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Differential Pulse Voltammetric Method for Determination of Acemetacin in Pharmaceutical Formulation using Glassy Carbon Electrode

Eman M. Moaaz*, Ahmed S. Fayed, Mamdouh R. Rezk, and Ezzat M. Abdel-Moety

Analytical Chemistry Department, Faculty of Pharmacy-Cairo University, Kasr El-Aini Street, ET-11562 Cairo, Egypt

*Corresponding Author, Tel.: +201114166929

E-Mail: eman.moaaz@pharma.cu.edu.eg

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Abstract- A new differential pulse voltammetric method was developed for determination of the non-steroidal anti-inflammatory drug; acemetacin. Various experimental parameters were studied, namely; electrode type, pH of the used buffer and scan rate on the reduction and oxidation peaks of acemetacin. The drug responded only to glassy carbon electrode among the studied working electrodes with higher peak current and sensitivity in the favour of the reduction side. The results also revealed that acemetacin best assayed through measuring its reduction peak current when prepared in Britton Robinson buffer solution at pH 8 when scanned at rate of 16 mV/s. The proposed method presented a bimodal calibration curve where each segment showed strong linearity. The linearity ranges were 1-100 µM for the lower segment and 0.1-3 mM for the higher one with detection limit of 0.1 µM. The proposed method was successfully applied for determination of acemetacin in its pharmaceutical dosage form.

Keywords- Acemetacin; Glassy carbon electrode; Bimodal calibration; Electrochemistry

1. INTRODUCTION

Acemetacin (ACE), is one of the non-steroidal anti-inflammatory drugs (NSAIDs) that chemically known as ([[[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetyl]oxy]acetic acid]) [1]. It acts by inhibition of cyclooxygenase (COX) enzymes and used in treatment of rheumatoid arthritis, osteoarthritis, back pain and post-operative pain [1].

Various methods have been developed for determination of ACE; namely, HPLC [2-10], TLC [10,11], UPLC [12], Spectroscopic [4,11,13] and Electrochemical methods. These electrochemical methods survey revealed five voltammetric methods, four of them are polarographic [14-17] and fifth, employed carbon microelectrode as working electrode modified with thin film of mercury [18].

Electroanalytical methods offer opportunity to rapidly assay different analytes with minimal sample preparations steps [19,20], they mainly consume water based solvents as supporting electrolyte which enhances the greenness of the developed methods [21,22]. Voltammetry is one of the electroanalytical methods through which the analyte is studied by measuring current in an electrochemical cell as a function of applied potential. The amount of developed current is directly proportional to the concentration of the studied analyte. In all voltammetric methods, commonly, three electrodes are used; a reference electrode whose potential remains constant during experiment (usually Ag/AgCl electrode for aqueous solution), working electrode at which the analyte is either oxidized or reduced and an auxiliary electrode which is used to collect the current between the working and auxiliary electrodes [23]. The three electrodes are immersed in an electrolytic cell containing the sample to be analyzed dissolved in a supporting electrolyte to ensure sufficient conductivity and minimize the *i*R drop [24]. Modern Voltammetric techniques replace the use of toxic mercury electrodes in polarography to other different working electrodes, such as: glassy carbon electrode (GCE), graphite, screen printed and carbon paste electrodes [25]. Voltammetry, as an electroanalytical method, requires minimal sample preparation and gives results with high sensitivity at optimum conditions.

In this work, we aimed to suggest using GCE coupled with differential pulse voltammetric (DPV) technique for the determination of ACE in its pharmaceutical preparation without involving mercury and mercuric salts in the working electrode materials excluding the disadvantages of variation in drop size and surface area of dropping mercury electrodes and the potential health and environmental hazards of mercury [26]. The proposed method was applied in determination of ACE in its pharmaceutical formulation.

2. Experimental

2.1. Chemicals and Reagents

Phosphoric acid, acetic acid, boric acid and sodium hydroxide obtained from (Adwic Co., Egypt). Ultra-pure HPLC grade water purified by New Human Power 1 device, Human Corporation (Seoul, Korea). Methanol was purchased from Sigma Aldrich (Missouri, USA). Acemetacin was kindly obtained from Multi-Apex pharmaceutical company (Cairo, Egypt). Ost-Map® capsules (BN: MT9291019) manufactured by Multi-Apex pharmaceutical company (Cairo, Egypt).

2.2. Instruments

Metrohm Autolab Potentiostat/Galvanostat, model PGSTAT204 was used for voltammetric measurements. A three-electrode system was utilized which included an Ag/AgCl reference electrode, the platinum electrode as the counter electrode and glassy carbon electrode (GCE) polished with 1.0, 0.3, 0.05-micron alumina slurry then rinsed with distilled water and sonicated to remove residual particles as working electrode. Jenway digital ion analyzer model 3330 with Jenway pH glass electrode (Essex, UK) was used for pH adjustments.

2.3. Optimization of the conditions

The effect of different experimental parameters affecting voltammetric responses of the studied drug such as: electrode type, scan rate (10 – 500) mV/s and pH (3 – 10) were carefully studied and optimized. Such factors were investigated individually while others were kept constant.

2.4. Solutions preparations

2.4.1. Buffer preparation

Britton–Robinson buffer (BRB) was prepared using different volumes of the same concentration (0.04 M) of phosphoric acid, acetic acid and boric acid. The pH was adjusted to the range of (3–10) using appropriate volumes of 0.2 M NaOH.

2.4.2. Standard stock solution of ACE 1×10^{-2} M

ACE stock solution of 1×10^{-2} M concentration was prepared transferring 20.8 mg of pure ACE powder to 50 mL volumetric flask and complete the volume with methanol, the solution was protected from light with aluminum foil and refrigerated at 8 ºC when not in use. The working solutions of descending concentrations (3×10^{-3} M to 1×10^{-7} M) were prepared by serial dilutions from stock solution and the volume was completed to 25 mL with BRB buffer at pH 8±0.2.

2.4.3. Pharmaceutical formulation stock solutions (1×10^{-2} M)

Ten capsules of Ost-map® were emptied, mixed well and weighted. An amount equivalent to 41.6 mg of ACE was transferred to 100 mL volumetric flask and the volume was completed with methanol.

2.4.4. Construction of calibration curves

Calibration solutions were prepared by transferring different aliquots of working solutions to 25 mL volumetric flasks and completing the volume with BRB at pH 8±0.2.

3. Results and discussion

3.1. Method Optimization

3.1.1. Type of electrode

Cyclic voltammetric scan (CV) and DPV scan were done on the surface of graphite pencil electrode (GPE), screen printed carbon electrode (C-SPE) and GCE as working electrodes. CV scans showed an oxidation peak at 0.82 V with no obvious reduction peak (Figure 1), while DPV showed oxidation peak at 0.78 V and reduction peak at -1.4 V with GCE as working electrode, other studied electrodes showed no distinctive peaks, therefore GCE was used as the working electrode in the rest of the experiments. Different conditions were optimized by CV and DPV for oxidation and reduction peaks, respectively.

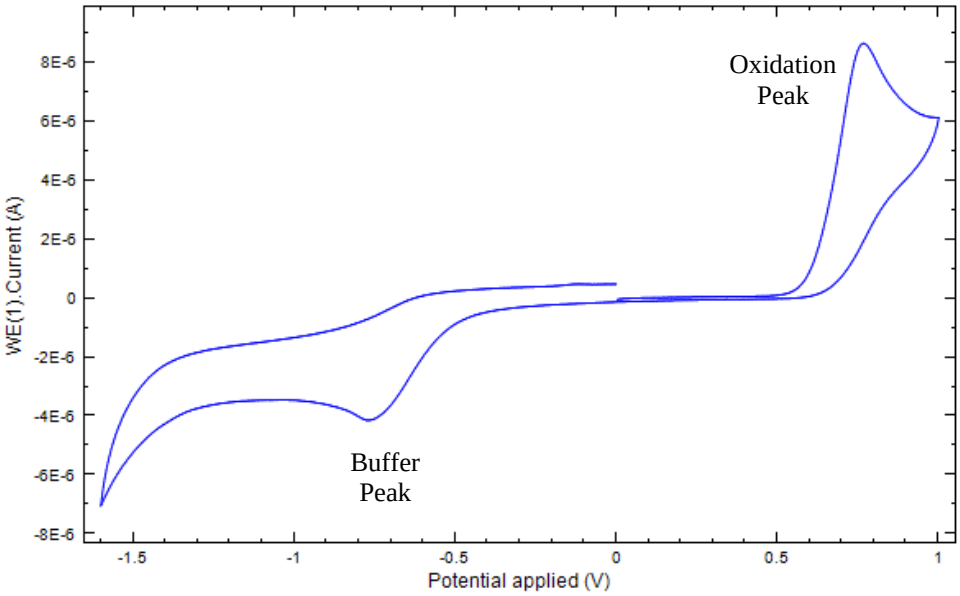

Figure 1. Cyclic voltammogram of 3 mM ACE on GCE vs Ag/AgCl reference electrode and BR buffer pH 8 as a supporting electrolyte.

3.1.2. Effect of pH

The CV of ACE oxidation peaks were recorded in BR buffer over pH range (3 – 10) at scan rate of 100 mV/s. The anodic peak current decreased at pH 4 then gradually increased as pH increased and reached its maximum at pH 8 and then started to decrease upon rising the pH to more basic values as shown in Figure 2.a so BR buffer of pH 8 was chosen for subsequent voltammetric study.

Increasing pH of the solution, anodic peak potential was shifted toward lower potential values indicating that protons have taken part in the electrode reaction process (Figure 2.b). This was expressed by the following equation:

$$E_p \text{ (V)} = 1.0677 - 0.0435 \text{ pH} \qquad r = 0.9966$$

The closeness of the slope value (0.0435 V/pH) to the theoretical value (0.059 V/pH) suggests that equal number of electrons and protons are involved in the oxidation of ACE on GCE surface.

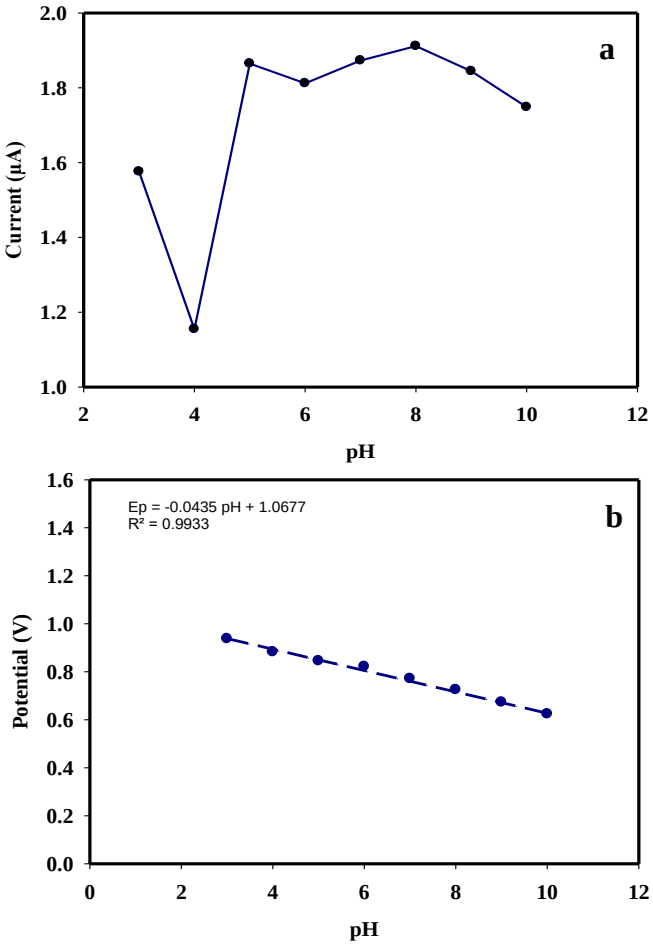

Figure 2. a) Effect of pH on the peak current (i_p) of ACE oxidation peak; **b)** Effect of pH on the potential of ACE oxidation peak (i_p)

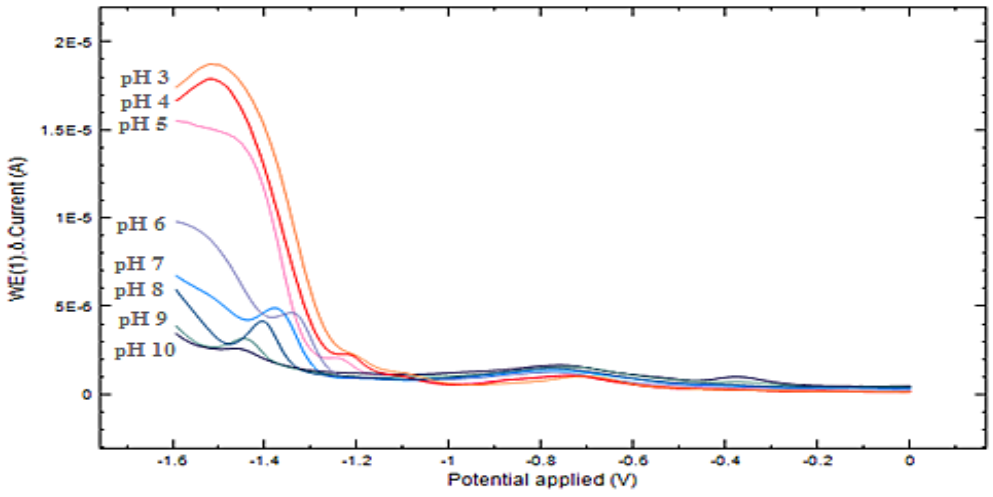

Figure 3. Effect of pH on the cathodic peak current (i_p)

The DPV of ACE reduction peak were recorded in BR buffer over pH range (3–10) at scan rate of 16 mV/s (Figure 3).

The cathodic peak current increased as pH increased and reached its maximum at pH 8 and then started to decrease upon rising the pH to more basic values as shown in Figure 4.a so BR buffer of pH 8 was selected as optimum solution pH.

Increasing pH of the solution, peak potential was shifted toward a more negative potential indicating that protons have taken part in the electrode reaction process (Figure 4.b). This was expressed by the following equation:

$$Ep\ (V) = 1.0434 - 0.0447\ pH \qquad r = 0.9921$$

The closeness of the slope value (0.0447 V/pH) to the theoretical value (0.059 V/pH) could suggest that equal number of electrons and protons are involved in the oxidation of ACE on GCE.

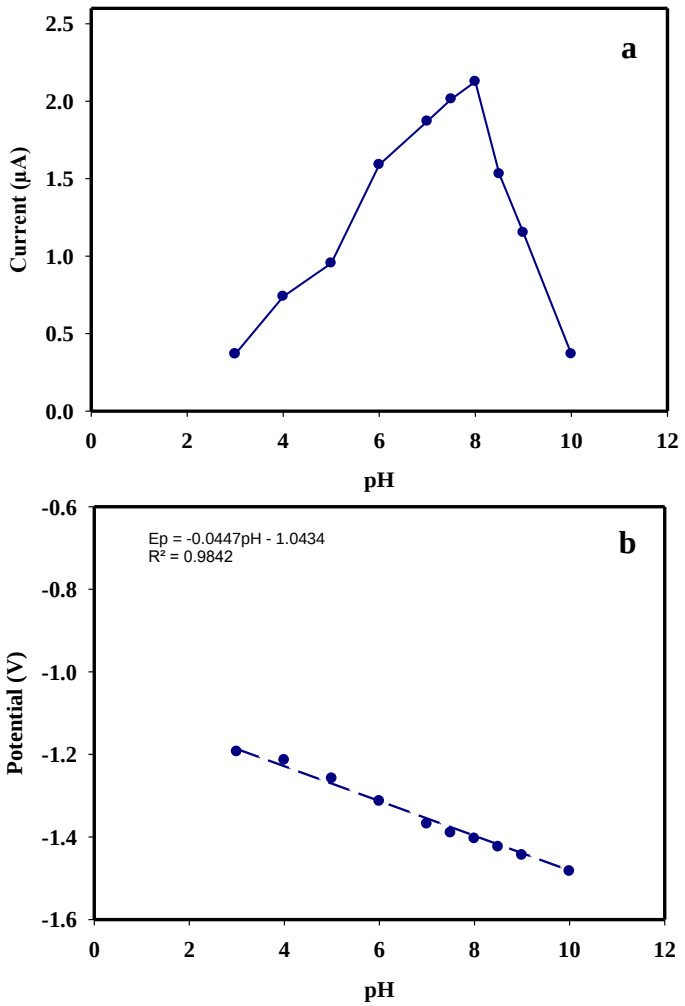

Figure 4. a) Effect of pH on the peak current of ACE reduction peak; **b)** Effect of pH on the potential of ACE reduction peak

3.1.3. Effect of scan rate (υ)

In CV, effect of scan rate on the anodic peak current of ACE was investigated within the range of 10-500 mV/s. The plot of log of peak current (log i_p) against log of scan rate (log υ) displayed linear correlation over the studied scan rate range, Figure 5 giving the following equation:

$$\log i_p\ (\mu A) = 0.3312 \log \upsilon\ (mV/s) + 0.4465 \qquad r = 0.9636$$

This slope of 0.3312 which is lower than the theoretical value indicated the ACE oxidation was a diffusion-controlled process.

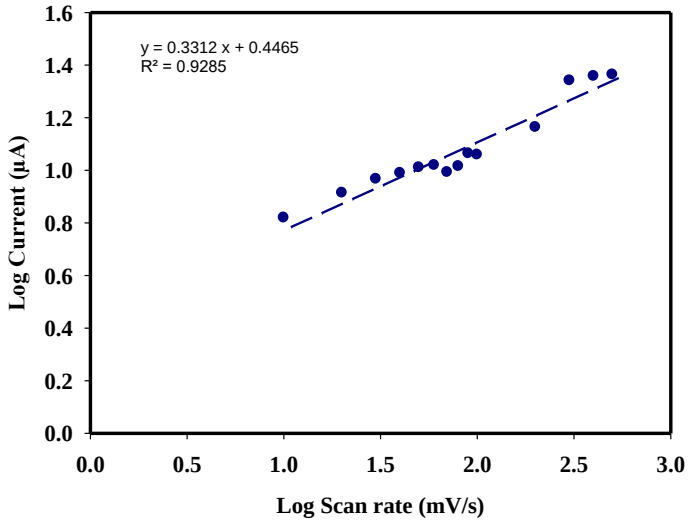

Figure 5. Log current against log scan rate of ACE oxidation peak

That was confirmed by the linear relationship obtained by plotting peak current against square root of scan rate as shown in figure 6 and represented by:

$$i_p\ (\mu A) = 0.9248\ \upsilon^{1/2}\ (mV/s) + 3.2954 \qquad r = 0.977$$

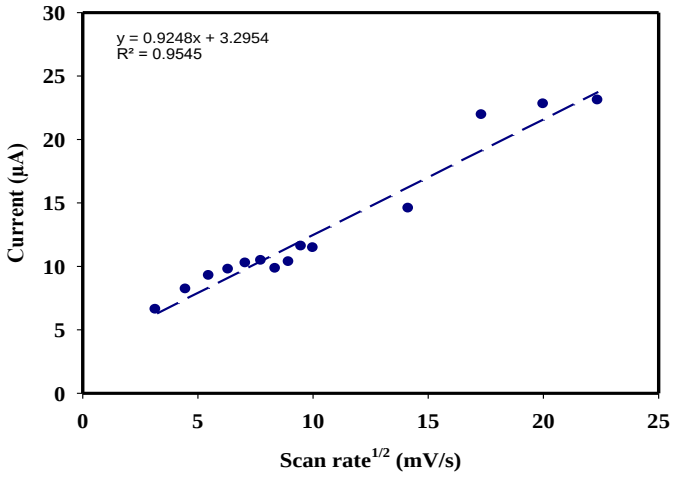

Figure 6. Peak current against square root of scan rate of ACE oxidation peak

There was a linear increase in the anodic peak current with positive shift in anodic peak potential when increasing the scan rate confirming the *irreversible* nature of the oxidation process as it was presented by plotting peak potential (E_p) against log of scan rate (log υ) (Figure 7) and represented by the following equation:

$$E_p = 0.074 \log \upsilon + 0.613 \qquad r = 0.9759$$

The slope of this equation was used to calculate the number of electrons involved in the oxidation process through Laviron equation [27]:

$$Ep = E^{\circ} + \left(\frac{2.303RT}{\alpha nF}\right) Log\left(\frac{RTK^{\circ}}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) Log\upsilon$$

where, R is the gas constant (8.314 J K mol^{-1}), T is the temperature (298 K), F is the faraday constant (96485 C mol^{-1}) and α is the electron transfer coefficient which presumed to be 0.5 [28]. The number of electrons (n) was calculated to be 1.6 ~ 2. From the information obtained from Laviron equation and from the plot of E_p vs. pH, the oxidation process of ACE involved two protons and two electrons in accordance with the reported literature of its active metabolite indomethacin [29].

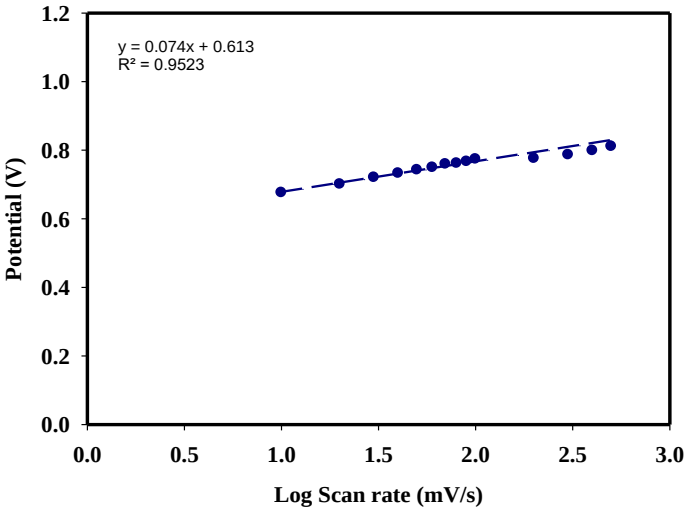

Figure 7. ACE oxidation peak potential against log scan rate

In DPV, although DPV is mainly a quantitative measure, we used it to study the effect of different scan rates to choose the best conditions for measurements of ACE reduction peak. Effect of scan rate on the cathodic peak current of ACE was investigated within the range of 10-200 mV/s. There was a linear decrease in peak current upon increasing scan rate and no peaks observed at scan rate higher than 40 mV/s (Figure 8). A scan rate of 16 mV/s was used for the quantitative determination of ACE as it showed well-shaped peak with relatively narrow peak width.

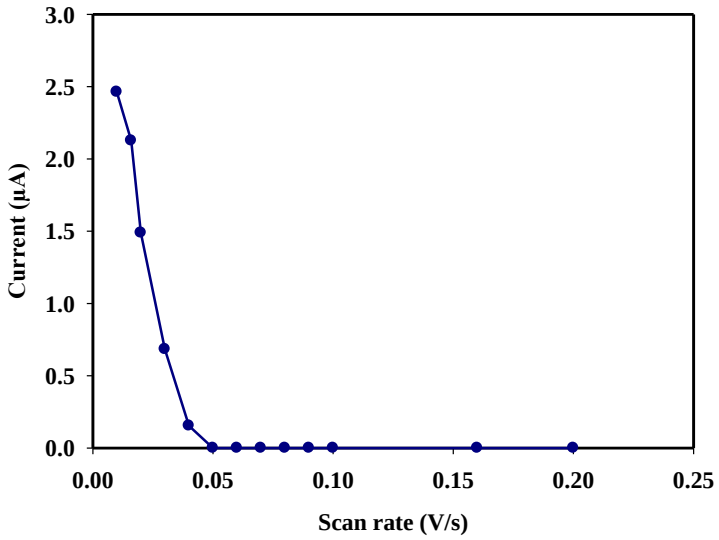

Figure 8. Effect of scan rate on ACE reduction peak current.

3.2. Method validation

Both oxidation and reduction peaks were scanned with DPV, reduction peak showed higher peak current at the same concentration, so we choose the reduction peak to complete the quantitative studies.

Validation was carried out according to ICH guidelines [30](Table 1).

Table 1. Validation of the Proposed DPV Method for the Determination of Acemetacin

Parameter	Lower Segment	Higher Segment
Range	1-100 (µM)	0.1-3 (mM)
Slope (b)[a]	0.0187	0.0023
Intercept (a)[a]	0.247	1.892
Correlation coefficient (r)	0.999	0.999
Accuracy (Mean ± SD)	100.30 ± 1.01	100.02 ± 1.65
Precision		
(% RSD)[b]	1.04	1.09
(% RSD)[c]	1.64	1.89

[a]Regression equation: A = a + bc, where 'A' is the peak current and 'c' is the concentration of ACE.
[b]Intraday precision [average of three different concentrations of three replicate each (n = 9) within the same day].
[c]Interday precision [average of three different concentrations of three replicate each (n = 9) repeated on three successive days].

3.2.1. Linearity and range

The linearity was studied by plotting the peak current i_p against drug concentration in the scanned range from 1 µM to 3 mM (Figure 9).

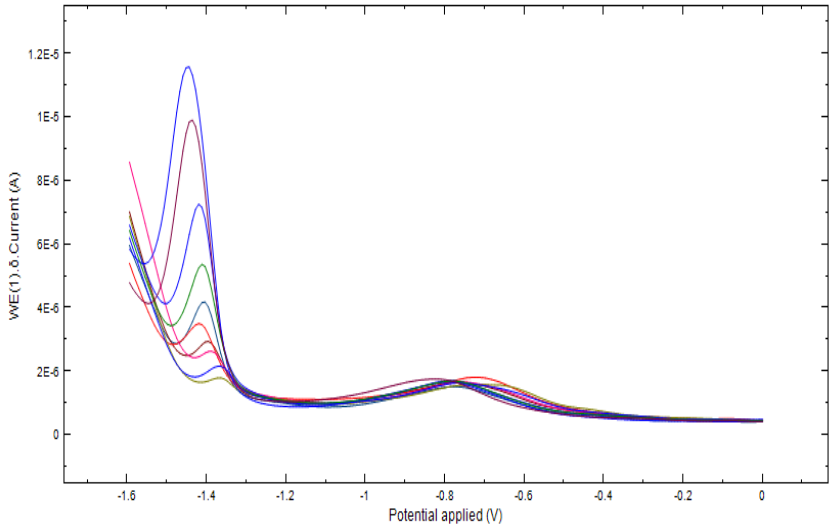

Figure 9. Peak current of ACE in range from 1 µM to 3 mM

The resulted calibration curves appeared to be bimodal (Figure 10) where each segment showed good linearity.

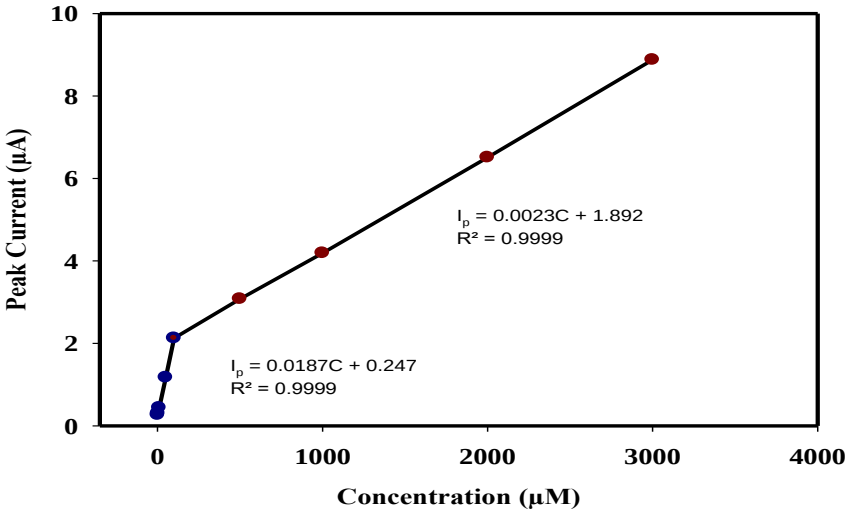

Figure 10. Bimodal calibration curves of reduction peak current against relative concentrations

The regression equation of the lower segment was I_p (µA) = 0.0187 C (µM) + 0.2473 and r = 0.9999 for concentration range 1 µM to 0.1 mM, while for the higher segment the equation was i_p (µA) = 0.0023 C (µM) + 1.892 and r = 0.9999 for concentration range 0.1 mM to 3 mM. The recovery of pure samples was 101.46% ± 1.69 and 101.37% ± 1.27, while precision, expressed as RSD, were 1.673 and 1.259, for the lower and the higher segments respectively.

Limit of detection (LOD) was 0.1 µM calculated by signal to noise ratio method and limit of quantification (LOQ) equal to 0.63 µM.

3.2.2. Accuracy and Precision

Accuracy for each linearity segment was tested by analysis of three different concentrations of pure ACE within linearity range for three times. The concentrations were calculated from their corresponding i_p using the regression and the average recoveries percentage were calculated.

Precision for each segment was determined by repeating three concentrations of standard ACE three times on the same day and three following days for each method (intraday and inter-day). The concentration calculated from the regression equation and the recoveries were obtained the relative standard deviation (RSD) were evaluated.

3.3. Pharmaceutical formulation analysis

The proposed method was successively able to determine ACE in its pharmaceutical preparation. Standard addition technique was performed to confirm the method accuracy (Table 2).

Table 2. Determination of Acemetacin in pharmaceutical dosage form by the proposed method and application of the standard addition technique

Product	**Lower Segment**				**Higher Segment**			
	Drug product	Standard addition			Drug product	Standard addition		
	Recovery% ± SD of the claimed amount*	Taken (µM)	Added (µM)	Recovery% from the added amount*	Recovery% ± SD of the claimed amount*	Taken (µM)	Added (µM)	Recovery% from the added amount*
Acemetacin Ost-Map® capsules each capsule is labeled to contain 60 mg, batch no. MT9291019	99.38 ± 1.43	10	10	98.50	99.32 ± 0.37	100	100	99.16
			20	100.27			200	99.09
			30	99.93			300	99.71
		Mean ± SD		99.57 ± 0.938		Mean ± SD		99.32 ± 0.341

3.4. Statistical analysis

Statistical comparisons between the analysis results of the pure compound analysis and the official method and no significant difference was found between the proposed and reported

methods at 95% confidence level regarding accuracy and precision by calculating student's t-test and the F value (Table 3).

Table 3. Statistical comparison of the results obtained by the proposed and official methods for the analysis of Acemetacin in its pure form

Value	Lower Segment	Higher Segment	Official method[a]
Mean	101.46	101.31	99.19
SD	1.698	1.275	1.812
%RSD	1.67	1.26	1.827
N	5	5	5
Variance	2.883	1.626	3.283
Student's t-test	2.044 (2.306)[b]	2.140 (2.306)[b]	
F value	1.139 (6.39)[b]	2.020 (6.39)[b]	

[a] Titrimetric method against 0.1 M NaOH with acetone and water as solvent with potentiometric determination of endpoint.
[b] The values in the parenthesis are the corresponding theoretical values of t and F at P = 0.05.

4. Conclusion

This work introduced a simple new differential pulse voltammetric method for determination of acemetacin by using GCE as working electrode. The proposed method showed accurate and precise results with acceptable sensitivity in range of 1 µM to 3 mM. The method successively assayed acemetacin in its formulated capsules without interference from the co-formulated excipients. These results suggest that the proposed method can be used in quality control labs for routine analysis of ACE.

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