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Surfactant Based Voltammetric Analysis of Anti-ulcer Drug in Real Samples

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Abstract- The effect of adding surfactants to electrolyte containing rabeprazole sodium (RAB sodium) on its voltammetric response at pencil graphite electrode (PGE) was explored. The current signal due to the oxidation process as a function of the amount of the cited drug, pH of the medium, type of surfactant and accumulation time at the electrode surface was evaluated. The use of sodium dodecyl sulphate in the presence of Britton-Robinson buffer (pH=6.0) for the electrochemical determination of RAB sodium using cyclic voltammetry (CV) and Square wave adsorptive stripping voltammetry (SWAdSV) at PGE was studied. The oxidation peak current has varied linearly with the drug concentration over the range of 0.006-2.5×10^{-7} M and 0.5-250 µM using SWAdSV and CV, respectively. The limit of detection was found to be 0.2 nM and 0.18 µM using SWAdSV and CV, respectively. The validity of the proposed method for the determination of the studied drug in pure, pharmaceutical formulation in addition to urine was conducted.

Keywords- Rabeprazole sodium, Pencil graphite electrode, Surfactant, Pharmaceutical formulations, Urine

1. INTRODUCTION

Rabeprazole sodium (RAB sodium) is a member of benzimidazole class of drugs. It is an important benzimidazole derivative which used in the treatment of gastric and duodenal ulcers and reflux esophagitis [1]. Its efficacy as antiulcer and anti-secretory agent has been

well established. Few methods were used to determine RAB sodium such as spectrophotometry [2,3], spectrofluorometry [4], thin layer chromatography [2] and high performance liquid chromatography [2,5]. One electrochemical method [6] has been applied for the determination of the above mentioned drug in pharmaceutical dosage form. Electrochemical sensors satisfy many of the requirements for analysis, particularly owing to their simplicity of preparation, high selectivity and sensitivity, and fast response [7-11].The utilization of pencil leads as electrodes is well documented [12-19].The pencil "lead" is actually a mixture of graphite, wax and clay, the proportions of which impart different properties to the pencil with increasing amounts of clay making the pencil harder, hence the designation 'H'. Increasing the levels of graphite make the pencil softer, and their marks darker or black and so the designation 'B'. For example, one of the darkest generally available pencils, the 6B is 84% graphite, 10% clay and 5% wax, compared to the common, lighter HB, or No. 2 pencil which has a composition of 68% graphite, 26% clay and 5% wax. Commonly, pencils have been used directly as the working electrode itself [12-19]. Only recently the possibility of using pencils to draw electrodes onto a suitable substrate has been reported. This type of electrode offers high electrochemical reactivity, high electrical conductivity, good mechanical rigidity, low cost, simple technology, ease of modification, renewable, low background current, and miniaturization, the pencil graphite electrode (PGE) can be applied in the analysis of drugs and in the detection of traces of metal ions. This electrode has a larger active surface area and is therefore able to detect low concentrations of the analyte. Also, it has been shown that surfactants play a vital role in electrode reactions, not only in solubilizing organic compounds [20,21] but also by providing specific orientation of the molecules at the electrode interface. To the best of our knowledge no electrochemical method for the determination of RAB sodium using PGE and surfactant was reported. Therefore, the present work was undertaken to apply PGE and surfactant for the first time for voltammetric analysis of RAB sodium in pharmaceutical formulations and human urine.

2. EXPERIMENTAL

2.1. Pharmaceuticals

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

2.2. Reagents

Methanol, sodium dodecyl sulphate (SDS), tween 80, cetyltrimethylammonium bromide (CTAB), glacial acetic acid, phosphoric acid, potassium chloride and ascorbic acid,

potassium ferricyanide were purchased from El Nasr Pharmaceutical Chemicals Co., Egypt, Boric acid was purchased from El-Gomhouria Co., Egypt. Uric acid and dopamine was purchased from Sigma Aldrich, Germany.

Britton-Robinson buffer (B.R.) as a supporting electrolyte (equal volumes of 0.04 M acetic acid, 0.04 M phosphoric acid and 0.04 M sodium acetate, adjusted to a desired pH by 2 N NaOH).

2.3. Instrumentation

A Princeton VersaSTAT MC (VersaSTAT 3, Model RE-1,Princeton Applied Research, AMETEK, USA) connected to a three-electrode cell was used. In all measurements, the reference electrode was an Ag/AgCl (3 M KCl), the auxiliary electrode was a platinum wire and the working electrode was PGE.A Pentel pencil, Model P205 (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. Unless stated otherwise, the pencil was fixed vertically with 10 mm of the pencil lead exposed outside and 3 mm of the pencil lead immersed into the solution. Measurements were performed in a glass cell containing 6 ml of supporting electrolyte solution. Stirring was achieved with a magnetic stirring bar.

The pH values of solutions were adjusted using Hanna pH meter (Hanna Instruments Brazil, Sa˜o Paulo, Brazil) with a combined electrode. The solutions were sonicated using Bransonic ultrasonic cleaner, Branson UL Transonics Corporation, Eagle Road, Danbury, CT 06813, USA.

2.4. Preparation of standard solutions

An accurately weighed amount of each standard drug was transferred into a 100-mL calibrated flask, and dissolved in about 10-mL methanol. The solution was completed to the mark with distilled water to provide a stock solution containing 1.0 mM of RAB sodium. The working standard solutions were prepared by further dilution of the suitable aliquots of the stock solution with B.R. buffer (pH=6.0).

2.5. Sample preparation

The contents of ten tablets were accurately weighed, finely powdered and thoroughly mixed in a mortar. Portions equivalent to about 1.0 mM of each drug were accurately weighed and dissolved in 20 mL methanol. The contents were sonicated for 20 min to assure complete solubility. The excipients were separated by centrifugation at 3000 rpm for 5 minutes. The residue was washed three times with distilled water.

Drug-free human urine samples were obtained from two healthy and non-smoking volunteers of different age and sex. The samples were stored at –20 ˚C and analyzed on the next day after collection without any further pretreatment. One milliliter of the corresponding urine sample was pipetted into 25 mL calibrated flask and completed to volume with B.R. buffer (pH=6.0). Each measurement was performed using a new pencil surface in a voltammetric glass cell containing 6 mL urine solution mixed with B.R. buffer (pH=6.0). The CV and SWAdSV were recorded after addition of each drug. The experimental protocol was approved by the Institutional human Ethics Committee, Assiut University, Assiut, Egypt. Informed consents were obtained from human participants of this study.

2.6. General procedure

The PGE surface was polished and pre-treated by applying a potential of +1.3 V for 30 seconds in the blank supporting electrolyte without stirring in order to increase the hydrophilic properties of the electrode surface through introduction of oxygenated functionalities. Each measurement was performed using a new pencil surface in glass cell containing 6 mL of B.R. buffer (pH=6.0) and 4.5×10^{-5} M SDS as a blank. The SWAdSV and CV were recorded after drug addition.

The optimum conditions for the determination of RAB sodium using CV and SWAdSV were: 4.5×10^{-5}M SDS, pH of B.R. buffer=6.0, adsorption time=210 sec., scan rate=300 mV s^{-1}, frequency=240 Hz, step potential=15 mV, potential amplitude=45 mV, adsorption potential for CV=0.0 volt while SWAdSV=+0.3volts.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of PGE using standard potassium ferricyanide system

Prior to voltammetric analysis, the PGE was evaluated. The CV was recorded on PGE wetted with 0.5 M KCl where no voltammetric peaks were recorded. Thus, no electro-active interfering species were appreciably released by all graphite sticks. Further, the CV was recorded again after wetting PGE with 10 mM potassium ferricyanide in 0.5 M KCl where a redox peak potential difference was recorded as 75 mV. This shows the electro-activity of PGE.

Randles –Sevick equation for a reversible process [22] was used to estimate effective surface area of the PGE ($A_{eff.}mm^2$) immersed in 10 mM potassium ferricyanide and 0.5 M KCl.

$$I_{pa}= (2.69\times10^5)\, n^{2/3} A_{eff.}\, D^{1/2}\, v^{1/2}\, C^o$$

Where D and C^o are the diffusion coefficient and bulk concentration of the redox probe, respectively. The A_{eff} was calculated to be 16.2 mm^2 (Fig. 1).

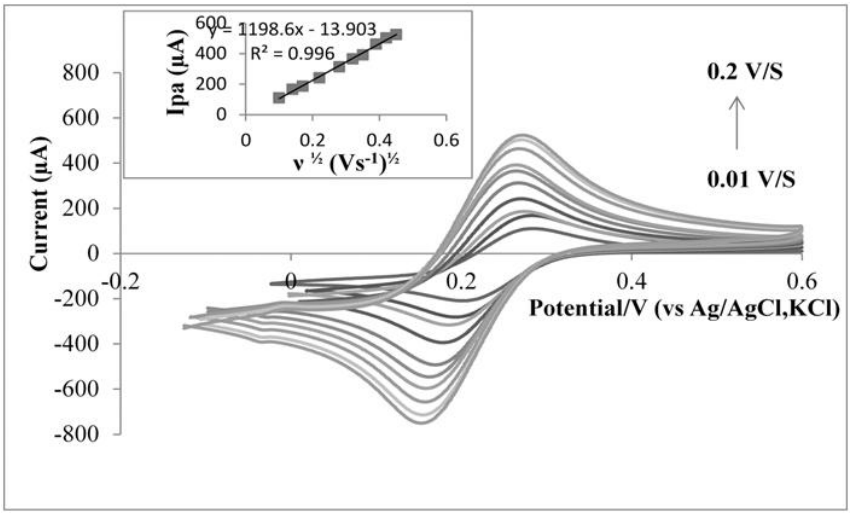


Fig. 1. The electroactive surface areas of the electrode evaluated by CV

3.2. Electrochemical behavior of RAB sodium using CV and SWAdSV

The electrochemical behavior of RAB sodium using SDS was investigated using three different types of carbon based electrodes, bare glassy carbon electrode (GCE), carbon paste electrode (CPE) and pencil graphite electrode (PGE). The best results were obtained with PGE (Fig. 1S).

3.3. Parameters affecting electrochemical oxidation process

3.3.1. The effect of pH

Within the range of pH 3.0–11.0, B.R. buffer was superior to other supporting electrolytes. Hence, B.R. buffer was chosen as a supporting electrolyte throughout this work. Sharp oxidation peaks were recorded only in the pH range 4.0-9.0 (Fig. 2). Lower than pH 4.0, the oxidation peak was not observed. With increasing the pH of the solution, the oxidation peak current increased up to pH 6.0 and then decreased continuously until pH 9.0. The peak potential was shifted towards less positive side with increasing pH, suggesting the involvement of protons in the oxidation reaction of RAB sodium. From the plot of the potential versus pH, the regression equation of RAB sodium can follow the following expression: E_{pa}=-0.07pH+1.39 (R^2=0.9915)

The optimum result with respect to sensitivity accompanied with sharper response was obtained at pH=6.0, so that this pH was selected for further experiments.

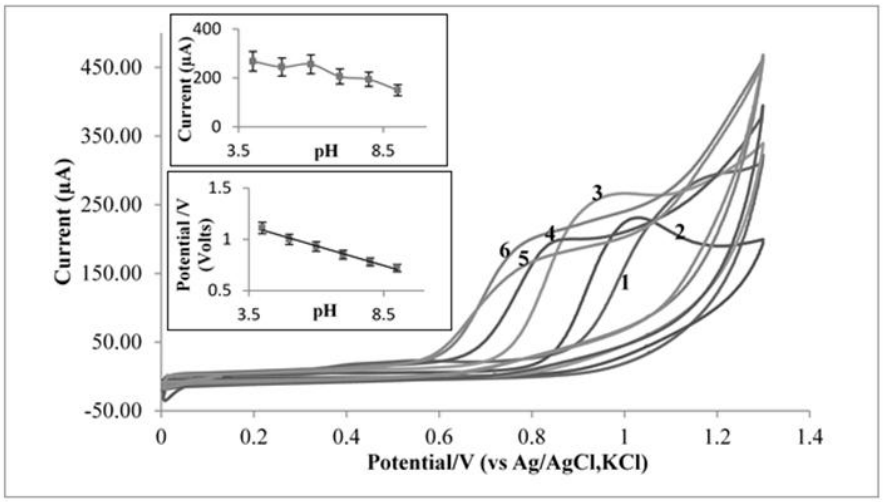


Fig. 2. The CV recorded on the PGE for 150 µM RAB sodium in B.R. buffer at (1) pH=4.0 , (2) pH=5.0, (3) pH=6.0, (4) pH=7.0, (5) pH=8.0 and (6) pH=9.0

3.3.2. The effect of surfactant

The effect of different types of surfactants using PGE at pH=6.0 on the CV of the RAB sodium was studied. A comparison between the effects of 4.5×10^{-5} M CTAB, SDS and tween 80 on the oxidation of RAB sodium was presented in (Fig. 2S). It is obvious that the anionic surfactant, SDS, has produced the maximum enhancement of the oxidation peak current. At pH 6.0, protonation of (N) in benzimidazole and pyridine rings leads to formation of cationic species. Accordingly, SDS would be adsorbed onto electrode surface forming a negatively charged hydrophilic film with the polar head group pointing to the bulk of the solution. This negatively charged hydrophilic layer facilitates reaching of RAB sodium to the electrode surface faster, and as a consequence, the reaction becomes easier and faster. It was found that optimum concentration of SDS that produced highest peak current was 4.5×10^{-5}M.

3.3.3. The effects of initial deposition potential and time

The effect of adsorption potential and time on the anodic peak current of RAB sodium was studied at various adsorption potentials between +0.3 V and +1.3 V for SWAdSV and 0 to +1.3 for CV in B.R. Buffer (pH 6.0) in presence of 4.5×10^{-5} M of SDS. The maximum peak current was obtained at adsorption potential of + 0.3 V and after 210 seconds for SWAdSV and at 0.0 V after the same time for CV.

3.3.4. The effect of scan rate on CV

In order to investigate the reaction mechanism of RAB sodium, the effect of scan rate was investigated and shown in (Fig. 3) The peak current (I_{pa}) varied linearly with the scan rates

(v) in the range of 30 to300 mV s^{-1} with the linear equation of: log I=0.641 log υ+0.843, r (correlation coefficient)=0.995.This has confirmed a typical adsorption controlled process. For an adsorption-controlled electrode reaction, the following equation could apply [23]:

$$Ipa = \frac{nFQv}{4RT}$$

Where, Q is the peak area that could be obtained under a given scan rate, n is the scan rate, and F, R and T are the constants. From the slope of I_{pa} *vs.* ν, the electron-transfer number (n) that was involved in the electrode reaction of RAB sodium was calculated to be 2.

3.4. Method validation

3.4.1. Linearity, LOD and LOQ

Figs 4A and 4B have shown linear calibration plots for the proposed SWAdSV and CV methods, respectively. Quantitative parameters for the proposed methods were listed in (Table 1) for RAB sodium which shows good values of the correlation coefficient (r) and low value of LOD and LOQ.

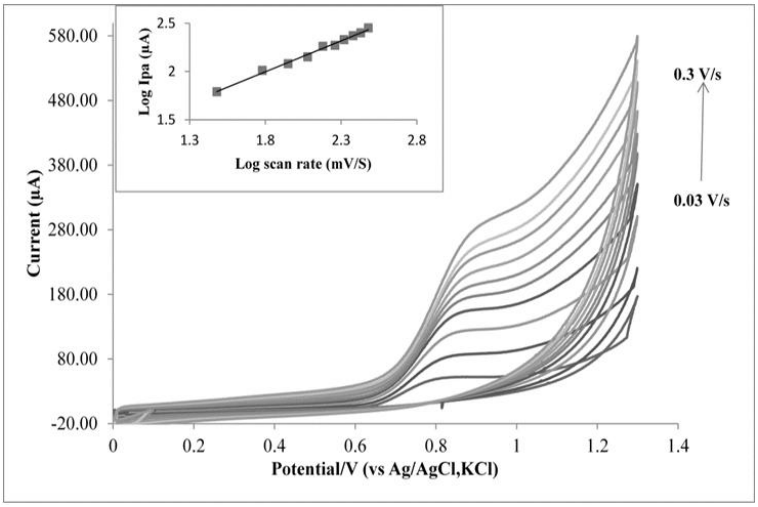


Fig. 3. The relationship between scan rate and I_{pa} for 150 µM RAB sodium. Insets are the linear relationship of log I_{pa} *vs*. log υ and E_{pa} *vs*. log υ

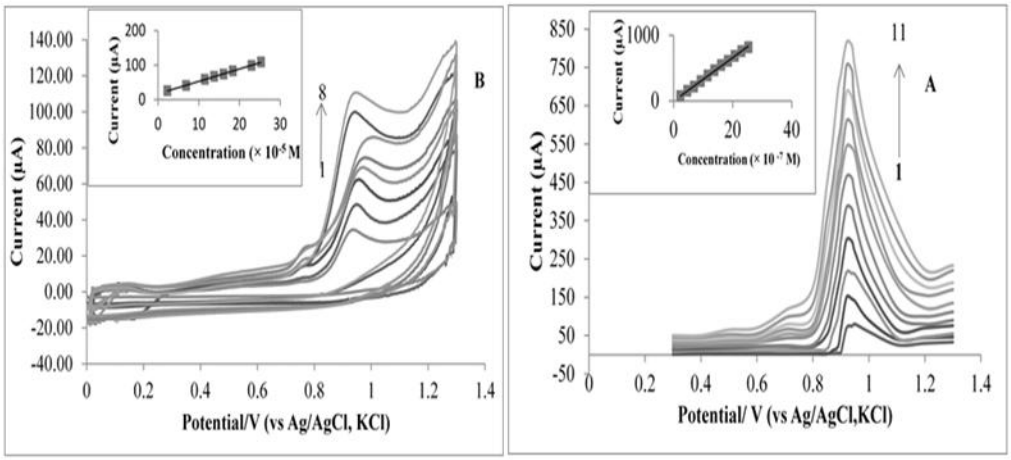


Fig. 4. The effect of different concentrations of RAB sodium on SWAdSV (A) (2-25×10^{-7} M) (1-11) and CV (B) (20-250 µM) (1-8) in B.R. buffer (pH=6.0) containing 4.5×10^{-5} M SDS. Insets are calibration curves

Table 1. The quantitative parameters of the CV and SWAdSV for RAB sodium in pure form

Parameters	**CV**	**SWAdSV**
Measured potential (volts)	0.910	0.915
Linearity [a]	20-250	2-25
Correlation coefficient (r± SD*)	0.9996± 0.001	0.9993±0.008
Intercept (± SD*)	17.1±0.2	0.7± 0.02
Slope (± SD*)	3.6±0.01	32.9± 0.08
LOD[b]	0.18	0.002
LOQ[c]	0.5	0.006

* Average of five replicates
[a] Linearity of SWAdSV expressed as 10^{-7} M while of CV expressed as µM
[b] Limit of detection of SWAdSV expressed as 10^{-7} M while of CV expressed as µM
[c] Limit of quantitation of SWAdSV expressed as 10^{-7} M while of CV expressed as µM

3.4.2. Accuracy and precision

The accuracy of the methods was determined by analysis of dosage form and calculating the recovery percentage. The results of the inter-day and intra-day precision of the proposed methods were ranged from 97.9-103.4% (±0.9-2.2%) and 97.1-103.2% (0.6-2.2%) for SWAdSV and CV, respectively. The inter-day and intra-day precisions were evaluated through replicate analysis of the studied drugs. The precision of the proposed methods was fairly high as indicated by the low values of %RSD.

Table 2. Assay of the studied RAB sodium in its pharmaceutical dosage form by proposed voltammetric methods

Pharmaceutical formulation	**CV % Recovery± SD***	**SWAdSV % Recovery± SD***	**Reported method**	**Student t-test**		**F-test**	
				CV	**SWAdSV**	**CV**	**SWAdSV**
Rabicid® tablets	99.6± 1.4	101.3± 1.2	99.3± 1.5	1.3	1.7	5.5	5.2

*Number of replicates was five determinations
**Tabulated F and t values at 95% confidence level: t-value=2.77 while F- value= 6.39

3.4.3. Selectivity of the method

The effects of common excipients, co-administered drugs, biologically active compounds and divalent metals were evaluated (Table 3). Clearly, the % signal change of RAB sodium upon addition of these potential interfering substances have not changed appreciably. This could indicate the selectivity of the method and hence its suitability for the determination of pharmaceuticals in complex matrices.

Table 3. The influence of potential components on the voltammetric response of RAB sodium

Common excipients		**Biological active substances**		**Co-administered drugs**		**Interfering cations**	
Amount (1 mM)	%signal change	Amount (1 mM)	%signal change	Amount (20 µM)	%signal change	Amount (0.3 µM)	%signal change
Starch	2.45	Ascorbic acid	2.66	Domperidone	5.03	Manganese	2.22
Glucose	2.09	Uric acid	3.22	Aceclofenac	1.90	Nickle	3.55
Gum acacia	3.98	Dopamine	2.00	Metronidazole	0.05	Copper	8.75
Lactose	4.12			Clarithromycin	1.22	Cadmium	5.43
Citric acid	2.33			Doxycycline	2.43	Zinc	6.55
						Chromium	3.22

3.4.4. Application to pharmaceutical dosage forms

The proposed methods were applied for the determination of the RAB sodium in tablets (Table 2). The results were compared with the other reported method [6]. The results of the proposed method were found to be comparable with those of the reported method as indicated by t-and F- tests.

3.4.5. Application to spiked urine sample

In order to evaluate the validity and practical applicability, the proposed methods were applied for the determination of the studied drugs in model human urine samples. The urine samples were collected from two healthy and non-smoking volunteers of different age and sex. The samples were analyzed by the proposed methods after simple dilution with B.R. buffer, pH=6.0. The recovery values have revealed the accuracy of the proposed methods and demonstrated the fact that there were no significant matrix interferences present in the model human urine samples (Table 4). Moreover, the highly positive oxidation potential of analyte in comparison with the possible interfering compounds has allowed a reliable quantitation of the RAB sodium under study.

Further, potential interference from biologically active reducing agents, ascorbic acid, uric acid and dopamine was explored.

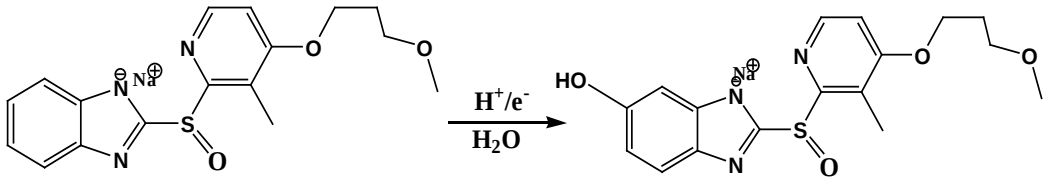
Table 4. The recovery analysis of RAB sodium in spiked human urine samples

Drug added*	SWAdSV				CV			
	Volunteer A		Volunteer B		Volunteer A		Volunteer B	
	Found**	Recovery%	Found**	Recovery%	Found**	Recovery%	Found**	Recovery%
50	48	96.0±1.7	51	102.0±1.4	48	100.0±0.6	49	98.0±1.2
140	143	102.1±1.4	141	100.7±1.2	139	99.3±1.0	143	102.4±1.4
240	230	95.8±0.9	236	102.7±0.7	238	99.2±0.9	241	100.4±1.8

* Concentration expressed as 10^{-7} M for SWAdSV and µM for CV
** Average of five replicates

3.5. Reaction mechanism

The suggested electrochemical oxidation of the studied RAB sodium by CV and SWAdSV is described in Scheme (1). This is based on the electrochemical data described, and supported by the RAB sodium structure, with two systems that are in resonance and separated by a methylene group.



Scheme 1. The proposed oxidation mechanism for RAB sodium

Because the benzimidazole moiety is planar, the first oxidation step in the ortho position would be favored, via resonance, by the stabilization of the radical formed. Preferential attack

is in the aromatic ring, where the extent of conjugation is greater, making removal of an electron easier. Two electrons are removed, followed by deprotonation to produce a cation radical, which would react with water and lead to the formation of hydroxylated species [24].

To raise the analytical performance of the proposed methods, a comparison with other reported electrochemical methods were conducted (Table 5). Clearly, the proposed method is simple with comparable and even more sensitive in terms of LOD and LOQ than the other reported methods.

Table 5. Comparison between the proposed methods and reported methods

Reported methods	**Linear range (µg/mL)**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**Ref.**
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]
HPTLC	4-20	0.025	0.076	[2]
	0.02-1.5	-----	0.02	[5]
Differential pulse voltammetry	1-20	0.4	1.0	[6]
SWAdSV	0.076-0.95	7.6×10^{-5}	2.28×10^{-4}	**This work**
CV	7.6-95.0	0.068	0.19	

4. CONCLUSION

In this study, PGE was applied for the first time as a simple and sensitive electrochemical sensor for rapid and direct determination of RAB sodium. The CV and SWAdSV were used for deeper investigation of the electrochemical behavior of RAB sodium with their quantification in pharmaceutical dosage forms and human urine. The proposed method is considerably time saving, economic and highly sensitive without any chemical modification of electrode surface. The method is sufficiently selective because common excipients, urine constituents have not interfered. These simple, rapid and inexpensive procedures could be a good alternative to other analytical approaches for pharmacokinetic and pharmacodynamic purposes. Taking into account the results obtained in this study, PGE would have future applications as a sensitive electrochemical sensor in drug analysis.

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