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Differential Pulse Voltammetric Determination of Propylthiouracil and Methylthiouracil Using their Catalytic Effects on the Electrochemical Oxidation of Catechol

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The electrochemical behavior of catechol in the presence of propylthiouracil (PTU) and methylthiouracil (MTU) at a glassy carbon electrode is thoroughly investigated. The cyclic voltammetric studies of the systems are performed in different pHs and sweep rates. The results of the voltammetric foundations show an electrocatalytic oxidation of catechol in the presence of PTU and MTU, leading to a remarkable increase in the anodic peak current of catechol together with the contemporaneous disappearance of the corresponding cathodic wave. The presented mechanism shows the nucleophilic addition/reduction of the electro-generated o-quinone by the PTU or MTU, which produced chemically reduced adducts whose subsequent re-oxidation at the electrode surface leads to a considerable amplification of the anodic peak current. The increasing of the anodic peak current shows a linear correlation with the concentration of PTU and MTU. The spectrophotometric investigations are used to confirm the interaction of considering thiols with the electrochemically produced o-quinone during the controlled-potential coulometry. The effect of various biological thiols, such as cysteine, penicillamine, N-acetyl cysteine, glutathione and captopril on the electrochemical behavior of catechol is investigated in the same conditions. The results represent the unique electrocatalytic effect of PTU and MTU for improving the anodic peak current of catechol and exhibit a very sensitive and relatively selective method for the voltammetric detection of the pointed thiols. A linear range from 0.1 to 10.0 μM with a detection limit of 0.05 μM is obtained for the determination of PTU. The linear range for MTU, using differential pulse voltammetry, was from 0.1 to 5.0 μM with a detection limit of 0.07 μM. The proposed method showed to be useful for the sensitive detection of PTU and MTU among pharmaceutical and biological thiols.

Keywords: Propylthiouracil; Methylthiouracil; Catechol; Cyclic voltammetry; Differential pulse voltammetry; Electrocatalysis

1. INTRODUCTION

The physiological significance of sulfhydryl compounds is well recognized with the authorized levels of these species within biological fluids such as plasma and urine serving as valuable diagnosis tests in a number of clinical investigations [1]. The antioxidant properties of many of these compounds, such as cysteine, penicillamine and glutathione are known to play an important role in various biochemical roles [2,3]. Depletion of these compounds has been noted to accompany premature, arteriosclerosis, leukemia and cervical cancer [4]. Thiouracils, as a group of sulfhydryl compounds, have attracted special interests in medicinal chemistry. These compounds are minor components of transfer RNA, which have anti-herpes virus activities. Propylthiouracil (PTU) is a thioamide drug to treat hyperthyroidism. It is a medicine that is used to decrease the amount of thyroid hormone produced by thyroid gland. On the other hand, using of PTU may cause some side effects such as, skin rash, itching, abnormal hair loss, upset stomach, vomiting, lose of taste, swelling, joint and muscle pain, drowsiness, dizziness, decreased the white blood cells and platelets [5,6]. Methylthiouracil is a thionamide anti-thyroid drug, introduced in the 1940s, which has been used in the treatment of hyperthyroidism [7].

Various analytical methods have been applied for the determination of such compounds in different samples. Main of these methods have been based on very complex, expensive and time consuming methods such as; liquid chromatography coupled with tandem mass spectrometry after derivatization with 3-iodobenzylbromide [8], HPLC for the determination of thyreostatic drugs (such as PTU and MTU) residue in cattle serum and thyroid tissue [9], determination in biological and food samples by matrix solid-phase dispersion and gas chromatography-mass spectrometry [10,11]. The electrochemical detection of thiols has been very successful for application in pharmaceutical and clinical preparations [12-15]. Chemically modified electrodes based on transition metal complexes, such as phthalocyanine and Schiff base complexes, have been used for the electrocatalytic detection of thiouracil [16] and PTU [17]. A serious problem in detection with these electrodes was the poor selectivity of the responses and interferences that created from the presence of other thiols (such as cysteine, N-acetyl cysteine, penicillamine, and glutathione), dopamine and ascorbic acid. Most of these compounds show voltammetric responses near to the thiouracils peaks at the surface of modified electrodes.

A number of strategies for improving the electrode response (sensitivity and selectivity) has been investigated which are typically based on application of reversible redox indicators such as hydroquinones, catechols and catechol amines [18-21] and also organic dyes (such as thionine) as a bulk modifier [22]. The detection methodology relies upon the electro-chemical oxidation of the indicator species to the corresponding quinoid intermediate. The electro-oxidation product subsequently undergoes a chemical addition-reduction process (e.g. 1+4 Michael addition) with thiol species as a nucleophilic agent to produce the reduced adduct compound. The presence of the thiol moiety in the structure of the product, due to its electron donating property, facile the electro-oxidation of the addition product and decreases the anodic oxidation potential. Therefore, the re-oxidation of the adduct leads to an increase in the oxidative current, the magnitude of which is proportion to the concentration of thiol present.

In the present work the electrochemical oxidation of catechol in various pHs in the presence of PTU and MTU is investigated at the surface of glassy carbon electrode. The behavior of the redox system in the presence of some biologically important sulfhydryl compounds (e.g. cysteine, N-acetyl cysteine, penicillamine, glutathione and captopril) is compared. The results are shown a unique effect of PTU and MTU in remarkable improvement of the anodic current for catechol together with disappearance of the corresponding cathodic peak. The increasing of the anodic current of catechol showed a linear relationship with the sulfhydryl concentration (PTU and MTU), which used for the determination of PTU and MTU with a detection limit less than 0.05 μM. The proposed methodology is successfully applied to the accurate determination of PTU and MTU in a wide range of concentration with a sub-micromolar detection limit.

2. EXPERIMENTS

2.1. Apparatus

Cyclic voltammetric experiments were performed with a Metrohm Computrace Voltammetric Analyzer Model 757VA. A conventional three-electrode system was used with a glassy carbon (GC) disc (2 mm diameter), a saturated Ag/AgCl reference electrode, and a platinum wire as the counter electrode. Controlled-potential coulometric experiments were performed by using a PC-controlled potentiostat/galvanostat EG&G Model 273A (Princeton Applied Research Corp. Princeton, NJ, USA). The working electrode used in controlled-potential coulometry was a graphite rod with a diameter of 15 mm and length 10 cm (EK20, Germany) and a platinum rod with a 3 mm in diameter and 10 cm in length was used as counter electrode. The working electrode potential was monitored vs. Ag/AgCl reference electrode (from Azar electrode, Iran).

A digital pH/mV/Ion meter (Cyberscan model 2500) was used for preparing of the buffer solutions, which were used as the supporting electrolyte in voltammetric, coulometric and spectrophotometric experiments. A photodiode array UV/Vis spectrophotometer (Sinco S-3500 series) with a standard 1 cm path length cuvette was used for obtaining the absorption spectra during the electrolysis.

2.2. Materials

Catechol, propylthiouracil (PTU), methylthiouracil (MTU), L-cysteine, N-acetyl-L-cysteine and glutathione (reduced) were prepared from Merck. Captopril was purchased from Fluka. All other chemicals were of the highest purity grade available and were purchased from Merck and used without further purification. . Aqueous buffered solutions for all voltammetric, coulometric and spectrophotometric studies were prepared with de-ionized double distillated water from dilute and alkaline solutions of potassium permanganate. Voltammetric experiments were carried out in buffered solutions of PTU, deaerated by purging the pure nitrogen (99.999% from Roham Gas Company) for 5 minutes.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetric and spectrophotometric studies

Cyclic voltammetric studies upon the effect of PTU and MTU on the electrochemical behavior of catechol were performed at the surface of glassy carbon (GC) electrode. Fig. 1 shows the cyclic voltammograms of 1 mM catechol in the absence and also, presence of 1 mM PTU and MTU in buffered solutions with pH 7.0 (0.1 M phosphate).

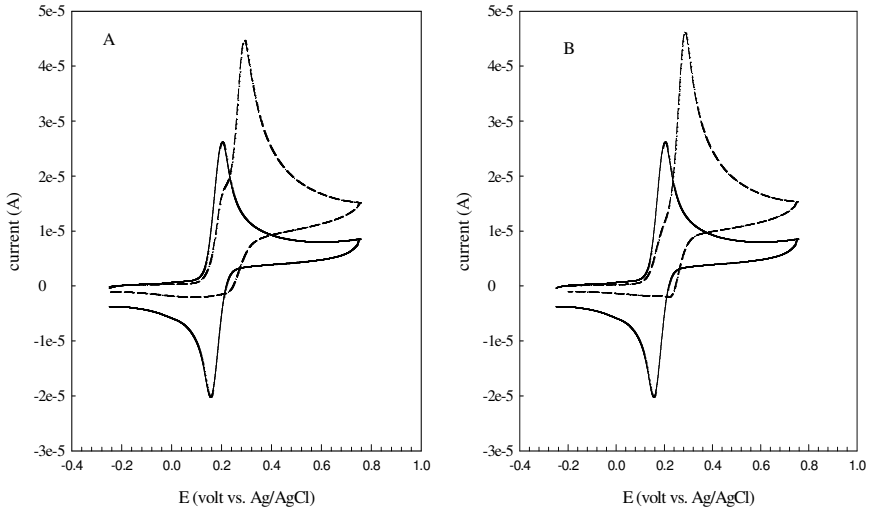

Figure 1. Cyclic voltammograms for 1 mM catechol in the absence (solid lines) and in the presence (dashed lines) of 1 mM (A) MTU and (B) PTU. Supporting electrolyte was 0.1 M phosphate buffer solution (pH 7) and sweep rate was 100 mVs^{-1}.

As can be seen, for catechol in the absence of the considering thiols a nearly reversible behavior is obtained. Results of the previous works confirm a two electron and two proton reversible process for catechol at the surface of GC electrode [23,24]. On the other hand, by introducing of PTU or MTU in catechol solution, an anodic wave with a slightly more positive peak potential and remarkably greater peak current is resulted. The results in Fig. 1 show that the cathodic wave in the reverse sweep is nearly disappeared. Such a behavior reveals that the oxidation product (o-quinone) is consumed in the chemical reaction with thiol species in a relatively fast kinetics fashion and therefore, is little available for the electro-reduction in the diffusion layer and at the electrode surface. Cyclic voltammetric studies for solutions containing only MTU and PTU do not show any electrochemical activity for these thiol species in the studied potential range. Previous studies on the nucleophilic addition of thiouracil (TU) to the electro-generated o-quinone showed that the addition of TU with a 1+4 Michael addition mechanisms resulted to a reduced product [23]. The anodic peak current shows a linear dependence to the square root of the potential sweep rate ($\upsilon^{1/2}$) in the range of 25-200 mVs^{-1}, suggest a diffusion-controlled process for the oxidation of catechol in the presence of PTU or MTU. The current function for the observed anodic wave ($I_{p,a}/\upsilon^{1/2}$) decreases with potential sweep rate. Such a behavior is adopted as indicative of a catalytic EC mechanism for the electrochemical oxidation of catechol in the presence

of PTU and MTU. The catalytic role of PTU and MTU in an addition/reduction fashion, leads to a remarkable increase in the anodic current. As a result, electrochemical oxidation of the reduced addition products in the region of catechol oxidation caused to a remarkable increase in the corresponding anodic peak, which can be correlated to the concentration of thiol present.

The cyclic voltammograms for catechol in the presence of PTU and MTU are obtained in the pH range of 3-7. These investigations showed that both anodic and cathodic peak potentials decreased linearly with pH with a slope between -56 to -58 (mV / pH unit) and correlation coefficients (R^2) greater than 0.99. These observations confirm the two electron and two proton processes for the oxidation of catechol in the presence of PTU and MTU. On the other hand, by decreasing the pH of the buffer solution a new cathodic peak in less positive potentials is resulted for both PTU and MTU (II_c in Fig. 2).

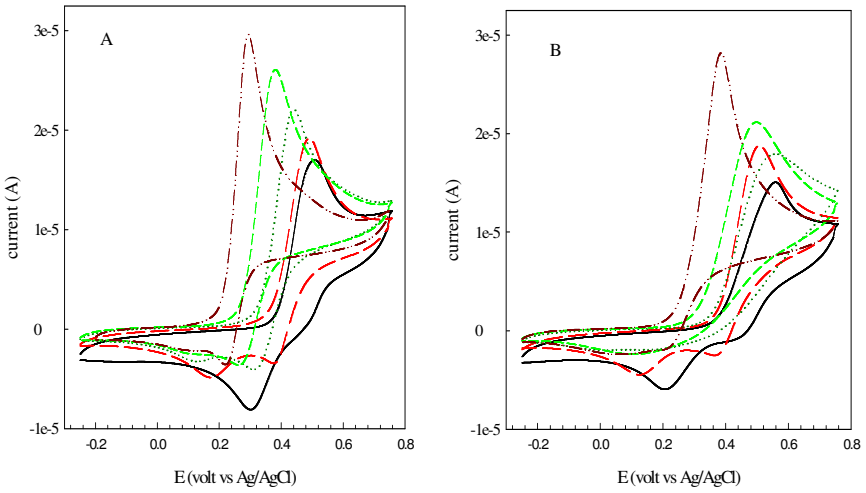

Figure 2. Cyclic voltammograms of 1 mM catechol in the presence of 1 mM (A) PTU and (B) MTU in different pH of buffer solution; (——) pH = 3, (– – -) pH = 4, (……) pH = 5, (----) pH = 6 and (_ . . _ . .) pH = 7.

The cyclic voltammograms of catechol in the presence of these thiol species in 0.1 M acetate buffer solution with pH 4.0 in various potential sweep rates (25-200 mVs^{-1}) are shown in Fig. 3. The current peak for this new cathodic peak shows a linear dependence to the sweep rate (υ). The results for solutions with pH 4.0, which is presented in Fig. 4, show the correlation coefficients of (R^2) 0.9997 and 0.9996 for catechol in the presence of MTU and PTU, respectively.

In order to investigate the interferences of other biological thiols on the determination of PTU and MTU, the cyclic voltammetric behavior of catechol is studied in the presence of various biological thiols, e.g., cysteine, N-acetyl cysteine, penicillamine, glutathione and captopril in solutions with pH 7.0 (0.1 M phosphate buffer). As can be seen in Fig.5, for catechol in the presence of cysteine and penicillamine the cathodic peak current is considerably decreased. However, the anodic peak is

broaded and transferred to more positive potentials. In the case of penicillamine, the anodic peak current is considerably decreased.

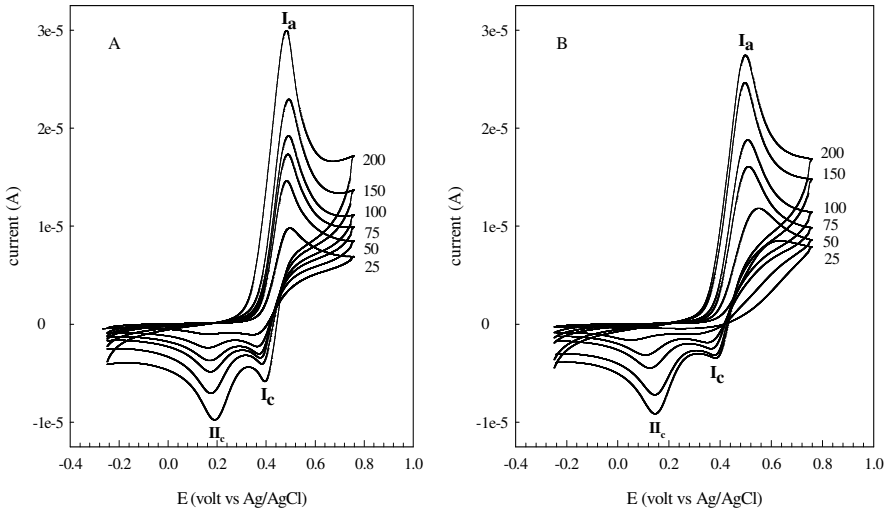

Figure 3. Cyclic voltammograms of 1 mM catechol in various potential sweep rates and in the presence of 1mM (A) PTU and (B) MTU. Supporting electrolyte was 0.1 M acetate buffer with pH 4.

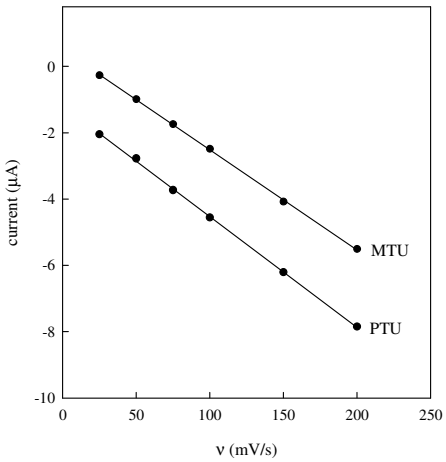

Figure 4. Dependence of the peak current for the second cathodic peak (II_c) to the potential sweep rate for solutions containing 1 mM catechol and 1 mM of PTU or MTU. Supporting electrolyte was 0.1 M acetate buffer with pH 4.

The presence of cysteine does not show a considerable effect on anodic peak current. Such behaviors reveal that, inverse to MTU and PTU, the kinetics of the anodic oxidation of the addition products is remarkably sluggish for cysteine and penicillamine. A previous study on the effect of

sulfhydryl compounds on the electrochemical behavior of catechol showed that the presence of cysteine caused to a slight increase in the anodic peak current and disappearance of the corresponding cathodic wave [25]. However, a unique effect for enhancement the sharpness and peak current of the anodic wave, via an effective catalytic fashion, is observed for catechol in the presence of PTU and MTU in the present work. These results showed the best sensitivity for variation of the anodic peak current with addition of PTU and MTU among biologically important sulfhydryl compounds.

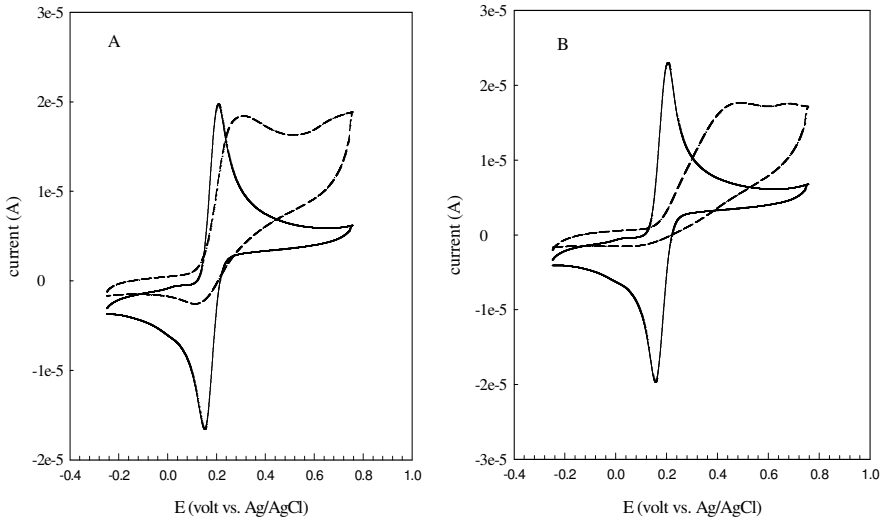

Figure 5. Cyclic voltammograms for 1 mM catechol in the absence (solid lines) and in the presence (dashed lines) of 1 mM (A) cysteine and (B) penicillamine. Supporting electrolyte was 0.1 M phosphate buffer solution (pH 7) and sweep rate was 100 mVs^{-1}.

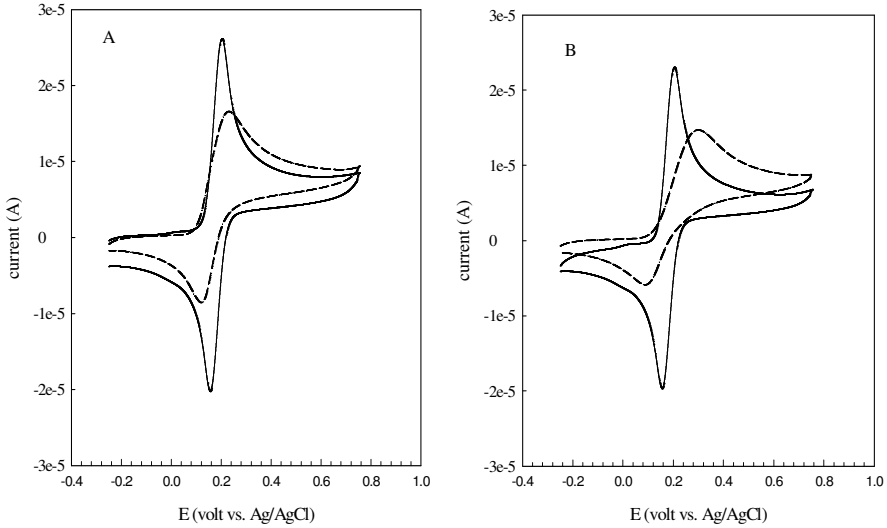

Figure 6. Cyclic voltammograms for 1 mM catechol in the absence (solid lines) and in the presence (dashed lines) of 1 mM (A) N-acetyl cysteine and (B) captopril. Supporting electrolyte was 0.1 M phosphate buffer solution (pH 7) and sweep rate was 100 mVs^{-1}.

These studies showed that N-acetyl cysteine and captopril have a very weak effect on the cyclic voltammograms of catechol (Fig. 6). In fact, in the presence of these thiols, the ratio of $I_{p,a}/I_{p,c}$ shows a very weak variation from 1, revealing the weak interaction of these compounds with electro-generated o-quinone and the stability of this species for reduction in the reverse sweep. A similar behavior is observed for catechol in the presence of glutathione. Previous studies on the nucleophilic addition of N-acetyl cysteine, glutathione and captopril to electrochemically produced dopaminoquinone [21] showed that the steric hindrance in the structure of these thiols is the main reason for decreasing their nucleophilic properties. Moreover, the keto-enol tautomerism between the –SH moiety with the carbonyl neighboring group caused that the thiol group cannot be accessed for nucleophilic addition to o-quinone in a 1+4 Michael fashion. The considerable and selective enhancement of the anodic current of catechol in the presence of MTU and PTU can also explained in a selective catalytic pathway, which provides a very sensitive and selective method for voltammetric determination of PTU and MTU with sub-micromolar detection limits.

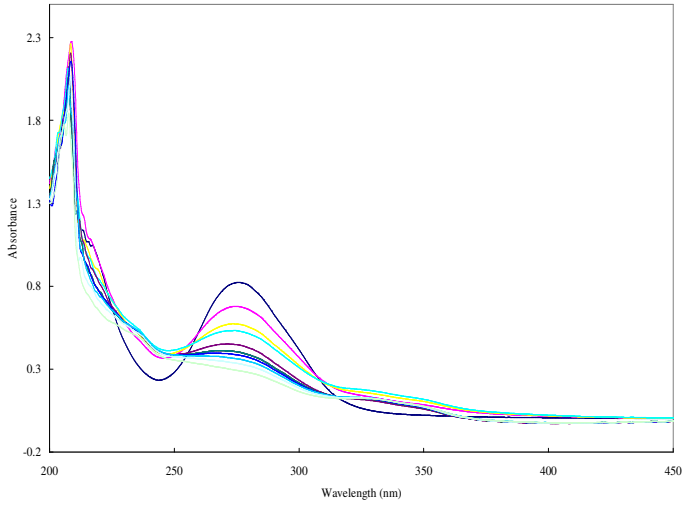

Figure 7. Absorption spectra for solutions containing 10 µM of both catechol and MTU in pH 7 at the start and after each 15 min of the controlled potential electrolysis time.

Spectrophotometric studies were performed during the controlled-potential electrolysis. In these experiments, a 100 ml solution of 0.01 M catechol containing 0.01 M PTU or MTU in 0.1 M phosphate buffer solution with pH 7.0 was transferred to an H-type divided cell in its anodic part. The electrolysis process was performed in a constant potential of 0.75 V (vs. Ag/AgCl as reference electrode). Aliquots (1ml) of this solution, at the start and in various times of the electrolysis process, were transferred to 50.0 ml volumetric flasks and dilute to volume with the same buffer solution and their absorption spectra were recorded. In these studies, an absorption band with the λ_{max} of 218 and 277 nm is obtained for catechol. By advances the electrolysis process, the intensity of the catechol absorption peak in 277 nm is decreases and a new broad band with a λ_{max} of nearly 340 nm corresponding to the addition product of thiol species to o-quinone is appeared. These results for the

controlled-potential electrolysis of catechol in the presence of MTU are typically shown in Fig. 7. Since the electrochemical oxidation of sulfhydryl compounds on the surface unmodified electrode is very sluggish and has very large amounts of overpotentials, application of the electron redox mediators have been proposed for improving the kinetics of the electron transfer and voltammetric sensitivity and of course, lowering the detection limit in determination of such compounds [1, 19-21].

3.2. Differential pulse voltammetric studies, analytical applications

It is shown that the voltammetric detection of electrochemically inactive species can be performed via the application of the electron redox mediators [1,20-22]. On the basis of the above mentioned mechanism, analytical determination of the trace amounts of PTU and MTU is performed.

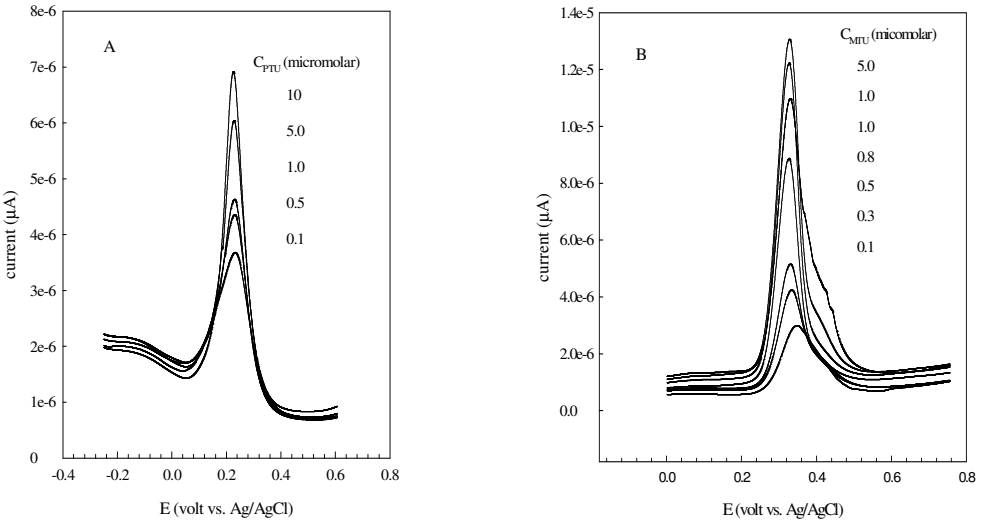

Figure 8. Differential pulse voltammograms of solutions containing 1×10^{-4} M catechol and different concentrations of (A) PTU, and (B) MTU. Supporting electrolyte was 0.1 M phosphate buffer with pH 7 and pulse amplitude was 50 mV.

The differential pulse voltammetry (DPV) was used as a very sensitive method with sub-micromolar detection limit for the determination of these sulfhydryl compounds, via their catalytic effects on the anodic current in catechol oxidation. DPV waves of the buffered solutions of 0.1 mM catechol (0.1 M phosphate, pH=7) were recorded in the presence of various concentrations of PTU and MTU. The results showed an increase in the anodic wave of catechol during the addition of these compounds. The DPVs for solutions containing 0.1 mM catechol and various concentrations of PTU and MTU are shown in Fig. 8. A linear dynamic range from 1×10^{-7} M to 1×10^{-5} M with a detection limit of 5×10^{-8} M is obtained for PTU with a slope of 2.6 μA/μM and a correlation coefficient (R^2) of 0.9958. The linear dynamic range for the detection of MTU using this method was from 1×10^{-7} M to 5×10^{-6} M with a detection limit of 7×10^{-8} M. The slope of the calibration curve for the detection of MTU was 5.23

μA/μM and correlation coefficient (R^2) was 0.9943. The results of the analytical determination of PTU and MTU by this method are very better than the previous reports based of the chemically modified electrodes using transition metal complexes. The detection of PTU using carbon-paste electrode modified by cobalt salophen complex has been shown a linear range from 5.0×10^{-6} M to 7.5×10^{-4} M with a detection limit of $2.\times10^{-6}$ M [17]. The voltammetric determination of MTU by the square wave voltammetry using carbon fiber microelectrode has be shown a linear dynamic range of 1.0×10^{-6} M to 1.0×10^{-4} M with a detection limit of 3.8×10^{-7} M [14]. The relatively expensive and time-consuming procedures based on HPLC methodology [9] or solid-phase dispersion coupled with gas chromatography-mass spectrometry [10] showed the detection limits greater than 1×10-7 m for the thyreostatic compounds (e.g. PTU and MTU). Another analytical importance feature of the present method, moreover to remarkable linear range and detection limit, is that presence of the other biological sulfhydryl compounds (such as, cysteine, N-acetyl cysteine, glutathione, penicillamine and captopril) does not show considerable effects on the anodic peak current of catechol. Therefore, the presented method can be considered as a very sensitive and reasonably selective method with a sub-micromolar detection limit for the detection of PTU and MTU in pharmaceutical preparations.

4. CONCLUSIONS

In the present work the electrochemical behavior of catechol in the presence of PTU and MTU is investigated by cyclic voltammetry. In these investigations, the effect of the pH of the buffer solution and potential sweep rate is described. The results revealed that, among the biological and pharmaceutically important sulfhydryl compounds, PTU and MTU (as thyreostatic substances) have unique effects on significant improvement of the anodic current of catechol. Since PTU and MTU, via a catalytic mechanism, remarkably increase the anodic peak current of catechol, such an effect cannot be observed for other biological thiols, e.g. cysteine, N-acetyl cysteine, penicillamine, glutathione and captopril. The proposed methodology, based on the application of catechol as a redox mediator, exhibit a very sensitive, and to some extent selective method for voltammetric detection of PTU and MTU. The DPV method is applied as an effective analytical method for detection the compounds. A linear dynamic range from 0.1 to 10 μM with a detection limit below 0.1 μM, provide an alternative for the determination of PTU and MTU in pharmaceutical and clinical preparations. The analytical performances obtained in the present work for these substances are very better than previous electrochemical and hyphenated methods such as gas chromatography-mass spectrometry.

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