Green Insight of High Performance UTGE Operation in Nano-Sensitive Electro-Analysis of Meropenem by Adsorptive Stripping Voltammetry

Ahmed S. Fayed,[1] Rania M. Youssif, [2,z] Hassan A. M. Hendawy,[2] Nahla N. Salama, [2] and Eman S. Elzanfaly[1]

[1]*Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Cairo 115562, Egypt*
[2]*Department of Pharmaceutical Chemistry, National Organization for Drug Control and Research (NODCAR), Giza, Egypt*

The present study concerns with investigation of the electrochemical activity of meropenem (MP) at ultra-trace graphite electrode (UTGE). The electrochemical measurements were performed in various buffer solutions in pH range (2.0–8.0). One irreversible anodic peak was observed in acidic medium. The effects of pH and scan rate on the peak current and potential were studied. The adsorption-controlled nature of MP peak was demonstrated. Therefore, it is directed to develop adsorptive stripping square wave voltammetric technique (AdS-SWV) for quantitative determination of MP in drug substance, pharmaceutical vials and in presence of interference substances. Under optimum experimental conditions, a linear dependence relationship was obtained over MP concentration range of 500.0–15000.0 nM. The limits of detection (LOD) and quantification (LOQ) were 160.0 and 470.0 nM respectively. Chemical safety is assessed at different aspects. Qualitative and quantitative metrics reveal excellent eco-friendly voltammetric method. In spite of high budget of UTGE; multiple merits of nano-sensitivity, selectivity, greenness evidence and high efficiency encourage wide application of our developed method in QC using UTGE in comparison to other chemically-modified electrodes.
© 2018 The Electrochemical Society. [DOI: 10.1149/2.0661814jes]

Meropenem (MP) is non-classical β-lactam drug; its chemical name is (4R, 5S, 6S)-3-[(3S, 5S)-5-(Dimethylcarbamoyl)-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-carboxylic acid, trihydrate,[1] having ultra-broad antibacterial spectrum.[2,3]
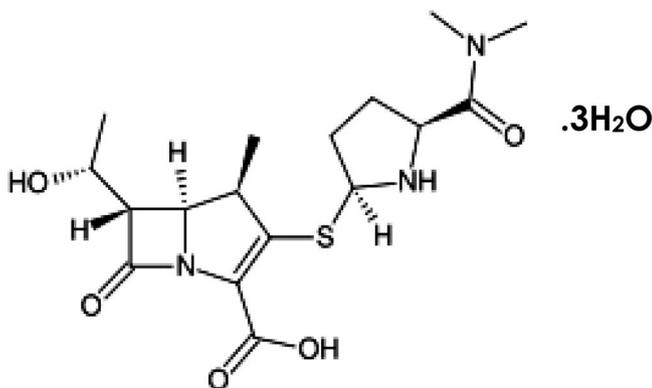
Chemical structure of MP

Currently reported methods in literature survey comprise various quantitative analytical techniques: colorimetry,[4–6] UV-spectroscopy,[7–10] spectrofluorimetry,[11] chemiluminescence,[12] chromatographic separation[13–16] and electrochemical voltammetric methods using classical,[17–19] and chemically modified electrode[20] for MP determination in pure form, commercial vials, human urine and plasma.

Ultra-Trace Graphite electrode (UTGE) is a kind of modification of GCE. It is ready fabricated and insulated electrode that has many advantages of high electrochemical performance properties, obvious potential stability, strong mechanical strength, less impurities, good vibration resistance and faster processing speed. It can be applied in ultra-levels of detection of many drugs in pharmaceuticals and biological fluids.[21–23]

The primary domain of adsorptive stripping voltammetry is the dependence on adsorption property of the cited drug in pre-concentration step. When the organic compound contains electrochemically oxidizable or reducible function groups, it reacts with oxidized electrode material and the formed compound is adsorbed producing either anodic or cathodic peaks, respectively. Adsorptive stripping voltammetry is employed in the trace analysis of a wide variety of organic and inorganic analytes.[24,25] It is also insensitive to matrix effects.[26]

Square wave voltammetry is the applied quantitative technique in our study because of its speed in analysis.[27]

Nowadays, Caring about Greenness insight at different methodology steps is considered implying qualitative National Environmental Methods Index (NEMI) label[28] and semi-quantitative Analytical Eco-Scale metrics.[29,30] Both metrics are sufficient enough to discuss the extent of chemical safety and the weakness points of any applied voltammetric method to minimize any harmful dominance to personnel or environment and to help in further improvement or modification.

The main concern of this study is directed toward investigation of the electrochemical behavior of MP at UTGE by cyclic and square wave voltammetry. Preliminary investigations using cyclic voltammograms shows the electrochemical mechanism of MP at UTGE and aids in mechanism postulation. The observed adsorptive character at UTGE was utilized in shifting toward application of adsorptive stripping voltammetry. Under optimized conditions, adsorptive stripping square wave voltammetry (AdS-SWV) was proposed for quantification of MP at nano-concentrations in drug substance and pharmaceutical vials. Moreover, selectivity of the proposed voltammetric method was discussed in presence of various interfering substances and degradants.

Safety consideration is the major point of interest presented in our study that appeal to many scientists before starting methodology application; in addition to the proposed method did not require sample pretreatment or time-consuming extraction. It is rapidly applied to collect data in few seconds without any sophisticated precautions.

Experimental

Instrumentation.—Metrohm computrace analyzer model 797 VA with Software Version 1.0 (Metrohm, Switzerland) for voltammetric analysis. Digital pH-meter Jenway 3310 pH-meter used to measure pH. All solutions were purged for 10 min with purified nitrogen gas.

Electrodes: Ultra-Trace Graphite Electrode (UTGE) is the working electrode: S.N. 6.1204.180UT, Metrohm, Switzerland. To improve the sensitivity and resolution of the voltammetric peaks; UTGE was

zE-mail: dr.ronica81@yahoo.com

polished manually with 0.5 μm alumina powder on a smooth polishing cloth. Then, it was thoroughly rinsed with distilled water. Ag/AgCl (3 M KCl) is used as the reference electrode and a platinum electrode is the auxiliary one.

Chemicals and reagents.—Meropenem trihydrate (99.9% on anhydrous basis) was obtained from Sumitomo Pharmaceuticals Co. Ltd., Japan. Meronem vials (1.140 gm of meropenem trihydrate) were manufactured by AstraZeneca, UK.

All chemicals and reagents used throughout this work were of analytical grade and used without purification. Boric acid (Polskie, Poland), Glacial acetic acid and orthophosphoric acid (Adwic Co., Egypt) were used to prepare Britton-Robinson (B-R) buffer solutions (0.04 M) over the pH range of 2.0–8.0.[31] pH was adjusted with 0.2 M NaOH. Bi-distilled water was used and indicated by the word "water" in the entire work.

Standard solutions.—*Standard stock solutions of MP.—*Fresh standard stock solution of (1.0×10^{-3} M) was prepared by accurately weighing 10.9 mg of MP certified material in 25.0-mL water; calculated on free acid form. Standard working solution of MP (1.0×10^{-4} M) was obtained by transferring an accurately measured volume of 10.0-mL of standard stock solution (1.0×10^{-3} M) into 100-mL volumetric flask and completed to volume with water.

*Stock solutions of the hydrolytic and oxidative-degradants.—*Hydrolytic and oxidative degradants of MP were prepared and their complete degradation was assured by TLC.[19] The final concentration of both prepared degradants is (1.0×10^{-3} M); dissolved in water. Working solution of each degradant (1.0×10^{-4} M) was prepared by separately transferring 10.0-mL of each stock solution (1.0×10^{-3} M) into two separate 100.0-mL volumetric flasks and completed to volume with water.

Procedures.—*Voltammetric behavior of MP at UTGE.—*The voltammetric behavior of MP at UTGE was collected by applying cyclic and stripping square wave voltammograms in 0.04 M B-R buffer solutions over pH range of (2.0–8.0). Ten milliliters of B-R buffer containing certain volume of MP was added to the voltammetric cup. UTGE was then immersed. The solution was stirred at 2000 rpm for the selected preconcentration period (5s) and accumulation potential (−0.40 V). Then, the stirrer was stopped for 5s for solution stabilization. Cyclic and anodic stripping square wave voltammograms of (1.0×10^{-4} M) of MP were recorded using an applied potential profile in the range from −0.1 to –0.8 V against 0.04 M B-R buffer solution as a supporting electrolyte. Meanwhile, the stripping square wave voltammograms of the hydrolytic and oxidative-degradants of MP were also collected and compared with those obtained from the intact drug at equal concentrations using UTGE. All data were collected at room temperature.

Construction of calibration curve.—Aliquots equivalent to, (5.0–150.0 μmol) of MP from its standard working solution (1.0×10^{-4} M), were transferred to a set of 10-mL volumetric flasks. The volume was completed to the mark with B-R buffer solution (pH 2.0) to give the concentration range of (500.0–15000.0 nM) MP. The peak current at UTGE was measured at amplitude 42.0 mV; frequency 20.0 Hz; voltage step 8.24 mV; and scan rate, 200 mV.s^{-1} using AdS-SWV method against blank of the same buffer. Calibration curve was plotted relating the anodic peak current (Ip) to the corresponding concentration of MP. The regression equation and correlation coefficient were calculated.

Applications.—*Analysis of laboratory prepared mixtures of MP in presence of its different degradants.—*Aliquots of MP, hydrolytic and oxidative-degradants were transferred, separately, from their stock working solutions (1.0×10^{-4} M) into two separate sets of 10-mL volumetric flasks. B-R buffer solution (pH 2.0) was used to complete
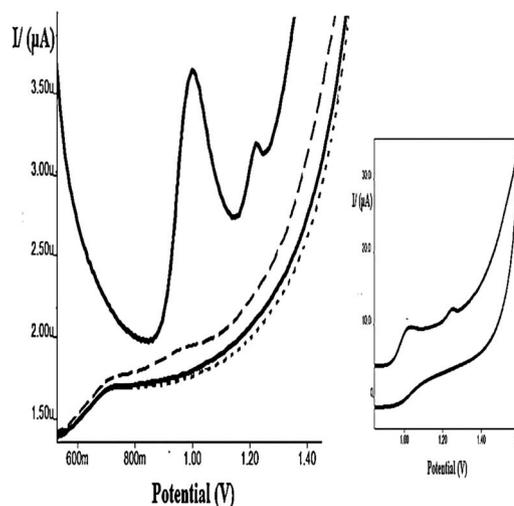

Figure 1. Anodic stripping square wave voltammograms of MP, the hydrolytic (____), oxidative (- - - -) degradants in 0.04 B-R buffer (pH 2.0) as a supporting electrolyte (. . . . .) using UTGE; scan rate = 200 mV.s^{-1}; [MP] = 1.0×10^{-4} M. The inset is the corresponding cyclic voltammograms of MP.

the volume. Laboratory mixtures containing different degradants percentages were prepared ranging from (1–40%). The peak current at UTGE was measured at pre-determined optimal conditions applying AdS-SWV method. MP concentration was calculated from the regression equation.

*Application to pharmaceutical vials.—*Five vials of Meronem (1.0 gm/vial) were accurately weighed and blended. A weight of 13.0 mg equivalent to 10.9 mg of MP anhydrous base was quantitatively transferred into 25-mL volumetric flask and then dissolved in water. Five milliliters of the previously prepared solution was transferred into 50-mL volumetric flask and the volume was completed with water.

Appropriate aliquot of the final prepared solution was added to 10.0-mL of 0.04 M B-R buffer (pH 2.0) to obtain 4.0 μM of MP to be measured. The prepared solution was quantitatively transferred to the voltammetric cup for measurement. The AdS-SW voltammograms were recorded implying the previously described voltammetric procedure to determine the concentration of MP in the pharmaceutical vials. The recoveries and RSD% were calculated from the regression equation.

For standard addition technique application; the vials were spiked with different known concentrations of standard MP. The AdS-SW voltammograms were collected and % recoveries were calculated by evaluating the collected peak currents.

Results and Discussion

Characterization of the utilized UTGE.—The active surface area of UTGE is calculated from Randles-Sevcik formula[32,33] for a reversible process. Cyclic voltammograms of (1.0×10^{-3} M) $K_3Fe(CN)_6$ at different scan rates were measured, where, n = 1, D_o = 7.6×10^{-6} cm^{2}s^{-1}. The plot of I_{pa} vs. $\upsilon^{1/2}$ was constructed and the slope was calculated. The electro-active area of UTGE was calculated from the slope and found to be 0.012 cm^2; hence, high responses were expected for MP at UTGE.

Voltammetric behavior of MP at UTGE.—The electrochemical behavior of MP was investigated at UTGE electrode applying both cyclic voltammetry and AdS-SWV. Cyclic voltammograms of MP exhibited a single anodic peak at 1.00 V with absence of a cathodic peak on the reverse scan signifying irreversible oxidation process at the electrode surface, (Figure 1). Meanwhile, the recorded (AdS-SW) voltammograms showed a single sharp anodic peak at 1.02 V at the same concentration and pH; as shown in (Figure 1). Upon comparing
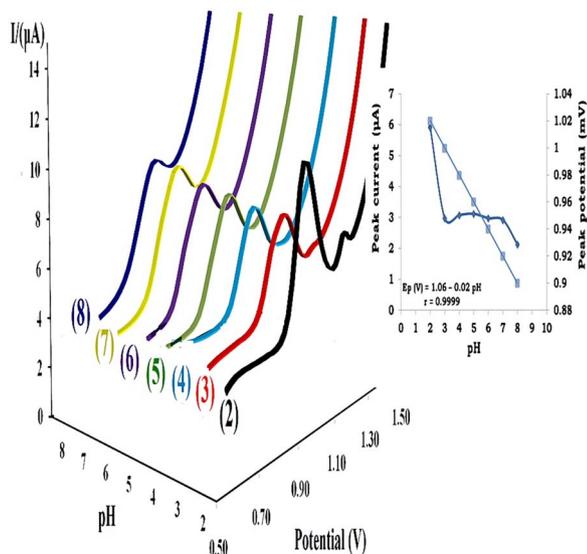
Figure 2. Cyclic voltammograms demonstrating the study of pH variations on the anodic peak current of MP. The inset is the relation between the peak potential, current and pH using UTGE; scan rate = 200 mV.s^{-1}, 0.04 M B-R buffer (pH 2.0); [MP] = 1.0×10^{-4} M.

the (AdS-SW) voltammograms of intact MP and different degradants in (Figure 1); it is clear that complete disappearance of the anodic peak of interest which successfully facilitated MP separation from its degradants. MP could be easily determined with ultra-high sensitivity and entire selectivity.

Optimization of experimental parameters.—Different experimental parameters were carefully discussed and optimized. Both cyclic and stripping square wave voltammetry were applied.

Effect of pH.—The effect of pH has a great influence on the oxidation peak of MP at the UTGE. Figure 2 shows the (AdS-SW) voltammograms of MP in B-R buffer in pH range (2.0 to 8.0).

From Figure 2; it is clear that the highest oxidation peak current was recorded at pH 2.0. On the contrary, at strong alkaline medium; the anodic peak current of MP is diminished. This is attributed to β-lactam ring hydrolysis at pH over 8.0. Therefore, B-R buffer (pH 2.0) was used in further SW voltammetric application.

The anodic peak potential shift toward negative direction upon pH rising. This signifies that the oxidation mechanism occurrence at UTGE is remarkably pH-dependent and that protons share at electrode reaction processes.

A linear relationship correlated peak potential (Ep) to pH over the range of (2.0–8.0); as presented in Figure 2. This was expressed by the following equation:

$$Ep\,(V) = 1.06 - 0.02pH \qquad r = 0.9999$$

Upon recognizing the adsorptive character of MP at UTGE; the study of accumulation time and potential effect is preferred to enhance drug response increase sensitivity and speed up the analysis.

Effect of accumulation time and potential.—The effect of accumulation time ranging from (0–30 s) has been studied. The peak current increases rapidly and remains constant as the accumulation time is increased from 0 to 20 sec. Above 20 sec, the peak current decreases slowly; as shown in Figure 3a. The peak current attains maximum value at 5s and this proves very fast analysis time to receive the required data.
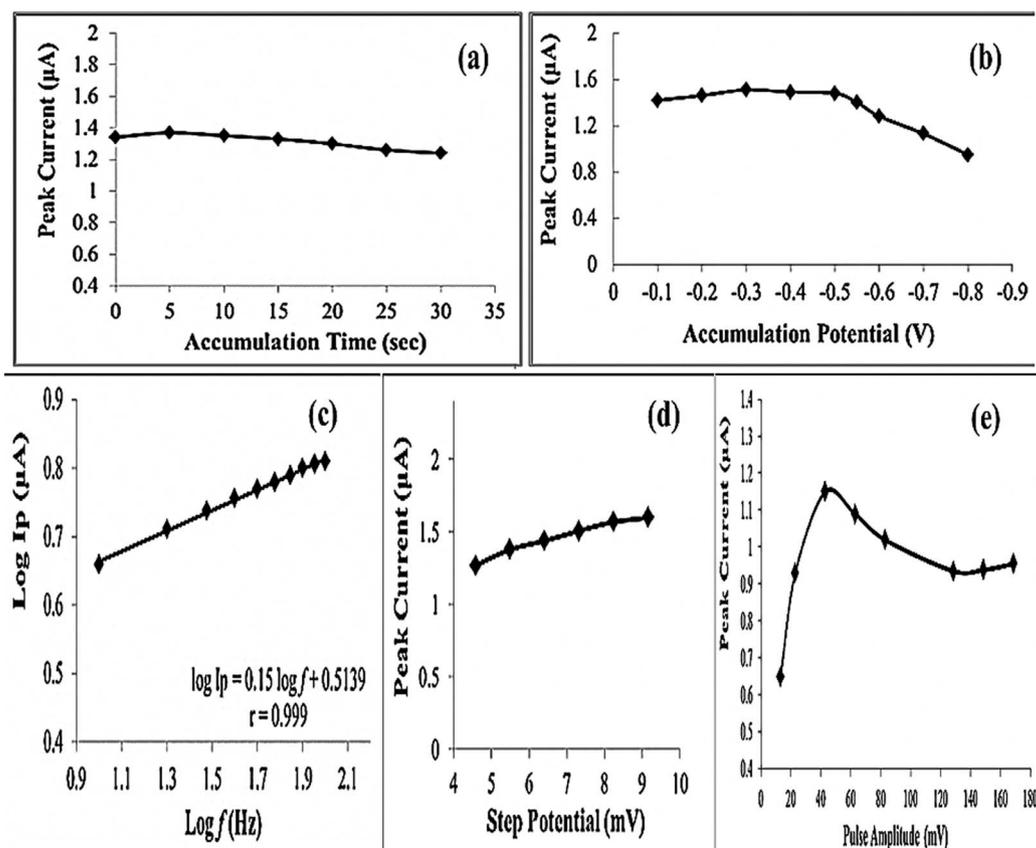

Figure 3. Effect of accumulation time (a), accumulation potential (b), SW frequency (c), SW voltage step potential (d) and SW pulse amplitude (e) on the anodic peak current of MP using UTGE; scan rate = 200 mV.s^{-1}, 0.04 M B-R buffer (pH 2.0); [MP] = 1.0×10^{-4} M.
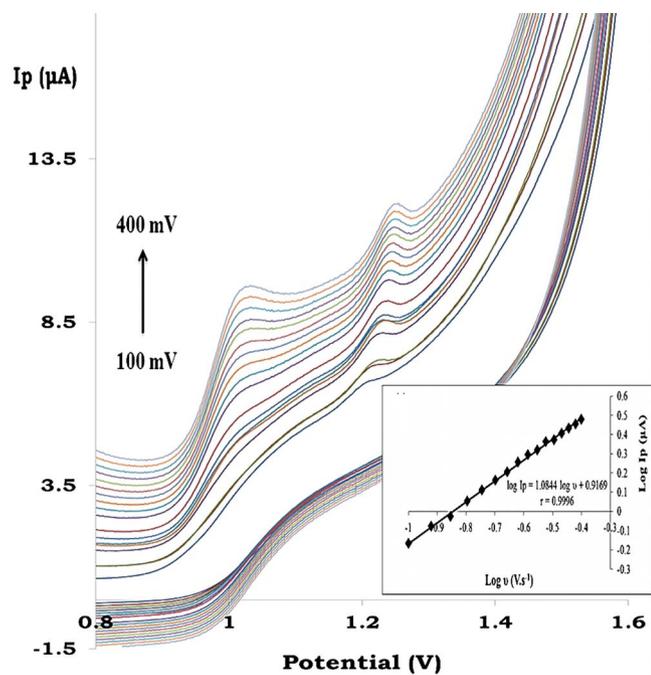
Figure 4. Cyclic voltammograms demonstrating the study of scan rate variations on the anodic peak current of MP. The inset is plot of log Ip vs. log υ using UTGE; 0.04 M B-R buffer (pH 2.0); [MP] = 1.0×10^{-4} M.

The starting potential was varied in the range (−0.1 to –0.8 V) and the peak current increases with increasing negativity of the starting potential in the range –0.1 to −0.5 V and a sharp decline was noticed above –0.6 V, Figure 3b. The maximum peak current came at accumulation potential of −0.4 V for UTGE which was used in the following voltammetric study.

Effect of SW frequency, voltage step potential and amplitude.— The study of frequency, voltage step potential and amplitude were performed. SW voltammograms were collected and recorded. Despite, the variation of the peak current of MP over the frequency range of (10–100 Hz) is linear, Figure 3c, as expressed by the following equation,

$$\log Ip\,(\mu A) = 0.15 \log f\,(Hz) + 0.5139 \qquad r = 0.999$$

The peaks broaden and become ill-defined at frequencies higher than 30.0 Hz. Therefore, 20.0 Hz was chosen as the optimum SW frequency.

The optimum voltage step potential of 8.24 mV was chosen as maximum response was obtained, Figure 3d.

The range of the amplitude studied over the range of 10.0–170.0 mV. As expected, the peak current increases as the amplitude increases and yields maximum value at about 40.0 mV. Then, the peak current decreases and peak distortion occurs over 70.0 mV resulting in a poorer resolution, Figure 3e. Thus, 42.0 mV was chosen as the optimum SW amplitude for further quantitative analysis.

Effect of scan rate.— The influence of scan rate (υ) on the anodic peak of MP is investigated in the range (40–400 mV.s^{-1}), Figure 4. Useful information includes electrochemical mechanism, the type of mass transport of MP at electrode surface and number of electrons involved in electrochemical mechanism which can be acquired from the relationship between the peak potential, current and scan rate.

Firstly, Figure 4 is the plot of logarithm of the anodic peak current of MP vs. logarithm of scan rate (*log Ip* vs. *log υ*) gave straight line as expressed by the following equation,

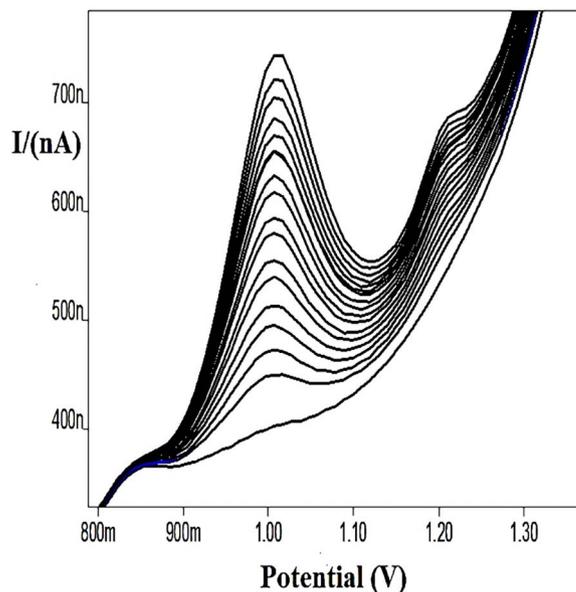$$\log Ip\,(\mu A) = 1.0844 \log υ\,(V.s^{-1}) + 0.9169 \qquad r = 0.9995$$

Figure 5. Anodic adsorptive stripping square wave voltammograms of MP range of (500.0–15000.0 nM) using UTGE in 0.04 M B-R buffer (pH 2.0), accumulation time 5 s, potential −0.4V, amplitude 42.0 mV, frequency 20.0 Hz, voltage step 8.24 mV and scan rate 200 mV.s^{-1}.

The obtained slope was found to be 1.0844; close to the theoretical value of 1.0.[34,35] It confirms that the process has a pure adsorptive character and declares the irreversibility of electro-oxidation of MP at UTGE by adsorption-controlled mass transport. This prefers the application of the adsorptive stripping voltammetry for MP quantification.

Moreover, while scan rate screening, the anodic peak potentials shifted to a positive direction indicating the irreversible nature of the oxidation process.

The optimum voltage step potential of 8.24 mV was chosen as maximum response was obtained, A linear correlation was deduced between the peak potential and scan rate (υ) in the range of (0.1 to 0.4 V.s^{-1}), Figure 4 and expressed as follows,

$$Ep\,(V) = 0.0658 \log υ\,(V.s^{-1}) + 1.0705 \qquad r = 0.998$$

This relation aids in determination of number of electrons involved in the electro-oxidation mechanism via applying Laviron equation[36,37] for an irreversible electrode process:

$$Ep = E^{o'} + \left(\frac{2.303RT}{\alpha nF}\right) \log\left(\frac{RT\,K0}{\alpha n F}\right) + \left(\frac{2.303\,RT}{\alpha n F}\right) \log υ$$

From Ep (V) vs. log υ plot, the calculated slope was 0.0658; then substitution in the slope of Laviron equation, taking T = 298 K and substituting the values of R and F, αn was calculated to be 0.9. Generally, α is assumed to be 0.5 in total irreversible electrode process.[36] Therefore, number of electrons (n) involved in the electro-oxidation of MP was assumed to be∼2.

k^o is the standard rate determining step constant of the reaction was also calculated using the intercept of Laviron equation if the value of E$^{0'}$ is known. E$^{0'}$ value in Laviron equation can be obtained from the intercept of Ep vs. log υ curve by extrapolating to the vertical axis at υ = 0.[38] The intercept for Ep vs. log υ plot were found to be 1.0705; E$^{0'}$ was calculated as 1.0056; k^0 was determined to be 0.34×10^3 cm.s^{-1}.

Validation of the proposed method.— The proposed (AdS-SWV) method was validated in accordance with ICH guidelines.[39]

Linearity and sensitivity.— In the current work, the dependence of peak current (Ip) on MP concentration in the analyzed solution was deduced under the above optimum experimental parameters, Figure 5.

Table I. Validation performance data of the proposed (AdS-SWV) method for determination of MP in drug substance at UTGE.

Parameters	(AdS-SWV) method	
Linearity		
Linearity range (nM)	500–15000.0	
Slope (a)	16.655	
SD of slope	0.089339	
Intercept (b)	20.433	
SD of intercept	0.786565	
Correlation coefficient (r)	0.9998	
Accuracy[a](Mean ± SD)	99.97 ± 1.365	
Specificity[a]	**Hydrolytic-degradant**	**Oxidative-degradants**
(Mean ± %RSD)	99.63 ± 1.272	100.17 ± 1.088
Precision (%RSD)		
Repeatability[b]	1.481	
Intermediate precision[b]	1.101	
LOD (nM)	160.0	
LOQ (nM)	470.0	

[a]n = 5.
[b]n = 9.

The developed method was linear over wide concentration range of (500.0–15000.0 nM) for MP. The regression equation was cited in (Table I). Ultra- high sensitivity is concluded at nano levels with wide sensitivity range starting from nano to micro levels; compared to other reported electrodes.[17,19]

Limits of detection and quantification.—Limit of detection (LOD) and quantification (LOQ) of MP were estimated at UTGE from the following equations: LOD = $3.3\sigma/S$, LOQ = $10 \sigma /S$ (where σ is the standard deviation of the residuals, and *S* is the slope of the calibration graph). Both LOD and LOQ values stated in (Table I), confirms the ultra-sensitivity of the proposed method at nano-level if compared with those calculated by the official[1] method.

Accuracy and precision.—Accuracy of five different concentrations in triplicates was calculated from the regression equation and permissible results were illustrated in (Table I). Statistical comparison[40] of the results obtained by the proposed and official method[1] using Student's *t*-test and variance ratio F-test revealed no prominent variation between the two methods as shown in (Table II).

Specificity and interference study.—AdS-SWV method was applied for MP determination in laboratory prepared mixtures at different percentages of its degradants (1–40%). Mean recovery and RSD% assures the absolute selectivity of the developed method upon investigation of the hydrolytic or oxidative degradation interference, (Table III).

The selectivity was attributed to mechanism of degradation which was characterized by the breakdown of the β-lactam[9,11] and that hin-

Table II. Statistical analysis of the results obtained by the proposed (AdS-SWV) method for determination of MP in drug substance at UTGE.

Parameters	(AdS-SWV)	Official method[1]
Mean	99.97	100.18
SD	1.365	1.322
Variance	1.863	1.747
n	5	5
t-test (1.860)[a]	0.248	-
F-test (6.39)[a]	1.066	-

[a]The values in parenthesis are the corresponding theoretical values of t and F at P = 0.05.

Table III. Determination of MP in laboratory prepared mixtures of the hydrolytic and oxidative-degradants by the proposed (AdS-SWV) method at UTGE.

Degradants Added%	Concentration (μM)	Hydrolytic-degradant	Oxidative-degradants
		% Recovery[a]	
1	6.93	99.60	98.73
5	6.65	98.70	101.60
10	6.30	100.68	99.16
20	5.60	98.00	100.70
30	4.90	99.32	99.97
40	4.20	101.45	100.86
Mean[a] ± %RSD		99.63 ± 1.272	100.17 ± 1.088

[a]Mean of three determinations.

dered the lone pair of electrons on the nitrogen of secondary amine in pyrrolidinyl ring localization. This prevented the oxidation mechanism to occur and thus, the anodic peak disappeared and the facility of selectivity was concluded.

The interference study of excipients present in pharmaceutical vials was realized by addition of ascorbic acid, uric acid, glucose, sodium carbonate or bicarbonate at different concentrations to a solution containing 2.80 μM of MP, separately. Ascorbic acid, uric acid and glucose show no interference to the oxidation peak. The obtained mean percentage recoveries and RSD% based on mean of five replicate determinations was 100.43 ± 0.130. While, sodium carbonate and bicarbonate interfere with obtained anodic peak up to 30%, (Table IV), due to alteration in the solution pH to more basic shift as both salts produce strong alkaline solutions. Actually, the amount of carbonate salt present in MP vials is unreachable to this level. So, the suggested results confirm devoid of interference by the most common excipients in the pharmaceutical products; thus, the proposed method is sufficiently selective.

Application on commercial vials and standard addition technique.—Application of the proposed (AdS-SWV) method was performed for MP quantification in commercial vials. The results were in good agreement with the nominal content of vials, as presented in (Table V). Recovery experiments were carried out by standard addition application. The collected recovery was not more than 101.34% which indicated the suitability of the proposed method for QC work.

Mechanism.—Oxidation mechanism of MP at UTGE was postulated from the number of electrons calculated by Laviron equation. Two electrons were deduced for the single obtained anodic peak at the same potential position. Therefore, the same oxidation mechanism as reported in Reference 19 was postulated by the loss of electrons on the nitrogen of secondary amine in pyrrolidinyl ring.

Greenness performance.—Greenness performance of any developed analytical method is taking major concern by many scientists, nowadays. In our study, we were interested in this new trend and

Table IV. Interference study of different interfering species in determination of 10.0 μM of MP by (AdS-SWV) method at UTGE.

| Interfering species	Tolerated interference/analyte ratio[a] (w/w)
Anhydrous glucose	100
Ascorbic acid	100
Uric acid	100
Sodium carbonate	30
Sodium bicarbonate	25

[a]Maximum ratio tested.

Table V. Determination of MP in pharmaceutical vials by the proposed (AdS-SWV) method at UTGE; Application of standard addition technique.

Pharmaceutical Vials	% Found ± SD[a]	Standard addition technique	
		Added (nM)	% Recovery[a]
Meronem Labeled (1gm MP/vial) B.N.: KF458	99.64 ± 1.300	4.0	102.00
		5.0	100.27
		6.0	101.67
		7.0	101.40
		8.0	101.38
		Mean ± %RSD	101.344 ± 0.642

[a]Mean of three determinations.

Table VI. Greenness scoring of the proposed (AdS-SWV) method using UTGE.

Reagents	Penalty points
Phosphoric acid	$1 \times 2 \times 1 = 2$
Glacial acetic acid	$2 \times 2 \times 1 = 4$
Boric acid	$1 \times 2 \times 1 = 2$
Sodium hydroxide	2
	$\Sigma = 10$
Instruments	
	Penalty points
Voltammetric device	0
Occupational hazard	3
Waste	$3 + 3 = 6$
	$\Sigma = 19$

Total penalty points: 10 + 3 + 6 = 19.
Analytical Eco-Scale total score: 100 − 19 = 81 (Excellent Green).
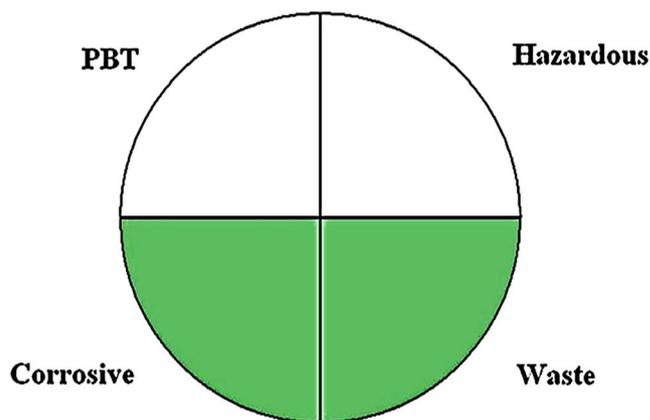
Figure 6. NEMI pictogram for the proposed (AdS-SWV) method.

recognized its importance. So, we performed a greenness evaluation of the proposed (AdS-SWV) method to measure the hazardous impact on the analyst and environment and to search for the weakest points to be improved to meet the green analytical chemistry requirements.

A comparative representation adopting two different metrics for assessment were performed namely; NEMI index labeling[28] and Analytical Eco-scale score[29,30] to visualize how different results can be obtained by implying these two metrics.

NEMI index labeling is a qualitative tool and represented by NEMI pictogram; divided into four quadrants. If the quadrant is filled in green, so it passes its requirement.

The first quadrant represents persistent, bioaccumulative and toxic chemicals (PBT); defined by the EPA's TRI list.[41] The second quadrant stands for hazardous reagents; defined by TRI[41] or RCRA's D, F, P or U hazardous waste list.[42] The third quadrant refers to corrosive effect to the environment during sample manipulation; defined by pH which is not less than 2 and not higher than 12. The last one stands for the

amount of waste generated during the whole analytical procedure are less than 50 g.

By deep search, it was found that phosphoric acid used in B-R buffer preparation is both PBT and hazardous reagent while, acetic acid is hazardous chemical only. Therefore, NEMI pictogram is represented as in Figure 6.

The second tool is the Analytical Eco-scale score assess method safety from other different aspects. It concerns with type and amount of the reagent that may cause environmental problems, occupational exposure of the analyst, the amount of energy consumed by the electrical devices and the way of analytical waste treatment. It is based on penalty points subtracted for the whole procedure from a base of 100. A penalty point is given for every hazard pictogram that corresponds to a chemical. The methods are ranked according to >75 represent excellent green analysis and <50 represent inadequate green analysis.

Here, we calculated the penalty points of our proposed method and found to be 19 as explained in details in (Table VI). Fortunately, the

Table VII. A comparative summary of various reported electrochemical methods and our developed (AdS-SWV) method at UTGE for MP quantification.

Used Electrode	Sensitivity	LOD	Application	References
Glassy Carbon Electrode	(1.0–40.0) µM	0.224 µM	Vials	17
Glassy Carbon Electrode	(1.0–40.0) µM	2.27 µM	Spiked human urine	18
	(1.0–40.0) µM	1.65 µM	Spiked human serum	
Disposable Graphite Pencil Electrode	(2.5–100.0) µM	1.23 µM	Vials	19
Polyaniline/Graphene modified Glassy Carbon Electrode	(0.25–35.0) µM	1.75 µM	MP metabolite determination in human urine and serum samples	20
UTGE	(500–15000.0) nM	160.0 nM	Vials	Our study

Eco-scale score is calculated to be 81 which represent an excellent analytical chemistry method from the green point of view.

Excellency of green analysis is obtained due to minimal number and amount of reagents used, small samples volume and waste generated if compared to other voltammetric methods utilizing chemically fabricated electrodes incorporating different nanostructured materials and aniline.[20]

Comparative summary between our proposed work and other published electrochemical methods.—The following comparison summarizes the main advantages and differences between our proposed electrochemical method utilizing the high performance UTGE and various published methods in literature, (Table VII).

The proposed (AdS-SWV) method for MP quantification at UTGE is obviously characterized by ultra-sensitivity at nano-levels, significant LOD and LOQ. It is also considered a stability indicating method with entire selectivity to different degradants and interferences.

Excellent green chemical analysis of our proposed method; accompanied with higher sensitivity is achieved if compared to other methods upon classical electrodes[17–19] and toxic polyaniline/graphene modified electrode[20] applications. Polyaniline is investigated as an environmentally toxic and teratogenic material. The previous limitations point out the privilege of our proposed methodology operating the high performance UTGE.

Conclusions

In the present work, UTGE was utilized in MP electro-oxidation behavior study. Sharp irreversible anodic response with adsorption controlled mass transport was detected. The results favor successful MP quantification via application of anodic adsorptive stripping square wave voltammetry. The proposed voltammetric method was highly rapid, ultra-high-sensitive at nano-level, accurate, precise and specific. It could be applied in drug substance and vials with no potential interference by presence of excipients or degadants. When we take into consideration, the greenness performance of the proposed method at sequential procedure implementation; the method was dedicated as an excellent green chemistry methodology to be safely applied without any sustainable hazard exposure to the analyst and environment.

ORCID

Rania M. Youssif ⓘ https://orcid.org/0000-0003-3785-8783
Nahla N. Salama ⓘ https://orcid.org/0000-0002-8783-3683

References

1. U. S. Pharmacopeia, General Notices and Requirements Applying to Standards, Tests, Assays, and Other Specifications of the United States Pharmacopeia USP 41, (2018).
2. L. L. Brunton, B. A. Chabner, and B. C. Knollmann, *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*, 12th Edition, Mc Grew Hill Companies, Inc., China, (2011).
3. R. Finkel, M. A. Clark, and L. X. Cubeddu, *Lippincott's Illustrated Reviews: Pharmacology*, Lippincott Williams & Wilkins, USA, (2009).
4. D. Singh and G. Maheshwari, *Med. Chem. Res.*, **22**, 5680 (2013).
5. L. Venkateswararao, S. Vardhan, and C. Rambabu, *Int. J. Pharm. Sci. Res.*, **4**(6), 100 (2013).
6. K. Babu and N. Kumari, *Int. J. Pharm. Sci. Res.*, **5**(5), 1963 (2014).
7. J. Cielecka-Piontek, M. Paczkowska, K. Lewandowska, B. Barszcz, and P. Garbacki Zalewski, *Chem. Cent. J.*, **7**, 98 (2013).
8. S. Shalvi, A. Nitasha, S. Jaskaran, and S. K. Shukla, *Res. J. Chem. Sci.*, **5**(7), 35 (2015).
9. N. Elragehy, N. Hassan, and M. Rezik, *Talanta*, **77**, 28 (2008).
10. V. Shah, S. Rathod, R. Parmar, and D. Shah, *Int. J. Inst. Pharm. and life sci.*, **2**, 503 (2012).
11. E. S. Elzanfaly, R. M. Youssif, N. N. Salama, A. S. Fayed, H. A. Hendawy, and M. Y. Salem, *Lumin.*, **32**(8), 1517 (2017).
12. A. Khataee, R. Lotfia, and A. Hasanzadeha, *Photochem. Photobiol. Sci.*, **16**, 170 (2017).
13. P. Zalewski, J. Cielecka-Piontek, and M. Paczkowska, *Acta Pol. Pharm. - Drug Res.*, **2**(71), 255 (2014).
14. P. Zalewski, A. Talaczyn´ska, P. Korban, P. Garbacki, M. Mizera, and J. Cielecka-Piontek, *Chromatographia*, **77**, 1483 (2014).
15. N. Deshmukh, R. Patel, and P. Das, *Pharm. Biol. Evaluations*, **3**(4), 431 (2014).
16. V. Negi, V. Chander, R. Singh, and K. Upadhaya, *Ind. J. chem. Technol.*, **24**, 441 (2017).
17. K. Jhankal, A. Sharma, S. Ramswaroop, and D. Sharma, *Int. J. Pharm. Sci. Res.*, **6**(7), 3014 (2015).
18. K. Jhankal and D. Sharma, *Chem. Sci. Trans.*, **5**(4), 1008 (2016).
19. H. A. Hendawy, R. M. Youssif, N. N. Salama, A. S. Fayed, and M. Y. Salem, *Electroanalysis*, **29**(12), 2708 (2017).
20. M. Sivaprasad, C. Swarupa, M. Dhananjayulu, M. Jayapal, and N. Sreedhar, *J. Anal. Bioanal. Tech.*, **5**(3), 192 (2014).
21. M. Sadikoglu, G. Saglikoglu, S. Yagmur, E. Orta, and S. Yilmaz, *Curr. Anal. Chem.*, **7**(2), 130 (2011).
22. S. Yilmaz, E. Baltaoglu, G. Saglikoglu, S. Yagmur, K. Polat, and M. Sadikoglu, *J. Serb. Chem. Soc.*, **78**(2), 295 (2013).
23. S. Yagmur, S. Yilmaz, G. Saclikoglu, B. Uslu, M. Sadikoglu, and S. Ozkan, *J. Serb. Chem. Soc.*, **76**(1), 53 (2014).
24. S. A. Özkan, B. Uslu, and H. Y. Aboul-Enein, *Crit. Rev. Anal. Chem.*, **33**, 155 (2003).
25. R. Kaldova and M. Kopanica, *Pure & Applied Chemistry*, **61**, 97 (1989).
26. V. K. Gupta, R. Jain, K. Radhapyari, N. Jadon, and S. Agarwal, *Anal. Biochem.*, **408**, 179 (2011).
27. S. Borman, *Anal. Chem.*, **54**, 698A (1982).
28. L. Keith, L. Gron, and J. Young, *Chem. Rev.*, **107**, 2695 (2007).
29. A. Gałuszka, P. Konieczka, Z. Migaszewski, and J. Namies´nik, *Trends Anal. Chem.*, **37**, 61 (2012).
30. M. Tobiszewski, *Anal. Methods*, **8**, 2993 (2016).
31. J. Heyrovský and P. Zuman, *Practical Polarography*, Academic Press, New York, **7**, 198 (1935).
32. A. J. MacLeod, *Applied Mathematics and Computation*, **57**(2–3), 305 (1993).
33. I. Streeter, G. Wildgoose, L. Shao, and R. Compton, *Sens Actuators B: Chem.*, **133**, 462 (2008).
34. D. Gosser, *Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms*, VCH: New York, (1994).
35. J. I. Gowda and S. T. Nandibewoor, *Asian J. Pharma. Sci.*, **9**(1), 42 (2014).
36. E. Laviron, *J. Electroanal. Chem. Interfacial Electrochem.*, **101**, 19 (1979).
37. E. Laviron, L. Roullier, and C. Degran, *J. Electroanal. And Chem. Interfacial Electrochem.*, **112**, 11 (1980).
38. J. C. Abbar and S. T. Nandibewoor, *Colloids and Surfaces B: Biointerfaces*, **106**, 158 (2013).
39. J. N. Miller and J. C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, 6th ed., Pearson Education Limited, Harlow, England, (2010).
40. *Guidance for industry; Q2(R1) of analytical procedure: methodology, International Conference on Harmonization (ICH)*, http://www.fda.gov/eder/guidance/1320fnl 2005.
41. Toxic Release Inventory (TRI) at (http://www.epa.gov/tri/chemical/).
42. Code of Federal Regulations, Title 40, Part 261 at (http://ecfr.gpoaccess.gov).