

Development of Square Wave Voltammetric Method for the Electrochemical Determination of Pregabalin as Pregabalin-Cu(II) Complex Using Graphite Electrode

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Abstract

In the present study, a square wave voltammetric (SWV) method was developed for the determination of pregabalin (PREG) in commercial drugs and biological samples as PREG-Cu(II) complex using graphite working electrode. A well-defined voltammetric signal of the complex was observed at nearly – 200 mV, by using Britton Robinson supporting electrolyte (B-R) at pH 7.5. There were some analytical conditions that have been evaluated through this research such as accumulation time and potential, scan rate, amplitude, frequency, and stirring rate to obtain a good voltammetric peak and high current without any overlap. In addition, some metal ions as potential interferences; such as Zn(II), Ni(II) and Cd(II) have been added at different concentrations to evaluate their effects on the voltammetric signal for PREG-Cu(II) complex. The SWV performance was investigated by monitoring parameters such as calibration curve, detection limit, reproducibility, stability and recovery. The calibration curve was studied over the range 5.0×10^{-8} – 1.0×10^{-6} mol L⁻¹ of (PREG) and 5.0×10^{-5} mol L⁻¹ of copper ion (n=8, $r^2 = 0.99$), yielded a linear relationship between voltammograms and complex concentrations. The sensitivity of SWV method was evaluated by calculating the detection limit which was found to be 6.5×10^{-9} mol L⁻¹. The reproducibility, stability and recovery were studied for 5.0×10^{-7} mol L⁻¹ of (PREG) and 5.0×10^{-5} mol L⁻¹ of copper ion, yielded a relative standard deviation (RSD%) that was calculated to be 0.4% and good stability for the voltammetric signal, while the recovery was recorded to be $93 \pm 1.23\%$. The analytical voltammetric technique (SWV) was applied to determine PREG-Cu(II) complex in pharmaceutical capsules and biological fluids, specifically urine and plasma, where it gave recoveries of $90 \pm 1.0\%$, $89 \pm 1.23\%$ and $86 \pm 1.23\%$, respectively

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Received 30Nov 2016,

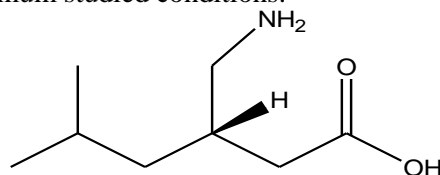
Revised 10Dec 2016,

Accepted 17Dec 2016

Keywords: Square wave voltammetry, pregabalin, graphite electrode, complexes, biological samples, interferences

1. INTRODUCTION

Pregabalin is known as β -isobutyl- γ -Aminobutyric acid (β -isobutyl-GABA) and it is sold under the trade name "Lyrica". It has been most used most commonly to treat epilepsy, neuropathic pain, fibromyalgia and generalized anxiety disorder. Its use for epilepsy is as an add-on therapy for partial seizures with or without secondary generalization in adults. The common side effects of pregabalin include: sleepiness, confusion, trouble with memory, poor coordination, dry mouth, problem with vision and weight gain. While potentially serious side effects include angioedema, drug misuse, and an increased suicide risk. The IUPAC nomenclature of Pregabalin is (3S)-3-(Aminomethyl)-5-methyl hexanoic acid [1,2] while its structure formula is illustrated as in Scheme 1. Pregabalin was studied and determined by several published articles using different analytical techniques such as HPLC [3-7], LC-MS [8-10], spectrophotometry [11-14], and electrochemistry [15,16]. There were many metal ions and organic compounds that were determined using square wave voltammetric method such as the determinations of eight heavy metals in soil, cadmium in natural water, and Cd, Pb and Ni in particulate matter collected in workplace atmosphere [17-19], acetylsalicylic acid and rutin in pharmaceutical formulations, 2-thiouracil in pharmaceuticals and real samples, captopril in reconstituted serum and pharmaceutical formulations, doxorubicin hydrochloride, anti-inflammatory drug meloxicam, anticancer drugs emodin and irinotecan, psychoactive 1,4-benzodiazepine drugs, dye marker, solvent blue 14, in kerosene and fuel alcohol, and eosin-Y dye [20-28]. The present research aims to optimize the experimental parameters for the developed square wave voltammetric method used for the determination of pregabalin as a complex with copper ion. The developed method (SWV) was successfully used to determine the pregabalin-Cu(II) complex in pharmaceuticals and biological fluids. There are no published articles that were reported for pregabalin determination using the same procedure under the optimum studied conditions.



Scheme 1. Structural formula of pregabalin

2. EXPERIMENTAL

2.1 Methods

Square wave voltammograms were obtained by 797 VA Metrohm (made in Switzerland) and controlled by computrace 2.0 software. All voltammograms were printed on a hp color LaserJet CP1215 printer (made in China). The square wave voltammetry approach used three electrode mode: graphite working electrode, Ag/AgCl reference electrode and platinum auxiliary electrode (Metrohm, made in Switzerland). Hanna instrument pH211 (made in Romania) was used to measure pH values. Micropipette (made in Ireland) was used to inject micro-liters of copper and pregabalin solutions. A labofuge 200 instrument (Heraeus Sepatech, made in Germany) was used for centrifuging pregabalin complex when it was determined in urine and plasma samples.

2.2 Reagents and solutions

2.2.1 Standard PREG Solution

Pregabalin standard was obtained from Qassim Pharmaceutical Plant, Buraydah, Spimaco Addwaeih- KSA. A stock solution of PREG (1×10^{-2} mol L⁻¹) was prepared by dissolving 0.0398 g PREG in 25 ml volumetric flasks with distilled water. This stock solution was used to prepare low concentration solutions by dilution with distilled water. Stock solutions were stored in the dark and at room temperature.

2.2.2 Supporting electrolyte and Cu(II) Solutions

The supporting electrolytes such as Britton-Robinson, phosphate, acetate and carbonate were prepared to evaluate the analytical parameters for the analysis of pregabalin drug[28]. A stock solution of copper ion was prepared by dissolving a suitable weight from $\text{CuSO}_4 \cdot 2\text{H}_2\text{O}$ in distilled water to obtain $1 \times 10^{-2} \text{ mol L}^{-1}$ of Cu(II), then it was diluted using distilled water again to obtain other copper solutions with different concentrations. The NaOH and HCl solutions (1.0 mol L^{-1}) were prepared to adjust the pH values to alkaline, acidic and neutral media by using supporting electrolyte solutions for the PREG-Cu(II) determination.

2.3 Procedures

2.3.1 Measurement procedure

Into the electrochemical cell, 10 ml of supporting electrolyte would be added immediately following the additions of PREG and Cu(II) solutions. Thereafter, these solutions were purged with nitrogen gas for 100 sec to remove oxygen. Then, the studied solutions were stirred during the measurement period. The cathodic scan was applied over the range $0.0 - -800 \text{ mV}$ for PREG-Cu(II) measurements at room temperature. The square wave voltammetric measurements were carried out using 0.0 V accumulation potential (E_{acc}), 30 sec accumulation time (t_{acc}), 20 Hz frequency, 10 mV amplitude potential and 100 mV s^{-1} scan rate for the determination of PREG-Cu(II) complex.

2.3.2 Preparation of lyrica capsules and biological fluids

Five capsules of "Lyrica® 300 mg pregabalin (Pfizer U.S. Pharmaceuticals Group)" were weighted using an analytical balance. Next, a $1 \times 10^{-3} \text{ mol L}^{-1}$ PREG solution was prepared by dissolving the appropriate weight of capsules in distilled water using a 50 ml volumetric flask. For the preparation of the biological samples, 1.0 ml of 5.0% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1ml of NaOH and 1.0 ml of ethanol were added to 0.5 ml of urine and plasma [29], in a centrifuge tube. Then the resulting solution was centrifuged 5000 rounds per minute for 8 min. Finally, the required concentrations of PREG and Cu(II) were added to the prepared biological samples, followed by the voltammetric procedure.

3. Results and discussion

3.1 Preliminary voltammetric study of PREG – Cu(II) complex

Pregabalin (PREG) was initially studied using SWV technique at different concentrations, and yielded no electrochemical reduction signal as anticipated (see figure 1- A).

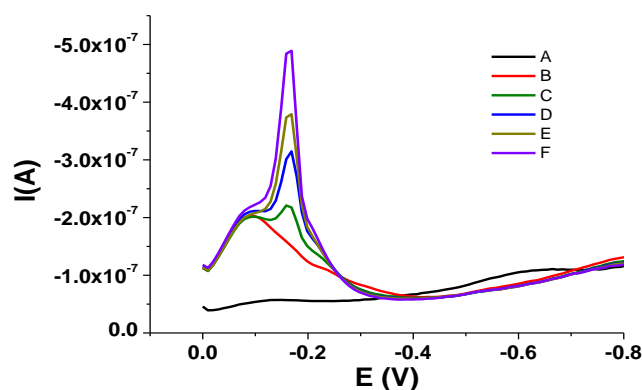
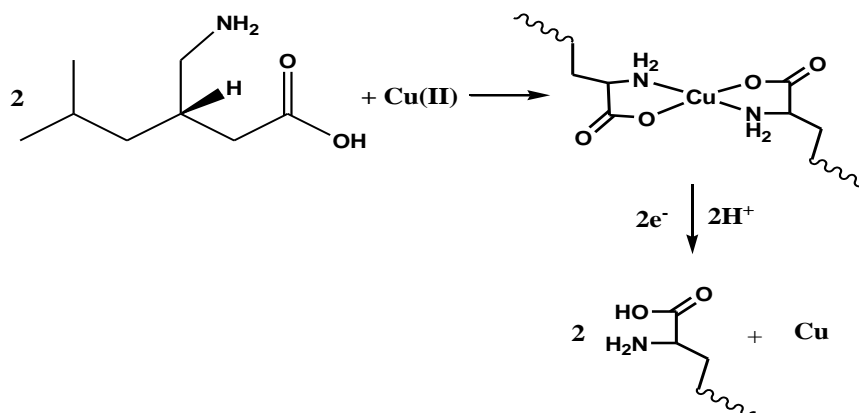


Fig. 1. Study of electrochemical behavior for pregabalin (PREG) with Cu(II): (A: 5×10^{-6} PREG, B: 3×10^{-5} Cu(II) + A, C: 8×10^{-6} PREG + B, D: 3.5×10^{-5} Cu(II) + C, E: 1.1×10^{-5} PREG + D, F: 4×10^{-5} Cu(II) + E, mol L^{-1}) at B-R supporting electrolyte, pH7.5, 0.0 V E_{acc} , 30 sec t_{acc} , 20 Hz, 100 mVs^{-1} and 10 mV amplitude potential

On the other hand, the copper ion displayed an electrochemical current at $E_{\text{red}} = -150$ mV (see figure 1-B). Upon mixing of the latter with the PREG solution, a complex formed as evident by the shift of the reduction peak to $E_{\text{red}} = -200$ V (see figure 1-C). It is noted that the current gradually increased by additions of PREG and Cu(II), respectively, as shown in figure 1 –D,E,F. The cathodic voltammetric responses were thought to result from the reduction process of PREG-Cu(II) complex which formed after alternating additions between PREG and copper ion. The proposed mechanism for the cathodic reduction is illustrated in Scheme 2.



Scheme 2. Proposed mechanism for the cathodic reduction of pregabalin – Cu(II) complex

3.2 Study of analytical conditions

In the current work, the electrochemical behavior was evaluated by examining analytical parameters to improve sensitivity and selectivity of the used technique and to obtain a high current with good shape. Certain analytical parameters that were studied gave overlaps and bad electrochemical signals shapes for PREG-Cu(II), and could not be reported. The following analytical parameters were used to obtain a high current and a good cathodic signal for the determination of PREG-Cu(II): 0.0 V E_{acc} , 30 sec t_{acc} , 100 mVs $^{-1}$, 20 Hz, 10 mV amplitude and 2000 rpm. Finally, several metals such as Zn, Pb, Cr, Ni, Al and Fe and various electrodes such as mercury, GC and Au; were used to study PREG electrochemical behaviour but exhibited no electrochemical signal or complex formation with PREG.

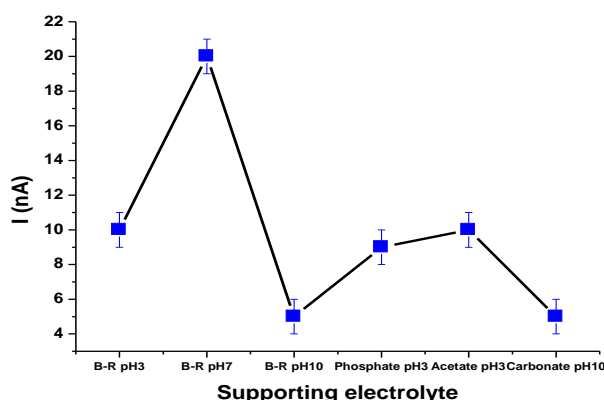


Fig. 2. Effect of supporting electrolyte on the voltammetric current of pregabalin – Cu(II) complex at 0.0 V E_{acc} , 30 sec t_{acc} , 20 Hz, 100 mVs $^{-1}$ and 10 mV amplitude potential

These conditions were selected as optimum parameters for all voltammetric measurements of PREG-Cu(II) onto the surface of graphite working electrode. While the study of effect of supporting electrolytes such as B-R, phosphate, acetate and carbonate with pH 3, 7 and 10, gave a high voltammetric response by using B-R supporting electrolyte with pH7 as shown in figure 2. On the other hand, pH was studied over the range 6 – 8 whereby a high current was

observed at pH 7.5 (see figure 3). So B-R pH 7.5 was chosen as the optimum parameter for further work. The effect of copper concentration was also evaluated over the range 1.5×10^{-5} to 4.5×10^{-5} mol L⁻¹ of Cu(II) with 5×10^{-6} mol L⁻¹ of PREG as shown in figure 4. This effect confirms a complex formation between copper ion and pregabalin drug.

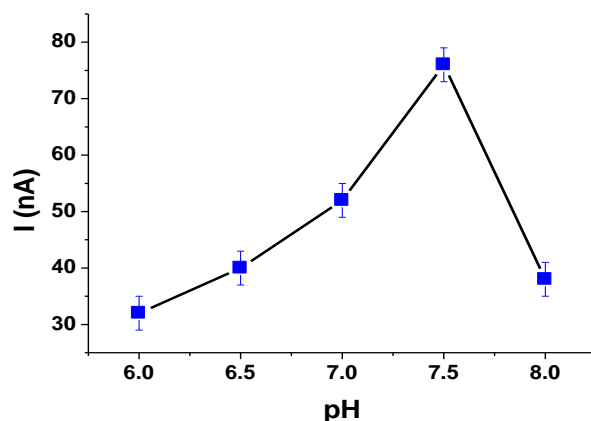


Fig. 3. Effect of pH on the voltammetric current of pregabalin – Cu(II) complex at B-R supporting electrolyte, 0.0V E_{acc} , 30 sec t_{acc} , 20 Hz, 100 mVs⁻¹ and 10 mV amplitude potential

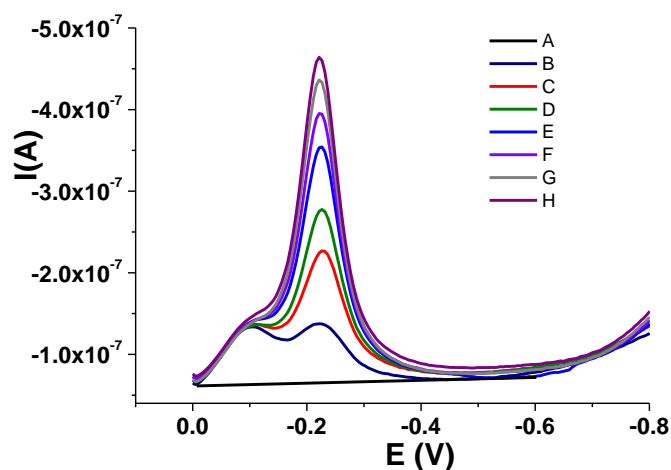


Fig. 4. Effect of copper concentrations on voltammetric current of PREG-Cu(II) complex, where: (A: 5×10^{-6} PREG, B: 1.5×10^{-5} Cu(II), C: 2×10^{-5} Cu(II), D: 2.5×10^{-5} Cu(II), E: 3×10^{-5} Cu(II), F: 3.5×10^{-5} Cu(II), G: 4×10^{-5} Cu(II), H: 4.5×10^{-5} Cu(II), mol L⁻¹) at B-R supporting electrolyte, pH7.5, 0.0 V E_{acc} , 30 sec t_{acc} , 20 Hz, 100 mVs⁻¹ and 10 mV amplitude potential

3.3 Validation of SWV method

3.3.1 Calibration curve

A linear relationship between SWV current and PREG-Cu(II) concentration was obtained over the range 2×10^{-7} – 1.6×10^{-6} mol L⁻¹, with Cu(II) concentration fixed at 5×10^{-5} mol L⁻¹; as shown in figures 5 and 6. Least square equation was used to evaluate calibration graph as shown in the following form:

$$I_{red} \text{ (nA)} = 1.85 \times 10^8 C + 184 \quad r^2 = 0.99, \quad n = 8$$

Where I_{red} is SWV current in nanoamperes (nA), C is PREG-Cu(II) concentration in mol L⁻¹, r^2 is the correlation coefficient and n is the cathodic measurements number.

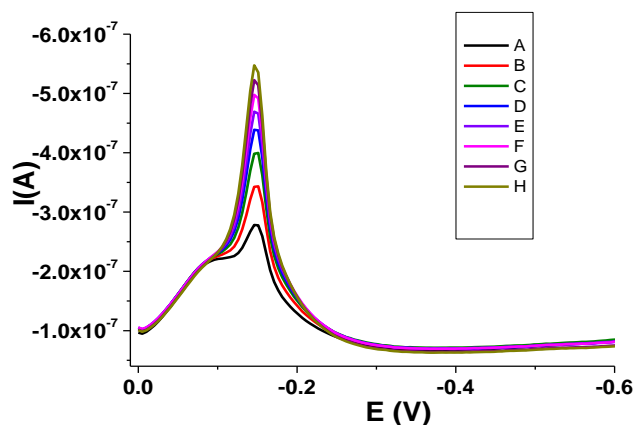


Fig. 5. Square wave voltammograms for PREG- Cu(II) complex over the range 2×10^{-7} to 1.6×10^{-6} mol L $^{-1}$ with 5×10^{-5} mol L $^{-1}$ Cu(II) at B-R buffer; pH 7.5, 0.0 V E_{acc} , 30 sec t_{acc} , 20 Hz, 100 mVs $^{-1}$ and 10 mV amplitude potential; where the PREG concentrations $2, 4, 6, 8 \times 10^{-7}$; $1, 1.2, 1.4, 1.6 \times 10^{-6}$ mol L $^{-1}$ for A, B, C, D, E, F, G, H, respectively

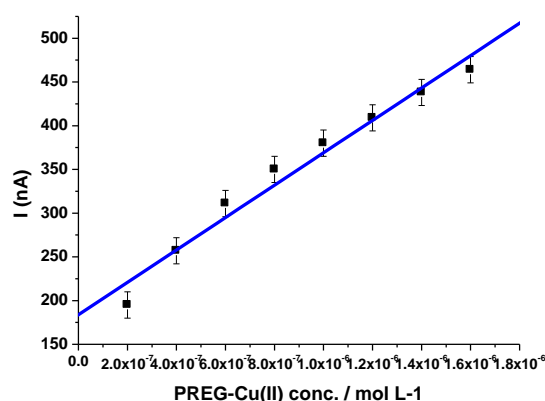


Fig. 6. Calibration curve of SWV for PREG- Cu(II) complex over the range 2×10^{-7} to 1.6×10^{-6} mol L $^{-1}$ with 5×10^{-5} mol L $^{-1}$ Cu(II) at B-R buffer; pH 7.5, 0.0 V E_{acc} , 30 sec t_{acc} , 20 Hz, 100 mVs $^{-1}$ and 10 mV amplitude potential

3.3.2 Detection limit ($C_{D,L}$)

The detection limit has been found by the equation: $3 \times \text{Standard deviation} / \text{Calibration curve slope (3S/m)}$. In this article, it was calculated to be 6.5×10^{-9} mol L $^{-1}$ (1.0335 ppb) under optimum conditions. This detection limit had high sensitivity compared to ion selective electrochemical method that was written 2.23×10^{-7} mol L $^{-1}$ (35.51 ppb) [15] and other methods which were reported for the determination of PREG by HPLC and spectrophotometry with $C_{D,L} = 8.10$ and 6 ppm, respectively [6, 12] as summarized in table 1.

3.3.3 Reproducibility, stability and recovery

The reproducibility of 5×10^{-6} mol L $^{-1}$ PREG with 5×10^{-5} mol L $^{-1}$ Cu(II) was evaluated from ten repeated measurements ($n=10$), yielded ± 1.55 SD and 0.4 % RSD. The same concentration was electrochemically monitored every 10 min for two hours which clearly confirmed a good stability for the complex current. In addition, accuracy of the developed SWV was investigated by study of the recovery approach for 5×10^{-7} mol L $^{-1}$ PREG with 5×10^{-5} mol L $^{-1}$ Cu(II) under optimum analytical conditions, yielded a good recovery of $93 \pm 1.23\%$.

Table 1. Analytical Comparison between the developed SWV technique and other methods

	<i>The developed SWV</i>	<i>Ion selective electrochemical Method^[15]</i>	<i>Spectrophotometry^[12]</i>	<i>HPLC^[6]</i>
Working Electrode	Graphite	ISE (PVC sensor)	-	-
Solvent Used	H ₂ O	0.1 N HCl	Phosphate pH7.4	H ₂ O
Supporting Electrolyte	B-R	B-R	-	-
Conc. Range (mol L⁻¹)	2×10^{-7} – 1.6×10^{-6}	1×10^{-6} – 1×10^{-5}	5×10^{-5} - 1×10^{-3}	5×10^{-5} - 3×10^{-3}
Detection Limit (ppb)	0.001	35.51	6000	8100

3.3.4 Interferences study

There are several ionic metals that could compete with the PREG-Cu(II) signal on the surface of graphite working electrode. These include but are not limited to Zn(II), Ni(II) and Cd(II). These metals were added in different concentrations (1 and 5 times) higher than PREG – Cu(II) complex's concentration (5×10^{-6} PREG and 5×10^{-5} Cu(II) mol L⁻¹). The addition of Zn(II) decreased the SWV current by about 36.4% and 73.3% respectively. While the addition of Ni(II) lowered the SWV current by about 30% and 58% respectively. Finally, the addition of Cd(II) decreased the SWV current by about 52% and 77% respectively, for the original voltammetric signal of the PREG-Cu(II) complex.

3.4 Square wave voltammetric applications

3.4.1 SWV applications in pharmaceutical capsules analysis

The electrochemical technique (SWV) was applied for the determination of PREG – Cu(II) complex in commercial capsules (Lyrica® 300 mg pregabalin, Pfizer, US). This application was carried out under the selected optimum parameters by standard addition method and recovery. Capsules were prepared according to section 2.3.2. A concentration of 2×10^{-7} mol L⁻¹ PREG was added to solution in five times that every addition was repeated to give five measurements while the concentration of copper ion was fixed (3×10^{-5} mol L⁻¹) during the electrochemical analysis period. The recovery was obtained in an average of $90 \pm 1.0\%$ SD for PREG – Cu(II) complex in the pharmaceutical capsules as reported in table 2, compared to ion selective electrochemical and spectrophotometric methods that determined PREG in pharmaceutical capsules which reported recoveries of 100.65 ± 0.83 and 101.03 ± 0.94 , respectively[15,11].

3.4.2 SWV applications in biological fluids analysis

PREG – Cu(II) complex was determined in urine and plasma after purification steps and preparation procedures as in section 2.3.2 as previously described. The recovered concentrations of PREG were 5×10^{-7} mol L⁻¹ with 3×10^{-5} mol L⁻¹ Cu(II) during the analysis period. A 2×10^{-7} mol L⁻¹ PREG was added to the recovered solution in five times that every addition was repeated to give five voltammetric measurements. The recoveries of PREG-Cu(II) were obtained in averages of $89 \pm 1.23\%$ and $86 \pm 1.23\%$ for urine and plasma respectively; as listed in table 3. In comparison ion selective electrochemical determination of REG in urine and plasma samples yielded 98.86 ± 0.61 and 98.32 ± 0.72 for 10^{-5} and 10^{-4} mol L⁻¹ as recovered PREG concentrations for urine and plasma, respectively [15].

Table 2. Square wave voltammetric determination of PREG – Cu(II) complex in pharmaceutical capsules

	(mg)	Recovery %
Lyrica capsules 300 mg PREG	267	89
	267	89
	270	90
	273	91
	273	91
Average	90 %	
SD	±1.0	

Table 3. Square wave voltammetric determination of PREG – Cu(II) complex in human urine and plasma

	Urine recovery %	Plasma recovery %
Recovered Conc (5x10 ⁻⁷ mol L ⁻¹ PREG	89	86
	90	86
	90	88
	87	85
	89	85
Average	89	86
SD	±1.23	±1.23

4. Conclusion

The presented SWV method was carried out for the determination of PREG as PREG–Cu(II) complex using B-R supporting electrolyte and pH 7.5. The developed method was applied to determine PREG–Cu(II) in pharmaceutical and biological samples. In this work, the developed SWV shows a high sensitivity determination for PREG–Cu(II) compared to other analytical methods, where the calculated detection limit was 6.5×10^{-9} mol L⁻¹ (1.033 ppb) by SWV using a graphite working electrode. No published articles have previously used SWV and graphite electrode for the determination of pregabalin as PREG – Cu(II) complex as in this research. The parameters; 0.0 V E_{acc} , 30 sec t_{acc} , 20 Hz, 100 mVs⁻¹ and 10 mV amplitude potential were used and selected as optimum conditions to obtain a well-defined cathodic voltammogram for PREG–Cu(II) complex determination. Other parameters for analytical performance including reproducibility, stability and recovery were also evaluated for PREG–Cu(II). In analytical applications, the recoveries of the developed SWV method ranged between 86 to 93% for the analysis of pure PREG solution, PREG in capsules, and PREG in human urine and plasma in the presence of Cu(II).

ACKNOWLEDGEMENT

The author would like to thank Mr. Awad Alqarni and Ahmad Alribdy at Qassim Pharmaceutical Plant Buraydah, Spimaco Addwaeih, Saudi Arabia. He also likes to thank Prof. Mustafa Abbodi , Dr. Moyawia Omer and Dr Ziad Moussa for their assistance and help in completing this research.

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