Egypt. J. Chem. **Vol. 66**, No. 1 pp. 143 – 154 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Altered Electrochemistry of Tropium Chloride on A Multiwalled Carbon Nanoelectrode: Rapid and Selective Detection in Authentic, Pharmaceutical and Biological Fluids by Square Wave Cathode Voltammetry

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Abstract

The voltammetric behavior of Trospium chloride (TRC) was studied using Cyclic (CV) and square wave (SWV) voltammetry. CV showed only one well-defined, irreversible, diffusion-controlled reduction peak using 0.04M Britton-Robinson buffer, PH 8.0 at modified with Mullet wall carbon Nano tube electrode (MWCEP). The peak current concentration relationship was rectilinear a much wider linear dynamic range of TRC determination was found over the range 0.3-2.6 µg/ml at MWCEP, with a minimum detectability of 0.07 µg/ml based on S/N = 3. And hence CV and SWV were conducted for the quantitative determination of (TRO) in its pure and pharmaceutical dosage form. The method was validated and the results were in good agreement with those obtained from the reported method. The Fabricated MWCEP exhibited many outstanding characteristics such as good stability, highly sensitivity, and notable repeatability. The designated sensor was used to determine TRC in biological fluid samples with good recovery. The proposed method was successfully applied for the estimation of TRC drugs in its combined dosage form and in human serum.

Keywords: Multi wall carbon Nano tube electrode, modified carbon nanotubes paste electrode Trospium chloride, Voltammetry.

1. Introduction

Trospium chloride (TRC) With IUPAC name 3-(2-Hydroxy-2,2-diphenylacetoxy) spiro [bicyclo [3.2.1] octane-8,1'-pyrrolidin]-1'-ium chloride belongs to a class of drugs known as antispasmodics agent used to treat symptoms of the overactive bladder[1, 2]. a condition that causes the bladder muscles to contract uncontrollably, an overactive bladder leads to an increased urge to urinate, frequent urination, and sometimes, loss of control over urination, is manufactured by Indevus Pharmaceutical Inc. and was granted FDA approval in 2007. Mechanism of action by relaxing the bladder muscles, TRC improves the ability to control urination. It helps reduce urine leakage, the feeling of needing to urinate right away, and the frequency of going to the bathroom. TRC belongs to a class of drugs known as antispasmodics. It is also known as an antimuscarinic[2-5]. It has the chemical formula (C25H30CINO3) and structure displayed in Fig. 1.

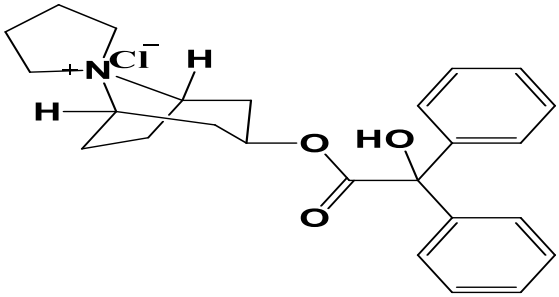

Fig. 1.: Structure of trospium chloride

Up to now, in the literature review, analytical methods have been reported for TRC determination in pharmaceutical and biological fluids,

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Receive Date: 05 February 2022, Revise Date: 26 March 2022, Accept Date: 07 April 2022.
DOI: 10.21608/EJCHEM.2022.120139.5396.

spectrophotometric estimation method,[6, 7] High performance liquid chromatographic (HPLC)[8-10], liquid chromatography-mass spectrometry (LCMS) determination method in human plasma and relative bioavailability of [11, 12], Fluorometric determination[13], potentiometry[14], Conductometric method[15], A survey of the literature reveals that no voltammetric analytical method has been reported to analysis TRC in surfactants.

The electroanalytical technique provides the advantages of simplicity, high sensitivity, cost-effective, relatively short analysis time and direct analysis, without any derivatization, extraction, or clean-up steps, rapid response, good reproducibility, and low detection limit[16-19].

Modified molecular surface and especially electrode nanoscale modifiers are of paramount importance for the development of electrochemical sensors with region-specific electron transfer potentials. Multiwall Carbon Nanotube (MWCNT) has been extensively used as functional modifiers for electrode surfaces in several electrochemical sensors[18, 20-24]. Indeed, MWCNT has been demonstrated to enhance the electrochemical activity of modified sensors and enhance electron transfer reactions of redox organic analytes compounds[25, 26]. So, this feature of MWCNT will well enable us to study the electrochemical behavior of TRC Therefore, MWCNT was chosen as the electrode modifier.

Hence, we herein report the voltammetric in this paper voltammetric behavior of TRC has been studied by employing cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The green preparation method was used as a modifier in all experiments. In addition, the voltammetric redox mechanism of TRC was first clarified in this study. The improved nano-rate sensor showed a faster response, sensitivity, and higher reproducibility and was applied for precise analysis of TRC in pure, pharmaceutical dosage forms and biological samples.

2. Experimental

2.1. Materials and Reagents

All reagents and solvents were of analytical reagent grade and used without further purification. graphite powder (spectroscopic grade, particle size < 20 mm) was obtained from Merck (Darmstadt, Germany). A carbon multiwalled nanotube (MWCNT), 3-20 nm OD, 1-3 nm ID, 0.1-10 micron long 95% powder, and (BMH) and paraffin oil were all obtained from Sigma Aldrich Egypt. The solutions were prepared with deionized water supplied by a Milli-Q Plus system (Millipore) with a resistivity of not less than 15.5 mΩ cm. Britton Robinson (B-R) buffer 0.04 M, was prepared by mixing the acid mixture containing 0.04 M phosphoric acid, 0.04M acetic acid, and 0.04M boric acid. Buffer solutions were adjusted with the appropriate amount of 0.2M sodium hydroxide to get the desired pH in the range of 2–12.0 [16, 27]. and it was obtained from Sigma-Aldrich. TRC was delivered from Hekma pharmaceutical company (B.N. 21787). The potency was certified to be 99.9%. Trospikan tablet was purchased from a local market and it's produced by Hikma Pharma, (Cairo, Egypt), B.N.003. Each tablet is claimed to contain 20 mg of TRC.

2.2. Instruments and apparatus

Voltammetric experiments (CV and SWV) were performed using Metrohm computrace Voltammetric analyzer model 797 VA. The measurements were recorded using a computrace version 1.3.1 (Metrohm), running under Windows 7. With three electrodes system consisted of a carbon paste electrode (CPE), and modified Mullet wall carbon nanotube (MWCNT) electrode as working electrode, Ag/AgCl (3 M KCl) electrode as reference electrode, and a platinum wire as the (counter) auxiliary electrode. A digital PH/mV meter JANEWAY 3510 (England) with a glass combination electrode was used for PH measurements. The PH was calibrated using standard buffer PH 4.0, PH 7.0, and PH 10.0. A micropipette (Eppendorf-multipette) was used throughout the present experimental work.

2.3. Standard Solutions

The standard stock solution of concentration (1x10-2 M) of studied drugs was freshly prepared by an appropriate amount of the studied pure drugs dissolving in water, the solution was then transferred to a 100 mL volumetric flask, and the volume was completed using the same solvent. The stock solutions were stored in a refrigerator. Serum samples, obtained from a healthy volunteer, were collected and stored frozen until assay.

2.4. Working electrode preparation

a. Carbon paste electrode (CPE):

The carbon paste was prepared by mixing 0.5g graphite powder with 0.3 mL of paraffin oil in an agate mortar with a pestle. Graphite powder, paraffin oil, and mortar were all provided by Sigma-Aldrich. A portion of composite carbon paste was packed into the hole of the insulin syringe body with a diameter of 3.0 mm which contain copper wire that contacted the apparatus. The tip of the electrode was polished with a weighing paper until it had a shiny appearance.

b. Preparation of MWCNT modified electrode

The MWCNT modified electrode (MWCPE) was prepared by mixing an appropriate weight of graphite powder and appropriate weight of mullet wall carbon nanotube (MWCNT) to prepare percentages 2, 5, 10, and 15% mixed well until homogeneous and then adding 0.3 ml of paraffin oil then Knead the paste well to reach homogenous. A portion of composite carbon paste was packed into the hole of the insulin syringe body with a diameter of 3.0 mm which contain copper wire contacted the apparatus and the tip of the electrode was polished will with a weighing paper until it had a shiny appearance[28-30].

2.5. The recommended procedure for measurements:

Assay of the pure form: Voltammetric measurements were studied at different working electrodes in 10.0 ml 0.04M B-R buffer electrolyte solution. An appropriate volume of TRC slandered prepared solution was transferred into an electrolytic measuring cell, and then complete to 10.0ml with a suitable electrolyte buffer solution. TRC calibration curves were generated using DPV by plotting response peak current I (μA) against TRC concentration (μg/ml) for the first cathodic peak, which resulted in a potential range at 0.0mV to -950.0mV Resulting in the reproducible current–potential curves in better, thinner, sharper, more consistent and smoother peaks without noise with DPV calibration curve.

2.6. Determination TRC in dosage forms

In the case of tablets, 5 tablets were weighed and the average mass per tablet was determined, then these tablets were powdered by mortar with pestle. An adequate portion of the powder needed to obtain the drug solution was accurately weighed and transferred into a 100 ml volumetric flask containing 50 ml bidistilled water. The prepared solution was sonicated for about 10 min. and then made up to the volume with the same solvent. An adequate portion of the solution was filtered by a syringe filter. Aliquots of the drug solution were introduced into the electrolytic cell and the slandered addition procedure was carried out.

2.7. Determination of TRC in serum

After withdrawing an amount of 0.5 ml of the serum sample was transferred to a 5.0 ml measuring flask, a suitable volume of the TRC solution was added and mix with 2.0ml methanol under manual Shaking for two minutes, the mixture of the solution was filtered by syringe filter. After separation of proteins, A portion of the filtrated solution was carefully taken out, and an appropriate volume of the filtrated was transferred to a 10 mL volumetric flask, diluted to the volume and the obtained solution was used for voltammetric determination by using a DPV method as described before for the pure drug.

2.8. Preparation of the degradation products

The Hydrolytic-degradants of TRC was prepared by TRC (100 mg) was refluxed for 2 hours with 100 ml 2M NaOH solution into an a100-ml round-bottom flask and the complete degradation was verified by TLC using acetonitrile / glacial acetic acid (5:5 by volume) as the mobile phase[10]. Two spots were observed not corresponding to TRC. One spot was visualized under a UV lamp at 254 nm, while the other spot was visualized after spraying with potassium iodobismuthate reagent. Then, hydrolytic degradants were neutralized with 0.2M HCl, all prepared degradants were evaporated on a thermostatic water-bath (60ºC) to dryness, cooled, and transferred quantitatively with methanol to a volumetric flask 25.0ml then the volume was completed to the mark to reach the obtained TRC concentration.

3. Results and Discussion

3.1. Evaluation of electroactive surface area

The voltammetric performance of the comparator working electrode is highly influenced by its electroactive area present at the surface. To study the electroactive surface area of CPE and MWCPE, The voltammogram response to a current peak by the CV of 1.0 mM K4[Fe(CN)6] in 0.1 M KCl at different scan rates was recorded at the prepared

electrodes, The area of the used electrodes was obtained by reversible reaction, Randles-Sevcik equation can be applied[31, 32]

Ipa = (2.69x105) A n3/2 DR1/2 C0 υ 1/2

where, Ipa discusses the anodic peak current, (n) number of electrons transferred, (A) surface area of the electrode, (DR) diffusion coefficient, (υ) scan rate, and (C0) concentration of K3Fe(CN)6, respectively. Using 10.0 mm K3Fe(CN)6 in 0.1 M KCl as an electrolyte solution, T=298 K, R=8.314 JK-1mol-1, F=96,480 Cmol-1, n=1, DR=7.6×10-6 cm2 s-1, by the obtained slope from the plot of Ipa vs. υ1/2, the calculated surface area of the electrodes was found to be, 0.065, and 0.121 cm2 for CPE and MWCNT respectively.

3.2. Cyclic voltammetric behavior of TRC

The voltammetric behavior of TRC at CPE and MWCPE electrodes was studied using cyclic voltammetry (CV) at pH = 9.0. The cyclic voltammograms obtained for TRC at a scan rate of 50 mVs-1 exhibit a well-defined irreversible cathodic peak at about -0.16 V at CPE and MWCPE. The results are displayed in Fig. 2. The cathodic peak that appeared was corresponding to the reduction of TRC. In addition, the oxidation-reduction product did not show any redox or reduced peak in the extended ranges of the potential, ensuring that the oxidized product might not be electroactive on the surface of the CPE and MWCPE. The voltammograms corresponding to the first cycle were generally recorded. It is also found that accumulation potential and time almost did not affect the peak current.

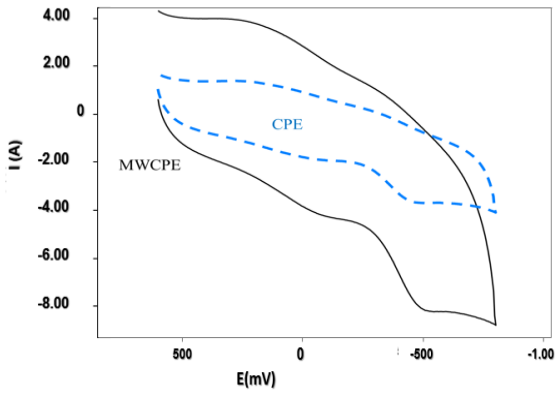

Fig. 2. Cyclic voltammograms obtained for 0.5µg/ml TRC on CPE and MWCPE in 0.04M buffer at pH =9.0 at υ = 50 mV/s1.

3.3. Optimization of Experimental parameters

3.3.1. Effect of supporting electrolyte on peak current

the influence of the type of the supporting electrolyte on the reduction peak current of the voltammetric redox reaction of TRC drug at MWCPE electrode was examined using the CV mode in various electrolytes, at PH 8.0. Some conventional supporting electrolytes of buffer solution were tested, such as 0.04 M of each acetate buffer, phosphate buffer, and Briton-Robinson (B-R) buffer as displayed Fig. 3. B-R buffer was selected as an optimum electrolyte due to giving higher reduction peak current at the working electrode, wide pH range, its higher buffer capacity sense of the composition of the electrolyte, and having no IP peak values.

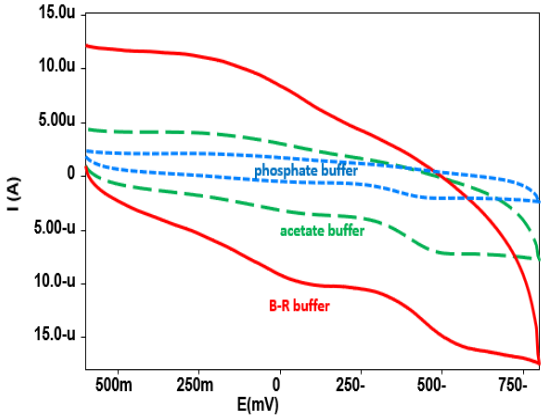

Fig. (3) CV voltammograms of 5.0 µg/mL TRC on MWCPE at scan rate of 100 mV.s-1in 0.04M B-R solution pH 8.0.

3.3.2.Comparison Voltammetric behavior of TRC

To improve the voltammetric behavior of the TRC signal, the MWCNT was investigated for surface modification. The SWV technique was applied to the blank, bare, and modified (MWCNT) electrodes to examine the effect of the modifier on CPE (Fig. 4) voltammetric peak currents of TRC increased on MWCPE showing that the MWCNT (high surface area) and nanoparticles created a synergistic effect together. The MWCNT modifier has been demonstrated to basis redox reaction and a notable catalytic effect, the modified with MWCNT enhanced the electrocatalytic effect by increasing the electron transfer rate at the MWCPE electrode surface.

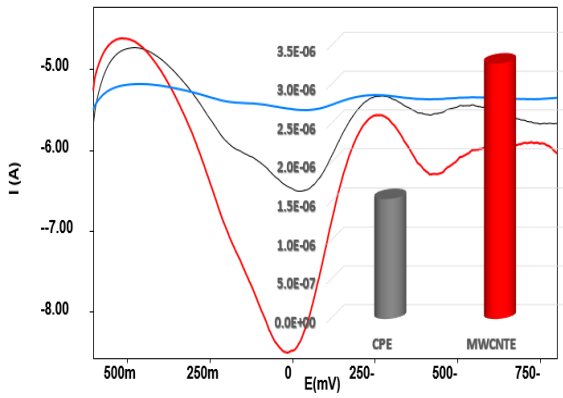

Fig. 4. SWV voltammograms of 2.0µg/ml TRC on the surface of bare CPE and MWCNT modified CPE in pH 8.0 B-R solution.

3.3.3. Effect of the pH value on peak current and peak potential.

The single peak of cathodic peak voltammograms for the redox of 2.0 µg/ml of TRC drug in pH range (2.0-10.0) at MWCPE are studied and given in fig. (5A). This is no anodic peak in the reverse sweep which indicates that the redaction of the studied drug is irreversible.

As illustrated before that the optimum and maximum peak height for TRC was obtained with 0.04 M B-R buffer of pH 8. fig (5B) shows the blot of peak current ip vs. pH for TRC drug. It was found from these figures that over the range the peak current increases with the increase of pH and reaches its maximum peak value at pH 8.0 for TRC.

Fig. (5) displays that the cathodic peak potential reliant on the pH values and in practice that it shifted when the pH values of buffer increased foremost to the formation of reducing species. This behavior can be attributed to the release of H+ ions during the redox process of TRC in buffer solution. The approximate relationship between the anodic peak potential (Ep) with the pH values can be described by the linear regression equation between the cathodic peak potential and the dielectric pH values, the calculated equation is as follows:

Ep= 0.0425 - 0.18 pH (r2= 0.995) for TRC

A plot of Ep against pH is liner with slope 0.059/n where n is the total number of electrons and the intercepted corresponding to standard cell potential (E0). The theoretical slop for the plot of Ep with PH for a classical Nernest corresponds to one electron[33-36].

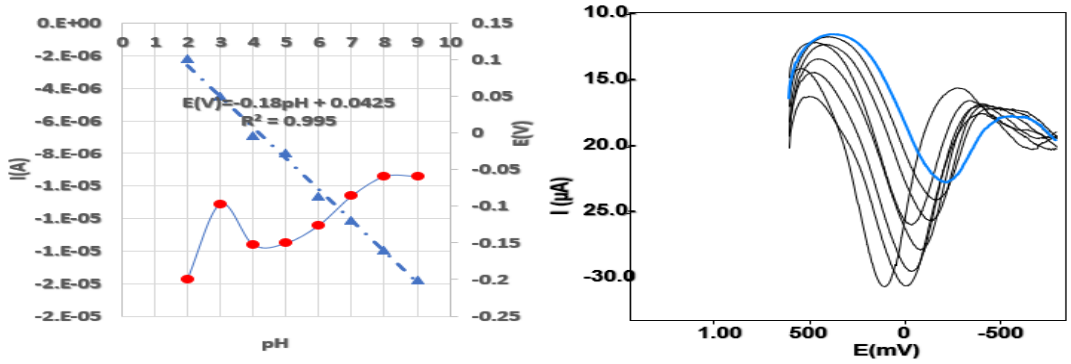

Fig. (5) (A) DPV voltammograms of 5.0 µg/mL TRC on MWCPE at scan rate of 100 mV.s-1 in 0.04 M BR buffer solution (B) Relation between different pH, peak current (●) and potential (▲) for TRC employing MWCPE at scan rate of 100 $mV.s^{-1}$ in 0.04 M BR buffer solution over pH range of 2.0–10.0.

3.3.4. Effect of potential scan rate

The outcome of scan rate influence on peak current and peak potential of TRC has been studied using CV by variation of scan rate. The CV of 5.0 µg/mL TRC solutions at MWCPE electrode over scan rate range 20-240 mVs-1 is shown in fig. (6). A positive shift in the peak potential of the anodic waves of the tested drugs is shown at the MWCEP electrode. The variation of the peak current (ip) with the voltage scan rate (υ) for the irreversible reaction at 298 ºk was given by the relation.

Ip= (2.69 x 105) n 3/2 A D 1/2 C υ 1/2

Where, ip= current (A), n= number of electrons, A=area of the electrode (Cm2), C = concentration (mol/cm3), D= diffusion coefficient (cm2/s), υ= scan

rate (V/s). indicating that these electrochemical processes are diffusion controlled at the MWCPE

In addition, we assessed the plots of log Ip vs log υ from CVs recorded expected slop are given in the range of 600mVto -750mV with slope 0.452, These confirming electrochemical processes are diffusion-controlled and the irreversibility of the electrochemical process with a simultaneous increase in the peak current in the range of 20-200 mVs-1. and give the regression equation as follow [34, 37, 38]:

Log ip= 5.4967 + 0.4521 log υ (r2= 0.9932) for TRC

The slop values obtained are closed to the theoretical value of 0.5 which confirmed that the electrode reaction is the ideal reaction of solution species. Thus, oxidation diffusion-controlled process over the studied scan rate at MWCEP. This indication can enhance by observing that the accumulation time and accumulation potential did not affect anodic peak current.

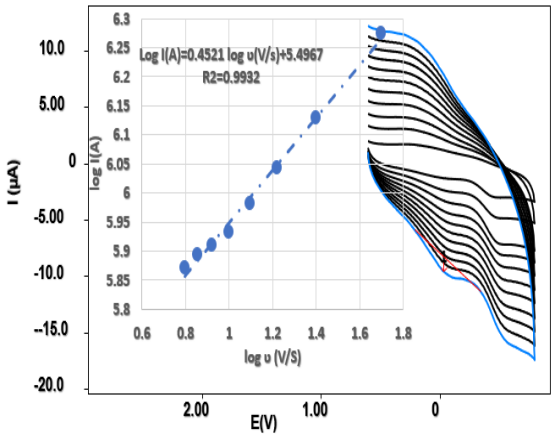

Fig. (6). (A) Relation between different scan rates and peak currents (log Ip vs. log υ) for TRC employing MWCPE in0.04 M BR buffer solution at pH 8.0; The (B)is the cyclic voltammograms of scan rates over the entire range of 20–240 mV. S^{-1}.

Moreover, by raising the scan rate, the peak voltage shifts to more positive values. This shift also elucidates the irreversibility of the electro-reduction process. The relation of cathodic peak potential and the log of scan rate also exhibited a linear relationship Fig. 6, following the equation:

in the range of 20–180 mV s-1, there is a line relationship between Epc and log υ (Fig. 3d). The linear regression equation is Epc = 0.111(±0.0061) + 0.059(±0.007) log υ; with a correlation coefficient of 0.9754. The Tafel slope (b) can be attained from the slope of Epc vs. log υ using the following equation

Epc = b/2 log υ + constant

The Tafel plots (log i vs E) were obtained with a scan rate of 5 mVs-1 beginning from a steady-state potential at pH 8.0 for MWCPE. The slope of the obtained Tafel curve is equal to (1− α) nF/RT. By substituting the numerical values of n, F, R, and T in the slope of the Tafel curve, the amount of α for the oxidation of naproxen obtained was 0.57.

3.3.5. The mechanism of TRC redaction

The mechanism of TRC redaction was illustrated by the CVs of TRC shown in Fig. 6. First, the TRC was reduced to ketone aliphatic a redaction peak at 50.0mV. This may be due to the reaction of C=O with intermediates. During the second scan, the reduction peak at the same 50.0mV, which were attributed to the reduction of C=O. From the above discussion, we conclude that an H+ and two electrons are involved in the electrochemical redaction of TRC. The possible mechanism of TRC redaction was shown in Fig. 7.

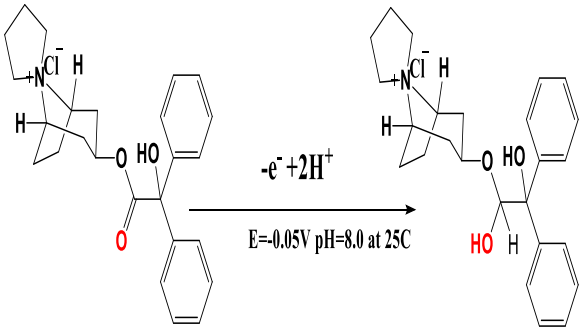

Fig. (7). Proposed mechanism for reduction processes of TRC at MWCEP and the generated product redaction in these potentials.

4. Voltammetric Analytical validation performance

The proposed (SWV) technique for TRC determination was validated in compliance with the ICH guidelines [27-28].

System suitability

To verify a consistent and constant response procedure, suitability tests (SST) were performed. A stable response is the main to the accomplishment of any analytical technique. The importance of the SST in this analytical technique is to confirm that the

entire analytical system (counting the instrument, reagents, and electrodes) is fitting for the envisioned application. Five replicate voltammetric analyses of Standard TRC solution (5.0/ml) were recorded at Operational parameters as listed in the table (1) and used to assess the suitability of the system. The RSD was calculated from five repeated voltmeter readings.

Table 1 Operational parameters of proposed voltammetric method.

Buffer	0.04M BR buffer
pH	8
Equilibration time (s)	5
Start potential (V)	0.6
End potential (V)	-0.8
Voltage step (V)	0.005
Voltage step time (s)	0.092
Sweep rate (V/s)	0.05
Pulse amplitude (V)	0.05
Pulse time (s)	0.04

4.1. Calibration curve Determination of TRC drugs by SWV at MWCEP.

Fig. 8A displayed the SWV responses of TRC with various concentrations from 0 to 50 M in in0.04 M BR buffer solution at pH 8.0 at the MWCEP electrode under the optimum conditions. As can be perceived, the redox peak current increased with cumulative TRC concentration. Fig. 8B presented the linear regression of peak height against TRC concentration on a scale over the range of 0.30 µg/ml to 2.60µg/ml. It is clear that the plot consists of two linear regions which Two linear regions could be discerned, as follows: the first range (a) was from 0.30µg/ml -1.2µg/ml with linear relation equation (I (A) = 0.93• C (µg/ml) + 2.2); the second range (b) was from 1.30µg to 2.6µg with linear relation equation I (A) = 0.47• C (µg/ml) + 2.79). The low detection limit was assessed to be 0.07 µg/ml based on a ratio of signal to noise of 3 (S/N = 3).

The low detection limit could be due to one or both of the following reasons which are the high surface-area-to-volume ratios of TRC and the low electron transfer resistance of MWCNT. The statistical parameters of the different data for the linear regression equation are listed in Table 2. Statistical data for calibration curves were obtained from the determination of the three different measurements.

The regression equation was computed and found to be:

Ip(mA) = 0.9321 C (µg/ml) + 2.1989 (r2= 0.9934) for TRC

Ip(mA) = 0.4708 C (µg/ml) + 0.2.7877 (r2= 0.9961) for TRC

Where ip = the peak current, C =concentration of drugs, r = correlation coefficient. The application of the proposed (ASWV) was further investigated for the quantitative determination of the TRC parameter as listed in the table (2).

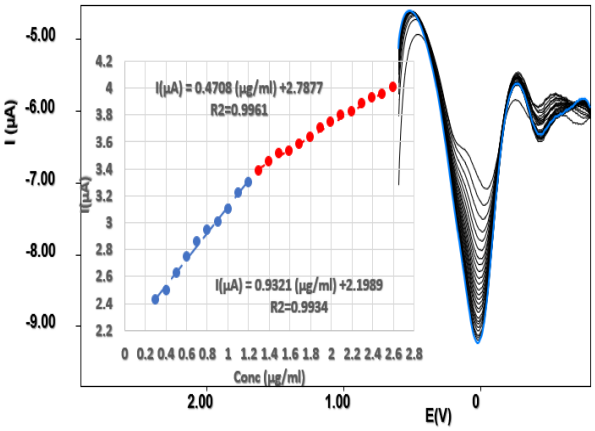

Fig. (8) SWV of TRC in series concentration Range (0.3 -2.6µg/ml) in 0.04M B-R at pH 8.0 from range 0.10 to - 0.800 V.

Table (2): Analytical parameters of TRC at MWCEP by using SWV technique.

Parameter	TRC	TRC
Linearity range (µg/ml)	0.3-1.2	1.3-2.6
Slop	0.975	0.471
SD of slope	0.025	0.01
Intercept (b)	2.144	2.79
SD of intercept	0.020	0.017
RSD%	0. 70	0.51
Correlation coefficient (r)	0.998	0.996
Accuracy [a] (mean± SD)	99.97±0.017	99.96±0.013
Specificity [b] (mean± RSD %)	100.00±0.054	100.00±0.071
LOD	0.07	
LOQ	0.21	
Repeatability	0.560	0.41
Intermediate precision	1.210	1.051

[a, b] Mean average of six determinations

4.2. Comparison of the proposed method with literature methods

Assay of TRC by reference reversed HPLC method is also used for comparison to assess the validity of the suggested SWV method. Table 3 shows the results obtained through the proposed SWV technique and reference HPLC measurement methods for three triplicate measurements. The obtained results are compared and there are no significant variances between the results obtained from both methods.

Table 3. Accurateness of the TRC voltammetric technique and comparison with reference HPLC method.

Parameter	Proposed method		Ref. method (39)	
	Amount taken (µg/ml)	Amount found (µg/mL)	found*	% Found
	2.5	2.49	99.6	99.88
	2	2.01	100.5	99.98
	1.5	1.503	100.2	100.5
Mean ± SD			100.1±0.001	100.12±0.04
Sample Variance			0.21	0.11
t-Test			0.477	
F-test			1.89	
t-Tabulated			2.77	
F-tabulated			19	

* Each result is the average of three separate determinations.

4.3.Specificity

For the potential analytical validation of the suggested technique, the influence of some common excipients used in the pharmaceutical formulation was studied. The specificity of the optimal technique for TRC analysis was investigated by detecting any interference encountered from endogenously substances existing in compound mediums such as biological fluids (e.g., urine and blood plasma) as displayed in table 4. The circumference of tolerance was defined as the maximal concentration of interfering substances that caused an error of less than 5% in the quantified TRC drug. So that the effect of interferes (uric acid, ascorbic acid, lactose monohydrate, and sucrose) was examined by implementing the determination of 3.0 µg/ml from TRC in the existence of different concentrations of interferes. It was observed that up to an additional 10-fold interferes there was no significant variance in the response of peak current. This demonstrates that the SWV technique in the MWCEP electrode under optimal conditions can be a process safely applied for the determination of TRC in biological fluids.

Table (4) Appraisal of the accuracy and precision of the projected technique for calculation of TRC in spiked serum samples

	Sample 1	Sample 2
Added	2	2.5
Found*	1.99	2.51
Recovery %	99.5	100.4
Bias (%)	0.01	0.01
SD	0.113	0.216
RSD %	0.89	1.12

SD = standard deviation; RSD = relative standard deviation, SE = standard error

* Mean of five measurements

4.4. Repeatability and intermediate precision

Reproducibility and intermediate precision were checked on the same day by taking triplicate measurements of the three different concentrations and for three separate days of standard TRC. The reproducibility of the result obtained by the proposed procedure was shown under linearity. The standard deviation (SD) values of inter-and intera day were

estimated and were less than 2%, It is indicated that the developed technique was accurate with a high degree of confidence as presented in (Table 5).

Table5. Table5. inter- and intera day SWV mode determination of TRC and recovery test using MWCEP.

Sample	Added amount (µg/ml)	Amount found (µg/ml)	Percentage recovery
Intraday precision	2.5	2.493	99.72
	2.5	2.507	100.28
	2.5	2.505	100.2
	2.5	2.52	100.8
Mean ± SD			100.25 ± 0.44
Interday precision			
	2.5	2.491	99.64
	2.5	2.513	100.52
	2.5	2.507	100.28
	2.5	2.504	100.16
Mean ± SD			100.15 ± 0.37

4.5. Application on pharmaceutical

The specificity of the determination technique for TRC assay in Trospikan tablet was assessed. No interfering peaks are found in presence of commonly excipients in their pharmaceutical formula under the above optimum conditions, additionally different. The result obtained was consistent with the content specified on the label (Table 6). Recovery experiments were performed by the standard addition scheme and the recovery range from 99.0 % to 100.2 %. These results indicated that the proposed method is suitable for the quality TRC drug in their pharmaceutical tablet. From these results, it is clear that the proposed SWV technique is appropriate for high selectivity and precision for the determination of TRC in their pharmaceutical tablets.

Table 6. Determination of Trospikan in pharmaceutical tablets and recovery test using MWCEP

Sample	Added (µM)	Found (µM)	Bais%	Recovery (%)	HPLC*
Tablet	2.4	2.40	0.08	100.08	100.10
	2	2.01	0.50	100.50	99.50
	1.3	1.30	0.15	99.85	99.73
Mean				100.14	99.78
Variance				0.11	0.09
F-test					1.96
F-tabulated					19
t-test					1.41
t-tabulated					2.78

* Each result is the average of three different separate determinations. Figures in parentheses are the tabulated t and F values respectively at P= (0.05).

Conclusions

The presented study employed accurate, simple, and sensitive cyclic and SW voltammetric techniques for the determination of TRC in pure, dosage forms and biological fluids using the most common and simplest working electrodes; CPE and MWCEP. A comparison was made between the behavior of the drug at the two electrodes where MWCEP proved to provide the highest sensitivity results for TRC drug, where LOD was equal to 0.09, and LOQ was equal to 0.265. The redox behavior of the drug was inspected and presented only one well-defined reduction irreversible peak. Different parameters were tested to

optimize the determination conditions. The analytical procedure was fully validated regarding linearity, precision, accuracy, reproducibility, sensitivity, and selectivity. The method developed was also compared with the reference one and was proved to be a satisfactory alternative for the fast, clean and simple quantitative determination of TRC.

Acknowledgments

The authors would like to express their gratitude to the Department of Analytical Chemistry at the Faculty of Science (Benha University) and the National Organization for Drug TRC and Research (NODCAR, Egypt) for providing instruments and the means necessary to accomplish this work.

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