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Ag/Au Modified Nafion Coated Glassy Carbon Electrode for the Detection of Metronidazole

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In pharmaceutical analysis, especially in Jordan, almost all of the attention is paid to the chromatographic and photometric methods of analysis. The utilization of electrochemistry as a tool for determination of the active pharmaceutical ingredients is not common. In this work, metronidazole (MNZ) was detected electrochemically in its aqueous buffered solution utilizing differential pulse voltammetry, which is one of the key electrochemical tools used for chemical analysis. For the detection purposes, nafion coated glassy carbon electrode was modified with gold, then silver by means of electrochemical deposition. The modified electrode has demonstrated significant performance toward the detection of metronidazole. Statistical evaluation of the reported performance shows that the detection is valid in the $1.00x10^{-4}$-$1.00x10^{-3}$ M range with limit of detection equals to $5.87x10^{-8}$ M and limit of quantitation equals to $1.96x10^{-7}$ M. The detection of metronidazole in solid pharmaceuticals provided locally was also investigated and recovery values in the 91-97% range were obtained. The modified electrode was also employed for the monitoring of metronidazole electrolytic degradation, usually detected by means of total organic carbon analysis or photometric instrumental methods. The utilized electrochemical monitoring have demonstrated efficiency that is comparable to the standard photometric method when the degradation was performed with platinum anode and boron-doped diamond cathode.

Keywords: Metronidazole, Modification, Differential Pulse Voltammetry, Scanning Electron Microscopy, Electrolytic Degradation

1. INTRODUCTION

Metronidazole (MNZ) or 2-(2-methyl-5-nitroimidazol-1-yl) ethanol is a slightly yellow fine crystalline solid that has molar mass of 171.16 $gmol^{-1}$. Structure of metronidazole is shown in Fig. 1. Metronidazole is commonly used as an anti-microbial agent against anaerobic bacterial infections in human and animals. [1,2] In human serum, metronidazole has an optimum efficiency when it is in the 2-8 µg/ml range. Therefore, detection of metronidazole concentrations in human and livestock serum is of critical value since most of the metronidazole metabolites are nitro anion radicals that interact with cellular DNA and as a consequence damage it. Therefore, these radicals are described as both carcinogenic and mutagenic.[3,4]

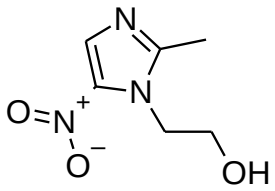


Figure 1. Chemical structure of metronidazole ($C_6H_9N_3O_3$)

Metronidazole solubility in water is about 10 mg/ml at 20°C. Therefore, it could be easily analyzed in aqueous solutions. For the analysis of MNZ in buffered solutions, it is worthy to mention that it has an acid dissociation constant equals to 2.6; therefore, structure of the anti-microbial could be adjusted by utilization of a buffer solution that maintains the desired pH value.

From a careful look at Fig. 1, one can easily understand that MNZ could be analyzed by the key instrumental methods usually used in pharmaceutical analysis; chromatography, spectroscopy, and electrochemistry. [5-8]The presence of different functional groups within the structure of MNZ, with different physical and chemical properties, affects its affinity to a wide range of stationary and mobile phases. Reverse phase chromatography has been widely used for MNZ analysis, either as individual active pharmaceutical ingredient (API) or as a component in mixtures of two or more APIs. [9,10]In addition, the conjugated system (alternating single and double bonds) in MNZ makes it an ultra violet light absorbing species. As a consequence, MNZ could be quantified simply by determining its absorbance, which is correlated to its concentration, via the calibration curve established based on Beer's law. The spectroscopic detection of MNZ could also be performed in the visible region since MNZ forms colored ion-pair complexes with electron acceptor compounds. In the visible photometric studies, MNZ is added to an electron acceptor species as limiting reactant to produce charge-transfer complex which color intensity depends on concentration of the added MNZ.[11-13]

Electrochemically, metronidazole has a reducible center, the nitro group, which is reduced to the corresponding hydroxyl amine group according to the equation shown in Fig. 2. The charge consumed in the reduction reaction is correlated to concentration of the reduced MNZ. As a result, assay of MNZ in its pharmaceutical forms and biological fluids could be performed.[14]

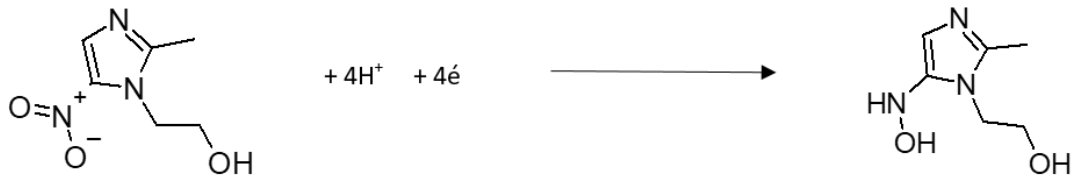


Figure 2. Electrochemical reduction of metronidazole

Two key electroanalytical methods are widely used for the quantification of pharmaceuticals; potential sweep voltammetry such as cyclic (CV) and linear scan voltammetry (LSV), and potential step methods such as differential pulse voltammetry (DPV) and differential pulse stripping voltammetry (DPSV). [15,16]The main advantage of the potential step methods is discrimination of the charging current that inherently flows beside the faradaic current produced from the analyte electrolysis (metronidazole reduction in this work). In all of the mentioned voltammetric methods, the electrolysis takes place at the working electrode that is an immersed in the analyte solution.

For metronidazole analysis, two kinds of working electrodes have been mainly used, carbon, and mercury. To enhance the detection selectivity and sensitivity, usually, working electrodes are modified with materials which have a high binding affinity toward the analyte and/or increase the charge transfer rate across the solution-electrode interface in the presence of the anayte. Different forms of carbon based electrodes have been employed for MNZ detection.[17-25] Glassy carbon, carbon fiber, single wall nanotubes, carbon paste, and screen printed electrodes have all been used for the detection of metronidazole. Mercury based electrodes used for the MNZ analysis could be divided into two groups, pure metallic mercury electrode, and amalgams. In the first class, mercury, as a working electrode is used as static hanging drop and the technique used for the analysis, is known as polarography. In the second category, mercury is combined with other metals to form an amalgam that has enhanced mechanical stability over the pure mercury electrode.[26-28]

This work has been performed with two objectives in mind. The first is the fabrication of an electrode qualified for the voltammetric detection of metronidazole. The second objective is the utilization of the prepared electrode for monitoring of MNZ electrolytic degradation as a function of time, in an approach similar to that utilized for kinetics studies of organics decomposition using high performance liquid chromatography (HPLC), total organic carbon (TOC) analysis, or Photometry.[29-31] All of the mentioned methods are either expensive, time-consuming, and need experienced operators, such as TOC and HPLC, or have high limits of detection (in the 1-10 µM range) such as photometry.

Electrochemical methods are simple, inexpensive, and have low detection limits, usually in the 10-1000 nM range. Therefore, in this study, glassy carbon electrode was coated with nafion (conductive and water-insoluble polymer) and modified with gold, then silver. The modification was performed electrochemically (i.e.) electrochemical deposition. The modified electrode has been imaged by scanning electron microscopy to confirm deposition of the precious metals, and then it was used for metronidazole detection. Performance of the analytical method was investigated by evaluating the corresponding statistical parameters such as linearity, limits of detection and quantitation, and recovery of local metronidazole commercial products. For comparison purposes, the decomposition of metronidazole was

also detected using UV spectrometry. Details of the analysis will be demonstrated within the context of the following sections.

2. EXPERIMENTAL

2.1 Chemicals and Solutions

Metronidazole, potassium nitrate, gold chloride, nafion (perfluoro sulfonic acid), silver nitrate, and boric acid were provided by Sigma-Aldrich Chemie GmbH, Darmstadt, Germany. Sodium hydroxide, potassium chloride, and phosphoric acid were all provided by VWR chemicals, NY, U.S.A. Glacial acetic acid was purchased from S D Fine-Chem Ltd., Mumbai, India. The commercial metronidazole products (Nidazole, Ramazol, and Dentagyl) were purchased from the local market.

All of the measurements were carried out using Britton-Robinson pH = 7.00 buffer solution. To prepare the buffer solution, the appropriate amounts of phosphoric, boric, and acetic acids were dissolved in ultrapure water (Ultra Max 372, Yong Lin Instrument Co., Ltd, Anyang, Korea). pH of the buffer was adjusted by addition of the required amount of 0.100 M NaOH. 1.00 mM of each of $AuCl_3$ and KCl, and 1.00 mM of each of $AgNO_3$ and KNO_3 solutions were prepared using the pH 7.00 BR buffer. These solutions were then used for the electrodeposition of gold and silver. The pH value was determined by SevenGo Duo pH meter supplied by Mettler-Toledo, AG, Analytical, Schwerzenbach, Switzerland.

2.2 Instruments and Measurements

All of the voltammetric experiments were performed in three compartment glass cell using Ag/AgCl and platinum as the reference and the counter electrodes, respectively. The glassy carbon working electrode (3.0 mm i.d.) and the rotator used were provided by Pine Research Instrumentation, NC, U.S.A. PGSTAT101 Autolab (Metrohm, Utrecht, The Netherlands) operated by NOVA 2.2 and connected to personal computer was used to perform the electrochemical experiments. For scanning electron microscopic imaging, VEGA3 microscope (Tescan, Brno, Czech Republic) was used. For the photometric measurements, Lambda 35 double beam spectrometer provided by Perkin-Elmer Instruments, MA, U.S.A. was employed.

The electrolytic degradation of MNZ was performed using 100x50x2 mm boron doped diamond electrode (Neocoat, La Chaux-de-Fonds, Switzerland) as the cathode, and 25x35 mm platinum electrode (ALS Co.,Ltd, Tokyo, Japan) as the anode. The degradation was performed in 250 ml teflon cylindrical home-made cell with a cover that has slots for the cathode and the anode. Tektronix PWS4305 DC power supply (Tektronix, Inc., OR, U.S.A.) was used for MNZ degradation. The applied voltage was 10.0 V. After every 10.0 minutes, the electrolysis was stopped, and portions of the electrolyzed solution were pipetted and used for the voltammetric and the photometric measurements. The electrolytic degradation (application of the 10.0 V between the anode and the cathode) was then resumed, and the degradation process was performed over 1.0 hour time period.

2.3 Electrode Fabrication

The glassy carbon electrode was polished, then rinsed with distilled water, and dried. After that, 3.0 µL of nafion were applied on the electrode surface; then the coated electrode was left to dry for 1 hour. The nafion coated electrode was then immersed in three compartment cell containing 1.00 mM of each of $AuCl_3$ and KCl dissolved in Britton-Robinson buffer solution (pH = 7.00). The electrode was modified with gold by holding its potential at -1.00 V for 3.00 minutes. After that, the electrode was rinsed with distilled water. Modification of the electrode with silver was performed by immersing the gold modified working electrode in the Britton-Robinson buffer solution that contains 1.00 mM $AgNO_3$ and 1.00 mM KNO_3, followed by holding its potential at -1.00 V for 3.00 minutes. The electrode was then rinsed with distilled water and oven-dried at 80°C for 15 minutes. The prepared electrode will be referred to in the following sections as Ag/Au-Nafion coated GCE. After the described modification, the modified electrode was imaged and used for the MNZ voltammetric detection.

2.4 Analytical Procedure

For the preparation of standard solutions of metronidazole, 5.00 mM metronidazole in pH 7.00 Britton-Robinson buffer was prepared and used as the stock solution. Standard solutions were then prepared from the stock by serial dilution. The prepared series was employed for establishing the calibration curve that correlates the reduction peak current to the metronidazole concentration.

The voltammetric measurements were performed using the differential pulse mode with the following parameters;

Potential Step	-0.005 V
Modulation amplitude	0.025 V
Modulation time	0.05 s
Interval time	0.5 s
Scan rate	0.01007 V/s
Rotation speed of the working electrode	50 rpm

Unless otherwise stated, all of the voltammetric measurements were performed in the hydrodynamic mode with rotation speed equals to 50 rpm. To establish the calibration curve, potential of the modified electrode was scanned between 0.00 and -1.00 V versus Ag/AgCl in each of the prepared standard solutions. The reduction peak current obtained from each measurement was plotted versus the corresponding solution concentration. To calculate the area under each DPV curve used for establishing the calibration curve, the charge, a tangent line was constructed between two maximum values before and after each peak. The area between this tangent line and the curve was integrated for each MNZ concentration and its replicate measurements. The area was integrated by using the trapezoidal rule in Matlab® software.

2.5 Samples Preparation

To obtain % recovery of metronidazole in the commercial drugs using the Ag/Au-Nafion coated GCE, one tablet of each of the three provided drugs was weighed and powdered with mortar and pestle. The powder was then accurately transferred to 100. ml volumetric flask and diluted to the mark with the prepared buffer solution. The solution was then sonicated for 10 minutes, filtered, and fraction of the filtrate was pipetted and used for the recovery evaluation.

% recovery was then estimated based on the following equation;

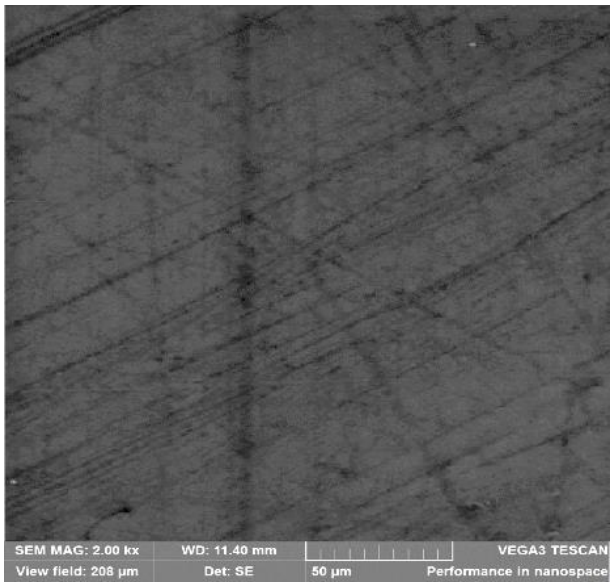
% recovery = ($[(S_{x+s}) - S_x]/S_s$) x 100%

Where S_x and S_s are the reduction peak current values for the sample and the standard MNZ solutions, respectively, and S_{x+s} is the reduction peak current for mixtures of equal volumes of the standard and the sample solutions.

3. RESULTS AND DISCUSSION

3.1 Modification of the bare electrode

Coating of the bare glassy carbon with nafion, followed by gold, then silver electrodeposition was followed step by step with the assistance of scanning electron microscopy (SEM). Fig. 3 presents SEM images of the glassy carbon surface before (Fig.3-A) and after the addition of nafion (Fig.3-B). Deposited gold and silver-gold particles are shown in Fig.3-C and Fig.3-D, respectively.



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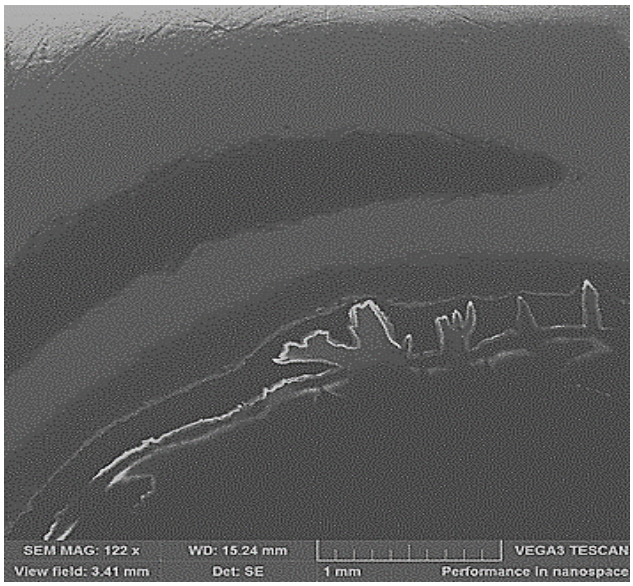

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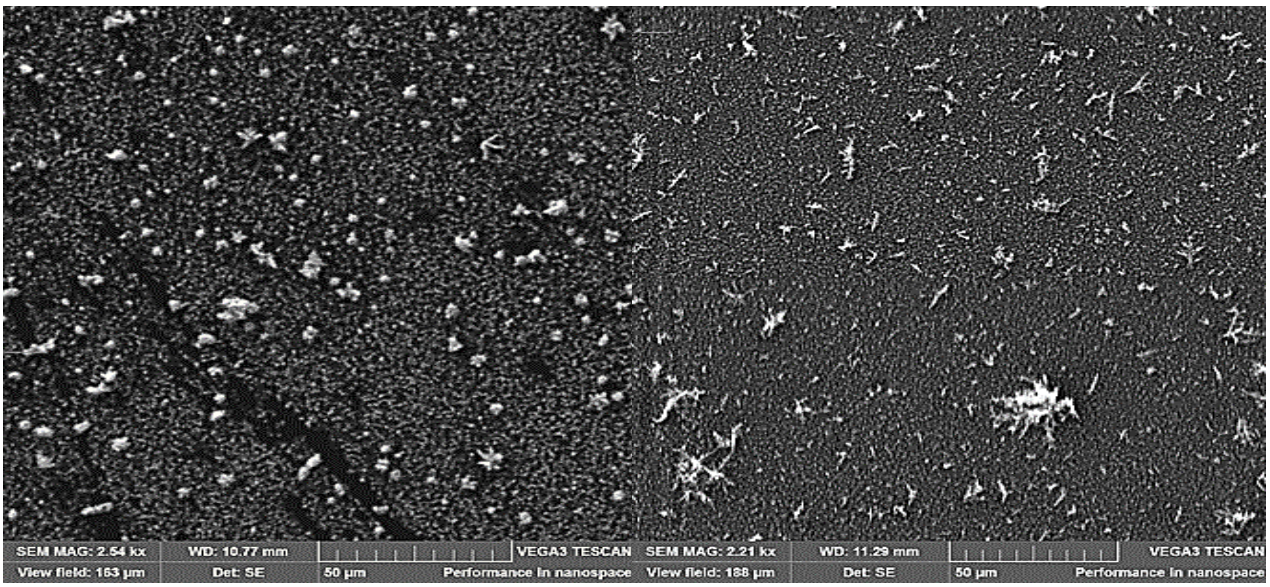

Figure 3. SEM images of bare glassy carbon electrode (A) nafion coated glassy carbon electrode (B) gold modified nafion coated electrode (C) silver/gold modified nafion coated glassy carbon electrode (D)

Figure 3-A shows 208µm x 208µm area of the bare glassy carbon electrode surface. The scratches in the carbon surface are originated from the continuous polishing of the electrode surface among the voltammetric measurements. The nafion layer over the carbon surface is shown in Fig. 3-B. The figure shows the teflon sleeve that surrounds the carbon electrode, in addition to the nafion, and the uncoated area of the carbon surface. As shown, the nafion covers almost all of the electrode surface and the revealed portion does not exceed 5% of the electrode surface area.

Deposition of gold on the nafion layer is presented in Fig. 3-C. As shown in the figure, the nafion layer is fully covered with the gold deposited particles and extension of the deposition was high enough to allow the growth of gold grains of different sizes and features, scattered over the electrodeposited gold layer. Deposition of the silver aggregates is presented in Fig.3-D. As shown in the figure, the gold grains have disappeared entirely after the silver electrodeposition, which could be attributed to deposition the silver on top of the gold grains.

3.2 Performance of the modified electrode

Britton-Robinson buffer was selected as the working buffer solution since it covers a wide range of pH values. pH 7.00 was selected because it is high enough to keep the analyte in its neutral, rather than acidic form. As a consequence, the reduction process becomes more feasible since as shown in Fig. 2, metronidazole must exist in the form of neutral species to be reduced efficiently. Performance of the modified electrode toward MNZ reduction is shown in Fig. 4. Reduction of MNZ at the modified surface occurs at a lower (less negative) potential with an enhancement in the reduction current. As shown in Fig. 4, reduction of MNZ at the modified surface occurs at -400 mV vs. Ag/AgCl. At the bare surface, the reduction peak appears at -700 mV.

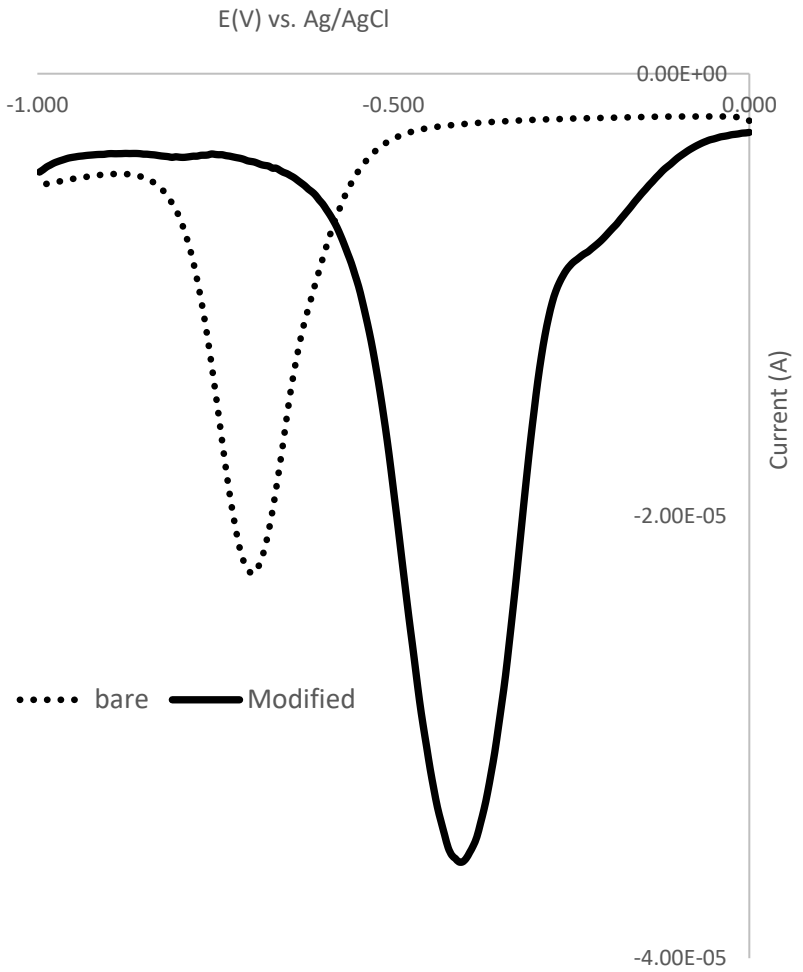

Figure 4. Differential pulse voltammograms of the bare and the modified glassy carbon electrodes in $2.50x10^{-3}$ M Metronidazole in pH 7.00 BR buffer solution.

The reported activity could be attributed to elevation of the electron transfer rate across the electrolyte-electrode interface because of the presence of two conductive metals (gold and silver) deposited on the working electrode surface which increases the electrode conductivity and hence, sensitivity.

In order to investigate nature of the metronidazole reduction process, as either diffusion or surface adsorbed controlled, the dependence of the metronidazole reduction peak current was plotted as a function of square root of the scan rate. As shown in Fig. 5, a correlation coefficient of 0.994 was reported, which confirms that the reduction process is diffusion controlled. MNZ diffuses from the electrolyte bulk to the electrode surface and is reduced at the modified surface.[32] Correlation of the reduction current to scan rate was also performed in this work (results not shown) and correlation coefficient with $R^2 = 0.937$ was obtained, which confirms that the electrolysis is diffusion, rather than surface adsorbed controlled process.

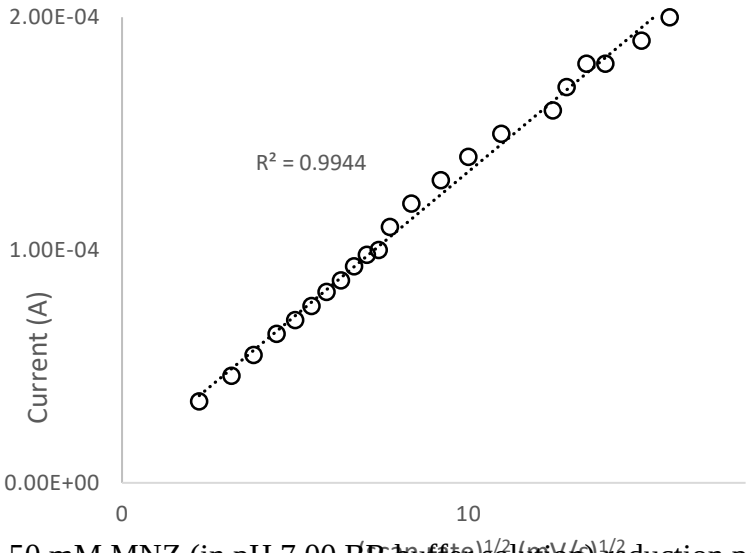

Figure 5. Dependence of 0.50 mM MNZ (in pH 7.00 BR buffer solution) reduction peak current, at the modified electrode, on square root of the scan rate. The dependence was investigated by linear scan voltammetry

The reduction current values shown in Fig.5 are relatively higher than those shown in the other figures since dependence of the reduction peak current on the square root of the scan rate was investigated by linear scan voltammetry. In linear scan voltammetry, the detected currents have two components, the faradaic component originates from the electrochemical reduction process, and the charging current that inherently exists in voltammetric sweep experiments such as cyclic and linear scan voltammetry. As a consequence, each of the current values presented in Fig. 5 is the combination of the faradic and the charging components. Rest of the experiments in this work were performed using differential pulse voltammetric signals. One of the inherent characteristics of the charging current, when compared to the faradaic current, is its fast decay. In differential pulse voltammetric measurements, a series of pulses is applied on the working electrode, and the current is sampled later in the pulse lifetime when almost all of charging current has decayed and the detected current is faradaic. As a consequence, all of the measured current values are mainly faradaic in nature and could be attributed to the investigated reduction process.

Dependence of the reduction current on metronidazole concentration is presented in Fig. 6. The figure demonstrates how the reduction peak current is directly proportional to the metronidazole concentration. The correlation between the reduction peak current and the concentration, obtained from Fig. 6, is shown in Fig. 7. As shown in Fig. 7, a straight line with a correlation coefficient of 0.997 was obtained (*N=5*), which confirms the linear adherence of the obtained current values to the corresponding metronidazole concentrations. Besides the correlation coefficient, the dynamic range, the limits of detection and quantitation were also evaluated. Detection of metronidazole at the modified electrode has been found linear in the $1.00x10^{-4}$-$1.00x10^{-3}$ M range with limit of detection equals to $5.87x10^{-8}$ M and limit of quantitation equals to $1.96x10^{-7}$ M.

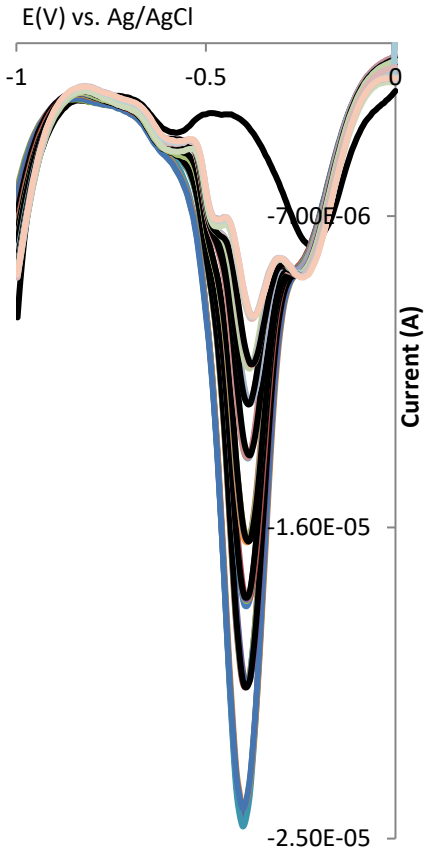

Figure 6. Differential pulse voltammograms of the modified electrode in metronidazole pH 7.00 BR buffer solutions in the 2.00×10^{-4} (pink curve) to 1.00×10^{-3} M (blue curve) concentration range

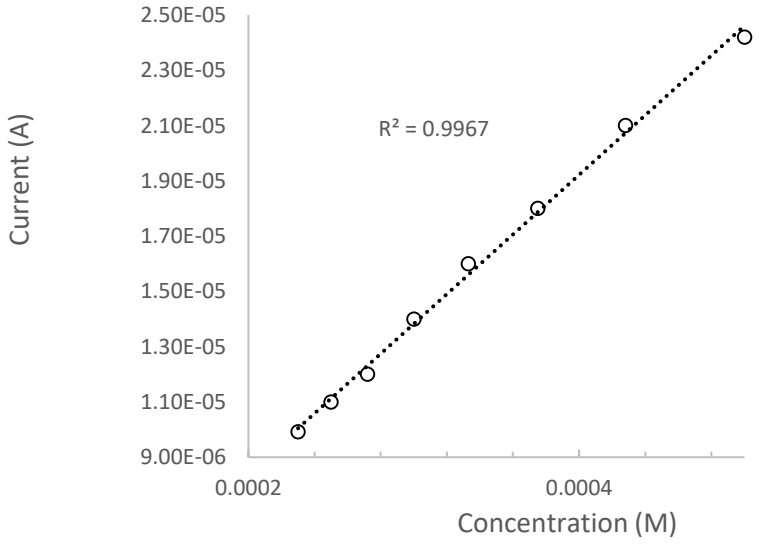

Figure 7. Dependence of the MNZ reduction peak current detected at the Ag/Au-Nafion coated GCE on MNZ concentration (in pH 7.00 BR buffer solution)

Dependence of the reduction charge on metronidazole concentration has also been studied in this work. The charge values were evaluated by calculating the area under each of the curves shown in Fig. 6, according to the procedure illustrated in section 2.5. The correlation between the reduction charge and the corresponding MNZ concentration is shown in Fig. 8. The correlation coefficient was found to be equal to 0.991, which is in good agreement with the coefficient calculated from dependence of the MNZ reduction peak current on the analyte concentration (i.e.) $R^2 = 0.997$ as shown in Fig. 7.

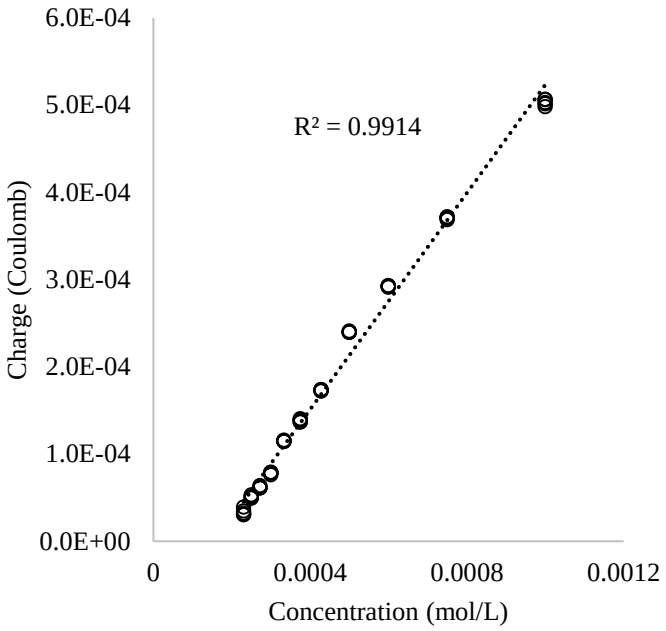

Figure 8. Dependence of the MNZ reduction charge on MNZ concentration (in pH 7.00 BR buffer solution)

Performance of the electrode modified in this work (Ag/Au-Nafion coated GCE) was compared to that of other electrodes, as demonstrated in table 1. The modified electrode exhibits a relatively low limit of detection, which could be attributed to the conductive nature of the deposited metals, and to the utilization of hydrodynamic voltammetry. As mentioned in the experimental section, the differential pulse voltammetric measurements were performed at 50 rpm, which secures convective mass transfer of the metronidazole molecules to the modified working electrode surface.

HPLC-UV is considered as the standard method for metronidazole evaluation in its biological and pharmaceutical samples. The key advantage of HPLC detection techniques is the ability of separation of the different analytes before their detection, which reduces the matrix effect and secures elimination of many of the interfering species (other analytes or impurities) that may mask or overlap with the target analyte signal. Values in the 0.88-6.9 µM concentration range have been reported when HPLC-UV technique has been employed for MNZ analysis.[9, 36-38] The limit of detection values shown in table 1 are relatively lower than those obtained by chromatography, which could be attributed to the high sensitivity of the potential step methods compared to the UV detection. In addition, in absorbance measurements, significant deviation from Beer's law is usually accompanying detection of

concentrations lower than 1.0 μM, as a consequence, the linear correlation between absorbance and concentration could not be obtained[39].

Table 1. Comparison of performance of the Ag/Au-Nafion coated GCE to other electrodes for the MNZ detection.

Working Electrode	Dynamic Range (μM)	Detection Limit (μM)	Voltammetric Signal	Reference
MWNT-GCE	$2.5x10^{-2}$-10	$6x10^{-3}$	DPV	21
Ag-NP/IL/SPCE	3.1-310 and 310-1300	0.4	DPV	22
Cys-Au SPE	50-300	2.6	SWV	23
Activated GCE	2-600	1.1	LSV	24
poly(*p*-ABSA)-modified GCE	3-70	0.373	DPV	33
Chit/CuTsPc modified GCE	$6.0x10^{-3}$-$8.0x10^{-2}$	$0.41x10^{-3}$	DPV	34
SDS-GR/CPE	0.08-200	$8.5x10^{-3}$	DPSV	35
Ag/Au-Nafion coated GCE	100-10^3	$5.87x10^{-2}$	DPV	This work

In this study, the modified electrode was employed for the detection of MNZ in pharmaceutical dosage forms, as shown in table 2. Evaluation of the % recovery is illustrated in section 2.5 and the reported values, listed in table 2, are within the acceptable recovery percentages between 80 and 120%.

Table 2. MNZ detection in drugs tablets at the Ag/Au-Nafion coated GCE (*N=3*)

Tablet	Recovery (%)	RSD (%)
Razamazol	95.86%	3.1
Nidazole	97.87%	2.5
Dentagyl	91.48%	4.4

3.3 Monitoring of the metronidazole degradation process

Efficiency of the modified electrode toward metronidazole reduction has been employed for probing of metronidazole electrolytic degradation. As mentioned in the experimental section, the electrolytic decomposition of metronidazole was performed with a platinum anode and boron-doped diamond cathode. Monitoring of the MNZ degradation utilizing differential pulse voltammetry is demonstrated in Fig. 9. The decomposition of metronidazole was carried out over an hour. After every 10 minutes, the degradation process was paused to detect the undecomposed MNZ at the Ag/Au-Nafion coated GCE. The curves shown in Fig. 9 present voltammograms of the undecomposed fractions of the anayte.

It is well known that the MNZ degradation follows pseudo first-order kinetics with a linear relationship between ln[MNZ] and the electrolysis time;

$$\ln[MNZ]_t/[MNZ]_o = -k_{obs}.t$$

Where $[MNZ]_t$ and $[MNZ]_o$ are the metronidazole molar concentrations at time t and the initial metronidazole concentration, respectively. k_{obs} is the reported pseudo first order decomposition constant in min^{-1}, and t is the degradation time in minutes.[40]

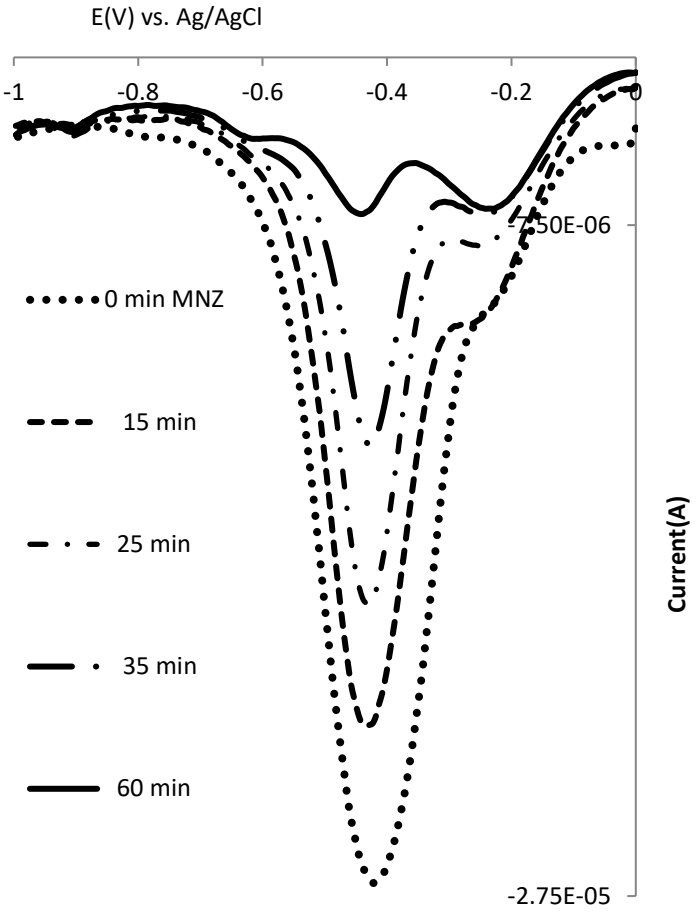

Figure 9. Differential pulse voltammograms of the Ag/Au-Nafion coated GCE in initially 1.0 mM MNZ in pH 7.00 BR buffer solution, after different time periods of electrolytic degradation

Fig. 10 presents the linear relationship between ln[MNZ] and the electrolysis time. The reported correlation coefficient was estimated and found to be equal to 0.994, which reflects the high performance of the modified surface toward monitoring of the target analyte degradation.

The degradation kinetics have also been studied by means of spectrometry. Metronidazole exhibits a significant absorption peak around 320 nm in the BR buffer solution. Fig. 11 presents the pseudo first-order decay of metronidazole (detected by UV spectrometry) over a period of 60 minutes. The reported correlation coefficient (0.996) is almost equal to that obtained from the electrochemical detection (i.e.) Fig. 10. That observation supports the utilization of the electrochemical analysis as an alternative for the HPLC, the TOC, or the spectrometric techniques commonly used for the detection of pharmaceuticals degradation processes.

The performed voltammetric monitoring has been done in an off-line mode. The voltammetric measurements were carried out among the electrolytic degradation time intervals over one hour. The

next step in that direction will be the design and utilization of a flow cell system where the degradation takes place in a reservoir that is connected to the voltammetric cell via a peristaltic pump. With that configuration, the degradation could be stopped, and the voltammetric measurements could be then performed with convenience, without transferring of the solution under investigation from the degradation reservoir to the three compartment voltammetric cell and vice versa.

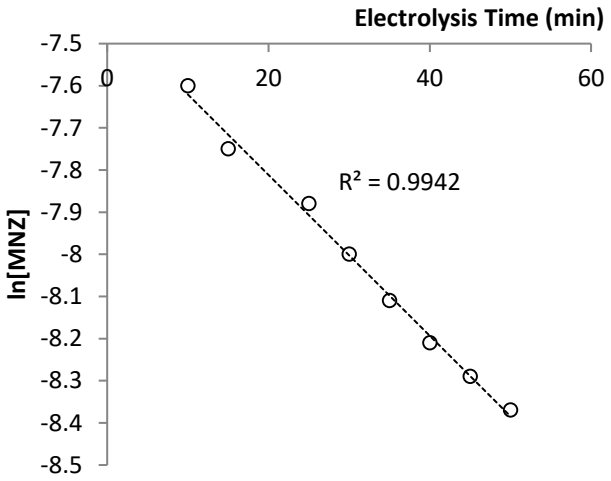

Figure 10. First-order electrolytic degradation of initially 1.00 mM MNZ in pH 7.00 BR buffer solution monitored by voltammetry using the modified electrode (Ag/Au-Nafion coated GCE)

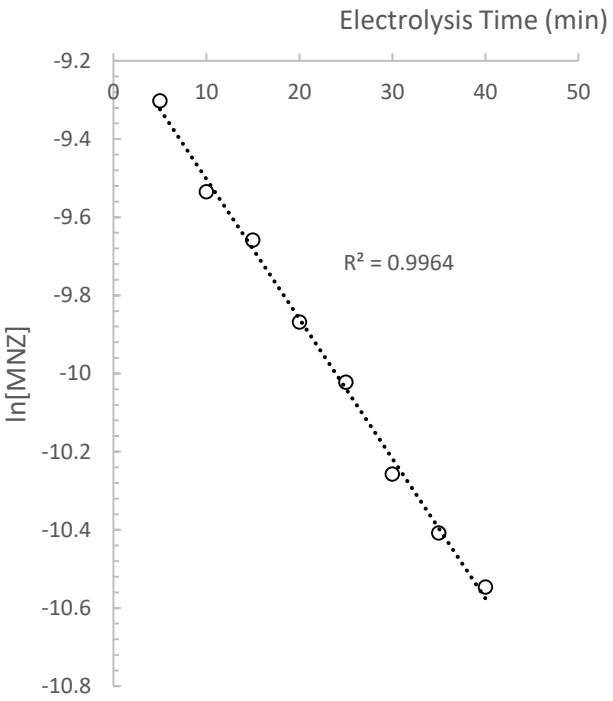

Figure 11. First-order electrolytic degradation of initially 1.00 mM MNZ in pH 7.00 BR buffer solution monitored by UV spectrometry at 320 nm

4. CONCLUSIONS

In this work, the modification of nafion coated glassy carbon electrode with metallic adsorbed layers was performed successfully. The modified electrode has shown significant activity toward the reduction of metronidazole. Another utilization of the modified glassy carbon electrode is monitoring of metronidazole mineralization, or degradation, that follows pseudo first order kinetics. This is the first step toward the design of continuous flow cell system that could be utilized to detect pharmaceuticals degradation in a manner similar to that obtained from HPLC, UV, or TOC methods of analysis.

The degradation process could be related to kinetics of the anodic oxidation of the target anayte and specifically to nature and activity of the utilized anode. Such kind of studies is of great value since it correlates structure of the utilized anode to kinetics of the decomposition process.

In general, there are two key disadvantages of electroanalytical methods; the inability of detecting electro-inactive species (that could not be oxidized or reduced) and the poor detection of electro-active analytes in complicated matrices that may contain a wide range of interfering or overlapping ingredients. The first problem could be solved by derivatization of electrochemically active products from the parent analyte while the impact of the second obstacle could be reduced by further purification of the investigated samples before performing the electrochemical measurements. Such obstacles have not been faced in the current work, but must be considered if inactive species are to be studied in the future.

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