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Furosemide Determination in Pharmaceutical Samples Using Differential Pulse Polarographic method

Zahrra A.Hussen, Salam A.H. Al-Ameri and Abbas A.Mohammed

Mustansiriyah University, College of Science, Department of Chemistry, Baghdad, Iraq

Abstract

The research includes the development of a simple, sensitive and accurate differential pulse polarography method for the quantitative determination of furosemide drugs in pharmaceutical preparations and in their pure and commercial forms, the electrochemical behavior using the dropping mercury electrode DME, as well as finding the values of the half-wave potential (E1/2) as a qualitative value, the number of electrons that were transferred and participating in the reduction process. The furosemide drug showed a clear reduction peak at a voltage of (-0.24) volts under optimal conditions. The correlation coefficient for furosemide at a concentration range of (2–24) µg.ml^{-1} was 0.9969, the LOD and LOQ of the drug were 2.139 and 7.129, respectively, and the average relative standard deviation (RSD) of the drug was 1.41%, with recovery of the f drug 97.58% and (n =5).

Introduction

Furosemide is commonly known as frusemide, which is a derivative of anthranilic acid inclusion to the class of compounds designated as high-ceiling diuretics [1]. Furosemide has different chemical names [5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethy1) amino) benzoic acid, 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid, 4-cliloro-N-(2-Furylmethyl-5-sulfamoylanthranilic acid, 4-chloro-2-furfurylamino-5-sulphamoyl benzoic acid] Fig (1) , Its CAS (Chemical Abstracts Service) number is (54-31-9). The empirical formula is

$C_{12}H_{11}ClN_2O_5S$ corresponds to molecular mass 330.77 g/mole . Furosemide is crystalline powder with melting point 206°C and white to slightly yellow color. The solubility of furosemide in water, chloroform and ether is slight, while it is soluble in acetone, methanol, dimethyl formamide. Also it is soluble in alkali hydroxide solutions [2]. Owing to its fast and powerful diuretic results, this drug has prolonged usages as a prevailing acidic diuretic in veterinary medicine and humans [3].

Its prime action is also classified as a loop diuretic, which prevents the vigorous reabsorption of chloride in the diluting segment of the loop of Henle [4].This drug was primarily used for the control of hypertension and later, it has found uses in the cure of edema related to nephrotic syndrome, heart failure, cirrhosis of renal and liver disease [5-6]. Prolonged administration of furosemide can lead to ototoxicity, hyperglycemia, and elevated blood LDL cholesterol and triglyceride levels [7].

Several techniques for determining furosemide in pharmaceutical preparations and biological fluids have been developed, these methods include spectroscopic methods[8,9,10,11], chromatographic methods [12,13,14], Voltammetric Methods[15,16,17,18]

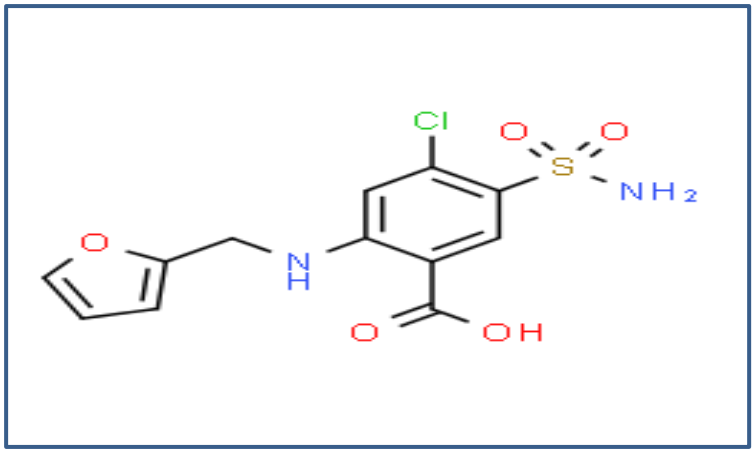

Fig. 1: Furosemide structural

Polarography is the study of the electrolysis of the oxidizable and reducible materials between a dropping mercury electrode (DME) and a reference electrode (RE) [19]. The distinguished characteristics of the polarographic wave are used in the quantitative and qualitative evaluation of the substance to be analyzed by measuring the diffusion current id(peak current)[20], which is directly proportional to the concentration of the studied substance as a quantitative estimation[21]. The half-wave potential (E1/2) is used for qualitative analysis of the matter. Among the many polarographic techniques, differential pulse polarography (DPP) method is the most commonly utilized [22]. In this study, the estimation of furosemide in its pure form and pharmaceuticals was done directly by differential pulsed polarography (DPP) method.

2.EXPERIMENTAL

2.1Apparatus

A polarographic analyzer type 797VA Computerize Metrohm, Herisau, Switzerland was used for electrochemical analysis. It was employed with an working electrode in DME mode, a reference electrode (RE) in Ag/AgCl mode and an auxiliary electrode in (Pt) wire mode .All of the trials were carried out at a temperature of 25 °C. The pH was measured using a WTW inoLab® IDS – Benchtop pH meter (Germany).

2.2Materials and Reagents

The analyses were achieved via applying analytical grade reagent, chemicals substances and solvents. Ethanol was used to preparing the standard solution and commercial drug sample. The pure form furosemide standard material was achieved from the state company for

drug industries - Samara Iraq (SDI). The furosemide tablet was obtained from local pharmacies.

100 mg of the furosemide was used to prepare a standard solution 1000 $\mu g.ml^{-1}$ by dissolving in 100 ml volumetric flask with 50/50 (water ethanol). The standard solutions were prepared by sequential dilution with 50/50(water ethanol) Acetate buffer solution, 0.1 M was prepared by mixing 9 mL of 0.1 M sodium acetate with 41 mL of 0.1 M acetic acid and completed to volume 100 mL with deionized water. B-R buffer, 0.04 M solution was prepared by dissolving 2.47 g of boric acid in 500 ml deionised water in 1000 mL volummetric flask then added 2.3 mL acetic acid and 2.7 mL phosphoric acid and complete the volume to the mark with deionized water [23] ,and Phosphate Buffer Solution It is prepared by dissolving 12 g of Sodium di-hydrogen phosphate 0.1 M in volumetric flask 1000 ml and then adds 2.3 ml of acetic acid and 2.7 mL of phosphoric acid and the volume was completed to the mark by deionised water [24].

2.3 Preparation furosemide standard calibration graph:

A aliquot volumes of 1000 $\mu g.ml^{-1}$ furosemide standard solution were transferred to 20 ml volumetric flasks, and 1 ml of 0.1M phosphate buffer a was added, along with 0.2 ml of KCl (0.01M) as a supporting electrolyte, and diluted to the mark with 50/50 (water ethanol). Each sample was transferred to a polarography cell and degassed with high purity nitrogen for 300 s to purge the oxygen and analysis at scan rate 5 mVs^{-1} with pulse amplitude 50.

2.4 Preparation of furosemide drug samples

A commercial sample of tablets was prepared by taking 10 tablets of the furosemide drug and grinding well in a fine powder with a mortar and a ceramic hammer and mixing homogeneously and taking a weight equal to the weight of one tablet in a 50 ml beaker A volume of 20 ml of 50/50 (water ethanol) was added with good stirring, then the mixture was filtered and a clear solution was obtained, and transferred to a volumetric bottle of 50 ml and the volume was completed to the mark by adding 50/50(water ethanol).

3 Results and Discussion

furosemide drug has been studied using the differential pulse polarography (DPP) method. In order to obtain the best performing conditions that provide the best results, by carrying out the analyses at different conditions and selecting the one that resulted in highest value for the diffusion current, the effect of pH solutions, buffers and supporting electrolyte. furosemide drug, preliminary experiments were conducted to evaluate the behavior of the active groups in it and the extent of the best effort to work using (DPP) technique ،Figure 2

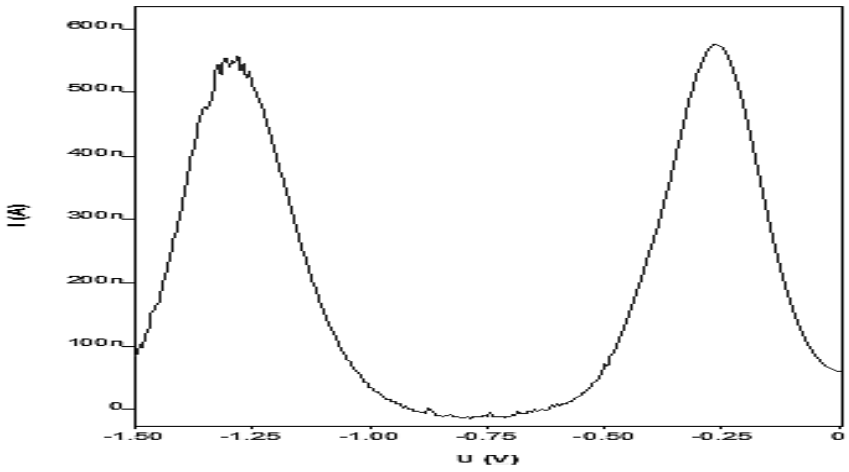

Fig (2) DPP polarograms of furosemide

3.1 The effect of pH

Changing the acidic function often leads to changing the reaction products, as well as adopting the value of E1/2 Organic compounds have a significant pH value , furosemide samples were analyzed With pH numbers 3, 5 and 7 according to the results, the work in PH 7 was, gave a clear peak with a constant half-wave voltage value E1/2 . The Polarograms shows that a pH of 7 is optimal for the analysis of furosemide and that The dependence of the diffusion current and peak voltage on the pH of its solution indicates the presence of a electrochemical reaction in which hydrogen ions are released ‹Figure 3

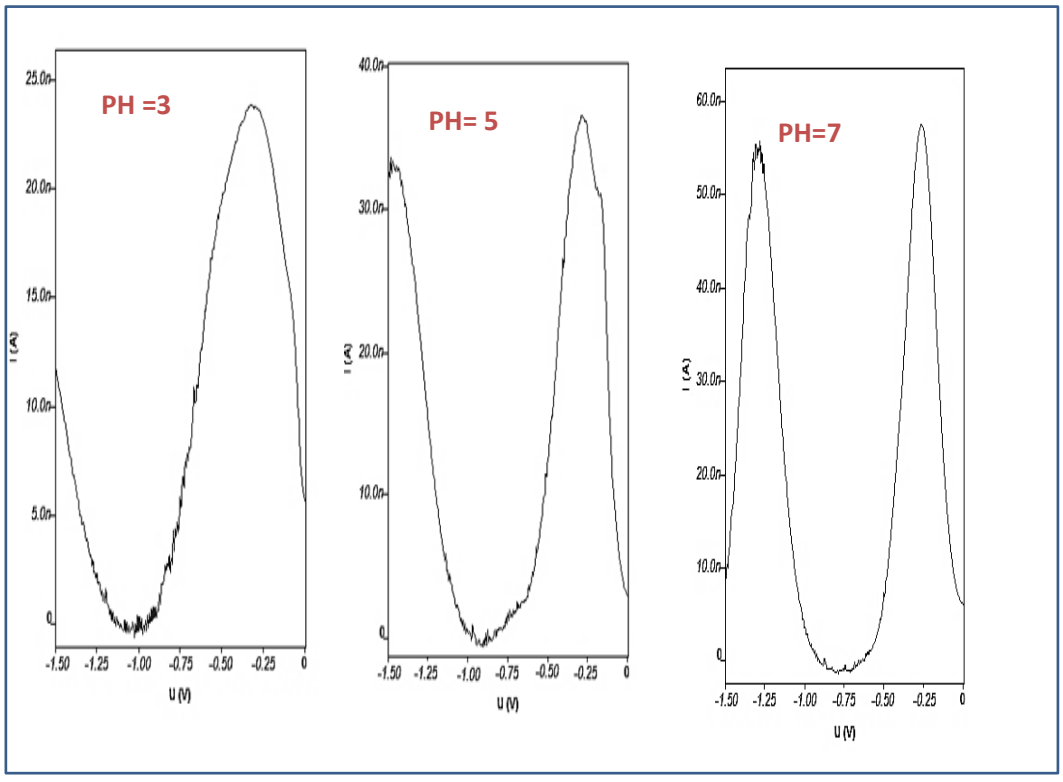

Fig (3) DPP polarograms of furosemide at different pH solutions

3.2Effect of Supporting Electrolyte

Three types of electrolytes were used: potassium chloride ,potassium nitrate and lithium chloride in the working conditions , the results showed better Signal for diffusion current using potassium chloride compared with potassium nitrate and lithium chloride , ،Figure (4) It was noted that the diffusion current value increased with the decreasing concentration of the supporting electrolyte, as well as the stability of the voltage values . The peak varies with the type and concentration of the supporting electrolyte.

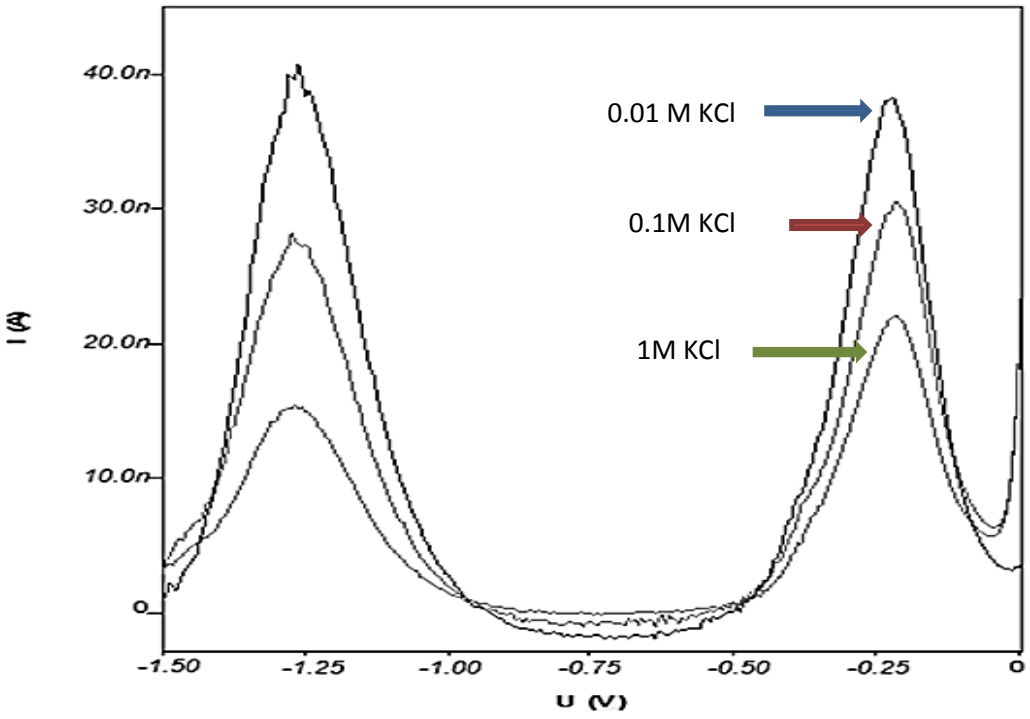

Fig (4) DPP polarograms of furosemide at different concentrations Supporting Electrolyte KCl

3.3 The effect of Temperature

By continuously measuring the Ip, the influence of temperature on the electrochemical reaction of furosemide was

investigated at various temperatures (20-45°C). The value of Ip was found to be unaffected by temperatures ranging from 20 to 45°C. The best temperature is from 25 °C.

3.4 Optimum conditions

Optimal experimental conditions that were used in all the performance studies and the analytical applications of this drug summarized in Table 1

Table (1) Optimum conditions for analyzing furosemide drug.

Experimental condition	Range	Appropriate conditions
Buffer	Britton – Robinson (B-R) buffer Phosphate buffer Acetate Buffer	Phosphate buffer
PH	3,5,7	7
Supporting Electrolyte	LiCl, KCl ,KNO_3	KCl
Solvent	Water -methanol –50 /50 water ethanol	50/ 50water ethanol
Temperature effect	20,25,30,35, 40 °C	25 °C

3.5 Calibration curve

Under optimal measurement conditions, a standard calibration curve prepared via measured diffusion current of furosemide with the corresponding drug concentration values within the concentration range 2 – 24 $\mu g.ml^{-1}$, Figure 6. The results showed a linear equation statistically treated using the least squares method

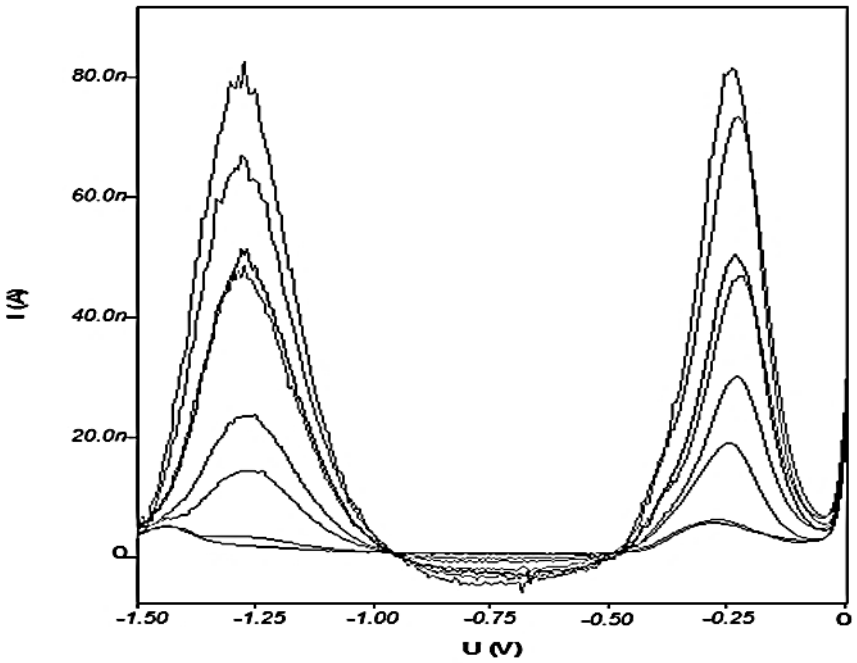

Fig (5)DPP polarograms of standard solutions of different concentrations of furosemide drug at pH 7 using buffer phosphate and KCl

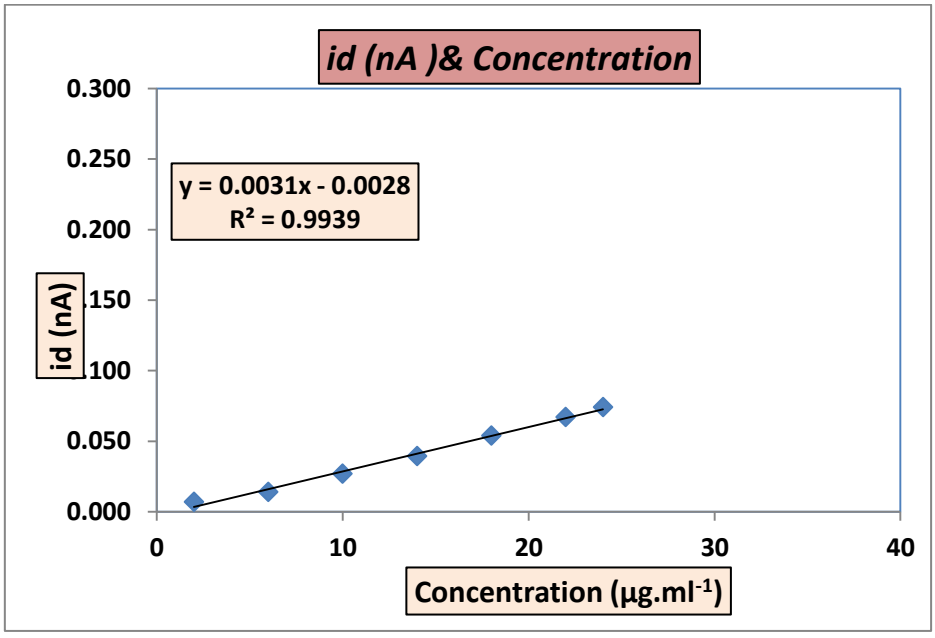

Figure (6) Standard calibration curve for furosemide drug

The statistical data for the standard curve showed a straight line equation well suited for the analysis of furosemide drug it was used to find the drug concentration in samples.

The results show that the LOD and LOQ found for furosemide drug was equal to 2.139 and 7.129 $\mu g.ml^{-1}$ Table 2

Table (2):Analytical data for the calibration curve for the standard furosemide drug

Parameters	Result
Peak potential, Ep, V	- 0.24
Concentration range, µg.ml-1	2- 24
Regression equation, y = bx ± a	**y = 0.0031x -0.0028**
Correlation coefficient, r	0.9969
Linearity, R^2	0.9939
Slope b, µA /µg.ml-1	0.0031
Intercept, a, µg.ml-1	0.0028
Standard error of regression line, $S_{Y/X}$	**0.00221**
Standard deviation of intercept, S_a a ± t(n-2)Sa at 95%	0.001726
Standard deviation of slope, S_b b ± t(n-2)Sb at 95%	0.000110
Limit of Detection, LOD, µg.ml-1	2.139
Limit of Quantitation, LOQ, µg.ml-1	**7.129**

The accuracy and precision of the method for the determination of furosemide were confirmed. Various standard samples were prepared and analysis (n=5), Table 3

Table (3) Analytical results of standard furosemide samples

Initial Conc. ($\mu g.ml^{-1}$)	Found Conc. ($\mu g.ml^{-1}$)	Absolute Error	Relative Error (%RE)	Recovery %	Standard Deviation (SD)	(RSD%)
3	2.89	-0.11	-3.67	96.33	0.06	2.08
12	11.63	-0.37	-3.08	96.92	0.22	1.89
15	14.92	-0.08	-0.53	99.47	0.04	0.268
n = 5	t_{n-2} = 2.57					

proposed DPP method was applied to the determination of the furosemide drug in the commercial pharmaceutical tablet The method demonstrated a good precision and accuracy and the pharmaceutical drug concentration was estimated in the range of 38.55 to 40.96 mg, which is within the actual concentration depending on the pharmaceutical drug composition and international standards for a value of 40 mg. The results of the analysis are summarized in Table (4)

Table (4) Results of the analysis of a commercial furosemide 40 mg tables.

Furosemide tablets , 40 mg						
Sample of drugs $\mu g.ml^{-1}$	Found Conc. $\mu g.ml^{-1}$	Amount Found (mg)	Recovery %	RE %	Standard Deviation (SD)	RSD %
10	9.94	39.76	99.40	-0.6	0.212	2.134
	10.24	40.96	102.4	2.4		
	9.64	38.55	96.4	-3.6		
	9.94	39.76	99.40	-0.6		
	9.94	39.76	99.40	-0.6		
mean	**9.940**	39.759	**99.40**	**-0.6**		

3.6 The Number of transferred electrons and actual $E_{1/2}$

The Ilkovic -Heyrovsky equation was used to calculate the number of electrons transferred during the reduction on the electrode and the actual value for the half-wave potential (E 1/2) at 25 °C. This equation explains the polarographic wave as reversible / irreversible reaction when the number of electrons is integer and irreversible while (n) is non-integer, Number of electrons (n) can be calculating from the plot of log (i/id -i) versus applied voltage (E) at set group concentrations[25] E applied = E1/2 - (0.0591/n) log (i/id –i) The standard solution of furosemide showed a peak reduction at -0.24v the number of electrons participating in the reduction process was determined and the true value was calculated of E1/2 half-wave voltage as a direct qualitative determination of de furosemide using Eq Alkovic - Hirovsky, figure (3-19), and the results showed a peak reduction at voltage -0.237 Volts and that the electrode operations are not reversible and share 2 electrons.

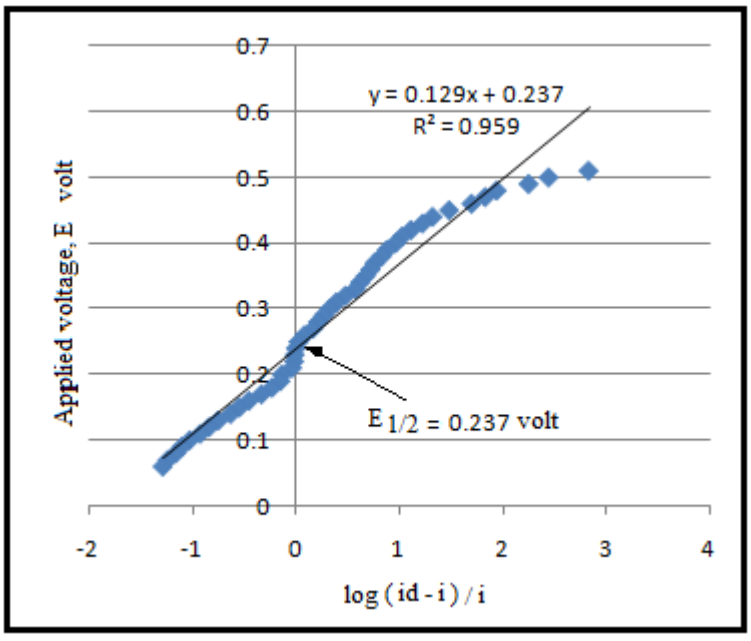

Fig (7) Effect of E applied on the variant log (i/id-i) by Heyrovsky-Ilkovic equation at 20 µg.mL^{-1} furosemide

Conclusion

Electrochemical behavior and differential pulse polarography of furosemide in pure form and in pharmaceutical preparations using DME with phosphate buffer pH 7.0 according to the optimal conditions was applied. Ip is linear over the range 2-24 $\mu g.mL^{-1}$ of furosemide. Regression analysis showed a good correlation coefficient (R2=0.9939) between Ip and concentration over the mentioned range. This method proved to be accurate, quick and precise, thus it might be used for pharmaceutical analysis in the future.

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