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Voltammetric Determination of Dicyclomine Hydrochloride by Carbon Paste Electrode Modified with Iron (III) Oxide Nanoparticles and Activated Glassy Carbon Electrode in Pharmaceutical Dosage Form, Human Plasma and Urine

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A carbon paste electrode modified with iron (III) oxide nanoparticles (MCPE) and an activated glassy carbon electrode (GCE) were constructed for quantitative voltammetric determination of dicyclomine hydrochloride (DH). The voltammetric response of DH was compared at both constructed electrodes. The electrochemical oxidation of the drug was established using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). For analytical purposes, a well-resolved irreversible diffusive controlled voltammetric peak was obtained at approximately 833 mV using Britton-Robinson (B-R) buffer solution of pH 8.00 using GCE. However, in the case of MCPE, a well-resolved irreversible adsorptive controlled voltammetric peak was obtained at approximately 821 mV at pH 6.00 using the same buffer. A linear relationship was attained between the peak current and DH concentration in the concentration range at 0.92-6.18 µg/mL and 0.13 $\times 10^{-6}$-1.93 $\times 10^{-6}$ µg/mL using GCE and MCPE, respectively. DPV and SWV techniques were developed for the quantitative voltammetric determination of DH in its pure form, in its pharmaceutical dosage form and in biological fluids. The method showed a minimum detectability (LOD) of 0.19 µg/mL and 0.39 $\times 10^{-6}$ µg/mL and a limit of quantitation (LOQ) of 0.59 µg/mL and 1.20 $\times 10^{-6}$ µg/mL with both GCE and MCPE, respectively. The proposed method was validated and compared with the reference method. It revealed good accuracy, precision and reproducible results.

Keywords: Dicyclomine hydrochloride (DH), voltammetry, glassy carbon electrode, nano iron (III) oxide modified carbon paste electrode, Spasmorest® tablets.

1. INTRODUCTION

Dicyclomine hydrochloride (DH), [bicyclohexyl]-1-carboxylic acid, 2-(diethylamino)ethyl ester hydrochloride, is anticholinergic/antispasmodic medication that is used to treat irritable bowel syndrome (IBS). IBS is a chronic disease that influences the colon, causing abdominal colics, bloating, diarrhea and constipation. DH acts by reducing the activity of the colon and by relaxing the muscles in the stomach and intestines, thus relaxing cramps of the stomach, bladder and intestine [1-3]. Animal studies suggest that DH acts via a dual mechanism. It involves a specific anticholinergic effect (antimuscarinic) at acetylcholine receptor sites and has a direct effect on smooth muscle (musculotropic). Its structural formula is shown in Fig. 1.

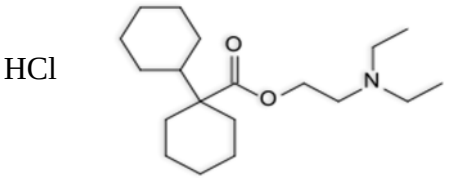

Figure 1. Chemical structure of dicyclomine hydrochloride

Many quantitative analytical methods have been given for the simultaneous determination of DH in its combined pharmaceutical dosage form. Among these are spectrophotometric [4-9], chromatographic [10-29] and other methods [30].

However, fewer analytical methods have been developed for the determination of DH alone in its pharmaceutical formula. Among these are a spectrophotometric method using pi- acceptors [31] and chromatographic [32-33], potentiometric [34-35], calorimetric [36] and voltammetric methods [37-39]

Electrochemical methods of analysis are very critical for the determination of many drugs and other ingredients present in pharmaceutical drug formulations [40-44]. The advancement in electrochemical techniques in the scope of examination and determination of drugs is because of their specificity, high sensitivity and short analysis times compared with other different techniques. The exploitation of carbon-based electrodes, especially the glassy carbon electrode, for electrochemical measurements has increased tremendously in recent years due to their convenience and simplicity for the determination of substances that are subjected to redox reactions, which is very important in the field of clinical and pharmaceutical analysis. The pharmaceutical activity and metabolic destiny of many drugs can easily be estimated by knowing their redox properties[45, 46].

Recent voltammetric techniques, such as differential pulse voltammetry (DPV) and square wave voltammetry (SWV), have been applied for estimation of a wide range of drugs with the advantages of high sensitivity, simplicity, low cost, and short analysis time compared to spectrophotometric and chromatographic techniques. There is no need for sample extraction, preparation, derivatization and purification steps [47, 48].

This work aimed to study the electrochemical oxidation of DH by using cyclic voltammetry (CV) as well as the determination of trace amounts of DH by DPV and SWV at GCE and MCPE electrodes. Different experimental conditions were studied, and then, the electrodes were applied

successfully for determination of DH in pure form, pharmaceutical dosage form and biological fluids. The results obtained can assist the application of the cited voltammetric method in the routine analysis of DH in quality control laboratories.

2. EXPERIMENTAL

2.1. Materials and Reagents

All reagents and solvents were of analytical reagent grade and used without further purification.

2.1.1 Pure Sample and Commercial Dosage Form

DH was of 99% purity and manufactured by Misr company for Pharmaceuticals, Cairo, Egypt.

The pharmaceutical preparation Spasmorest®, in 10 mg tablets (*Batch No*. 126066, *Manuf. by* Misr company for Pharmaceuticals, Cairo, Egypt), was purchased from a local pharmacy.

Nano iron (III) oxide (average crystal diameter 6 nm) was obtained from Sigma-Aldrich.

2.1.2. Reagents

Britton Robinson (BR) buffer, 0.04 M, was prepared by blending 0.04 M orthophosphoric acid with 0.04 M glacial acetic acid and 0.04 M boric acid [49]. Buffer solutions were adjusted with a suitable amount of 0.2 M sodium hydroxide to obtain the required pH in the range of 2–11. All reagents used were supplied by Sigma-Aldrich.

2.1.3 Standard Solutions

A standard stock solution of 200.0 µg/mL DH was freshly prepared by weighing 0.02 g of pure DH and dissolving it in ~20 mL of double distilled water. The solution was then transferred to a 100 mL volumetric flask, and the volume was completed to the mark using double distilled water.

2.2. Apparatus

Voltammetric measurements were carried out using the electrochemical analyzer Computrace system with 797 VA Computrace software (1.0) from Metrohm, Switzerland. A three-electrode cell was employed. The working electrodes were a glassy carbon stationary electrode and a nano ferric oxide modified carbon paste electrode. Electrical contact with the working electrodes was achieved by soldering a copper wire to the metallic part of the apparatus. Ag/AgCl (3 mole L^{-1} KCl) was used as a reference electrode and platinum wire as a counter electrode. The pH measurements were performed

using a Jenway 3330 Research pH meter. Deionized water used throughout the present study was supplied from a Hamilton-Aqua-Metric deionized water system. All the experiments were performed at a temperature of 25°C.

2.3. General Procedure

2.3.1 Preparation of Working Electrodes

a) Glassy carbon electrode (GCE): Mini glassy carbon disk electrode of active zone 2.8 mm, ELCD 641/656. To improve the sensitivity and resolution of the voltammetric peaks, the GCE was polished manually with 0.5 mm alumina slurry on a smooth polishing cloth prior to each electrochemical measurement. Then, it was rinsed with methanol and double distilled water and dried with a tissue paper.

b) Carbon paste electrode modified with iron (III) oxide nanoparticles (MCPE) was prepared as follows: the carbon paste was prepared by mixing of 20% (400 mg graphite + 100 mg nano iron (III) oxide), 30% (350 mg graphite + 150 mg nano iron (III) oxide) and 40% (300 mg graphite + 200 mg nano iron (III) oxide) with 0.3 mL of paraffin oil in a mortar with a pestle until a uniform homogenous paste was obtained. Analytical-grade graphite (particle dimension 20 μm, Sigma-Aldrich) and nano iron (III) oxide (average crystal diameter of 6 nm) were used. A portion of the uniform modified carbon paste was packed into the hole of an insulin syringe body with diameter 3.0 mm that contained a copper wire contacted the apparatus, and the tip of the electrode was polished manually with a weighing paper until it had a shiny appearance.

2.3.2 Surface Area of the Electrode

The active area of GCE and MCPE was determined by applying the cyclic voltammetric method using 20.0 mM $K_3Fe(CN)_6$ at different scan rates. For a reversible process, the following Randles-Sevcik formula was used:

$$I_{pa} = (2.69 \times 10^5)\, n^{3/2} A_0 D_0^{1/2} C_0^{*} \upsilon^{1/2}, \quad (1)$$

where I_{pa} refers to the anodic peak current, n is the number of electrons transferred, A_0 is the surface area of the electrode, D_0 is the diffusion coefficient, υ is the scan rate, and C_0* is the concentration of $K_3Fe(CN)_6$. For 20.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, n = 1, and $D_0 = 7.6 \times 10^{-6}$ cm^2 s^{-1}; then, from the slope of the plot of I_{pa} versus $\upsilon^{1/2}$, the electroactive area was calculated. In our experiment, the electroactive area calculated from Randles-Sevcik equation was found to be 0.112 and 0.056 cm^2 for MCPE and GCE, respectively. The electroactive area of MCPE had an almost doubled active area compared to GCE, so there was a greater response of MCPE than GCE on peak current that resulted from DH.

2.3.3 Construction of Calibration Curve

Appropriate aliquots of DH standard stock solution were transferred to a 10 mL volumetric flask, and the solution was completed to the mark using 0.04 M BR buffer, pH 6-8, to cover the final

concentration ranges of 0.92 – 6.18 µg/mL and 0.13 $\times 10^{-6}$ – 1.93 $\times 10^{-6}$ µg/mL at GCE and MCPE, respectively. DP and SW voltammograms were recorded using different electrodes, applying pulse amplitude (ΔE) = 60 mV, pulse time = 0.06 s and scan rate (υ) = 50 mV/s in the case of DPV, while in the case of SWV, ΔE =50 mV and υ = 60 mV/s, over an oxidation potential range from +400 to +1300 mV. The average of triplicate measurements at room temperature of content in the sample was calculated.

2.3.4 Application to Pharmaceutical Formulations

The content of five tablets of Spasmorest® (10 mg) was transferred into a mortar and ground with a pestle. A portion of the resulting fine powder was accurately weighed, and an equivalent was added to a solution to give a concentration of 1.0×10^{-3} M DH. This was then transferred into a 50 mL volumetric flask and diluted to the mark with double distilled water. The measuring flask was sonicated for 20 min to complete the dissolution of the solution. The clear supernatant liquid was transmitted into a 100 mL measuring flask and then diluted to the mark with double distilled water. An aliquot of the solution was taken, and the above procedure was repeated.

2.3.5. Application to Human Serum

A mixture of 0.4 mL ethanol, 0.4 mL 5% $ZnSO_4$ and 0.4 mL if serum was centrifuged for 25 min at 14000 rpm. One mL of the centrifuge supernatant was then transferred into the voltammetric cell and diluted to the desired volume with Britton-Robinson buffer at pH 8.00 and 6.00 for GCE and MCPE, respectively. This was subsequently spiked with 20 µg mL^{-1} of DH and then analyzed as described above in section 2.3.

2.3.6. Application to Human Urine

A mixture of 0.5 mL urine, 0.5 mL ethanol and 0.5 mL of 5% $ZnSO_4$ was centrifuged for 25 min at 14000 rpm. An aliquot (1 mL) of the clear solution was then transferred into the voltammetric cell, diluted to the desired volume with Britton-Robinson buffer at pH 8.00 and 6.00 for GCE and MCPE, respectively, subsequently spiked with 20 µg mL^{-1} of DH, and then analyzed as described in section 2.3.

3. RESULTS AND DISCUSSION:

3.1. Electrochemical Oxidation of DH

The cyclic voltammetric behavior of DH at GCE and MCPE was studied (Fig. 2). DH gave only one oxidation peak. On the reverse scan, no reduction peak was observed, indicating that oxidation of DH is an irreversible process at GCE and MCPE.

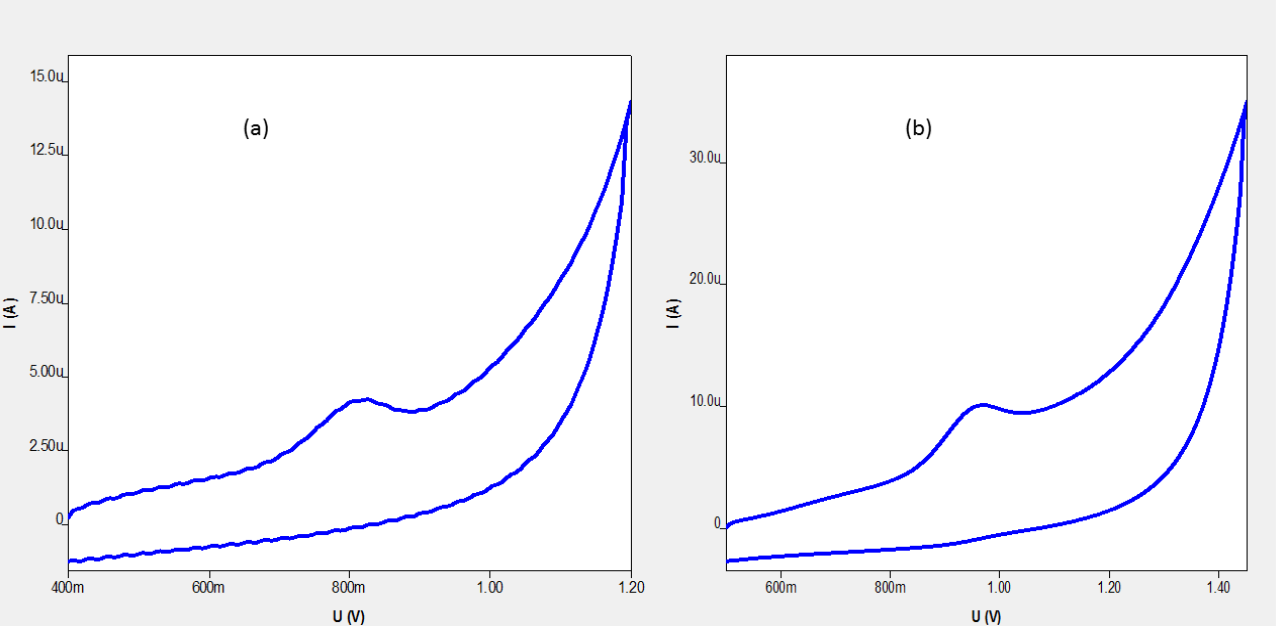

Figure 2. Cyclic voltammograms of DH using (a) 4 µg/mL at GCE and (b) 0.9 µg/mL at the nano iron (III) oxide electrode, at υ = 50 mV/s, pre-concentration time = 5 s, and E_{app} = 50 mV.

3.2 Optimization of Experimental Conditions

3.2.1 Effect of scan rate:

The influence of scan rate (υ) on the oxidation peak currents of DH was studied with GCE and MCPE at the optimum pH for each electrode. The linear variation in the logarithm peak current (Ip) with the logarithm of scan rate (υ) for the irreversible electrode reaction was given by the following equation (Randles-Sevcik equation) [50]

$$Ip = (2.69 \times 10^5) n^{3/2} A D^{1/2} v^{1/2} C^*$$

where n is the number of electrons exchanged during the redox process; A (cm^2) is the active area of the working electrode; D ($cm^2\ s^{-1}$) and C* (mol cm^{-3}) are the diffusion coefficient and the bulk concentration of the electroactive species; and υ is the scan rate (V s^{-1}). In the present work, the data are plotted as a log-log graph. This confirmed the irreversibility of the electrochemical processes with a simultaneous increase in the peak current at a high scan rate. A good linearity between log I_p and the log scan rate (υ) was obtained from the range of 20 ~ 200 mV/s as shown in Fig. 3. The slope value obtained in case of GCE was 0.414, which is close to the theoretical value (0.5). This demonstrated that the electrode reaction is the ideal reaction of the diffusion-controlled electrode process [51]. However, in the case of MCPE, the slope (0.88) was close to the theoretically expected value (1.0), which demonstrated that the electrode reaction is the ideal reaction for the adsorptive controlled electrode process [51].

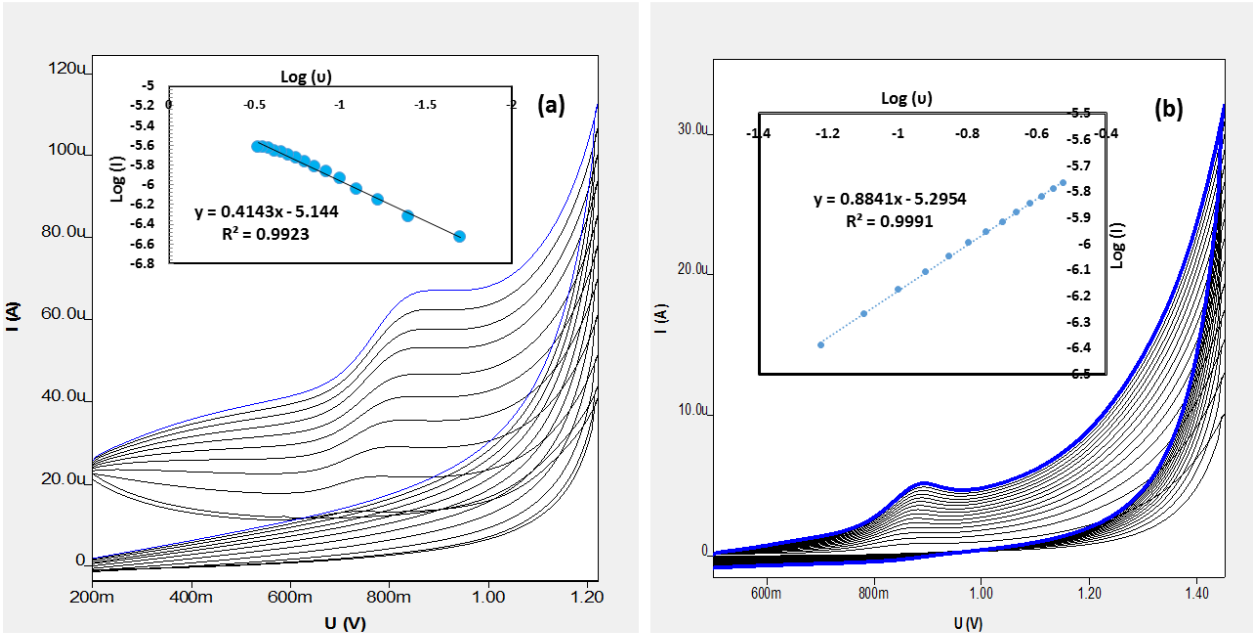

Figure 3. Cyclic voltammograms of DH in BR buffer of pH 8 at (a) GCE and pH 6 at (b) MCPE and the relation between log I (μA) and log υ (V/s) for oxidation of DH at (a) GCE and (b) MCPE.

3.2.2 Effect of Accumulation Potential and Accumulation Time (in the Case of MCPE)

The effect of accumulation potential on peak current was studied over the range -1 to 0.2 V for 1.0×10^{-3} M DH at pH 6. It is obvious from Fig. 4 that the peak current reached its maximum value at -0.9 V and the anodic current decreased as the value of accumulation potential increased. Therefore, the results obtained showed that the Ip attained shows the maximum value at accumulation potential -0.9 V. Additionally, the influence of accumulation time on peak current was studied. The anodic peak current increased as the accumulation time increased and reached its maximum value at an accumulation time of 40 seconds. After this, the value of the anodic current decreased. An accumulation time of 40 seconds was chosen as the optimum accumulation time as shown in Fig. 5.

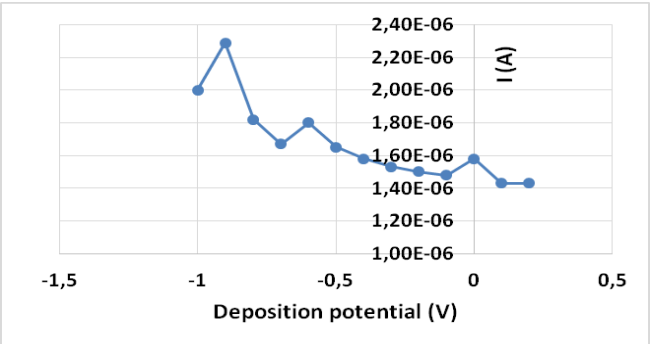

Figure 4. Effect of accumulation potential on the peak current of 1.0×10^{-3} M DH in BR buffer, pH 6, at MCPE.

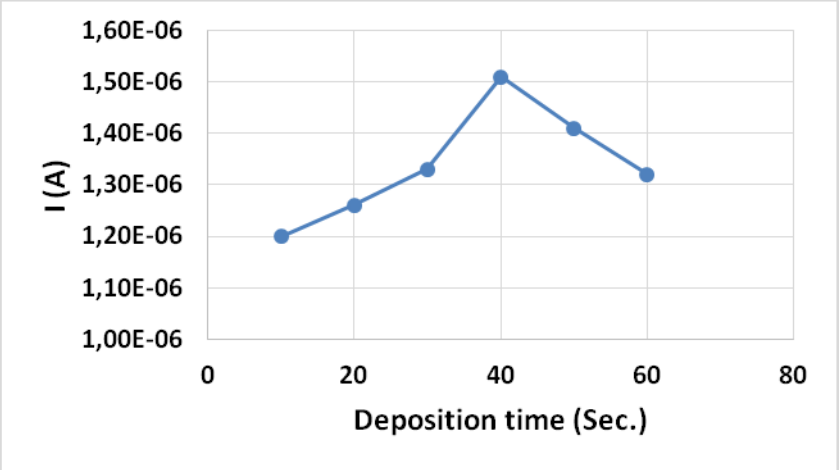

Figure 5. Effect of accumulation time on the peak current of 1.0×10^{-3} M DH in BR buffer, pH 6, at MCPE.

3.2.3 Effect of pH:

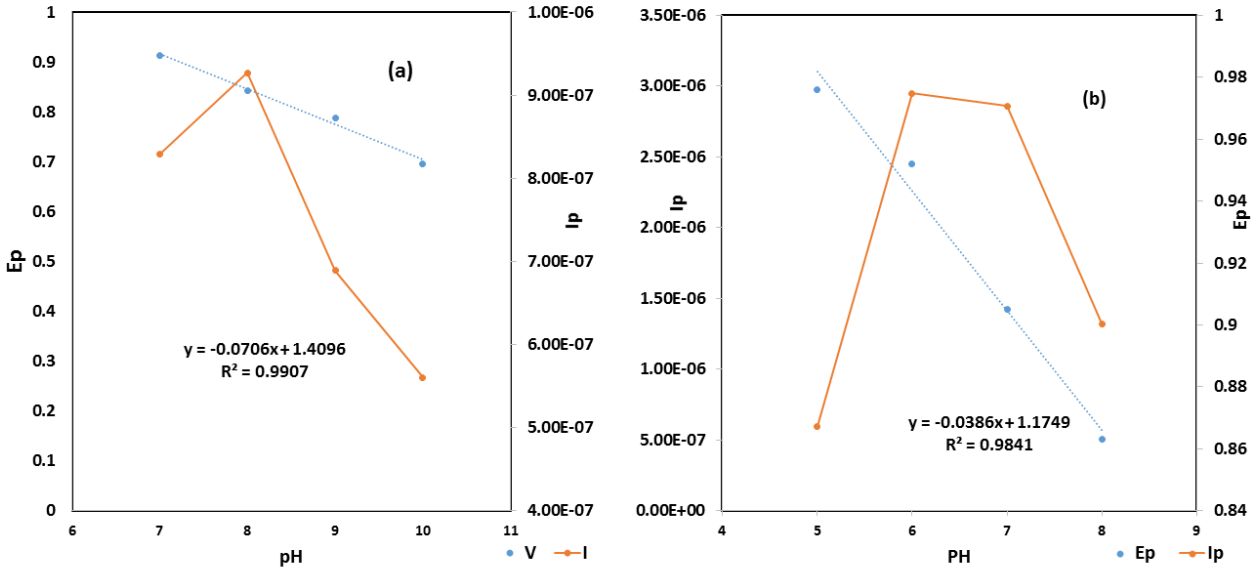

Figure 6. (a) The effect of pH on the peak current (I_p) and potential (E_p) of DH at GCE. (b) The effect of pH on the peak current and potential of DH at MCPE.

The influence of pH on the electro oxidation was measured by cyclic voltammograms using Britton–Robinson buffers within the pH range of 2–11. It was found that the electrochemical behavior of DH was dependent on the pH value of the aqueous solution, and the pH of the solution had a remarkable effect on the peak current and potential of the oxidation of DH as shown in Fig. 6. The maximum current response of DH was observed at pH 8.0 at GCE and pH 6 at MCPE. The anodic

peak potential (Ep) of DH was found to be dependent on pH and shifted to less positive potential with increasing pH, suggesting the participation of protons in the oxidation reaction of DH at GCE and MCPE electrodes. From the plot of Ep vs. pH (Fig. 6), it is obvious that the oxidation peak potential differed linearly with pH and was shifted to become more negative by 0.0706 and 0.0386 V/pH for DH for GCE and MCPE electrodes, respectively. This is illustrated by the following regression equations:

Ep (V) = 1.4096 − 0.0706 pH (R^2 = 0.9907)

Ep (V) = 1.1749 − 0.0386 pH (R^2= 0.9841)

3.2.4 Proposed Oxidation Mechanism of DH:

By applying the Nernst equation using the formula ΔEp/ΔpH (slope) = 0.059/n, we can conclude that the number of electrons transferred is equal to 2, which is greater than number of protons transferred [52, 53], and the reaction is totally irreversible as discussed above. Based on this conclusion, the following mechanism is suggested (Fig. 7). It is obvious that oxidation occurred on the nitrogen atom, which is surrounded by two electron donating groups (2 ethyl groups), thus increasing the basicity and facilitating the loss of electrons and the oxidation reaction.

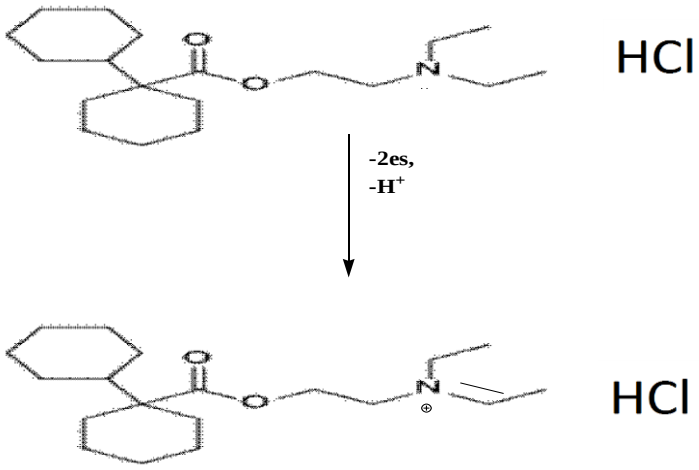

Figure 7. The suggested oxidation reaction of DH.

3.2.5 Effect of Iron (III) Nano Particle Contents

To estimate the ultimate composition of the modified carbon paste electrode towards DH drug, three electrodes containing 20, 30 and 40% of iron (III) oxide nano particles were constructed and tested for their application in the determination of DH at pH 6 using the DPV and SWV techniques. The electrode containing 30% iron (III) oxide nano particles was chosen for further studies as it produced the highest peak current (Figure 8).

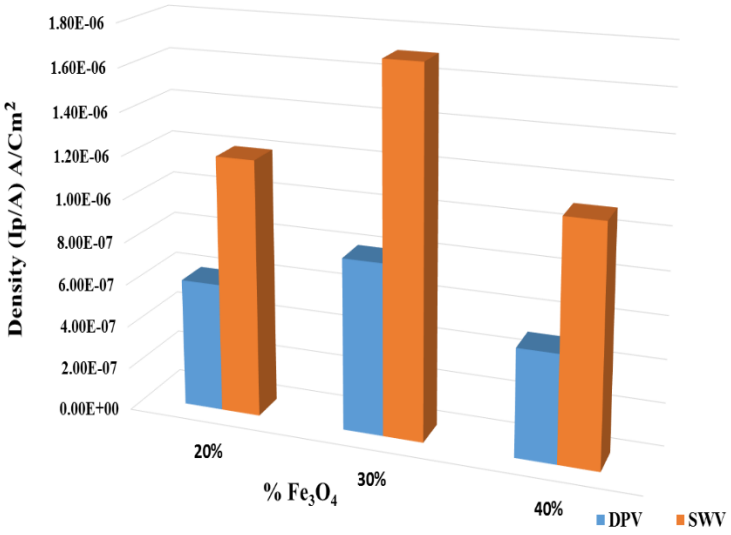

Figure 8. Relationship between the percent of iron (III) oxide nano particles and the anodic peak current

3.2.5 Calibration Graph of DPV

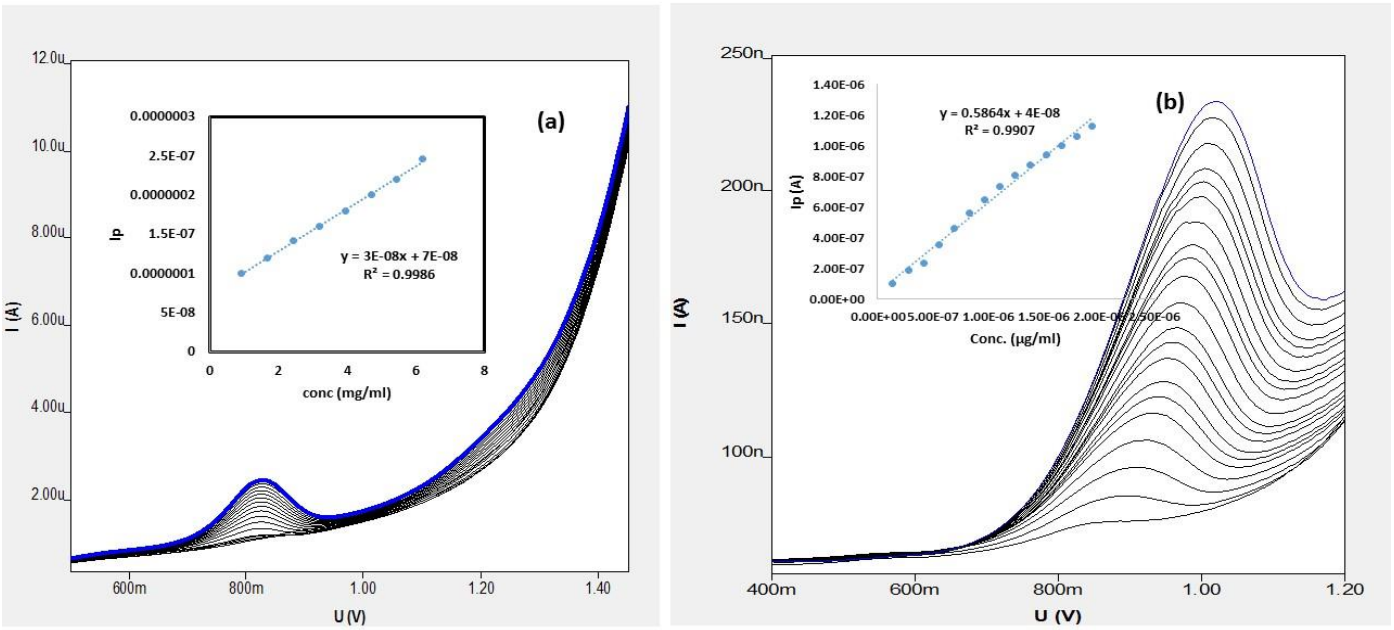

Figure 9. DP voltammograms of DH at (a) GCE and (b) MCPE at υ = 50 mV/s, preconcentration time = 5 s, pulse amplitude (V) = 0.05, pulse time = 0.04 s, voltage step (V) = 0.006561 and voltage step time = 0.06561 s. Inset: The corresponding calibration plots.

Differential pulse voltammograms obtained with an increasing amount of DH revealed that the peak current increased linearly with increasing concentration. The results showed good linearity with regression parameters calculated according to Miller and Miller [54] and were compared with the

reference method [55] as given in Table 1. Fig. 9 shows the dependence of peak current on the concentration of DH at GCE and MCPE, respectively.

3.2.6 Calibration Graph of SWV

Square wave voltammograms obtained with an increasing amount of DH revealed that the peak current increased linearly with increasing concentration [56]. The results showed good linearity with regression parameters also calculated according to Miller and Miller [54] as given in Table 1. Fig. 10. shows the dependence of the peak current on the concentration of DH at GCE and MCPE.

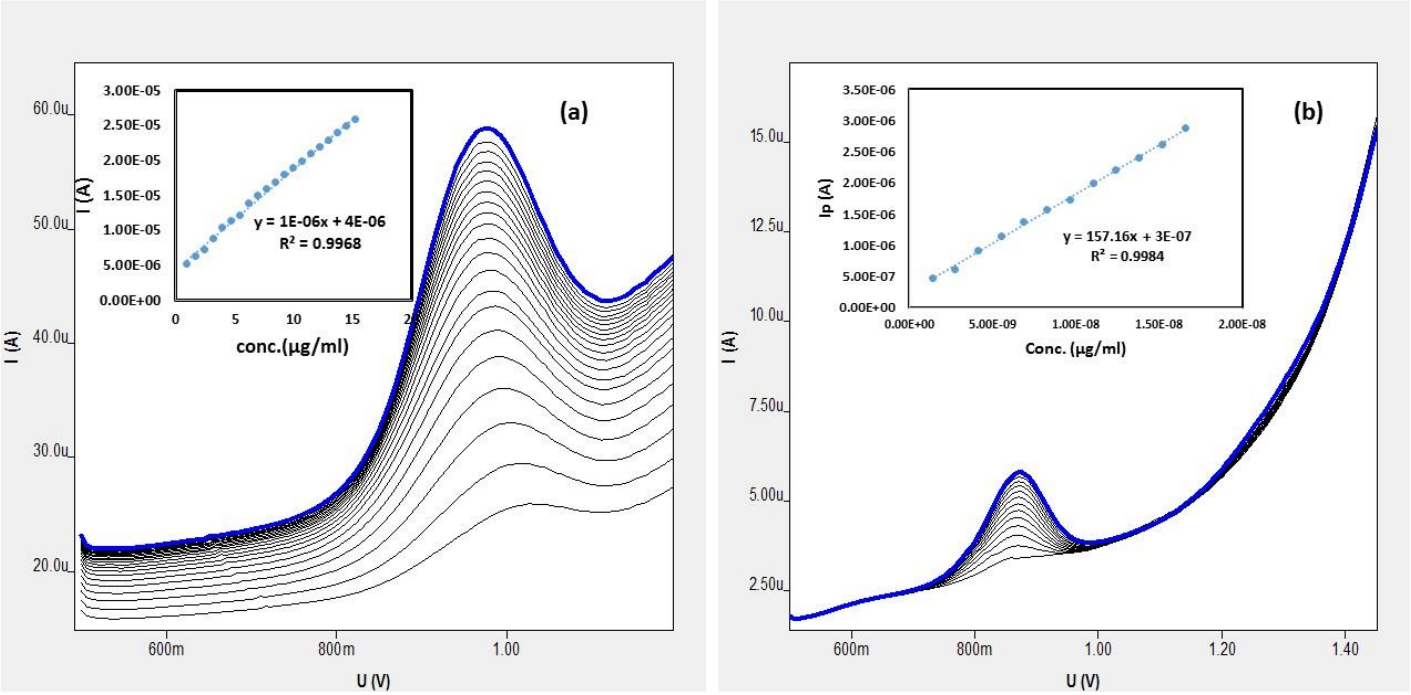

Figure 10. SW voltammograms of DH at (a) GCE and (b) MCPE at υ = 50 mV/s, preconcentration time = 5 s, voltage step (V) = 0.006561, amplitude (V) = 0.019, sweep rate = 0.328 V/s. Inset: The corresponding calibration plots.

Table 1. Regression parameters obtained from calibration curves of DH at GCE and MCPE

Parameter	**DPV**		**SWV**		**Reference Method** [55]
	GCE	**MCPE**	**GCE**	**MCPE**	
Anodic peak potential Ep (V)	0.100	0.100	0.995	0.995	0.930
Linearity range (µg/mL)	0.920 – 6.180	0.13×10^{-6} - 1.93×10^{-6}	0.922 - 15.220	0.13×10^{-8}- 1.66×10^{-8}	0.2×10^{-3}- 0.8×10^{-3}
SD	0.316	0.170	0.556	0.220	
% RSD*	0.315	0.170	0.558	0.220	
Slope (a)	2.7×10^{-8}	0.586	1.44×10^{-6}	157.15	

Intercept (b)	7.4×10^{-8}	4.38×10^{-8}	4.34×10^{-6}	2.52×10^{-7}	
Correlation coefficient (R)	0.9993	0.9950	0.9984	0.9970	0.9981
SE	2.00×10^{-9}	7.07×10^{-8}	3.72×10^{-7}	3.49×10^{-8}	
LOD (µg/mL)	0.198	0.39×10^{-6}	0.004	0.73×10^{-9}	0.12×10^{-3}
LOQ (µg/mL)	0.600	1.2×10^{-6}	0.011	2.22×10^{-9}	0.43×10^{-3}

* Five different concentrations of DH; number of replicates (n) = 5

4. METHOD VALIDATION

The validity of the cited voltammetric method was estimated by determining the following parameters: linearity, range, LOD, LOQ, precision, accuracy, robustness and specificity [57], according to the International Conference on Harmonization (ICH) guidelines [58].

4.1 Linearity and Range

Linearity was verified over the concentration ranges indicated in Table 1 for both DPV and SWV techniques as shown in Figs. 9 and 10. Statistical analysis of the data gave high values of the squared correlation coefficient (R^2) and small values of standard deviation (SD) and relative standard deviation (RSD), resulting from the low scattering of the points around the calibration graph and proved linearity of the method over the specified concentration range (Table 1).

4.2 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LODs and LOQs were calculated according to ICH guidelines [58]. LOD was determined by evaluating the lowest amount of analyte that could be detected but not necessarily quantified.

LOQ was determined by establishing the lowest amount of analyte that could be quantified with suitable accuracy and precision. The results are shown in Table 1.

4.3 Accuracy

Table 2. Accuracy of the proposed method for determination of DH in its pure form using SWV.

Parameter		**Proposed method**		**Reference method [59]**
		Amount taken (µg/mL)	**% found***	**% found***
GCE		2.00	99.5	99.44
		4.00	100	99.25
		6.00	99.2	99.09
	Mean ± SD		99.5±0.4	99.2±0.17
	t-test		0.14	
	F-test		5.32	
		2.00	100	

		4.00	101.2
MCPE		6.00	100.8
	Mean ± SD		100.6±0.6
	t-test		0.01
	F-test		12
	t-tabulated at p = 0.05		2.77
	F-tabulated at p = 0.05		19.00

*each result is the average of three separate determinations.

To prove the accuracy of the cited method, the results of the determination of DH in pure form were compared with those obtained using the reference chromatographic method [59]. Statistical comparison of the results obtained by the proposed method and those obtained by the reference method using Student's t-test and variance ratio F-test revealed no significant difference between the two methods as demonstrated in Table 2.

4.4 Precision (Repeatability and Reproducibility)

The intra- and inter-day precision were evaluated by assaying freshly prepared samples in triplicate on the same day and on three different days, using the cited method. The repeatability (intra-day) and reproducibility (inter-day) of the results achieved by the SWV procedure were examined, and the results revealed high accuracy and precision of the proposed method and proved to be appropriate for quality control of DH (Table 3).

Table 3. Inter- and intra-day regression parameters SWV for determination of DH.

parameter	**GCE**			**MCPE**		
	5.00	10.00	15.00	2.00	4.00	6.00
Intra-day*	99.9±2.00	99.8±0.26	99.3±0.70	100.1±0.76	99.9±1.25	99.6±0.47
Inter-day*	100.2±0.64	100.3±0.60	99.9±0.65	100.5±0.50	100.5±0.60	100.4±0.40

*each result is the average of three separate determinations.

4.5 Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by small, but deliberate variations in method parameters [60]. The robustness of the proposed method was demonstrated by constancy of the peak current with deliberate minor changes in the experimental parameters. The studied variables included the change in pH (±0.2) and the time considered before each measurement (10 s±5 s). These minor changes that may occur during the experimental operation did not affect the peak current of DH, indicating the reliability of the cited method during normal usage.

4.6 Specificity

The specificity of the cited voltammetric method was proven by its ability to determine DH in a pharmaceutical formulation without interference from excipients and additives that are commonly present. This was assured by carrying out the method on a placebo sample.

4.7. Application

The cited voltammetric method was applied to the quantitative determination of DH in pure form, pharmaceutical dosage form and biological fluids. The results and recoveries of known amounts of DH are given in Table 4 for tablets, in Table 5 for spiked plasma and in Table 6 for spiked urine. The accuracy of the cited voltammetric method was determined by its recovery during spiked experiments. The results proved the validity of the cited method for the determination of DH in tablets and biological fluids. These results revealed that both DPV and SWV methods had adequate accuracy and precision and consequently can be applied to the determination of DH in pharmaceutical dosage forms and biological fluids without any interference. No interference from other additives occurred in the potential range where the analytical peak appeared.

Table 4. Analytical performance data of Spasmorest tablets at GC and MCPE electrodes

	GCE		**MCPE**	
	DPV	**SWV**	**DPV**	**SWV**
Added	20	20	20	20
Found [a]	19.95	19.965	19.975	20
Recovery	99.75	99.82	99.8	100
Bias (%)	-0.25	-0.175	-0.125	0
SD	0.023	0.018	0.018	0.014
SE	0.009	0.007	0.007	0.005
CL (p = 0.05)	19.95±0.024	19.965±0.019	19.975±0.019	20±0.014
CV	0.117	0.09	0.09	0.07

SD = standard deviation; SE = standard error; CL = confidence level; and CV = coefficient of variation.

[a] Mean of six measurements.

Table 5. Analytical performance data of spiked plasma at GC and MCPE electrodes

	GCE		**MCPE**	
	DPV	**SWV**	**DPV**	**SWV**
Added	20	20	20	20
Found [a]	19.935	19.958	19.98	20.05
Recovery	99.675	99.79	99.9	100.25
Bias (%)	-0.325	-0.21	-0.1	0.25
SD	0.017	0.025	0.008	0.12
SE	0.007	0.01	0.003	0.049

CL (p = 0.05)	19.935±0.018	19.958±0.027	19.98±0.009	100.25±0.12
CV	0.089	0.129	0.044	0.6

SD = standard deviation; SE = standard error; CL = confidence level; and CV = coefficient of variation.

[a] Mean of six measurements.

Table 6. Analytical performance data of spiked urine at GC and MNFOCP electrodes

	GCE		**MCPE**	
	DPV	**SWV**	**DPV**	**SWV**
Added	20	20	20	20
Found [a]	19.95	19.97	19.98	20.0075
Recovery	99.75	99.88	99.9	100.03
Bias (%)	-0.25	-0.11	-0.01	0.037
SD	0.023	0.009	0.01	0.015
SE	0.009	0.003	0.004	0.006
CL (p = 0.05)	19.95±0.024	19.97±0.009	19.98±0.01	20.0075±0.016
CV	0.11	0.04	0.05	0.07

SD = standard deviation; SE = standard error; CL = confidence level; and CV = coefficient of variation.

[a] Mean of six measurements.

5. CONCLUSION

Due to the simplicity and high sensitivity of electrochemical methods, the determination of DH has been a subject of enormous interest. The voltammetric technique is one of the best-known analytical quantitative methods. This technique has the advantage of extremely low detection limits and is suitable for the routine analysis of drugs in quality control laboratories. The modified carbon paste electrode revealed very low detection limits compared to GCE. It also showed a fast response, high stability and good reliability. This work also offers further details concerning the mechanism of electrochemical oxidation of DH. Moreover, the results obtained from the application of the cited method for estimation of DH in real samples confirm the high accuracy and precision of our cited method.

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