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Electrochemical Behavior of Oxytocin Hormone through its Cysteine Reduction Peak Using Glassy Carbon Electrode Modified with PolyFurfurylamine and Multi-Walled Carbon Nanotubes

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Abstract

Electrochemical behavior of oxytocin was studied using differential pulse voltammetric technique coupled with electrochemical sensor based on glassy carbon electrode (GCE) modified with multi-walled carbon nanotubes and polyfurfurylamine.

The electrochemical behavior of oxytocin was investigated on the GCE and modified GCE through its cysteine (oxytocin component) reduction peak. Cysteine gives well defiened reduction peak at (-0.4) V versus Ag/AgCl.sat.KCl.

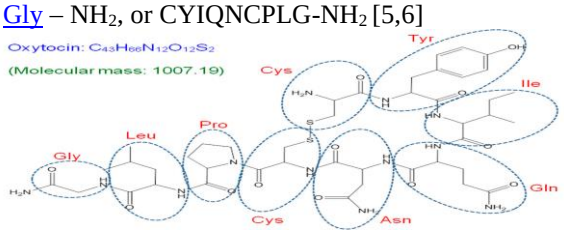
The optimum conditions were tested, the effect of pH was examined and the calibration curves were contructed, a plot of current versus concentrations gives a straight line with r^2 values 0.9938, 0.9835 and 0.9924, 0.9791 for GCE, Glassy Carbon Electrode/Poly Furfurylamine (GCE/PFA) and Glassy Carbon/ Multi-Walled Carbon Nanotubes/ poly Furfuryl Amine (GCE/MWCNTs/PFA) modified electrodes respectively. The GCE/MWCNTs/ PFA gives about three folds as much current as the GCE for a given oxytocin concentration .

Keywords: Oxytocin**,** Cysteine, Differential Pulse Voltammetric Technique, Conducting Polymer, Glassy Carbon Electrode, Multi- Walled Carbon Nanotubes, PolyFurfurylamine, Modifid Electrodes.

1. Introduction

Oxytocin a neuropeptide composed of nine amino acids, it is produced mainly in hypothalamic nuclei and released into central and peripheral tissues including the uterus, placenta, corpus leteum and heart [1]. In addition to its functions as a hormone in facilitating uterine contractions and mammalian milk ejection, it was also discovered that oxytocin works as a neurotransmitter and is considered to mediate social behavior, such as pair bonding and instinctive maternal aggression[2,3,4]. Oxytocin is derived by enzymatic splitting from the peptide precursor encoded by the human *OXT* gene. The deduced structure of the active nonapeptide is:

Cys – Tyr – Ile – Gln – Asn – Cys – Pro – Leu – Gly – NH_2, or CYIQNCPLG-NH_2 [5,6]



Oxytocin in biological fluids has been measured by different methods radioimmunoassay [7], enzyme immunoassay[8], high performance liquid chromatography (HPLC) [9] and LC plus tandem mass spectrometry (MS/MS) [10,11]. Although immunological methods are sensitive, they are relatively non-specific due to the cross-reactivity of the antibodies with structurally related peptides. In addition, HPLC methods with UV detection have low sensitivity in biological samples. Most of the above methods are time-consuming and needs sample preparation procedures, such as protein precipitation[12], solvent extraction [13], evaporation to dryness[12], immunoaffinity purification[7,9]. Gel chromatography[8,14], microdialysis[11] and solid-phase extraction (SPE) to concentrate oxytocin in plasma[8,12,20] .

Furthermore, of the twenty standard proteinogenic amino acids, only cysteine, tryptophan and tyrosine are known to be electrochemically active at moderate potential[15-18]. Therefore, peptides or proteins that contain at least one of these three amino acids in their structure can, theoretically, be detected

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electrochemically. However, only a few reports have been published regarding to the direct electrochemical detection of peptides or proteins[15,16,19,20], which might be due to the lower, passivation of the electrode surface due to adsorption of oxidized or polymerized protein molecules causes low reproducibility of the measurements[21].
In this work, the electrochemical behavior of oxytocin through its cysteine reduction peak has been examined on modified GCE in an attempt to improve the detection ability of bare GCE.

Experimental:

2. MATERIALS AND METHODS:

2.1. Apparatus:

Al elctrochemical measurements were performed using a797VA Computrace voltammetric analyzer supplied by Metrohm company, Switzerland, coupled with a three electrode cell, consists of solid electrodes GCE, GCE/PFA and GCE/MWCNTs/PFA as working electrodes, Ag/AgCl/sat.KCl as reference electrode and 1mm Pt-wire as an auxliary electrode. pH measurements were performed using HANNA digital pH meter model pH211 with accurate to ±0.05 supplied by HANNA Company, Portugal.

2.2. Reagents and Chemicals*:*

Oxytocin was purchased from Hopking and Williams British LTD. Freshly standard solution of Oxytocine was prepared in water. Phosphate buffer supporting electrolyte solution (PBs) (pH7) was prepared by mixing 30.5ml of K_2HPO_4 (0.2M) with 1.5ml of KH_2PO_4 (0.2M) then the volume completed to 100 ml in volumetric flask .

2.3.Procedure:

5ml Phosphate buffer solution (pH7) was added to voltammetric cell and dissolved oxygen was removed by passing N_2 gas for a period of 5 minutes prior the measurements, then the voltammogram was recorded. under the optimum conditions a voltamograms were recorded for sequence additions of Oxytocin stock solution and the calibration curves were constructed.

2.4.Fabrication of modified GCE:

To prepare modified GCE sensors, first" the bare GCE was polished with 0.05 µm of aluminium oxide powder, a polishing cloth, rinsed and ultrasonicated in water for 1min. [22,23].The poly furfurylamine was prepared in acetonitrile supporting electrolyte containing furfurylamine (0.01 mol L^{-1}), lithium perchlorate (0.06 mol L^{-1}) and sodium hydroxide(0.06 mol L^{-1}) [24].The electropolymerization performed using the cyclic voltammetric technique by scanning the potential between (0.5-1.8)V versus Ag/AgCl.sat.KCl for 5cycles with scan rate equal to 0.14 V/s, then the modified electrode was washed with water .

2.5.Construction of GCE/MWCNTs /PFA Electrode:

The cleaned GCE was electrochemically activated in a 0.1 µmol L^{-1} H_2SO_4 solution by scanning the potential 20 cycles between (-0.5 – 2.0)V. A 20 µL of the freshly prepared (5mg/5ml) dispersion of MWCNTs /DMF was dropped onto the GCE surface and the solvent was evaporated with IR lamp[25].Then MWCNTs –modified electrode was further modified with a thin film by placing 10µL of PFA solution over the surface of the electrode and evaporating the solvent in air .

3.RESULTS AND DISCUSSION*:*

3.1 Oxytocine Electrochemical Behaviour on GCE:

A voltammogram of (31.37×10^{-9} M) oxytocin was recorded using DPV under the default conditions of the instrument . Oxytocin gives reduction peak at (-0.4) V which belongs to its cysteine component (figure 3-2a) [26].

Table 3-1: Default conditions of 797-VA Computrace

Parameters	Default Condition Values
Deposition Potential	-0.9 V
Deposition Time	60s
Equilibration Time	5s
Pulse Amplitude	0.05V
Pulse Time	0.04s
Voltage Step	0.006V
Voltage Step Time	0.4s
Sweep rate	0.0150V/s

3.1.1 Effect of pH:

The effect of pH on the cysteine reduction peak potential (a component of oxytocin) (Ep) and peak current (Ip) has been examined using different pHs (6-8), the default conditions and 31.37×10^{-9} M of oxytocin concentration. The results (table 3-2) shows that the higher diffusion current was at pH7. The plot of pH versus Ep (figure 3-2) gives a straight line with intercept value equal to (-0.0606) which is close to the thioretical value calculated by Hillson, this indicates that the reduction process involved one electron reduction[27] .

Table 3-2

pH	Ep(V)	Ip(µA)
6	-0.260	9.39
6.5	-0.272	10.2
7	**-0.301**	**12.8**
7.5	-0.337	11.7
8	-0.379	12.4

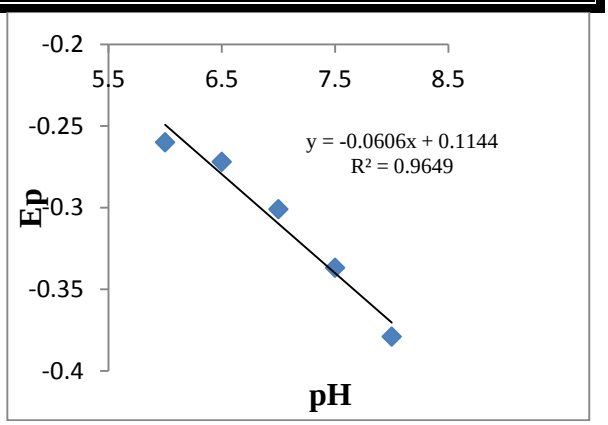


Figure 3-1

3.1.2 Optimum conditions :

To increase the sensitivity of the sensor, optimum conditions were examined using 31.37×10^{-9} M oxytocin in phosphate buffer (pH7) , the results are shown in table (3-3) and the voltammograms before and after optimum conditions are shown in figure (3-2) .

3.1.3 Stability of oxytocine at GCE:

The stability of cystein-oxytocin reduction peak was tested by the recording of voltammogam in different time intervals under the mentioned optimum conditions (Table3-2), the results obtained are shown in (Table 3-4) , it is clear that the reduction peak current is stable within the measurement time.

Table (3-3):oxytocin optimum conditions in phosphate buffer solution (pH7) at GCE

Parameters	Condition Values
Deposition Potential	-0.8 V
Deposition Time	60s
Equilibration Time	15s
Pulse Amplitude	0.08V
Pulse Time	0.02s
Voltage Step	0.007V
Voltage Step Time	0.4s
Sweep rate	0.0175V/s

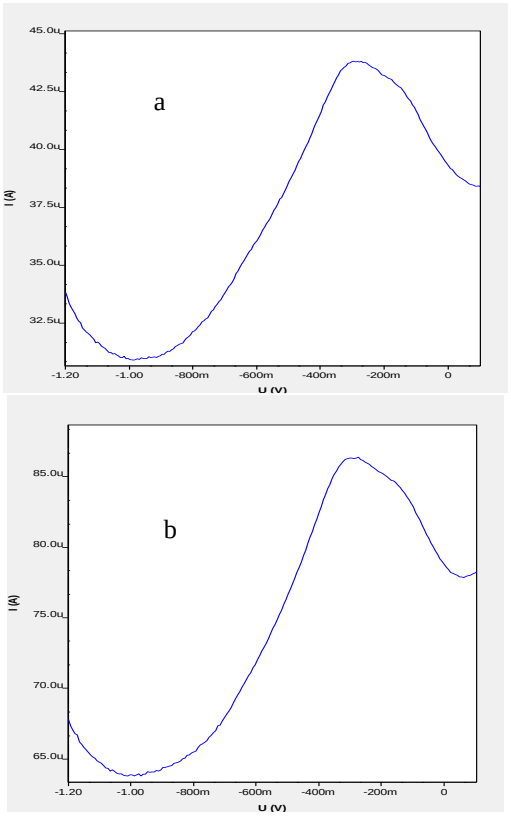


Figure 3-2 voltammogram of oxytocin in phosphate buffer solution (pH7) at GCE
a-before the optimum Conditions b- after the optimum Conditions

Table 3-4: stability of oxytocin peak at GCE

Time (min.)	Ip(µA)
0	11.3
5	11.4
10	11.6
15	11.7
20	11.9
25	11.9
30	12.0
S.D	±0.26726

3.1.4.Calibration Curve :

The DP-Vltammograms were recorded for sequence additions of oxytocin (47.8-329) $\times10^{-10}$ M under the previous mentioned optimum conditions.The responses of peak current towards Oxytocin concentrations were linear in the studied concentrations range (figure 3-3) by a calibration equation Ip = 0.0039 $C_{Oxytocin}$ – 0.0155 and regression coefficient of r^2 =0.9938.

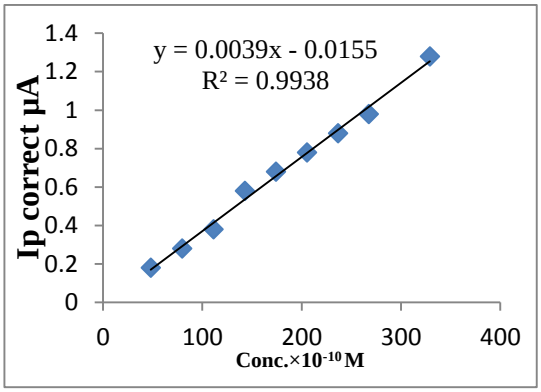
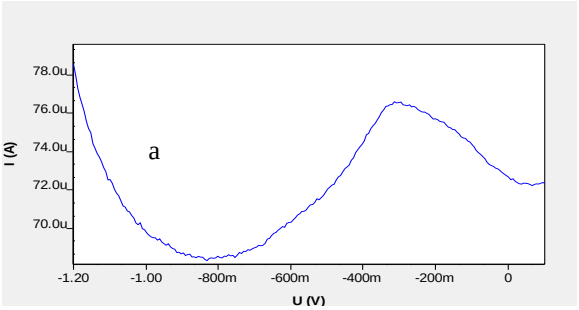


Figure 3-3 calibration curve of oxytocin

Electrochemical Behavior of Oxytocin at GCE/PFA Modified Electrode:

3.1.5 Optimum conditions :

To optimize the conditions of measurements, various instrumental and experimental variables were tested using 31.37×10^{-9} M of oxytocin in phosphate buffer (pH7), the results are shown in table (3-6) and the voltammogram before and after optimum conditions is shown in figure (3-4), it is clear that the current at GCE/PFA under the optimum conditions is higher than that before the optimum conditions .



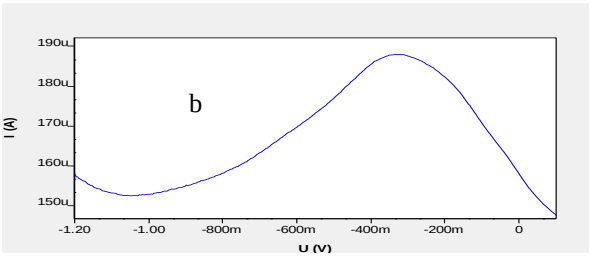


Figure 3-4 voltammogram of oxytocin in phosphate buffer solution (pH7) at GCE/PFA
a - before the optimum Conditions
b- after the optimum Conditions

3.1.6. Stability of oxytocin at GCE/PFA:

The stability of the modified electrodes was examined for a period of time. DPVs showed that the reduction peak of cystein–oxytocin is stable within the studied time which indicates suitable stability of the performed sensors.

Table (3-6):oxytocin optimum conditions in phosphate buffer solution (pH7) at GCE/PFA

Parameters	Condition Values
Deposition Potential	0.7 V
Deposition Time	10s
Equilibration Time	30s
Pulse Amplitude	0.11V
Pulse Time	0.05s
Voltage Step	0.004V
Voltage Step Time	0.1s
Sweep rate	0.04V/s

Table 3-6: stability of oxytocin peak at GCE/PFA

Time (min.)	Ip(µA)
0	39.5
5	39.7
10	39.9
15	39.5
20	40.0
25	40.1
30	40.2
35	40.3
40	40.4
45	40.5
50	40.5
55	40.7
60	40.7
S.D	±0.41556

3.1.7. Calibration Curve :

The DP-Vltammograms were recorded for sequence additions of oxytocin (31.9-375) $\times10^{-10}$ M under the measured optimum conditions, The responses of peak current towards oxytocin concentrations were linear in the studied concentrations range (figure 3-5) by a calibration equation Ip = 0.016 $C_{Oxytocin}$ + 0.0004 and regression coefficient of r^2 =0.9835.

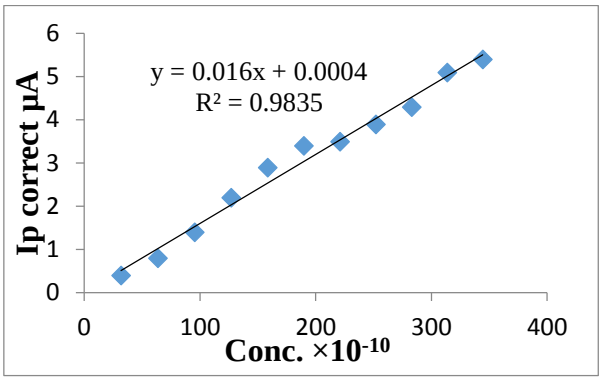


Figure 3-5 calibration curve of oxytocin peak

Electrochemical Behavior of Oxytocin at GCE/MWCNs/PFA Modified Electrode:

3.1.8 Optimum conditions :

To optimize the conditions of measurements, various instrumental and experimental variables were tested using 31.37×10^{-9} M of oxytocin in phosphate buffer (pH7), the results are shown in table (3-9) and the voltammogram before and after optimum conditions is shown in figure (3-6)

.Table (3-9):oxytocin optimum conditions in phosphate buffer solution (pH7) at GCE/MWCNTs/PFA

Parameters	Condition Values
Deposition Potential	0.5V
Deposition Time	35s
Equilibration Time	15s
Pulse Amplitude	0.1V
Pulse Time	0.05s
Voltage Step	0.005V
Voltage Step Time	0.1s
Sweep rate	0.05V/s

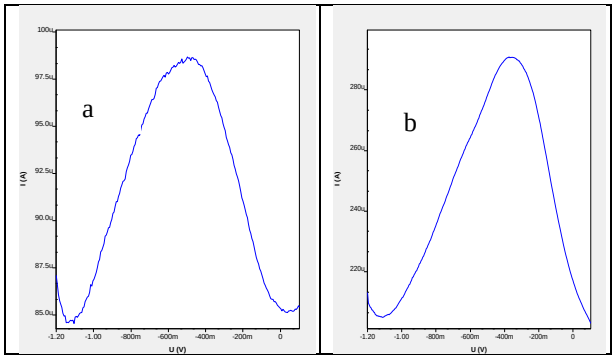


Figure 3-6 voltammogram of oxytocin in phosphate buffer solution (pH7) at GCE /MWCNTs/PFA

a-before the optimum Conditions b-after the optimum Conditions

3.1.9 Stability of oxytocine at CE/MWCNTs/PFA:

Table : 3-10 stability of oxytocin peak at GCE/MWCNTs/PFA

Time (min.)	Ip(μA)
0	85.9
5	86.3
10	86.4
15	87.0
20	87.4
25	87.7
30	88.6
35	87.9
40	87.5
45	87.4
50	87.4
55	87.6
60	87.6
S.D	±0.72554

3.1.10. Calibration Curve :

The DP-Vltammograms were recorded for sequence additions of oxytocin (15.9-298.3) $\times10^{-10}$ M under the measured optimum conditions. A plot of current ($Ip_{correct}$) versus concentration gives two straight lines, first at low concentrations range (15.9-79.6)$\times10^{-10}$ M fig(3-7a) and second at higher concentrations range (95.4-298.3) $\times10^{-10}$ M fig(3-7b) by a calibration equations Ip = 0.0942 $C_{Oxytocin}$ - 0.0005 with regression coefficient of r^2 =0.9924 and Ip = 0.0455$C_{Oxytocin}$ + 0.0005 with regression coefficient of r^2 =0.9641 respectively .

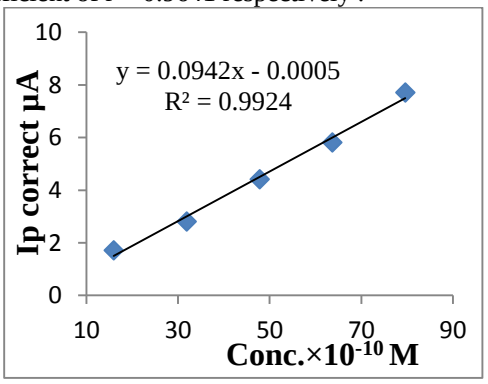


Figure :(3-7a) calibration curve of oxytocine peack at low concentratios

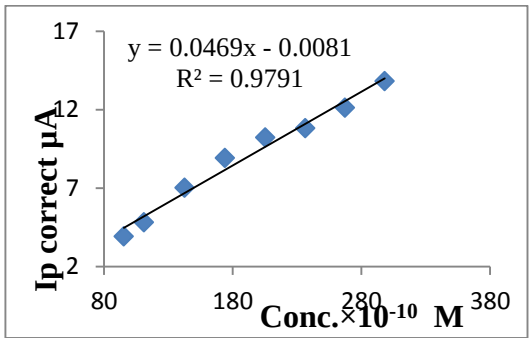


Figure: (3-7b) calibration curve of oxytocine peak at high concentrations

Conclusions:

1- The modification of GCE leads to improve in electrode response towards the cysteine –oxytocin reduction peak.

2- According to the table (3-11) ,the slope of calibration equation increase from GCE to GCE/MWCNTs/PFA that means the sensitivity of the measurement also increased in the same order.

3- The GCE/MWCNTs/PFA about three times as much sensitive as the GCE .

4- The GCE/MWCNTs/PFA gives about ten times as much current as the GCE for a given oxytocin concentration.

Table : 3-11 Comparison between GCE and modified GCE

Oxytocin	CGE	CGE/PFA	GCE/MWCNTs/PFA
Equation	y = 0.0039x -0.0155	y= 0.016x +0.0004	y = 0.0942x - 0.0005 y = 0.0469x - 0.0081
r^2	r^2 = 0.9938	r^2 = 0.9835	r^2 = 0.9924 r^2 = 0.9791
Concentration	(47.8-329) $\times 10^{-10}$ M	(31.9-375) $\times 10^{-10}$ M	(15.9-79.6)$\times 10^{-10}$ M (95.4-298.3) $\times 10^{-10}$M

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