Electrochemical Determination of Rifampicin Based on Its Oxidation Using Multiwalled Carbon Nanotubes Modified Glassy Carbon Electrode

Çok Duvarlı Karbon Nanotüp Modifiye Camı Karbon Elektrot Kullanılarak Rifampisin Oksidasyonuna Dayalı Elektrokimyasal Tayini

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INTRODUCTION: The aim of the study was to investigate the electrochemical behavior of rifampicin in the anodic direction using multiwalled carbon nanotube modified glassy carbon electrodes.

METHODS: The anodic investigation of rifampicin was carried out with cyclic, differential pulse and square wave voltammetry techniques. The three-electrode system consisting of a glassy carbon electrode with a modification of MWCNT as working electrode, a platinum wire as counter electrode, and an Ag/AgCl electrode as reference was used for the experiments.

RESULTS: The anodic process of rifampicin was irreversible and diffusion controlled. Linear responses were obtained between 0.04 and 10 μM for both techniques in acetate buffer at pH 3.5 as supporting electrolyte. Limit of detection values were 7.51 and 11.3 nM for differential pulse and square wave voltammetry techniques, respectively. The repeatability, reproducibility, precision, and accuracy of the proposed methods were also investigated. Determination of rifampicin was carried out from its pharmaceutical dosage forms and the results were compared with other electrochemical sensors and also the liquid chromatographic and spectrophotometric methods in the literature.

DISCUSSION AND CONCLUSION: These validated techniques provided selective, rapid, sensitive, precise, and cheap determination of rifampicin as alternative techniques to the liquid chromatographic and spectrophotometric methods in therapeutic drug monitoring.

Keywords: Rifampicin, voltammetry, multiwalled carbon nanotubes, glassy carbon electrode, pharmaceuticals

GİRİŞ ve AMAÇ: Bu çalışmanın amacı, rifampisin elektrokimyasal davranışını çok duvarlı karbon nanotüp ile modifiye edilmiş camı karbon elektrotlar kullanarak anodik yönde incelemektir.

YÖNTEM ve GEREÇLER: Rifampisin anodik incelemesi dönüşümlü, diferansiyel puls ve kare dalga voltametri teknikleri ile yapıldı. Çalışma elektrotu olarak MWCNT'nin bir modifikasyonuna sahip camı bir karbon elektrot, karşı elektrot olarak bir platin tel ve referans olarak bir Ag/AgCl elektrotundan oluşan üç elektrotlu sistem, deneyler için kullanıldı.

BULGULAR: Rifampisin anodik süreci geri dönüşümsüzdü ve difüzyon kontrollü idi. Destek elektrolit olarak pH 3.5'te asetat tamponunda her iki teknik için de 0.04 ila 10 μM arasında doğrusal cevaplar elde edildi. Tespit limiti değerleri diferansiyel puls ve kare dalga voltametri teknikleri için sırasıyla 7.51 ve 11.3 nM olarak bulundu. Önerilen yöntemlerin tekrarlanabilirliği, tekrar üretilebilirliği, kesinliği ve doğruluğu da incelendi. Rifampisin tayini, farmasötik dozaj formlarından yapıldı ve sonuçlar, literatürdeki diğer elektrokimyasal sensörler ve ayrıca sıvı kromatografik ve spektrofotometrik yöntemlerle karşılaştırıldı.

TARTIŞMA ve SONUÇ: Bu valide edilmiş teknikler, terapötik ilaç izlemede sıvı kromatografik ve spektrofotometrik yöntemlere alternatif teknikler olarak rifampisin seçici, hızlı, hassas, kesin ve ucuz bir şekilde tayinini sağladı.

Anahtar Kelimeler: Rifampisin, voltametri, çok duvarlı karbon nanotüp, camı karbon elektrot, farmasötikler

INTRODUCTION

Rifampicin (RIF), a semisynthetic derivative of rifamycin, is a macrocyclic antibiotic used to treat bacterial infections such as tuberculosis, leprosy, and Legionnaire's disease.¹ This drug, whose major activity is against mycobacteria, inhibits DNA-dependent RNA polymerase in susceptible bacteria by forming a stable complex with the enzyme. Inhibition of the initiation of RNA synthesis by binding to β -subunit of RNA polymerase results in the death of the cell.² It is generally used with other antibiotics to expand the antibacterial effect and, thus, various infections originating from both intracellular and extracellular organisms can be treated.^{3,4} Although RIF is one of the most potent antibiotics used for the treatment of anti-tuberculosis, the bacteria quickly develop resistance to RIF. In order to prevent the development of resistance, RIF is preferred to use in combination with other effective antimycobacterial agents such as isoniazid and ethambutol.² RIF is not used for the treatment of viral infections such as cold and flu.

Rifampicin has some adverse effects such as nausea, vomiting, loss of appetite, diarrhea, liver problems, and allergy. The most serious adverse effect of RIF is hepatotoxicity causing liver damage. In addition, RIF causes orange coloration of body fluids such as urine, sweat, and tears.

Rifampicin has a heterocyclic structure containing naphthoquinone giving its characteristic orange color. Its chemical name is 3-(4-methylpiperazinyl)dimethylrifamycin SV (Figure 1). RIF binds the bacterial RNA polymerase with the four hydroxyl groups forming hydrogen bonds with amino acid residues on the protein.²

Rifampicin is usually administered orally, rapidly absorbed, and distributed to body. The half-life of RIF is 2-3 hours. Taking RIF with meals significantly decreases its absorption. RIF is quickly hydrolyzed after the absorption and completely eliminated from the body through urine and mostly feces after about six hours.⁵

Fig. 1

Some studies are found in the literature for the determination of RIF using HPLC,⁶⁻¹⁰ LC-MS/MS,¹¹ Raman spectroscopy,¹² NMR spectroscopy,¹³ and UV-Vis spectrophotometry.¹⁴ Few electrochemical studies of RIF have also been found studied both in the direction of oxidation using some unmodified^{15,16} and modified electrodes¹⁷⁻²¹ and in the direction of reduction.^{22,23} The older studies of RIF were based on the electrochemical reduction of RIF using mercury drop electrode by differential pulse polarography²² and differential pulse adsorptive stripping voltammetry (DPAdSV)²³. In later years (between 2004-2017), RIF was studied in the direction of oxidation. In a literature¹⁵, carbon paste electrode was used for the determination of RIF and isoniazid by cyclic and square wave voltammetry. Adsorptive stripping voltammetry was also used for both RIF and rifamycin at renewable pencil graphite electrodes¹⁶. Some modifications such as a hollow manganese oxide@mesoporous silica oxide core-shell nanohybrid at a disposable carbon paste microelectrode¹⁷, nickel hydroxide nanoparticles-reduced graphene oxide nanosheets film at glassy carbon electrode¹⁹, polyvinyl pyrrolidone capped CoFe₂O₄@CdSe core shell at a glassy carbon electrode²⁰ were also used for the sensitive determination of RIF.

Most of the chromatographic and spectrophotometric methods in the literature require expensive instrumentation and high running costs. Electrochemical methods ensure simple, sensitive, cheap, and fast analysis and determination of the drugs. In addition, electrochemical properties of drugs help to understand its metabolic fate or in vivo redox processes and pharmacological activity. Although modified electrodes provide highly sensitive determination of drugs, it is important that modification procedures of the electrodes are easy to prepare and stable. For this reason, new modification methods of the electrodes and the voltammetric methods are constantly investigated to find the best results for the electroactive components.

Carbon nanotubes (CNTs) were discovered in 1991 and served fascinating electronic, magnetic, and mechanical properties. Their unique structure making them strong, and rigid makes them suitable for use in the fields of

medicine (drug delivery, treatments of diseases, monitoring of cells), manufacturing, electronics, optics, nanotechnology, and other materials science and technology.²⁴ In addition, electrically conductive CNTs can also be used as a carrier of some specific drugs by attaching to the drugs and targeting a certain types of cells such as cancer cells.²⁵ Carbon nanotubes are generally categorized as single-walled (SWCNT) and multi-walled (MWCNT) carbon nanotubes.²⁶ MWCNT has been preferred in this study since they are well-known and widely used materials since 1991 due to their superior chemical and physical properties mentioned above. In addition, MWCNTs have a very strong and elastic structure due to the sp^2 bonds between carbon atoms. These strong bonds ensure that MWCNTs have a very high electric conductivity and resistance to high temperatures.²⁶ This study aims to achieve electrochemical analysis and sensitive determination of antibacterial drug rifampicin through its oxidation with the functionalized multi-walled carbon nanotubes (MWCNT) modified glassy carbon electrode (GC) by using cyclic (CV), differential pulse (DPV), and square wave (SWV) voltammetry techniques. Developing fully validated and rapid methods using a simply modified glassy carbon electrode will ensure more sensitive determination of RIF based on its oxidation compared to the studies in the literature providing the direct determination of RIF from pharmaceutical dosage forms without any sample pre-treatment.

MATERIALS AND METHODS

Materials

Rifampicin and its pharmaceutical dosage form (Rifcap[®], including 300 mg of RIF per capsule) were kindly supplied from Koçak Farma (Istanbul, Turkey). COOH Functionalized multi-walled carbon nanotubes were from NanoLab, U.S.A., with >95% purity, 15 ± 5 nm diameter, and 1-5 μ m length. The other chemicals were analytical grade (Merck or Sigma) and used without any purification.

Stock solutions of RIF (1.0×10^{-3} M) were prepared in ultrapure water and stored in the dark at +4 °C. Voltammetric experiments of RIF were done using working solutions of RIF prepared by direct dilution of the stock solution with the selected supporting electrolyte. The supporting electrolytes were phosphate buffer (PB, 0.1 M $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$; 0.1 M Na_2HPO_4 ; pH 5.5-8.0), Britton-Robinson buffer (BRB, 0.04 M H_3BO_3 ; 0.04 M H_3PO_4 ; 0.04 M CH_3COOH ; pH 2.0-8.0), and acetate buffer (AcB, 0.5 M CH_3COOH ; pH 3.5-5.5).

Apparatus

Voltammetric measurements were recorded using a computer-controlled Autolab Type II potentiostat/galvanostat with Nova 1.10 software (Metrohm, The Netherlands) at room temperature, $25 \pm$ °C. The three-electrode system consisted of a glassy carbon electrode (GC, BASi, ϕ : 3.0 mm diameter) with a modification of MWCNT as working electrode, a platinum wire as counter electrode (BASi), and an Ag/AgCl electrode (BASi, 3.0 M KCl) as reference. Bare GC electrode was polished with aqueous slurry of alumina powder (ϕ : 0.01 μ m) on a damp smooth polishing cloth before the modification.

Operating conditions for DPV were: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate, 2 mV s^{-1} ; for SWV were: pulse amplitude, 25 mV; frequency, 10 Hz; step potential, 1 mV.

Surface morphology of bare GC and MWCNT modified GC electrodes was carried out using scanning electron microscopy (SEM) with LEO 438 VP (LEO Instruments, UK) in high vacuum mode at 20 keV.

The pH measurements were made using a model HI2211 pH-meter (Hanna, Romania) with a combined electrode (Glass-reference electrodes) with an accuracy of ± 0.05 pH at room temperature.

Sartorius Arium® ProUV ultrapure water (resistivity $\geq 18 \text{ M}\Omega \text{ cm}$) was used for the preparation of all solutions.

Preparation of MWCNT Modified Electrodes

Functionalized MWCNTs were weighted and dispersed in dimethyl formamide (DMF) as $0.2\% \text{ (mg mL}^{-1}\text{)}$. The dispersion was sonicated for 4 hours to ensure a homogenous mixture. Before the coating, bare GC electrode was polished with Al_2O_3 slurry on a polishing pad and rinsed with nanopure water. Then, a specified amount of MWCNT/DMF dispersion, 1.5, 2.5, 3.5, and $5.0 \mu\text{L}$, was dropped on the surface of GC electrode and left to dry overnight at room temperature to obtain MWCNT modified GC electrode, labeled as MWCNT/GC electrode.

Voltammetric Studies

All working solutions of RIF were prepared freshly just before the experiments and protected from the light. The calibration equations were obtained from both DP and SW voltammograms by plotting the peak current against RIF concentration. The ruggedness, precision, and accuracy of the methods were checked by assaying five replicate samples on the same day and different days over a week. The ruggedness and the precision of the methods were checked with relative standard deviations (RSD%). The relative errors (Bias%) which describe the deviation from the expected results were also calculated to check the accuracy and the precision of the developed methods.^{27,28}

Capsule Assay Procedure

The interior of ten capsules of Rifcap®, including 300 mg of RIF per capsule, were weighted. An appropriate amount of the interior was taken and diluted to 25 mL with ultrapure water to prepare a stock solution of $1.0 \times 10^{-3} \text{ M}$ RIF. The solution was sonicated for 15 min to complete dissolution and then left for 10 min to settle the insoluble portions to the bottom. The working solutions were prepared by taking from the clear supernatant liquor and diluting with the selected supporting electrolyte.²⁹ The RIF amount per capsule was calculated using the corresponding calibration plots of both DPV and SWV techniques.

The other components of the matrix of the pharmaceutical dosage forms can show any interference during the determination of RIF. For this purpose, recovery studies were carried out to check the accuracy of the developed methods using standard addition method.²⁷ A known amount of RIF was added to the pre-analyzed RIF capsule and then, the calculations were done using the related regression equations of the calibration plots for both techniques.

RESULTS AND DISCUSSION

An easily modified glassy carbon was used to ensure more sensitive determination of RIF based on its oxidation comparing to the studies in the literature. As a first step, coating of GC electrode was performed using $0.2\% \text{ (mg mL}^{-1}\text{)}$ of MWCNT/DMF dispersion. For this purpose, an amount of the dispersion was dropped using a micropipette on the surface of GC electrode. After the electrode dried, the surface was activated by cycling the potential in the region from -0.8 to $+1.2 \text{ V vs. Ag/AgCl}$ at a scan rate of 100 mV s^{-1} for 15 cycles in BRB solution at pH 5.0.

The electrochemical responses of $20 \mu\text{M}$ RIF were compared for both bare GC electrode and $2.5 \mu\text{L}$ of 0.2% MWCNT/DMF coated GC electrode using CV and DPV in BRB solution at pH 5.0. The peak currents of RIF

obtained with MWCNT/GC electrode were 1.86 times and 4.52 times higher than those bare GC electrodes for CV and DPV (Figure 2), respectively.

The coating amount of MWCNT/DMF dispersion was selected for the best electrochemical analysis of RIF. For this purpose, 1.5, 2.5, 3.5, and 5.0 μL of the 0.2% MWCNT/DMF dispersion were dropped on the surface of GC electrode and the peak currents of RIF were analyzed by CV, DPV, and SWV techniques. The highest peak current with the best peak shape was obtained with 1.5 μL of 0.2% MWCNT/DMF dispersion, thus, GC electrode was modified with this amount for the further studies of RIF.

Surface Characterization

SEM was used to investigate the surface morphology of both bare GC and MWCNT modified GC electrodes. Figure 3A and 3B showed SEM images of bare GC electrode and GC electrode coated with 1.5 μL of 0.2% MWCNT/DMF dispersion, respectively. As can be seen in Figure 3B, densely packed and popcorn-like MWCNT was coated and formed a porous structure onto the surface of GC electrode.

Fig. 3

Effect of pH and Scan Rate

Electrochemical characterization of RIF was carried out with MWCNT/GC electrode. For this purpose, cyclic voltammograms of RIF were obtained at a scan rate of 100 mV s^{-1} in the potential range from -0.6 to +1.1 V in different buffer solutions at pH values between 2.0 and 8.0. The buffer solutions at higher pH values than 8.0 were not used in order to prevent the loss of MWCNT coatings on the surface of GC electrode.

According to the cyclic voltammograms (Figure 4A), an anodic peak having a high peak current at all pHs was observed at around +0.9 V (labeled as Ox1), presenting an irreversible oxidation process. At around +0.3 V, another anodic peak having relatively high peak current was also seen (labeled as Ox2), showing a reversible redox process. With increasing of pH, additional anodic and cathodic waves were appeared at the peak potentials between -0.2 and +0.2 V. Differential pulse (Figure 4B) and square wave (Figure 4C) voltammograms were also obtained at all pHs to investigate the anodic peaks of RIF for its quantitative determination study. The Ox1 peak was not observed at around +0.9 V in both DP and SW voltammograms. However, the Ox2 peak at around +0.3 V could be seen as a symmetrical and well-defined anodic peak with a high peak current. Another anodic peak separated well from Ox2 peak was observed at around -0.1 V at lower pHs than 5.0, whereas this peak intercalated with Ox2 peak and observed as a shoulder at higher pHs than 5.0.

The reversible redox process including Ox2 anodic peak (Figure 4A) may be due to the hydroquinone-quinone redox system of the 6,9-dihydroxynaphtalene moiety to the corresponding naphthoquinone of RIF.¹⁵ The irreversible oxidation peak labeled Ox1 (Figure 4A) may be attributed to the irreversible oxidation of the piperazinyl-imino moiety.²¹

Peak potentials of both Ox1 and Ox2 peaks shifted to less positive potentials with increasing pH from 2.0 to 8.0 for all voltammetric techniques. The shift values were 0.285 V for Ox1 peak with CV technique and 0.256 V, 0.266 V, and 0.275 V for Ox2 peak with CV, DPV, and SWV techniques, respectively. These negative shifts showed that the decrease in the concentration of H_3O^+ in the buffer solutions allowed easier oxidation of RIF due to its weak acidic nature.

The relationship between pH and peak potential and peak current of 40 μM RIF was studied using CV for Ox1 peak and using CV, DPV, and SWV for Ox2 peak. Graphs of E_p -pH for both Ox1 and Ox2 peaks using CV were

Fig. 4

given in Figure 5A and 5B, respectively. The graphs obtained with DPV and SWV for Ox2 peak were similar, thus, their graphs were not shown. According to all graphs, linear plots were obtained for both Ox1 and Ox2 peaks at pHs between 2.0 and 8.0, expressed by the equations (1-4).

$$E_p \text{ (V)} = 1.030 - 0.048 \text{ pH}; \quad r = 0.990, n = 10 \text{ (for Ox1, obtained with CV)} \quad (1)$$

$$E_p \text{ (V)} = 0.365 - 0.041 \text{ pH}; \quad r = 0.991, n = 10 \text{ (for Ox2, obtained with CV)} \quad (2)$$

$$E_p \text{ (V)} = 0.335 - 0.042 \text{ pH}; \quad r = 0.996, n = 10 \text{ (for Ox2, obtained with DPV)} \quad (3)$$

$$E_p \text{ (V)} = 0.345 - 0.042 \text{ pH}; \quad r = 0.992, n = 10 \text{ (for Ox2, obtained with SWV)} \quad (4)$$

Fig. 5

The negative slope values of the linear plots of E_p -pH graphs in the pH range between 2.0 and 8.0 were between -41 and -48 mV pH⁻¹, showing that the numbers of electrons and protons might not equal in the redox process of RIF. The slope of 59 mV pH⁻¹ is the theoretical value presenting that the numbers of protons and electrons are equal.²⁹

The effect of pH on the peak current (I_p) of RIF was also investigated using CV, DPV and SWV for Ox2 peak. Since Ox1 peak was not observed in DP and SW voltammograms, quantitative determination of RIF was studied with only Ox2 peak. According to the I_p -pH graphs of Ox2 peak, a single, well-defined, sharp, and symmetrical anodic peak (Figure 4B and 4C) having the highest peak current was obtained in AcB at pH 3.5 for both DPV (Figure 5C) and SWV techniques. For this reason, AcB solution at pH 3.5 was selected as supporting electrolyte for the further studies.

The redox process of RIF was determined with scan rate studies using CV in the sweep range from 5 to 200 mV s⁻¹ for 40 µM RIF in AcB at pH 3.5. The linear relationship between the square root of the scan rate ($v^{1/2}$, mV s⁻¹) and the peak current (I_p , µA) of RIF for Ox2 peak demonstrated the diffusional behavior of RIF on the MWCNT/GC electrode (Eq. 5). The graph between the logarithm of scan rate ($\log v$) and the logarithm of peak current ($\log I_p$) gave a straight line with a slope of 0.667 for Ox2 peak (Eq. 6). Theoretical values of 0.5 and 1.0 for $\log v$ vs. $\log I_p$ graph express diffusion and adsorption controlled electrode process, respectively.³⁰ The obtained slope of 0.667 showed that the process was diffusion controlled under some adsorptive effects. Due to the adsorptive effects, differential pulse adsorptive stripping voltammetry and square wave adsorptive stripping voltammetry techniques were tested with the parameters of accumulation potential and accumulation time for the determination of RIF. However, these parameters could not be optimized and linear calibration graphs could not be obtained. For this reason, DPV and SWV techniques were used for the determination studies of RIF.

$$I_p \text{ (}\mu\text{A)} = 0.172 v^{1/2} \text{ (mV s}^{-1}\text{)} - 0.197; \quad r = 0.996, n = 8 \text{ (for Ox2)} \quad (5)$$

$$\log I_p \text{ (}\mu\text{A)} = 0.667 \log v \text{ (mV s}^{-1}\text{)} - 1.154; \quad r = 0.993, n = 8 \text{ (for Ox2)} \quad (6)$$

Determination of RIF by MWCNT/GC Electrode

Determination of RIF was studied with MWCNT/GC electrode using both DP and SW voltammetry techniques serving good selectivity, high sensitivity, and low detection limits. All measurements were done for the anodic Ox2 peak in AcB at pH 3.5 as the supporting electrolyte. Calibration graphs were obtained between the concentration of RIF and the peak current of Ox2 for DPV and SWV. Figure 6 shows DP and SW voltammograms obtained with different concentrations of RIF.

Fig. 6

The plots of the calibration graphs were linear in the range between 0.04 and 10 μM for both DPV and SWV. At higher concentrations than 10 μM , the linearity was lost probably due to the increase of adsorption effect of RIF on the surface of MWCNT/GC electrode. Besides linearity ranges; the values of slope, correlation coefficient, limits of detection (LOD) and quantification (LOQ), repeatability (within day), reproducibility (between days), and precision were calculated for both techniques at MWCNT/GC electrodes.^{27,28} All values were listed in Table 1. Calibration graphs gave a linear plot with a slope of 0.799 $\mu\text{A } \mu\text{M}^{-1}$ for DPV and two linear plots with slope values of 1.503 $\mu\text{A } \mu\text{M}^{-1}$ and 0.714 $\mu\text{A } \mu\text{M}^{-1}$ for SWV.

The LOD and LOQ values were calculated from the peak current of RIF using $\text{LOD} = 3s/m$ and $\text{LOQ} = 10s/m$ equations, where s is the standard deviation of the peak currents (three runs) and m is the slope of the related calibration equation.^{27,31} The repetitive five DPV and SWV experiments in same day (repeatability) and different days over a week (reproducibility) obtained from different solutions containing RIF at the same concentration (4 μM) gave the precision of the techniques. These within day and between day precision, accuracy, and reproducibility values were presented as RSD% (Table 1). Lower RSD% values than 1.5% demonstrated good precision, accuracy, and reproducibility.

Stability of RIF

Stability of RIF solutions were also studied for a month period. Stock solution of RIF dissolved in ultrapure water was stored in the dark at +4 °C. DP and SW voltammograms of the prepared solutions containing the same RIF concentration in AcB at pH 3.5 were obtained. According to the voltammograms, a decrease of 2.68% for DPV and 2.37% for SWV was observed at the peak currents of RIF after a four-week storage. These low decrease values show that the solutions of RIF prepared by dissolving in ultrapure water can be used for up to four weeks. However, all solutions were freshly prepared every week to ensure the stability of RIF in the working solutions.

Table 1

Determination of RIF from Pharmaceutical Dosage Forms

Determination of RIF from its pharmaceutical dosage forms was studied to investigate accuracy, selectivity, and precision of the developed voltammetric techniques with MWCNT/GC electrodes. For this purpose, the proposed DPV and SWV techniques were used to determine RIF in its capsules (300 mg RIF per capsule) for the prepared MWCNT/GC electrode. Pretreatment steps such as extraction and evaporation were not used before the voltammetric measurements. Recovery studies showed whether the excipients in pharmaceutical dosage forms caused any interference in the analysis of RIF. These studies were carried out by adding known amounts of standard RIF solution to the pre-analyzed capsule solutions. The results are listed in Table 2. These results showed that the proposed methods for the prepared MWCNT/GC electrode could be applied for the sensitive anodic determination of RIF from pharmaceutical dosage forms without any interference of the excipients.

Table 2

The bias% values were calculated to see the differences between the expected values obtained with DPV and SWV measurements and the true value (labeled RIF amount per capsule). Positive values of bias% indicated overestimation bias, meaning that the true value was slightly lower than the calculated values for both DPV and SWV techniques (Table 2).

Comparison of The Proposed Methods

The parameters of the validated DPV and SWV techniques for the MWCNT/GC electrode were compared with other sensors in the literature obtained for the determination of RIF both in the anodic^{15-18,21,32-35} and cathodic^{21,22,36-38} directions (Table 3). According to the results obtained in the anodic direction, the widest linear range was obtained in this study compared to the studies in Table 3. Lower LODs were only in^{18,32}, however linear ranges of those studies were narrower. In addition, the electrode preparation procedures were long and complicated especially in^{17,33-35} compared to the MWCNT/GC electrode used in this study.

The results of RIF obtained in the direction of reduction in Table 3 showed that the wider linear range was in this study compared to the studies in the literature.^{21,37,38} Although carbon paste electrode gave a wider linear range in²², LOD value was higher than in this study. In³⁶, there was no information about the linear range and the value of LOD was at the nM level as in this study. The detection limits of the other studies in Table 3^{37,38} were higher compared MWCNT/GC electrode in this study.

The linear range and LOD values obtained in this study were also compared with the liquid chromatographic and spectrophotometric methods used for the determination of RIF (Table 4). According to the results, linear range was wider and LOD values were lower for both DPV and SWV techniques compared to the studies in Table 4.³⁹⁻⁴⁴

Table 3

Table 4

CONCLUSIONS

The electrochemical behavior of rifampicin was studied in the anodic direction by using multiwalled carbon nanotube modified glassy carbon electrodes. Modification was done by coating of 1.5 μL of 0.2% (mg mL^{-1}) multiwalled carbon nanotube/dimethyl formamide dispersion on the surface of glassy carbon electrode. The anodic process of rifampicin was irreversible and diffusion controlled in acetate buffer at pH 3.5 as supporting electrolyte. Determination studies were carried out with differential pulse and square wave voltammetry techniques. These validated techniques enabled selective, rapid, sensitive, and cheap determination of rifampicin. Rifampicin also sensitively determined in its pharmaceutical dosage forms without any separation steps. The results showed that the inactive excipients showed no interference during the voltammetric measurements. The results obtained in this study were compared with both other electrochemical sensors and the liquid chromatographic and spectrophotometric methods proposed for rifampicin. The proposed differential pulse and square wave voltammetry techniques using multiwalled carbon nanotube modified glassy carbon electrodes demonstrated more sensitive, cheaper, simpler, and faster determination methods for rifampicin. They might be alternatives to the liquid chromatographic and spectrophotometric methods in therapeutic drug monitoring.

Conflict of Interest: No conflict of interest was declared by the author.

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Figure 1. Molecular structure of rifampicin

Figure 2. (A) Cyclic and (B) differential pulse voltammograms of 20 μM of RIF in BRB solution at pH 5.0 obtained at (a) bare GC and (b) 2.5 μL of 0.2% MWCNT/DMF coated GC electrodes

Figure 3. SEM images of (A) bare GC and (B) 1.5 μL of 0.2% MWCNT/DMF coated GC electrodes. Scale bar: 1 μm

Figure 4. Voltammograms of 40 μM RIF obtained with (A) CV, (B) DPV (for Ox2 peak, baseline corrected), and (C) SWV (for Ox2 peak, baseline corrected) in (a) BRB at pH 2.0, (b) AcB at pH 3.5, (c) PB at pH 5.5, (d) PB at pH 7.0, and (e) BRB at pH 8.0 at MWCNT/GC electrode. Scan rate: 100 mV s^{-1} for CV

Figure 5. Effects of pH on the peak potentials for Ox1 (A) and Ox2 (B) and the peak currents for Ox2 (C) of RIF obtained with CV (A and B) and DPV (C) at MWCNT/GC electrode. RIF concentration 40 μ M in 0.1 M PB (o), 0.5 M AcB (x), and 0.04 M BRB ($\blacklozenge$)

Figure 6. Baseline corrected (A) differential pulse and (C) square wave voltammograms obtained for the determination of (a) Blank, (b) 0.06 μM , (c) 0.2 μM , (d) 0.4 μM , (e) 0.8 μM , (f) 2.0 μM , (g) 4.0 μM , (h) 6.0 μM , and (i) 8.0 μM of RIF in pH 3.5 AcB. Plots of the peak current (I_p) vs. the concentration of RIF with error bars representing standard deviations for each concentration, calculated from (B) differential pulse and (D) square wave voltammograms

Table 1. Regression data of the calibration lines for quantitative determination of RIF in pH 3.5 AcB with DPV and SWV at MWCNT/GC electrode

	DPV	SWV	
Measured potential (V)	0.193	0.203	
Linearity range (μM)	0.04-10	0.04-1.6 and 1.6-10	
Slope of the calibration graph (μA μM ⁻¹)	0.799 ± 0.01	1.503 ± 0.04 and 0.714 ± 0.01	
Intercept (μA)	-0.059 ± 0.04	-0.078 ± 0.02 and 1.169 ± 0.11	
Correlation coefficient	0.997	0.994 and 0.998	
LOD (μM)	7.51×10 ⁻³	1.13×10 ⁻²	
LOQ (μM)	2.50×10 ⁻²	3.78×10 ⁻²	
4 μM RIF	Repeatability of peak current (RSD%)	1.01	0.84
	Repeatability of peak potential (RSD%)	0.56	0.35
	Reproducibility of peak current (RSD%)	1.07	1.42
	Reproducibility of peak potential (RSD%)	0.60	0.42

Table 2. The results for the determination of RIF from capsule form and recovery experiments achieved in pH 3.5 AcB using MWCNT/GC electrode

Parameters	DPV	SWV
Labeled claim (mg)	300.00	300.00
Amount found* (mg)	300.32	300.36
RSD%	1.11	1.38
Bias %	0.11	0.12
Added (mg)	1.640×10^{-2}	1.640×10^{-2}
Found* (mg)	1.656×10^{-2}	1.660×10^{-2}
Average recovered (%)	100.98	101.22
RSD% of recovery	1.68	2.40
Bias %	0.98	1.22

*Obtained from five experiments

Table 3. Compared parameters obtained using different electrochemical sensors for the determination of RIF in the anodic direction

	Electrode	Method	Medium	Linear range, μM	LOD, μM
Anodic direction	Carbon paste electrode ¹⁵	SWV	Britton Robinson buffer, pH 4.0	0.5 – 50	0.235
	Renewable pencil graphite electrode ¹⁶	DPAdSV	-	-	0.013
	Disposable carbon paste microelectrode modified with a hollow manganese oxide@mesoporous silica oxide core-shell nanohybrid ¹⁷	SWAdSV	Citric acid - Na_2HPO_4 buffer, pH 6.0	0.03 – 3.0	10.8×10^{-3}
	Lead film modified glassy carbon electrode ¹⁸	AdSV	Acetate buffer, pH 5.0	2.5×10^{-4} – 1.0×10^{-2}	9.0×10^{-5}
	Surfactant modified carbon paste electrode ²¹	DPAdSV	0.1 M KCl, pH 2.0 or 0.2 M HCl	3.5×10^{-4} – 5.4×10^{-3}	-
	Carbon paste electrode ³²	AdSV	Acetate buffer, pH 4.9	0.1 - 2	5×10^{-3}
	Iron oxide nanoparticles (Fe_3O_4 NPs) and multiwalled carbon nanotubes (MWCNTs) composite modified glassy carbon electrode ³³	DPV	Phosphate buffer, pH 7.5	2 - 20	0.032
	Poly-melamine and gold nanoparticles modified glassy carbon electrode ³⁴	LSV	Phosphate buffer, pH 7.0	0.08 - 15	0.030
Cathodic direction	Carbon-dots@ CuFe_2O_4 nanocomposite modified carbon paste electrode ³⁵	SWV	Britton Robinson buffer, pH 7.0	0.07 – 8.0	0.022
	Surfactant modified carbon paste electrode ²¹	DPAdSV	0.1 M KCl, pH 2.0 or 0.2 M HCl	9.0×10^{-5} – 2.9×10^{-3}	-
	Carbon paste electrode ²²	DPP	Acetate buffer, pH 4.5	0.1 – 100	0.010
	Hanging mercury drop electrode ³⁶	DPAdSV	-	-	6.14×10^{-3}
		SWAdSV	-	-	9.83×10^{-3}

Hanging mercury drop electrode ³⁷	DPAAdSV	Carbonate buffer, pH 9.5	1.99 – 2.78	0.170
Hanging mercury drop electrode ³⁸	DPV	McIlvaine buffer, pH 7.0	0.49 – 2.4	85×10 ⁻³
MWCNT/GCE (this work)	DPV	AcB, pH 3.5	0.04-10	7.51×10 ⁻³
	SWV			1.13×10 ⁻²

AdSV: Adsorptive stripping voltammetry; DPAAdSV: Differential pulse adsorptive stripping voltammetry; SWAdSV: Square wave adsorptive stripping voltammetry; LSV: Linear sweep voltammetry; DPP: Differential pulse polarography

Table 4. Compared parameters obtained using different analytical methods for the determination of RIF

Method	Medium	Linear range, µg/mL	LOD, µg/mL
RP-HPLC ³⁹	Methanol:acetonitrile: water (60:20:20, v/v)	40 - 100	0.5
HPLC ⁴⁰	20 mM monobasic sodium phosphate buffer with 0.2% triethylamine (pH 7.0):acetonitrile (96+4, v/v)	105 - 195	-
RP-HPLC ⁴¹	Acetonitrile:methanol:water (30:5:65, v/v, pH 5.2)	60 - 150	0.13
UV Spectrophotometry ⁴²	Folin–Ciocalteu reagent (FCR)	1.0 - 35	0.32

UV spectrophotometry ⁴³	Methanol	5 - 50	2.30
UV spectrophotometry ⁴⁴	Ethyl acetate solution	2.5 - 35.0	0.83
DPV (this work)	AcB, pH 3.5	0.033-8.23	6.18×10^{-3}
SWV			9.11×10^{-3}