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Electrochemical Oxidation of 4-Hydroxyindole and Effects of Its Oxidation Products on Blood Parameters of Albino Mice

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Electrochemical oxidation of 4-hydroxyindole has been studied at pyrolytic graphite electrode in aqueous phsophate buffers of pH 2.1–10.1. Oxidation has been found to proceed in a single well-defined peak. The number of electrons involved under CV conditions were found as 1.3 ± 0.1 and were different than CPE (2.7 ± 0.2). The spectral studies during oxidation indicated that no UV-absorbing intermediate is generated in the reaction. The products of oxidation were separated by HPLC and characterized by m.p., ^{1}H NMR, and mass spectra as a hydroxy tetramer. A single dose of the oxidation product at LD_{50} value on intracranial injection in albino mice produced substantial changes in blood parameters and indicated liver disorder and hyperthyroidism conditions.

Key Words: electroxidation; 4-hydroxyindole; toxicity.

1. INTRODUCTION

Indole and its derivatives have been reported to produce long-lasting depletions of serotonin in brain tissues (*1,2*). Biochemical evidences suggest that depletion occurs mainly due to degeneration of axons and terminals of serotonin containing neurons (*3,4*). Hydroxy derivatives of indole, particularly 5,6-dihydroxytryptamine, have shown an affinity to serotonin uptake system and produce cytotoxic effects (*5*), which have been assigned to the formation of aminochrome of the indole type by oxidation (*6*). In spite of their toxicity, indole derivatives have been used in the treatment of osteoporosis (*7*) and antithromotics (*8*) and as allergy inhibitors (*8*). Audia *et al.* (*9*) used 4-hydroxyindole in the preparation of 3-(4-indolyloxy)-2-hydroxypropanamines, as a serotonin I_A receptor antagonist and partial agonist, and found it useful for alleviating the symptoms of nicotine and tabacco withdrawal and the treatment of depression, anxiety, hypertension, etc. Hydroxyindoles are the precursor of indolic neurotransmitter 5-hydroxytryptamine, and abnormal oxidation chemistry or biochemistry of indolic neurotransmitters has been implicated in the etiology of several psychotic diseases, such as schizophrenia, major depression, and neurodegenerative Alzheimer's disease. Recently our laboratory initiated electrochemical oxidation studies on indole (*10*). It was observed that oxidation of indole yields trimer as the major

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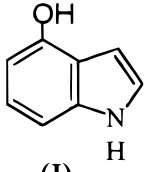
product of oxidation in aqueous phosphate buffers. Since many hydroxyindole derivatives are formed during the oxidation of indolic neurotransmitters, it was considered interesting to study the oxidation behavior of hydroxyindoles.

This paper presents oxidation chemistry of 4-hydroxyindole (I), used as a precursor in the composition of oxidative hair dyes. It is anticipated that the results obtained will provide deep insights into oxidative chemistry of indole derivatives used as neurotransmitters.



(I)

2. EXPERIMENTAL

4-Hydroxyindole was obtained from Sigma Chemical Co. (U.S.A.) and was used as received. The diagnostic kits for the determination of urea, cholesterol, alanine transaminase (ALT), aspartate transaminase (AST), blood sugar, and alkaline phosphatase of serum were obtained from SPAN Diagnostics Ltd. (Surat), India. Phosphate buffers used in this investigation were prepared by the method reported in literature (*11*) and had an ionic strength of 0.5 M. The equipments used for linear and cyclic voltammetry, controlled potential electrolysis and coulometry were essentially the same as reported earlier (*12,13*). UV spectral studies during oxidation of 4-hydroxyindole were carried out using Hitachi-3200 spectrophotometer.

Pyrolytic graphite electrode (area $\sim$0.4 cm^2), used as a working electrode, was prepared in the laboratory by the method reported earlier (*14*). Platinum wire and SCE were used as auxiliary and reference electrode, respectively. The value of *n*, the number of electrons involved in the oxidation were determined by connecting a coulometer in series during controlled potential electrolysis, whereas, to achieve complete oxidation in a short period of time electrolysis was carried out in thin layer cell (volume 258 μl) as described earlier (*15*).

3. PROCEDURE

A stock solution of 4-hydroxyindole (1.0 mM) was prepared in double-distilled water. For recording voltammograms, 5.0 ml of the stock solution was mixed with 5.0 ml of phosphate buffer (ionic strength = 1.0 M) of appropriate pH, so that the overall ionic strength of the solution became 0.5 M. Voltammograms were recorded after passing nitrogen gas for 10–12 min.

Controlled potential electrolysis was carried out in a conventional three compartment cell using platinum gauge as auxiliary electrode, SCE as reference electrode, and pyrolytic graphite plate (area 6.0 $\times$ 1.0 cm^2) as a working electrode. Nitrogen atmosphere was also continously maintained during the course of controlled potential electrolysis.

The products of electrooxidation of compound I were analyzed using High performence liquid chromatography (HPLC), employing a Perkin–Elmer Unit (Model

SDD 10A). For this purpose, exhaustively electrolyzed solution of 4-hydroxyindole was lyophilized and the freeze dried material was extracted with methanol (AR, 2 × 10 ml). The buffer constituents were insoluble and were separated by filtration using millipore membrane filter (4 μm). The methanolic solution of the oxidation products was once again filtered and 10 μl of the solution was injected in a reversed phase (C_{18}) column to which precolumn was attached. The mobile phase solvent used was HPLC grade methanol (E. Merck) at the rate of 0.5 ml/min. The absorbance of the eluent was determined at 260 nm. The column was equilibrated for 10 min before the next injection was made. The volume collected under HPLC peak 3 after several injections was again lyophilized and the greenish black coloured material obtained was characterized using FT-IR, ^{1}HNMR, and mass spectra. The mass spectrum of the material was recorded using a JEOL, JMS D 300 mass spectrometer and ^{1}HNMR spectrum was recorded with a Bruker AC 300 F instrument.

4. BIOLOGICAL STUDIES

In vivo experiments employed outbred male mice (25–30 g) of ICR albino strain, which were obtained from the animal house of Indian Drugs and Pharmaceuticals Ltd. (Rishikesh). These mice were housed six per cage and allowed access to food and water *ad libitum*. The mice were allowed to adjust in the laboratory environment for 7 days and then were used in experiments. Control animals were treated with 10 μl of isotonic saline (0.9% NaCl) containing 1 mg/ml of ascorbic acid, whereas various doses of product of oxidation were administered by dissolving them in isotonic saline. Light ether anesthesia was given before injection and all injections were made intracranially in the vicinity of the left lateral ventricle using a 10-μl Hamilton syringe. A teflon stopper was placed on the injection needle so that the insertion depth remained constant (3 mm) in all the injections.

5. RESULTS AND DISCUSSION

Linear sweep voltammetry of 4-hydroxyindole (0.5 mM) at a sweep rate of 10 mVs^{-1} exhibited a well-defined oxidation peak (1_a) in the entire pH range of 2.1–10.1. The peak potential of peak 1_a was found to be dependent on pH and shifted to less positive potential with increase in pH. The E_p vs pH plot was linear (Fig. 1) and the dependence of E_p on pH can be expressed by the relation

$$E_p \text{ (pH 2.1–10.6)} = [560 - 50 \text{ pH}] \text{ mV vs SCE}$$

In cyclic sweep voltammetry at a sweep rate of 100 mVs^{-1}, 4-hydroxyindole exhibited one well-defined peak 1_a when sweep was initiated in the positive direction. When the direction of sweep was reversed after scanning peak 1_a, a cathodic peak (11_c) was noticed which formed a quasireversible couple with peak 11_a observed in the subsequent sweep toward positive potentials. Some typical cyclic voltammograms of 4-hydroxyindole are presented in Fig. 2. Peak potentials of peaks 11_a and 11_c were also found to be linearly dependent on pH and can be expressed by the relations

$$E_p \text{ (pH 2.1–10.1)} = [170 - 60 \text{ pH}] \text{ mV vs SCE (for peak } 11_a)$$

$$E_p \text{ (pH 2.1–6.6)} = [115 - 53 \text{ pH}] \text{ mV vs SCE (for peak } 11_c)$$

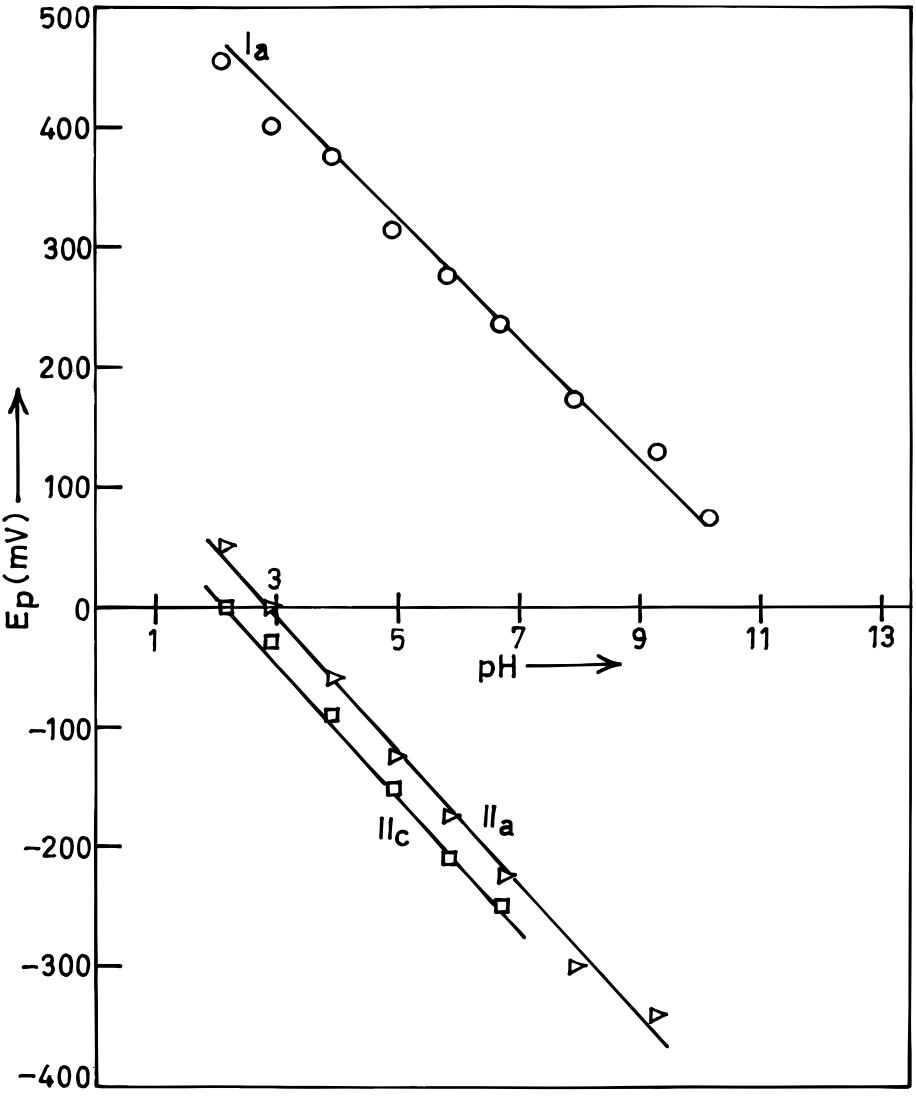


FIG. 1. Observed dependence of E_p on pH at the PGE for the voltammetric peaks of 0.5 mM 4-hydroxyindole.

The peak potentials separation for peaks $11_a/11_c$ was close to ~25 mV in the entire pH range. The i_p values for redox couple $11_a/11_c$ decreased with increase in pH. However, the peak current ratio indicated a value close to 0.8 in the entire pH range and hence further indicated quasireversible nature of the redox couple $11_a/11_c$.

To elucidate the behavior of redox couple $11_a/11_c$ cyclic voltammograms were also recorded after changing the direction of positive sweep at different potentials. It was interesting to observe that if sweep is reversed just before peak 1_a potentials, a new

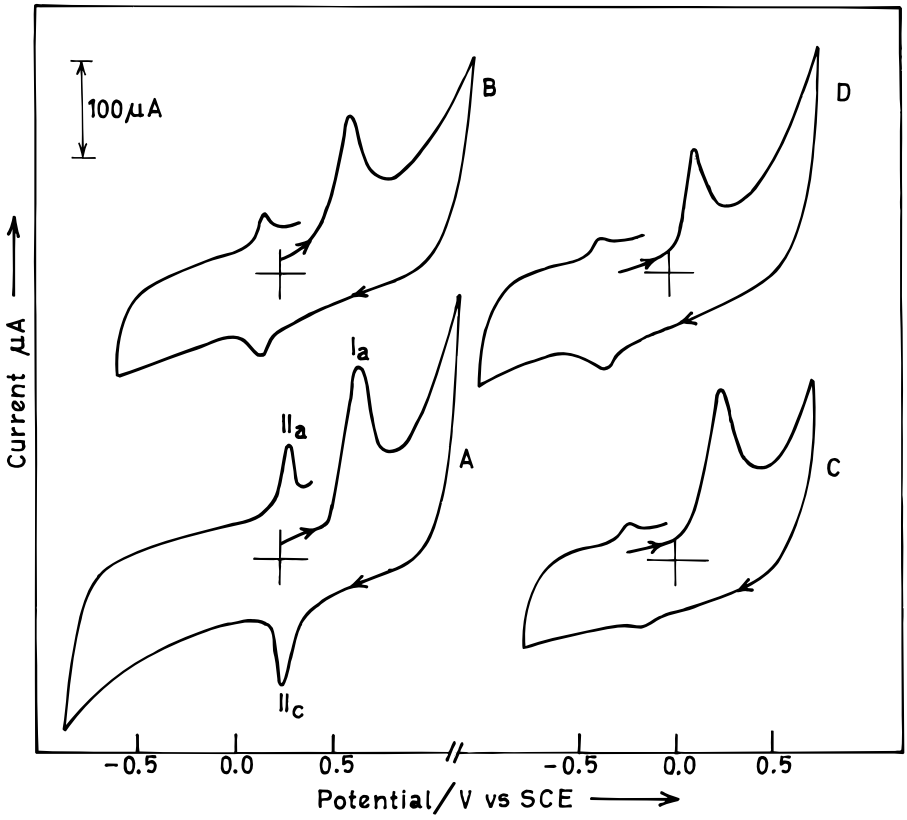


FIG. 2. Typical cyclic voltammograms of 4-hydroxyindole at pH (A) 3.0, (B) 6.7, (C) 7.9, and (D) 10.1, Sweep rate 100 mVs^{-1}.

redox couple $111_c/111_a$ is observed (Curve A, Fig. 3) at a sweep rate $\geq$ 100 mVs^{-1}. When the sweep is extended to more positive potentials the redox couple $111_c/111_a$ increases and another couple $11_c/11_a$ started appearing at more negative potentials (curve C). With further sweep extension couple $11_c/11_a$ increased and $111_c/111_a$ decreased as shown in curves D and E. Appearance of the couple $111_c/111_a$ clearly indicated that oxidation peak 1_a is composed of two overlapping oxidation steps (E_1 and E_2), whose E_p are very close to each other. The couple $111_c/111_a$ is responsbile for the product generated in reaction E_1 as it appears even before reaching peak potential of peak 1_a and couple $11_c/11_a$ is due to reaction E_2 whose peak potential is close to E_1. The decrease in peaks 111_c, 111_a, and increase in 11_c, 11_a with extended sweep further suggested that the species responsible for couple $111_c/111_a$ further oxidised in E_2 reaction to generate a product responsible for couple $11_c/11_a$.

Cyclic voltammograms were also recorded at different times by holding the potential soon after recording peak 1_a. These studies clearly indicate that redox couple $111_c/111_a$ decreases with increase in potential holding time. Interestingly the second redox couple $11_c/11_a$ increased systematically up to 10 min and then started decreasing.

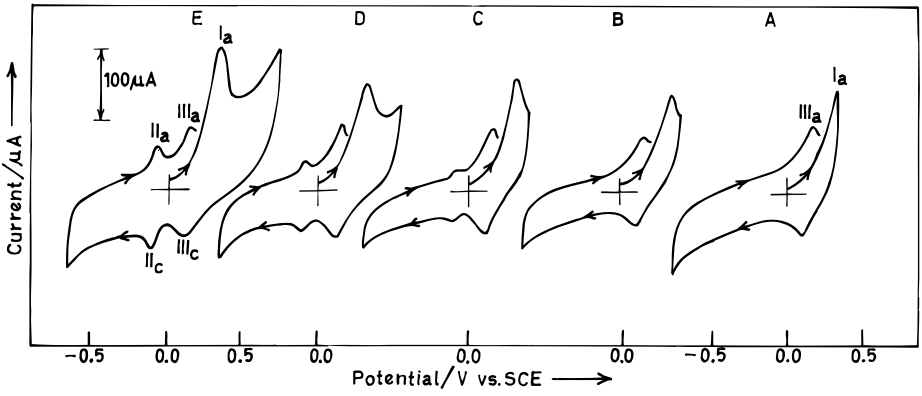


FIG. 3. Effect of potential of reversal observed on oxidation peak 1_a for 0.5 mM 4-hydroxyindole at pH 6.7, sweep rate 100 mVs^{-1}.

The exhaustively electrolyzed solution did not exhibit any reduction peaks. Thus, it is concluded that species responsible for peaks $111_c/111_a$ is unstable and further oxidized. As exhaustively electrolyzed solution did not exhibit any reduction peaks, it is reasonable to conclude that products formed in CPE are different than observed under cyclic voltammetric conditions.

The peak current of peak 1_a, was found to increase with increase in concentration of 4-hydroxyindole in the range 0.05–1.5 mM. The i_p vs concentration plot was practically linear up to ~1.0 mM, and at concentrations greater than 1.0 mM, the peak current had a tendency to limit (Fig. 4). This behavior indicated strong adsorption of reactant at the surface of PGE (*16,17*).

Controlled potential electrolysis of 4-hydroxyindole was carried out in phosphate buffer of different pH. The coulometric *n* values were calculated from the total coulombs passed and the value of *n* was found as 2.7 ± 0.2 in the entire pH range studied. On the other hand the values of *n* observed from *i* vs *t* curves during electrolysis in thin layer cell were always found as 1.3 ± 0.1.

6. SPECTRAL STUDIES

The UV spectra of 4-hydroxyindole were recorded in the entire pH range studied and two absorption maxima at 262 and 288 nm with a shoulder at 278 nm were observed. The spectral changes during electrooxidation of 4-hydroxyindole were monitored at pH 3.0 and 7.0 to detect the formation of UV absorbing intermediate generated during oxidation. Curve 1 in Fig. 5 presents a UV-spectrum of 4-hydroxyindole at pH 7.0 just before the oxidation and two well-defined λ_{max} at 262 and 288 nm were noticed. When potential corresponding to peak 1_a was applied, a systematic decrease in absorbance at both the λ_{max} was noticed (Curves 2–10). Curve 11 was recorded after 90 min of electrolysis and the absorbance at λ_{max} reduced to less than 40%. If potential at any stage of electrolysis is switched off to zero volt, the absorbance changes immediately cease, and hence it is concluded that no UV-absorbing intermediate is generated during the oxidation of 4-hydroxyindole. The decrease in absorbance with time at different wavelengths was found to be exponential.

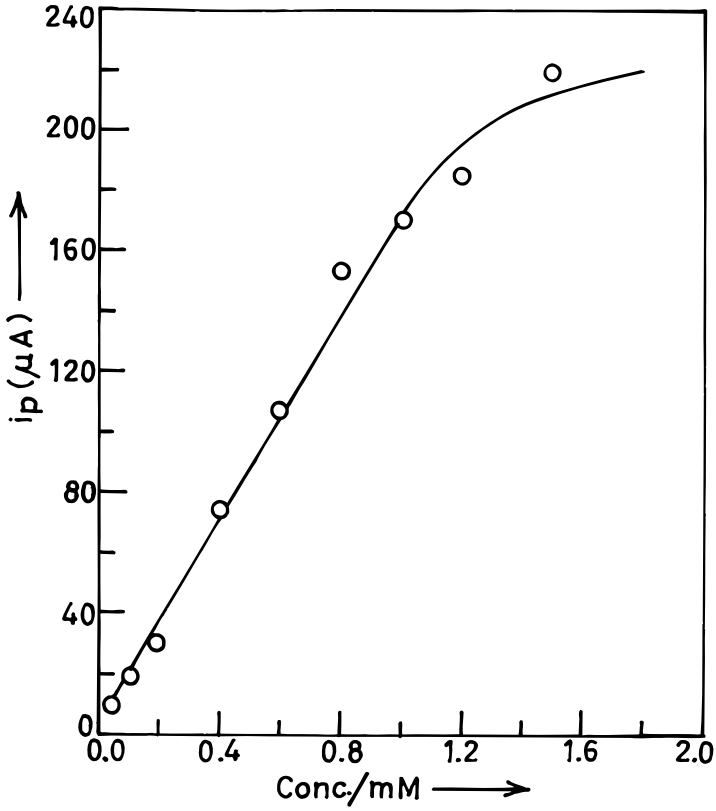


FIG. 4. Observed dependence of peak current for peak 1_a on concentration of 4-hydroxyindole at pH 6.7. Sweep rate 100 mVs^{-1}.

7. PRODUCT CHARACTERIZATION

The products of oxidation of 4-hydroxyindole were separated by using HPLC. Curve A in Fig. 6 presents the chromatogram observed for compound I just before the oxidation was initiated and a peak at $R_t \sim 5.23$ min was noticed. With progress of electrolysis the peak at $R_t \sim 5.23$ systematically decreased and at least four new peaks emerged in the HPLC chromatogram. Curve B presents a chromatogram observed for exhaustively electrolysed solution of 4-hydroxyindole and peaks at 4.96 (P_1), 6.92 (P_3), 8.80 (P_4), and 9.90 (P_5) min were observed. The material called under peaks P_1, P_4, and P_5 was never enough to permit complete characterization and hence the greenish-black colored material obtained under peak P_3 was analyzed by m.p., ^{1}HNMR, and mass spectrum.

The greenish-black material obtained had a m.p. 58°C. It gave prominent peaks in FT-IR spectrum at 3068, 1624, 830, 770 [aromatic C-H]; 1624, 1532 [C=C]; 3430, 3350, 1291 [secondary > NH]; 1130 [C-O-C]; 3563 [−OH]; 1342 [C-O] cm^{-1}. The mass spectrum of the material exhibited a clear peak at m/e 575 (4.8%, P+1) and suggested the molar mass of the material as 574. Other prominent high mass peaks