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Full Paper

Ultrasensitive and Economical Voltammetric Sensor for the Rapid Determination of Anti-Covid Drug Roflumilast and Co-administered Drug Salmeterol in Pharmaceutical Formulation and Human Plasma

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Abstract- COVID-19, a novel coronavirus, was identified as the primary source of pneumonia and severe acute respiratory syndrome. Roflumilast (ROF) is a widely used anti-inflammatory medication for controlling severe inflammatory pulmonary conditions. A novel sensor of sodium polystyrene sulfonate has been constructed to get an economic modifier for developing sensitive and swift voltammetric determination of ROF by square wave voltammetry in pharmaceutical dosage form and biological fluid. For obtaining best results, a universal buffer was used at pH=7. The calibration range was found to be 30×10^{-9} - 3×10^{-4} M and the correlation coefficient was computed to be 0.9995, under optimum conditions, while LOD and LOQ were found to be $9\times10^{-9,}$ 27×10^{-9} M respectively. Sensitive simultaneous determination of ROF and Salmeterol (SAL), the co-administered drug, was achieved efficiently by the sodium

polystyrene sulfonate sensor. The modified electrode was proved to be the method of choice for analysis of ROF in the dosage forms and in human biological fluids due to high sensitivity, good accuracy, and specificity. Both the number of transported electrons and the potential for an electro-oxidation pathway were examined. Greenness assessment of the presented method was checked by applying NEMI, ESA, and GAPI tools and the method proved to be acceptably green.

Keywords- Square-wave voltammetry; Coronavirus;Sodium polystyrene sulfonate; Biological fluids; Electro-oxidation pathway; Ecological evaluation

1. INTRODUCTION

In Wuhan, Hubei Province, China, outbreaks of bronchopneumonia cases with no apparent reason surfaced in the last months of 2019 year [1]. A new Corona virus called COVID-19 was thought to be the culprit behind a serious case of acute respiratory distress and pneumonia [2]. Impairment of the pulmonary oxygenation that needs mechanical ventilation is the main distinguishing feature of severe acute respiratory syndrome (SARS) which is considered as severe respiratory problem that could be fatal [3]. Release of inflammatory cytokines and genetic vulnerability are directly linked to the incidence of SARS. The genetic factor and the liberal production of inflammatory cytokines have significant impacts on the probability of infection with SARS and the severity of the symptoms. The pathogenesis and outcome of SARS are associated with tumor necrosis factor (TNF), ACE2, vascular endothelial growth factor, and interleukin IL-6, IL-8, and IL0 [3]. Airways are immediately obstructed by inflammatory cells, tissue swelling, and deposited mucus leading to reduced gas transfer, increased air trapping, asthma, and obstructed pulmonary complications [4].

Developing of novel anti-inflammatory drugs, particularly phosphodiesterase inhibitors, is the main goal of many studies. Roflumilast (ROF) has a potent anti-inflammatory action through selective inhibition of phosphodiesterase-4 enzyme targeting the systemic inflammation related to chronic obstructive pulmonary disease (COPD) [5]. The anti-inflammatory effects of ROF depend on many factors such as decreasing inflammatory mediators, inhibition of apoptosis, and cell surface markers expression [6]. In serious cases, Salmeterol (SAL) is used as a co-administered drug with ROF as it is prescribed for the treatment of asthma and COPD. The co-administration of both active ingredients is due to the action of SAL as it is a long-acting beta 2 adrenoreceptor agonist. Subsequently, co-administration of both drugs induces a synergistic effect [7].

3-cyclopropylmethoxy-4-difluoromethoxy-N-[3,5-dichloropyrid-4-yl]- benzamide is the chemical name of roflumilast (ROF) drug which inhibits the PDE-4 enzyme specifically and long-actingly. With the potential to greatly slow the progression of respiratory inflammatory problems, it possesses anti-inflammatory properties and is being developed as an oral medication to treat inflammatory lung disorders like asthma and COPD. Mast cells,

eosinophils, neutrophils, T lymphocytes, and macrophages are examples of inflammatory cells that ROF effectively targets. [8,9].

Salmeterol (SAL) is a long-acting beta-2 adrenergic receptor agonist. SAL, (2-(hydroxymethyl)-4-[1-hydroxy2[6phenylbutoxy)hexylamino]ethyl]phenol), is currently prescribed to control asthma and pulmonary diseases. For managing asthma SAL is co-adminstrated with an inhaled corticosteroid, minimizing exercise-induced bronchospasm, maintaining airflow restriction, and preventing complications of chronic obstructive pulmonary disease [10].

From the literature survey, it could be concluded that there were many analytical methods that had been established for the estimation of ROF. ROF has been previously studied alone by HPLC [11-19], HPTLC [20], and spectrophotometry [21-24]. Few methods were found for the determination of roflumilast and salmeterol in their synthetic mixture by HPTLC [25] and by UV spectroscopic methods [26].

Electrochemical techniques are known to be fast, sensitive, simple, and economic analytical methods. They are regarded as a great substitute for traditional analytical techniques since they allow for the identification of medications that are undergoing oxidation or reduction processes. Successful application to pharmaceutical formulations and biological fluids could be also achieved [27,28].

Chemically modified electrodes (CME) have garnered a lot of interest lately because of their many benefits, which include high sensitivity, improvement of the mass transfer velocity, stability, and anti-interference properties, and improved detection performance of electrochemical techniques [29,30].

A polymer called sodium polystyrene sulfonate is created by adding sulfonate functional groups to polystyrene. The formula for polystyrene sulfonic acid is $(CH_2CHC_6H_4SO_3H)_n$. Its salts are polystyrene sulfonates in sodium form. Polystyrene sulfonates are used as super plastifiers in cement and cotton dye enhancers. Sodium polystyrene sulfonate salts is used in fuel cell applications as proton exchange membranes owing to their water solubility and ion exchange capabilities. When the resin is in its acidic condition, it is used as a solid acid catalyst in organic synthesis [31,32].

Only HPTLC analytical method was found for the simultaneous determination of ROF and SAL in their synthetic mixtures [33]. The previously published HPTLC used several harmful reagents such as toluene which is a carcinogenic chemical and environmentally toxic reagent [34] and biological analysis was missed in the published HPTLC study. Accordingly, the goal of the introduced research is to establish a new, economic, accurate, selective, precise and validated analytical technique for the simultaneous determination of ROF with SAL in their synthetic mixture and in spiked human plasma as well. To guarantee the effectiveness, safety, and quality of multi-component medications in their final marketed dose form, the proposed approach was verified and complied with International Conference on Harmonization (ICH)

requirements [35]. Ecological evaluation study was implemented to investigate the greenness of the introduced method [36-38].

2. EXPERIMENTAL SECTION

2.1. Instruments

A Bio-logic SP 150 electrochemical workstation was consumed in all voltammetric experiments. The electrochemical workstation was connected to a single compartment cell with a triple electrode set-up by C3-stand from BAS (USA). The auxiliary electrode was a platinum wire from BAS (USA). All the cell potentials were assessed with respect to Ag/AgCl reference electrode (3.0M NaCl) from BAS (USA). Measurements of pH were performed using a digital pH meter, Cyberscan 500, from EUTECH Instruments, USA equipped with a glass-based electrode. Transmission electron microscopy (TEM) measurements were applied with TEM Model Quanta 250 FEG (Field Transmission Gun) connected to EDX Unit (Energy Dispersive X-ray Analyses), applying accelerating voltage 30 K.V., magnification 14× up to 1,000,000 and resolution for Gun.1n (FEI company, Netherlands). At 25 °C (room temperature), all the electrochemical trials were carried out.

2.2. Reagents and chemicals

All reagents and chemicals consumed during application of the developed method were of analytical grade. Universal buffer was obtained through mixing various quantities of boric acid (2.47 g) which was purchased form Polski EODZNN Chemiczne S.A. Co., Poland, orthophosphoric acid (2.73 mL) from Loba-Chemic Co., India; and glacial acetic acid (2.29 mL) from Adwic Co., Egypt; with the suitable volume of 0.2M NaOH from Adwia Co., Egypt; to get the required pH range (2.0–11.0).

Roflumilast was gifted by (Nodcar, Cairo) with certified purity of 99.8%. Salmeterol was kindly supplied by (Nodcar, Cairo) with certified purity of 99.7%. Laboratory Chemicals Drugs (LED), the British drug houses, was the supplier of Sodium polystyrene. Westabreath® tablets were burchased from the local pharmacy and labeled to release 500 mcg of Roflumilast with batch number of 104334.

Biological Products and Vaccines holding company (VACSERA, Egypt) provided human plasma samples, which were stored at -4°C. Volunteers in good health were the source of human plasma samples.

2.3. Working solutions and standard solutions

To get the stock standard solution of ROF (1×10^{-3} M), 20.1 mg of the authentic drug was mixed with ethanol, and the volume was filled to 50 mL in a volumetric flask.

After dissolving 20.7 mg of the pure SAL with ethanol and filling a volumetric flask to the full 50 mL, Salmeterol stock standard solution (1×10^{-3} M) was completed. Using ethanol, the stock solution was diluted appropriately to provide working solutions (1.0×10^{-5} - 1.0×10^{-4}M). For over a week, these solutions were stored well at 4 °C in refrigerators.

2.4. Procedures

2.4.1. Construction of the bare (CPE)

Carbon paste unmodified electrode (CPE) was made by adding graphite powder (0.50 g) to paraffin oil (0.3 mL) in a mortar and good mixing was applied using the pestle.

2.4. 2. Construction of the modified carbon-paste electrode

Different quantities of sodium polystyrene were mixed with graphite powder (0.5%), 1%, and 3% to create a sodium polystyrene modified paste electrode (PSMCPE). For optimal results, mixing 50 mg of sodium polystyrene with 950 mg of graphite powder was done in a mortar with a pestle and mortar to get a final concentration of 5%. To obtain greater uniformity, diethyl ether was added to the resultant composite and stirred with a magnetic stirrer continuously to ensure complete solvent evaporation. Subsequently, 0.6 milliliters of paraffin oil were poured. Shiny appearance was achieved after the paste was smoothed over a filter paper surface and packed into the electrode body's hole.

2.4.3. Electrochemical behavior of ROF by square wave voltammetry

The drug's electrochemical behavior was investigated using square wave voltammetry (SWV).

After addition of a portion of 4.5 mL of universal buffer in the cell, 0.5 mL of standard ROF solution (1.0×10^{-3} M) was added and applied with the scan with a speed of 100 mV/s and pH range of 2–11 for both modified and bare carbon paste electrode as the test solution.
At room temperature, all scans were performed in +ve direction using an applied potential range of +0.4 to +1.4 V.

2.4.4. Recommended procedure for calibration curve via square wave voltammetry

A range of 5-mL volumetric flasks was completed with precisely measured portions that corresponded to (3×10-8 - 3×10-4 M) ROF, which were taken from its stock solutions. With universal buffer (pH 7.0), the required accurate volumes were obtained. The prepared solutions were delivered into the electrolytic cell. Utilizing SWV, the anodic peak current (I_p) was assessed with 100 mV/s scanning speed. By plotting the peak current (I_p) versus the ROF concentration (M), the calibration curve was produced, and the regression equation was obtained automatically.

2.4.5. Application of the developed square-wave voltammetric method on the commercial dosage form and the human-spiked plasma samples

2.4.5.1. Pharmaceutical preparation.

25 Westabreath® tablets were accurately weighed and then ground into a fine powder. 5.0 mL of methanol was used to extract a precisely weighed amount of the powder containing 1250 µg/mL of ROF in a 10.0 mL volumetric flask. 25 minutes of sonication were required to ensure complete dissolving of the active ingredients followed by filling the flask to the mark. Mixing and filtration were necessary to get clear solutions without any excipients.
Ethanol was further added to produce a solution with 1×10^{-3} M of ROF. Next, applying the steps outlined in Section 2.4.4, the concentration of ROF was determined using the regression equation.

2.4.5.2. Preparation of biological samples.

.5 mL aliquots of plasma were moved into 10 mL centrifuge tube. 0.5 mL of (1×10^{-2} M) ROF and SAL solutions were then added. 4 mL of acetonitrile was added to precipitate the plasma protein, then continuous centrifugation for fifteen minutes at 4000 rpm was done, and the careful addition of the supernatant was 5 mL graduated glass vials was done. Aliquots of supernatant equivalent to (1×10^{-3}M) of ROF and SAL were mixed with 5 mL of universal buffer of pH 7. Finally, to obtain the calibration curve, the peak current (I_p) was plotted versus the drug concentration (M).

3. RESULTS AND DISCUSSION

3.1. Morphologies of sodium polystyrene sulfonate

The response of the electrochemical sensor depends on its physical characteristics. The PSMCPE's morphology was shown in Figure 1.

Polystyrene sulfonate was prepared by sulfonating polystyrene obtained by emulsion polymerization of styrene. Sulfonate groups were introduced on the phenyl groups of polystyrene. Polystyrene sulfonate has a negative charge on its surface, so that chemical attraction occurs between the drug and polystyrene sulfonate resulting in increasing the current.

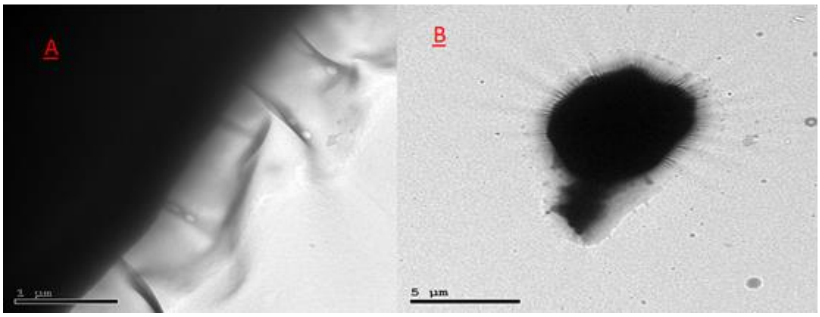
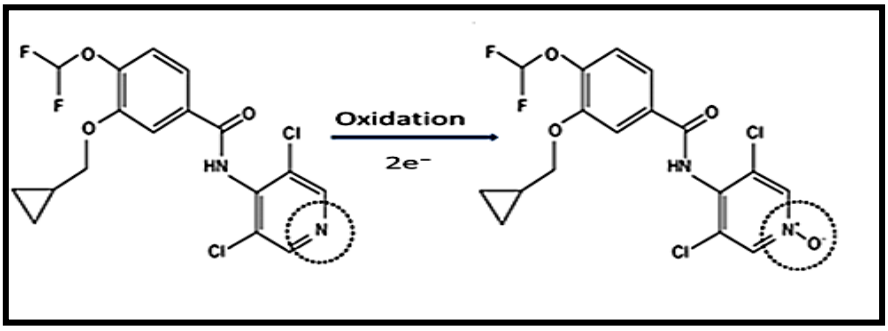


Figure 1. Transmission electron microscopy image of PSMCPE

The transmission electron microscopy (TEM) image of PSMCPE, TEM A is image with more magnification while TEM B is an image of low magnification to show the physical character of PSMCPE which showed colloids that have compressed spherically conformation thought to be the effect of cation condensation—either Na^+ or H^+—along the ionized polymer chain.

3.2. Electrochemistry of ROF

The oxidation of ROF is due to oxidation of the nitrogen group to nitrogen oxide (N-Oxide). The tertiary nitrogen of pyridine ring in ROF is oxidized by oxygen that can be bubbled through a solution of pyridine group to ROF N-Oxide [39]. The resulting anodic peak current and the loss of a cathodic peak in the reverse scan confirm irreversible oxidation of the drug under test, Scheme 1.



Scheme 1. Electrochemical oxidation reaction of ROF to ROF N-Oxide

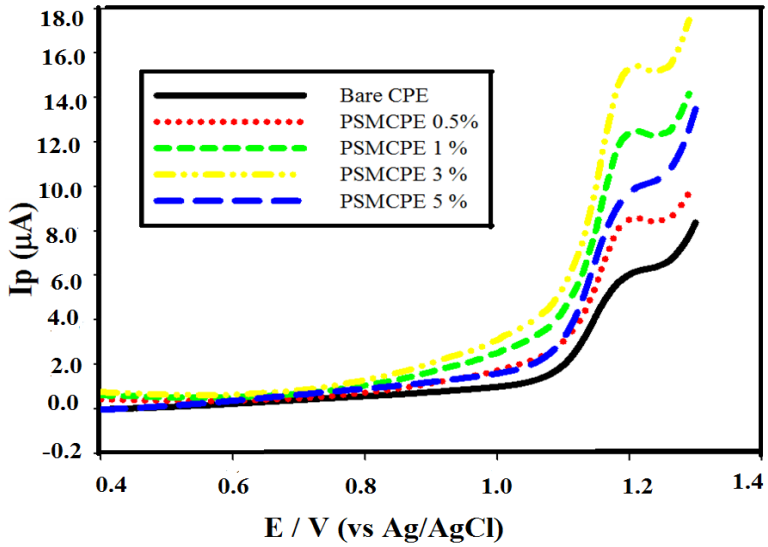


Figure 2. Square wave voltammetric actions of ROF (1.00×10^{-4} M) in universal buffer with pH 7.0 and scanning rate of 100 mV s^{-1} applying various sodium polystyrene sulfonate concentrations % (w/w)

3.3. Optimization of experimental parameters

3.3.1. Impact of sodium polystyrene sulfonate concentration

In carbon paste, different percentages of sodium polystyrene sulfonate (0.5–5.0%) were blended. In universal buffer pH 7, the square wave voltammogram of 1.0×10^{-4} M ROF was scanned with a rate of 100 mV s^{-1} as illustrated in Figure 2. When the anodic peak current reached 3.0% sodium polystyrene sulfonate concentration, it was stabilized, allowing 3.0% to be used for implementing the modified electrode PSMCPE.

3.3.2. Effect of pH

The oxidation procedure of the studied drug at (PSMCPE) was checked by investigating the pH effect in the pH range (2-11) utilizing a universal buffer.

The oxidation of ROF was found to be pH-dependent as the peak potentials moved in a less positive direction when the pH was raised. Figure 3 shows the linear relationship between potential (Ep) and pH for both electrodes:

Ep (V) = 1.5558 - 0.0509 pH r^2= 0.9993 for PSMCPE

A completely detailed follow-up was done to assess the impact of pH on the peak currents at PSMCPE. The produced outcomes proved that the ideal graphs and the highest peak currents occurred when the pH 7.0 buffer solutions were used for PSMCPE. Subsequently, the ideal pH for further experiments was determined to be 7.0.

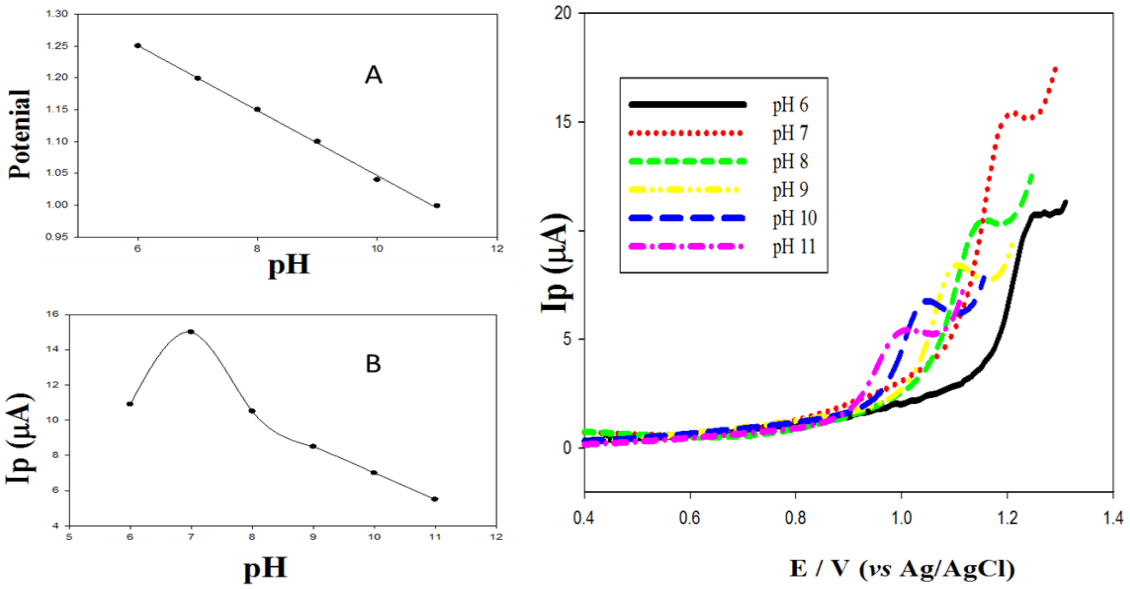


Figure 3. Square wave voltammetric behavior of 1.0×10^{-4} M ROF at various pH values utilizing PSMCPE with 100 mV s^{-1} scan rate. Inset A: the relationship between anodic peak potential and pH recorded at PSMCPE. Inset B: the relationship between peak current and pH recorded at PSMCPE

3.3.3. Effect of scan rate

Square wave voltammetry was used for PSMCPE to study the impact of scan rate (υ) (20-150) mV s^{-1} at pH 7 on ROF oxidation peak current in order to comprehend the electrode reaction mechanism. An optimum linear relationship was detected between the peak current (I_{pa}) and the square root of the scan rate ($\upsilon^{1/2}$), as shown in Figure 4 A. It was confirmed that there were kinetic limitations in the drug-redox site reaction of the modified electrode when the catalytic oxidation peak current increased gradually with increasing scan rate. The outcome shows that the diffusion stage controls the sensor process [40]. Ip (μA) = 1.8249 υ 1/2(V s^{-1}) - 3.2818 is the corresponding equation that is calculated with a correlation coefficient of 0.9992.

Moreover, an exponential relationship was observed between the logarithm of oxidation peak currents and scan rates, as demonstrated by the equation presented in Figure 4 B. The regression equation is Log I_p = -0.1961+ 0.6889 log υ with a correlation coefficient of 0.9993.

The modified electrode plays a key role in indicating the diffusion-controlled transport of electroactive species, as indicated by the slope of the obtained linear relations, which has a value of approximately 0.5 [41].

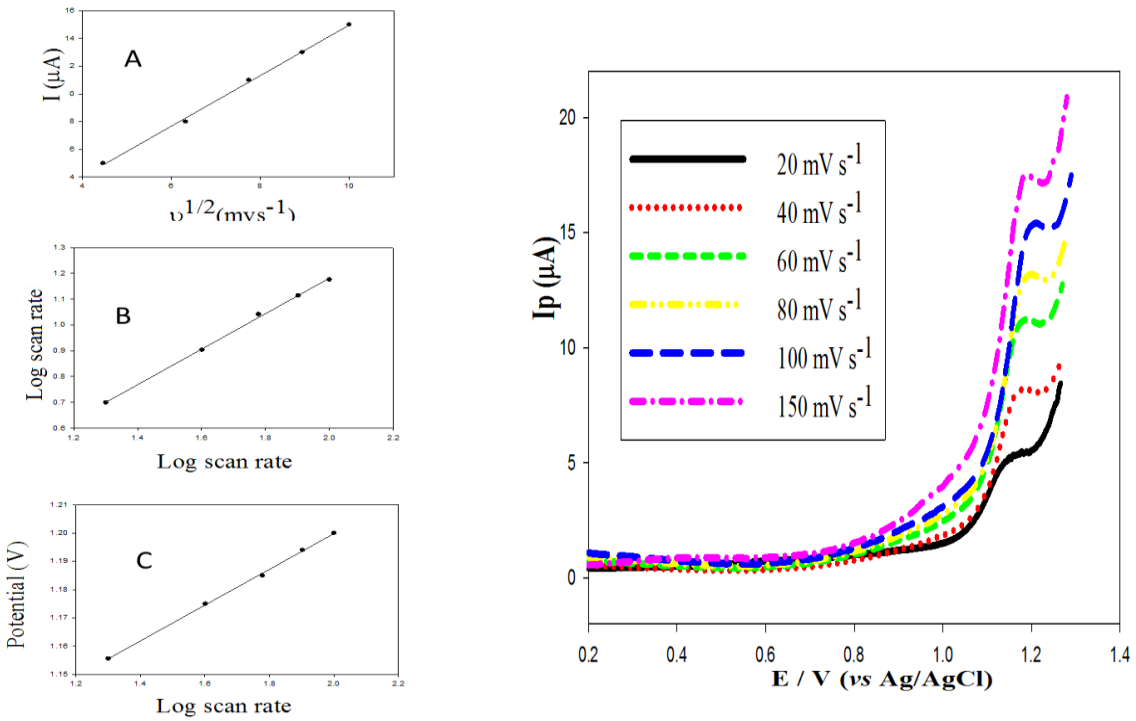


Figure 4. Square wave voltammetric results of 1.00×10^{-4} M of ROF at various scan rates (20.0–150.0 mV s^{-1}) in universal buffer (pH 7.0) using PSMCPE, inset (A) describes relation between anodic peak current and square root of scan rate, while inset (B) presents relation between log scan rate and log anodic peak current and inset (C) illustrates relation between log scan rate and peak potential

Additionally, the scan rate affected the electrochemical oxidation peak potential (Ep), with higher scan rates resulting in a shift toward higher positive potentials, as shown in Figure 4 C.

$$E_p\ (V) = 1.0732 + 0.0633 \log \upsilon\ (V\ s^{-1}) \qquad r^2 = 0.9996$$

The number of electrons transported was calculated using Laviron's theory [42] for irreversible processes in order to ascertain the kinetic parameters of the electron-transfer process for the DTP oxidation on the PSMCPE. $E = E^o + 2.303\ RT/\ \alpha\ nF\ [\log RTK^o/\ \alpha\ nF] + 2.303RT/\ \alpha\ nF\ (\log \upsilon)$ R refers to the gas constant which is 8.314 J K mol^{-1}, F represents the Faraday constant which is 96.485C KJ, T is the temperature (298 K), n is the number of electrons and α refers to the electron transfer coefficient. αn can be calculated using the slope of potential versus log scan rate. The slope in this system is 0.0633, while αn was determined to be 0.942. Considering α has 0.5 value, since for a totally irreversible electron transport, n was computed to be 1.88 indicating electrons were incorporated within the oxidation process of ROF.

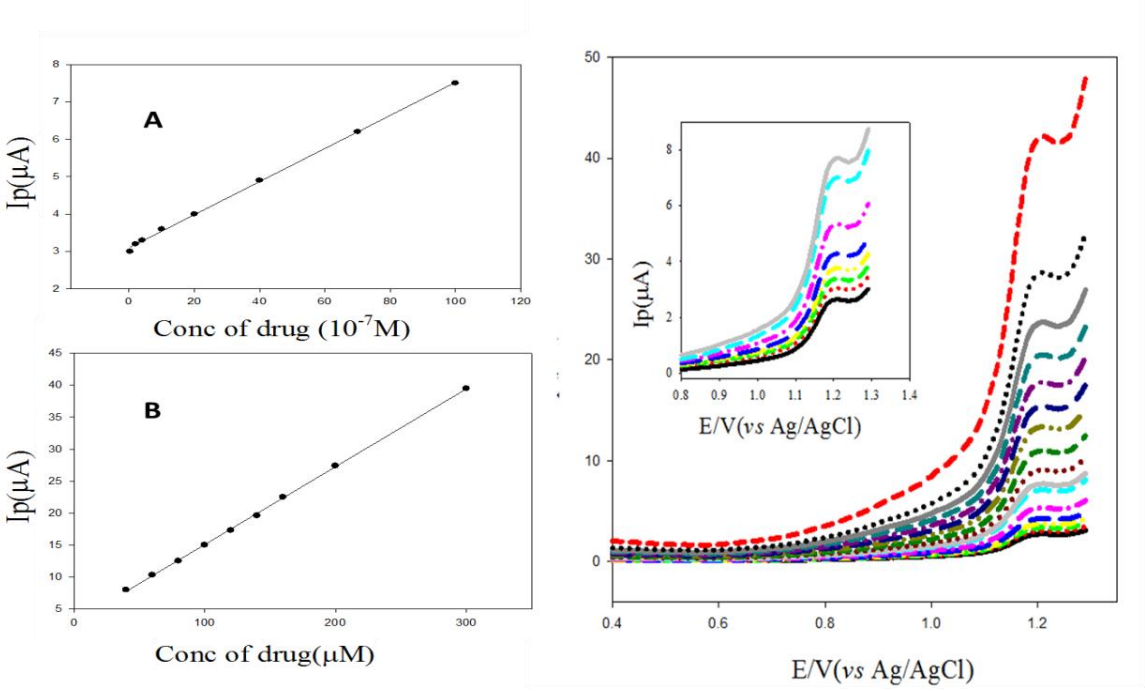


Figure 5. Square-wave voltammograms for various concentrations of ROF (3.0×10^{-8}-3.0×10^{-4} M) in universal buffer (pH 7.0) at an amplitude of 25.0 mV, scan rates 100 mV s^{-1}, a frequency of 100 Hz and a step potential of 5 mV applying PSMCPE. Inset (A) illustrates a relation between current I_p against low concentrations rang 3×10^{-8}–1×10^{-5} M, and inset (B) illustrates a relation between current Ip against high concentrations rang 4×10^{-5}– 3×10^{-4} M

3.4. Method validation

Validation was carried out according to the ICH guidelines [35] as follows:

3.4.1. Linearity

Using SWV, the linearity of ROF was attained after achieving the aforementioned ideal conditions. The experimental parameters were scan rate (υ) = 100 mV s^{-1}, amplitude of 25.0 mV, step potential of 5 mV, and frequency of 100 Hz. As shown in Figure 5, a perfect relation between peak current and concentration was verified in the (3.0×10^{-8} – 3.0×10^{-4} M) range. The parameters are included in Table 1.

3.4.2. Limits of detection and quantification

LOD and LOQ were found to be 1.9×10^{-8}, 5.8×10^{-8} M, and all results were indexed in Table 1.

3.4.3. Accuracy and precision

Three distinct concentrations were measured three times in order to calculate the accuracy of the suggested method to determine ROF in authentic form. After evaluating the mean % recoveries, good outcomes were obtained, as indicated in Tables 1 and 2. To determine the inter-day precision, repeating three assay runs with the same concentrations measured in duplicate was done. Table 1 indicated that the calculated RSD was less than 2%. In a single drug assay run, three concentration levels were examined in triplicate to ascertain the intra-day precision, which was subsequently calculated as RSD.

Table 1. Validation parameters of the suggested voltammetric method for analysis of ROF in universal buffer (pH 7.0) in bulk powder and spiked human plasma

Parameters		**Pure sample**	**Plasma**
Linearity (M)	**Low range**	3×10^{-8}– 1×10^{-5} M	3×10^{-8}–1×10^{-5} M
	High range	4×10^{-5}– 3×10^{-4} M	
Intercept		3.1008	0.056
Slope		0.0442	0.0907
Correlation coefficient (r)		0.9995	0.9996
Accuracy[a]			
Mean±SD		99.65±1.71	101.13±0.44
Precision[b] **(RSD %)**			
Repeatability		±0.56	±0.43
Intermediate Precision		±0.57	±0.63
LOD		1.9×10^{-8}	1.1×10^{-6}
LOQ		5.8×10^{-8}	3.5×10^{-6}

[a]n=3
[b]n=9

3.4.4. Robustness

The stability of the peak current even after purposeful, slight modifications to the experimental parameter showed how robust the suggested approach was. The pH change (7.0 ± 0.2) and the change of time (20 s ± 4 s) preceding as each measurement were among the variables under investigation. The peak current intensity of the drug under study was unaffected by these small adjustments that might have happened during the trial operation, demonstrating the validity of the suggested approach under normal operating circumstances.

3.4.5. Selectivity and specificity

The specificity of the presented voltammetric approach was shown by its ability to identify ROF in its dosage form regardless the impact of any excipient.

Table 2. Accuracy of the developed SWV technique for the assessment of ROF

Taken concentration M ($\times10^{-7}$)	**Found concentration M ($\times10^{-7}$)**	**% Recovery[a]**
4.00	4.06	101.50
6.00	5.96	99.33
8.00	7.85	98.12
Mean		99.65
SD		1.71
RSD		1.72

[a]Average of three determinations

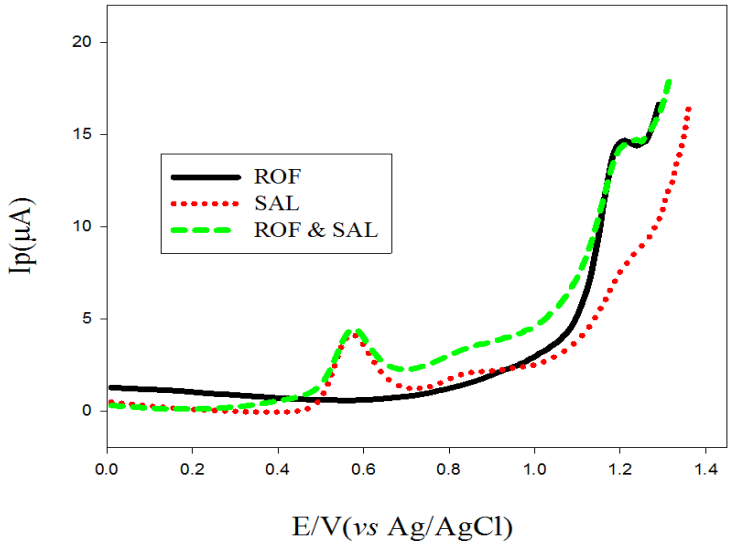


Figure 6. The simultaneous determination of ROF and SAL in universal buffer pH 7.0 using SWV at PSMCPE

3.5. Effect of co- formulated drug

The electrochemical oxidation of ROF in the presence of co-administered medication SAL in universal buffer pH 7 was done to demonstrate the selectivity and sensitivity of the suggested sensor for ROF. SAL has no influence on the suggested electrode's sensitivity or selectivity, as Figure 6 illustrates. The SAL and ROF oxidation peaks are well separated from one another. Figure 6 represents the simultaneous assessment of ROF and SAL in a universal buffer pH 7.0 using SWV at PSMCPE.

3.6. Application in pharmaceutical preparation

3.6.1. Pharmaceutical formulation

The suggested technique was implemented on Westabreath ® tablets, and the recoveries were satisfactory. Employing the standard addition technique, the suggested method's validity was evaluated, and the outcomes are displayed in Table 3 with a good recovery percentage (99.93±1.13).

Table 3. Application of the proposed SWV method for the determination of ROF in pharmaceutical formulation

Product	**Claimed amount (µg/mL)**	**Found% [b] ±SD**	**Standard addition technique**				
			Claimed taken ×10^{-8} µg/mL	**Amount added ×10^{-8} µg/mL**	**Total found[a] ×10^{-8} µg/mL**	**Standard found[a] ×10^{-8} µg/mL**	**%Recovery of added[a]**
Westabreath® tablet (B.N104334 **)**	6.20×10^{-8}	100.53±1.60	6.20	-----	6.08	------	-----
			6.20	3.10	9.15	3.07	99.03
			6.20	6.20	12.18	6.10	98.38
			6.20	12.40	18.32	12.24	98.70
			Mean ± SD				**98.70±0.32**

[a]Average of three determinations

3.6.2. Application of co-administered drugs in real plasma

Linear electrode response in plasma for ROF and SAL was observed in the range 3×10^{-8} - 1×10^{-5} M for ROF and SAL. The regression equations were found to be:

For ROF I_p (µA) = 0.0443C +1.5978 with correlation coefficient, r=0.9994 and for SAL I_p (µA) = 0.0166 C + 0.5415 with correlation coefficient, r=0.9990 as shown in Figure 7.

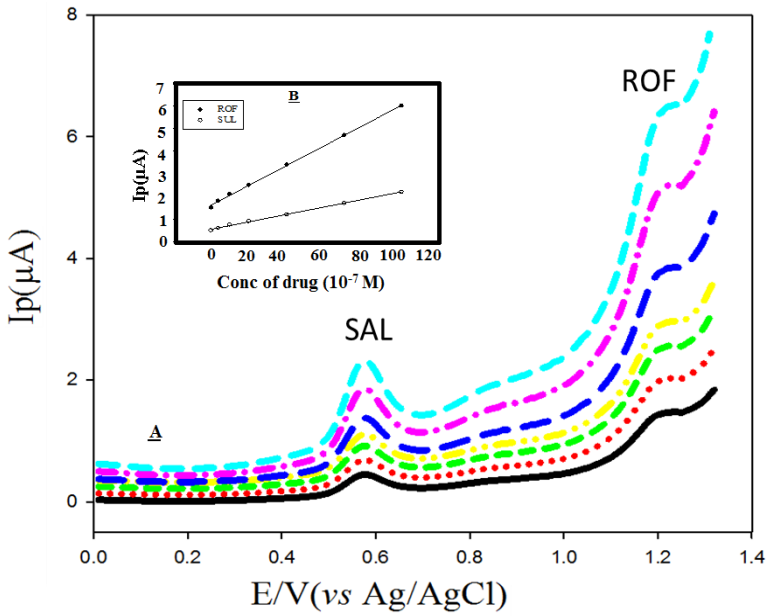


Figure 7. Square-wave voltammograms of various concentrations of ROF and SAL (3×10^{-8} - 1×10^{-5} M) applying Britton-Robinson buffer (pH 7.0) in spiked human plasma with scanning rates of 100 mV s^{-1} utilizing PSMCPE (A). The inset plots of I_p versus concentrations**.** Inset (B) illustrates a relation between current I_p against ROF and SAL concentrations ranging from 3×10^{-8}-1×10^{-5} M

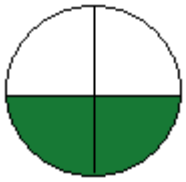
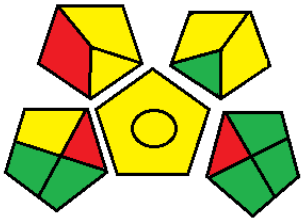
3.7. Greenness Assessment of the proposed method

Our nature is exposed to many threats due to the risks caused by harmful chemicals used in chemical analysis in pharmaceutical companies. For this reason, many studies have been published concerned with measuring the convenience of the presented analysis methods for the environment. NEMI (National Environmental Method Index) is the simplest method could be applied to determine the environmental efficiency of the presented method initially [36].

Despite its simplicity and ease of application, it lacks much important information. Therefore, the ESA method was used, which relies on score-based evaluation of the studied method [37].

GAPI; the green analytical procedure index; is a quick visual tool for evaluating greenness. [38]. The previously published research [43] contains more information and details regarding how to apply the three ecological assessment methods. Acceptable resultant values obtained after application of the three ecological assessment tools referring that the suggested method is environmentally benign and can be applied safely, Table 4.

Table 4. Ecological evaluation of the suggested method applying NEMI, ESA, and GAPI Tools

NEMI	**ESA**		**GAPI**
	Orthophosphoric acid Glacial acetic acid Boric acid 0.2M NaOH Methnol Ethanol Acetonitrile Energy consumption Waste production Occupational Risk Penalty points **Analytical Eco-scale**	4 4 1 2 6 4 4 2 0 0 23 **77**	

4. CONCLUSION

In order to study ROF employing SWV, the current work introduces a novel, sensitive, straightforward, and accurate sensor—PSMCPE—which has improved the electrode's electrical conductivity. Due to the improved electron transfer procedure and enhanced substantial surface area of the adjusted electrode, the analytical process has been extensively validated in terms of sensitivity, accuracy, linearity and precision. The present study introduces a simple, sensitive, and precise method for assessing the drug's pure dosage form and applies it to biological fluids, especially plasma. Additionally, this sensor offers a very useful and efficient technique to measure ROF simultaneously when SAL is being prescribed as a co-administered drug. Greenness assessment of the introduced method was done implementing NEMI, ESA and GAPI tools and the method proved to be acceptably green.

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Authors' Declarations

Authors' contributions

The paper has been read and approved by all authors. Sh. A. and N. N.gathered all of the information and wrote the primer draft of the article. A. A. took part in the article's authoring and prepared all figures. F. F. was responsible of ecological assessment of the presented

research and prepared Table 4. A. M, H. E, and I.A. were responsible for supervision. The entire article was edited and reviewed by A. M., H. E., I. A., and F. F.

Declarations Conflict of interest

Regarding the current study, the authors declare that they have no competing interests.

Availability of data and material

Upon request, the corresponding author will provide the data used to support the study's conclusions.

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