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Voltammetric Analysis of ATPase-inhibitor Using Redox Mediator Platform: Application in Biological Fluids

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ABSTRACT

In this study, the oxidation of rabeprazole sodium (RAB Na^+), a widely used anti-ulcer drug, was investigated at a modified poly (bromocresol green)/ pencil graphite electrode (poly (BCRG)/PGE). A disposable, sensitive and selective electrochemical platform is proposed for the determination of RAB Na^+ by recording its cyclic voltammetry (CV) and square wave adsorptive voltammetry (SWV) in Britton-Robinson buffer solution at pH of 7.0 using the modified PGE. The poly BCRG/PGE displayed a good electrochemical behaviour with significant enhancement of the peak current compared to the bare PGE. Under experimental conditions, the modified electrode had a linear response range from 10-180 × 10^{-8} M and 3-330 × 10^{-7} M for SWV and CV, respectively. The detection limit was found to be 3 × 10^{-8} M and 1 × 10^{-7} M for SWV and CV, respectively. These voltammetric methods were successfully applied for the direct determination of RAB Na^+ in real samples. The effect of various interfering substances on the RAB Na^+ peak current was also investigated.

Keywords: Bromocresol green, Pencil graphite electrode, Rabeprazole sodium, Real samples

INTRODUCTION

Peptic ulcers form painful ulcers in the lining of the stomach or in the first part of the small intestine (duodenum) produced by excessive secretion of HCl. These ulcers can cause serious health problems including bleeding, stomach and duodenal perforation or swelling. The newest group discovered to treat peptic ulcers are proton pump inhibitors (PPIs) such as rabeprazole sodium (RAB Na^+) that irreversibly inhibit ATPase enzyme [1]. Few methods were used to determine RAB Na^+ as spectrophotometry [2,3], spectrofluorometry [4], thin layer chromatography [2] and high-resolution liquid chromatography [2,5]. Two electrochemical methods [6,7] have been applied for the determination of RAB Na^+ in the bulk drug and pharmaceutical dosage form. Electrochemical techniques have attracted more attention in recent times for the analysis of electro-active compounds with biological, clinical, and environmental application [8-10]. The use of modified pencil electrodes is well documented [11-18]. These electrochemically treated electrodes are compact, inexpensive, easy to prepare and easy to use as working electrodes for both laboratory analysis and on-pot detection analysis. The ability to modify these electrodes in one step is very important. The pencil graphite electrode (PGE) can be applied for analysis of drugs and in trace detection of metal ions [19]. This electrode has a larger active surface area and is therefore capable of detecting low concentrations of the analyte. Polymer-modified electrodes have the advantages of improving electro catalysis, the absence of surface fouling and the prevention of undesirable reactions that compete kinetically with the desired electrode procedure [20-22]. It has been shown that the electrodes modified with polymers show excellent stability, reproducibility and homogeneity [23-25]. Most redox dyes are artificial electron donors [26,27] and are capable of electro-polymerization to generate active redox layers [28].

In this work, bromocresol green was chosen as monomer to obtain poly (BCRG)/PGE by electrochemical polymerization for the first time where the process was simple and rapid. In addition, because of the high hydroxyl (OH) groups in the bromocresol green molecule made the polymer dye to have good concentrations of negatively charged surface functional groups. The modified electrode showed excellent electro-catalytic properties in the determination of RAB

Na^+ making it suitable for the analytical purpose. Therefore, the present work was carried out for the first time to apply poly (BCRG)/PGE to determine RAB Na^+ electrochemically in tablets and biological fluids.

MATERIALS AND METHODS

Pharmaceutical products

RAB sodium was supplied as a gift from Global Napi, 6th October city, Giza, Egypt. Rabicid® tablets (Sigma, Quesna, El-Menoufia, Egypt) were labeled to contain 40 mg RAB sodium. Domperidone was supplied as a gift from EIPICO, 10th Ramadan city, El-Sharquia, Egypt. Aceclofenac, tinidazole, and clarithromycin were obtained as gifts from NODCAR, El-Giza, Egypt. Doxycycline was supplied as a gift from CID, Assiut, Egypt.

Reagents and solvents

Glacial acetic acid, phosphoric acid, boric acid, potassium ferricyanide, potassium chloride and ascorbic acid were purchased from El Nasr Pharmaceutical Chemicals Co., Egypt. Bromocresol green, uric acid and dopamine were purchased from Sigma-Aldrich, Germany. Double distilled water was used in all the steps.

Instrumentation

For electrochemical measurements a Princeton VersaSTAT MC (VersaSTAT 3, Princeton Applied Research, AMETEK, USA) was used to connect to a three-electrode cell. In all measurements, the reference electrode was Ag / AgCl, KCl the auxiliary electrode was a platinum wire and PGE as a working electrode. The electrical contact with the wire was obtained by welding a wire to the metal part holding the cable in place within the pencil. Unless otherwise indicated, the pen was fixed so that about 3 mm of its length would be immersed in the solution. The measurements were carried out in a 10 ml glass cell containing 6 ml of electrolytic support solution.

The stirring was obtained with a magnetic bar. pH values were measured using a Hanna pH meter (Hanna Instruments Brazil, São Paulo, Brazil). The solutions were ultrasound using a Bransonic ultrasonic cleaner, Branson UL, Eagle Road, Danbury, CT 06813, USA. Surface morphological superficial studies of the modified electrode were performed using scanning electron microscopy (SEM), JEV JSM-5400 LV (Oxford, USA) instrument. A Nicolet 6700 FTIR Advanced Gold Spectrometer, supported with OMNIC software (Thermo Electron Scientific Instruments Corp., WI USA) for data processing.

Preparation of standard solutions

1.0 mM RAB Na^+ was prepared by dissolving the appropriate amount of the drug in 20 ml of double distilled water for five minutes to ensure complete solubility of the drug. The volume was completed to 100 ml using double distilled water. Standard work solutions were prepared by further diluting the stock solution with B.R. buffer (pH=7.0).

Sample preparation

The contents of ten tablets were carefully weighed, finely powdered and thoroughly mixed in a mortar. Parts equivalent to about 1.0 mM of the drug were carefully weighed and dissolved in 20 mL of distilled water. The contents were subjected to sonication for about 20 minutes to ensure complete solubility. The excipients were removed by centrifugation at 3000 rpm for 5 minutes. The residue was washed three times with double distilled water. The volume was completed to 100 ml using double distilled water.

Human blood free of drug has been obtained by healthy volunteers. In order to remove serum proteins, acetonitrile (0.75 ml) was added to 1.0 ml of the serum sample and diluted to B.R. (pH 7.0) in a 10 ml volumetric flask and the mixture was centrifuged for 10 min at 5000 rpm. The supernatant was then carefully taken and used for further analysis.

Drug-free urine samples were obtained from healthy volunteers and non-smokers of different age and sex. Samples were stored at -20°C and analyzed the day after collection without further pretreatment. One milliliter of the corresponding urine sample is pipetted into a 50 ml calibrated flask and packed in volume with B.R. buffer (pH=7.0).

RESULTS AND DISCUSSION

Deposition of poly (BCRG) films on the surface of PGE and parameters that influence the deposition

Preparation of poly (BCRG)/PGE

Figure 1 shows the CV of BCRG electro-polymerization on the surface of PGE. The results have shown that the oxidation peak for BCRG is gradually diminished during multiple successive cycles (**Scheme 1**).

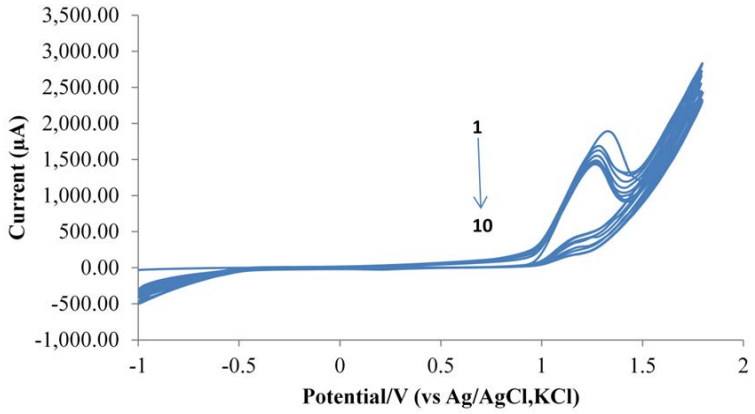
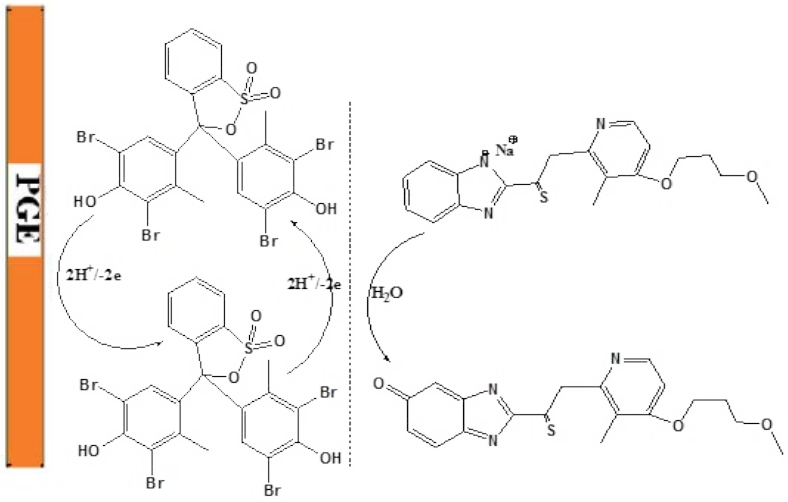


Figure 1: Multiple CV for polymerization of BCG on PGE surface. Conditions were 0.4 mM of BCRG in 0.1 M NaOH, scan rate of 100



Scheme 1: Proposed mechanism for the oxidation of RAB Na^+ on the surface of the developed platform.

Poly (BCRG) Film Study on PGE

The Electron Scanning Microscope (SEM) was used to study the interfacing morphology of the electrode surfaces. **Figures 2A** and **2B** show the surface morphology of PGE bare and poly (BCRG)/PGE, respectively. **Figure 2B** shows that the surface of PGE was coated with a film of a uniform scale, indicating that the BCRG film deposited successively on the surface of the electrode and can surprisingly increase the interaction between the modified electrode and the analyte. **Figure 2C** illustrates the FTIR spectra of the monomer BCG (a) and poly (BCG) (b). The peak at 3446 cm^{-1} is O-H stretching vibration peak and the peaks at 1584 and 1628 cm^{-1} are benzene skeleton vibration peaks. As shown in curve b, it can be seen that the peaks of S=O stretching vibration (1050 cm^{-1}), the peaks of C-Br stretching vibration (620 cm^{-1}) and the peaks of benzene skeleton vibration (1520 cm^{-1}).

Factors affecting the deposition of BCRG in PGE

The maximum current of the drug was increased with a growing deposition of BCRG which in turn increased with an increasing concentration of BCRG up to 0.4 mM (**Figure 3A**). The effect of BCRG polymerization cycles on RAB Na^+ oxidation current has been studied. Peak current has been reached to maximum after cycles increased to 10 cycles after that the current decreased (**Figure 3B**). This may due to blocking the PGE surface by excessive amount of the dye. The effect of scan rate on redox behaviour of the poly (BCRG) film was investigated in the range of 25-100 mVs^{-1} (**Figure 4**).

Electrochemical characterization of the poly (BCRG/PGE) using standard potassium ferricyanide

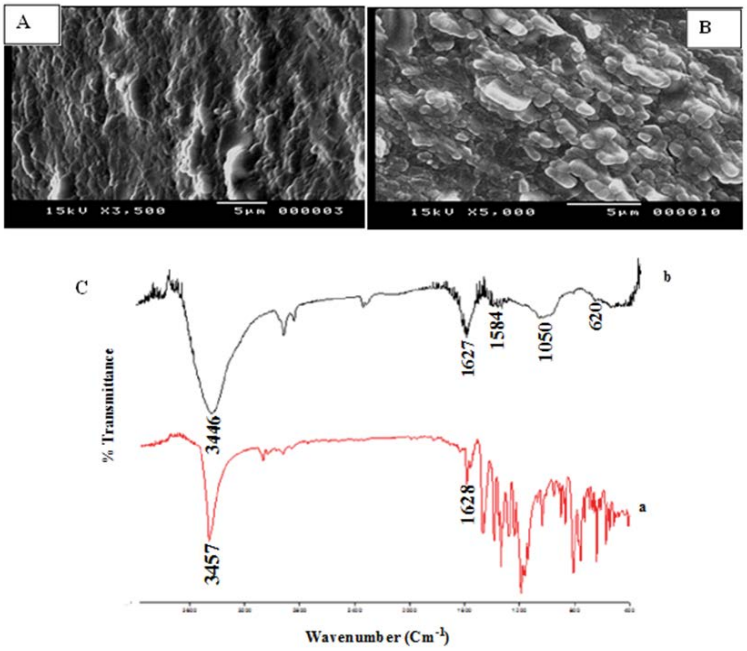


Figure 2A-2C: SEM of bare PGE (A) and BCRG/PGE (B), and (C) FTIR spectra of BCRG (a) and poly (BCRG) film (b).

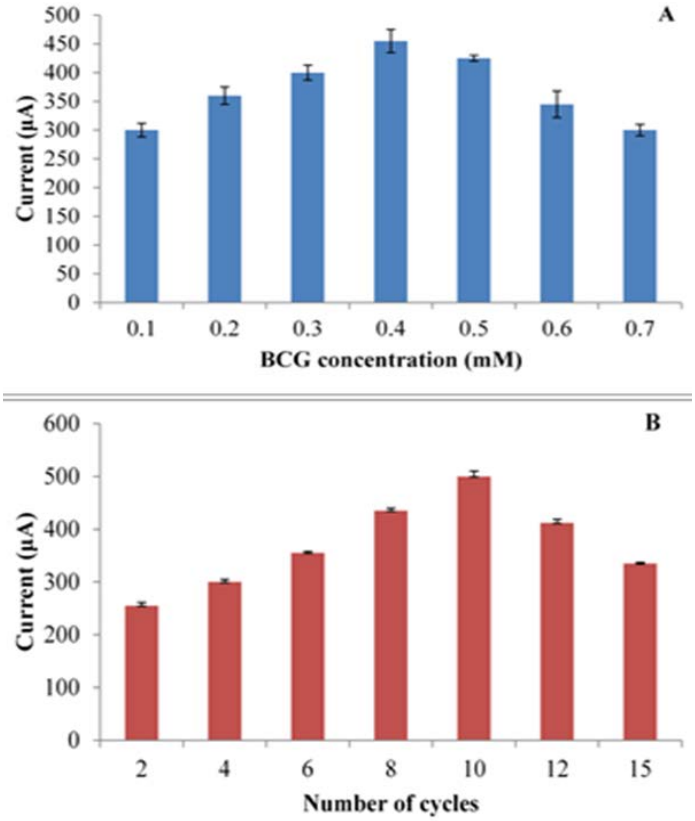


Figure 3: Effect of BCRG concentration (A) and number of cycles (B) on anodic oxidation of RAB Na+.

Before the voltammetric analysis, the surface activity of PGE was evaluated before and after modification using cyclic voltammetry (CV). The CV was recorded after immersion of PGE/modified PGE in 1 mM ferricyanide potassium mixed with 0.5 M KCl. Randles -Sevcik equation for a reversible process [29] was used to estimate the effective surface (Aeff.mm^2) of the electrodes.

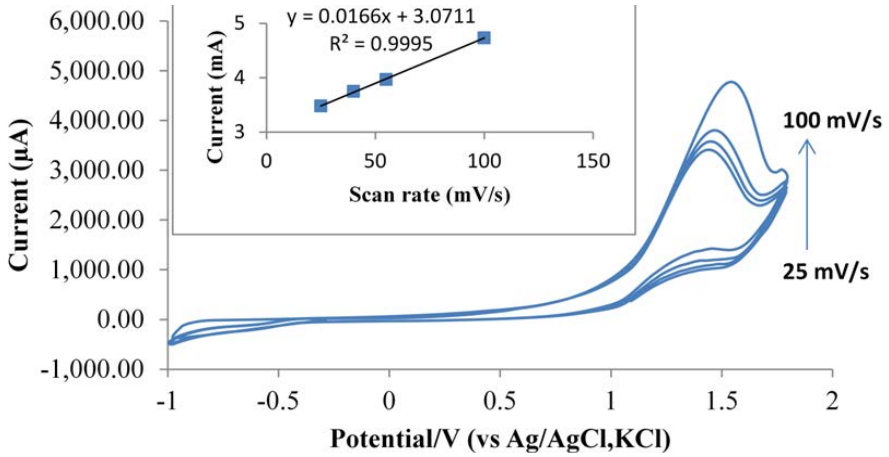


Figure 4: The CV of poly (BCRG)/ PGE at different scan rates. (a) 25 mV/s; (b) 40 mV/s; (c) 55 mV/s; (d) 100 mV/s. Inset is relationship between peak current and scan rate.

Where D and C° are the diffusion coefficient and ferricyanide mass concentration, respectively. The surface area of the bare PGE was 16.5 mm² (**Figure 5A**), while the poly (BCRG)/PGE was 30.3 mm² (**Figure 5B**).

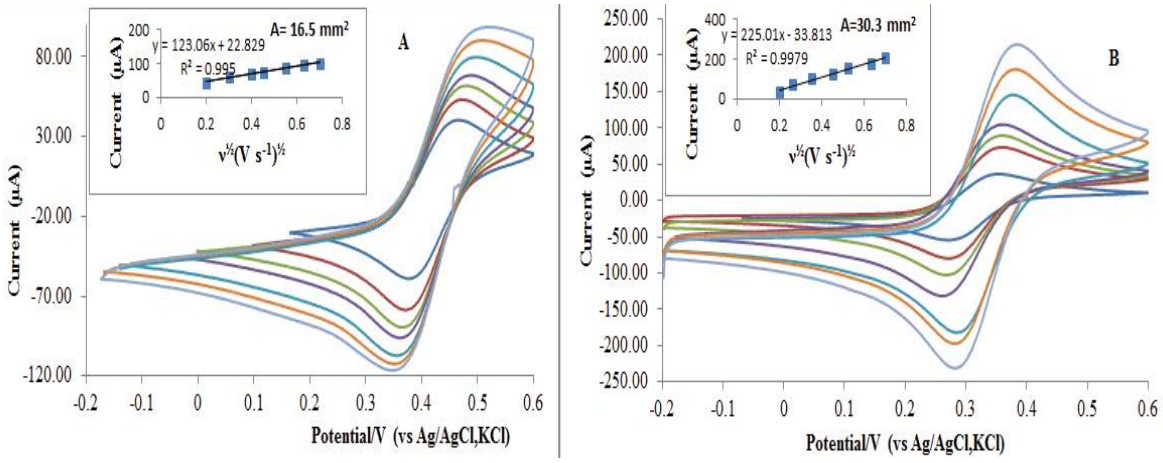


Figure 5: The electro-activity of bare PGE (A) vs poly (BCRG)/PGE (B) using CV.

Electrochemical characterization of RAB Na⁺ on bare electrode against modified electrode

The CV and SWV were presented in (**Figures 6A and 6B**). The CV of RAB Na⁺ showed only an irreversible anode peak at +850 mV and no cathodic peak was recorded in reverse scanning.

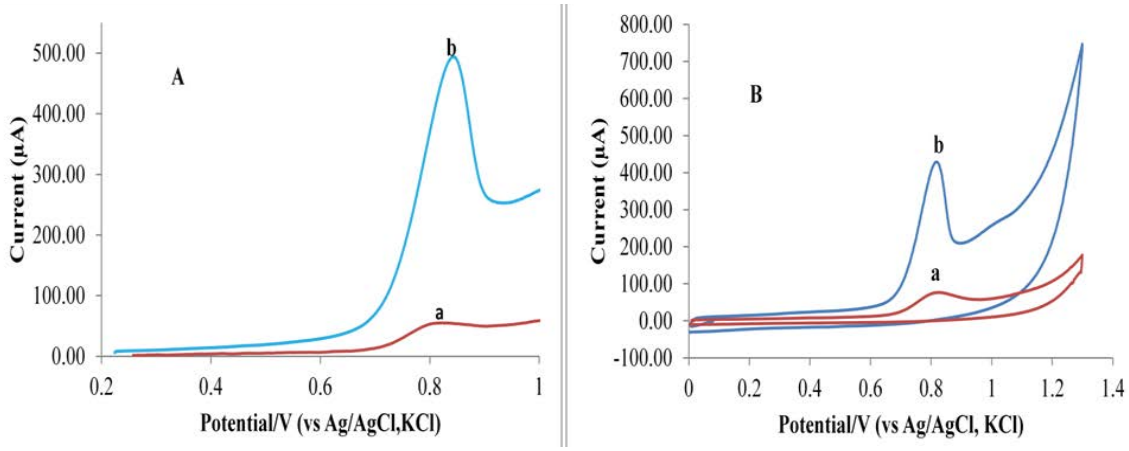


Figure 6: The SWV (A) and CV (B) of RAB Na⁺ at bare PGE (a) and poly (BCRG)/PGE (b).

Analytical parameters involved in the determination of RAB Na^+

The effect of pH

Britton-Robinson buffer compared to other supporting electrolytes produced better results. Therefore, B.R. buffer was chosen as a suitable supporting electrolyte. A well-defined oxidation peak was recorded at pH 7.0. The maximum potential (E_{pa}) was shifted to the less positive side with a pH increase. The optimal result with respect to the sensitivity was obtained at pH 7.0 (**Figure 7A**).

The effect of the scan rate on CV of RAB Na^+

Figure 7B shows the effect of the scan rate (υ) in the range of 20-120 mV s^{-1} on the oxidation peak of RAB Na^+. It was found that the logarithm of the peak oxidation current (log I) is linear to the logarithm of the scan speed (log υ), with the linear regression equation: log I=0.787 + 1.989 log υ. From the slope value, it can be deduced that the oxidation process of RAB Na^+ is a controlled diffusion adsorption with a contribution process [30].

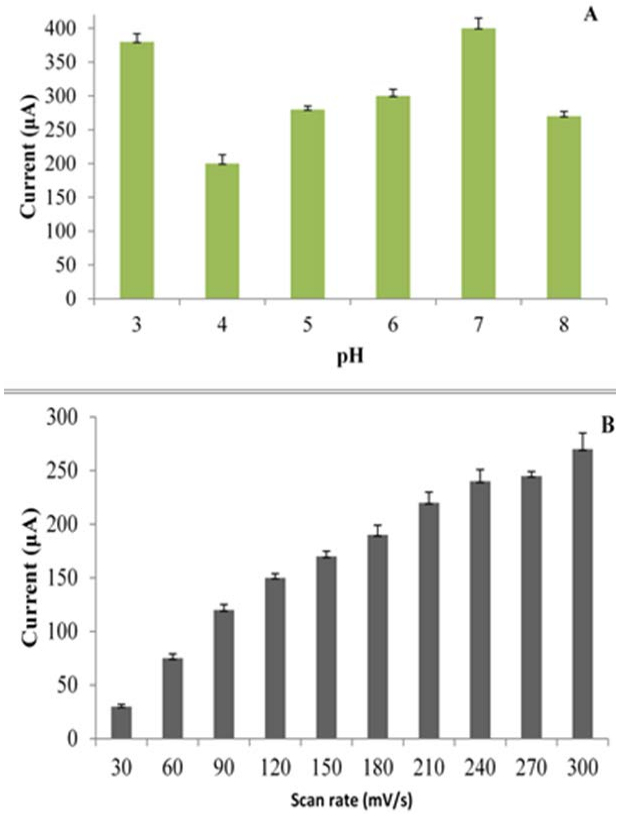


Figure 7: (A) The effect of pH on SWV of RAB Na^+: (1) pH=3.0, (2) pH=4.0, (3) pH= 5.0, (4) pH= 6.0, (5) pH= 7.0 and (6) pH= 8.0. B) The effect of scan rate on CV of RAB Na^+. Inset is a relationship between log scan rate and log current.

Method validation

The optimum conditions for the determination of RAB Na^+ using SWV were: adsorption potential=0.2 V, adsorption time=20 seconds, frequency=180 Hz, step potential=20 mV and pulse amplitude=55 mV while CV conditions were adsorption potential=0 V, adsorption time=20 seconds and scan rate=300 mVs^{-1}.

Linearity, LOD and LOQ

The spontaneous adsorption of RAB Na^+ on the surface of modified PGE can be exploited as a high sensitivity possibility for its determination because of the effective accumulation stage before its voltammetric measurement. **Figure 8** shows the linear relationship between the concentration and the anodic current. From **Table 1**, it is evident that CV and SWV methods are sensitive to the determination of RAB Na^+ in LOD and LOQ terms.

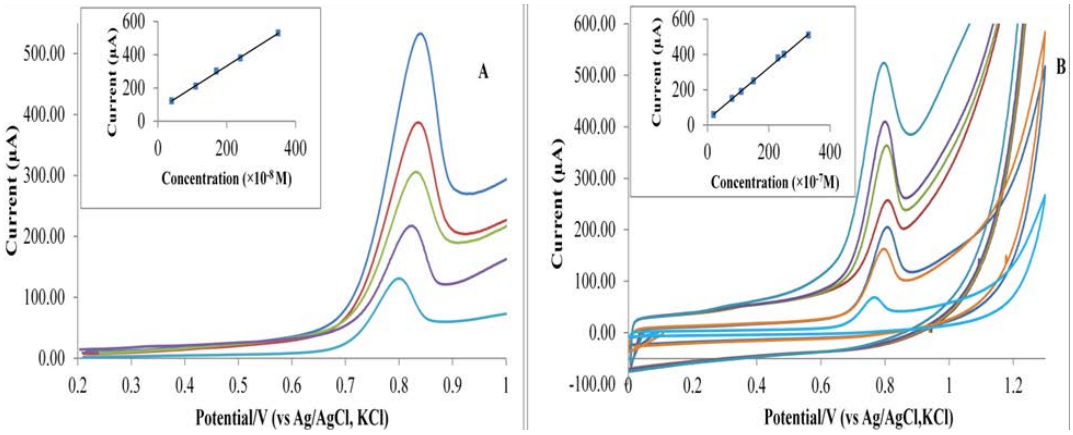


Figure 8: The effect of various concentrations of RAB Na^+ on SWV (A) and CV (B).

Table 1: The quantitative parameters of the proposed SWV and CV methods.

Analytical parameters	SWV	CV
Linearity range[a]	15-180	20-330
Correlation coefficient (r) ± SD*	0.9993 ± 0.02	0.9991 ± 0.01
Intercept (a) ± SD*	106.5 ± 2.1	31 ± 0.5
Slope (b) ± SD*	2.1 ± 0.02	1.5 ± 0.015
LOQ[a]	10	3
LOD[a]	3	1
[a]Concentration in SWV expressed as 10^{-8} M while in CV as $\times 10^{-7}$ M. *Average of five replicates.		

Accuracy and precision of the proposed platform

The accuracy of the proposed platform has been confirmed by the standard addition method and calculation of recovery percentages. In addition, the results of intra-day and intra-day precision demonstrate the reproducibility of the proposed methods (**Table 2**).

Table 2: Analysis of Rabicid® tablets by standard addition method and precision of the proposed methods.

Voltammetric method	Standard addition method			Precision		
	Amount added[a]	Found ± SD*	% Recovery ± SD*	Amount [a] taken	Inter-day	Intra-day
CV	50	51	102 ± 2	80	99 ± 1.5	100 ± 2
	100	98	98 ± 2	110	101 ±1	103 ± 2
	200	197	98.5 ± 1	140	98 ± 1	101 ± 1.5
SWV	50	50.5	101 ± 1.5	100	100 ± 1.5	100 ± 1
	80	81	101 ± 1.5	150	102 ± 1	101 ± 1.5
	120	116	97 ± 2	180	98 ± 2	100 ± 1
[a]Concentration in SWV expressed as 10^{-8} M while in CV as $\times 10^{-7}$ M. *Average of five replicates.						

Proposed platform selectivity

The effects of excipients, administered drugs, biological compounds and divalent cations were evaluated (**Table 3**). It is obvious that the % change in the RAB Na^+ signal after the addition of these potential interfering substances has not been significantly altered. This may indicate the selectivity of the method.

Table 3: The effect of potential components on the voltammetric response of RAB Na^+.

Common excipients		Biological active substances		Co-administered drugs		Divalent metals	
Amount (1 mM)	%signal change	Amount (1 mM)	%signal change	Amount (20 µM)	%signal change	Amount (0.3 µM)	%signal change
Starch	2.5	Ascorbic acid	3.5	Domperidone	5	Manganese	3
Glucose	3	Uric acid	4	Aceclofenac	3	Nickle	3

Gum acacia	4	Dopamine	2	Metronidazole	0.5	Copper	9
Lactose	5	-	-	Clarithromycin	1	Cadmium	6
Citric acid	2.5	-	-	Doxycycline	4	Zinc	4
-	-	-	-	-	-	Chromium	6

Real sample application

The proposed methods were applied for the determination of RAB Na^+ using the standard addition method in tablets (**Table 2**), urine samples and serum (**Table 4**) where the results obtained confirmed that there were no interferences with biological active compounds.

Table 4: Recovery of the proposed methods in human serum and urine.

Amount added[a]	Human blood serum				Human urine			
	SWV		CV		SWV		CV	
	Found	% Recovery ± SD*	Found	% Recovery ± SD*	Found	% Recovery ± SD*	Found	% Recovery ± SD*
50	48	96 ± 2	50.5	101 ± 2.5	52	104 ± 2	51	102 ± 1.5
100	103	103 ± 1.5	101	101 ± 2	102	102 ± 4	101	99 ± 3
130	133	102 ± 2	128	98.5 ± 1.5	132	101.5 ± 2	131	101 ± 2

[a]Concentration in SWV expressed as 10^{-7} M while in CV expressed as µM.
*Average of five replicates.

Modified electrode stability

The modified electrode stability built has also been studied in 0.1 M NaOH solution at room temperature and electrode measurements were measured for a period of 26 days. The electrode retained 95% of its original signal for the determination of RAB Na^+ for about three weeks. This revealed that there were no surface deterioration for a period of three weeks if stored in NaOH solution (0.1 M) (**Figure 9**).

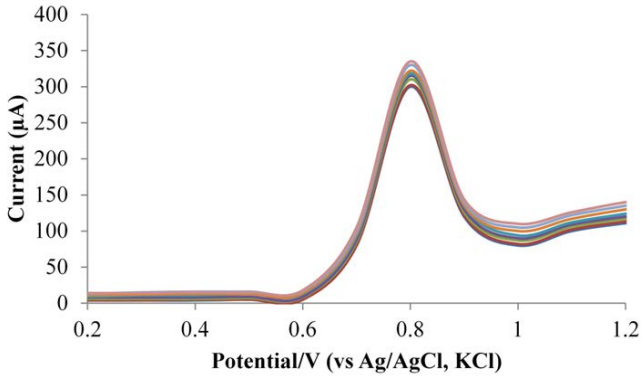


Figure 9: The stability of the sensor over 22 days for determination of RAB Na^+ by SWV.

Comparison with other reported methods

The newly developed method for determining RAB Na^+ was compared with other reported methods. Obviously, the developed methods had shown high sensitivity and applicability with respect to other methods (**Table 5**).

Table 5: Comparison between the proposed methods and reported methods.

Reported methods	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Reference
Spectrophotometry	10-30	0.019	0.058	[2]
	5-40	0.19	0.57	[3]
Spectrofluorometry	10-85	2.99	9.07	[4]
HPTLC	0.5-2.5	0.1	0.31	[2]
	4-20	0.025	0.076	[2]
HPLC	0.02-1.5	-----	0.02	[5]
	1-20	0.4	1	[6]
Differential pulse voltammetry				

SWV	0.06-0.7	0.01	0.04	This work
CV	0.76-12.6	0.03	0.1	

The proposed mechanism

The proposed oxidation mechanism in **Scheme 1** is based on the electrochemical data described and supported by the RAB Na^+ structure. The oxidation phase in the ortho position will be favored (by resonance) from stabilizing the radical size. The preferred attack is on the aromatic ring, where the degree of conjugation is greater, making it easier to eliminate an electron. Two electrons were removed, followed by deprotonation to produce a cationic radical, which reacts with water and leads to the formation of quinone species [31].

CONCLUSION

A modified pencil graphite electrode was successfully fabricated using the bromocresol green (BCRG). Poly (BCRG)/ PGE has good catalytic activity for RAB Na^+ oxidation. It could be seen that electrochemical oxidation was greatly improved due to the bromocresol green with respect to the bare electrode. The modified platform showed superior performance in peak current, linear field of operation, detection limit. The sensor was applied for the determination of RAB Na^+ in real samples such as pharmaceutical tables, human urine and blood serum samples. The selective determination of RAB Na^+ is possible in the presence of potentially interfering species since they do not alter the actual reaction of the drug. The proposed method is simple, precise and does not require any pre-treatment phase such as extracting an analyte from pharmaceutical and biological samples. In addition, the sensor offered minimal interference, good reproducibility and stability. The proposed method is efficient and more sensitive than other methods reported.

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