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Electrochemical preparation of sodium dodecylsulfate doped over-oxidized polypyrrole/multi-walled carbon nanotube composite on glassy carbon electrode and its application on sensitive and selective determination of anticancer drug: Pemetrexed

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ABSTRACT

Electrochemical oxidation of pemetrexed (PMX) was studied on bare, carboxylic acid functionalized multi-walled carbon nanotubes and over-oxidized polypyrrole modified (oo-PPy/MWCNTs-COOH/GCE) glassy carbon electrodes by cyclic and adsorptive stripping differential pulse voltammetric techniques. The oo-PPy/MWCNTs-COOH/GCE is very sensitive to the oxidation of PMX. The results proved that the over-oxidation of the PPy film gave a negative charge density on porous layer that improved the adsorption for PMX. The effects of pH, concentrations of MWCNTs and monomer, the number of cycles for the electropolymerization and the scan rate for sensor preparation were optimized. The MWCNTs-COOH and oo-PPy based sensor showed an excellent recognition capacity toward PMX. The linear responses have been obtained in the range from 8.00×10^{-7} M to 1.00×10^{-4} M with 2.04×10^{-7} M detection limit for the bare GCE and from 1.00×10^{-8} M to 1.00×10^{-7} M with 3.28×10^{-9} M detection limit for the modified GCE. The oxidation of PMX was controlled by the adsorption process on both types of electrode surfaces. The proposed methods were compared with the literature on UV spectrophotometric assay, which was carried out at an absorption maximum of 225 nm. The proposed method and the designed sensors have been successfully applied for the determination of PMX in pharmaceuticals.

1. Introduction

Pemetrexed (PMX), *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo [2,3-*d*] pyrimidin-5-yl) ethyl] benzoyl]-L-glutamic acid disodium salt is a novel multi-targeted anti-cancer antifolate agent that exerts its action by disrupting crucial folate-dependent metabolic processes essential for cell replication [1–3]. Its primary target is thymidylate synthase but it also inhibits folate-dependent enzymes involved in purine synthesis. PMX has shown good activity in preclinical models with human tumor cells and xenografts. In the majority of clinical trials of PMX, its dose-limiting toxic effects can be reduced by dietory folate, resulting in an improved theropeutic index. Low folate status is also associated with higher levels of toxicity in patients. As a single agent, PMX has shown good activity against non-small-cell lung cancer, squamous-cell carcinoma of head and neck, colon cancer and breast cancer, and it particularly appears to be active in combination with cisplatin against non-small-cell lung cancer and mesothelioma [4–7] Scheme 1.

Chemical sensors designed by conductive polymers are commonly used for analytical purposes [8]. Polypyrrole (PPy) is one of the most extensively used conductive polymers for developing modified electrodes [9]. PPy has many properties such as possibility of growing in aqueous media, capacity to form adhesive coatings, high electronic conductivity, high chemical stability, ease of electrochemical polymerization, high porosity that enables fast kinetics ion exchange with the surrounding medium, controllability of thickness and good reversibility between its conductive and insulative forms. These features make the PPy highly suitable for modification of electrodes. PPy undergoes overoxidation at positive potentials or at more basic media [10]. Destructive overoxidation process of PPy occurs due to the nucleophilic attacks [11] and results show that the carbonyl functionality is added to the pyrrolic rings [12]. Small doping anions can be ejected from oo-PPy and forms a porous structure [13].

MWCNTs are attractive modifiers in the design of electrochemical sensors for their high conductivity, unique structure, high surface-to-volume ratio, high stability, low resistance, and ability

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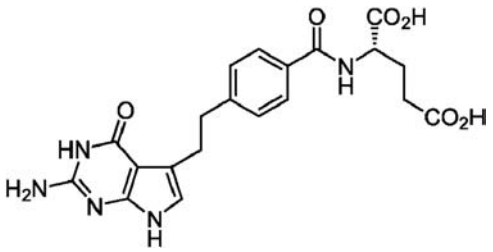

Scheme 1. The structure of PMX disodium.

to facilitate electron transfers [14–17]. Recently, conductive polymer/CNTs composites [18–20] have been intensively studied to improve the conductivity, electronic transport and electromagnetic properties for applications of nanoelectronic elements and electro-optical devices [21–25].

In the literature, there are several analytical studies on the determination of PMX in pharmaceuticals or biological fluids using UV [26], LC with UV [27–31], MS [32] and MS/MS [33] detections. The limit of detection and the limit of quantification have been obtained as 3.28×10^{-9} M and 9.94×10^{-9} M, respectively, with the proposed voltammetric method. Our method is more sensitive than previous published methods according to the obtained LOQ values. The already published methods were influenced by interference of endogenous substances and potential loss of drugs in the re-extraction procedure and involved lengthy, tedious and time-consuming sample preparation and extraction processes and required a sophisticated and expensive instrumentation.

PMX is an electroactive molecule, but nothing seems to have been published concerning neither its electrochemical behavior nor the electroanalytical assay in its dosage form. In this work, a glassy carbon electrode coated with oo-PPy/MWCNTs-COOH film for the determination of PMX has been developed. The aim of the present study is to investigate detailed voltammetric behavior and sensitive, rapid, simple and new assay of PMX at oo-PPy/MWCNTs-COOH film modified glassy carbon electrode (GCE) using cyclic voltammetry (CV) and adsorptive stripping differential pulse voltammetry (AdSDPV). The developed nanosensor exhibits high sensitivity, rapid response and good reproducibility. This nanosensor is successfully used in the sensitive and selective analysis of PMX.

2. Experimental

2.1. Reagents

The injectable dosage forms of PMX and its pharmaceutical form Pemtrex® were kindly supplied from Kocak (Istanbul, Turkey). Pemtrex® contains pemetrexed disodium equivalent to 500 mg pemetrexed and 500 mg mannitol. Pyrrole was obtained from Aldrich and purified twice by distillation under the protection of high-purity nitrogen and then kept in a refrigerator before use. Dodecylsulfate sodium (SDS) was obtained from Sigma-Aldrich. All other chemicals were of at least analytical reagent grade, double distilled deionized water was used for all solutions. The standard solution of 1×10^{-3} M PMX was prepared by dissolving PMX in distilled water and then stored in the refrigerator. –COOH grup functionalized and non-functionalized MWCNTs were purchased from DropSens. H_2SO_4 solution (0.1 and 0.5 M), Britton-Robinson buffer (0.04 M, H_3BO_3; H_3PO_4 and CH_3COOH; pH∼2.0–12.0), Phosphate-buffered saline (PBS; 0.1 M) solutions were used for the preparation of buffer solutions with pH: 2.0, 3.0, 6.0, 7.0, 8.0. Glacial acetic acid was used for pH 3.7, 4.7 and 5.7 acetate buffer solutions. The pH values were adjusted to the related pH by sodium hydroxide (Fluka) under the pH-meter. Distilled water was used in accordance with the literature on the UV spectrophotometric method development and validation [26].

The ruggedness and precision were checked at different days. The results were given as repeatability (within day) and reproducibility (between days). Relative standard deviations (RSD) were calculated to check the ruggedness and precision of the method [34–37]

2.2. Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and adsorptive stripping differential pulse voltammetry (AdSDPV) were performed using AUTOLAB-PGSTAT302 (Eco Chemie, Utrecht, The Netherlands) electrochemical and electroanalytical instrument having General Purpose Electrochemical Software (GPES) 4.9, with a conventional three-electrode system, a glassy carbon (ϕ=3.0 mm) working electrode, platinum wire counter electrode and Ag/AgCl reference electrode. All pH measurements were carried out using a pH meter Model 538 (WTW, Austria) with a combined electrode with an accuracy of pH ± 0.05.

DP voltammetric experiments were conducted under the instrumental conditions of 0.00795 V step potential, 50 mV modulation amplitude, 50 ms modulation time and 500 ms time interval. Average baseline correction was defined using a 'peak width' of 10 mV. Optimum AdSDPV conditions for bare glassy carbon electrode were; accumulation potential (E_{acc}): 600 mV and accumulation time (t_{acc}):150 s. For oo-PPy/MWCNTs-COOH/GCE E_{acc} and t_{acc} were 100 mV and 180 s, respectively.

For checking the accuracy of our developed voltammetric assay, UV spectrophotometric method was used on double beam UV–visible spectrophotometer (Shimadzu, model 1601) having two matched quartz cells with 1 cm light path according to literature [26].

2.3. Fabrication of the oo-PPy/MWCNTs-COOH/GCE

The glassy carbon electrode was polished with 0.05 μm alumina in water slurry using polish pad. The polished GCE was rinsed with distilled water, and ultrasonicated in ethanol and doubly distilled water for 5 min, successively, in order to remove unpurities or any adsorbed substances on the electrode surface. Prior to modification, it was dried under nitrogen flow.

A 0.5 g MWCNTs-COOH was dispersed in 0.5 mL dimethylformamide solution and sonicated for 2 h. Different dropping volumes of MWCNTs-COOH solutions were dropped on GCE surface. MWCNTs-COOH/GCE was compared with non functionalized MWCNTs/GCE and SWCNTs-COOH/GCE.

Pyrrole monomer was dissolved in 0.001 M SDS solution. The optimization of pyrrole concentration, overoxidation potential and thickness were made in 0.1 M PBS (pH: 2.0). 0.02 M pyrrole was polymerized in 0.1 M PBS solution with 0.001 M SDS by CV from −0.2 to 1.0 V at 20 mV/s. Polymerization cycle number of pyrrole was optimized as 1 with 20 mV/s scan rate. Then, the negatively charged SDS doped oo-PPy film was formed on MWCNTs-COOH/GCE by CV from −0.2 V to 1.2 V at 0.1 V/s scan rate for 20 cycles. The obtained electrode, denoted as oo-PPy/MWCNTs-COOH/GCE was gently washed with distilled water to remove non-adsorbed species.

2.4. Injectable dosage form and recovery assay procedure

For voltammetric analysis, adequate volume of Pemtrex® (containing 500 mg/20 mL PMX in 0.9% NaCl solution) was transferred to a 50 mL of calibrated flask, and completed to the volume with bi-distilled water. The concentration of the prepared solution is equivalent to 1.0×10^{-3} M. Analyzed solutions were prepared by taking aliquots of the clear supernatant and diluting with the selected supporting electrolytes. In order to investigate the effects

of interferences by the excipients, known amounts of the pure drug was added into the pre-analyzed dosage formulation. The recovery results were determined based on five parallel analyses. The nominal content of amounts was calculated from the corresponding regression equations of previously plotted calibration plots.

3. Result and discussion

3.1. Electrochemical behavior of PMX

Voltammetric responses of PMX solutions have been investigated in detail on the surface of modified electrodes. Fig. 1 shows DPV results for the electrochemical oxidation of 5×10^{-5} M PMX in a 0.1 M PBS with pH 2.0. The anodic response of PMX is not high for the sensitive assay on the surface of bare GCE and presents an increase on the surface of MWCNTs-COOH/GCE. Optimum volume was obtained as 5 μL for MWCNTs solution. Carboxylic acid functional group affected the answer of PMX comparing to non-functional MWCNTs. Also, the response of PMX decreased on the SWCNTs-COOH/GCE. However, a substantial increment is observed for the oxidation peak current on oo-PPy/MWCNTs-COOH/GCE surface (Fig. 1). After the over-oxidation process, a remarkable enhancement in the oxidation current of PMX can be seen, due to the increase in the film porosity and creation of more active surface area.

3.2. Effect of concentration, cycle and over-oxidation potential of pyrrole monomer

The optimization conditions for the polymerization of pyrrole monomer on MWCNTs-COOH/GCE were investigated. The oo-PPy/MWCNTs-COOH/GCE was chosen as the modified electrode in voltammetric measurements. The obtained data showed that the response of the modified electrode can be affected by the amount of pyrrole concentration. The DP voltammograms of 5×10^{-5} M PMX in 0.1 M PBS (pH 2.0) were measured at various concentrations (0.01–0.08 M) of pyrrole modified MWCNTs-COOH/GCE. According to the obtained results, 0.02 M pyrrole was chosen as best concentration of monomer to form well and sensitive modified electrode.

The amount and thickness of the PPy were controlled through changing the cycle number (1–5) in electropolymerization process. The optimum current was obtained when the cycle number was 1. When cycle number was above 1, the currents decreased gradually with further increasing cyclenumber. So, one cycle was chosen as the optimum cycle number for the electropolymerization of pyrrole on the MWCNTs-COOH/GCE surface.

PPy films over-oxidized at different potentials (between 1.1 V and 1.3 V) to increase the sensitivity of the modified electrode. 1.2 V was chosen as the optimum potential to over-oxidize the PPy. During the over-oxidation process, higher density carbonyl groups such as C=O and COO– can be generated at the backbone of oo-PPy film. This is favorable for PMX in the buffer solution to be accumulated onto the film through ion-exchange process. To achieve sensitive modified electrode, 0.02 M pyrrole was polymerized (one cycle) in 0.1 M PBS solution with 0.001 M SDS at 20 mV/s scan rate (Fig. 2).

3.3. Influence of pH solution and scan rate on bare GCE and oo-PPy/MWCNTs-COOH/GCE

The effect of pH on the peak current and potential was investigated between pH 0.3 and 12.0 for bare GCE and at pH 2.0 and 8.0 for oo-PPy/MWCNTs-COOH/GCE using CV and DPV techniques.

The peak potentials of the responses were shifted to less positive potentials by increased pH and a good linear relationship was observed between the E_p and pH values for both electrodes with the following equations;

$$E_p(\mathrm{mV}) = 720.4 - 46.9\ \mathrm{pH};\ r = 0.994 \text{ for bare GCE (between pH 0.3 and 10.0)}$$

and

$$E_p(\mathrm{mV}) = 710.9 - 46.4\ \mathrm{pH};\ r = 0.997 \text{ for oo-PPy/MWCNTs-COOH/GCE (between pH 2.0 and 8.0)}$$

The obtained slope values – 46.9 mV and – 46.4 mV per pH unit in the redox behavior of PMX on the bare GCE and oo-PPy/MWCNTs-COOH/GCE. The effect of pH on the anodic peak current of PMX shows that the maximum response value and well peak shape was obtained at pH 0.3 with bare GCE and at pH 7.0 for modified GCE. Therefore, all voltammetric measurements and further studies were realized in the 0.5 M H_2SO_4 solution for bare GCE and at pH 7.0 PBS for oo-PPy/MWCNTs-COOH/GCE.

Electrochemical responses from the redox properties of drugs and biomolecules might have profound effects on the understanding of the redox mechanism related to the activity. The PMX molecule is extensively metabolized in vivo [1–7]. In order to identify the possible functional group responsible from the electro oxidation of PMX, the CV and DPV results of PMX were compared to those of some selected model compounds. Although the exact oxidation mechanism was not determined, some conclusions about the potential electroactive centers under working conditions could be reached. As model compounds, indole,

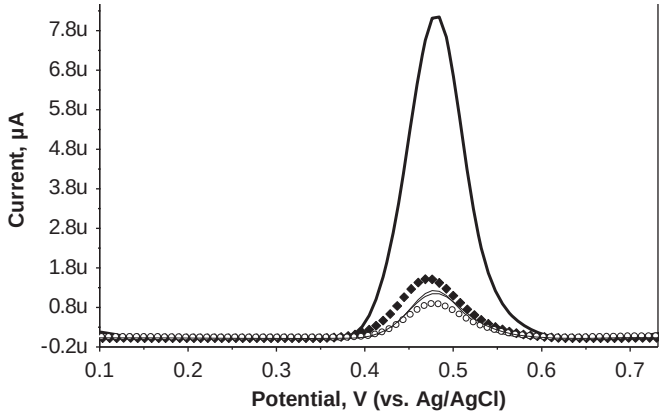

Fig. 1. DP voltammograms of the bare GCE (—), MWCNTs/GCE (○), MWCNTs-COOH/GCE (♦) SWCNTs-COOH/GCE (–), oo-PPy/MWCNTs-COOH/GCE (▪) in 0.1 M PBS (pH 2.0) with 5×10^{-5} M PMX.

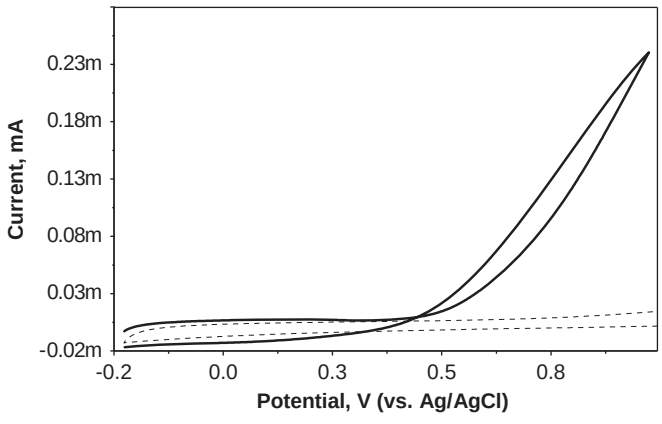

Fig. 2. Cyclic voltammograms of bare GCE in 0.001 M SDS with (▪) 0.02 M pyrrole monomer and (——) without monomer in (pH 7.0) 0.1 M PBS at scan rate 0.100 V/s.

guanine and folic acid were used for understanding the electro-oxidation mechanism of PMX. The results showed that the similar anodic behavior and oxidation peak potential was obtained with indole compound. It is assumed that the oxidation occurred on the nitrogen atom of indole ring of the molecule. The anodic oxidative behavior of PMX is also comparable to indole oxidations that were reported previously [37–42]. Considering the above comparison and the position of the break E_p versus pH plot of PMX, which is obtained about pH 5.5 and 7.50 bearing in mind the oxidative process of the nitrogen atom in the indole ring, it was assumed that the oxidation step is located on the indole ring, similarly to model compounds main peak, and attributed to the oxidation of the nitrogen atom. Our results on model compounds confirm that the electroactive center corresponding to the anodic peak was the nitrogen atom on the indole ring [37–42]. According to the literature [43], it is expected that the acidic group of PMX will be permanently charged at the pH of 7.4 and 5.5. However, at the acidic pH of 5.5, the basic group of PMX was expected to undergo protonation of its pteridine ring. In our pH scanning results, the break was obtained more clearly at pH 7.4.

The influence of scan rate on the oxidation peak potential and current of PMX at the bare GCE in 0.5M H_2SO_4 solution and at the oo-PPy/MWCNTs-COOH/GCE in pH 7.0 PBS (0.1 M) were studied by cyclic voltammetry. The scan rate studies were carried out to understand whether the process was diffusion or adsorption controlled. The peak potential of 5×10^{-5} M PMX solution is shifted to the anodic direction when scan rate increased. I_p showed a linear relationship with the scan rate. The variation of the logarithm of the peak current as a function of the logarithm of the scan rate in the range between 5 mV/s and 500 mV/s showed a linear dependence on slopes, which were found to be very close to the theoretical value of 1.0. Hence, it can be stated that the process is an adsorption controlled electrode process for both electrodes; that is the electrochemical process is controlled by the adsorption of the PMX to the electrode surfaces. [44]

I_p (μA)=0.021υ+0.531 (r=0.998) for bare GCE in 0.5 M H_2SO_4 solution;
I_p (μA)=0.040 υ−0.184 (r=0.999) for modified GCE in pH 7.0 PBS (0.1 M);
log (I_p)=0.714 log υ−0.899 (r=0.999) for bare GCE in 0.5 M H_2SO_4 solution;
log (I_p)=0.910 log υ−1.141(r=0.994) for modified GCE in pH 7.0 PBS (0.1 M).

The electron transfer coefficient and electron number 'αn' is calculated from the difference between the peak potential (E_p) and half wave potential ($E_{p/2}$) according to equation given below [45]:

$$\Delta E_p = E_p - E_{p/2} = (47.7/\alpha n)\ \text{mV (for irreversible process, at25 } ^\circ C)$$

The transfer coefficient (α) and the number of electrons (n) involved in the rate determining step were calculated as 0.88 for bare GCE and 1.08 for modified electrode. If α was assumed equal to 0.50, n is equaled 1.78 and 2.17 for bare and modified GCEs that are close to 2 in the working media.

3.4. Effect of accumulation potential and time

The effects of E_{acc} and t_{acc} on the anodic response were studied by AdSDPV on bare GCE and oo-PPy/MWCNTs-COOH/GCE in 1×10^{-6} M PMX solution. It is important to fix the E_{acc} and t_{acc} during the adsorption of PMX at the electrode surfaces. As can be seen in Fig. 3, for the bare and modified GC electrodes, the maximum anodic peak current was obtained at 600 mV using 150 s accumulation time and 100 mV using 180 s accumulation time by AdSDPV method, respectively, therefore further studies were carried out to obtain these E_{acc} and t_{acc} values. With optimized AdSDPV, sensitivity was increased about 30.47 times with the modified electrode when compared to the response of DPV method in pH 7.0 PBS (Fig. 4).

3.5. Analytical characterizations

Under optimum conditions, anodic peak currents of AdSDPV linearly depended on PMX concentrations (Fig. 5). The limit of detection (LOD) and limit of quantification (LOQ) were obtained according to the 3 s/m and 10 s/m criteria, respectively, where m is the slope of related calibration graph and s is the standard deviation of the anodic peak currents (five measurements) [46,47].

The bare GCE showed a linear response in the range from 8.00×10^{-7} M to 1.00×10^{-4} M (r=0.9950) with 2.04×10^{-7} M LOD and 6.19×10^{-7} M LOQ with AdSDPV method in 0.5 M H_2SO_4 solution. The oo-PPy/MWCNTs-COOH/GCE showed a linear dynamic range of 1.00×10^{-8} M to 1.00×10^{-7} M (r=0.9995) with 3.28×10^{-9} M LOD and 9.94×10^{-9} M LOQ values by AdSDPV in pH 7.0 PBS.

The characteristics of the calibration curves and its related validation parameters are presented in Table 1.

The relative standard deviation (RSD) values were calculated to investigate repeatability and reproducibility of the bare GCE and modified GCE. The RSD values for five repetitive measurements during 1 day were 0.51% and 0.92% for peak potentials and 0.88% and 1.66% for peak currents for the bare GCE and modified GCE, respectively. The evalution of the reproducibility of the electrodes which are obtained in different days, based on five measurements, the RSD values of bare GCE and modified GCE were obtained as 0.51% and 1.11% for peak potentials and 1.43% and 1.75% for peak currents, respectively.

3.6. Analytical applications of developed method

Determination of PMX from injectable dosage forms was studied in detail. For checking the accuracy, selectivity and precision of the proposed electroanalytical method and in order to know whether the excipient dosage form shows any interference with the analysis, the recovery studies were evaluated after adding known amounts of PMX. Recovery studies showed that the absence of interference from commonly used pharmaceutical dosage form presented its excipients and proved that the proposed methods had adequate precision and accuracy.

Table 2 shows that the AdSDPV method could be applied successfully for the assay in injectable dosage form without any interference. Standard addition method was applied for recovery study.

The proposed AdSDPV results, which were obtained from tablet assay, were statistically compared with the results determined in the previously published literature [26]. The linearity range and other validation parameters were reported in the related tables. Statistical comparisons were performed on data from both AdSDPV and UV-spectrophotometric assays. According to the Student's t- and variance ratio F-test, the calculated t- and F-values were less than the theoretical values in either test at the 95% confidence level. This indicates that there is no significant statistical difference between the performance of the proposed AdSDPV and UV-spectrophotometric method with regard to mean values and standard deviations (Table 2). Student's t- and F-tests revealed that there was no significant statistical difference between AdSDPV and UV [26] methods with regard to accuracy and precision. However, the proposed AdSDPV method is more accurate, precise, sensitive and selective than the previously published UV-spectrophotometric method [26].

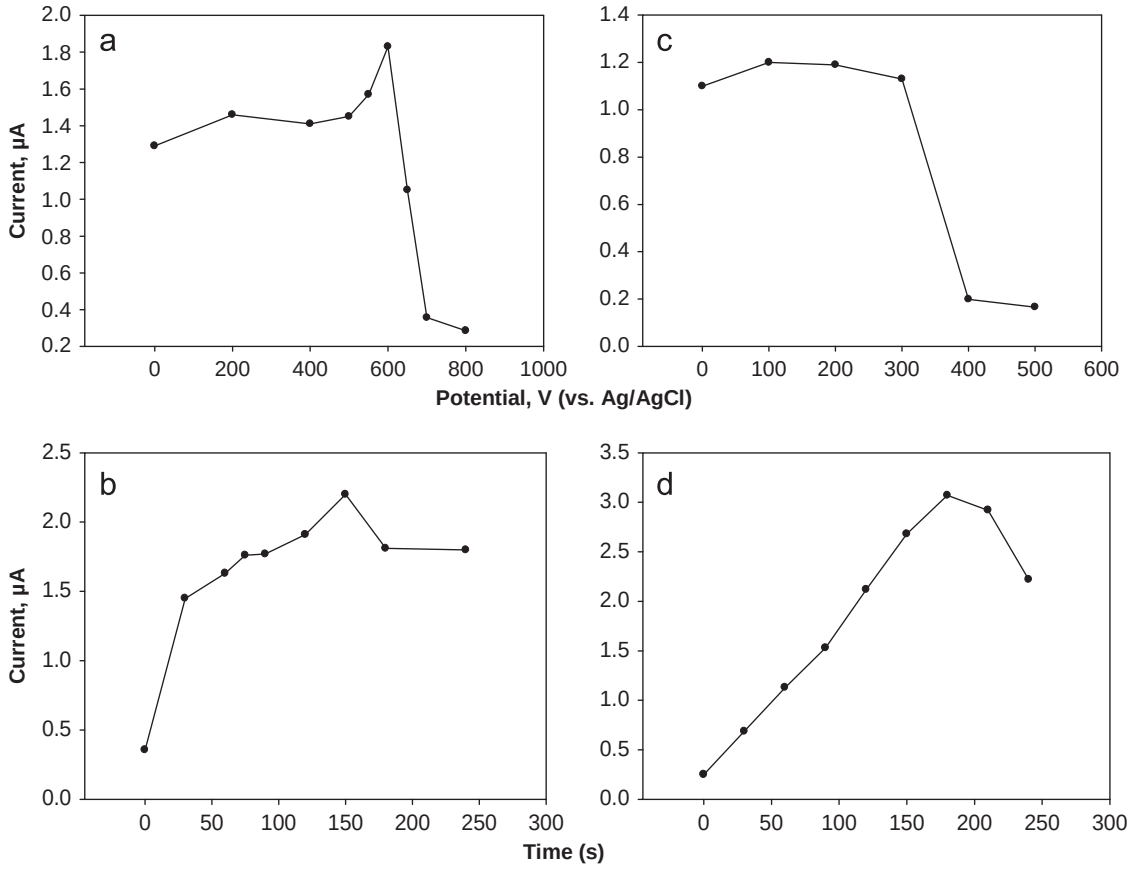

Fig. 3. Influence of E_{acc} (t_{acc}: 60 s) (a) and t_{acc} (E_{acc}: 600 mV) (b) at bare GCE, E_{acc} (t_{acc}: 60 s) (c) and t_{acc} (E_{acc}: 100 mV) (d) on the 1.00×10^{-6} M PMX peak current in pH 7.0 0.1 M PBS at oo-PPy/MWCNTs-COOH/GCE.

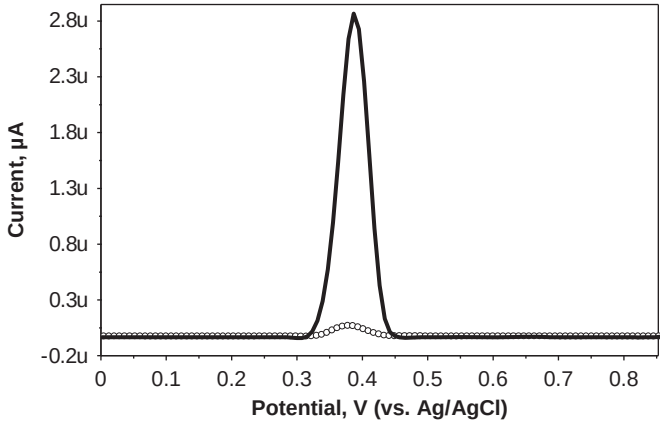

Fig. 4. DP (○) and AdSDP(▪) voltammograms of oo-PPy/MWCNTs-COOH/GCE in 0.1 M PBS (PH 7.0) containing 1×10^{-6} M PMX.

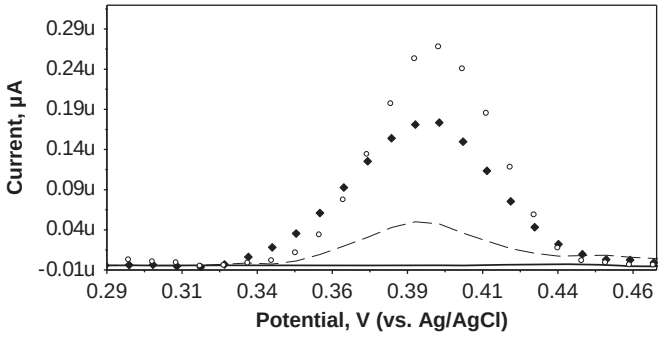

Fig. 5. AdSDP voltammograms of different PMX concentrations at oo-PPy/MWCNTs-COOH/ GCE in pH 7.0 PBS: blank (–), 2.00×10^{-8} M (—), 8.00×10^{-8} M (♦) 1.00×10^{-7} M (○).

3.7. *Interference study and stability of modified electrode*

The research of interference study aims to investigate the effects of possible co-exiting species on the determination of PMX. Therefore, some metal ions and possible biological molecules were chosen such as K^+, Ca^{2+}, Na^+, SO_4^{2-}, Cl^-, NO_3^-, dopamine, ascorbic acid, uric acid. Meanwhile, 1×10^{-5} M PMX was used as sample. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ± 10% relative error in the determination [48]. The results showed that the concentrations of K^+, Ca^{2+}, Na^+, SO_4^{2-}, Cl^-, NO_3^- have not significantly influenced the height of the peak currents. The tolerated concentration of foreign substances was shown in Table 3.

The stability of the oo-PPy/MWCNTs-COOH/GCE was examined by measuring the current response of 4×10^{-8} M PMX during a week period. The modified electrode was stored at 4 °C. The RSD 2.1% was obtained for seven independent measurements in PMX solution during a week. For the modification of GCEs with oo-PPy/MWCNTs-COOH, different GCEs were used and the reproducibility was calculated using the results, which were obtained from different electrode modifications. With five different GCEs, the measurements were taken in 4×10^{-8} M PMX solution and RSD for peak currents and peak potentials were obtained as 1.93% and

Table 1
Regression data of the calibration lines of PMX on bare GCE and modified GCE by AdSDPV and UV–visible method in standard solutions.

	GCE	oo-PPy/MWCNTs-COOH/GCE	UV method
Measured potential (V)/λ_{max}	0.704	0.390	225 nm
Linearity range (M)	8.00×10^{-7}–1.00×10^{-4}	1.00×10^{-8}–1.00×10^{-7}	4.68×10^{-6}–3.74×10^{-5}
Slope	3.154×10^{4}	2.789×10^{6}	0.0545
Intercept (μA)	2.010	-8.310×10^{-4}	−0.0119
Correlation coefficient	0.999	0.999	0.999
SE of slope	5.845×10^{2}	3.753×10^{4}	3.17×10^{-4}
SE of intercept	3.047×10^{-2}	2.278×10^{-3}	3.20×10^{-3}
LOD (M)	2.04×10^{-7}	3.28×10^{-9}	8.17×10^{-8}
LOQ (M)	6.19×10^{-7}	9.94×10^{-9}	2.48×10^{-7}
Repeatability of peak current/absorbance (RSD%)[a]	0.885	1.66	0.084
Repeatability of peak potential/wavelength (RSD%)[a]	0.508	0.916	0.040
Reproducibility of peak current/absorbance (RSD%)[a]	1.43	1.75	0.132
Reproducibility of peak potential/wavelength (RSD%)[a]	0.508	1.11	0.047

[a] Obtained from five experiments.

Table 2
Results obtained for the UV–visible and AdSDPV determination and recovery experiments in PEMTREX® injectable dosage form on bare and modified electrodes.

	GCE	oo-PPy/MWCNTs-COOH/GCE	UV method
Labeled claim (mg)	500.00	500.00	500.00
Amount found [a] (mg)	499.57	502.55	501.20
RSD%	0.256	0.367	0.129
Bias%	0.087	−0.505	−0.240
t_{value}	0.034	0.160	$t_{theoretical}$: 2.31
F_{value}	0.216	0.067	$F_{theoretical}$: 6.39
Added (mg)	500.00	500.00	250.00
Found [a] (mg)	507.56	505.61	240.18
Average recovered%	101.51	101.13	96.08
RSD% of recovery	0.996	0.753	0.114
Bias%	−1.51	−0.50	3.93

[a] Obtained from five experiments.

Table 3
Tolerance of interferences on the determination of 1×10^{-5} M PMX using modified GCE.

	Tolerance level ratio of (Foreign substance:PMX)	± % Error
K^+	1000:1	2.54
Na^+	100:1	5.08
Ca^{2+}	1000:1	5.93
Cl^-	1000:1	4.24
SO_4^{2-}	100:1	10.01
NO_3^-	1000:1	2.54
Dopamine	1:1	10.0
Ascorbic acid	10:1	6.78
Uric acid	10:1	5.08

1.12%, respectively. These results indicate that oo-PPy/MWCNTs-COOH/GCE has good stability and could be used for PMX analyses.

4. Conclusion

In the present study, a bare glassy carbon electrode compared with a carboxylic acid functionalized multi-walled carbon nanotubes and sodium dodecyl sulfate doped over oxidized polypyrrole modified glassy carbon electrode. The negatively charged modified electrode showed good properties. The electrochemical behavior of PMX was examined for the first time with this study. The voltammetric oxidation step of PMX in different buffer solutions of pH 0.3–10.0 and pH 2.0–8.0 have been elucidated with bare and modified GCE, respectively. The LOD value was obtained as 3.28×10^{-9} M with the developed modified electrode. The electrooxidation of PMX at both electrodes was investigated in detail. The behavior of PMX at carbon based electrodes indicates that these electrodes might be used for analytical purposes, particularly as a sensor. The obtained results may possibly clarify the oxidation pathways of PMX. A linear relationship between the PMX concentration and the current response was obtained with excellent features, like low detection limit, high reproducibility and repeatability. Fully validated, highly selective and sensitive, simple and precise voltammetric procedures were described for determination of PMX in bulk form and pharmaceutical dosage form without the necessity of sample pre-treatment or any time-consuming extraction and evaporation steps prior to the analysis. Once the instrument is set, just by changing the analyte, within about 3 min, the amount of PMX can be determined, indicating its potential in high throughput analysis of large number of samples. The modified electrode results showed higher selectivity and sensitivity when compared to the bare GCE. This method provides a new way to construct a modified electrode for fast, simple and low cost analysis of anticancer drug pemetrexed from its dosage forms so that the proposed methods can be easily and directly applied to the analysis of pharmaceutical dosage forms without the necessity of any separation or complex sample preparation, since there was no interference from the excipients.

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References

[1] I.D. Goldman, R. Zhao, Semin. Oncol. 29 (2002) 3–17.
[2] A. Lansiaux, F. Lokiec, Bull. Cancer 94 (2007) 134–138.
[3] A. Gangjee, H.D. Jain, J. Phan, X. Guo, S.F. Queener, R.L. Kisliuk, Bioorg. Med. Chem. 18 (2010) 953–961.
[4] D.W. Miles, I.E. Smith, R.E. Coleman, A.H. Calvert, M. Lind, Eur. J. Cancer 37 (2001) 1366–1371.
[5] N.J. Curtin, A.N. Hughes, Lancet Oncol. 2 (2001) 298–306.
[6] G. Scagliotti, D. Shin, H. Kindler, D. Johson, U. Keppler, Eur. J. Cancer 37 (2001) 20–21.
[7] A.R. Hanauske, V. Chen, P. Paoletti, C. Niyikiza, Oncologist 6 (2001) 363–373.
[8] U. Lange, N.V. Roznyatovskaya, V.M. Mirsky, Anal. Chim. Acta 614 (2008) 1–26.
[9] A. Ramanavicius, A. Ramanaviciene, A. Malinauskos, Electrochim. Acta 51 (2006) 6025–6037.
[10] S. Asavapiriyanont, G.K. Chandler, G.A. Guanawardena, D. Pletcher, J. Electroanal. Chem. 177 (1984) 229–244.

[11] F. Beck, P. Braun, M. Oberst, Chem. Phys. 91 (1987) 967–974.
[12] P.A. Christensen, A. Hamnett, Electrochim. Acta 36 (1991) 1263–1286.
[13] F. Beck, P. Braun, F. Schloten, J. Electroanal. Chem. 267 (1989) 141–148.
[14] M. Pumera, Chem. Eur. J. 15 (2009) 4970–4978.
[15] H. Beitollahi, H. Karimi-Maleh, H. Khabazzadeh, Anal. Chem. 80 (2008) 9848–9851.
[16] A. Benvidi, P. Kakoolaki, H.R Zare, R. Vafazadeh, Electrochim. Acta. 56 (2011) 2045–2050.
[17] R.T. Kachoosangi, M.M. Musameh, I. Abu-Yousef, J.M.Y.S.M. Kanan, L. Xiao, S. G. Davies, A. Russel, R.G. Compton, Anal. Chem. 81 (2009) 435–442.
[18] Y. Li, P. Wang, L. Wang, X. Lin, Biosens. Bioelect. 22 (2007) 3120–3125.
[19] S. Shahrokhian, E. Asadian, J. Electroanal. Chem. 636 (2009) 40–46.
[20] X. Lu, Y. Li, J. Du, X. Zhou, Z. Xue, X. Liu, Z. Wang, Electrochim. Acta. 56 (2011) 7261–7266.
[21] K.H. An, K.K. Jeon, J.K. Heo, S.C. Lim, D.J. Bae, Y.H. Lee, J. Electrochem. Soc. 149 (2002) 1058–1062.
[22] G.Z. Chen, M.S.P. Shaffer, D. Coleby, G. Dixon, W. Zhou, D.J. Fray, A.H Windle, Adv. Mater. 12 (2000) 522–526.
[23] M. Hughes, G.Z. Chen, M.S.P. Shaffer, D.J. Fray, A.H. Windle, Chem. Mater. 14 (2002) 1610–1613.
[24] K. Ramanathan, M.A. Bangar, M. Yun, W. Chen, N.V. Myung, A. Mulchandani, J. Am. Chem. Soc. 127 (2005) 496–497.
[25] X. Zhang, J. Zhang, R. Wang, Z. Liu, Carbon 42 (2004) 1455–1461.
[26] A.D. Patel, S.K. Parikh, Dr.D.J. Sen, Dr.C.N. Patel, Int. J. Drug Dev. Res. 3 (2011) 301–307.
[27] D. Ouellet, A.P. Periclou, R.D. Johnson, J.R. Woodworth, R.L. Lalonde, Cancer Chemother. Pharmacol. 46 (2000) 227–234.
[28] J.M. Woodland, C.J. Barnett, D.E. Dorman, J.M. Gruber, C. Shih, L.A. Spangle, T. M. Wilson, W.J. Ehlhardt, Drug Metab. Dispos. 25 (1997) 693–700.
[29] C.L. Hamilton, J.A. Kirkwood, J. Chromatogr. B Biomed. Appl. 654 (1994) 297–303.
[30] D.A. Rinaldi, H.A. Burris, F.A. Dorr, J.R. Woodworth, J.G. Kuhn, J.R. Eckardt, G. Rodriguez, S.W. Corso, S.M. Fields, C. Langley, J. Clin. Oncol. 13 (1995) 2842–2850.
[31] L.P. Rivory, S.J. Clarke, M. Boyer, J.F. Bishop, J. Chromatogr. B. 765 (2001) 135–140.
[32] C. Bobin-Dubigeon, M.B. Amiand, C. Herrenknecht, J.M. Bard, J. Chromatogr. B. 877 (2009) 2451–2456.
[33] J. Zhou, S. Gao, F. Zhang, B. Jiang, Q. Zhan, F. Cai, J. Li, W. Chen, J. Chromatogr. B. 906 (2012) 1–8.
[34] J. Ermer, Method Validation in Pharmaceutical Analysis, in: J.H McB., Miller (Eds.), Wiley-VCH Publication, Weinheim, 2005.
[35] P. De Bievre, H. Günzler (Eds.), Springer Publication, Berlin, 2005.
[36] I.R. Berry, D. Harpaz, Validation of Active Pharmaceutical Ingredients, second ed., CRC Press, Washington, 2001.
[37] Y. Altun, B. Uslu, B.D. Topal, S.A Ozkan, Electrochim. Acta 54 (2009) 1893–1903.
[38] B. Dogan, S. Tuncel, B. Uslu, S.A. Ozkan, Diamond Relat. Mater. 16 (2007) 1695–1704.
[39] S. Yılmaz, B. Uslu, S.A. Ozkan, Talanta 54 (2001) 351–360.
[40] S. Suzen, B.T. Demircigil, E. Buyukbıngol, S.A. Ozkan, New J. Chem. 27 (2003) 1007–1011.
[41] H. Lund, O. Hammerich (Eds.), fourth ed.,Marcel Dekker Inc. Publication, 2001.
[42] J. Grimshaw, Electrochemical Reactions and Mechanism in Organic Chemistry, first ed, Elsevier Science Publication, 2000.
[43] L Li, Y.Y. Sham, Z. Bikadi, W.F. Elmquist, Drug Metab. Dispos. 39 (2011) 1478–1485.
[44] E. Hammam, J. Pharm. Biomed. Anal. 30 (2002) 651–659.
[45] J.A. Bard, L.R Feulkner, Electrochemical methods Fundamentals and Applications, Wiley, New York, 1980.
[46] S.A. Ozkan, Electroanalytical Methods in Pharmaceutical Analysis and Their Validation, HNB Publishing, New York, 2012.
[47] N. Karadas, S. Sanli, M. Gumustas, S.A. Ozkan, J. Pharm. Biomed. Anal. 66 (2012) 116–125.
[48] H. Lin, G. Li, K. Wu, Food Chem. 107 (2008) 531–536.