CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.166021

Available online at: http://www.iajps.com *Research Article*

HIGHLY SENSITIVE VOLTAMMETRIC DETERMINATION OF DANTROLENE SODIUM IN PURE FORM, PHARMACEUTICALS, HUMAN BREAST MILK AND URINE AT PENCIL GRAPHITE AND GLASSY CARBON ELECTRODES

Hassan A. Hendawy[1], Ehab F. El Kady[2], Hoda M. El Qudaby[1], Marwa A.Omran[1*]

[1]National Organization for Drug Control and Research (NODCAR), Giza P.O. 29, Egypt.

[2]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, 11562 Cairo, Egypt.

Abstract:

Voltammetric behavior of dantrolene sodium (Dan) was comparatively investigated in Britton Robinson (BR) buffer as electrolyte at pencil graphite (PGE) and glassy carbon (GCE) electrodes, cyclic voltammetry (CV) gave one reversible cathodic anodic peak at -0.302, -0.249 V at PGE and -0.257, -0.178 V at GCE respectively and another irreversible cathodic one at -0.748 at PGE and -0.694 V at GCE. Differential pulse (DPV) and square wave (SWV) voltammetry were investigated for the reversible cathodic peak and gave at PGE, linearity range from 0.395-2.955 and 0.395-1.9 µg/mL with correlation coefficient 0.999 for both, LOD 0.09 and 0.052µg/mL, LOQ 0.273 and 0.158 µg/mL, respectively and at GCE, linearity range from 0.974- 2.44 and 0.199- 2.96 µg/mL with correlation coefficient 0.999 for both, LOD 0.167 and 0.095 µg/mL, LOQ 0.507 and 0.289 µg/mL, respectively. The proposed procedures were successfully applied to the determination of Dan with good recovery in pharmaceutical dosage form, human mother milk and urine directly without any pretreatment at both electrodes. The techniques were validated and revealed good accuracy and reproducible results.

Keywords: *Dantrolene Sodium, Biological fluids, pencil graphite electrode, glassy carbon electrode, Pharmaceuticals, Voltammetry.*

Corresponding author:

Marwa A. Omran,

4 Ali Abd El-Ghaffar street, Said El-Awam street,
Al-Arbeen street, Kaabeesh, Tawabek,
Faisal street, Giza, Egypt.

NODCAR address: 6 Akeed Ahmed Mandoor street,
Abo Hazem street (previously), Al-Ahram street, Giza, Egypt.

E-mail address: marwa251285@yahoo.com

Tel.: (+2)01006731250

QR code

Please cite this article in press as Marwa A. Omran *et al*, **Highly Sensitive Voltammetric Determination of Dantrolene Sodium in Pure Form, Pharmaceuticals, Human Breast Milk and Urine at Pencil Graphite and Glassy Carbon Electrodes** , *Indo Am. J. P. Sci, 2016; 3(10).*

1. INTRODUCTION:

Dantrolene sodium; sodium salt of 1-{[5-(4-nitrophenyl)-2 furyl]methylideneamino}imidazolidine-2,4-dione[1](Fig.1), is a skeletal muscle relaxant drug that acts by abolishing excitation-contraction coupling in muscle cells, probably by acting on the ryanodine receptor and decreasing free intracellular calcium concentration. It is the only specific and effective treatment for malignant hyperthermia, a rare, life threatening disorder triggered by general anesthesia[2]. It is also used for the management of neuroleptic malignant syndrome and muscle spasticity[3]. The bioavailability of Dan is 70% and is excreted through both biliary and renal pathways. It's reported to be prohibited during lactation[4].

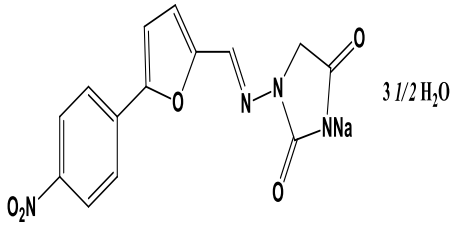


Fig. 1: Chemical Structure of dantrolene sodium

Literature survey shows that several techniques have been used to determine Dan including spectrophotometry [5,6], liquid chromatography[6-10], polarography [11,12,13], stability characterization and kinetics[14]. These reported techniques are sophisticated, need expensive instrumentations, elaborated and tedious extraction and polarographic one has the danger of toxicity due to using mercury electrode [15].

Thus, the development of new voltammetric techniques such as CV, DPV and SWV are proved to be more simple, rapid, selective, sensitive, inexpensive with the advantages of no need for derivatization and less sensitivity to matrix effects than other analytical techniques and capable of determining the drug in pure, pharmaceuticals and biological fluids. Additionally, application of electrochemistry includes the determination of electrode mechanism and redox properties of the drug under study. Redox properties of drugs can give insights into their metabolic fates, in-vivo redox processes or pharmacological activity and great importance that serve clinical investigations such as pharmacokinetic studies [16-21].

The aim of this work is the development of new voltammetric techniques for direct determination of Dan in pure, pharmaceuticals, spiked human mother milk and urine samples that introduces a good reference for the study of its toxic effect as it is excreted by kidney and reported to be contraindicated during lactation moreover, PGE [22-27] and GCE provide the needed advantages as simplicity, cheapness, availability, being solid and environmental friendship over the reported techniques for the determination of Dan.

2. Experimental:

2.1 Instrumental and experimental set-up

Voltammetric measurements were obtained using the electrochemical analyzer Computrace system with 797VA Computrace software (1.0) from Metrohm, Switzerland. A three-electrode cell was employed. The working electrodes were PGE, a HB Rotring hi polymer pencil graphite with a diameter of 0.7 mm identifiable with (S0312690, R505708N) and batch number (4 006856 505733), electrical contact with the pencil electrode was achieved by soldering a copper wire to the metallic part of the apparatus fixing the leads [28] and GCE, a mini glassy carbon disk electrode of the active zone: 2.8 mm, for 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 thoroughly rinsed with methanol and doubled distilled water, and dried with a piece of paper[29]. Ag/AgCl (3 mol.L-1KCl) was used as a reference electrode and a platinum wire as a counter electrode. A Mettler balance (Toledo-AB104) was used for weighing the solid materials, U.S.A. A micropipette (Eppendorf- multipette plus) was used throughout the present experimental work, German. The pH measurements were performed using Jenway3330 Research pH meter, U.K. Ultrasonic Cleaner, United Jeveiry Tool Supplies, model UTA-60, 6L capacity, Italy. Deionized water used throughout the present study was supplied from a burette still plus deionized connected to a Hamilton-Aqua-Metric deionized water system, U.K. All the experiments were performed at room temperature.

2.2 Chemicals and reagents

Dan as a hydrate sodium salt was supplied from Chemipharm company, potency was certified to be 101.7 %. Dantrelax capsules labeled to contain 25 mg Dan from Chemipharm, Cairo, Egypt were purchased from local market. A stock solution of 39.9 µg/mL Dan was freshly prepared by dissolving the weighed amount 1.9 mg in 50 ml dimethylformamide (DMF), and stored in refrigerator 4ºC. Britton- Robinson (B-R buffer) of concentration 0.04 M was prepared by mixing phosphoric acid, acetic acid and boric acid[30] with appropriate amount of 0.2 M NaOH to obtain the desired pH range (2-12). All solutions were prepared from analytical grade chemicals and deionized water. All materials and reagents were used as received without further purification.

2.3 Recommended experimental procedure

2.3.1 Assay of pure form

In the electrochemical measurements, Voltammetric analyses were performed in 10 and

20 ml of B-R buffer solution at PGE and GCE, respectively. Appropriate aliquots of the drug solution of Dan were introduced into the electrolytic cell while 8 mm of PGE was immersed into the supporting electrolyte, the calibration curves of Dan using DPV and SWV were constructed by plotting the peak current I(µA) against drug concentration (µg/mL) for the first cathodic peak which appeared nearly at -0.3 V (PGE) and -0.2 V (GCE) as it introduced better, narrower, sharper symmetrical and smooth peaks without noise with the calibration experiment.

2.3.2 Analysis of Pharmaceutical dosage form

The contents of ten dantrelax capsules were emptied and carefully mixed in a mortar. A weighed portion of the powder equivalent to 39.9 µg/mL of Dan was dissolved in DMF by sonication for 15 min to achieve complete dissolution, filtered and the residue was washed several times with DMF added to a 200 mL volumetric calibrated flask then diluted to the mark with the same solvent. The amount of Dan per capsule was calculated using the linear regression equation obtained from the calibration curve of pure Dan[31].

2.3.3 Application to human mother milk and urine samples

The bioavailability of Dan is 70%, hence direct application to human mother milk and urine without any sample pretreatment was carried out to save time and introduce a new technique for the direct determination of Dan in the biological fluids because Dan undergoes in vivo N-hydroxylation and acetylation metabolism [8], moreover real samples were made before. Milk was used as an alternative to blood serum to be used as it is, contrasting to serum that needs blood centrifugation and separation. Milk and urine samples were supplied from healthy volunteers from the team work, allowed to settle for about 30 minutes and then used for the experiments. The biological sample constitutes 10% of the electrolyte in the voltammetric cell then we added Dan solution with concentration of applicable range subsequently, analyzed according to the recommended in the general analytical procedure.

3. RESULTS AND DISCUSSION:

As a result of Dan excretion in urine and prohibition during lactation, the application of the developed techniques in the biological samples gives a great opportunity for the rapid and earlier detection of very small amounts of the cited drug helping in prevention of its side effects and intoxication. The voltammetric behavior of Dan was studied at PGE and GCE using 0.04M B-R buffer solution as supporting electrolyte investigating different chemical and electrochemical parameters.

3.1 Mechanism

Applying CV technique, the forward scan Dan showed one reversible cathodic peak for PGE at -0.302 and GCE at -0.257 V may be owing to the reduction of nitro group to hydroxyl amine group in a first step, another irreversible cathodic one at -0.748 V (PGE) and -0.694 V (GCE) may be owing to the reduction of the hydroxyl amine group to amino group in a second step and in the reverse sweep an anodic one at -0.249 V (PGE) and -0.186 V (GCE) may be owing to oxidation of hydroxyl amine group to nitro group in a third step as depicted by the cyclic voltammograms given in (Fig.2) and proposed mechanism in (Fig.3)[10].

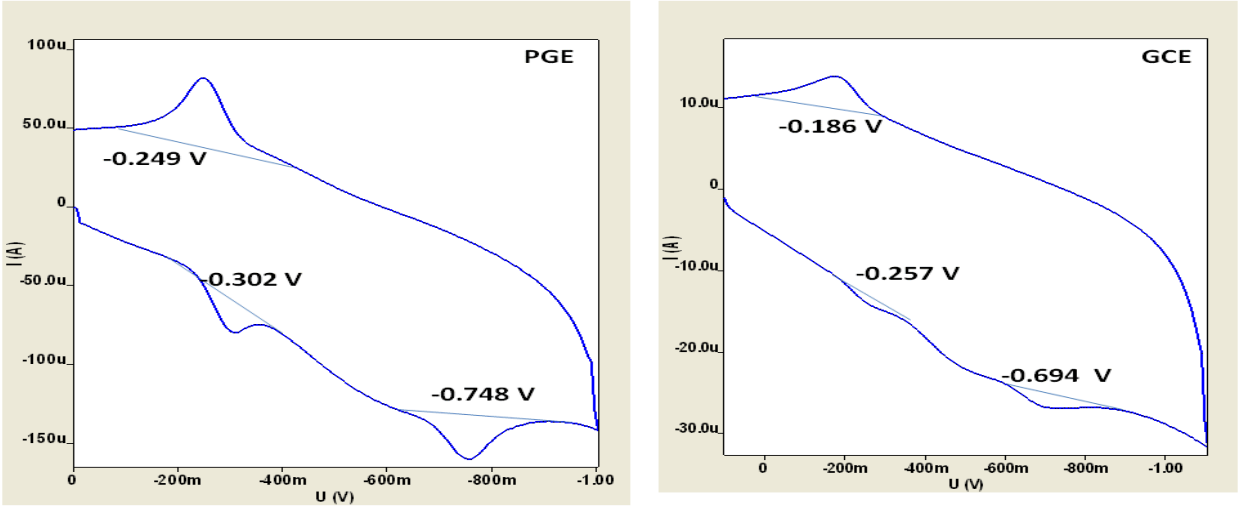


Fig.2: Cyclic voltammograms of 2.95 µg/mL Dan at 0.04 M BR buffer pH 9.0 at PGE and GCE, scan rate 280 mV/s.

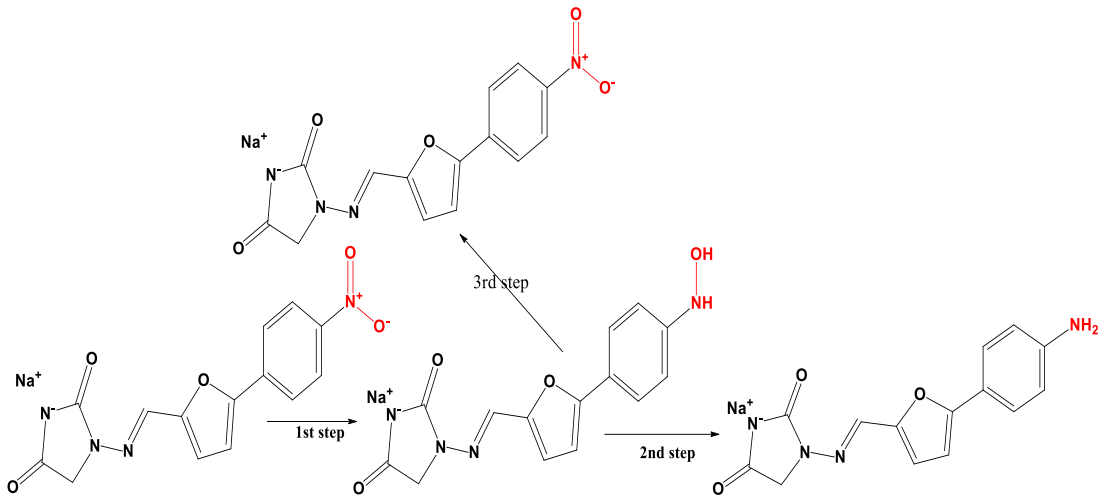


Fig.3: Proposed mechanism of the electrochemical reaction of Dan at 0.04 M BR buffer pH 9.0 at PGE and GCE.

3.2 Influence of the pH of supporting electrolyte

The pH of the electrolyte medium is one of the variables that commonly and strongly influence the shape of the voltammogram, and therefore it was important to investigate the effect of the pH on the electrochemical behavior of the drug. The influence of pH on the peak current was examined using SWV. The change of peak current with different pH values was evaluated at 1.9 µg/mL Dan solution in 0.04 M B-R buffer. It was found that, the maximum peak current was obtained at pH 9.0 at PGE .Hence it was used as an optimum pH value throughout the whole study. At GCE, pH 6.0 was the maximum value but pH 9.0 was used for the calibration experiments where the symmetrical peaks were obtained (Fig 4).

The effect of solution pH on peak potentials of Dan at both electrodes was also investigated (Fig.4) it was found that negative shifting by increasing pH values and linear correlation between the peak potential and solution pH revealing that a proton has taken part in the reaction and were obtained as shown with a linear equation and correlation coefficient:[32, 33]

$$E(V) = -0.0598pH + 0.2467$$

$$E(V) = -0.0422pH - 0.272$$

At PGE, the slope was found to be (-0.0598 mV/pH) which close to the Nernst equation, suggested that the number of proton taking part in the electrode reaction is equal to the number of electrons.

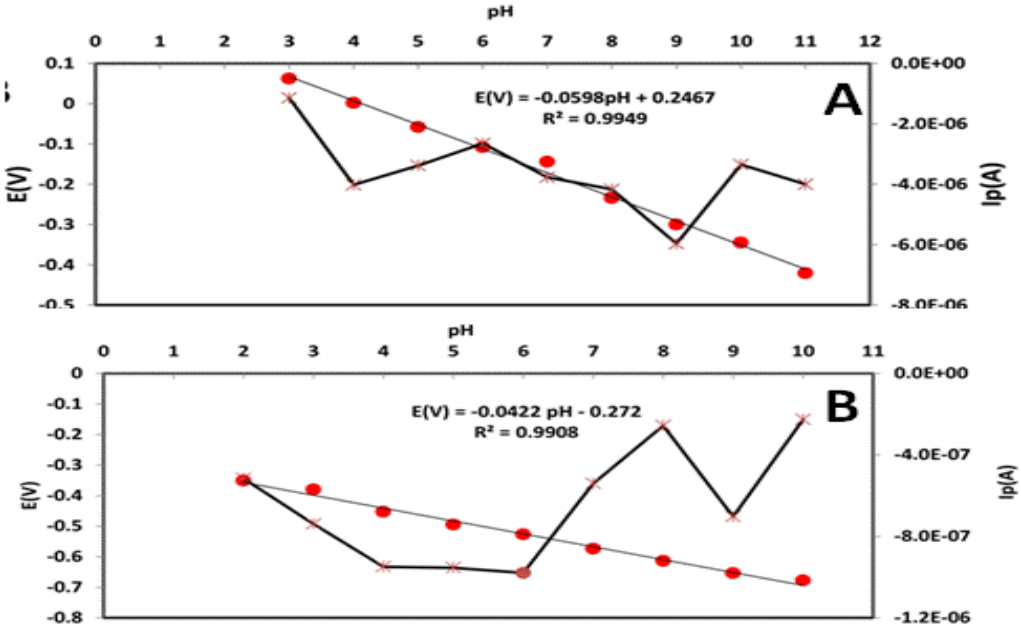
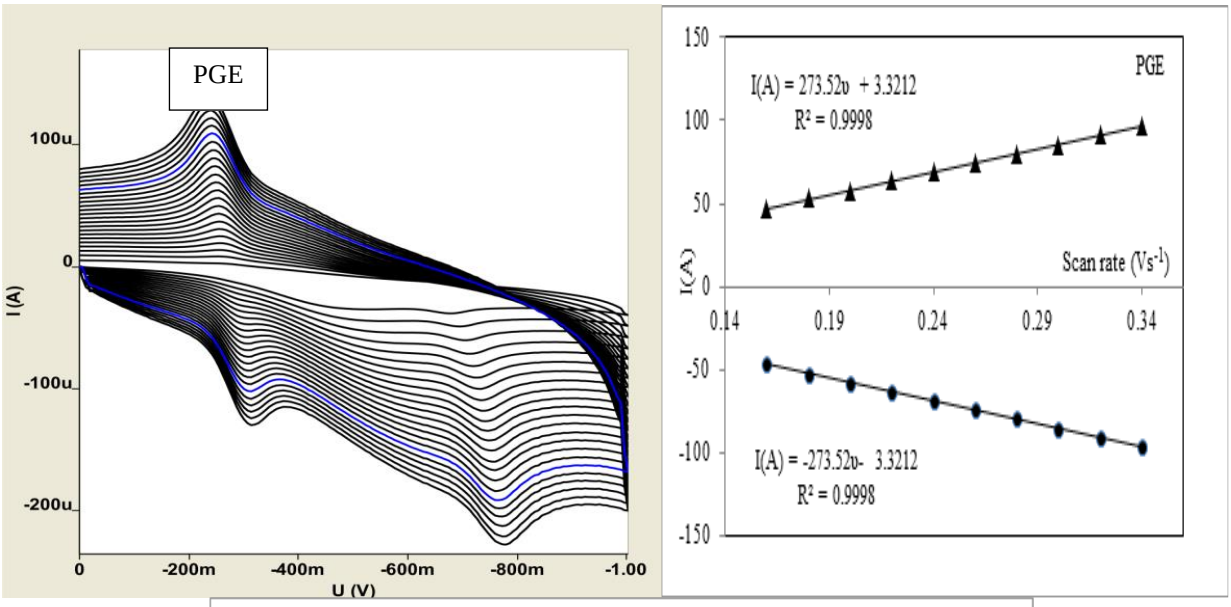


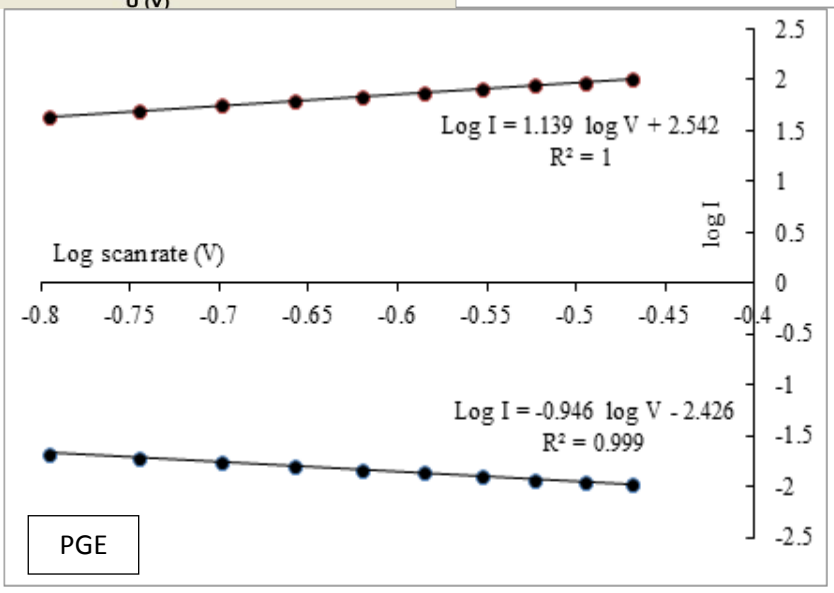
Fig. 4: Effect of pH on the peak current and potential for 1.9 µg/mL of Dan in 0.04 M BR buffer at (A) PGE and (B)GCE.

3.3 The effect of adsorption character

3.3.1 Effect of scan rate

The effect of scan rates on the peak current as a function of electro-reduction of Dan at PGE and GCE in 0.04M B-R buffer of pH 9.0 was investigated by cyclic voltammetry. Scan rate studies were carried out to assess whether the electrochemical process was under diffusion or adsorption controlled process. Fig.5 illustrates that Dan was under adsorption process that is supported by a plot of logarithm of peak current(for the reversible peak, cathodic/anodic for PGE and anodic for GCE) versus logarithm of scan rate that gave a straight line with a slope very close to the theoretical value of 1.0, which is expressed for an ideal reaction of adsorption controlled electrode process for Dan at PGE, the sharp form of the main cathodic peak and by the dependence of the peak current on the scan rate, moreover the higher values of correlation coefficient R^2[33]. The anodic and cathodic peak potentials of the electrode were slightly shifted towards positive and negative potentials, respectively. This may be attributed to the accumulation of the oxidation or reduction products on the electrode surface[34].





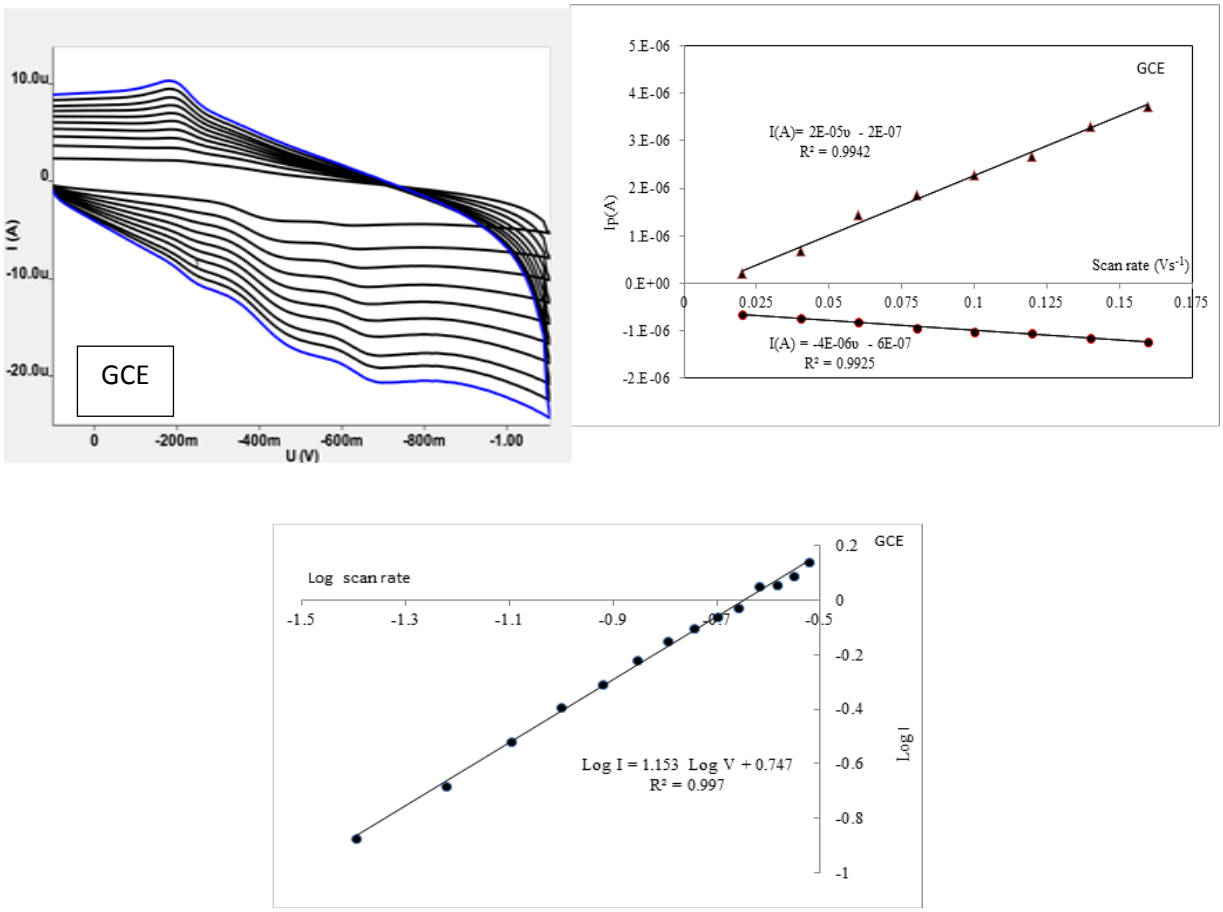
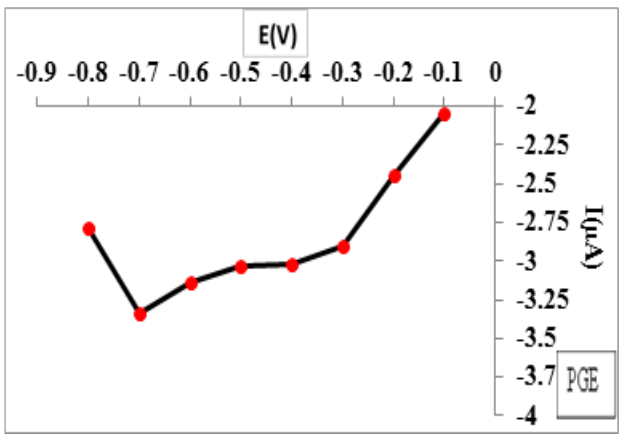


Fig. 5: Cyclic voltammograms of 2.95 µg/mL of Dan in 0.04 M BR buffer at PGE and GCE (pH 9.0) with different scan rates from 20 to 460 mV/s. The curves show the plot of peak current I(µA) of Dan VS scan rate(vs^{-1}) and the plot of log peak current VS log scan rate.

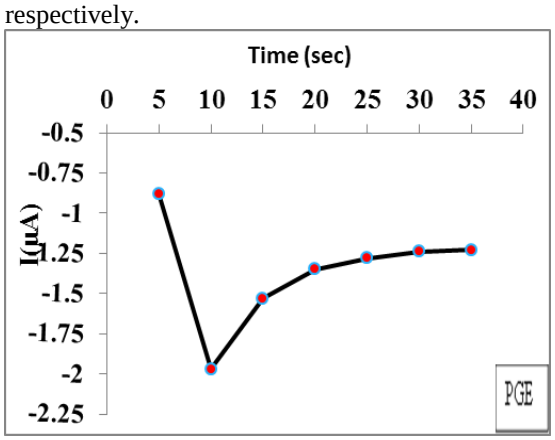
3.3.2 Effect of deposition potential

The interfacial adsorptive character of Dan onto PGE and GCE was studied by SWV technique. The effect of deposition potential as a function of the peak current was evaluated at a concentration level of 1.9 µg/mL showing -0.7 and 0.8 to be the best values to be applied in whole experiments for PGE and GCE, respectively as shown in (Fig.6). The adsorptive peak current at the electrodes' surfaces appear to be dependent on the deposition potential.



3.3.3 Effect of deposition time

The dependence of peak current developed in B-R buffer at the selected pH on the deposition time was also investigated and shown in (Fig.6) at a concentration level of 1.9 µg/mL Dan. From the plot it is clear that a short deposition time of the drug resulted in large cathodic peak current. A full surface coverage was established after a certain accumulation time, 10 and 30 seconds are the selected deposition time values at PGE and GCE, respectively.



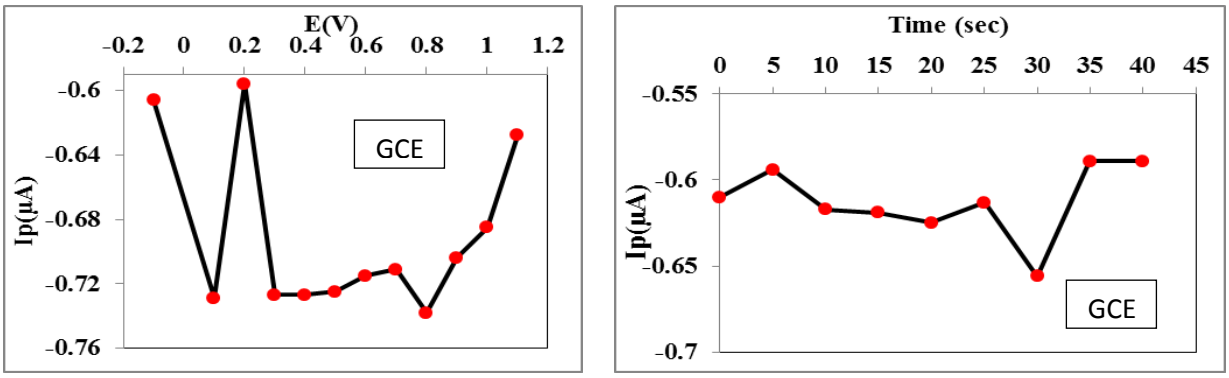


Fig.6: Effect of deposition potential and deposition time on the peak current of 1.9 µg/mL of Dan in 0.04 M B-R buffer at PGE and GCE.

3.4 Determination of Dan in pure form

In order to develop a voltammetric methodology for determining the drug, we selected DPV and SWV techniques, since the peaks are sharper with much higher current sensitivity and better resolution at lower concentration of Dan than those obtained by CV[33], in 0.04 M BR buffer pH 9.0 at both PGE and GCE, calibration graphs of the drug concentration (µg/mL) against the peak current I(µA) were constructed [35] (Fig.7,8). Statistical parameters are illustrated in (Table 1).

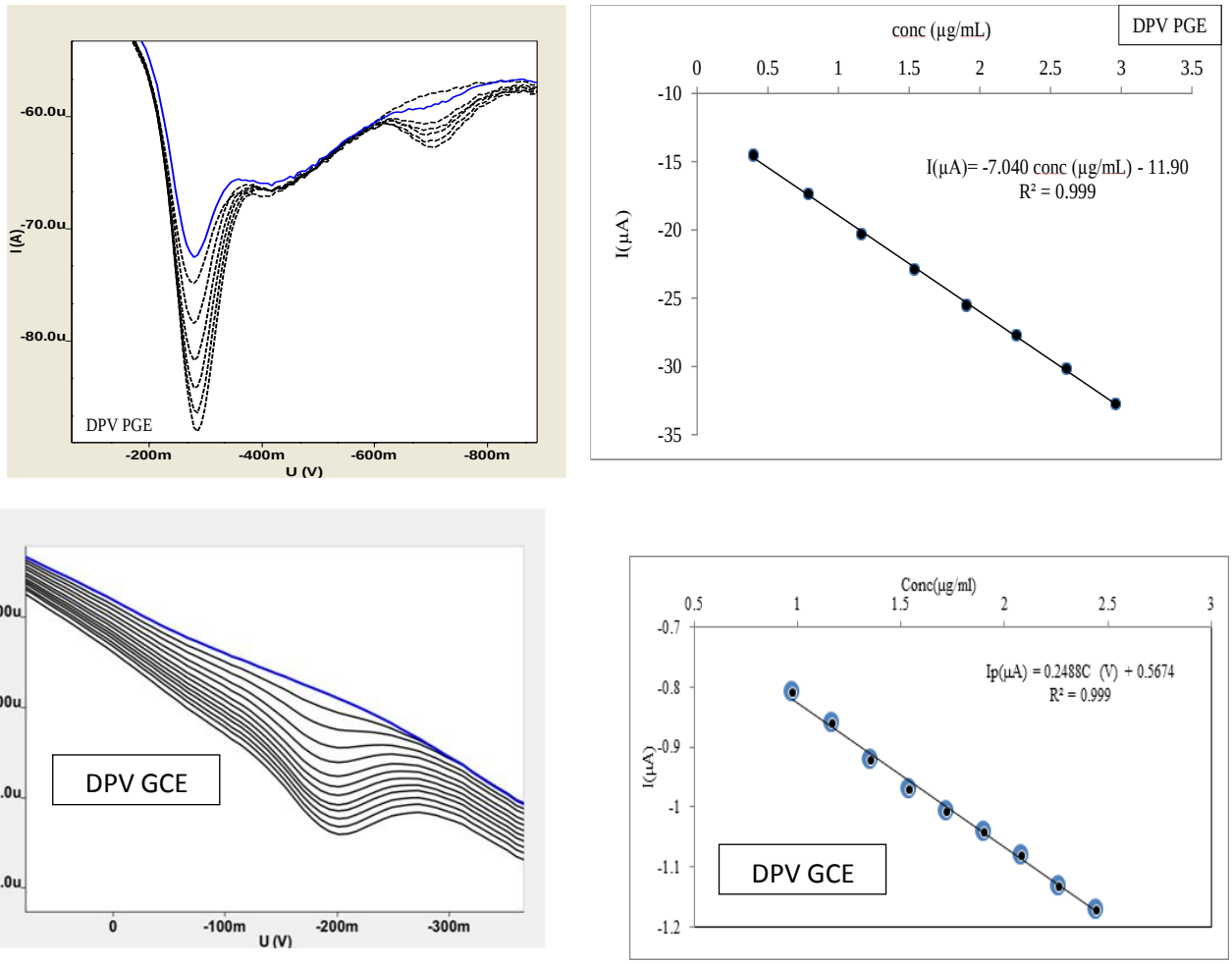


Fig. 7: Differential pulse Voltammograms and the corresponding calibration curves responding for successive additions of Dan in 0.04 M BR buffer pH 9.0 at pulse amplitude(V) 0.05005, Pulse time(s) 0.04 at both PGE and GCE, at PGE: Voltage step(V) 0.006561, Voltage step time(s) 0.1, Sweep rate(V/s) 0.0656, deposition time 10 s and deposition potential -0.7 V. At GCE: Voltage step(V) 0.007935, Voltage step time(s) 0.2, Sweep rate(V/s) 0.0397, deposition time 30 s and deposition potential 0.8 V.

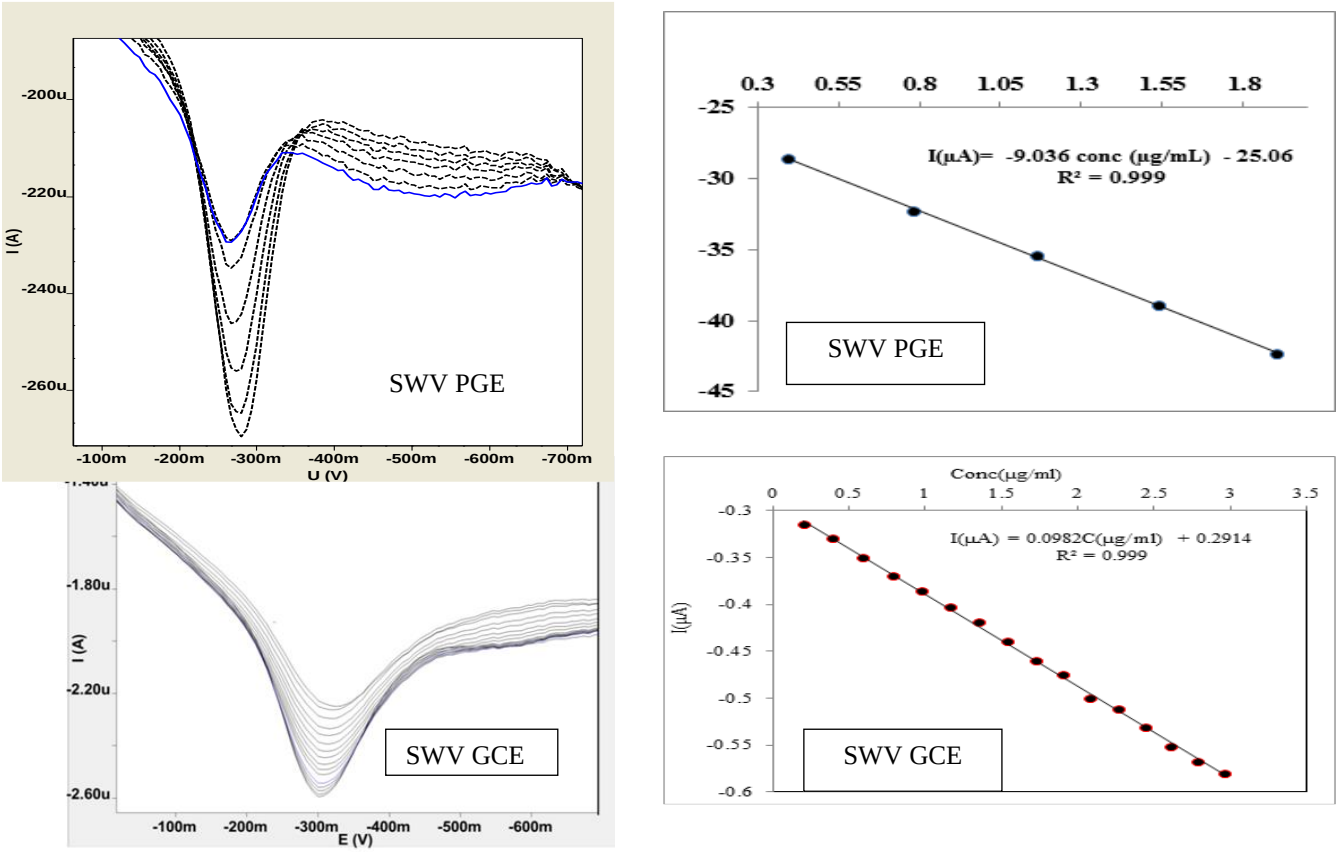


Fig. 8: Square Wave Voltammograms and the corresponding calibration curves responding for successive additions of Dan in 0.04 M BR buffer pH 9.0 at PGE: Voltage step(V) 0.006561, Amplitude(V) 0.01999, Frequency(Hz) 50, Sweep rate (V/s) 0.3281, deposition time 10 s and deposition potential -0.7 V. At GCE: Voltage step(V)0.007931, Amplitude(V)0.01999, Frequency(Hz) 2.5, Sweep rate (V/s)0.0198, deposition time 30 s and deposition potential 0.8 V.

Table 1: Statistical parameters of pure form of Dan using DPV and SWV at PGE and GCE.

Electrode	PGE		GCE	
Parameter	DPV	SWV	DPV	SWV
Potential value E(V)	-0.282	-0.287	-0.202	-0.3
Linear range conc(µg/mL)	0.395-2.955	0.395-1.9	0.974-2.44	0.199-2.958
SD	0.543	0.319	0.038	0.0112
%recovery	99.3	100.9	99.7	100.1
Correlation coefficient (R^2)	0.999	0.999	0.999	0.999
slope	-7.040	-9.036	0.2488	0.0982
Intercept of regression line (µA)	-11.90	-25.06	0.5674	0.2914
LOD (µg/mL)	0.254	0.116	0.502	0.376
LOQ (µg/mL)	0.771	0.353	1.52	1.14

Table 2: Recovery values of pharmaceutical form of Dan using DPV at PGE and SWV at GCE.

	DPV/ PGE			SWV/ GCE		
Added conc (µg/mL)	1.53	2.258	3.29	0.395	1.35	2.44
Found conc (µg/mL)	1.52	2.264	3.28	0.392	1.33	2.48
Recovery(%)*	99.3	100.3	99.6	99.2	98.5	102.0

3.5 Determination of Dan in the pharmaceutical dosage form (capsules)

On the basis of above results, DPV and SWV were applied for the direct determination of Dan in capsules at PGE and GCE, respectively in 0.04 M BR buffer pH 9.0 at both electrodes. This application reveals good recovery (Table 2).

3.6 Determination of Dan in both spiked human mother milk and urine samples

The best results were obtained using the biological fluids constituting 10% of the electrolyte one at a time. The measurement of Dan in milk and urine samples was performed directly without pretreatment at PGE but, at GCE, application in milk wasn't available that reveals a distinct advantage of PGE over GCE. The applicability of the DPV for the analysis of human milk and urine samples introduced calibration curves (Fig.9) and the statistical parameters are shown in (Table 3)

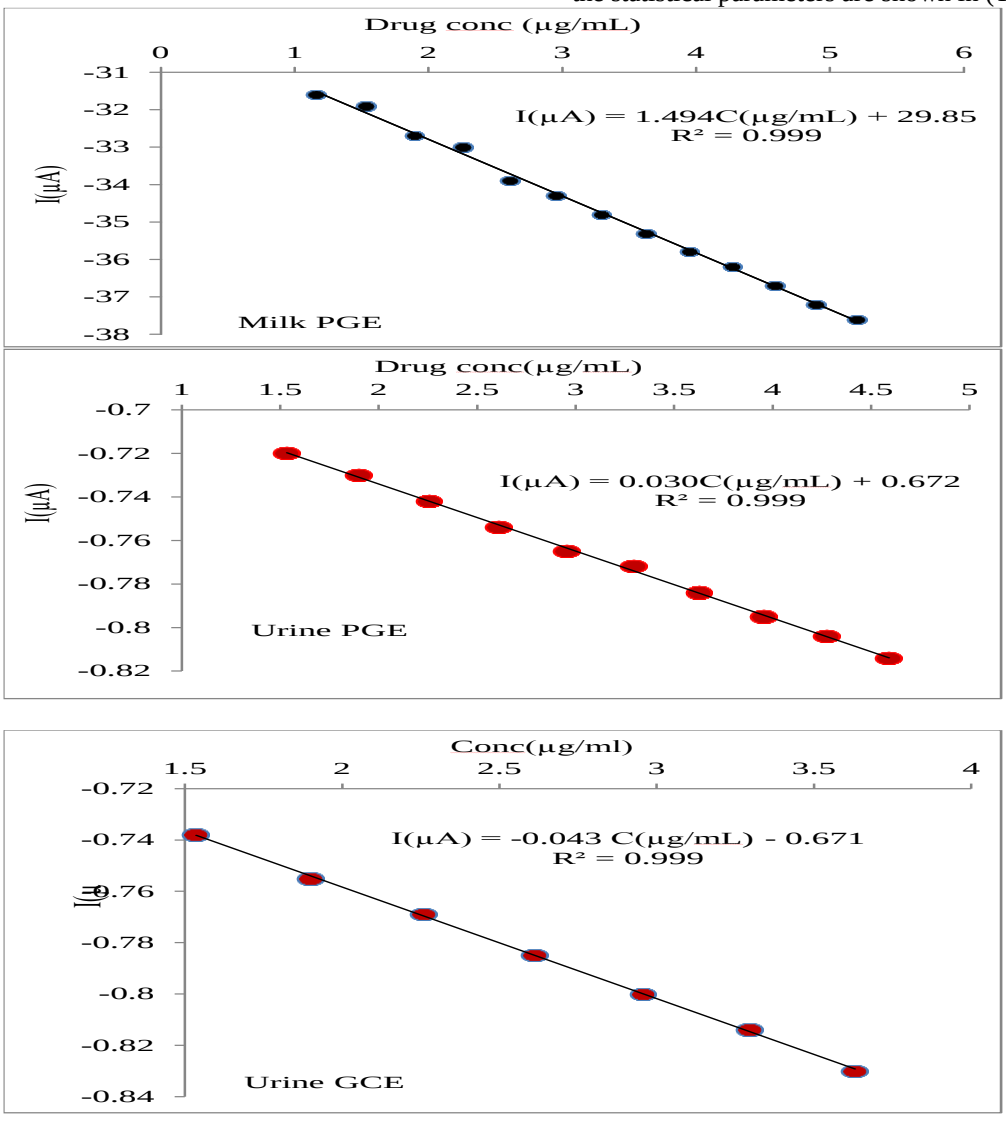


Fig. 9: Calibration curves of Dan in biological fluids at optimum conditions at PGE and GCE.

Table 3: Statistical parameters of Dan in human mother milk and urine at PGE and GCE.

Electrode	PGE		GCE
Parameter	MILK	URINE	URINE
Linear range conc (µg/mL)	1.16-5.204	1.53-4.59	1.53-3.63
SD	0.385	0.003	0.0018
%recovery	98.9	98.9	98.3
Regression Correlation coefficient (R^2)	0.999	0.999	0.999
Slope	1.494	0.03	0.043
Intercept of regression line (µA)	29.85	0.672	0.671
LOD (µg/mL)	0.85	0.347	0.143
LOQ (µg/mL)	2.576	1.0	0.419

Usually, analysis of drugs in biological samples requires extensive time-consuming, sample preparation, use of expensive organic solvents and in sometimes use of other chemicals. In this study using DPV, no sample pre-treatment was required. DPV was the technique of choice due to its higher sensitivity, sharper and narrower peaks with good symmetrical pattern that made it preferred than other ones.

Furthermore, no extra noise peaks were present in biological material peaks occurred in the potential range where the analytical peak appeared. Stability of the milk and urine samples kept in refrigerator (+4 ˚C) was tested by making three consecutive analyses of the samples over a period of approximately 5h. There was no significant change in the peak currents and potentials between the first and last measurements.

4. Method validation

4.1 Linearity and range

Two calibration graphs from the standard solution of Dan according to the procedure described above were constructed by using DPV and SWV techniques for pure form as shown in (Fig.7,8) Table 1 and for human mother milk and urine (Fig 9) Table 3. Above these concentrations a loss of linearity was probably due to the saturation of the electrodes' surfaces with the drug.

4.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were measured for pure DPV, pure SWV (Table 1), milk and urine (Table 3) at PGE and GCE.

These values are calculated on the peak current using the following equations[36,37]:

LOD= 3.3s/m LOQ= 10s/m

Where(s) is the standard deviation and (m) is the slope of the calibration curves.

4.3 Accuracy

Good recovery results were obtained for the determination of Dan in pure DPV, SWV, breast milk and urine (Tables 1,3). Standard addition technique was applied to the determination of Dan in dosage form and good results were obtained (Table 2).Statistical comparison of the results obtained by the proposed method of Dan drug and those obtained by the official and reference ones using t-test and F-test revealed no significant difference between the two values (Table 4).

Table 4: Statistical parameters of pharmaceutical dosage form assay of Dan by the proposed DPV at PGE and SWV at GCE, Official and reference methods.

	PGE/DPV	GCE/SWV	Official method	Reference method
Added Conc (µg/mL)	%Found			
2.0 2.5 3.0	98.9 98.8 100.6	99.0 99.6 100.3	99.01 98.46 99.05	98.5 99.5 99.9
Mean±SD	99.43% ± 1.49	99.63% ± 0.79	98.84% ± 1.2	99.3±0.82
t-test	0.445	0.18		
F-test	1.35	1.71		

Each concentration value is average of three replicates.
Tabulated t-value at 95% confidence limit = 2.3, Tabulated F-value at 95% confidence limit = 6.38, n = 3,
Official method: B.P 2011.
Reference method: spectrophotometric method[5].

4.3 Precision (Repeatability and reproducibility)

In order to check the repeatability and reproducibility of peak potentials and peak currents, multiple measurements of 1.9 µg/mL of the cited drug were performed for three replicates measurements (n=3) intra and inter-day respectively, using DPV and SWV. A mean recovery of 99.3 ± 0.192 at PGE and 100.1 ± 0.0028 at GCE were achieved indicated a high precision of the proposed method for determination of Dan.

4.5 Robustness and ruggedness

The robustness of the results of the procedure is the ability to remain unaffected by small but deliberate change in its operational parameters. In the present work this was examined by studying the effect of the variation of pH and deposition time. The values of pH 9 (±0.2) and deposition time 10 sec (±2) at PGE and pH 6 (±0.2) and deposition time 30 sec (±2) at GCE were used to achieve the robustness parameter (table 5). Only one factor was changed at a time, while the other was kept constant. These changes had little effect on the peaks' shape of Dan. The peak current values were not significantly affected by these variations and consequently the optimized procedure was reliable for the assay of the drug.

The ruggedness is the degree of reproducibility of the results obtained by analysis of the same sample under a variety of normal test conditions, such as different laboratories, analysts and lots of reagents. Dan was also investigated by different analysts and showed no significant difference between both analysts; this ensures that our method is rugged (Table 5).

Table 5: The robustness and the ruggedness of the conditions of the proposed techniques for the determination of Dan at PGE and GCE.

PGE		GCE	
pH	I(µA)	pH	I(µA)
8.8	-0.79	5.8	-0.975
9	-0.8	6	-0.979
9.2	-0.77	6.2	-0.976
Time (Sec)	I(µA)	Time (Sec)	I(µA)
8	-1.95	28	-0.651
10	-1.97	30	-0.656
12	-1.96	32	-0.652
RSD (%)			
Ruggedness Analyst 1	Ruggedness Analyst 2	Ruggedness Analyst 1	Ruggedness Analyst 2
0.89	0.91	0.93	0.95

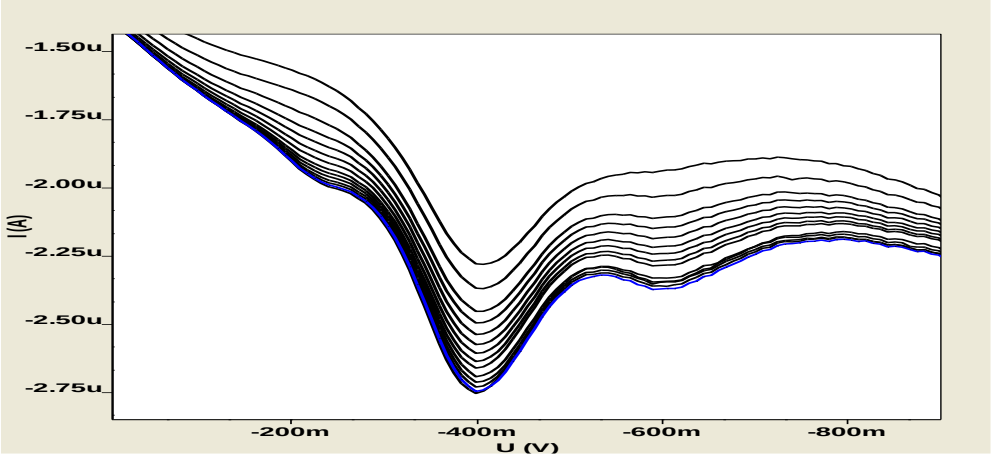


Fig. 10: Square Wave Voltammogram responding for successive additions of Dan from pharmaceutical dosage form in 0.04 M BR buffer at GCE pH 6.0, Voltage step(V)0.007931, Amplitude(V)0.01999, Frequency(Hz) 2.5, Sweep rate (V/s)0.0198, deposition time 30 s and deposition potential 0.8 V.

4.6 Specificity

No placebo was available thus specificity was proved by comparing the voltammograms of the pharmaceutical preparation to that of the pure form, they were found similar (Fig 10). Besides, good results for the recovered concentrations of the pharmaceutical preparation prove specificity (Table 2). Accordingly, excipients present in capsule did not interfere with the analysis.

5. CONCLUSION:

Validated differential pulse and square wave voltammetric techniques have been developed and successfully applied to the estimation of Dan in pure, pharmaceutical formulation, human mother milk and urine samples at both PG and GC electrodes. PGE introduces several advantages over GCE constituting in higher sensitivity that is clearly illustrated from the LOD and LOQ values for pure SWV, narrower and sharper peaks, lower deposition time, direct application in milk samples in contrast to GCE that didn't supply satisfied results, moreover, being disposable, availability and cheapness more than GCE. The proposed techniques are direct, more safe, sensitive and inexpensive than other reported techniques. Furthermore, the proposed techniques are simple, accurate, precise and do not need the elaborated treatment and tedious extractions required in spectrophotometric and chromatographic techniques. The main advantage of the techniques is rapidity as it requires less than 5 min to run sample. Applicability of our new methodology at biological samples is the stone of the corner of our aim of this study to help in rapid detection and prevention of Dan intoxication that help in clinical investigations.

REFERENCES:

1.Commission B.P., Council G.M., Commission G.B.M., B p. 2015. Her Majesty's Stationery Office.

2.Krause T., Gerbershagen M., Fiege M., Weisshorn R., Wappler F. Dantrolene--a review of its pharmacology, therapeutic use and new developments. J Anaes, 2004; 59:364-373.

3.Snyder H. R., Davis C. S., Bickerton R. K.and Halliday R. P., 1-[5Arylfurfurylidene)amino] Hydantoins: A New Class of Muscle Relaxants. J Med Chem, 1967; 10:807-810.

4.Pinder R. M. , Brogden R. N. , Speight T. M., Avery G. S., Dantrolene Sodium A Review of its Pharmacological Properties and Therapeutic Efficacy in Spasticity, 1977; 13: 3-23.

5. El-Bagary R.I., El-kady E. F., Hegazi, M A, Nour Eldin A.,Spectrophotometric methods for the simultaneous determination of paracetamol and dantrolene sodium in pharmaceutical dosage form. Europ J Chem, 2014; 5:96-101.

6.Rashed N.S., Abdallah O.M., Farag R.S., Awad S.S., Validated Bivariate Calibration Spectrophotometric and High Performance Liquid Chromatographic Methods for Simultaneous Determination of Dantrolene Sodium and Paracetamol in Pharmaceutical Dosage Form, Adv Anal Chem, 2014; 4:1-8.

7.Hadad G.M., Emara S., Mahmoud W.M., Development and validation of a stability-indicating RP-HPLC method for the determination of paracetamol with dantrolene or/and cetirizine and pseudoephedrine in two pharmaceutical dosage forms. Talanta, 2009; 79: 1360-1367.

8.Wuis E.W., Grutters A.C.L.M., Vree T.B., Van Der Kleyn E., Simultaneous determination of dantrolene and its metabolites, 5-hydroxydantrolene and nitro-reduced acetylated dantrolene (F 490), in plasma and urine of man and dog by high-performance liquid chromatography. J Chrom B: Biomed Sci App, 1982; 231:401-409.

9.Saxena S., Honigberg I., Stewart J., Keene G., Vallner J., Liquid chromatography in pharmaceutical analysis VI: determination of dantrolene sodium in a dosage form. J pharm sci, 1977; 66:286-288.

10.Mobin A. Tawakkula, Patrick J. Faustinoa, Vilayat A. Sayeedb, Mansoor A. Khana & Saeed R. Khan, Development and

application of a validated stability-indicating Ultra-Performance Liquid Chromatography (UPLC) method for the determination of dantrolene and its related impurities. Clin Res Reg Aff, 2010; 27:21-29.
11.Cox P.L., Heotis J., Polin D., Rose G.M., Quantitative determination of dantrolene sodium and its metabolites by differential pulse polarography, J pharm sci, 1969; 58:987-989.
12. Ghoneim E.M., Electroreduction of the muscle relaxant drug dantrolene sodium at the mercury electrode and its determination in bulk form and pharmaceutical formulation. J Chem Pharm Bul, 2007; 55:1483-1488.
13.Livertoux M.H., Jayyosi Z., Batt A.M., Study of the physicochemical properties aqueous dantrolene solutions by differential pulse polarography. Talanta, 1988; 35:613-619.
14.Khan S. R., Tawakkul M., Sayeed V. A., Faustino P., Khan M.A., Stability Characterization, Kinetics and Mechanism of Degradation of Dantrolene in Aqueous Solution: Effect of pH and Temperature. J Pharm & Pharm, 2012; 3:281-290.
15. Mohd Dzul Hakim Wirzal, Abdull Rahim Mohd Yusoff, Jiri Zima, Jiri Barek, Voltammetric Determination of Nifedipine at a Hanging Mercury Drop Electrode and a Mercury Meniscus Modified Silver Amalgam Electrode. Int J Electrochem Sci, 2015; 10:4571 - 4584,.
16.Uslu B., Özkan S.A., Electroanalysis in Biomedical and Pharmaceutical Sciences: Voltammetry. Electrochim acta, 2004; 49:4321-4329.
17.Shalaby A., Hassan W.S., Hendawy H.A., Ibrahim A., Electrochemical oxidation behavior of itraconazole at different electrodes and its anodic stripping determination in pharmaceuticals and biological fluids. J Electroanalyt Chem, 2016; 763:51-62.
18.Özkan S.A., Uslu B., Sentürk Z., Electroanalytical Characteristics of Amisulpride and Voltammetric Determination of the Drug in Pharmaceuticals and Biological Media. Electroanal, 2004; 16: 231-237.
19.Kauffmann J.-M., Vire J.-C., Pharmaceutical and biomedical applications of electroanalysis: A critical review. Anal chim act, 1993; 273:329-334.
20.Ronkainen N.J., Halsall H.B., Heineman W.R., Electrochemical biosensors, Chem Soc Rev, 2010; 3:1747-1763.
21.Wang J., Electroanalytical techniques in clinical chemistry and laboratory medicine, 1988. John Wiley & Sons.
22.Levent A., Yardim Y., Senturk Z., Voltammetric behavior of nicotine at pencil graphite electrode and its enhancement determination in the presence of anionic surfactant. Electrochim Act, 2009; 55:190–195.
23. Gao W., Song J., Wu N., Voltammetric behavior and square-wave voltammetric determination of trepibutone at a pencil graphite electrode, J Electroanal Chem, 2005; 576: 1–7.
24.Demetriades D., Economou A., Voulgaropoulos A., A study of pencil-lead bismuth-filmelectrodes for the determination of trace metals by anodic stripping voltammetry, Anal Chim Act, 2004; 519:167–172.
25.Karadeniz H., Gulmez B., Sahinci F. et al., Disposable electrochemical biosensor for the detection of the interaction between DNA and lycorine based on guanine and adenine signals, J Pharm Biomed Anal, 2003; 33:295–302.
26.Bond A. M., Mahon P. J., Schiewe J., Vicente-Beckett V., An inexpensive and renewable pencil electrode for use in fieldbased stripping voltammetry. Anal Chem Act, 1997; 345:67–74.
27.Dede E., Sa˘glam¨ O., Dilgin Y., Sensitive voltammetric determination of niclosamide at a disposable pencil graphite electrode. Electrochim Act, 2014; 127:20–26.
28.Rizk M., Hendawy H.A., El-Alamin M.M.A., Moawad M.I., Sensitive anodic voltammetric determination of methylergometrine maleate in bulk and pharmaceutical dosage forms using differential pulse voltammetry. J Electroanal Chem, 2015; 749:53-61.
29.Rizk M., Abou El-Alamin M.M., Hendawy H.A., Moawad M.I., Highly Sensitive Differential Pulse and Square Wave Voltammetric Methods for Determination of Strontium Ranelate in Bulk and Pharmaceutical Dosage Form. Electroanal, 2015; 27:1-10.
30.Britton H.T.S., Robinson R.A. J Chem Soc (Resumed), 1931; 1456-1462.
31.Adhoum N., Monser L., Determination of trimebutine in pharmaceuticals by differential pulse voltammetry at a glassy carbon electrode. J pharm biomed anal, 2005; 38:619-623.
32.Elqudaby H. M., Mohamed G. G., El Din G. M. G., Electrochemical behaviour of trimebutine at activated glassy carbon electrode and its direct determination in urine and pharmaceutics by square wave and differential pulse voltammetry. Int J Electrochem Sci, 2014; 9: 856–869.
33. Özkan S., Uslu B., Aboul-Enein H., Analysis of Pharmaceuticals and Biological Fluids Using Modern Electroanalytical Techniques. Crit Rev Anal Chem, 2003; 33:155-181.
34. Elqudaby H.M., Mohamed G. G., Ali F. A., Eid S.M., Validated voltammetric method for the determination of some antiprotozoa drugs based on the reduction at an activated glassy carbon electrode. Arab J Chem, 2013; 6:327–333.
35.Uslu B., Özkan S.A., Electrochemical characterisation of nefazodone hydrochloride and voltammetric determination of the drug in pharmaceuticals and human serum. Anal Chim Act, 2002; 462:49-57.
36.Swartz E., Krull I. S. 1997. Analytical Method Development and Validation. New York, USA: Marcel Dekker.
37.Riley C.M., Rosanske T.W. 1996. Development and validation of analytical techniques. Elsevier.