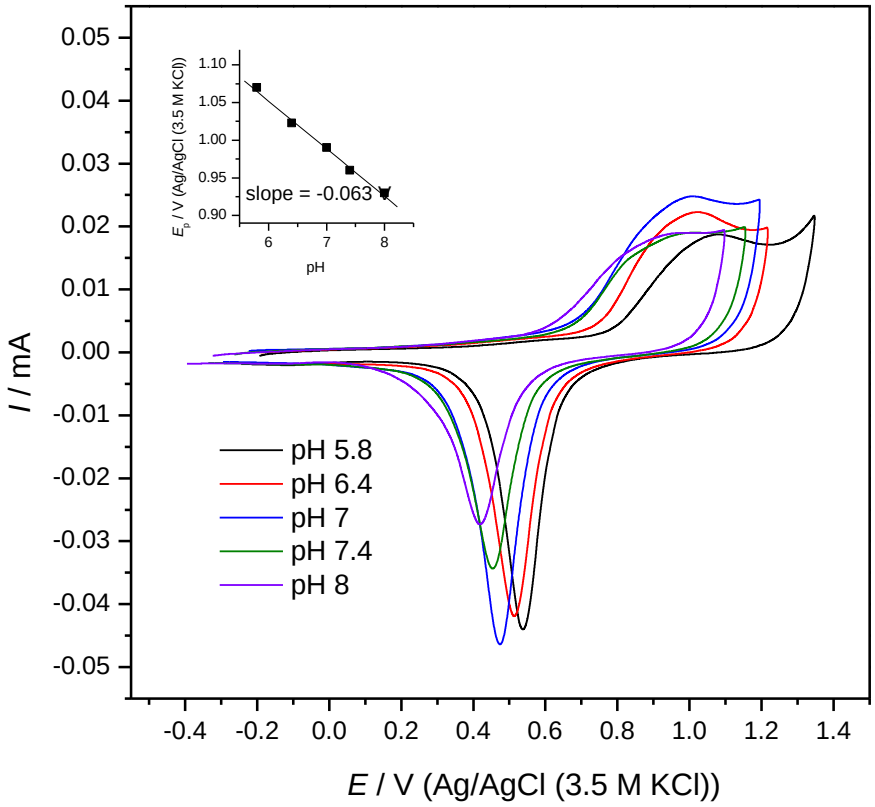


**Figure 5.** CVs of the Au electrode in the solution of TCG standard $c$ = 1 ×$10^{-5}$ mol $ml^{-1}$ in phosphate buffer solutions of presented pH, $v$ = 50 mV $s^{-1}$. Insert: the linear dependency of anodic peak potential on pH.

For a quasi-reversible system difference between peak potential and half- peak potential is equal to $|Ep - Ep_{1/2}| = 0.048/(\alpha n_a)$, where α is the charge transfer coefficient, and $n_a$ the number of the electrons in the rate determining step [40]. Considering that α = 0.5 the number of the transferred electrons is calculated. The obtained values for $n_a$ are in the range from 0.59 to 0.88, depending on pH, what leads to the conclusion that one electron is involved in the rate determining step of the investigated process.

The obtained result is in accordance with the mechanism proposed by El-Desoky et al. [34] in which the oxidation proceeds at sulfur atom by removal of one electron (slow step, rate determining). In the next, fast step, formed radical cation loose another electron and sulfoxide is formed (water molecule as nucleophile is involved).

*3.3 Testing of TCG electrochemical stability and possibility of its degradation at Au electrode*

It was interesting to examine the electrochemical stability of TCG and the possibility of its degradation by its electrooxidation at the Au electrode. In order to test it, under the experimental conditions presented in Fig. 2, the potential was cycled during 4.5 h and the results are presented in Fig 6. From Fig. 6 it is clear that after 4.5 h, the new anodic reaction occurs coinciding with the beginning of the oxide formation (much differs from the first scan), two small peaks appear coinciding with OH adsorption/desorption (not existing during the first scan) and the peak for the oxide reduction is increased (in the first scan it is decreased).

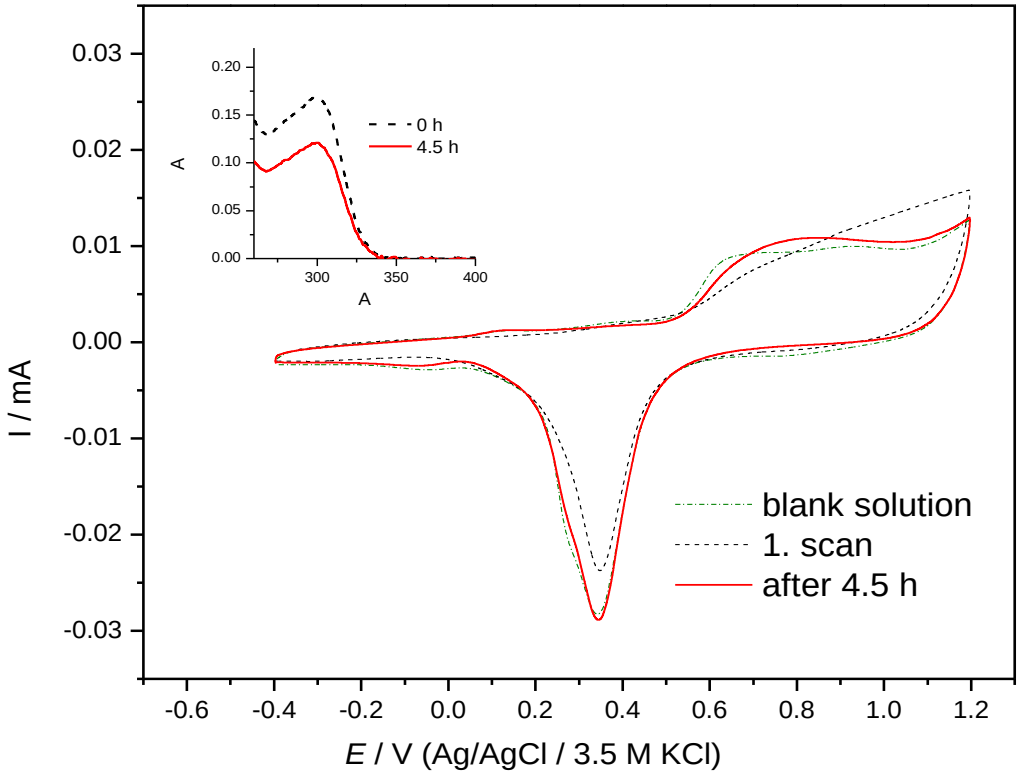


**Figure 6.** CVs of TCG $c = 5 \times 10^{-6}$ mol $l^{-1}$ on Au electrode using 0.05 M $NaHCO_3$, 1. scan (dot line) and after 4.5 h (solid line), scan rate: 50 $mVs^{-1}$. In inset: UV spectra of electrolyte before the electrochemical TCG oxidation (dot line) and after 4.5 h of the potential cycling (solid line).

All the observed changes in CV of TCG after 4.5 h of potential cycling indicate that under presented experimental conditions (Fig. 6) it is not stable and that its electrochemical degradation occurred. In order to check it, the UV experiments are performed. UV spectra of electrolyte before the electrochemical TCG oxidation and after 4.5 h of the potential cycling show significant decrease (26%) of initial TCG concentration indicating that TCG is degraded (Inset in Fig. 6).

## 4. CONCLUSION

The electroanalysis of TCG via its oxidation on the solid Au electrode was performed with DPV, enabling the drug determination in 0.05 M $NaHCO_3$ in a concentration range found in human serum after its administration ($3 \times 10^{-7}$ mol $l^{-1}$ to $1 \times 10^{-5}$ mol $l^{-1}$) with the LOD and the LOQ: 0.288 μmol $l^{-1}$ and 0.96 μmol $l^{-1}$, respectively. The applicability of the developed method was confirmed by the analysis of Ticagrex® tablets and human serum spiked with standard TCG. Apparent electrochemical activity of Au electrode in TCG electrooxidation was noted in the region of surface oxide formation suggesting the oxidation of thiol-containing compounds. Mechanistic study in a phosphate buffer solution of various pH suggests that one electron is involved in the rate determining step as proposed in the literature. Stability during electrochemical oxidation of TCG was tested by continuously potential cycling. It was noticed that after 4.5 h of potential cycling, 26 % of TCG degradation occurs as confirmed by UV spectroscopy.

ACKNOWLEDGEMENTS
This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia for the financial support (Contracts No. 451-03-68/2022-14/200026 and No. 451-03-68/2022-14/200135).

## References

1. I. Jaffer, J. I. Weitz, *Hematology*, Chapter 149 – Antithrombotic Drugs, (2018), Elsevier, Netherlands.
2. I. Meena, H. G. Sowmya, J. Gnana Babu, *Research and Reviews: Journal of Pharmaceutical Analysis*, 10 (2021) 1.
3. N. C. Sanderson, W. A. E. Parker, R. F. Storey, *Rev. Cardiovasc. Med*. 22 (2021) 373.
4. C. H. Lee, C. L. Cheng, Y. H. Kao Yang, T. H. Chao, J. Y. Chen, Y. H. Li, *Circ. J*., 82 (2018) 747.
5. R. Teng, Clin Pharmacokinet. 2015 Nov;54(11):1125-38. doi: 10.1007/s40262-015-0290-2. PMID: 26063049; PMCID: PMC4621714.
6. Dhillon S. Ticagrelor: a review of its use in adults with acute coronary syndromes. Am J Cardiovasc Drugs. 2015 Feb;15(1):51-68. doi: 10.1007/s40256-015-0108-5. PMID: 25672642.
7. P. Amarenco, H. Denison, S. R. Evans, A. Himmelmann, S. James, M. Knutsson, *Stroke*, 51 (2020) 3504.
8. S. C. Johnston, P. Amarenco, H. Denison, S. R. Evans, A. Himmelmann, S. James, *The New England Journal of Medicine*, 383 (2020) 207.
9. H. Kelemen, G. Hancu, L. A. Papp, *Biomed. Chromatogr*., 33 (2019) 4528.
10. G. Nikitha, A. Ajitha, *Int. J. Pharm. Sci. Res*., 12 (2021) 6260.
11. P. Ravisankar, M. Sireesha, P. S. Babu, C. P. Vyshnavi, K. D. Raju, *Int. J. Pharm. Sci. Rev. Res*. 62 (2020) 135.
12. P. P. Reddy, G. T. Rani, N. Pal, B. Navya, A. Prathyusha, *Indo American J of Pharmaceutical Sciences*, 5 (2018) 6874.
13. H. Narware, K. Malviya, B. Sirohi, L. K. Omray, *Asian J. Pharm. Edu. Res*. 4 (2018) 94.
14. K. M. Bhameshan, S. H. Rizwan, A. Sultana Mehnaaz, *International Journal of Innovative Pharmaceutical Science and Research*, 5 (2017) 43.
15. L. M. Bueno, J. Wittckind Manoel, C. F. Alves Giordani, A. S. Loureiro Mendez, N. M. Volpato

Elfrides, E. Scherman Schapoval, M. Steppe, C. V. Garcia, *Eur. J. Pharm. Sci*., 97 (2017) 22.
16. N. R. Wingert, J.B. Ellwanger, L. M. Bueno, C. Gobetti, C. V. Garcia, M. Steppe, E. E. S. Schapoval, *Eur. J. Pharm. Sci*. 118 (2018) 208.
17. N. Marsousi, S. Rudaz, J.A. Desmeules, Y. Daali, *Curr. Anal. Chem.*, 16 (2020) 602.
18. D. Danielak, P. Gorzycka, K. Kruszyna, M. Karazniewicz - Kada F, Glowka, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1105 (2019) 113.
19. S. U. Chae, K. Min, C.B. Lee, Z. Huang, M. J. Chang, S. K. Bae, *Translational and Clinical Pharmacology*, 27(2019) 98.
20. J. Lagoutte-Renosi, B. Royer, V. Rabani, S. Davani, *Molecules* 26 (2021) 278.
21. X. Xu, X. Ding, B. Yuan, W. Lim, Y. Wang, Y. Jin, and H. Xu, *Biomed. Chromatogr.*, 33 (2019) 4498.
22. H. Sillén, M. Cook, P. Davis, *J. Chromatogr. B*, 878, (2010) 2299.
23. H. Sillén, M. Cook, P. Davis, *J. Chromatogr. B*, 879, (2011) 2315.
24. P. Kaleab, Y. Agrawal, G. Soni, P. Patel, *World J. Pharm. Sci*., 3 (2015) 37.
25. R. Teng, K. Butler, *Eur J. Clin. Pharmacol*., 66 (2010) 487.
26. C. Gobetti, R. L. Pereira, A. S. L. Mendez, C. V. Garcia, *Curr. Pharm. Anal*., 10 (2014) 279
27. P. R. Kulkarni, G. K. Gajare, *Int. J. Res. Pharm. Chem*., 6 (2016) 733.
28. L. Kalyani, A. L. Rao, *Int. J. Pharm*., 3 (2013) 634.
29. B. S. Muddukrishna, *J. Global Pharma. Tech*., 8 (2016) 1.
30. M. A. Ambasana, N. P. Kapuriya, K. M. Mangtani, K. D. Ladva, *Int. J. Pharm. Sci. Res*., 7 (2016) 2009.
31. E. Joshy, A. Babu, D. D'cruz, and T. P. Aneesh, *Der Pharmacia Lettre*, 8 (2016) 206.
32. D. Delma, B. Anu, J. Eena, T. P. Aneesh, *Int. J. App. Pharm*., 951 (2017) 51.
33. M. Risk, M. A. Sultan, E. A. Taha, A. K. Attia, Y. M. Abdallah, *J. Electrochem. Soc*., 164 (2017) 12.
34. H. El-Desoky, M. Ghoneim, M. Gado, M. Abdel-Galeilz, *J. Electrochem. Soc*. 167 (2020) 067510.
35. H.P S Makkar , P Siddhuraju, K. Becker, Plant secondary metabolites, Methods Mol Biol. 2007;393:1-122. doi: 10.1007/978-1-59745-425-4_1.
36. K.M. Drljević-Djurić, V.D. Jović, U.Č. Lačnjevac, M.L. Avramov Ivić, S.D. Petrović, D.Ž. Mijin, S.B. Djordjević, Electrochim. Acta, 56, (2010), 47.
37. T. A. Enache and A. M. Oliveira-Brett, *Bioelectrochemistry*, 81 (2011) 46.
38. J.C. Miller, J.N. Miller, *Statistics for Analytical Chemistry*, 2nd ed. Wiley, (1984) New York, USA.
39. A. J. Bard and L. R. Faulkner, *Electrochemical methods: fundamentals and applications*, 2nd ed. John Wiley and Sons, Inc.,(2001), New York, USA.
40. C. M. A. Brett and A.M. Oliveira Brett, *Electrochemistry. Principles, Methods and Applications*, Oxford University Press, (1993), UK.