

Investigation of 5-Hydroxytryptophol Electrooxidation

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In the present study the electrochemical oxidation of 5-hydroxytryptophol, a normal metabolite of the indol amines 5-hydroxytryptophan and 5-hydroxytryptamine was investigated using various electrodes in different electrolyte solutions. It was concluded that the electrooxidation mechanism depended on the electrolyte, pH, scan rate and the nature of the electrode.

Key Words : Electrooxidation, 5-Hydroxytryptophol, Solid electrodes.

The aromatic amino acid L-tryptophan can be metabolized in animals by hydroxylation to 5-hydroxytryptophan (5-HTPP) by the enzyme tryptophan hydroxylase. Then 5-HTPP is decarboxylated by 5-hydroxytryptophan decarboxylase to produce 5-hydroxytryptamine(5-HT) . This mechanism is called 5-hydroxyindole route¹. 5-hydroxytryptophol (5-HTOL) is a normal metabolite of the above indolamines .According to the recent reports a faulty mechanism in the 5-hydroxyindole route in the central nervous system might results severe depression and schizophrenia².

The electrochemical oxidations of 5-HTPP, 5-HT and 5-HTOL had been investigated by Dryhurst et all.^{1,3,4} to have some information about their biochemical oxidations . The results of Dryhurst's investigations show that the oxidation mechanisms of these substances vary depending on the conditions ie in the case of. 5-HTOL it was found that in acidic solutions of pH 2 with pyrolytic graphite electrode the major product of the electrooxidation was defined by the initial concentration of 5-HTOL . In the present study the effects of the electrolyte, pH of the solution , electrode material and the potential scan rate on the electrooxidation of 5-HTOL were investigated.

EXPERIMENTAL

5-Hydroxytryptophol (5-HTOL) was obtained from Sigma. A Tacussel PRG-3 polarograph with an EPL-2 recorder was used for recording the cyclic voltammograms for the scan rates up to 100 mVs^{-1} and a Wenking 81-PGS potentiogalvano Scan potentiometer with Hewlett Packard 7003-B XY recorder was used for the higher scan rates.

The tests were carried out in a three compartment cell containing a platinum counter electrode and a saturated calomel reference electrode (Tacussel XR-100), a Pt wire 1 mm in diameter, 15.7 mm in length, a Ru (Ruthenium) wire 1 mm in diameter, 17.9 mm in length, a G.C.(glassy carbon) rod (Tacussel XM-540) 3mm in diameter and 4.2 mm in length, and a C.P.(carbon paste) disc electrode (75.42 mm^2) were used as working electrodes. The carbon paste electrode and modified carbon paste electrode were prepared in our laboratory, the former was prepared by mixing nujol oil (Sigma) with graphite (Aldrich 1 to 2μ) and the later was obtained by the addition of poly vinyl imidazol to the carbon paste mixture.

All the chemicals were of analytical grade. The test solution was deaerated with nitrogen before each test. All potentials were referred to the normal hydrogen electrode.

Pretreatment of the electrodes:

To obtain reproducible results working electrodes were electrochemically pretreated in $0.5 \text{ M H}_2\text{SO}_4$ solution. Pt electrode was oxidised by the application of 1750 mV for 5 minutes at the first step and then a potential of 150 mV was applied until the current became zero.

The surface of the Ru electrode was prepared for the experiment by keeping the electrode at -270 mV for 5 minutes and then by applying a potential of $+150 \text{ mV}$ for 15 minutes.

Glassy carbon electrode was pretreated at two main steps. The first one which contains a few steps, among them the application of high frequency (3500Hz) triangular potential pulse was the most important step, produced an active and permanent surface state (nearly for 40 experiment). The detailed explanation of this procedure was given elsewhere⁵. The second main step consisted of the application of potentials of 1500 mV for 5 minutes and -1000 mV for 2-3 second respectively in 0.1 M KNO_3 solution before each experiment.

RESULTS and DISCUSSION

Fu Chan Cheng et al³ investigated the oxidation of 5-HTOL by cyclic voltammetry using a pyrolytic graphite electrode and applying constant potential electrolysis in acidic solutions and they identified the reaction products spectroscopically and on the bases of the data they proposed three different mechanisms. Two of them involves two successive deprotonation steps and two successive reduction steps each involves one electron and then a dimerisation occurs. According to the third mechanism an initial one electron abstraction is followed by water addition and a deprotonation step, finally an equilibrium state related to quinoid and benzoid structures is reached. Since relative yields of the oxidation products depend on the concentration of 5-HTOL the favoured mechanism is determined by 5-HTOL concentration.

In the present study voltammograms were recorded with various scan rates.

A. Voltammograms recorded with the scan rate of lower than 200 mVs^{-1} :

In Fig.1 a,b,c and d the curves obtained using Pt, Ru, G.C, CP and modified C.P were shown respectively. The scan rate was 100 mVs^{-1} . It is clear that Ru is not a suitable electrode for the oxidation of 5-HTOL, because the oxidation partly takes place at the potentials that the structure of the chemisorbed film on the Ru surface changed⁶. With Pt electrode two oxidation steps occur. The first one is at about 800 mV where the formation of chemisorbed PtO film begins and the second is at 1150 mV where the stable PtO film forms⁶. Similar voltammograms were obtained with the scan rate of 50 mVs^{-1} . It can be deduced that chemisorbed oxide films don't effect the reduction steps since no reduction step appears related to the reduction of 5-HTOL itself and its oxidation products. There are two oxidation steps on the curves obtained with G.C electrode. The potential of the first step varies depending on the nature of the anion in the solution. But the beginning of the second step is at 1150 mV as in the case of Pt electrode and independent on the composition of the solution. With the C.P and modified CP electrodes only one oxidation step with a long broad limiting current region occur and no reduction step forms.

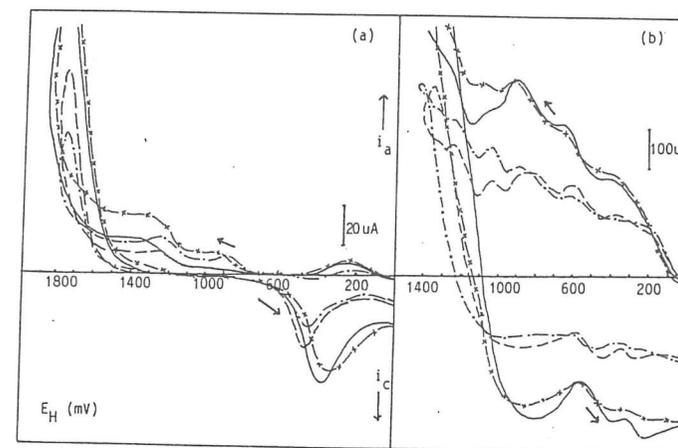


Figure 1 : Voltammograms obtained using (a) a Pt electrode in H_2SO_4 solution (pH 1.8) (b) a Ru electrode in HCl supporting electrolyte (pH 2). (----) 50 mVs^{-1} , (—) 100 mVs^{-1} in supporting electrolyte. (---) 50 mVs^{-1} , (-x-x-) 100 mVs^{-1} in the solutions containing 10^{-3} M 5-HTOL.

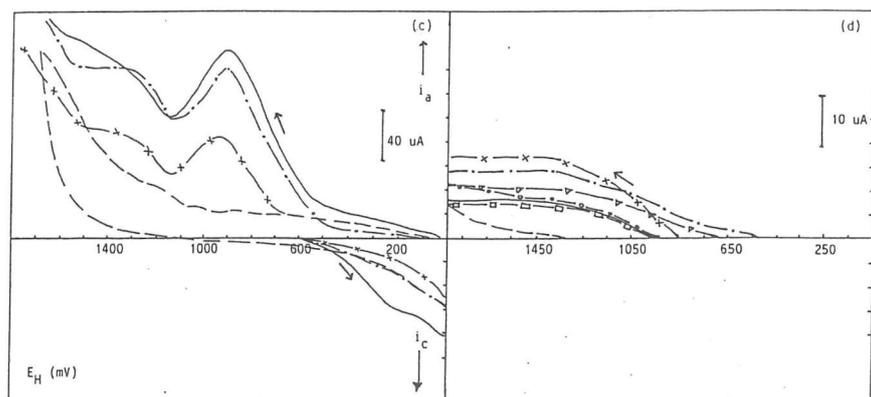


Figure 1 : Voltammograms obtained in different supporting electrolytes using (c) a G.C. electrode at a scan rate of 100 mVs^{-1} . (----) supporting electrolyte; (-x-x-) pH 2 phosphate buffer, (—) HCl solutions (pH 2), (-·-·-) H_2SO_4 solution (pH 1.8) , in the solutions containing 10^{-3} M 5-HTOL. (d) C.P. and modified C.P. electrode at a scan rate of 100 mVs^{-1} . (----) supporting electrolyte; (—) HCl solutions (pH 2), (-x-x-) H_2SO_4 solution (pH 1.8) , (-Δ-Δ-) pH 2 phosphate buffer, in the solutions containing 10^{-3} M 5-HTOL with C.P. electrode. (-□-□) HCl solution (pH 2), (-o-o-) H_2SO_4 solution (pH 1.8), (-·-·-) pH 2 phosphate buffer, in the solutions containing 10^{-3} M 5-HTOL with modified C.P. electrode.

B. Voltammograms at the sweep rate of $\geq 200 \text{ mVs}^{-1}$:

The examination of the curves in Fig 2 reveals that 200 mVs^{-1} is a convenient scan rate for the investigation of the intermediate steps for the oxidation of 5-HTOL with C.P. electrode. Although at the scan rate of 100 mVs^{-1} there is only one step the beginning potential of which changes with the nature of the electrolyte (Fig 1d) at a scan rate of 200 mVs^{-1} (Fig 2a) the voltammograms have two peaks at the anodic branch at the more positive potentials than 600 mV. After scan reversal two reduction peaks appear (R_1, R_2). On the second anodic sweep two new oxidation peaks (O_2, O'_2) which are symmetrical according to the R_1 and R_2 , appear before the first oxidation peak O_1 on the first scan.

The curves obtained with G.C. electrode at the same scan rate with those in curve 2 a are given in Fig 2 b. Here O_2, O'_2 appear on the second sweep as with C.P. electrode (Fig 2a) and peak O_1 (Fig 2c) is also similar to the peak O_1 on the curves of C.P. electrode (Fig 2a). When the initial anodic scan was reversed at the foot of peak O_1 two peaks R_1 and R_2 appear (Fig 2c). This reveals that R_1 and R_2 must be related to the reduction of the products formed on the anodic sweep at O_1 . Fig 2d and 2e were obtained with C.P. electrode as in Fig 2a and at the same scan rate but in different electrolyte solutions to show the effect of the nature of the electrolyte on the oxidation of 5-HTOL. Comparison of Fig 2a, 2d and 2e reveals that well defined successive steps can be obtained in phosphate and H_2SO_4 solutions. With G.C. electrode in H_2SO_4 solution on the second sweep only one peak appears before O_1 (Fig 2f).

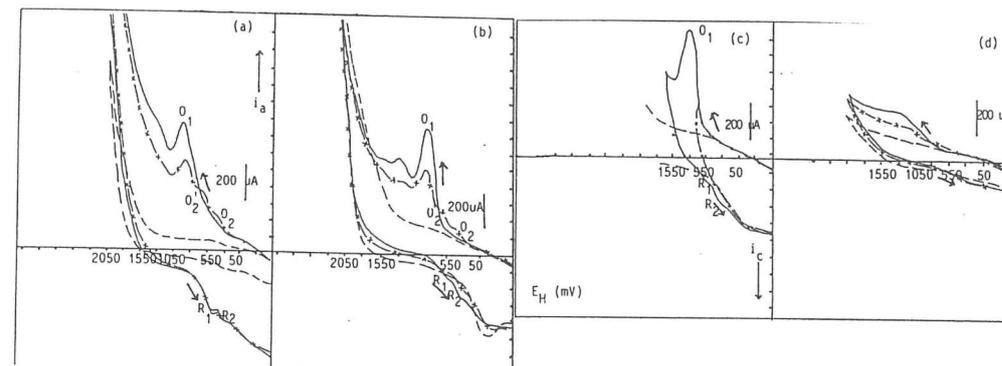


Figure 2 : Voltammograms obtained in pH 2 phosphate buffer using (a) a C.P. electrode (b) a G.C. electrode at a scan rate of 200 mVs^{-1} . (----) supporting electrolyte; (—) 10^{-3} M 5-HTOL (first sweep) (-x-x-) 10^{-3} M 5-HTOL (second sweep) (c) a G.C. electrode at a scan rate of 200 mVs^{-1} . (----) supporting electrolyte; (—) reversed at 1100 mV (-·-·-) reversed at 700 mV (d) Voltammograms obtained using C.P. electrode in HCl supporting electrolyte (pH 2) at a scan rate of 200 mVs^{-1} . (----) supporting electrolyte; (—) 10^{-3} M 5-HTOL (first sweep) (-x-x-) 10^{-3} M 5-HTOL (second sweep)

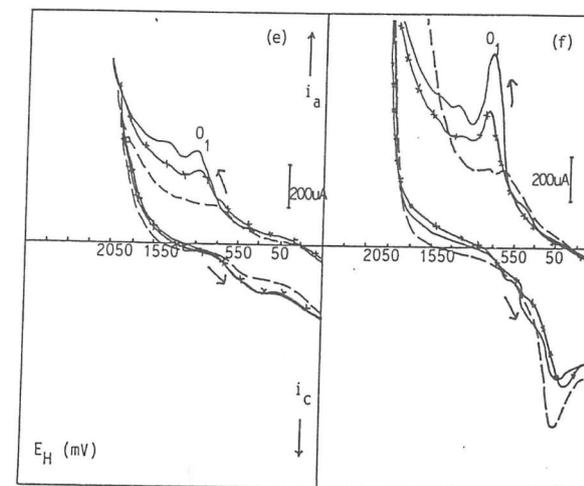


Figure 2 : Voltammograms obtained in H_2SO_4 supporting electrolyte (pH 1.8) using (e) a C.P. electrode (f) a G.C. electrode at a scan rate of 200 mVs^{-1} . (----) supporting electrolyte; (—) 10^{-3} M 5-HTOL (first sweep) (-x-x-) 10^{-3} M 5-HTOL (second sweep)

The curves in Fig 2g was obtained with Pt electrode. Also at this sweep rate it is clearly seen that the oxidation takes place at the potentials of chemisorbed oxide film formation⁶. The voltammograms obtained with G.C electrode at the sweep rates of 400 mVs⁻¹ and 600 mVs⁻¹ are shown in Fig 3. With C.P electrode under the same conditions similar curves were obtained. The linear dependence of the peak current of O₁, on the first sweep, on the square root of scan rate ($\gamma^{1/2}$) indicates that the first oxidation step of 5-HTOL is diffusion controlled.

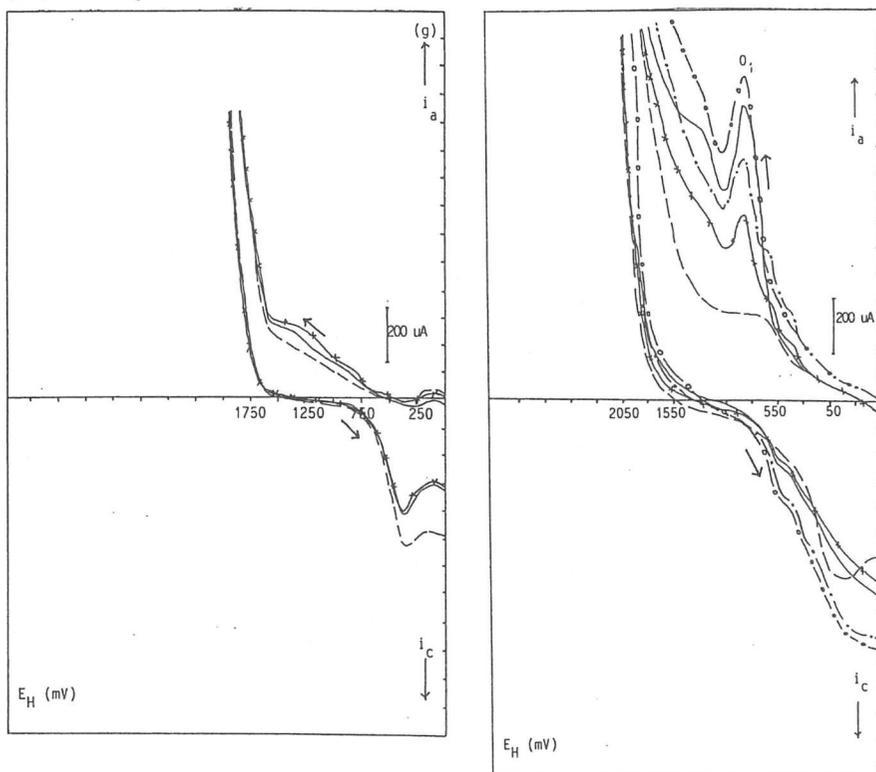


Figure 2 g: Voltammograms obtained using Pt electrode in pH 2 phosphate buffer at a scan rate of 200 mVs⁻¹. (----) supporting electrolyte; (—) 10⁻³ M 5-HTOL (first sweep) (-x-x) 10⁻³ M 5-HTOL (second sweep).

Figure 3: Voltammograms obtained G.C. electrode in pH 2 phosphate buffer at the scan rates of 400 mVs⁻¹ and 600 mVs⁻¹. (----) supporting electrolyte; (—) 10⁻³ M 5-HTOL (first sweep for 400 mVs⁻¹) (-x-x) 10⁻³ M 5-HTOL (second sweep for 400 mVs⁻¹) (-o-o-) 10⁻³ M 5-HTOL (first sweep for 600 mVs⁻¹) (-.-.-) 10⁻³ M 5-HTOL (second sweep for 600 mVs⁻¹).

Effect of the solution pH:

The curves in Fig 4a obtained with C.P electrode and in Fig 4b obtained with Pt electrode at a scan rate of 100 mVs⁻¹ in the solutions of various pHs. With C.P electrode in the solutions of pH < 6.4 limiting currents appear. These one step oxidation curves must show a potential shift depending on the pH if the reaction mechanism depends on pH.

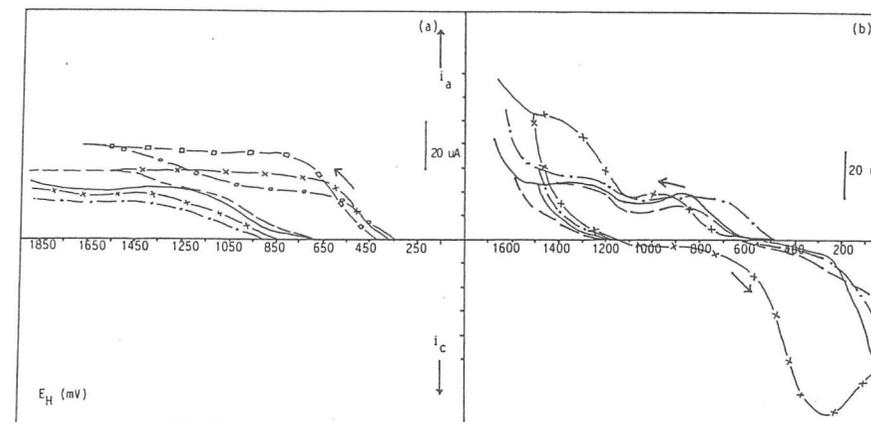
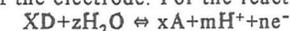


Figure 4: Voltammetric curves obtained in different supporting electrolytes using (a) a Carbon paste and modified carbon paste electrode at a scan rate of 100 mVs⁻¹ (—) pH 3.7 acetate buffer, (-□-□) pH 5.2 acetate buffer, (-x-x-) pH 2 phosphate buffer, (-o-o-) pH 6.4 phosphate buffer, in the solutions containing 10⁻³ M 5-HTOL with C.P. electrode. (-.-.-) pH 3.7 acetate buffer, (----) pH 2 phosphate buffer, (*-*) pH 6.4 phosphate buffer, in the solutions containing 10⁻³ M 5-HTOL with modified C.P. electrode. (b) a Pt electrode at a scan rate of 100 mVs⁻¹. (-x-x) pH 2 HCl, (—) pH 5.2 acetate buffer, (----) pH 6.4 phosphate buffer, (-.-.-) pH 7.8 phosphate buffer in the solutions containing 10⁻³ M 5-HTOL.

The changes in the step potentials may be related to the anion adsorption which changes the catalytic activity of the electrode. For the reactions represented by



type electrochemical equilibrium the electrode potential must shift to less positive values as $n/m \times 0.059$ V for unit increase in pH.

This shift was observed in the case of Pt electrode (Fig.4b). The kinetics of chemisorbed film formation on Pt electrode surface can be shown by the chemical equation of⁶



if the oxidation of 5-HTOL takes place via PtO the shift in oxidation step potential depending on the pH can be explained. Fig. 5a and 5b show the curves obtained using G.C. and C.P. electrodes in the solutions of different pHs at a sweep rate of 200 mVs⁻¹. On the curves obtained in the solutions having pH > 2 no well defined reduction steps can be observed. And the potential of the first peak O₁ doesn't show a regular shift with pH. This can be explained by the change in mechanism with pH. When pH of the solution exceeds 9, 5-HTOL decomposes.

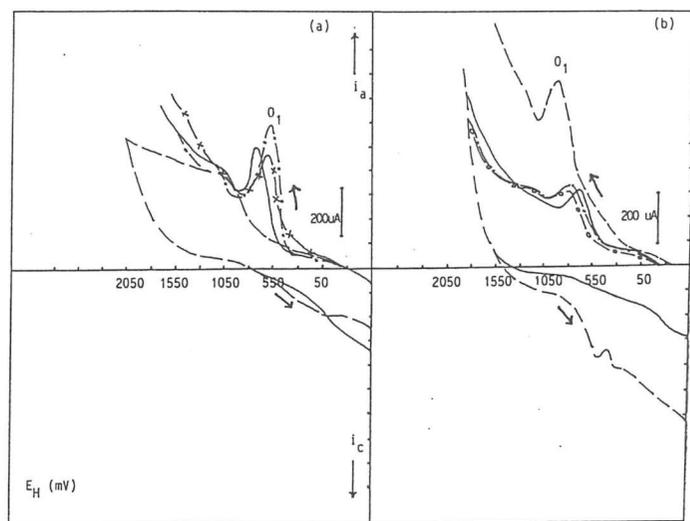


Figure 5 : Voltammograms obtained in different supporting electrolytes using (a) a G.C. electrode at a scan rate of 200 mVs⁻¹. (----) pH 3.7 acetate buffer, (—) pH 5.2 acetate buffer, (-·-·-) pH 6.4 phosphate buffer, (-x-x-) pH 7.8 phosphate buffer, in the solutions containing 10⁻³ M 5-HTOL. (b) a C.P. electrode at a scan rate of 200 mVs⁻¹. (----) pH 2 HCl, (—) pH 3.7 acetate buffer, (-o-o-) pH 5.2 acetate buffer, (-·-·-) pH 6.4 phosphate buffer, in the solutions containing 10⁻³ M 5-HTOL.

CONCLUSION

The examination of the voltammograms reveals that the oxidation mechanism of 5-HTOL is very complicated. Among the electrodes used in the present study the most convenient ones are GC and CP and the proper pH is 2.

The disappearance of the reduction steps with the increase in pH suggests that in these media the oxidation products become inactive in the potential range under study.

In the solutions having pHs of higher than 9, 5-HTOL decomposes.

The graph of the peak potential of O₁, on the curves obtained in H₂SO₄ solutions of pH 1.8 with G.C electrode against, log γ (log sweep rate) gives a straight line the slope of which is 85 mV.

Chu Cheng et al³ similarly obtained a linear relation between E_p and log γ in phosphate buffer of pH 2 with pyrolytic graphite electrode but they found a slope smaller than in our case.

This difference may be because of difference in the electrodes and the electrolytes in the two studies.

With G.C electrode in H₂SO₄ solution of pH 1.8 (scan rate 200 mVs⁻¹) the peak current of the oxidation peak of O₁ is found as linearly depended on the concentration of 5-HTOL (10⁻⁴-10⁻³ M) the statistical analysis of this dependence is given in Table-I. It is clear that the quantitative determination of 5-HTOL can be made under these conditions.

Table I: Result of the linear regression analysis of concentration-peak current relations of 5-HTOL obtained with G.C. electrode in H₂SO₄ solution (pH 1.8).

Correlation coefficient :	0.9976
Slope :	5.38 x 10 ⁵
y-Intercept :	106.85

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