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Optimization of conditions for voltametric determination of tryptophan in pharmaceutical formulations using overoxidized polypyrrole glassy carbon electrode

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ABSTRACT

Trp was preferentially concentrated to the electrode under an open circuit condition, and was determined by the cyclic voltammetric technique .The electrocatalytic ability of the modified surface of the electrode allows the possibility of applying moderate potential and current for the voltammetric detection of Trp. The electrocatalytic behavior of Trp is elucidated with respect to solution pH, operating potential and other variables as well as the chemically modified electrode(CME) preparation conditions. Optimized conditions allowed the determination of Trp with detection limit of 6.9µM in liner dynamic rang 25 to 225µM (n=7, R^2=0.9992) at overoxidaized polypyrrole/GC electrod. The optimized condition was obtained via two consective stages: (a) electrochemical stage where two cycles of cyclic voltametry were applies at 0.30 to 0.85V range and the scan rate of 200mV/s and (b) overoxidation stage where the electrode was placed in 0.50M NaOH solution and 7 cycles were applied at 0.30 to 1.10V range and the scan rate of 20mV/s.

KEYWORDS

Tryptophan analysis;
Polypyrrole;
Overoxidized film;
Electrochemical detection.

INTRODUCTION

A simple, fast and sensitive method for tryptophan (Trp) determination in pharmaceutical formulations is the use of a glassy carbon(GC) electrode modified with overoxidized poly-pyrrole(OPPy) film as voltammetric sensor. Out of 21 protein forming amino acids, only tryptophan and tyrosine exhibited an oxidative voltammetric response with this electrode[1].

The determination of amino acids and their related compounds is of great significance in clinical, nutritional and biotechnological fields and many efforts have been devoted to develop a simple and sensitive analytical method[2].

L-Tryptophan is one of 10 essential amino acids and plays an important role in protein synthesis and as precursor of many biologically active substances such as serotonin 5-hydroxy- indoleacetic acid (5-HT).

Trp is the only amino acid in the blood which is bound to a large extent to albumin. Several studies have demonstrated that the degree of binding of Trp to serum albumin is an important factor in the unidirectional uptake of Trp into the brain[3,4,5].

Methods for the determination of Trp are mainly based on HPLC[6,7] and spectrophotometric procedures[8,9]. Most of the spectrophotometric methods involve laborious and slow procedures with the modification of Trp by numerous reagents.

Several liquid chromatography(LC) methods for detection and quantitation of Trp and its metabolites in various biological samples have been described. These methods have employed electrochemical[10], ultraviolet[11], fluorometric[12], and mass spectrometric[13] detection.

Electrochemical detection following liquid chromatography (LC-ED) represent a very attractive detection possibility for Trp[14].

Unfortunately Trp exhibit irreversible oxidation requiring extreme positive overpotential at most conventional electrode surfaces and consequently, are not ideally suitable for quantification via conventional electrochemical approaches following liquid chromatography [15].

Although the chemical oxidation of Trp has been studied quite extensively, the reactions are not well understood in terms of mechanisms or even products. During recent years, advances in modern electrochemical techniques have been made which facilitates the more specific electrochemical oxidation of Trp to be utilized in the study of oxidation pathways[16]. Electrochemical detection of those species is usually performed at mercury[17], or mercury amalgam[18] electrodes at which the formed mercury sulfide species can be oxidized at comparatively modest values of applied potential.

Much efforts have been made toward decreasing the overpotential and increasing the oxidation current[19,20,21,22].

Sugawara et al.[23] relate the possibility of the determination of Trp and some other amino acids at a level of 10^{-6}M by a chemical modified carbon paste electrode with copper (I) cyclohexylbutyrate and reverse linear sweep voltammetry after reduction of Cu(I).

Differential pulse voltammetry (DPV) using modified carbon paste electrode has been also reported[23] for the direct determination of Trp.

Electropolymerized polypyrrole (ppy) films have been widely used in recent years as a means to modify electrode surface[24]. It is founded that when this polymer is overoxidized, the electrocatalytic activity is increased in some cases in selectivity.

Overoxidized poly pyrrole(OPPY) film modified electrodes have been studied previously[25,26,27]. These electrodes are easily prepared by electro-polymerization of pyrrole monomer with subsequent overoxidation treatment.

In this context, the use of overoxidized polypyrrole (PPY) as a surface modifier of glassy carbon(GC) electrodes has been demonstrated to give rise to voltammetric sensor with important analytical advantages with respect to bare electrodes. The most interesting property of this modified electrode is that for determination of 100μM Trp, interference from a 15-fold excess of tyrosine gave an positive error of 7.5%, while the other amino acids did not exhibit any detectable interference.

EXPERIMENTAL

Apparatus

All electrochemical experiments were performed with BEH-PAJOOH (Model BHP2063+ potentiostat/ galvanostat, iran) and Hewlett Packard X-Y recorder (USA). An electrochemical cell with three electrodes was used for both the electropolymerization and electrochemical determination. A bare or OPPY-film-coated GC disc electrode with an area of $3.14mm^2$ was used as a working electrode. Prior to surface modification, the carbon electrode was polished successively using 0.05μm alumina powder suspension on a smooth cloth and then thoroughly rinsed with water. This electrode is referred to as fresh glassy carbon electrode. A platinum foil was used as auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. All potentials were recorded relative to the SCE.

Reagents

Pyrrole(Merk, Darmstadt, Germany) was purified by double distillation and was stored in a refrigerator. Stock 0.05M pyrrole solutions were prepared in deionized water containing 0.1M lithium perchlorate(Merk, Darmstadt, Germany). Other solutions were prepared from analytical- reagent grade chemicals without further purification and by using double distilled and deionized water. Stock Trp (Merk, Darmstadt, Germany), were prepared in 0.1M phosphate buffer solutions of pH 6.5, potassium phosphate mono basic and dibasic, sodium hydroxide(NaOH) were obtained from Merck company. 0.5M NaOH solution was used for overoxidation of PPy/GC. All experiments were carried out at ambient temperature. All chemicals were of reagent grade.

Electropolymerization

Electrochemical polymerization was carried out in 0.05M pyrrole solutions in water containing 0.1M lithium perchlorate. Film growth was accomplished under cyclic voltammetric conditions in the potential range –0.2 to 0.85V[28]. Three cycles were carried out at a scan rate of 200mV/s. The thickness was controlled by the amount of charge passed and was estimated to be 0.1μm by passing $24mC/cm^2$

Overoxidization of polypyrrole films on glassy carbon electrodes

Ultra thin OPPY film electrodes were prepared in two polymerization/overoxidation steps. PPy was first electrodeposited on the GC by cyclic voltammetric as mentioned above. PPy was then overoxidized by applying cyclic voltammetry program in solution of 0.5M NaOH with 7 cycles in the range of –0.3 to 1.1V and the scan rate of 20mV/s. This is the optimum condition for overoxidation of that polymer with respect to responsibility and sensitivity to the solution of tryptophan.

Stabilization of OPPy/GC electrode

The most important problem in electrochemical determination of Trp and other amino acids is that the oxidation products, are adsorbed on electrode surface and hence the electrodes could not useable more. So, for each measurement, the modified electrode must be prepared again.

To eliminate the problem of adsorbing amino acids to the surface of the electrode, a new method was proposed in the present study. Using this method, the need to the electropolymerization and the subsequent oxidation of the electrode is eliminated. The procedure includes washing of the electrode using distilled water after each measurement and immersed in 0.5M NaOH buffer solution together with the reference and auxiliary electrodes. Applying cyclic voltametric method where a 20 mV/s cycle is used, just at the identical range where oxidation of polymer was carried out, the surface of the electrode reactivated. Upon using such an electrode in an analyte solution, the same result was obtained after multiple use. So the significance of this method is to introduce a new technique where the response of the electrode would be stable for at least 12 hrs periode.

RESULTS AND DISCUSSION

Electrochemical preparation and characterization of PPy/GC electrode

The cyclic voltametric condition for electropoly merization was chosen due to higher stable voltametric measurements of analyts with respect to responsibility and sensitivity to solution of Trp. Moreover, it has been suggested that this procedure allows coating to be obtained with a higher homogeneity[29].

Figure 1 shows the cyclic voltammogram obtained in free monomer solution at PPy-coated GC(a), bare electrode (b) and OOPPy-coated electrode (c) in free monomer containing prepared by the following procedure as described in the Experimental section.

As shown in figure 1, the oxidation peak was appeared at around –0.2V and reduction peak appeared at around -0.5V corresponding to the polymer film dogin-dedoping process in the supporting electrolyte. A decrease in the oxidation signal due to the polymer relaxation[29], was observed in second and successive cycles. The peak currents depend on the thickness of the prepared polymer.

Effect of the experimental variables used in the polymer electro synthesis on the trp voltammetric

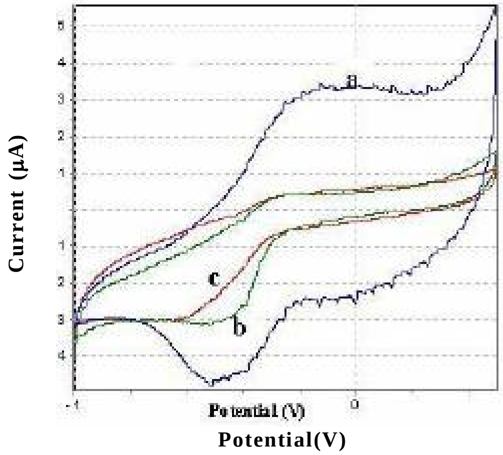

Figure 1 : Cyclic voltamogram of PPy-coated GC(a), bare electrode(b) and OPPycoated electrode(c) in free monomer (pyrrole) containing 0.1M LiCLO4, v=100mV/s (the first cycle is shown)

response

The experimental conditions used for the preparation of polymer-coated electrodes may considerably affect the voltammetric response of the analyte under investigation. Thus, the effect of the different variables involved in the PPy electro synthesis on the Trp voltammetric signal has been considered in order to achieve the best Trp electroanalytical response.

Since the type of doping anion can affect on the Trp voltammetric response, some of the anions were selected to find the best doping anion, and after polymerization by each doping anions, each electrode was overoxidized in exactly similar parameters, and then immersed in solution of 0.1M $H_2PO_4^-$, HPO_4^{2-}buffer (pH=7), containing 4×10^{-5}M Trp and by differential pulse voltammetry (DPV) program in the rang of 0.4 to 0.9V. The results are shown in TABLE 1.

In contrast to above results, it is founded that for doping of polymer with ClO_4^- and $Fe(CN)_6^{4-}$ anions, the modified electrode have maximum responsibility and sensitivity to solution of Trp (with respect to maximum of Ip and minimum of Ep). Since $LiClO_4$ is a cheap electrolyte, it is used for further investigations.

The number of potential cycles applied for the polymer growth was tested regarding its influence on the Trp electrochemical response. As shown in figure 2, the peak current is constant with number of cycles up to five, and then leveled off, which has been attributed to the achievement of a homogeneous coating of the glassy carbon electrode.

A very slight decrease in Ip was observed from three cycles onwards, which might be related to a degradation of the polymer due to overoxidation in the polymer formation process. Consequently, the slight decrease in Ip may be attributed to a change in the coating morphology, in the sense of a greater compactness. The number of two cycles was selected as an optimum value under the working conditions used.

The potential scan rate at which cycling was carried out also had an influence on the Trp peak current, a decrease being observed with slower, and with faster scan rates used, probably because at higher scan rate lees monomer is oxidized during each cycle and consequently lees polymer is produced. 200mV/s was chosen as an appropriate scan rate.

TABLE 1: The result of differential pulse votammograms for 1.0×10^{-4}M Trp at OPPy-coated GC doped with different anions

Ep/mV	Ip/ìA	Dopping anion	Number
580	3.1	SO_4^{2-}	1
591	3.7	CL^-	2
580	3.5	$B_2O_7^{2-}$	3
580	2.6	HCO_3^-	4
581	3.5	$H_2PO_4^-$	5
573	3.3	HPO_4^{2-}	6
610	1.8	Molibdat	7
574	2.9	NO_3^-	8
582	4.0	EDTA	9
573	4.0	Dodecyl solfat	10
570	4.1	$Fe(CN)_6^{4-}$	11
570	4.1	CLO_4^-	12

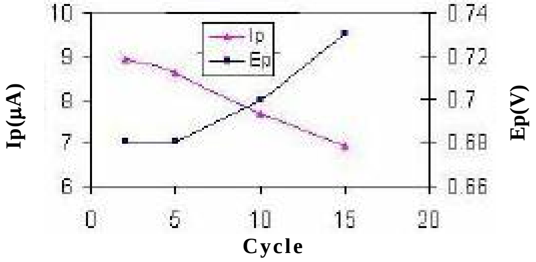

Figure 2 : The consequent of variety electropolymerization cycle numbers on electrochemical response of obtained OPPy electrodes to the Trp. The overoxidation conditions were constant for each electrode. The obtained electrode in each case immersed in a Trp solution and the result related to the cyclic voltametric determination of Trp

Regarding chemical variables of the monomer-water solution, both background electrolyte and monomer concentration were considered. A relatively high concentration of doping anions is needed in the polymer electro synthesis to ensure electro neutrality of the polymer phase in the oxidized state. Moreover, a higher conductivity of the polymeric coating has been observed as the supporting electrolyte concentration increases. The decrease of the signal observed for $LiClO_4$ concentrations higher than 0.1M can be explained by taking into account morphological changes of the coating in the presence of a very high electrolyte concentration, giving rise to a more compact structure of the polymer, and then to a decrease in the charge-transfer rate at the conducting polymer[29].

Figure 3 shows the effect of concentration of monomer on response of obtained polymer to Trp.

As shown in figure 3, in concentration values less than 0.025M and more than 1M, decrease of the peak

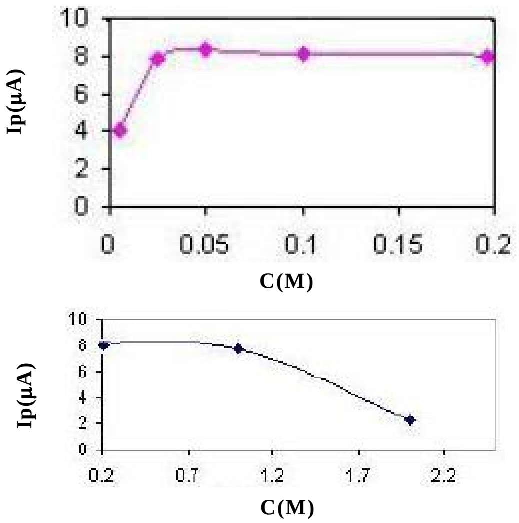

Figure 3 : The monomer concentration effect (Pyrrole) in electropolymerization stage on final response of OPPy electrode to cyclic Voltametric measurement of Trp. Other conditions are fixed

current is induced, while in the range of 0.025M to 1M, the peak current remains constant. Hence the concentration of 0.05M was selected in this investigation.

Overoxidation of polypyrrole film placed on glassy carbon electrode

As mentioned above, it is founded that when PPy-coated electrode be overoxidized, the electro catalytic activity is increased in some cases of selectivity. overoxidation of PPy is a destructive process whereby the conducting PPy is oxidized to a higher oxidation state after which it becomes susceptible to nucleophilic attack [30].

Figure 4 shows the overoxidation mechanism of Ppy in basic medium. Az shown in figure 4, during overoxidation, carbonyl groups are introduced into the polymer backbone. This process usually results in the addition of a carbonyl functionality to the pyrrolic rings by losing of conjugation and hence the inherent electronic conductivity. After undergoing oxidation a net electronegative character is imparted to the polymer film and it then undergoes cation perm selective behavior resulting in overoxidized (OPPy) film and derivative films[30]. Since the OPPy repels anions with the neutral carbonyl group instead of negative charge site, electrostatic binding does not occur, and the OPPy has fast response time and fewer memory effects and is free from the problem of saturation of the binding sites. A thinner ion-selective film is always desirable due to an expected faster response time and higher sensitivity[31].

In this work, for overoxidation of PPy film, cyclic voltammetry program is used in the rang of –0.3 to 1.1V with number of 7 cycles at scan rate of 20mV/s and in solution 0.5M of NaOH. The mentioned conditions are optimized.

Effect of the experimental variables used in over oxidation of polymer on the Trp voltammetric response

The experimental parameter for overoxidation of PPy-coated electrode have main effect on voltammetric response of obtained modified electrode and therefore on response to analyte. In the present work it is attempted that these parameters become optimum to achieve best response for Trp. Thus, the effect of the different variables involved in the overoxidation on the Trp voltammetric signal has been considered in order to achieve the best Trp electroanalytical response.

There are two methods for overoxidation of poly pyrrole-coated GC electrode: cyclic voltammetric and potentiostatic programs, and hence both of them have investigated in different parameters to give the best re-

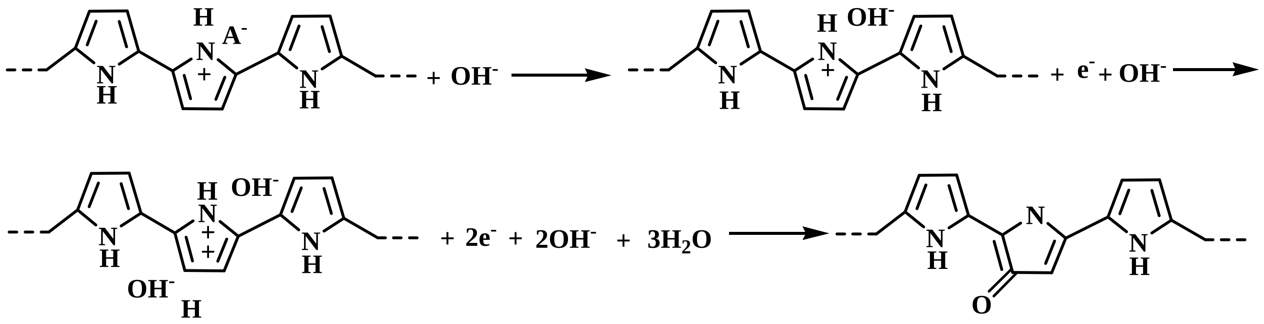

Figure 4 : The overoxidation mechanism of Ppy in basic medium

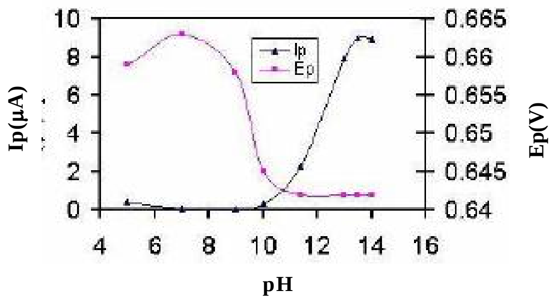

Figure 5 : The effect of overoxidation stage pH on response of the obtained electrode in cyclic Voltametric measurement of Trp. other conditions are fixed

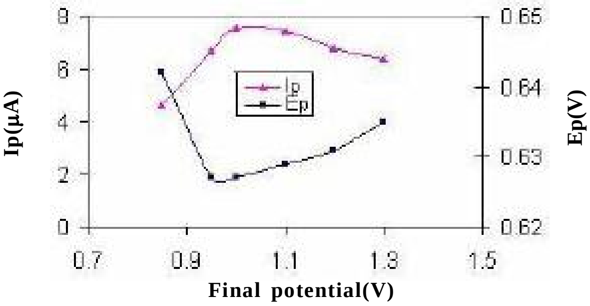

Figure 6 : The effect of final potential quantity in overoxidation stage on the obtained electrode to cyclic voltammetric response of Trp. other conditions are fixed

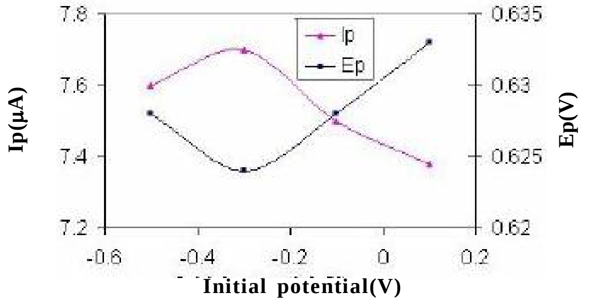

Figure 7 : The effect of initial potential quantity in overoxidation stage on the obtained electrode to cyclic voltammetric response of Trp. other conditions are fixed

sponse for Trp. The result have shown that overoxidation with cyclic voltammetric has more stability and repeatability response for Trp in comparison to the other methods. Hence the cyclic voltammetric program was used to overoxidation of polymer coated GC electrode.

The solvent pH plays an important role in the overoxidation. The different in solvent that overoxidation take place , induce a considerable affect on voltammetric response of the obtained electrode in the analyte solution. Figure 5 shows the effect of electrolyte (at different pH values), in overoxidation stage of PPy-coated GC electrode, that effect on responsibility of obtained electrode.

As it seems in figure 5, overoxidation in different pH values induce different responses for obtained electrode. Up to pH=10 no response was observed, however for overoxidation at pH values more than 10, the peak current (Ip) increases sharply by raise in pH. Maximum peak current is visible at pH=13.5 that appertain to 0.5M of NaOH. So the solution of 0.5M NaOH was selected as better solvent to overoxidation.

In order to obtain the best range to overoxidation, either the initial or the last potentials were selected as the variable and the other constant. Thereby the electro polymerization conditions of monomer and other experimental conditions were selected in constant and in optimal state as mentioned before. So the potential values of 0.85, 0.95, 1, 1.1, 1.2, 1.3 V was selected as respectively the final potential ongoing of overoxidation of PPy-coated electrode with cyclic voltammetric program. Each of the electrodes that obtained, immersed in solution of Trp to gain best response with cyclic voltammetric program in the rang of 0.4 to 0.9V and with scan rate of 100mV/s.

Figure 6 shows the effect of final potential quantity in response of the obtained electrode in cyclic voltammetric measurement of Trp.

The optimum initial potential in overoxidation is obtained as mentioned for final potential. So the potential values of –0.5, -0.3, -0.1, 0.1V was selected respectively as the initial potential ongoing of overoxidation of PPy-coated electrode with cyclic voltammetric program. The result is illustrated in figure 7.

As shown in figure 6 and figure 7 the final potential of 1.1V and the initial potential of –0.3V in overoxidation of polymer, produce maximum responsibility and sensitivity to Trp solution, so it was selected as better range to overoxidation of PPy-coated electrode.

The scan rate in overoxidation of PPy-coated electrode, have important role in responsibility and sensitivity of obtained electrode. Figure 8 shows the effect of scan rate in overoxidation on response of the obtained electrode in cyclic voltammetric measurement of Trp.

As shown in figure 8. the scan rate of 20mV/s in overoxidation of polymer produce maximum responsibility and sensitivity of obtained electrode, and was se-

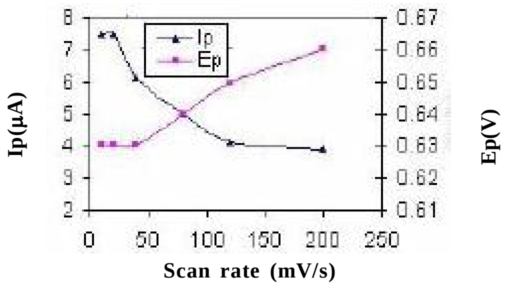

Figure 8 : The effect of scan rate in overoxidation stage on response of the obtained electrode to cyclic voltammetric measurement of Trp. other conditions are fixed

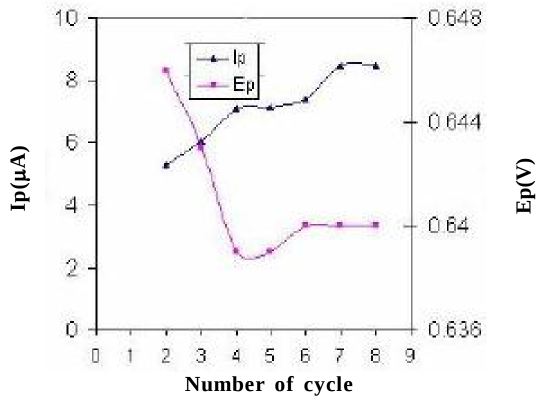

Figure 9 : The effect of overoxidation cycle numbers on response of the obtained electrode to cyclic voltammetric measurement of Trp. Other conditions are fixed and optimized

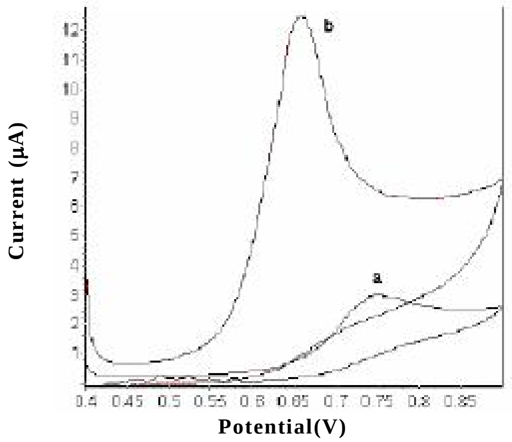

Figure 10 : Cyclic voltammograms of 1.0×10^{-4} M Trp at (a) bare GC electrode (b) OPPy-coated modified GC electrode. Other conditions are fixed and optimized

lected as better scan rate to overoxidation of PPy-coated electrode.

The number of potential cycles applied for overoxidation of polymer as well as polymer growth was tested regarding its influence on the Trp electrochemical response (Figure 9).

Figure 9, shows that the peak current increased sharply with number of cycles up to 7, and then by increase of cycle be humdrum. This is probably due to that before 7 cycles in overoxidation, the amount of polymer that overoxidized, is not the total amount of the polymer, and by increasing the number of cycles, the amount of polymer that is overoxidized becomes more and that is, the responsibility and sensitivity of obtained electrode are increased. However after 7 cycles, since the total amount of polymer is overoxidized, increasing the number of cycles do not induce any increase in responsibility and sensitivity of the obtained electrode. So the number of 7 cycles was selected as the optimum number of cycles for overoxidation of PPy-coated GC electrode.

Electroanalytical studies of the Trp oxidation process at OOPPy-coated GC electrode

The objective of the present study is to propose a simple and fast method for the determination of Trp. Thus a preliminary study has been developed in order to compare the response of CME and glassy carbon (GC) electrode. For Trp low sensitivity and resolution was observed for bare GC, pointing to the CME as the best choice for the determination of this analyte in the further studies. The differences should be related with the higher active area of the CME when compared with GC.

For CV, the potential window ranging was between 0.4 and 0.9V (versus SCE) and scan rate of 100mV/s for the first cycle.

Figure 10 shows the different ability of a bare GC and OOPPy-coated modified GC electrodes in measurement of Trp. It was found that modified electrode have an increase (and shift) in peak current. As it is shown in figure 10, OOPPy-coated modified GC electrode has an effect of increasing the magnitude of the background current. Provided CV from supporting alone electrolyte confirms this state.

The influence of pH on the electroanalytical response of Trp was studied by cyclic voltammetry using a 0.1M phosphate buffer solution as background electrolyte. Plot of peak current and peak potential versus pH are shown in figure11. It is clearly manifested that protons

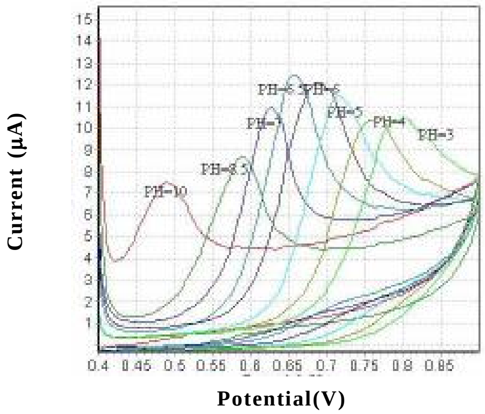

Figure 11: The effect of buffer pH on cyclic voltammetric response of obtained electrode to cyclic voltammetric measurement of 1.0×10^{-4}M Trp in 0.1M phosphate buffer solution with scan rate;100mV/s. other conditions are fixed and optimized

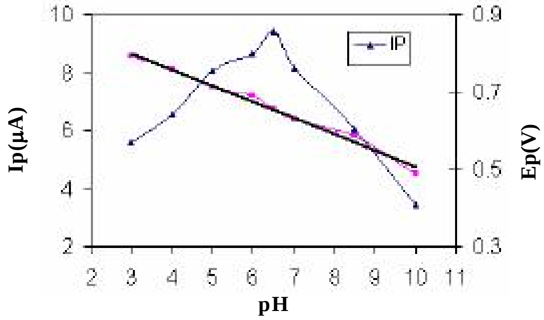

Figure 12 : The obtained result from cyclic voltammetric analysis of Trp in different pH 25 conditions are the same as figure 11

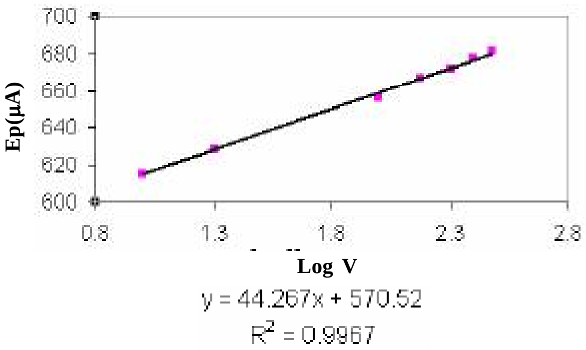

Figure 13 : The relation between Ep and log v(v=scan rate) in phosphate buffer solution of Trp (pH=6.5). The electropolymerization and overoxidation conditions are optimized

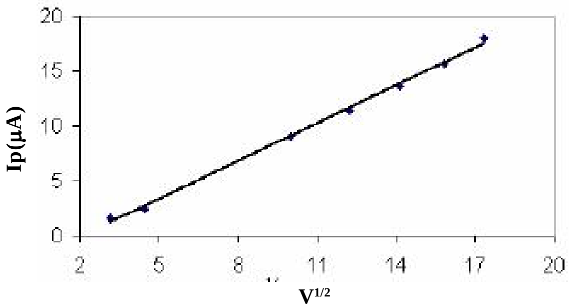

Figure 14 : The relation between Ip and v1/2(v=scan rate) in phosphate buffer solution of Trp (pH=6.5). The electropolymerization and over-oxidation and other conditions are optimized

are involved in the oxidation processes of this compound at OOPPy-coated GC electrode. In all cases more positive peak potential values were obtained as pH decreases, thus the electrochemical oxidation being easier in basic media, but despite increasing of peak potential, the peak current is varied with increasing of pH as shown in figure 11. The result of cyclic voltammetric analysis of Trp in different pH values are illustrated in figure 12.

As found in figure12, the peak current for neutral pH values is larger than those for acidic or basic pH values. So the pH of 6.5 was selected owing to maximum amount of Ip.

Cyclic volttammograms of 1.2×10^{-4}M Trp solutions were performed at OOPPy-coated GC electrode at potential scan rates between 10 and 300mVs^{-1}. The E_p slope vs. log ν plot(R^2=0.9967) was 44.267, thus demonstrating that the Trp oxidation is irreversible reaction, and its current is diffusion controlled. Moreover, the peak potential was shifted to more positive values as the scan rate increased, indicating the irreversibility of the electrodic process. This results shown in figure 13.

In contrast, cyclic voltammograms under the same experimental conditions at OOPPy-coated GC electrode shows that I_p change linearly with $\nu^{1/2}$ (R^2=0.9975) over the whole scan rate range tested(10 to 300mVs^{-1}) as shown in figure 14.

All these data clearly demonstrate that, for the OOPPy-coated GC electrode, the current was purely diffusion-controlled.

A linear calibration graph (R^2=0.9992) was obtained for Trp in the 25 to 225μM concentration range, with slop and intercept values 0.0444 and 2.3707, respectively, as shown in figure 15. The resulted detection limit is 6.9×10^{-6}M.

DPV determination of Trp in pharmaceutical samples

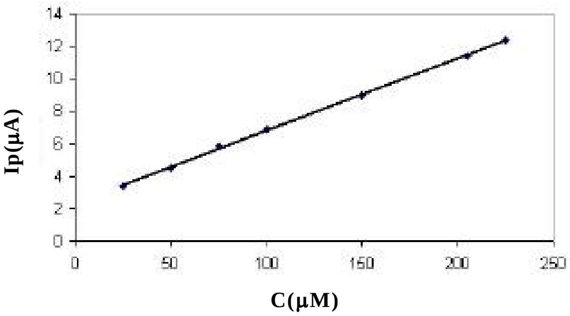

Figure15 : The relation between Ip and analyte concentration in phosphate buffer solution of Trp (pH=6.5). The electropolymerization and over-oxidation and other conditions are optimized

TABLE 2: DPV determination of Trp in pharmaceutical samples

Sample	Composition	Tryptophan content(m<)		Error %
		DPV	UV	
S1[a]	Trp	2.48	2.50	-0.8
S2[b]	Tyrosine(2200mg) Trytophan(510mg) Phenylalanin(20mg)	2.60	2.51	+3.5
S3[c]	cysteine(240mg) Cysteine chloridrate(2mg) L-lysine(300mg) Citric acid(5mg) Pyridoxine choloridrate(20mg) Buclizine, Chloridrate(10mg) Cyanocobalamice(50mg) Sodium Saccharinate[d] Citric acid[d], Tryptophan[d]	13.3	13.2	+0.7

a : Trypthophan tablet(purchased from biogenesis laboratories, South Africa); b: Amino acid drug (purchased from razi company, Iran); c: Purchased in drugstores; d: Quantity not labeled

Since the sensitive of OPPy (that gained in optimum conditions) for Trp in differential pulse voltammetry (DPV) is several times higher than in CV, hence DPV was used to determination of this analyt in pharmaceutical samples. The results are shown in TABLE 2.

As shown in TABLE 2, the content of Trp in such samples determined by the present method using DPV at OPPy are in good agreement with those determined by indirect spectrophotometric method.

The main advantage is that the present method is faster and easier than the spectrophotometric procedure, which takes 50min just for the tryptophan derivatization step.

CONCLUSION

It is demonstrated that the electrochemical response for the oxidation of tryptophan is dramatically improved for electrodes coated with overoxidized poly-pyrrole coated glassy carbon when compared to bare glassy carbon electrodes.

The obtained results demonstrate that the electrode coated with overoxidized poly-pyrrole can be successfully used for the monitoring of some biological compound such as tryptophan. These modified electrode show advantageous electrochemical properties due to the electrocatalytic ability of the modified surface, which gives rise to the possibility of applying low potential and large current values for the voltammetric detection.

The advantage of the proposed method is the use of optimized conditions to fast, inexpensive and sensitive procedure for the determination of tryptophan in pharmaceutical formulations.

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