Differential-Pulse Polarography Method for Evaluation of Diclofenac Sodium in Pharmaceuticals

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Abstract

A reliable DPP process has been developed and applied for the estimation of Diclofenac sodium in pharmaceuticals form with DME versus Ag/AgCl. The best peak response was established a cathodic peak -1.14V in 0.002 M acetate buffer at pH5and 0.1M KCl as supporting electrolyte with four electrons transferred in irreversible process. To attain the highest sensitivity, instrumental and experimental and parameters were examined including the type and concentration of supporting electrolyte, pH, buffer solutions, pulse amplitude, and voltage step time. Diffusion current was in direct proportion to Diclofenac sodium concentration and with a linear relationship with the drug concentration in the range of (5 - 30 µg. mL^{-1}) i.e. (1.57×10^{-5}-9.4×10^{-5} mol·L^{-1}) with a correlation coefficient (r= 0.9993), detection limit (S/N = 3) was 0.85 µg. mL^{-1}(i.e.2.67×10^{-6} mol·L^{-1}) and the values of precision, in terms of RSD% ranged between 1.28- 4.11%.The established DPP methods offers excellent analytical figures of merits as well as applied to examine the commercial drug olfen capsule and Diclofenac sodium injection for the determination of Diclofenac sodium and found to be 99.8 mg, 76.9 mg compared to the stated value of 100mg and 75 mg respectively.

Keywords: *Differential-pulse polarography; Diclofenac sodium.*

Introduction

Diclofenac sodium (DCL) is drug prescribed with different trade names, such as Zorvolex, Olfen, Voltaren, Cataflam, Pennsaid, Zipsorand Solaraze.

DCL,$C_{14}H_{10}Cl_2NNaO_2$,318.129g.moL^{-1}, is a 1-sodium [o-(2,6-dichloroanilino) phenyl] acetate [1], Figure 1.
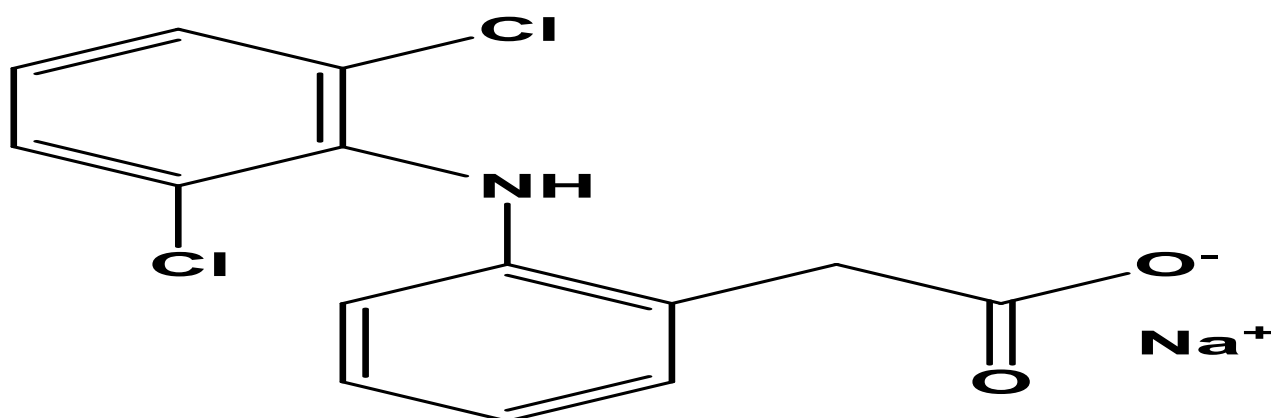

Figure 1: The structural formula of Diclofenac sodium.

A brand name of Voltaren was the first name given to DCL Diclofenac by the Food and Drug Administration (FDA) in 1998, which was firstly produced by Novartis [2]. The drug is classified as non- steroidal anti-inflammatory drug (NSAID) and is typically prescribe to treat pain, swelling, or as anti-inflammatory medicine especially for injuries and for conditions such as rheumatoid, osteoarthritis, painful menstruation periods,

arthritis, migraines, ankylosingsplondylitis, post-surgery analgesia in human and veterinary, and for musculoskeletal injuries [3].The action of the drug is to the inhibit the synthesis of arachidonate metabolites via cyclooxygenase inhibition.

The drug inhibits the formation of prostaglandins from arachidonic acid, hence prevents the inflammatory process. Usually the NSAIDs such as DLC, flurbiprofen and indomethacin which inhibits the action of COX-1 and COX-2 have undesirable gastrointestinal side effects mostly ascribed to the inhibition of COX-1 [4]. Several techniques have been used for the determination of Diclofenac include spectrophotometry [5, 6], HPLC [7], GC and voltammetry [8, 9]. This work aims to confirm the polarographic response of the cited drug and to introduce a new simple voltametric procedure for its determination in pharmaceuticals.

Experimental

Apparatus

Electrochemical measurements were performed by a 797VA Computrace Metrohm, Herisau, Switzerland polarographic analyser. It was used with DME mode as a indicating electrode and Ag/AgCl as a reference electrode with Pt wire while auxiliary electrode. All experiments were performed at 25°C. Hanna model pH 211 pH-meter (Romania) was used to accomplish pH measurements.

Materials and Reagents

All experiments were achieved with analytical grade reagent, chemicals and solvents. Deionised water was employ for preparation the standard and samples. Pure form Diclofenac standard material was received as a powder from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). olfen capsul100 mg was obtained from local pharmacies. 0.1g of the diclofenac sodium was used to prepare standard solution (1000 $\mu g.mL^{-1}$) by dissolving it in 100 mL with water.

Working solutions were freshly obtained by serial dilutions. 1.68 g of $C_2H_3NaO_2$ was mixed with 1.12 ml of CH_3COOH in 500 mL of distilled water; the mixture was then diluted to one liter with same solvent. On the other hand, to obtain pH $\approx$ 2 of Britton-Robinson buffer, 2.47 g of H_3BO_3, 2.3 mL of glacial CH_3COOH and 2.7 ml of ortho-phosphoric acid were mixed and diluted to 1 liter with distilled water (i.e. 0.04 M in each constituent). One liter of phosphate buffer was prepared by dissolving 12 g of NaH_2PO_4 (0.1 mole) and 0.1 M 6.78 g of H_3PO_4 (0.1 mole) in distilled water [10]. 1M solutions of potassium chloride, lithium chloride and potassium nitrate were prepared by dissolved 7.450g, 4.239g and 10.100g of the mentioned salts respectively in 100 ml of deionized water.

General DPP Procedure

An aliquot volume of Diclofenac samples was transferred to 20ml volumetric flasks, then 2 ml of 0.02 M acetate buffer at pH 5 was added with 2ml of (1M) KCl as supporting electrolyte and diluted to the mark with deionized water.Each sample was transfered to a polarographic cell and degassed with high purity nitrogen for 300 sec to remove the oxygen and analysis atscan rate 3 mV s^{-1},voltage step time 2sec, voltage step6mV,pulse time 0.04sec,pulse amplitude 160mV and equlibrium time 10 sec .

Preparation the Calibration Curve of Diclofenac Sodium

A series of six standard solutions ranged between (5-30) $\mu g.mL^{-1}$daily prepared by transfeer volumes 1-6 ml of $100\mu g.mL^{-1}$(DCL) standard solutionto 20 ml volumetric flask with 2 ml of 0.02M acetate buffer at pH 5 and 2ml of 1M KCl as supporting electrolyte, then diluted to the mark with deionized water. Each standard solution was analysis using suggested DPP method, paragraph 2.3, at the optimal conditions. A standard calibration graph were prepared between i_d obtained for Diclofena sodium against the concentration using the Least Squares Method[11].

Analysis Capsul and Injection Samples

The contents of 10 capsules were separately weighed and mixed well. To prepares 1000 μg. ML^{-1} of DCL solution, a measure quantity of the powder containing 100mg of the DLC was weighted and dissolved in a small amount of distilled water. The mixture was stirred for10 minutes before dilution to 100 mL in a calibrated flask. The solution was filtered off with suitable filter paper to remove any UN dissolved particle. Finally,

appropriate dilutions of stock solution were made to obtain fresh working solutions for analysis under the recommended procedure.

Results andDiscussion

Preliminary Observations

Typical differential pulse polarogram of (25µg.mL⁻¹) diclofenac in acetate buffer at (pH = 5.0) is shown in Fig. 2 the polarograms shows a well-defined peak appeared at (-1.14) V versus (Ag/AgCl/sat KCl) electrode.
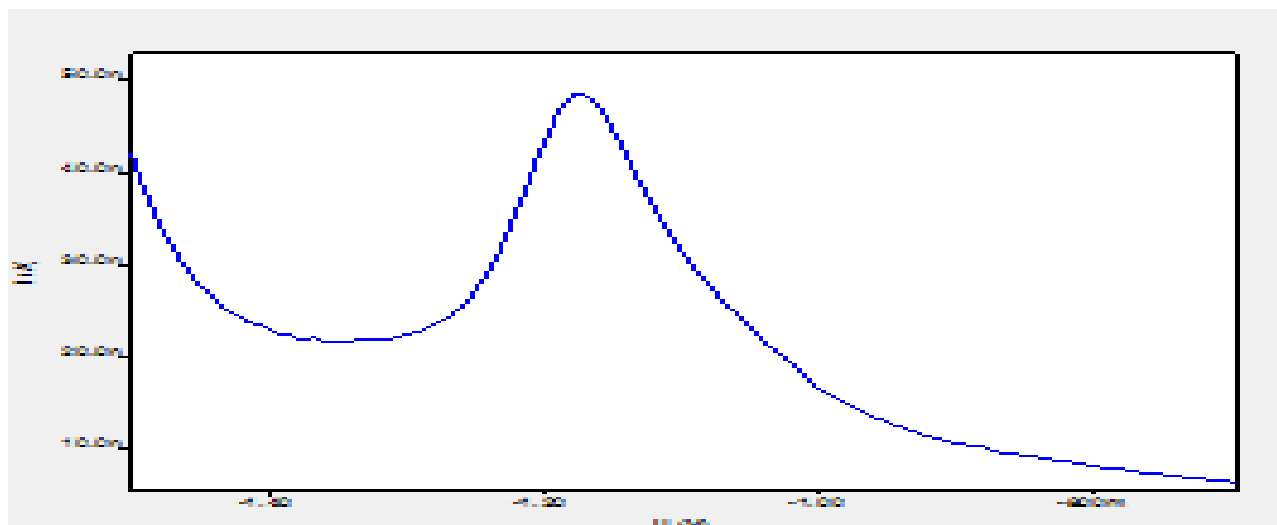
Optimization of DPP Method

DPP technique was applied for the analysis of diclofenac and The effects of a number of experimental factors and instumental parameters which impact the DPP effectiveness were carried out by one variable at a time optimization, whereas just one factor is changed and the others are kept at a steady rank. In this work the effect of buffers with different pH , supporting electrolyte with different concentrations ,influence of solvent system, pulse amplitude and the voltge step time (scan rate) were studied. The optimum conditions represent both the

highest peak current and the best peak shape.

Effect of Buffers and PH

The differential-pulse polarogram of 25µg.mL-1of diclofenac was investigated at different pH values ranged between (3 to 6) with three buffer solutions such as Britton-Robinson, phosphate, and acetate buffer and The polarographic response of diclofenac has been appear as a one distinguished reduction peak at −1.14V applied potential versus Ag/AgCl in 0.002M acetate buffer at pH 5 as a best buffer solution, this peak shifted to more posative potientialwith pH increasement, Fig.3.
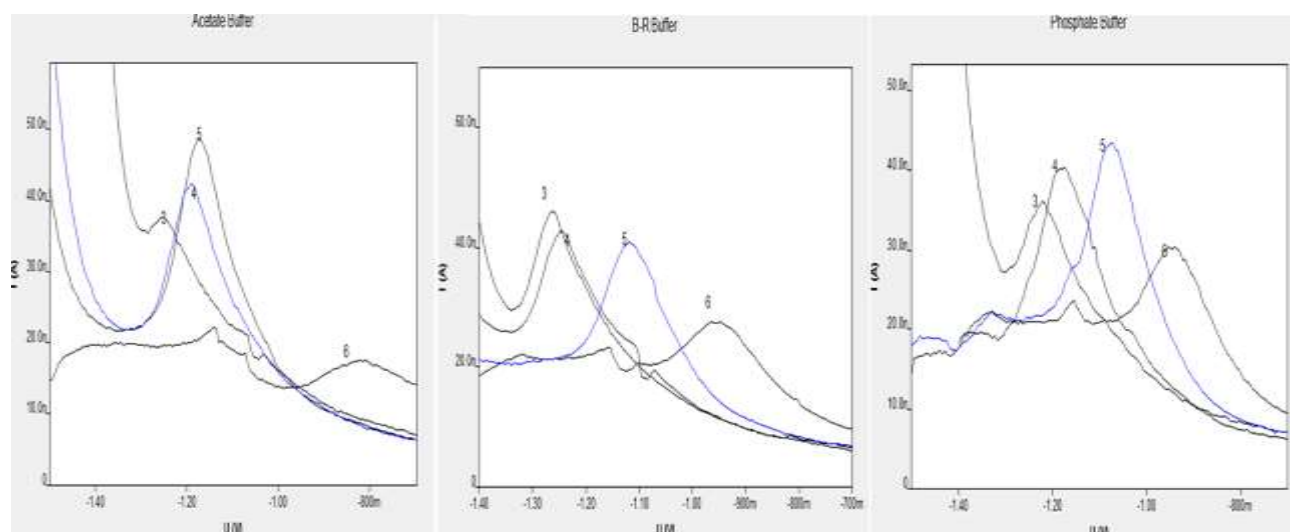

Figure 3: Polarograms of diclofenac at different pH and different buffer solutions

Effect of Supporting Electrolytes

The differential pulse polarograms of 25µg.mL⁻¹ (diclofenac) were measured in

three different supporting electrolytes (KCl, KNO₃, LiCl) with four different concentration of each one(0.025,0.05,0.1,0.15)M.

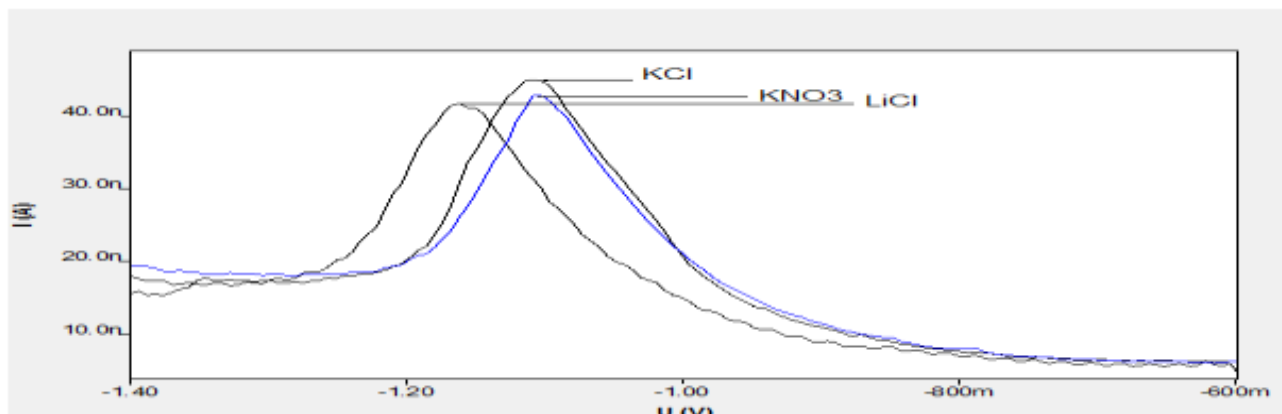The maximum peak current (Ip) was found in 0.1 M of KCl. as Fig.4 shows that.

Figure 4: Polarograms of 25μg.mL⁻¹diclofenac at different supporting electrolytes solutions

Effect of Solvent

While the water was the preferred solvent since it has a high current peak and best shape of peak compare with ethanol and methanol.

Effect of Instrumental Parameters

Pulse Amplitude Effect

The effect of different values of pulse amplitude (viz: 50, 60, 80,100,120,140,160,180,200 mV) on the peak current was carefully investigated. The results showed that the value of peak current was in direct proportion to the applied amplitude up to 160 mV, the after it starts to decrease. Therefore, an amplitude of 160 mV was applied in the subsequent studies snice it gave the best peak Figure 4.
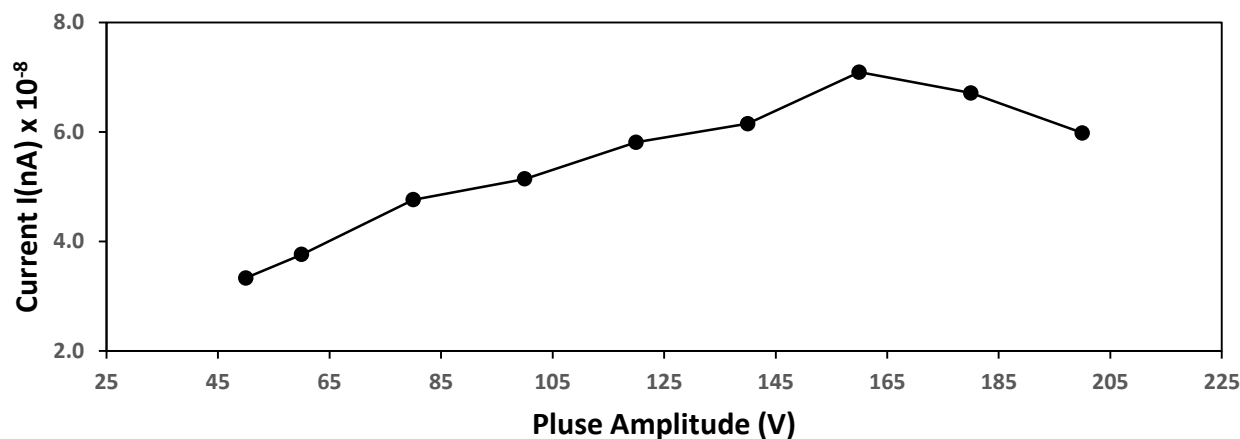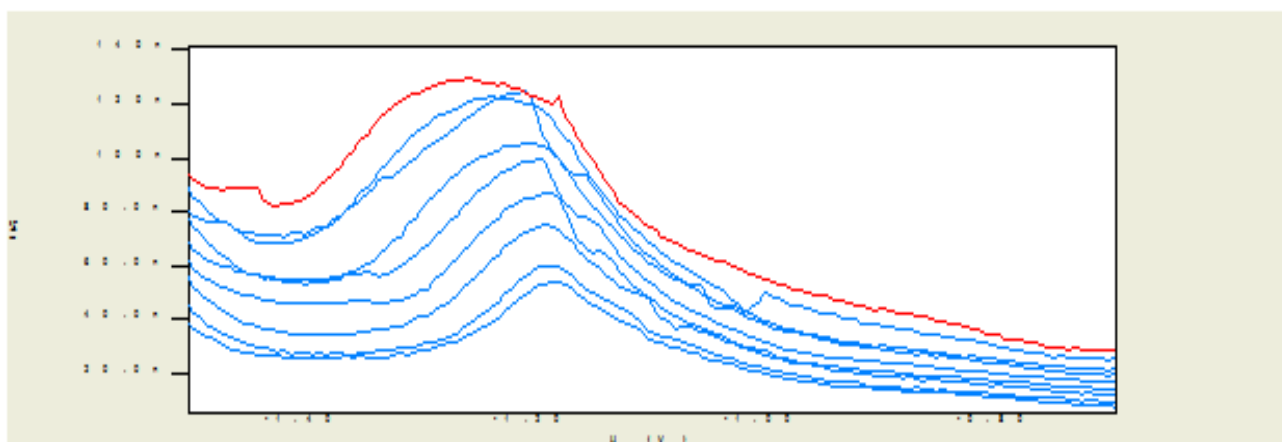

Figure 4: Effect of pulse amplitude on the peak current of 25μg.mL⁻¹ diclofenac in pH 5 Acetate buffer, scan rate (0.015V/s)

Effect of Drop time (Voltage Step Time)

Peak current increases with increasing voltage step time at values (0.6, 0.8, 1.0, 1.2, 1.4 and 2.0 s), while Ep remains quasi-static. The value of the preferred was 2 s, Figure 5.
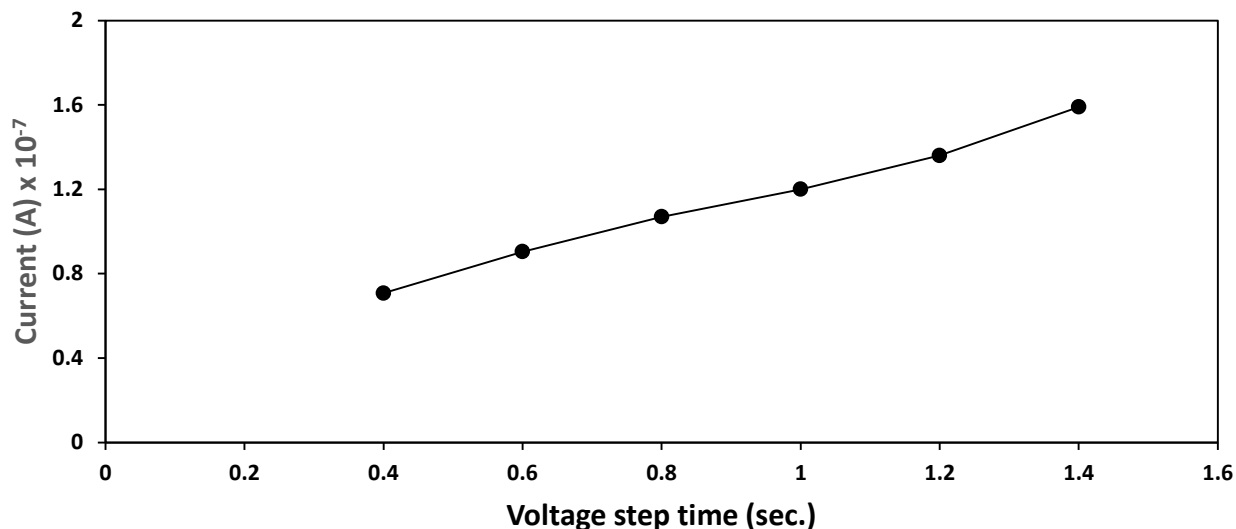

Figure 5: Effect of voltage step time on the peak current of 25µg.mL⁻¹ diclofenac in pH 5 Acetate buffer, pulse amplitude (160) mV scan rate (0.003V/s)

Table1: The optimum parameters established for determination of diclofenac

Condition	Value
Initial potential	-1.5V
Final potential	-0.7V
Pulse time	0.04sec
Buffer	Acetate buffer
PH	5
Supporting electrolytes	KCl (0.1M)
Solvent	Water
Voltage step time	2sec
Pulse Amplitude	160 mV
Voltage step	0.006v
Scan rate	0.003 V/s
Equilibrium time	10 sec
Initial purge time	300sec

Analytical Consideration

Using the optimum condition shown in Table (1), the calibration curve was constructed using a serial dilution of a standard diclofenac in aqueous acetate buffer. (PH = 5.0) Figure 6.
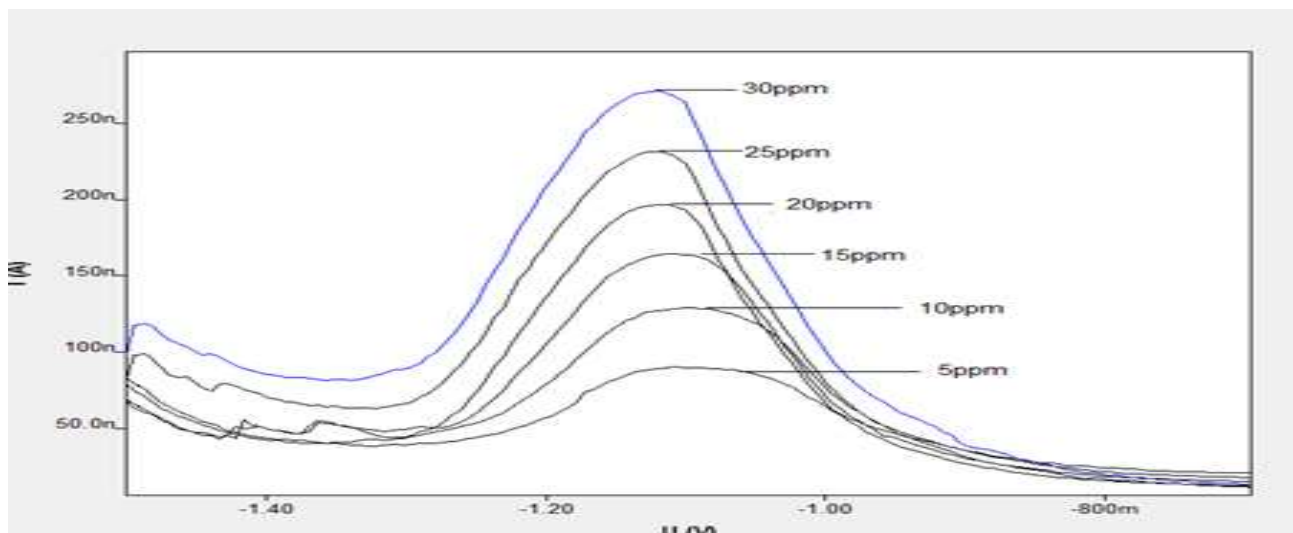

Figure 6: DPP polarograms of Diclofenac at different concentrations

Method Validation

Calibration Graph

Applying the recommended conditions, an excellent linear relationship between the measured polarographic peak current and the concentration of DLC in the range $(1.57 \times 10^{-5}$ to $9.4 \times 10^{-5})$ mol$\cdot$L^{-1} was obtained, Figure7.
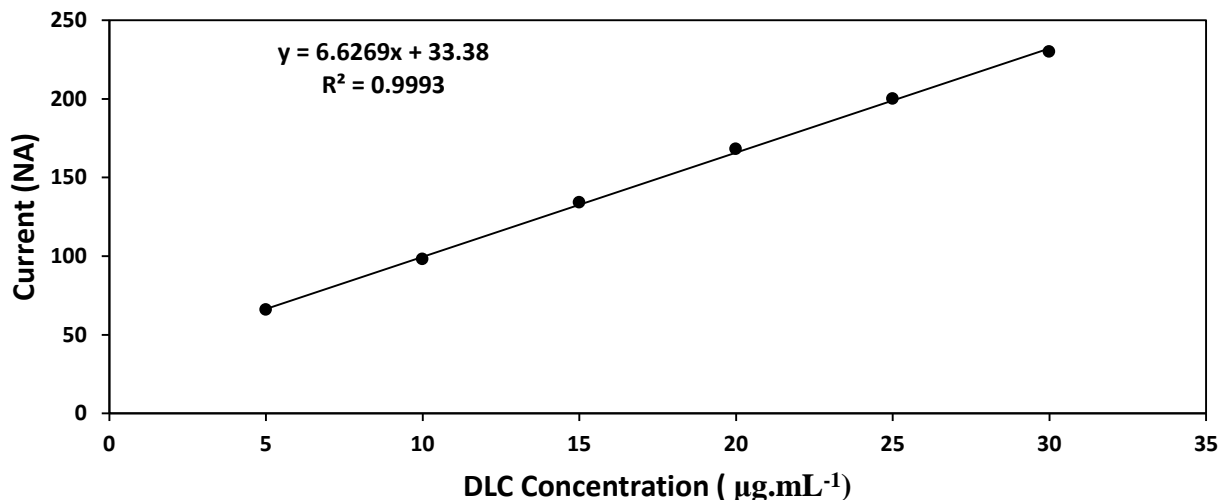

y = 6.6269x + 33.38
R² = 0.9993

Figure 7: The relation between peak current and concentration of $(1.57 \times 10^{-5}$ to $9.4 \times 10^{-5})$ mol·L^{-1}

The numerical estimate for the calibration graph exposed that the linear regression equations for analyte are statistically suitable. The prediction based on the regression line is acceptable as listed in Tables2 this regression line is used to estimate the Diclofenac concentrations in the selected sample which appear justified on statistical basis. The values of limit of detection (LOD) also limit of quantification (LOQ) were calculated based on the standard deviation of the response, residual standard deviation $S_{y/x}$ and the slope b of the calibration curve using the equations (a + $3S_{y/x}$) and (a + $10S_{y/x}$) respectively (11). The results obtained by the projected methods is in agreement with many methods, the results show that the LOD and LOQ found was equal to 0.85 and 2.55 µg.mL^{-1}.

Table 2: Analytical numbers of merit of the Diclofenac estimation using DPP methods

Parameter	Values
Peak potential, E_p (V)	-1.13
Concentration range (µg.mL^{-1})	5- 30
Regression equation (y = bx - a)	Y=6.6269x+33.38
Correlation coefficient (r)	0.9993
Linearity (R²)	0.9986
Slope (b)	6.6269
Intercept (a)	33.38
Standard deviation of regression line ($S_{y/x}$)	1.88
Standard deviation of intercept (S_a)	4.87
Standard deviation of slope (S_b)	0.25
C.L. for the intercept (a ±ts_a) at 95%	33.38± 4.87
C.L. for the slope (b ±ts_b) at 95%	6.6269± 0.25
Limit of Detection- LOD, (µg.mL^{-1})	0.85
Limit of Quantitation - LOQ, (µg.mL^{-1})	2.55

The proposed DPP method was applied for the determination of diclofenac in commercial olfencapsule, 100 mganddiclofenac ampoule 75 mg. the pharmaceutical samples were treated and examined according to the recommended DPP procedure. The results show that the actual amounts of the cited drug in commercial 100 mg olfen capsule, ranged between 98.5 to 102.5 mg,while its mount in the 75mg diclofenac injection, ranged between 74.5 to 79.5 mg. these values are in good agreement with those values fixed on the original products. The results are presented in Table 3.

Table 3: Proposed method Application for the estimation of diclofenac sodium in pharmaceuticals

| Sample	Conc. (µg. mL⁻¹)		Weight fond (mg)	% Recovery	Average % recovery	% RSD
	Taken	Found				
Diclo china Ampoule (**75mg**) (MEHECO) Corp.)	25	24.80	74.50	99.33	102.57	3.32
		25.59	76.78	102.37		
		26.50	79.50	106.00		
Olfencapsule(**100mg**)	20	19.70	98.50	98.500	99.83	2.57
		19.70	98.50	98.50		
		20.59	102.50	102.50		

Number of Transferred Electrons and Actual E½

The real number of electrons involved in a reversible/irrevrsible electrode procedure and the value ofhalf wave potential (E½) was found depending on the equation of Heyrovsky–Ilkovic which shows that the produced wave due to the cathodic reduction of the druge is a reversible process at 25°C [12].

$$E_{applied} = E_{1/2} - \frac{0.0591}{n} \log(\frac{i}{i_d - 1})$$

The given equation ascribs the obveious relation the value of i_d and applied potential for a reversible/irrevrsible reaction. Therefore , the real number of electrons involved in the process could be obtained by plotting the values of $\log(\frac{i}{i_d-1})$ aganest the applied voltage (E) for a set group ofdrug concentrations. The processe assumed to be reversible when an exact number of electrons (n) is obtained, while incomplete number of n indicats irreversible process[13]. The actual peaks voltage Ep calculated were -1.14 V and four electrons were required for the reduction, Figs. 9
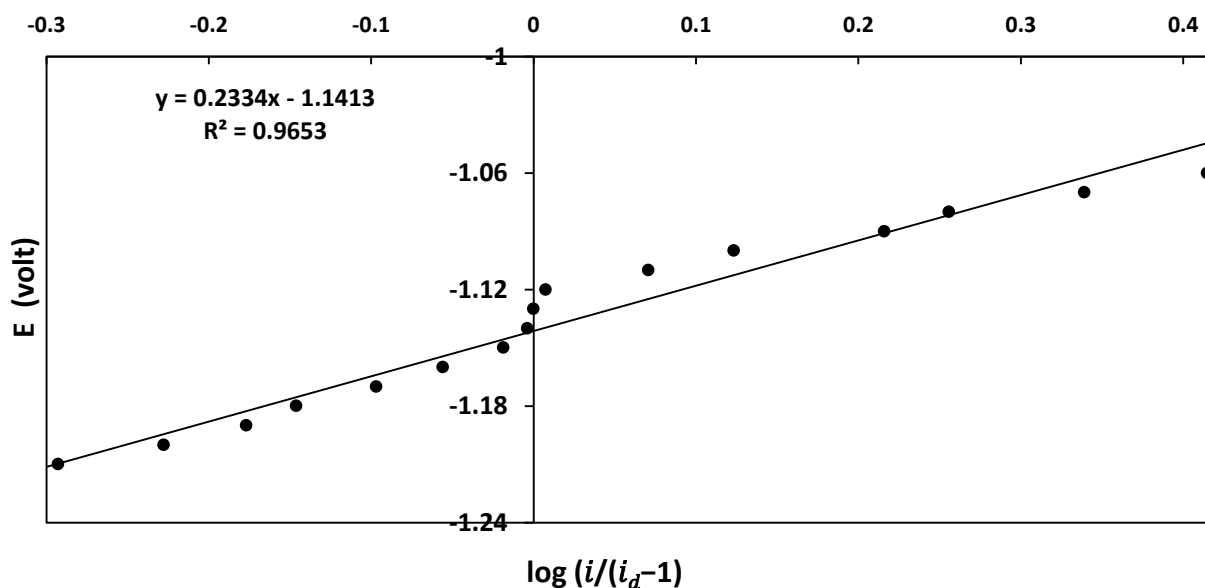
Figure.9: The plot of the applied potential versus log($i/(id - 1)$) according to Heyrovsky-Ilkovic equation at 30µg.mL⁻¹ of diclofenac

Conclusion

Building on the results, it is concluded that this method can be used successfully to determine the diclofenac conc. in pharmaceuticals preparations. Moreover, the advantages of the proposed procedure are direct method, simple, fast; sensitive needs no extra time-consuming steps or sample pretreatment prior to analysis.

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