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Electrochemical stability of metformin in NaHCO₃ and Na₂SO₄ water solution at Au, GC and IrO_x electrodes

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Abstract: In this study the electrochemical behavior of metformin (MET), oral antihyperglycaemic agent, was assayed at three different electrodes. The drug standard was investigated by cyclic voltammetry and square wave voltammetry via its electrooxidation at Au and glassy carbon electrode in 0.05 M NaHCO₃. Under these conditions transformation of MET to corresponding *N*-carbonyl guanidine via oxime intermediate is suggested. The stability of MET was tested under directed stress conditions using IrO_x electrode with sodium sulphate as an electrolyte and cyclic 4-amino-2-imino-1-methyl-1,2-dihydro-1,3,5-triazine appeared as the main end-product. The courses of the electrochemical processes at three electrodes were followed by UV spectroscopy and evaluated by total organic carbon analysis.

Keywords: cyclic voltammetry; square wave voltammetry; Au electrode; GC electrode; IrO_x electrode; total organic carbon analysis.

INTRODUCTION

Metformin (MET, 1,1-dimethylbiguanide, Fig. 1), has been used in the treatment of type 2 diabetes for several decades.¹ Due to the basic nature of the guanidine part of the molecule, it is present as positively charged protonated form in human plasma,² while in the lower pH values, MET exists as double-charged molecule.³ Clinical investigations show that MET also exhibits anticancer effect due to its involvement in the metabolism of glucose.⁴ The consumption of this pharmaceutical is assumed to be more than 1 g per person and year in EU and as such represents one of the most prescribed drugs.⁵ In the human body, MET is

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not metabolized and is eliminated by renal excretion.² Thus, the occurrence of this drug in waste and surface waters is considerable and its behavior in an aqueous environment is a topic of the numerous publications.^{3,6,7}

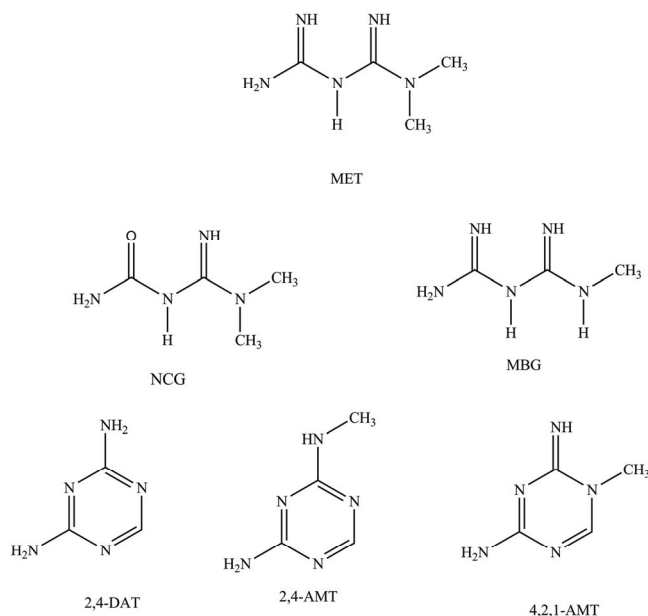


Fig. 1. Chemical structure of MET and end-products of electrochemical oxidation at different electrodes.

Electrochemical behavior and sensitive determination of MET has been intensively examined in pharmaceutical dosage forms and biological fluids using different electrodes.^{8,9} Electrochemical sensor involving metals are developed for the determination of MET due to their pronounced catalytic effect towards the oxidation of this drug.^{10–13} Some sensors provide improved sensitivity and selectivity for the electrochemical stripping assay of MET in real samples.^{12,13} Electrooxidation of MET at modified carbon electrode includes the transformation of imino group to *N*-carbonyl imino *via* corresponding oxime intermediate to obtain *N*-carbonyl guanidinium (NCG, Fig. 1).^{10,11,13} Also, the electrochemical behavior of MET at gold and glassy carbon electrodes are performed exhibiting better electrochemical response at GC electrode.¹⁴

The degradation of MET promoted by non-electrochemical methods such as photocatalytic degradation,^{15,16} hydroxyl free radical-induced oxidation by gamma radiolysis,¹⁷ or thermal stress degradations¹⁸ is presented in the literature. For the purpose of identification of by-products, a variety of separation techniques have been proposed. These include mass spectrometry detection and high-performance liquid chromatography.^{16,17,19}

Electrochemical oxidation of MET was also reported.⁷ The study was performed using a boron-doped-diamond (BDD) electrode and two-compartment cell at pH 3 and 7 in ammonium fluoride solution. Analysis showed that four products were formed (Fig. 1): 2,4-diamino-1,3,5-triazine (2,4-DAT), 2-amino-4-methylamino-1,3,5-triazine (2,4-AMT), 4-amino-2-imino-1-methyl-1,2-dihydro-1,3,5-triazine (4,2,1-AIMT) and methylbiguanide (MBG). At lower pH, the formation of 4,2,1-AIMT was more pronounced in comparison to 2,4-AMT (100 times). Higher pH favors the formation of 2,4-AMT,¹⁷ but 4,2,1-AIMT is still the main product at pH 7.⁷

In this work, the electrochemical behavior of MET at gold and glassy (GC) electrodes was investigated using cyclic voltammetry (CV) and square-wave voltammetry (SWV) in bicarbonate solution. Also, the stability of MET was tested under directed stress conditions using IrOx electrode with sodium sulphate as an electrolyte. The investigation of MET at three electrodes was coupled with total organic carbon and UV analyses. In the case of IrOx, the reaction residue was analyzed by Fourier transform infrared spectroscopy (FT-IR).

EXPERIMENTAL

Metformin, as reference standard, was kindly provided by Hemofarm Stada A.D. (Vršac, Serbia). All chemicals used were of p.a. grade. The deionized water was obtained by a Millipore Waters Milli-Q purification unit and used in experiments. CV and SWV measurements of MET electrooxidation during three hours were carried out on 402 Volta Lab (Radiometer Analytical, Lyon, France). The three electrode electrochemical cell with Au and GC (Pine, surface area 0.5 cm²) as working electrode, gold wire as counter electrode and saturated calomel electrode (SCE) as reference one, was used. All the potentials are given vs. SCE. As electrolyte served 0.05 M NaHCO₃ (pH 8.4). Au electrode was mechanically polished on diamond paste, washed in mixture (50 % sulphuric acid, 50 % water) and washed with deionized water.²⁰ GC electrode was prepared by mechanical treatment, which involves abrasion with emery paper, polishing with Al₂O₃ suspension (particle size 1, 0.3 and 0.05 μm), washed in mixture 50 % sulphuric acid/50 % water and washed with deionized water. Both electrodes were then electrochemically treated. For that purpose Au electrode was cycled in the potential window from 0.2 to 1.2 V during 30 min. GC electrode was activated by holding the potential at 1.2 V during 30 min. The operating parameters for SWV measurements were: step size 5 mV, pulse size 75 mV, frequency 10 Hz and scan rate 50 mV s⁻¹. Accumulation time was 0.2 s at $E = -0.45$ V giving the maximal peak current values. After each hour of potential cycling, SWV was recorded.

The electrochemical oxidation experiments of MET (4 mg) under stress conditions were also performed in a glass reactor (250 ml of solution) at room temperature using the IrOx electrode (De Nora, 5 cm²). Na₂SO₄ (0.5 g) and deionized water were used for electrolyte preparation. A PAR M273 potentiostat/galvanostat and the ampere meter (both obtained from Iskra). The cathode (10 cm²) was made from stainless steel (18Cr/8Ni). The gap between electrodes was 3 mm. The current was maintained at 250 mA. A Heidolph magnetic stirrer was used during these experiments. The UV analysis was performed on a UV-Vis Shimadzu 1700 spectrophotometer. TOC analysis was performed using ZellwegerLabTOC 2100. Residue sample after the direct electrochemical oxidation at IrOx was prepared for FT-IR spectroscopy

as follows: the reaction mixture after 3 h was extracted with dichloromethane and the solvent evaporated. FT-IR spectra of MET and degradation residue were recorded in absorbance mode using a Nicolet™ iS™ 10 FT-IR spectrometer (Thermo Fisher Scientific) spectrometer with Smart iTR™ attenuated total reflectance (ATR) sampling accessories. The ATR FT-IR spectra were recorded in the 400–4000 cm^{-1} range with 32 scans per spectrum.

RESULTS AND DISCUSSION

The CVs during 3 h of MET oxidation on gold electrode in 0.05 M NaHCO_3 alongside the voltammetric response of Au electrode in blank solution are presented in Fig. 2A. In the presence of MET, the initial CV was changed so that for the first two hours a slight decline of anodic currents in the area of the oxide formation was noticed. In the last hour of electrode cycling the anodic currents increased suggesting simultaneous oxidation of MET and the products of its oxidation. SWV (Fig. 2B) confirms that effect showing the increasing currents in the potential area of the first anodic peak that appears from -0.45 up to 0 V. At $E > 0$ V, initial SWV show higher currents comparing to that obtained after long term potential cycling.

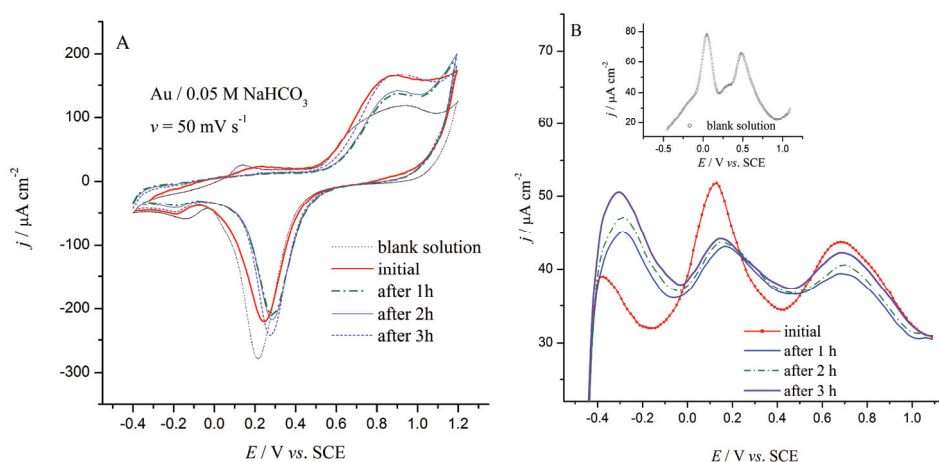


Fig. 2. Cyclic voltammograms (A) and square wave voltammograms (B) of Au electrode in 0.05 M NaHCO_3 and with 7 mg MET, after 1, 2 and 3 h of cycling, scan rate 50 mV s^{-1} .

Square wave parameters: step size 5 mV, pulse size 75 mV, frequency 10 Hz and scan rate 50 mV s^{-1} , accumulation time 200 ms at -0.45 V.

The electrochemical behavior of MET in the course of long term potential cycling was examined also on GC electrode as is presented in Fig. 3.

Based on CV measurements (Fig. 3A) it can be noticed that anodic reaction currents for $E > 0.8$ V slightly decreased during long term potential cycling. The appearance of small reversible oxidoreduction peaks at ~ 0.2 V was also observed with the increasing tendency after two and three hours.

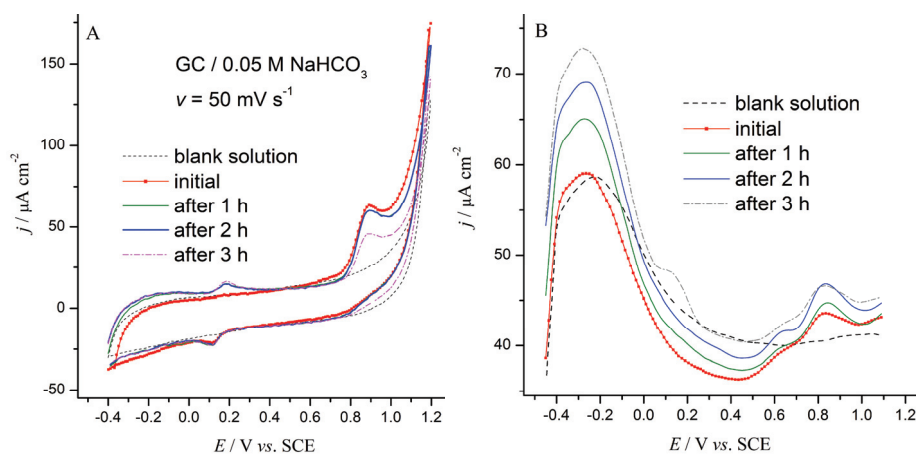


Fig. 3. Cyclic voltammograms (A) and square wave voltammograms (B) of GC electrode in 0.05 M NaHCO₃ and with 7 mg MET, after 1, 2 and 3 h of cycling, scan rate 50 mV s⁻¹. Square wave parameters: step size 5 mV, pulse size 75 mV, frequency 10 Hz and scan rate 50 mV s⁻¹, accumulation time 200 ms at -0.45 V.

Fig. 3 strongly suggests that continuous cycling after two and three hours enables the increasing currents of MET transformation products in a reversible reaction. Considering SWV measurements of MET oxidation on GC electrode (Fig. 3B), the increasing currents of the anodic peak at -0.2 V in continuously cycling was recorded. The development of smaller peaks at $E > 0$ V is present suggesting the oxidation of reaction intermediates that were formed after 2 and 3 h of cycling.

According to the similar behavior of MET on Au and GC electrodes to the previously reported electrooxidation on carbon paste electrodes,^{10,11,13} it could be assumed that electrochemical transformation on these two electrodes proceeds in two steps: oxidation of MET to the corresponding oxime and reversible reaction between the obtained oxime and NCG (Fig. 1).

The stability of MET was also tested under the electrochemical oxidation conditions using Na₂SO₄ as electrolyte and IrOx as electrode.²¹

The course of the electrochemical processes on all three electrodes was followed by UV spectroscopy at 232 nm. Changes in UV spectra are displayed in Fig. 4 for GC (Fig. 4A) and IrOx (Fig. 4B) electrodes. For Au electrode, the same trend is observed as in the case of Au electrode and thus is not presented. For process at GC electrode, no clear dependence of absorbance after 1, 3 and 3 h is observed highly suggesting the reversible process. The increase of the absorbance after 3 h with respect to MET could be explained by the higher extinction coefficient of the transformation product. From the UV spectra presented in Fig. 4B, it can be observed that the absorbance gradually decreases during 3 h due to electrooxidative degradation of MET at IrOx electrode.

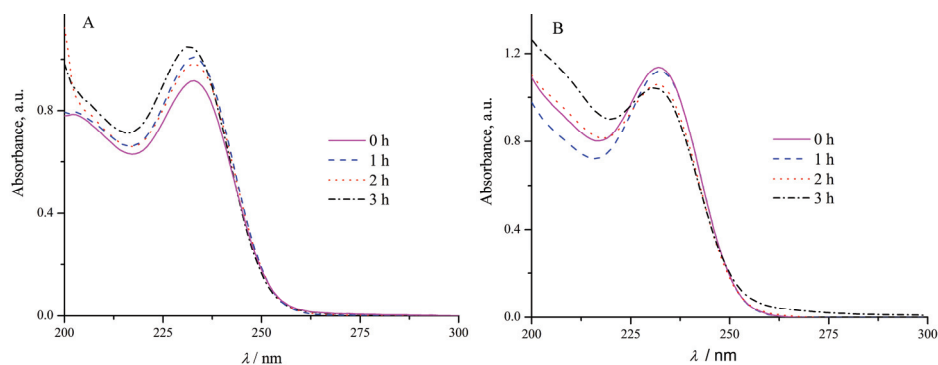


Fig. 4. UV spectra of MET and the reaction solution after 1, 2 and 3 h at different electrodes: A) at GC; B) at IrOx.

TOC analysis was performed in order to evaluate the degree of MET mineralization during electrochemical oxidation. The samples were taken at 0 h and after 3 h of electrochemical treatment. Negligible change in TOC was recorded for Au (less than 1 %) after 3 of CV cycling. CV cycling using GC electrode gave small TOC reduction (2 %). The highest TOC reduction was recorded when the direct electrochemical oxidation was applied (10.6 %). The results of TOC reduction of MET for Au and GC electrodes are in accordance with the proposed mechanism of transformation for these electrodes whereas no carbonyl atoms are lost during the electrochemical process. Since IrOx electrode was used in the direct electrochemical oxidation,²² this could be expected, since this electrode generates hydroxyl radicals. The formed radicals attack MET molecules which lead to mineralization. Thus, in order to determine the degradation products after the direct electrochemical oxidation at IrOx, ATR FT-IR spectra of MET and the residue were recorded (Fig. 5).

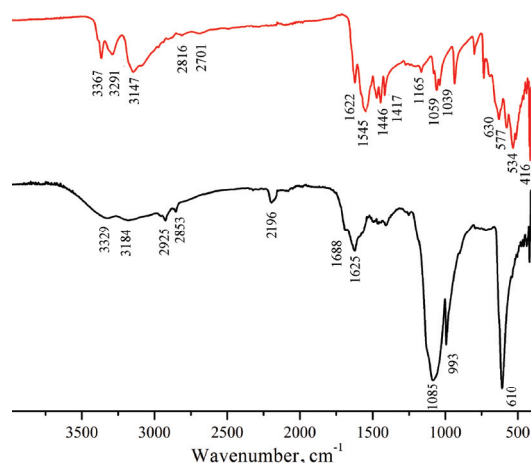


Fig. 5. ATR FT-IR spectra of MET (up) and degradation residue (down) after 3h at IrOx electrode.

FT-IR spectrum of MET exhibits some characteristic vibrations: N–H stretching vibrations at 3367, 3291 and 3147 cm^{-1} and N–H deformation in the range 1622–1417 cm^{-1} . The weak bands at 2816 and 2701 cm^{-1} are ascribed to C–H stretching modes of $-\text{CH}_3$ group attached to N atom. Peaks originating from C–N stretching vibrations are observed in the range 1165, 1059 and 1039 cm^{-1} . Out of plane vibrations of C–H bond is observed at 630 cm^{-1} , while peaks from C–N–C deformations are positioned at 577, 534 and 416 cm^{-1} .²³ If the residue mainly consists of demethylation product, MBG, than the characteristic vibrations should not vary in great extent. However, the FTIR spectrum of the degradation residue shows significant difference. Two dominant bands at 1085 and 610 cm^{-1} appear from Na_2SO_4 since it was used as an electrolyte. Observed peaks at 3329, 3184 and 1625 cm^{-1} are ascribed to N–H vibrations, while bands at 2925 and 2853 cm^{-1} originate from C–H stretching vibrations of $-\text{CH}_3$ group. The strong band at 993 cm^{-1} could be ascribed to C–N stretching vibrations which is shifted to lower wavelength than in FTIR spectrum of the MET indicating significant structural rearrangement. Also, in the spectrum of the residue, no C–N–C deformations are observed strongly indicating the formation of the rigid cyclic ring. A new peak at 1688 cm^{-1} appeared and could be ascribed to C=N–H stretching vibrations of the possible cyclic degradation product 4,2,1-AMT. The obvious peak at 2196 cm^{-1} is ascribed to N=C–N(sp^2) vibrations, additionally supporting the formation of the cyclic 4,2,1-AMT.

CONCLUSIONS

CV and SWV measurements of MET at Au and GC electrodes in 0.05 M NaHCO_3 showed that the anodic currents increased after a long term potential cycling. Under these conditions transformation of MET to corresponding *N*-carbonyl guanidine *via* oxime intermediate is suggested. The course of the electrochemical processes on Au and GC electrodes was followed by UV spectroscopy. The increase of the absorbance after 3 h with respect to MET could be explained by the higher extinction coefficient of the transformation product. The stability of MET was also tested under the direct electrochemical oxidation conditions using Na_2SO_4 as electrolyte and IrOx as an electrode. The reaction was also followed by UV spectroscopy. Here the absorbance decreased after 3 h due to electrooxidative degradation of MET. Negligible change in TOC was recorded for Au (less than 1 %) and small for GC (2 %) after 3 h of CV cycling. The highest TOC reduction was recorded using the IrOx electrode (10.6 %). The obtained results are in accordance with the proposed mechanisms. ATR FT-IR spectra of MET and the residue showed the possible formation of cyclic degradation product 4,2,1-AMT.

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ИЗВОД

ЕЛЕКТРОХЕМИЈСКА СТАБИЛНОСТ МЕТФОРМИНА У ВОДЕНИМ РАСТВОРИМА
 NaHCO_3 И Na_2SO_4 НА АУ, GС И IrO_x ЕЛЕКТРОДАМА

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У оквиру рада изучавано је електрохемијско понашање метформина, лека са антихипергликемијским дејством, на три различите електроде. Стандард лека испитан је циклчном волтаметријом и волтаметријом са правоугаоним импулсима у 0,05 М раствору NaHCO_3 на електроди од злата и електроди од стакластог угљеника. Под овим условима, предложена је трансформација лека до одговарајућег *N*-карбонил гуанидина преко оксима као интермеђијера. Стабилност метформина тестирана је и под условима електрохемијске оксидације на IrO_x електроди у присуству натријум-сулфата као електролита. У овом случају, предложено је настајање циклчног 4-амино-2-имино-1-метил-1,2-дихидро-1,3,5-триазина као главног производа. Ток електрохемијских процеса на све три електроде праћен је UV спектроскопијом. Степен минерализације утврђен је анализом укупног органског угљеника.

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