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Adsorptive Stripping Voltammetry of Antibiotics Rifamycin SV and Rifampicin at Renewable Pencil Electrodes

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Abstract

Adsorptive stripping voltammetry of antibiotics of rifamycin SV (RSV) and rifampicin (RIF) was investigated by cyclic voltammetry and differential pulse voltammetry using a renewable pencil graphite electrode (PGE). The nature of the oxidation process of RSV and RIF taking place at the PGE was characterized. The results show that the determination of highly sensitive oxidation peak current is the basis of a simple, accurate and rapid method for quantification of RSV and RIF in bulk forms, pharmaceutical formulations and biological fluids by differential pulse adsorptive stripping voltammetry (DPASV). Factors influencing the trace measurement of RSV and RIF at PGE are assessed. The limits of detection for the determination of RSV and RIF in bulk forms are 6.0×10^{-8} mol/L and 1.3×10^{-8} mol/L, respectively. Moreover, the proposed procedure was successfully applied to assay both RSV and RIF in pharmaceutical formulations and in biological fluids. The capability of the proposed procedure for simultaneous assay of antibiotics RSV-isoniazid and RIF-isoniazid was achieved. The statistical analysis and calibration curve data for trace determination of RSV and RIF are reported.

Keywords: Rifamycin SV, rifampicin, stripping voltammetry, pharmaceuticals, biological fluids, pencil electrode

1. Introduction

Ansamycins are a very specific class of macrocyclic antibiotics of which the rifamycin SV and rifampicin (Figure 1) are among the better known members. Rifampicin 3-[(4-methyl-1-piperazinyl) imino] methyl rifamycin SV is administrated together with isoniazid, isonicotinic hydrazide, in treatment of tuberculosis and other infections. Combined formulations were introduced to improve acceptability and compliance, while intermittent short course therapy was used to reduce adverse reactions and improve the quality of life. In this context drug monitoring in patients during antituberculosis therapy is important, especially in AIDS patients, owing to a global increase in the prevalence of drug-resistant and toxicity[1].

The analytical methods reported for the assay of RSV and RIF include: high-performance liquid chromatography,[2–15] spectrophotometry,[16,17] flow-injection chemiluminescence,[18] electrochemiluminescence[19] and electrochemical detection.[20–25] The combination of RIF and isoniazid (INH) drugs was determined in pharmaceutical mixtures and biological fluids using spectrophotometry,[26–28] micellar electrokinetic capillary chromatography with spectrophotometric detection,[29] reversed-phase-high performance thin-layer chromatography,[30] liquid chromatography with diode array detection,[31–33] differential pulse polarography and square-wave voltammetry.[34–36] The compendial method available for the assay of the combined RIF-INH drugs in capsules employs a HPLC method for determination of RIF and a titrimetric method, which requires a separation step, for determination of INH. Up to now, there is no report on the utilization of inexpensive and disposable pencil graphite electrode for trace determination of antibiotics RSV and RIF by using DPASV. Therefore, in the present investigation a sensitive adsorptive stripping voltammetric procedure was developed for trace determination of RSV and RIF in bulk forms, pharmaceutical formulations and biological fluids. The developed method permits the screening of two antibiotic drugs RSV-INH and RIF-INH in a single voltammetric run with accuracy and precision.

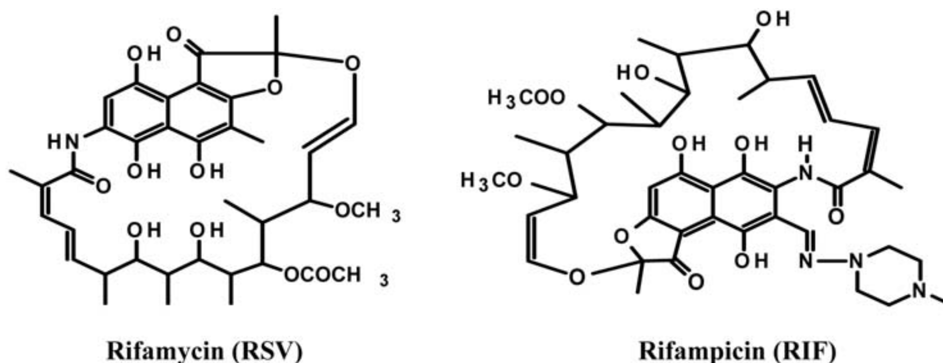

Figure 1: Molecular structures of RSV and RIF.

2. Experimental

2. 1. Instrumentation

A PAR Model 264A polarographic analyzer/stripping voltammetry was employed for differential pulse adsorptive stripping voltammetric and cyclic voltammetric measurements. The electrode system consisted of the pencil working electrode, an Ag/AgCl (3M NaCl) reference electrode (Model RE-1, BAS) and a platinum counter electrode. A Pentel pencil Model P250 (Japan) was used as a holder for the pencil lead. Electrical contact with the lead was achieved by soldering a metallic wire to the metallic part that holds the lead in place inside the pencil. The pencil was fixed vertically with 8 mm of the pencil lead extruded outside, and 6 mm of the lead immersed in the solution. Such length corresponds to an active electrode area of 9.82 mm^2. Details of the pencil electrode were described earlier.[37]

2. 2. Chemicals and Reagents

RSV, RIF and INH were obtained from Sigma (U.S.A.), and used without further purification. Stock solutions of RSV, RIF and INH were prepared by dissolving an appropriate amount of the compound in double distilled deionized water. The supporting electrolyte was Britton-Robinson (BR) prepared in the usual way, by adding an appropriate amount of sodium hydroxide (0.4 mol/L) to an orthophosphoric acid, boric acid and acetic acid mixture (0.08 mol/L). The BR buffer was brought to a constant ionic strength by the addition of 0.5 mol/L NaX (X: Cl^-, NO_3^- and SO_4^{2-}). The pH values of the buffer solutions were measured with a digital radiometer pH meter, Jenway 3310 accurate to ±0.05 unit. All chemicals used were reagent grade, (E. Merck, Darmstadt). Double distilled deionized water was used to prepare the solutions.

2. 3. Voltammetric Measurements

Cyclic voltammetric response was obtained using scan rate 100 mV/s (unless otherwise stated). For anodic stripping experiments an accumulation potential (E_{acc}) was applied for a certain accumulation time (t_{acc}) while the solution was stirred at 400 rev/min. At the end of the accumulation period, the stirrerwas stopped, and the solution was allowed to become quiescent for 15 s prior to the voltammetric scan.

2. 4. Urine and Serum Treatment

Human urine and serum samples were taken from healthy donors and used shortly after collection. Urine samples were centrifuged and filtered before use. A 0.45 ml aliquot of the serum sample was treated with 0.9 ml methanol as serum protein precipitating agent. The precipitated proteins were separated by centrifugation for 20 min at 1400 rpm. The clear supernatant layer was filtered through a 0.45 μm milli-pore filter to produce a protein – free spiked human serum.

2. 5. Pharmaceutical Formulations Treatment

A stock standard solution of each of the pharmaceutical formulations rifactine® and rimactazid® was prepared in double distilled deionized water. The contents of one tablet of rifactine® (300 mg RIF, Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt (MUP)), and one tablet of rimactazid® (150 mg isoniazid + 300 mg Rifampicin, Novartis pharma, Cairo, Egypt) were separately weighed and the average mass per tablet was determined. Then each of these formulations was separately ground to a homogeneous fine powder in a mortar. A quantity of each of these powders equivalent to 200–300 mg was transferred accurately into a separate 100 ml calibrated flask containing 70 ml double distilled deionized water. The content of each flask was sonicated for approximately 15 min then completed to volume with double distilled deionized water. Each of these solutions was then filtered through a 0.45 μm mili-pore filter (Gelman, Germany) to separate out the insoluble excipients, rejecting the first

portion of the filtrate. The desired concentrations (1×10^{-4} – 1×10^{-6} mol/L) of each of these drugs were obtained by accurate dilution with double distilled deionized water and used as a standard solution. An aliquot of the standard solution of the drug was added to BR buffer in the electrolysis cell for assay of its drug content according to the general analytical procedure.

3. Results and Discussion

3. 1. Anodic Voltammetric Studies of RSV and RIF

The electrochemical oxidation of both RSV and RIF drugs was investigated by cyclic voltammetry using a pencil graphite electrode (Figure 2). The oxidation of both RSV and RIF generated first anodic peak corresponding to a mechanism involving the transfer of two electrons/two protons typical to that of hydroquinones,[35,36] in addition to that an irreversible second anodic peak at more positive potential which may be due to the oxidation of phenolic hydroxyl group. The peak potentials of two anodic peaks shifted linearly to less positive values with the increase of pH. The current of first and second anodic peaks is much more developed over pH range 2–3 and then decreased upon the increase of pH. Taking into account that RIF is a zwitterionic species (with pK_a value of 1.7 related to the 4-hydroxy and pK_a value of 7.9 related to the 3-piperazine nitrogen[25]), whereas RSV is mainly in its unprotonated at pH higher than 3, it can be concluded that higher efficiency of the preconcentration step for accumulation of RIF and RSV on the electrode surface is obtained at pH 2 and 3, respectively. A much more developed peak current was achieved after pre-concentration of the investigated drugs onto the electrode surface for 60 s at an accumulation potential of 0.0 V (vs. Ag/AgCl sat. KCl) indicating the adsorptive character of the RSV and RIF on the PGE surface.

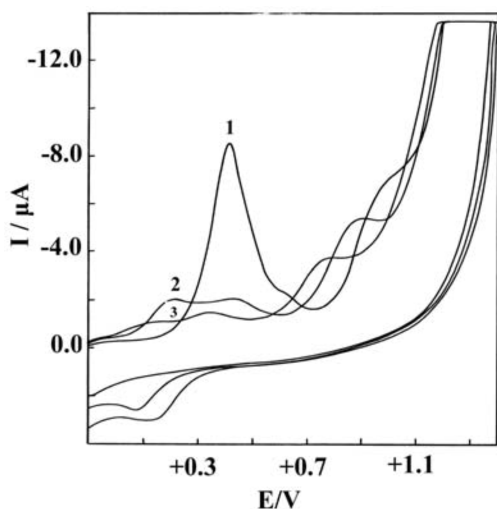

Figure 2: Cyclic voltammograms of 74 μmol/L RIF at different pH values: (1) 2.0; (2) 4.0; (3) 6.0. Accumulation time: 60 s, accumulation potential: 0.0 V, scan rate: 100 mV/s.

The dependence of the peak current and potential on the scan rate in the cyclic voltammograms of RSV and RIF was studied. In this context the relation between the anodic peak current ($i_{p/}\mu A$), the diffusion coefficient of the electroactive species, D_0 ($cm^2 s^{-1}$) and the sweep rate, ν (mV/s) is given by the following expression:

$$i_p/\mu A = (2.99 \times 10^5)\, n\alpha^{1/2} A C_0^* D_0^{1/2} \nu^{1/2} \quad (1)$$

where n is the number of electrons exchanged in the oxidation process, α is the electron-transfer coefficient for irreversible process, A is the surface area of the working electrode (cm^2) and C_0^* is the concentration of electro-active species.

It was found that the anodic peak potential shifted to more positive direction and the current height increased as the scan rate increased from 50 to 500 mV/s. The peak currents (i_p) of RSV and RIF were plotted against square root of the scan rate ($\nu^{1/2}$) and log i were also plotted against log ν showing that the dependence of the anodic peak current on both $\nu^{1/2}$ or log ν was linear at scan rates in the range from 50 to 500 mV/s. This suggests adsorption of the investigated drugs prior to oxidation. Adsorption of RSV and RIF on the surface of PGE can hence be used as an effective pre-concentration step for the anodic stripping experiments. These findings suggested the development of a sensitive differential pulse adsorptive stripping voltammetric method for trace determination of RSV and RIF at a PGE. DPASV of 1.99×10^{-6} mol/L RSV and RIF in BR buffer solution of varying pH values is shown in Figure 3. The effect of anions of the indifferent supporting electrolyte such as NO_3^-, Cl^- and SO_4^{2-} on peak height RSV and RIF is studied. The oxidation peak decreases in the order $NO_3^- > Cl^- > SO_4^{2-}$ and it is assumed that these differences are due to specific adsorption of the anions on the electrode surface. A well-defined peak is observed at pH 3 for RSV and pH 2 for RIF in BR buffer containing 0.5 M NO_3^-. This indicates that NO_3^- exhibits the lowest tendency for specific adsorption forces on a PGE. Therefore, BR buffer containing 0.5 mol/L NO_3^- was chosen for the stripping analysis of RSV and RIF.

The DPASV response of RSV and RIF using a PGE markedly depends on parameters of excitement signal. In order to obtain a much more developed stripping peak current, the optimum instrumental conditions were studied. The dependence of peak height on the scan rate in the range 5–20 mV/s was studied for RSV and RIF solution (1.99×10^{-6} mol/L). The maximum response was found at 5 mV/s. Due to an increase in the scan rate resulting in bigger peaks, the best signal was achieved at 5 mV/s. This value was adopted for the stripping analysis of investigated drugs. The effect of the pulse amplitude on

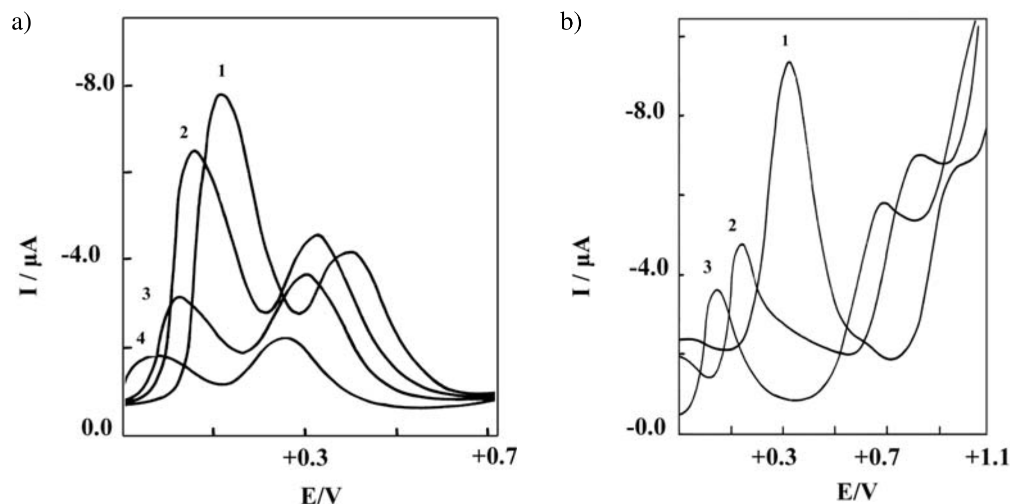

Figure 3: (a) DPASV of 56 µmol/L RSV at different pH values (1) 3.0, (2) 4.0, (3) 5.0, (4) 7.0 (b) DPASV of 74 µmol/L RIF at different pH values (1) 2.0 , (2) 4.0 , (3) 6.0, Accumulation time: 60 s, accumulation potential : 0.0 V, scan rate: 10 mV/s, pulse height: 50 mV_{pp}.

height of DPASV peak was also studied. The dependence of the peak height on the pulse amplitude indicates that the more sensitive anodic peak of RSV and RIF was recorded at 50 mV_{PP} whereas at 100 mVpp the width of anodic peak is broader. Therefore the analytical determination of RSV and RIF was performed at 50 mVpp.

3. 2. Effect of Accumulation Parameters

The effect of varying accumulation potential (E_{acc}) on the anodic peak current response of RSV and RIF using DPASV at a PGE was investigated from 0.0 V to 0.3 V in BR buffer at pH 3 and 2 , respectively. The dependence of the height of DPASV peaks of the investigated drugs on the accumulation potential showed a rapid decrease at more positive potential. A potential of 0.0 V was adopted as the optimum accumulation potential for the determination of both of RSV and RIF drugs. The dependence of the anodic peak current on the accumulation time (t_{acc}) was evaluated over wide concentration range for both RSV and RIF. As expected, the peak height increases with the accumulation time in the form of an adsorption isotherm. At t_{acc} 180 s an equilibrium surface concentration was reached and the peak height became constant. Hence t_{acc} 180 s was chosen for the determination of RSV and RIF at lower concentrations while t_{acc} had to be reduced for higher concentrations. This behavior is consistent with a process that is limited by adsorption of reactant at electrode surface due to saturation of the surface.

3. 3. Validation of the DPASV Procedure for Trace Assay of RSV and RIF

The spontaneous adsorption of RSV and RIF onto the PGE surface can be exploited as a high sensitive possibility for the trace determination the investigated drugs due to the effective accumulation prior to the voltammetric measurements. Validation of proposed differential pulse adsorptive stripping voltammetric procedure for the assay of RSV and RIF was examined through estimation of the linearity range, limit of detection and limit of quan-

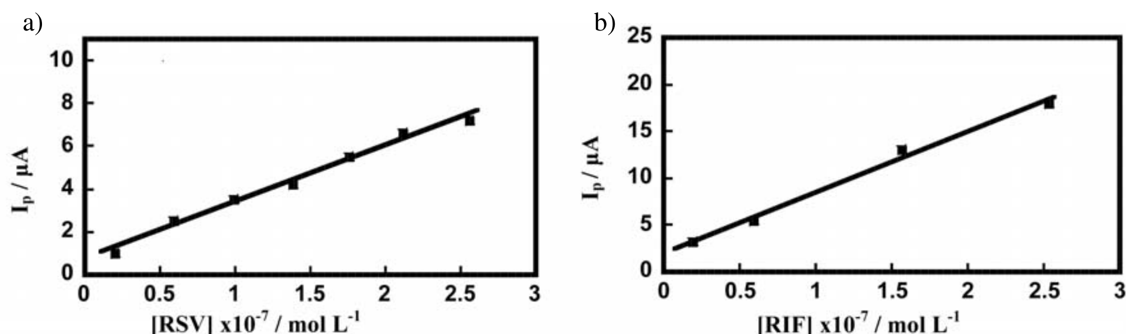

Figure 4: (a) Plot of I_p as a function of RSV concentration (b) Plot of I_p as a function of RIF concentration.

titation. Under the optimum conditions the anodic peaks of RSV and RIF were found to show a linear dependence on concentration over range 1.9×10^{-8} to 4.10×10^{-7} mol/L and 1.99×10^{-8} to 11.99×10^{-7} mol/L, respectively. The calibration curves for RSV and RIF present good linear responses as shown in Figure 4.

The variation of i_p with the concentration of RSV and RIF is represented by the straight line equation $i_p = ac + b$ where a and b are the slope and intercept of straight line respectively. The data for three to five replicated measurements are subject to a least square refinement and the values of regression coefficient (R) are computed and assembled together with the straight line constants (Tables 1 and 2). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the relation $k.SD_a$ / b where k = 3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept and **b** is the slope of the calibration curve. The obtained limit detection value for RIF in bulk solution (1.30×10^{-8} mol/L) using DPASV at a PGE is similar to those reported of this drug using the carbon paste electrode (1.72×10^{-8} mol/L),[35] mercury electrode (1.0×10^{-8} mol/L)[21] and electrochemiluminescence method (3.90×10^{-8} mol/L).[19] However an improved detection limit was achieved for the determination of RIF at a PGE using the proposed procedure compared with data reported previously by HPLC method (2×10^{-7} mol/L)[4] and the amperometric detection (5.06×10^{-6} mol/L) [24]. The LOD for RSV (6.0×10^{-8} mol/L) by means of the proposed procedure is lower than obtained by HPLC method (2×10^{-7} mol/L)[4]. Although the determination of rifamycins by using the surfactant modified carbon paste electrode[25] has lower detection limit than the developed method, the practical applications of the reported method for determination of RSV and RIF in commercial formulations and in biological fluids were not studied. Moreover, the capability of the reported method for simultaneous assay of antibiotics RSV-INH and RIF-INH was not achieved. The aforementioned results indicate that the developed adsorption stripping voltammetry using inexpensive and renewable graphite electrode provides a convenient and efficient method for quantitation of RSV and RIF.

3. 4. Simultaneous Analysis of RSV-INH and RIF-INH

Preliminary DPASV experiments for anodic oxidation of 2.99×10^{-6} mol/L INH at a PGE showed a single well-defined irreversible anodic peak in acidic solutions (pH<5). The anodic peak was attributed to oxidation of the amide moiety of isoniazid molecule.[35] The DPASV response of a binary mixture of RSV-INH at pH 3 is studied and showed a well peak at +0.1 V (vs. Ag/AgCl sat. KCl) for RSV while that of INH indicates an anodic peak at +0.69 V. The separate determination of RSV in concentration range 1.99×10^{-6} mol/L to 9.99×10^{-6} mol/L was accomplished in solution containing INH at a fixed concentration of 1.99×10^{-6} mol/L (Figure 5). On the other hand, the separate determination of INH in concentration range over 1.99×10^{-7} mol/L to 6.95×10^{-6} mol/L was achieved in solution of RSV at a fixed concentration of 1.99×10^{-6} mol/L (Figure 6). A current of RSV increases regularly as its concentration is increased at a fixed concentration of INH (its peak reduction remains constant). Similarly, as shown in Figure 6, the peak current reduction of INH increases regularly as its concentration increases at a fixed concentration of RSV (its peak reduction current remains constants).

Table 1: Calibration curve data for determination of RSV in different systems using DPASV at a PGE

System	Straight line equation I(μA) = a + bC	Regression coefficient (R)	Standard deviation (SD)	LOD (mol/L)	LOQ (mol/L)
RSV in bulk form	$Y = 1.24 + 2.19 \times 10^7X$	0.999	0.050	6.0×10^{-8}	2.31×10^{-7}
RSV–urine	$Y = 1.53 + 4.55 \times 10^5X$	0.989	0.059	1.3×10^{-7}	5.60×10^{-6}
RSV–serum	$Y = 1.04 + 5.79 \times 10^6X$	0.999	0.035	1.8×10^{-8}	3.50×10^{-7}

Table 2: Calibration curve data for determination of RIF in different systems using DPASV at a PGE

System	Straight line equation I(μA) = a + bC	Regression coefficient (R)	Standard deviation (SD)	LOD (mol/L)	LOQ (mol/L)
RIF in a bulk form	$Y = 4.78 +3.68 \times 10^7X$	0.989	0.054	1.30×10^{-8}	4.80×10^{-8}
RIF in Rifactine tablet	$Y = -3.05 + 5.9625 \times 10^4X$	0.999	0.100	5.03×10^{-6}	1.67×10^{-5}
RIF in Rimactazid tablet	$Y = 0.324 + 2.3292 \times 10^4X$	0.999	0.041	5.28×10^{-6}	1.76×10^{-5}
RIF in Rifactine – urine	$Y = 0.124 +3.4909 \times 10^4X$	0.999	0.060	5.15×10^{-6}	1.71×10^{-5}
RIF in Rifactine – serum	$Y = 0.196 + 2.0259 \times 10^4X$	0.999	0.035	5.18×10^{-6}	1.72×10^{-5}
RIF in Rimactazid – urine	$Y = 0.143 + 7.997 \times 10^3X$	0.998	0.090	3.37×10^{-5}	1.12×10^{-4}
RIF in Rimactazid – serum	$Y = 0.52 + 6.889 \times 10^3X$	0.998	0.090	3.91×10^{-5}	1.30×10^{-4}

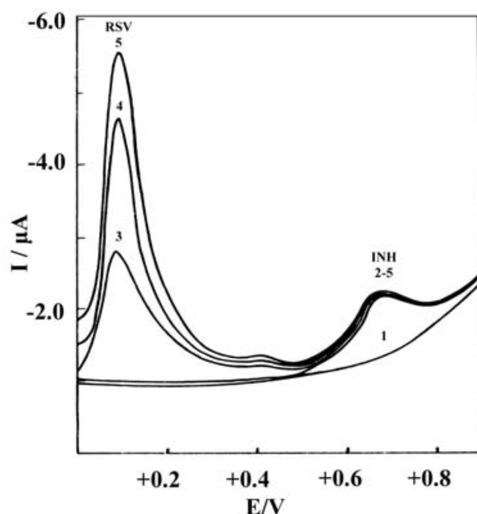

Figure 5: DPASV of RSV at different concentrations in presence of 1.99 µmol/L INH at pH 3.0; (1) Blank solution, (2) 1.99 µmol/ L INH, [RSV]: (3) 1.99 µmol/ L, (4) 5.96 µmol/ L, (5) 9.99 µmol/ L. Other conditions are the same as in Figure 3.

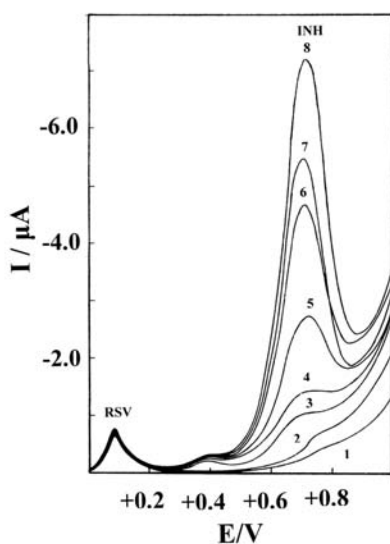

Figure 6: DPASV of INH at different concentrations in presence of 1.99 µmol/L RSV at pH 3.0, (1) Blank solution + 1.99 µmol/L RSV, [INH] : (2) 0.19 µmol/L, (3) 0.56 µmol/L, (4) 0.59 µmol/L, (5) 0.99 µmol/L, (6) 2.99 µmol/L, (7) 4.97 µmol/L, (8) 6.95 µmol/L.

The DPASV of a binary mixture of RIF-INH in BR of pH 2.0 at a PGE exhibited two anodic peaks at +0.34 V and +0.94 V (vs. Ag/AgCl sat. KCl) corresponding to the oxidation of RIF, while that of INH showed an anodic peak at +0.69 V. Figure 7 shows differential pulse voltammograms for different concentrations of RIF keeping the concentration of INH constant. It clearly depicts that RIF signal increases with the increase in its concentration without affecting the INH signal. This indicates that the difference in E_p for RIF and INH at pH 2 and the pulse amplitude 50 mVpp permit the analysis of a binary mixture of these two drugs. Individuals DPASV of RIF and INH are identical to the voltammetric curves observed in the mixture of two drugs. It was found that neither RIF nor INH interfered with the reduction signals of each other. This means that the anodic peaks generated for both drugs in BR buffer of pH 2.0 are potentially separated and therefore the DPASV form using a PGE can be considered as efficient mode for the simultaneous assay of RIF and INH in pharmaceutical and biological fluids.

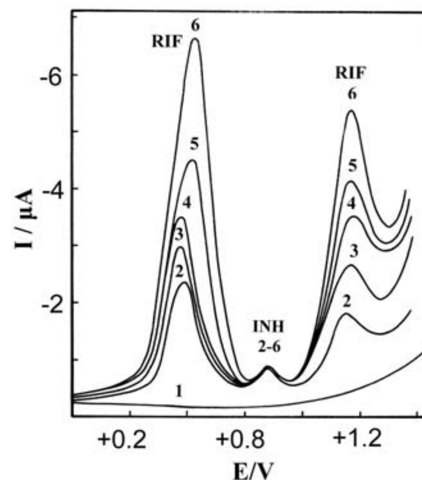

Figure 7: DPASV of RIF at different concentrations in presence of 1.49×10^{-8} mol/L INH at pH 2.0, [RIF], (1) Blank solution, (2) 2.49 µmol/ L, (3) 5.57 µmol/ L, (4) 8.34 µmol/ L, (5) 11.1 µmol/L and (6) 13.8 µmol/ L. Other conditions are the same as in Figure 3.

4. Analytical Applications

4. 1. Pharmaceutical Formulations

4. 1. 1. Analysis of Rifactine Tablets

The proposed analytical procedure was applied to assay RIF in rifactine drug without necessity for sample pretreatment or any time-consuming extraction or evaporation step prior to the analysis. Five samples from different dissolved rifactine (300 mg) tablets were analyzed using DPASV at a PGE. The two anodic peaks at +0.34 V and +0.94 V were obtained upon successive additions of pharmaceutical formulation rifactine. The variation of the anodic peak is represented by a straight line following the equation: $Y = 11.78 + 4.02 \times 10^7 X$ (Table 2). The mean percentage recovery of 95.32 % was achieved for RIF in the analysis of rifactine tablet. The achieved LOD and LOQ of RIF in rifactine drug were found to equal 5.03×10^{-6} mol/L and 1.67×10^{-6} mol/L, respectively.

4. 1. 2. Analysis of Rimactazid Tablets

The DPASV of rimactazid drug in BR of pH 2.0 at the PGE exhibited two anodic peaks at +0.34 V and +0.94

V corresponding to the oxidation of RIF, while that of INH showed an anodic peak at +0.69 V. The anodic peaks at +0.34 V, +0.94 V and +0.66 V were obtained in a single voltammetric run corresponding to the oxidation of RIF and INH respectively and were used as detection signals for their assay in rimactazid tablets**.** This means that the anodic peaks generated for RIF in presence of INH are well defined. The mean percentage recovery of 95.00 % was achieved, for RIF in the analysis of rimactazid tablet. The achieved LOD and LOQ of RIF were calculated and cited in Table 2.

4. 2. Biological Fluids

4. 2. 1. Analysis of RSV Spiked in Human Urine and Serum Samples

DPASV using a pencil graphite electrode was applied for determination of RSV spiked in human urine and serum samples. The variation of the anodic peak current vs. the RSV concentration was represented by a straight line and the values of regression coefficients (R) are computed and assembled together with the straight line constants (Table 1). The achieved LOD and LOQ of RSV in urine samples were found to equal 1.30×10^{-7} mol/L and 5.60×10^{-6} mol/L, respectively. Detection and quantitation limits for RSV in serum samples were 1.80×10^{-8} mol/L and 3.50×10^{-7} mol/L, respectively.

4. 2. 2. Analysis of Rifactine Drug in Human Urine and Serum Samples

Direct analysis of rifactine drug in human urine and serum samples was achieved using DPASV at a PGE. In this context the DPASV of rifactine drug exhibits two anodic peaks at +0.34 V and +0.94 V corresponding to the oxidation of RIF and were used as detection signals for determination of RIF in rifactine drug. The mean percentage recovery of 93.00% was obtained for RIF. The achieved LOD and LOQ values of RIF in the analysis of rifactine drug spiked in human urine and serum were given in Table 2. Since potentially interfering compounds such as ascorbic acid, amino acids, phenol and polysaccharides are always present in biological fluids,[38] the decrease of sensitivity in presence of the biological matrix can attributed to a weaker adsorption of the interfering compounds on the electrode surface which affects to some extent the analytical signals of the investigated antibiotic drugs. The detection limits of RSV and RIF in biological fluids are hence higher than in pure buffer solutions.

4. 2. 3. Analysis of Rimactazid Drug in Human Urine and Serum Samples

The anodic stripping voltammetry of successive additions of rimactazid tablet in urine and serum samples at pH 2.0 is studied. The DPASV of rimactazid drug at a PGE showed two anodic peaks at +0.34 V and +0.94 V corresponding the oxidation of RIF, while that of INH showed an anodic peak at +0.69 V. This means that the anodic peaks generated for both RIF and INH are potentially separated and therefore the DPASV is suitable mode for the simultaneous voltammetric assay of RIF and INH in biological samples. The LOD and LOQ values for determination of RIF in urine and serum samples are cited in Table 2. The simplicity, sensitivity, selectivity, and short time of analysis are main advantages of developed procedure for routine analysis.

5. Conclusions

The anodic stripping performance for the oxidation and determination of RSV and RIF using the renewable pencil graphite electrode was demonstrated without generation of hazardous wastes. The developed adsorption stripping voltammetric procedure provides a convenient and efficient method for determination of antibiotics RSV and RIF in bulk solution, biological fluids and commercial pharmaceutical products. Hence, it can be recommended for the routine quality control of these drugs by the developed procedure. Moreover the proposed procedure was successfully applied to a simultaneous assay of two antibiotic drugs RSV-INH and RIF-INH. This work opens a new possibility in the application of the stripping performance at a PGE to a simultaneous analysis of more than two drugs.

6. Acknowledgement

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Povzetek

S pomočjo ciklične voltametrije in diferencialne pulzne voltametrije na obnovljivih elektrodah iz grafitnega svinčnika (PGE) smo raziskovali adsorptivno *stripping* voltametrijo antibiotikov rifamicina SV (RSV) in rifampicina (RIF). Okarakterizirali smo naravo oksidacijskega procesa RSV in RIF na PGE. Rezultati kažejo, da je visoko občutljiva določitev maksimalnega oksidacijskega toka osnova za preprosto, točno in hitro metodo za kvantifikacijo RSV in RIF v surovem proizvodu, farmacevtskih oblikah in bioloških tekočinah z diferencialno pulzno adsorptivno *stripping* voltametrijo (DPASV). Preizkusili smo, kateri faktorji vplivajo na določitev RSV in RIF v sledovih na PGE. Meje zaznave za določitev v surovem proizvodu so bile 6.0×10^{-8} mol/L za RSV in 1.3×10^{-8} mol/L za RIF. Predlagani postopek smo uspešno uporabili tudi za določitev RSV in RIF v farmacevtskih oblikah in v bioloških tekočinah. S predlaganim postopkom je možno hkrati določati antibiotike RSV in isoniazid ali RIF in isoniazid. Prilagamo statistično analizo in umeritvene krivulje za določitev RSV in RIF v sledovih.