Int. J. Electrochem. Sci., 16 **(2021)** Article ID: 210746, doi: 10.20964/2021.07.09

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Detection of α-Ethinyl Estradiol in Water by Voltammetry: Mechanistic Study and Practical Implications

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Received: 31 January 2021 / *Accepted:* 29 March 2021 / *Published:* 31 May 2021

Residue amounts of synthetic estradiol; an active ingredient of oral contraceptives finds its way into water bodies. Estrogenic effects on aquatic life as well as on humans and other land animals are of great health concerns. A carbon-based screen-printed disposable electrode was used to detect α-Ethinyl Estradiol by cyclic voltammetry. The data analysis shows that the observed one electron oxidation peak is irreversible. The mechanism of reaction primarily involves adsorption of 17-α-Ethinyl Estradiol (EE2) on the surface of the electrode. The rate constant and adsorption capacity were estimated to be 1.46 s^{-1} and 1.3×10^{-10} moles/cm^2. The peak current vs concentration relationship was found to be linear over the range of concentration investigated. In addition, data on repeatability, sensitivity and detection limits are provided.

Keywords: Cyclic voltammetry, estradiol, biosensor, disposable electrode

1. INTRODUCTION

One of the important components of the combined oral contraceptive pill that is extensively used worldwide is 17-α-Ethinyl Estradiol (EE2). EE2 is a synthetic derivative of estradiol, which is not easy to degrade and varies from the naturally occurring estradiol due to its metabolic resistance. Thus, EE2 is found increasingly in wastewater because of human and animal excretion. Some reports have found links between this difficult to degrade estrogen and various human reproductive issues such as low sperm counts in adult males, postponement in sexual maturity and decline in secondary sexual characteristics even at low concentrations corresponding to a few ng/L. Thus, it is important to detect EE2 in waste water.

A number of investigations involve different ways to detect EE2. Some of the techniques involve spectrophotometry (1), liquid or gas chromatography(2, 3) , isotope dilution mass spectrometry (4), Solid-Phase Extraction (5), and immunoassay (6). Although sensitive, accurate and selective, these

methods are not only time-consuming but they are also quite expensive and involve complicated operational procedures. In order to operate these instruments, which often involve multistep methods, a skilled technician is needed. Therefore, research is also directed at finding solutions that are relatively quick and easy to carry out while being convenient and inexpensive.

Electrochemical methods offer a viable route for the detection of EE2 given that these techniques are not only rapid in nature but they are inexpensive and easily deployable even in the field. Many hand-held sensors are built around electrochemical principles. Smajdor et al (7) carried out its determination on carbon black glassy electrode using differential pulse voltammetry and obtained a detection limit of the order of 10^{-5} to 10^{-6} g/L. They proposed the following oxidation mechanism shown in Figure 1 involves the loss of one electron.

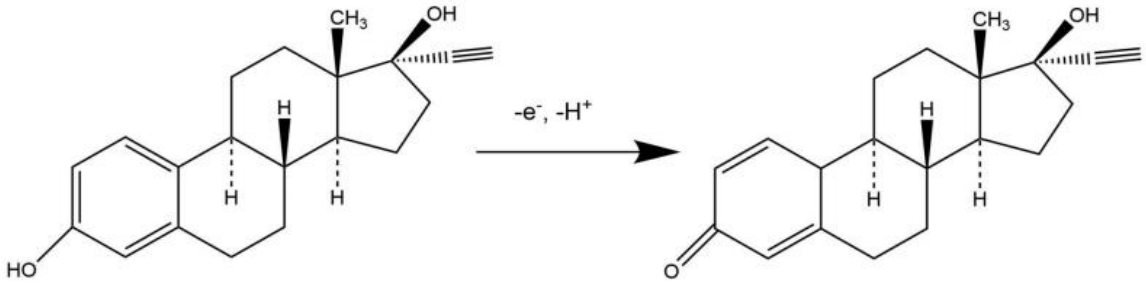

Figure 1. Oxidation Mechanism of EE2 proposed by Smajdor [7]

Using a carbon paste electrode, Li et al (8) determined that during voltammetry the oxidation peak of EE2 shifts in a negative direction when cetyl pyridine bromine is added to the solution. The detection limit of EE2 was found to be of the order of 10^{-6} g/L. They showed that EE2 is adsorbed on the surface of the electrode based upon the cyclic voltammetry experiments. Similar detection limits were obtained in results published by Nunes et al (9) who employed a hanging drop mercury electrode in their experiments.

Various electrodes have been described in a number of papers for the determination of EE2. Singh et al (10) have used ZnO nanorods for detection. Duan et al (11) have employed molecularly imprinted sensor based on sensitization by EOF/CNTs containing Prussian blue for detection. Namgehi et al (12) have decorated a gold electrode with split DNA aptamers as recognizing agents. Antoniazzi et al (13) determined estradiol concentration in humans and in buttermilk by conducting voltammetry on a copper (II) oxide-modified carbon electrode. Wen at al (14) used nanowall-based gold film electrodes to detect estradiol in food samples. Wong et al (15) employed scree-printed disposable electrodes made of carbon ink modified with CuPc, P6LC, and Nafion film. Li et al (16) used Pd-decorated N-doped reduced graphene oxide electrodes in their investigation. Trivino et al (17) used hanging drop mercury electrodes as well as the screen-printed carbon and the screen-printed carbon nanotube electrodes for estradiol concentration determination in solution. In addition, they have compiled a summary of detection limit of estradiol by different researchers.

This study explores a commercial disposable screen printed carbon electrode for EE2 detection. In addition to the ease of use, and given the fact that no surface preparation is required, it is expected

that these electrodes will entail a greater degree of standardization and will thus result in greater extent of reproducibility than the ones prepared in individual research labs. There is relatively very little information available in the published literature regarding the use of commercial electrodes for EE2 determination, which provides for additional motivation for this research.

2. EXPERIMENTAL

Disposable, commercially available screen-printed carbon electrodes which are used as working electrode and are purchased from Metrohm Dropsens were used to detect EE2 by cyclic voltammetry using GAMRY Interface 5000E Potentiostat/Galvanostat. The counter and reference electrodes are platinum and silver, respectively.

EE2 was obtained from Fisher Scientific. The phosphate buffer solution was prepared from potassium phosphate monobasic and sodium phosphate dibasic, which were obtained from Sigma-Aldrich. Other chemicals used were of as analytical grade. All the experiments are done under room temperature.

The supporting electrolyte phosphate buffer solution was prepared by adding KH_2PO_4 and Na_2HPO_4 to deionized water. The concentration of phosphate buffer solution prepared was 0.067 M. A stock solution of 17-α-EthinylEstradiol of 5×10^{-3} M concentration was prepared with pure ethanol and it was stored in a refrigerator at around 4^oC.. The electrolyte used for conducting cyclic voltametric experiments consisted of the phosphate buffer solution to which the desired amount of EES was added using the stock solution.

In some experiments, the pH of the phosphate buffer was adjusted by adding small amounts of concentrated 2 M NaOH and 2 M HCl to avoid solution volume change. Solutions were prepared by thorough mixing of chemicals in solvent using sonication.

The scan rate used in cyclic voltammetry experiments was varied from 100 to 700 mV/s in order to investigate the relationship between the peak current and peakpotential with the scan rate.

3. RESULTS AND DISCUSSION

Effect of Scan Rate on Peak current and Peak potential

Figure 2 is the cyclic voltammogram obtained on solutions containing 3×10^{-6} M EE2 in 0.067 M phosphate buffer solution with varying scan rates that ranged from 100 to 700 mV/s. Only the anodic peaks are obtained. The data shows that the peak current increases with the scan rate. The absence of any cathodic peak implies that the electrochemical reaction is irreversible(18). The data also show that the peak potential shifts to a more positive value with the scan rate.

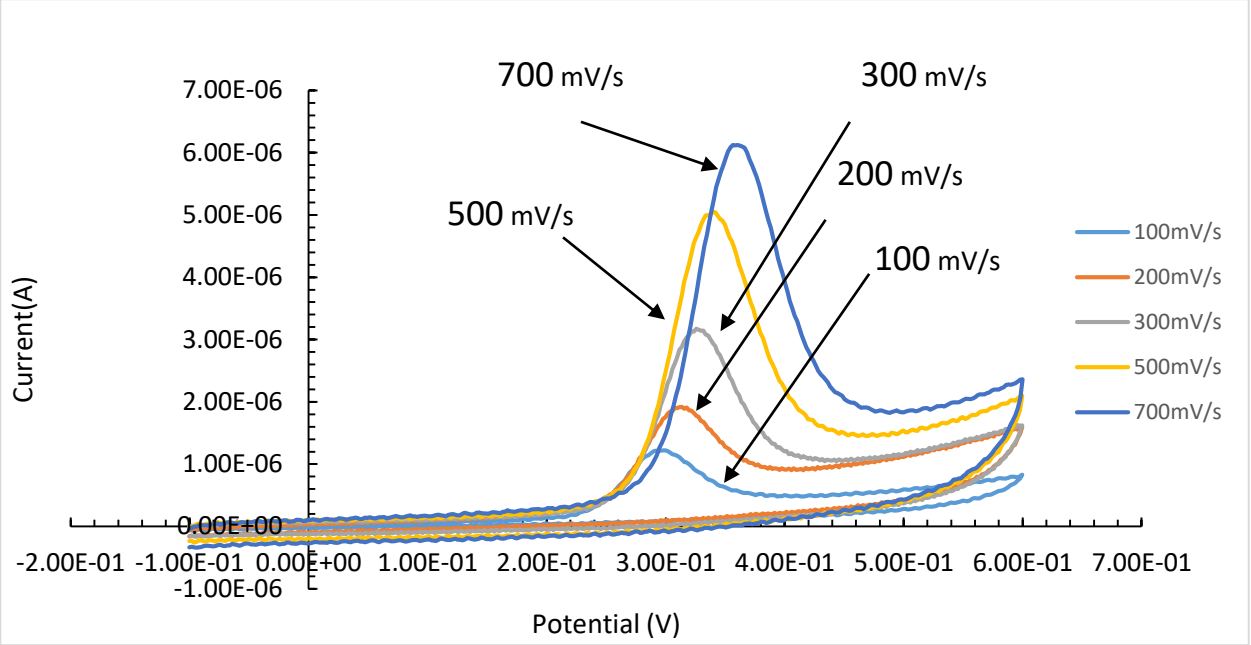

Figure 2. Cyclic Voltammograms obtained on a solution containing 0.067M phosphate buffer solution (pH 6.8) with 3×10^{-6} M EE2 measured for the scan rate of: 100, 200, 300, 500 and 700 mV/s.

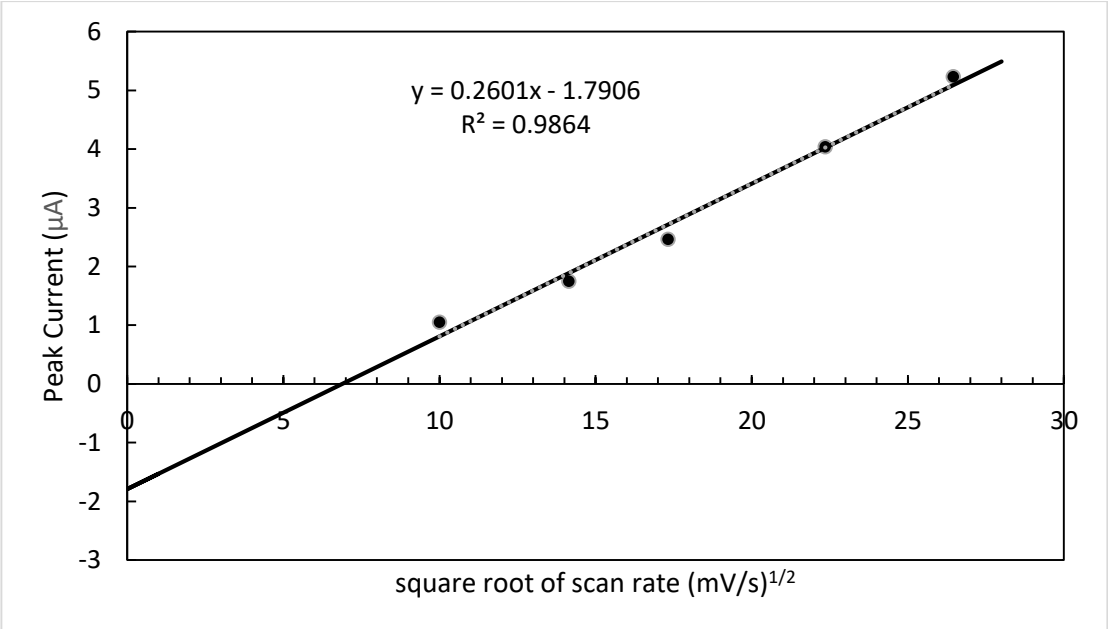

Figure 3. Peak current vs. the square root of the scan rate

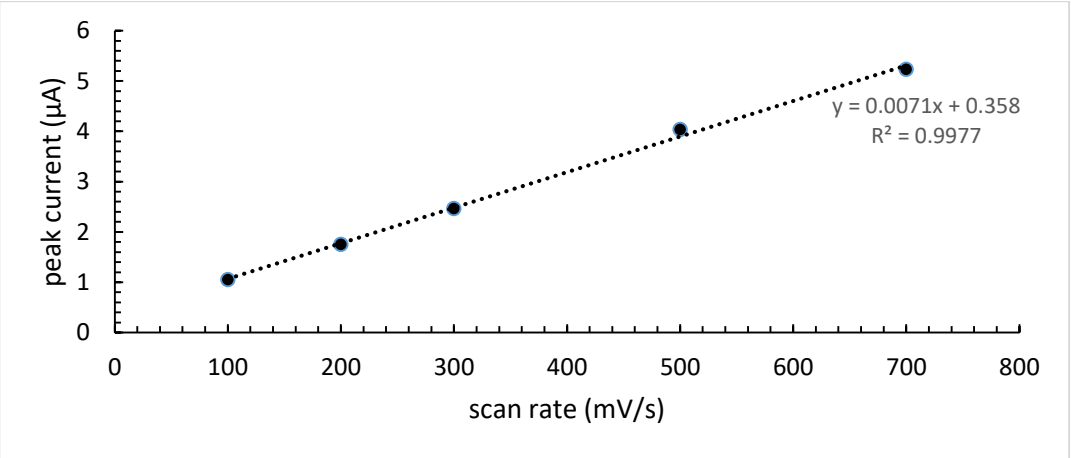

Figure 4. Peak current vs. the scan rate

In order to elucidate the mechanism of the reaction, as a first step the peak current was plotted against the square root of the scan rate (Figure 3). Although the resulting plot is a straight line and the correlation factor is relatively high (R^2= 0.9864), the line does not pass through the origin and a significantly negative (1.7906 µA) y-axis intercept is observed. Another plot of the peak current vs. the scan rate was prepared (Figure 4). A near perfect correlation factor of 0.9977 is obtained while the y-axis intercept is fairly small (0.358 µA) lending support to the hypothesis that the peaks obtained during cyclic voltammetry correspond to adsorption of estradiol on the electrode surface. In order to further test this conjecture, the electrode was placed in a 3×10^{-5} M EE2 solution for 24 hours and then rinsed with deionized water. After drying, the electrode was placed in a 0.067 M phosphate buffer solution in the absence of any estradiol and a cyclic voltammetry was conducted.

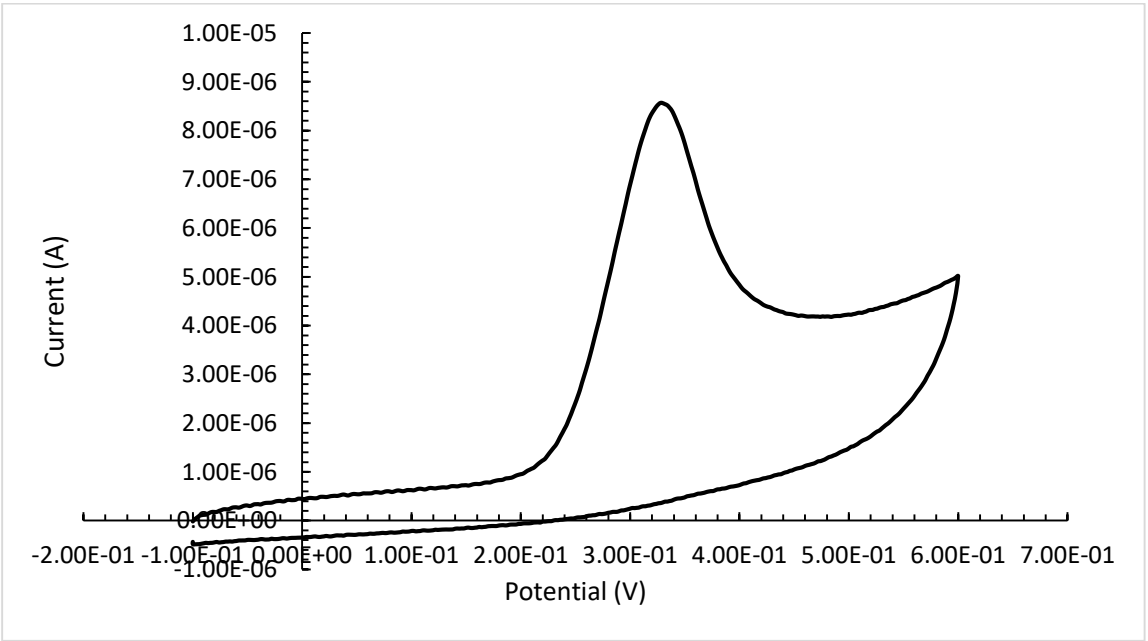

Figure 5. Cyclic voltammogram obtained in the absence of estradiol in the solution. The electrode was immersed in a solution containing estradiol prior to electrochemical analysis.

Figure 5 shows the presence of an oxidation peak at about -0.34 V (vs the reference electrode) when the electrode was scanned at 100 mV/s. This peak corresponds to the one, which was obtained when the electrode was scanned in the presence of estradiol as shown in Figure 2. The data supports the fact that adsorption of estradiol plays an important role in determination of the oxidation peak. The very nature of the asymmetrical shape of the voltammogram (Figure 2) also lends credence to the fact that adsorption of EE2 plays a very significant part in the process(18). To further investigate the role of diffusion vs. adsorption, a plot of natural log of the peak current vs the natural log of the scan rate was created (Figure 6). The slope of the straight line obtained is 0.82. The slope of 0.5 implies diffusion control while the corresponding value of 1.0 indicates complete adsorption control. The intermediate value (0.82) of the slope indicates mixed control between diffusion and adsorption.

The adsorption characteristics of estradiol were examined using the following correlation by modifying the adsorption related treatment presented by Bard (19) as shown below with further related material provided in the Appendix:

$$i_p = \frac{(1-\alpha)F^2 A v \Gamma}{2.718RT} \qquad (1)$$

$$E_p = E^{0'} - \frac{RT}{(1-\alpha)F}\ln\left(\frac{RT}{(1-\alpha)F}\frac{k^0}{v}\right) \qquad (2)$$

Where F is the faraday constant, A is the electrode area (0.126 cm^2), v is the scan rate, R is the universal gas constant, α is the electron transfer coefficient, and T is the temperature.

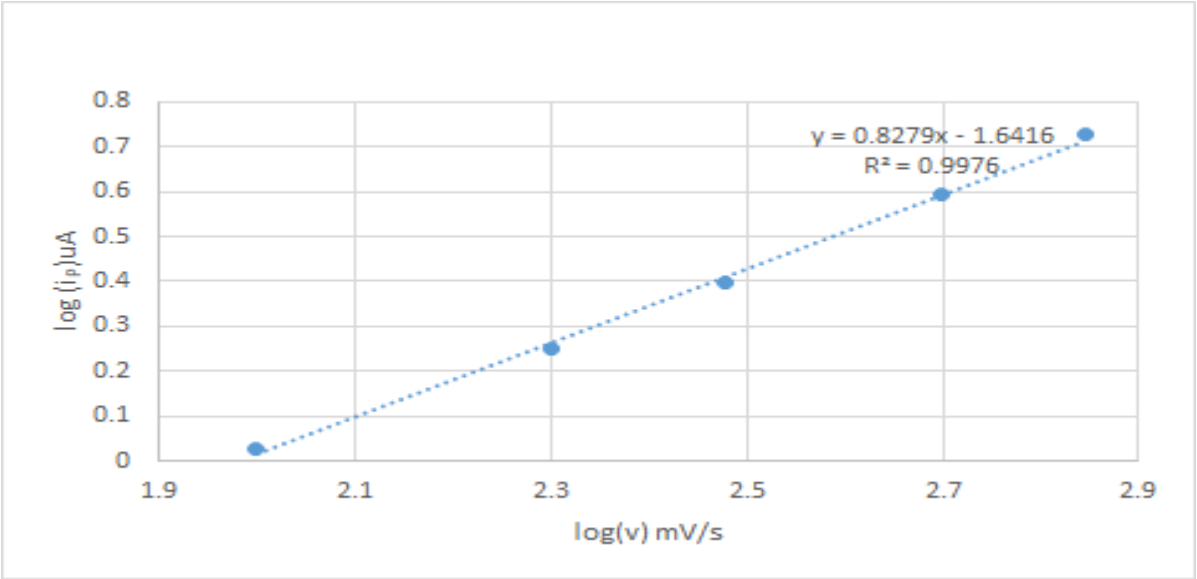

Figure 6. Log of the anodic peak current vs. the log of the scan rate

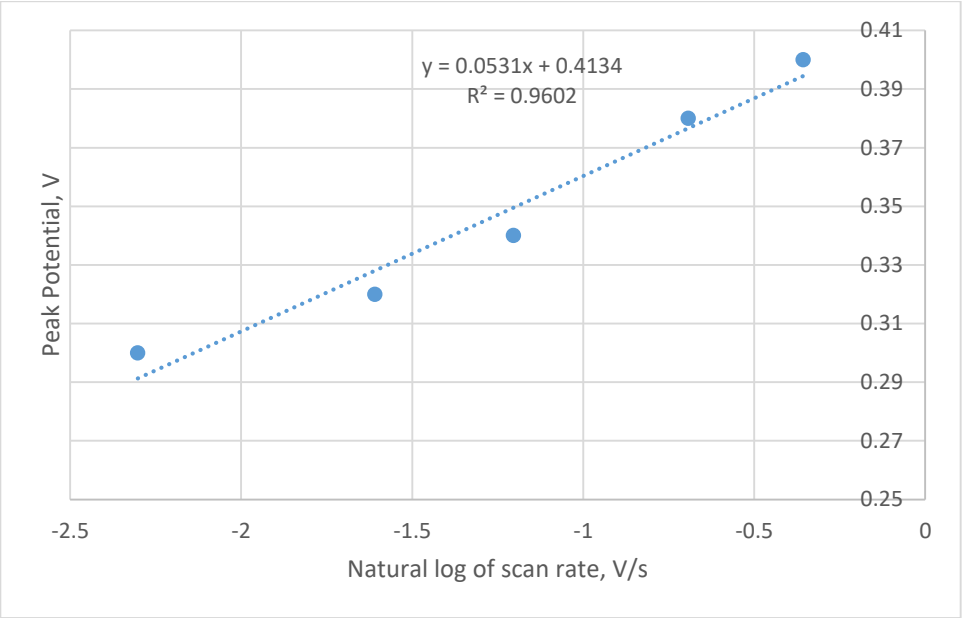

Figure 7. Peak potential vs scan rate

A plot of the peak potential, E_p vs. the natural log of v results in a straight line (Figure 7) with a slope of $\frac{RT}{(1-\alpha)F}$, which gives us $\alpha = 0.516$. The y-axis intercept of the line is:

$$0.4315 = E^{0'} - \frac{RT}{(1-\alpha)F}\ln\left(\frac{RTk^0}{(1-\alpha)F}\right) \qquad (2-a)$$

The formal potential $\left(E^{0'}\right)$along with the rate constant (k^0) are calculated using the following equation for the anodic current as a function of time, t (or potential) as listed below:

$$i = FAK_f\Gamma \exp\left(\frac{RT}{(1-\alpha)F}\frac{K_f}{v}\right) \qquad (3)$$

Where the forward rate constant is are given by:

$$K_f = K_{fi}\exp\left(\frac{(1-\alpha)Fvt}{RT}\right) \qquad (3-a)$$

Where $K_{fi}$is expressed as:

$$K_{fi} = k^0\exp\left(\frac{(1-\alpha)F}{RT}\left(E_i - E^{0'}\right)\right) \qquad (3-b)$$

A non-linear regression analysis of the data, which is fitted to equation (3), gives us the following values:

$E^{0'} = 0.278\,\text{V}, \text{and}\ k^0 = 1.456s^{-1}$

The surface coverage (Γ) is then obtained from equation (1), which is found to be equal to 1.3×10^{-10} moles/cm^2.

Effect of EE2 concentration on Peak Current

Voltammograms obtained on a solution containing 0.067 M phosphate buffer solution (pH 6.8) with varying concentrations of EE2 at a scan rate of 100mV/s are shown in Figure 8.

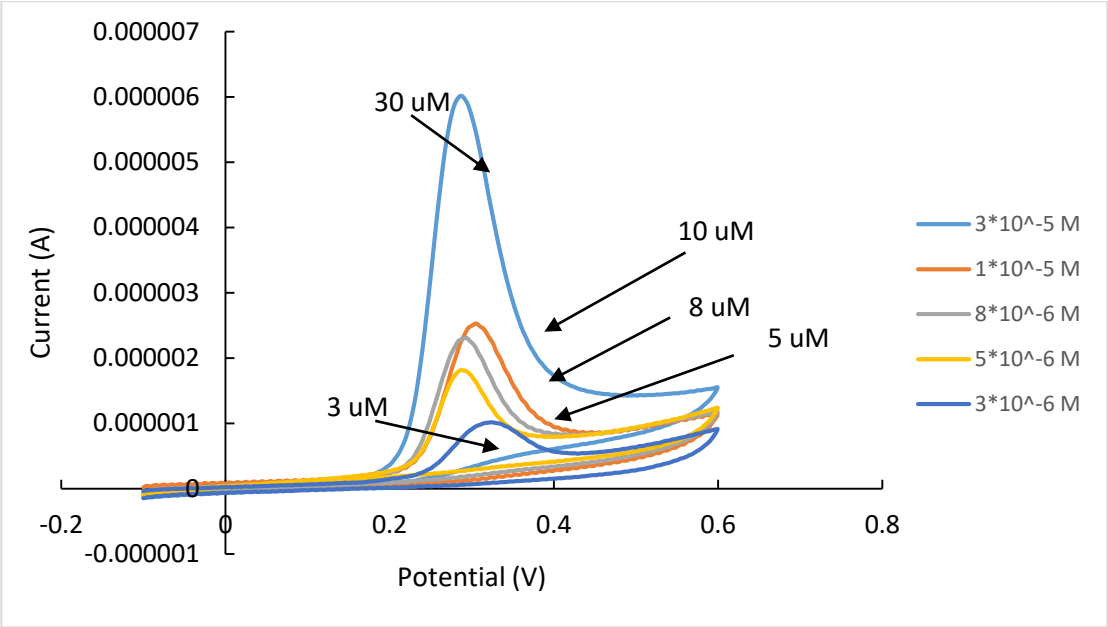

Figure 8. Voltammograms obtained on a solution containing 0.067M phosphate buffer solution (pH 6.8) as a function of EE2 concentration at a scan rate 100 mV/s

As expected, the peak current increases with the concentration. A plot of the peak current vs. EE2 concentration is shown in Figure 9. The linearity (peak current = 0.1714×Concentration + 0.5741) of the plot is evidenced by the calculated high value (R^2 = 0.9981) of the correlation factor. A number of experiments were conducted to determine the reproducibility of the experiments. The data show that at the highest value of EE2 examined, the experimental reproducibility is rather low because solubility is rather low for EE2 concentration corresponding to 3.0×10^{-5} M.

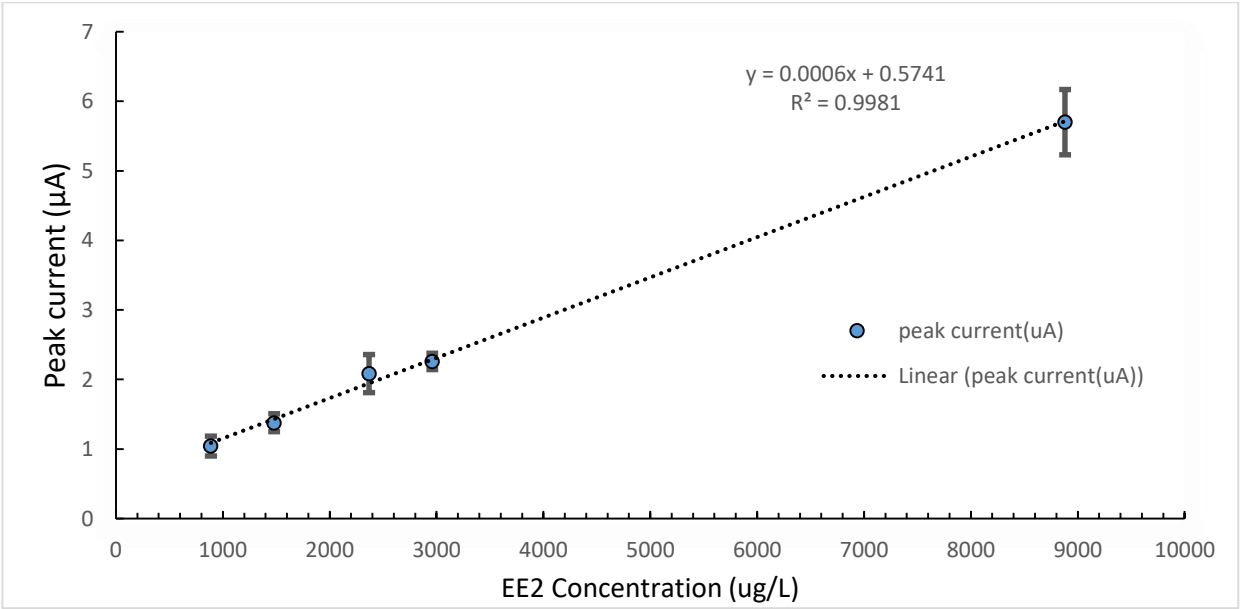

Figure 9. Peak current vs concentration Experimental conditions as in Figure 8. Error bar corresponds to ± one standard deviation.

Influence of Solution pH

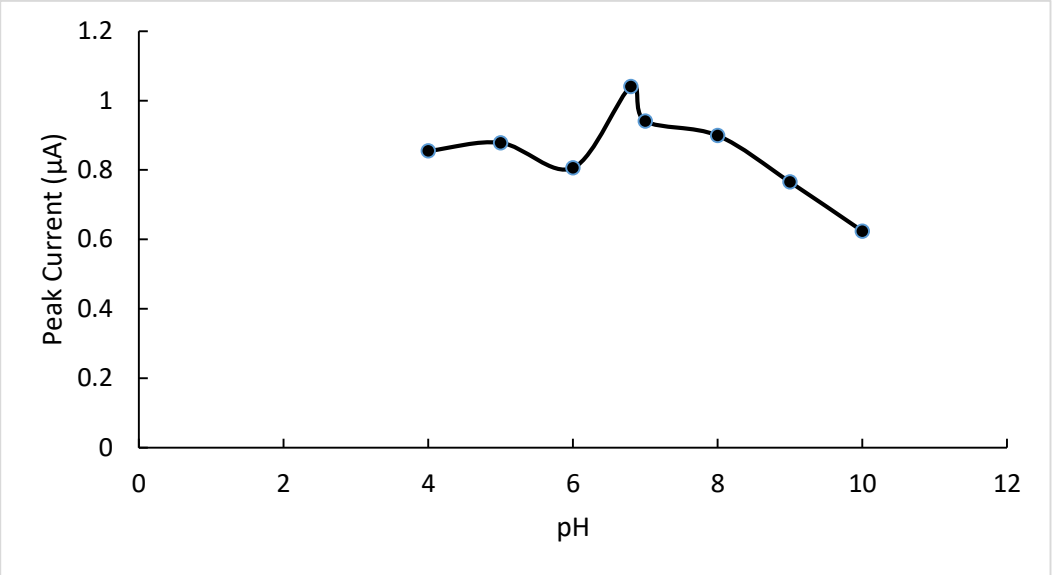

Figure 10. Peak current vs. solution pH

The original pH value of the phosphate buffer solution is 6.8. The solution pH prior to conducting cyclic voltammetry at scan rate of 100 mV/s was adjusted to desired value by adding the required amount of 2 M HCl or 2 M NaOH. In all the experiments described below, the estradiol concentration of 3×10^{-6} M was used. Figure 10 is a plot of the peak current vs. solution pH. The data show that maximum peak current is obtained at solution pH of 6.8, which happens to be the non-adjusted pH of the phosphate buffer solution.

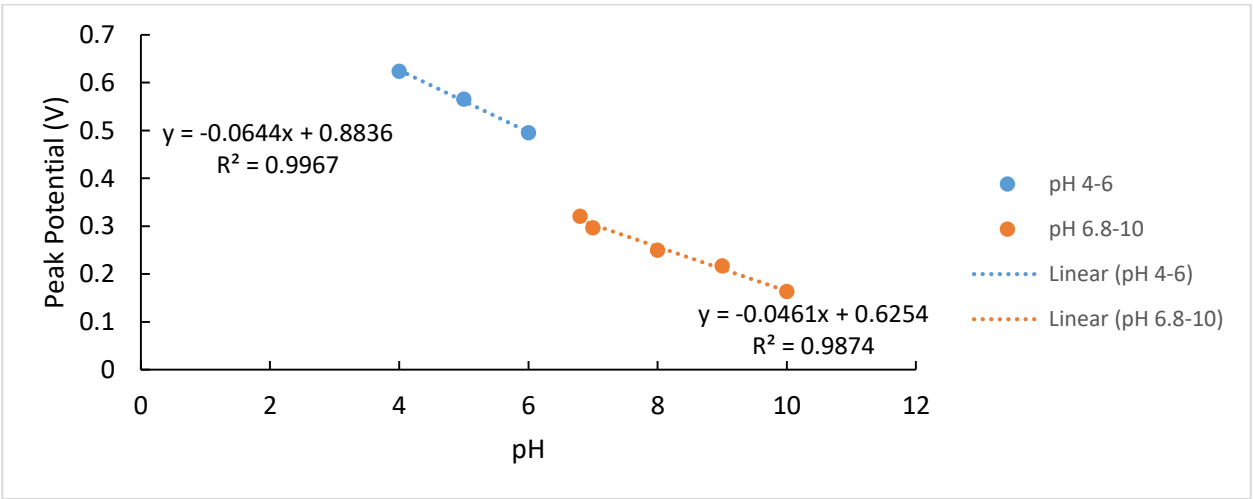

Figure 11. Peak potential vs. pH

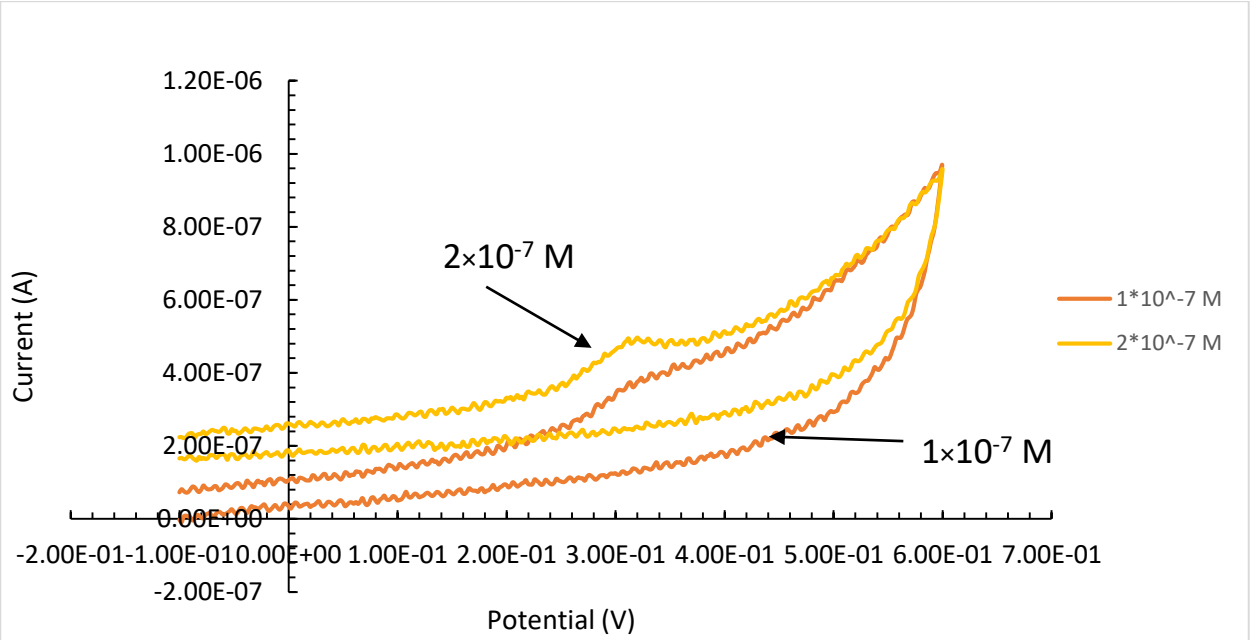

Figure 12. Cyclic Voltammograms obtained on a solution containing 0.067M phosphate buffer solution (pH 6.8) with 2×10^{-7} M and 1×10^{-7} M EE2 at a scan rate of 100 mV/s

The peak potential is plotted vs the solution pH (Figure 11). The data are divided into two subgroups: pH values corresponding to 4-6, and 6.8-10. Visual examination suggests that the slopes of the two lines are just about the same. The data are quantified as follows.

$E_{p,a}$ = -0.0644 pH + 0.8836, (R^2=0.9967) for pH between 4-6, and
$E_{p,a}$ = -0.0461 pH + 0.6254, (R^2=0.9874) for pH between 6.8-10

The Nernst equation is rewritten as follows:

$$E = E^{0'} + \frac{RT}{nF} ln \frac{[C_O^0][H^+]^m}{[C_R^0]} = E^{0'} + \frac{RT}{nF} ln \frac{[C_O^0]}{[C_R^0]} - 2.303 \frac{RT}{F} \left(\frac{m}{n}\right) pH \qquad (4)$$

The slopes for both the lines are nearly equal to 0.059 V/pH ($= \frac{RT}{F}$ at room temperature), which suggests the number of electrons (n) and protons transferred (m) are about equal to one another.

In experiments described below, data reported in Figure 9 served as a calibration curve to relate the peak current to the estimated estradiol concentration in the solution.

Repeatability

In order to establish the consistency of results obtained, experiments were carried out to determine repeatability. The conditions of repeatability consist of the same measurement procedure, operators, measuring system, operating conditions and locations and repeat the same measurement on the same object in a short period of time (17). Five measurements (n=5, confidence level = 95%) on

each solution containing 3.0×10^{-6}, 5.0×10^{-6}, 8.0×10^{-6},1.0×10^{-5}, and 3.0×10^{-5} M EE2 were prepared over the course of 24 hours. The results of repeatability are expressed in terms of relative standard deviation (RSD %) using the following expression. The data obtained are reported in Table 1.

$$\text{RSD}(\%) = \frac{\text{Standard Deviation of five measurements}}{\text{Mean of five measurements}} \text{X}100$$

The highest RSD% and lowest RSD% obtained are 13.72% and 5.11% for solutions containing EE2 concentrations of 3.0×10^{-6} M and 1.0×10^{-5} M, respectively.

Table 1. Repeatability vs. EE2 concentration

Concentration (M)	Mean i_p (μA)	Standard Deviation	RSD%
3.0×10^{-5}	5.70	0.47	8.24%
1.0×10^{-5}	2.26	0.12	5.11%
8.0×10^{-6}	2.09	0.27	13.09%
5.0×10^{-6}	1.46	0.13	8.84%
3.0×10^{-6}	1.04	0.14	13.72%

Even the highest RSD% value obtained is lower than 20%, which indicates the repeatability of proposed method is acceptable in the EE2 concentration range investigated in this study.

Limit of Detection

The limit of detection is the ability to measure the lowest concentration of the desired substance. The data in Figure 10 shows that a distinct anodic peak is discernible for EE2 concentration of 2×10^{-7} M, however, it is no longer true when the solution concentration is lowered to 1×10^{-7} M. Thus, it is concluded that the detection limit lies in the range of 2×10^{-7} M and 1×10^{-7} M.

Selectivity

Selectivity is an ability to discriminate the target analyte in the mixture of other interferences. Synthetic municipal wastewater is a good sample to carry out the selectivity test of EE2 as this has implications for human health. The municipal water contains several different metals and organic compounds, which are possible candidates for causing interference in detection of EE2. It must be noted that human urine is one of those chemicals. Synthetic municipal wastewater includes NaCl, $CaCl_2$, $MgSO_4$, K_2HPO_4, urea, peptone, and meat extract. In addition, L-ascorbic acid and citric acid were also added as the model compounds for organics present in municipal water (20). The data presented in Table

2 lists the error calculated in measurement of the EE2 concentration due to the interference caused by the chemicals present in a typical municipal water sample (with 3×10^{-6} M EE2 in 0.067 M phosphate buffer solution).

The Na^+ ions and L-ascorbic acid do not appear to interfere with EE2 measurement. Ca^+ ions, K_2HPO_4, citric acid and urea did slightly interfere with the EE2 detection signal. The beef extract caused a decrease of EE2 anodic peak by 10.65% while the presence of Mg^+ ions caused 16.25% decrease in the reported concentration of EE2. The presence of peptone resulted in no detection of EE2 due to the absence of the anodic peak during the voltammetric determination. The solution containing all the interfering agents listed in Table 2 (except peptone) resulted in a reasonably acceptable level of selectivity using the commercially available disposable screen-printed electrode.

Table 2. Selectivity of EE2 detection as a function of impurities

Chemicals	Concentration of impurity (mg/L)	Error in detection%
NaCl	7	±0%
$CaCl_2$	4	+8.89%
$MgSO_4$	2	-16.25%
K_2HPO_4	28	-6.25%
Urea	30	-3.64%
Peptone	160	N/A
Beef Extract	110	-10.65%
L-ascorbic acid	4.4	±0%
citric acid	4.8	+7.23%
All Mixture	All chemicals	N/A
All mixture without peptone	All chemicals except peptone	-8.58%

4. CONCLUSIONS

Carbon-based electrodes have been investigated to detect EE2 in aqueous solution through voltammetric methods. Data reported in the literature employing carbon paste electrode in the presence of cetyl pyridine bromine (8) and carbon black modified electrode (7) are compared with the results obtained in this study. A summary of the comparison of the results obtained using three types of carbon-based electrodes is shown in Table 3.

Table 3. Comparison of detection, sensitivity and repeatability data with the one reported in the literature

Type of electrode	Measurement method	Electrode area (cm^2)	limit of detection (M)	Sensitivity (μA/μM)	Repeatability (RSD%)	Selectivity
Carbon paste electrode in the presence of cetyl pyridine bromine (8)	DPV	0.00785	3×10^{-8}	0.125	4.24% for 5×10^{-6} M	Fe^{+}, dopamine, acetaminophen, adrenaline, uric acid and vitamin C
Carbon black modified electrode (7)	DPV	N/A Glassy carbon electrode (standard area:0.071)	1.3×10^{-7}	N/A	2.5% for 5×10^{-7} M	Mg^{2+}, Pb^{2+}, Mn^{2+}, glucose, ascorbic acid
Disposable screen-printed carbon electrode (this investigation)	CV	0.126	Between $1\times10^{-7}$and 2×10^{-7}	0.1714	5.11% for 1×10^{-5} M and 13.72% for 3×10^{-6}M	Peptone, Mg^{2+} ,,beef extract

The presence of cetyl pyridine bromine results in lowering of the detection limit, however, it must be noted that differential pulse voltammetry, a very sensitive technique was deployed to make these measurements(7, 8). With respect to sensitivity, the disposable screen-printed carbon electrode in this study seems to be the best among three studies that are reported. The presence of Mg^{+} ions and some organic compounds in the solution was found to cause interference in EE2 determination for every single investigation. Although the screen-printed carbon electrode is not the most optimal choice yet, its suitable modification along with the use of differential pulse voltammetry instead of cyclic voltammetry deployed in this study could greatly enhance EE2 detection limit. In addition, it is disposable and easily deployable in the field due to the presence of all three electrodes in one hand-held assembly that requires only a small quantity of the analyte for making measurements.

Appendix

The reduction peak current and peak potential are given by the following two equations:

$$i_p = \frac{\alpha F^2 A v \Gamma}{2.718RT} \qquad \text{A-1}$$

$E_p = E^{0'} + \frac{RT}{\alpha F}\ln(\frac{RT}{\alpha F}\frac{k^0}{v})$ A-2

The cathodic current, according to the Butler Volmer equation is given by:

$i_c = k^0 e^{-\alpha f(E-E^{0'})}$ A-3

Where f = RT/f

While the anodic current is given by:

$i_a = k^0 e^{(1-\alpha) f(E-E^{0'})}$ A-4

The cathodic potential during the scan is given by:

$E = E_i - vt$ A-5

While the anodic potential is given by:

$E = E_i + vt$ A-6

In order to express them as the peak anodic and peak anodic potentials, the values for α and v in equations A-1 and A-2 are replaced by -(1- α) and – v respectively.

ACKNOWLEDGEMENT
The authors would like to thank the funding support from Southwestern Ohio Council For Higher Education (SOCHE) Dayton Area Institute of Graduate School (DAGSI) program for RX19-4 and RH20-5.

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