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An Eco-Friendly Method for Voltametric Determination of Prucalopride Succinate on Simple Nanoparticles Modified Carbon Paste Electrode

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Abstract

An eco-friendly, novel, rapid, accurate, and precise voltametric method for the determination of prucalopride succinate (PRU) in drug substance and its dosage form. The drug showed an anodic oxidation peak at a potential of 0.989 V using zirconium oxide nanoparticles modified carbon paste electrode (2% ZrO_2/CPE) as a suitable electrochemical transducer in phosphate buffer of pH 6. Voltametric peaks which were diffusion-controlled, have been recorded and the experimental conditions including change in pH, different electrode modifiers, different buffer solutions, the effect of different types of surfactants and scan rates were all optimized. The peak current showed good linearity and sensitivity over the concentration range of 4.03×10^{-7} to 2.20×10^{-6} mol L^{-1} with a 0.9992 correlation coefficient. The limit of detection and limit of quantitation were found to be 9.23×10^{-8} mol L^{-1} and 2.80×10^{-7} mol L^{-1}, respectively. The prospective validated method was successfully used for the evaluation of (PRU) in dosage form without the interference of the excipients with recovery (%) ± % RSD of 100.52 ± 1.29. All validation parameters were measured according to ICH guidelines.

Keywords: Prucalopride, zirconium oxide, nanoparticles, carbon paste electrode, voltammetry, AGREE assessment

1. Introduction

Prucalopride succinate (PRU) (Figure 1) is considered a selective type 4 serotonin (5-HT4) receptor agonist; GI pro-kinetic agent. This medication is approved by European Medicines Agency [1]. It is commonly used to treat chronic constipation. Moreover, it is used when other laxatives have not provided relief. It is known that (PRU) works by improving the movement of food in the stomach and intestines through the bowels during digestion. Pharmacokinetic steady-state is attained within 3 to 4 days for 2 mg per day one dose administration, and plasma concentrations steady-state fluctuate between trough and peak values of 2.3 and 7.7 ng mL^{-1}, respectively, with mean plasma AUC^0- 24 h of 109 ng h mL^{-1} [2]. Minor side effects like abdominal pain, nausea, diarrhea, or headache have commonly occurred during oral administration of (PRU) from the first 1 to 2 days of treatment. Other side effects including dizziness or tiredness may also occur[3].

Few pharmacological, pharmacokinetic, and analytical information about the drug have been reported in the literature includes prucalopride succinate synthesis [4] evaluation of its effects on cholinergic neurotransmission through 5-HT4 receptor [5], and its effect on the pharmacokinetics of oral contraceptives [6]. Upon going on the literature survey, few published methods have been described for the determination of (PRU) through selective separation and characterization of stress degradation products and process impurities of (PRU) by LC-QTOF-MS/MS [7], RP-HPLC methods [8-10]. Also, spectroscopic methods [11, 12] have been reported for the estimation of PRU in a pharmaceutical dosage form. Moreover, (PRU) quantitation in rat plasma and its application to pharmacokinetics study was successfully done [13] .

Carbon paste electrodes (CPEs) are well known for their wide potential window, ease of fabrication, response stability, and ability to be modified with various modifying agents to control selectivity and sensitivity.[14-16], it was used as a bare and modified electrode in studying the electrochemical behavior of the drug. Additionally, the electrochemical technique has the privilege of its simplicity, timesaving, low cost,

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and simple sample preparation without the need for sample pre-treatment.

Recently, metal oxide nanoparticles (NPs) have displayed various types of photochemical and electrical traits due to their size, stability, and high surface area[17] Nowadays, metal oxide nanoparticles have an intense scientific activity due to a variety of potential applications in several fields[18], as they widely employed in different research areas, ranging from analytical chemistry and environmental science to medicine, the agriculture and pharmaceutical industry. This is mainly due to the unique characteristics of NPs and the novelty they introduce in such applications The main functions of metal nanoparticles in electroanalysis include increasing the surface area of the electrode leading to an increase the electroactive species loading on it, in addition, they have good catalytic activities as they function as ultra -microelectrode clusters for large amount substrate catalysis and they catalyse the deposition of reactive species [19].

Among the transition metal oxide nanoparticles, zirconium oxide nanoparticles (ZrO_2 NPs) have attracted major research interest due to its unique thermal, catalytic, electrical, sensing, optical, mechanical, and biocompatible characters[20-22].

However, ZrO_2 NPs is a known p-type semiconductor with piezoelectric characteristics due to its acidic and basic nature. Therefore, ZrO_2 NPs have been widely used in myriad applications including bone implants[23], dental[24], gas sensor[25], in energy storage and water treatment [26] and solar cells [27, 28].

In addition to the previous applications, ZrO_2 nanoparticles showed improvement in current and sensitivity in several electrochemical analysis[29-35].

AGREE approach [36] is the simplest automated dependable software. The greenness score of this approach is to specify the hazards of analytical methods for analysts and the environment.

Hitherto, no electrochemical method has been detailed for estimation of PRU in both bulk and pharmaceutical formulation. Therefore, the aim of this work is to present a new voltametric validated, green, and sensitive method for the determination of (PRU) in bulk. Besides, to apply this method on the pharmaceutical dosage form.

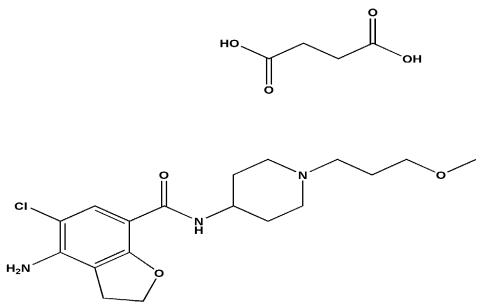

Fig. 1: Structure of prucalopride succinate

2.Experimental

2.1. Apparatus

All the voltametric parameters were optimized on a computer-controlled explanatory electrochemistry workstation (Metrohm), and the information was analyzed using the Viva 884 built-in software. Three electrodes consisted of (modified carbon paste electrode) as working electrode, the auxiliary electrode used was a platinum electrode and the reference electrode used was Ag/AgCl (3 mol L^{-1} NaCl). The electrochemical measurements were obtained using a glass cell (10 mL) containing the buffer solution and sample to be measured. Measuring the pH values of the prepared solutions was done using HANNA (HI 2211pH/ORP Meter) with a combined electrode (glass-reference electrode).

2.2. Materials and Reagents

Prucalopride succinate (PRU) authentic drug was kindly supplied by Macryl pharmaceutical industries, of purity 9.51% (El Obour city, Egypt). Prucasoft®, provided by Macryl pharmaceutical industries, (El Obour city, Egypt), which is labelled to contain 2.64 mg of prucalopride succinate equivalent to 2 mg prucalopride. Both Graphite powder of 1-2 μm and zirconium oxide (ZrO2) nano particles of particle size (<100 nm) were purchased from (Sigma, Aldrich, Germany). And for carbon paste preparation, paraffin oil was used from (Fluka, Germany).

Chemicals including boric acid, acetic acid, phosphoric acid, citric acid, sodium dihydrogen phosphate and sodium hydroxide all were of analytical grade purchased from (Sigma, Aldrich, Germany). Anionic surfactants including sodium dodecyl sulphate (SDS), cationic surfactant such as cetyltrimethylammonium bromide (CTAB), non-ionic surfactants like tween 80, all were purchased from (Sigma-Aldrich, Germany). An accurately weighed amount of each surfactant was dissolved separately in double distilled water to obtain solutions with concentration of 5.0×10^{-3} mol L^{-1}.

Britton-Robinson (BR) buffer solutions were prepared by mixing 10 mL volumes of 0.04 mol L^{-1} for each (boric acid, acetic acid, and phosphoric acid) 100 mL volumetric flask mixed with 50 mL double distilled water, followed by drop wise addition of 0.2 mol.L^{-1} NaOH solution to obtain the desired pH (2-11), then the volume was completed with the same solvent.

Phosphate buffer was prepared to obtain final buffer solutions of ionic strength 0.05, 0.1 and 0.2 M by dissolving definite weights of potassium dihydrogen phosphate in 70 mL double distilled water in 100 mL volumetric flask, adjust pH with 0.2 mol L^{-1} NaOH then complete to the total volume with the same solvent.

2.3. Preparation of working electrodes

Carbon paste electrode (CPE) was prepared by mixing (0.50 g) graphite powder with portion amount of

paraffin oil (≈ 0.3 mL) in a mortar till obtaining homogenous paste. This paste was filled into the hole of the electrode body, polished on a filter paper until its appearance became shiny, then connected to the apparatus as a working electrode through a copper wire. The carbon paste modified with zirconium oxide nanoparticles (1%, 2%, 3% ZrO_2/CPE) were prepared by mixing graphite powder with 1%, 2%, 3% (w/w) of its weight with zirconium oxide nanoparticles using ethyl ether with continuous stirring to get homogeneity, then the mixture was left in air to dry. Paraffin oil was included to the previous mixture drop wise till obtaining homogenous paste. MWCNT-SPEs were supplied from DROPSENS (DRP-110). PGE (pencil graphite electrode) HB pencil lead of 0.9 mm diameter, supplied from local library supported on plastic sheet with 1 cm length exposed surface. GCE (glassy carbon electrode) supplied by Metrohm®.

2.4. Standard solutions

A stock solution of 2.06×10^{-3} mol L^{-1} PRU was prepared in double distilled water and was stored refrigerated at 2-4 °C . Working solutions were processed from the stock standard solution.

2.5. Electrochemical assay and construction of a calibration curve

A calibration curve was constructed by adding aliquots of PRU working solution (2.06×10^{-5} mol L^{-1}) to the glass electrolytic cell containing 10 mL phosphate buffer (0.2 M, pH 6). The concentration of drug in the buffer solution covers the range of 4.03×10^{-7} to 2.20×10^{-6} mol L^{-1}. The voltammograms were recorded from potential 0.25 to 1.4 V, pulse amplitude 50 mV, and a potential scan rate of 100 mVs^{-1}. A blank signal (I_{pb}) voltammogram was first obtained. Then, aliquots of PRU solutions were added to the electrolytic cell and the differential pulse (DP) voltammograms were recorded to obtain the anodic peak current of the sample. Measuring of the anodic peak current was done and consequently recorded as the signal of the sample (I_{ps}). The net current (ΔI_p) for each determination is constructed by calculating the difference between the two currents (I_{ps} - I_{pb}). The calibration curve was constructed by plotting the (ΔI_p) against the concentration of PRU in the solution.

2.6. Pharmaceutical dosage form preparation and analysis

Ten tablets of Prucasoft® were crushed and mixed well. An accurately weighed amount of the powder which is equivalent to the weight of one tablet was transferred into a 10-mL volumetric flask followed by addition of 5 mL doubled distilled water. The solution was sonicated for about 30 min, then completed to the final volume with the distilled water to obtain a stock solution of (5.43×10^{-4} mol L^{-1}). The stock solution was then filtered through 0.45 µm filter, take 5 mL of filtrate into 100-mL volumetric flask and complete to the total volume with distilled water to obtain a working solution of concentration (2.72×10^{-5} mol L^{-1}). Different portions from the working solution were transferred into the electrolytic cell and analyzed according to the proposed voltametric method as described before.

3.Results and discussion

3.1. Electrochemical oxidation of PRU

In order to measure the electrochemical behaviour of PRU**,** cyclic voltammetry (CV) technique was performed on PRU solution (2.0×10^{-5} mol L^{-1}) in phosphate buffer (0.2 M, pH 6) at CPE as showed in (Figure 2). One anodic peak current showing no cathodic peak upon scanning in the reverse scan, which confirms the irreversible nature of the electrode reaction.

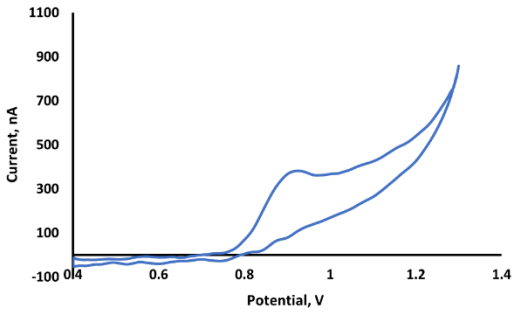

Fig. 2: Cyclic voltammogram of PRU solution (2×10^{-5} mol L^{-1}) in phosphate buffer (0.2 M, pH 6) at CPE.

By studying the effect of pH over the range of 2 to 11 of BR buffer on the electrochemical behaviour of the drug, using differential pulse voltammetry (DPV) at bare CPE on scan rate 100 mVs^{-1} as shown in (Figure 3), well-defined anodic peaks were observed in the pH range 2-11, associated with negative shift in potential by increasing the pH value which ascertained that the oxidation behaviour of PRU is pH-dependent. The maximum peak current reached when the pH value of BR buffer was 6.

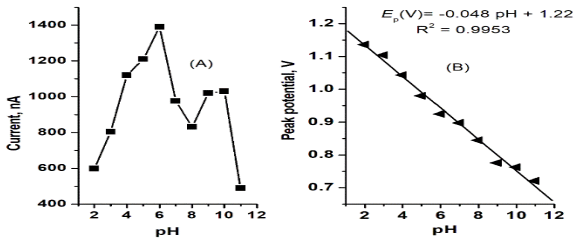

Fig. 3: Plot of different pH (2-11) BR buffer as a function of peak potential E_p(V) (A) and current, nA (B) of (4.11×10^{-6} mol L^{-1}) PRU at scan rate 100 mVs^{-1}

According to the following equation, $E_p(V) = K - (0.059\ y/n)$ pH [37], where ***y*** is the number of hydrogen ions H^+

which is involved in the reaction of electrode and ***n*** is the number of electrons. As demonstrated in (Figure 3), the oxidation anodic peak potential of PRU shifted directly to less positive potential in a linear pattern with pH change (through the pH range 2-11) resulting in the regression equation of E_p(V) = -0.048 pH + 1.22, with correlation coefficient of 0.9953. As the slope was found to be 0.048 which is quietly near to the slope value of Nernst equation (0.059), this indicates that the protons involved in this reaction are equal to the conveyed electrons. By trying different buffer of pH 6, as phosphate buffer of different molarities (0.05, 0.1 and 0.2 M) as shown in (Figure 4), the voltametric signals which showed the best in terms of peak height (sensitivity) and shape of the peak (resolution) were fulfilled by the usage of 0.2 M phosphate buffer which showed the highest current, so it was used for further optimizations.

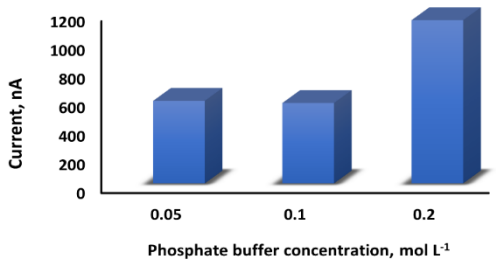

Fig. 4: Different concentrations in (mol L^{-1}) of phosphate buffer, pH 6 at scan rate 100 mV s^{-1}

3.2. Effect of different electrodes

The ease of CPE preparation, its wide window of potential, the simple surface renewable process and it's easy to be modified to increase its sensitivity, all of these factors provide advantages for CPE to be used for electrochemical investigations [38].

Modified CP electrodes were examined to find out the best electrode that gives the highest peak current and well-defined peaks. Due to the advantages of metal oxide nanoparticles [19], so modification with different ratios (1%, 2% and 3% (w/w)) of ZrO_2 nanoparticles were examined. 2% ZrO_2/CPE showed the highest peak current by (84.39%) increment in current when compared to the bare CPE as shown in (Figure 5).

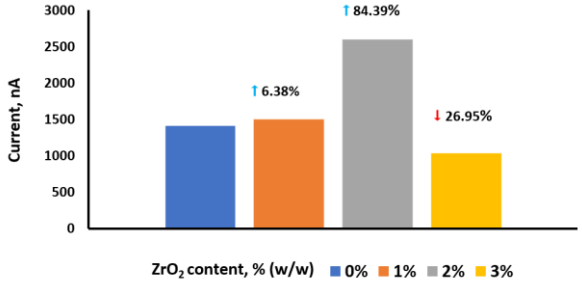

Fig. 5: Effect of different contents of %ZrO_2 (1%, 2% and 3% (w/w)) modified CPE on PRU, scan rate 100 mVs^{-1}, in (0.2 M, pH 6) phosphate buffer

By comparing the peak current of pencil graphite electrode (PGE), glassy carbon electrode (GCE), and MWCNT/SPE with that of bare CPE and 2%ZrO_2/CPE. As shown in (Figure 6), 2%ZrO_2/CPE showed the highest current. This may be owing to the chemical and thermal stability, non- toxicity and propensity towards analytes having oxygen groups [39]. In addition, limited orientations of ZrO_2 provides a feasible electron transfer between the electrode surface and analyte [40] as it was proved by our previous work on mosapride [35] and for simultaneous determination of mebendazole and levamisole hydrochloride[34] . Therefore, it was the electrode of choice which was used in subsequent experiments as it showed well-defined peaks.

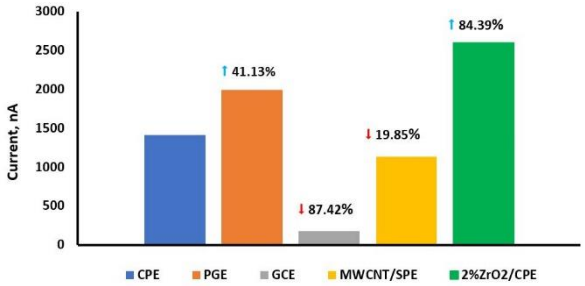

Fig. 6: Effect of different types of electrodes on PRU, scan rate 100 mVs^{-1}, in (0.2 M, pH 6) phosphate buffer

3.3. Effect of the scan rate

The behaviour of the anodic peaks of PRU was studied at different scan rates of (20 - 200) mV s^{-1} in 0.2M phosphate buffer of pH 6 at 2% ZrO_2/CPE. As illustrated in (Figure 7A), a linear relationship was obtained from the plot of logarithmic anodic peak currents (log I_p) versus the logarithm of the scan rate (log υ) resulting in a linear regression equation of log I_p = 0.407 log υ - 0.553 (R^2 = 0.9957). From the slope value of 0.407 which is quite related to the theoretical slope value of 0.5, it was concluded that the oxidation behaviour was a diffusion-controlled processes.[41]

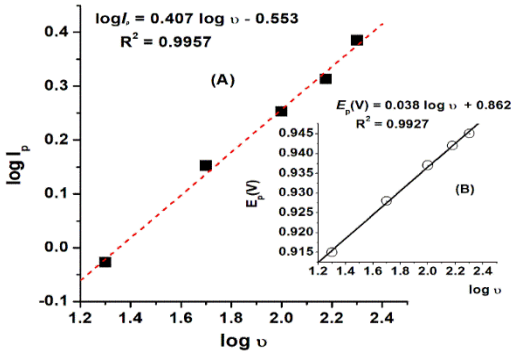

Fig. 7: Plot of (A) log current (log I_p) and (B) E_p(V) versus log scan rate (log υ) for 4.11 × 10^{-6} mol L^{-1} PRU in 0.2 M, pH 6 phosphate buffer.

The involved electrons in the oxidation reaction can be estimated by applying Laviron's equation, which is illustrated as following [42]

$$E_p(V) = E^0 + 2.303\ RT/\alpha nF\ [\log RTK^0 / \alpha nF + \log \upsilon]$$

Where, $\boldsymbol{\alpha}$ is the coefficient of electron transfer, $\boldsymbol{n}$ is the electrons number, $\boldsymbol{R}$ is the gas constant (8.314 J K mol^{-1}), $\boldsymbol{T}$ is the temperature (298 K) and $\boldsymbol{F}$ is the Faraday constant (96 485 C mol^{-1}). By plotting peak potential E_p(V) against logarithm of scan rate (log υ) as shown in (Figure 7 B), resulting in the equation E_p(V) = 0.038 log υ + 0.862 (R^2 = 0.9927), and from the slope value of 0.038, $\boldsymbol{\alpha n}$ could be evaluated. And by assuming $\boldsymbol{\alpha}$ (coefficient of electron transfer) to be 0.6, consequently the number of the electrons were (n ≈ 2) supposed that PRU was oxidized according to the proposed mechanism as shown in (Figure 8) which involves oxidation of primary amine to oxime in this suitable pH, involving two electrons and two protons and this is in acceptance with the oxidation mechanism of sulfaguanidine [43]. In addition, scan rate of 100 mV s^{-1} was selected for the proposed method as it showed the least SD in comparison with the other scan rates.

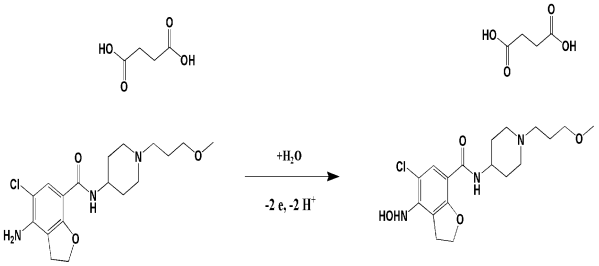

Fig. 8: Proposed scheme of prucalopride succinate oxidation

3.4. Effect of surfactants

Different successive additions of 5.0 × 10^{-3} mol L^{-1} anionic, cationic, and non-ionic surfactants, as (SDS, cetrimide and tween 80), respectively, were added to 10 mL voltametric cell which contains 4.11 × 10^{-6} mol L^{-1} of PRU in 0.2 M/ pH 6 phosphate buffer and the DPV were reported at 2% ZrO_2/CPE. Usage of various classes of surfactants which varying in lengths and charges of hydrocarbon chain did not influence the redox conduct of electroactive species of PRU and subsequently its corresponding voltametric response.

3.5. Working electrode Area

Working electrode area was obtained by using 20.0 mmol L^{-1} $K_4Fe(CN)_6$ as a probe, utilizing different scan rates ranging from 20 to 200 mV. Randles- Sevcik equation [44] was applied:

$$I_{pa} = (2.69 \times 10^5)\ A\ n^{3/2} D^{1/2} C_0 \upsilon^{1/2}$$

Where, $\boldsymbol{I_{pa}}$ is the oxidation peak current, $\boldsymbol{n}$ refers to the number of electrons transferred, $\boldsymbol{A}$ of (cm^2) is the electrode surface area, $\boldsymbol{D}$ (cm^2 s^{-1}) diffusion coefficient of the electro active species, $\boldsymbol{C}$ of (mmol L^{-1}) is the electro active species concentration and $\boldsymbol{\upsilon}$ (Vs^{-1}) is the scan rate. In a 0.1 M KCl solution, 20.0 mmol.L^{-1} of $K_4Fe(CN)_6$ was applied with $\boldsymbol{D}$ of 7.6 × 10^{-6} and n=1. By plotting $\boldsymbol{I_{pa}}$ of ferrocyanide against $\boldsymbol{\upsilon^{1/2}}$ and from the slope value, we can get the electrode surface area which was found to be 0.205 cm^2.

3.6. Validation of the method

According to ICH Q2 (R1) recommendation [45], the validation of the intended method was performed. It includes linearity and range, precision and accuracy, detection, and quantitation limits.

3.6.1. Linearity and range

Calibration curve was composed as a function of standard PRU concentrations. Differential pulse voltametric peaks current increase linearly by increasing concentration of PRU, as illustrated in (Figure 9).

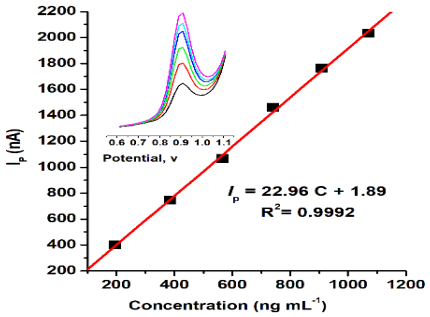

Fig. 9: Plot of concentration range against peak currents of prucalopride succinate, phosphate buffer (0.2 M, pH 6), scan rate 100 mVs^{-1}

The regression parameters of linearity were calculated according to ICH guidelines and presented in **Table 1**.

Table 1: Performance data of the proposed method for estimation of prucalopride in pure form parameters

Concentration range	96.10 - 1071.43 ng mL^{-1} equivalent to (4.03 × 10^{-7} to 2.20 × 10^{-6} mol L^{-1})
Slope (b)	1.89
Intercept (a)	22.97
Correlation coefficient	0.9992
Mean % recovery ± %RSD	100.05 ± 1.92
standard error of slope S_b	0.036
LOD (mol L^{-1})	9.23 ×10^{-8}
LOQ (mol L^{-1})	2.80 × 10^{-7}

3.6.2. Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined according to ICH guidelines. The standard deviation of Y-intercept of regression line was utilized and the LOD and LOQ values were calculated, using the following formulas

$$\mathbf{LOD = 3.3\ \sigma/S}$$
$$\mathbf{LOQ = 10\ \sigma/S}$$

Where, $\boldsymbol{\sigma}$ = the standard deviation of Y-intercept of regression line and $\boldsymbol{S}$ = slope of the calibration curve. The calculated LOD and LOQ were found to be 9.23 $\times 10^{-8}$ mol L^{-1} and 2.80 $\times 10^{-7}$ mole L^{-1}, respectively, as shown in **Table 1**.

3.6.3. Precision and accuracy

Assessment of repeatability was done by replicate analysis of three concentration levels of PRU; 7.91× 10^{-7}, 1.16 $\times 10^{-6}$ and 1.52 $\times 10^{-6}$ mol L^{-1} by analysis of each concentration for three times per day, while the intermediate precision was measured through replicate analysis of the same concentrations for three successive days. The results of repeatability and intermediate precision were summarized in **Table 2**. The high percent recovery and low value of relative standard deviation (%RSD) show that the method was accurate and precise, respectively, for the determination of PRU. The standard addition technique was used to assess the accuracy of the prospective method. A known concentration of PRU solution was spiked into the cell containing a sample solution of known concentration of Prucasoft ® sample solution. High accuracy of the method was expressed by high% recoveries as shown in **Table 3.**

3.6.4. Robustness

The constancy of the anodic peak current with minor changes in the experimental parameters such as the electrolyte pH (6 ± 0.2) indicated robustness of our evaluated method.

3.7. Method applications

3.7.1. Application of pharmaceutical preparation

PRU was successfully analysed in its pharmaceutical preparation Prucasoft® tablets by using the proposed method. No interferences showed by well-defined peaks and acceptable results of high % recoveries with no significance difference between our proposed method and the reported method [8] which were proved by *t*-test and F- value as illustrated in (**Table 4**). The method wasn't applied for determination of drug in plasma as the plasma concentrations steady-state fluctuate between trough and peak values of 2.3 and 7.7 ng mL^{-1}, respectively, with mean plasma AUC^{0}- 24 h of 109 ng h mL^{-1} [2] **,** in addition there is no toxic effect of prucalopride on human health as is not extensively metabolised in body[46].

3.8. Comparison with previous work

By comparing our estimated voltametric method with the previously published analytical methods, it was shown that our method is highly sensitive, simple, rapid, and more economic if compared to the previously other methods.

Table 2: Precision data for the evaluation of prucalopride in drug substance by the proposed DPV method

Parameters	**PRU concentration (mol L^{-1})**		
	7.91×10^{-7}	**1.16×10^{-6}**	**1.52×10^{-6}**
Repeatability (recovery %)	101.62 101.09 101.68	100.61 100.33 99.35	101.07 100.91 101.69
Mean ±%RSD	101.10 ± 0.41	100.78 ± 0.32	100.91 ± 1.09
Intermediate precision (recovery %)	101.66 100.95 100.42	101.00 101.58 101.75	102.26 101.81 101.65
Mean ±%RSD	101.01 ± 0.50	101.44 ± 0.32	101.91 ± 0.26

Table 3: Quantitative determination of prucalopride in pharmaceutical dosage form (Prucasoft®) by the proposed DPV method using the standard addition technique. a

	Proposed method			
parameters	**Amount taken (mol L^{-1})**	**Amount added (mol L^{-1})**	**Amount found (mol L^{-1})**	**Recovery %**
Prucasoft®	4.03×10^{-7}	3.87×10^{-7}	7.99×10^{-7}	101.14
		7.61×10^{-7}	1.14×10^{-6}	98.27
		1.12×10^{-6}	1.50×10^{-6}	98.68
Mean ± %RSD				99.36 ± 1.25

a Each result is an average of three determinations.

Table 4: Quantitative determination of prucalopride in pharmaceutical dosage form by the proposed DPV method a

Parameters	Amount taken (mol L^{-1})	Amount found (mol L^{-1})	Recovery %	Reported method [8*]
Prucasoft®	7.91×10^{-7} 1.16×10^{-6} 1.52×10^{-6}	7.90×10^{-7} 1.17×10^{-6} 1.55×10^{-6}	99.86 100.11 101.95	98.80 98.11 99.24
Mean ± %RSD			100.64 ± 1.14	98.72 ± 0.58
t-Student test			2.61(3.18)[b]	
F-value			4.03 (19.00)[b]	

[a] Each result is an average of three determinations [b], the tabulate t and F values at p = 0.05
*Reported method is Stability Indicating RP-HPLC

Table 5: Comparison of our proposed method with the other reported analytical methods for analysis of prucalopride

Method	Applications	Linearity range	References
LC-QTOF-MS/MS	Stability study	80 - 120 µg mL^{-1}	[7]
UHPLC-MS/MS	Rat plasma	0.1 - 100 ng mL^{-1}	[13]
RP-HPLC	Tablets Stability study	2 - 12 µg mL^{-1}	[8]
RP-HPLC	Tablets	10-50 µg mL^{-1}	[9]
HPLC/UPLC	Forced degradation study	20.0 to 80.0 µg $mL^{-1}$for HPLC and 8.0 to 32.0 µg mL^{-1} for UPLC	[10]
Spectrophotometry	Tablets	5 - 60 µg mL^{-1}	[11]
Spectrophotometry	Tablets	2 -10 µg.mL^{-1}	[12]
Voltammetry	Tablets	196.10 - 1071.43 ng mL^{-1}	**Our proposed method**

3.*9. Estimation for the greenness of the proposed electrochemical method*

AGREE approach is simple and reliable for the assessment of eco-friendly characters of the analytical method [47, 48]. It was selected due to its automation, simplicity, and integration. The final greenness numerical value was 0.73 with a relatively pale green colour inside the pictogram as demonstrated in (Figure 10) which illustrates the eco-friendly characters for the novel method. The use of the organic free solvent is one of the merits of the method because of its known hazards to the environment. The most hazardous red subsections in the pictogram are sectors 3 and 10. Sector 3 denotes off-line sampling while sector 10 refers to the used reagents are not bio-based. The analysis of PRU in 1 minute only which permits the analysis of many samples per hour is understood from full green sector 8 in the pictogram. Sector 7 denotes the amount of analytical waste.

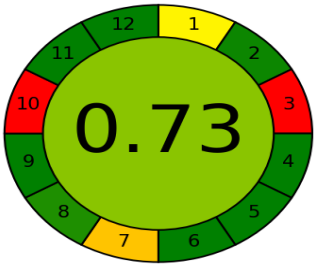

Fig. 10: AGREE approach for estimation of new electrochemical method greenness for PRU

4.Conclusion

A novel, precise, accurate, time saving, and simple voltametric method was developed for estimation of PRU with high efficiency in bulk and dosage form. Electrochemical behaviour using DPV technique which is based on oxidation of PRU on nanoparticles modified CPE gave good sensitivity and acceptable reproducibility of the voltametric responses. Modification with zirconium oxide nanoparticles leading to increase the surface area of the electrode which provides a useful tool for the detection of PRU at lower concentration levels in addition to its cheapness if it is compared to alternative methods such as HPLC. Our method showed that it is eco-friendly with the high greenness numerical value and the green colour of pictogram. Also, our method is sensitive and simple by comparing to the other literature analytical methods for determination of the drug.

Our suggested approach has the privilege of being sensitive and there was no need for sample pre-treatment so it can be extended to the routine detection of PRU in quality control labs.

5. Conflicts of interest

The authors have no conflicts of interest to declare, and there has been no significant financial support for this work that could have influenced its outcome. As a corresponding author, I confirm that the manuscript has been read and approved for submission by all named authors. We certify that the submission is

original work and is not under review at any other publication.

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