Voltammetric determination of wedelolactone, an anti-HIV herbal drug, at boron-doped diamond electrode

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Abstract. Boron-doped diamond electrode has been utilized for the study of electrochemical behaviour of an anti-HIV herbal drug wedelolactone in Britton-Robinson buffer (pH-2.5) by square-wave and cyclic voltammetry techniques. The response characteristics of cyclic voltammetry and square wave voltammetry showed a remarkable increase in the anodic peak current and electrochemical impedance spectroscopy revealed a lowering in charge transfer resistance at the boron-doped diamond electrode as compared to the glassy carbon electrode that can be attributed to the higher sensitivity of boron-doped diamond sensor. Cyclic voltammetry at the boron-doped diamond surface revealed the oxidation of wedelolactone with two oxidation peaks (P_1 and P_2) with $E_{p1} = 0.4V$ and $E_{p2} = 1.00V$ with scan rate varying from 10 - 220 mV/s and exhibits diffusion-controlled process. Based on the electrochemical measurements, a probable oxidation mechanism has been deduced and the electrode dynamics parameters have been evaluated. The effect of concentration on the peak currents of wedelolactone was found to have a linear relationship within the concentration range of 50–700 ng/mL. The LOD and LOQ were found to be 43.87 and 132.93 ng/mL respectively. The applicability of the proposed method was further scrutinized by the successful determination of wedelolactone in real plant samples.

Keywords. Wedelolactone; boron-doped diamond electrode; impedance; voltammetry.

1. Introduction

Herbs are considered as the storehouse of various secondary metabolites having pharmacological potentials and their chemical partnership leads to favourable synergistic or additive effects with minimum side effects. Their bioactive principles tend to normalize physiological function and provide rational means for the treatment of many obstinate and incurable diseases.^{1–3}

Wedelolactone (WDL), 7-Methoxy-5, 11, 12-trihydroxycoumestan, (scheme 1) forms the major constituent of the traditional medicinal plant *Eclipta alba* Hassk. (Asteracea), which is a perennial herb grown throughout India and Southwestern U.S, in moist and damp land.^{4,5} The literature reveals that WDL is known to show anti-HIV activity against HIV-1 integrase (IN), exhibiting high inhibitory effect, and possesses a wide range of biological activities and therapeutic properties.^{6–11} It was further found to suppress caspase-11 which plays an important role in regulating apoptosis by inhibiting IKK complex.¹² These reasons fasten the development of an efficient and reliable analytical method for the quantification of WDL.

Different analytical methods have been explored for the estimation and quantification of WDL which includes HPLC,^{13–15} HPTLC,^{16,17} UV spectrophotometry,¹⁸ ICP-MS analysis, IR, EPR.¹⁹ However, these methods are time consuming and involve derivatization steps prior to the final analysis. Apart from this, the aforementioned procedures involve excess amount of solvents, reagents and sophisticated instrumentation. On the other hand, electrochemical techniques are easy to use, highly sensitive, inexpensive, and provide quick response. Its application includes determination of redox properties of chemical constituents which provides insights into their metabolic fate and *in vivo* redox processes.^{20–30} An extensive literature survey revealed that the electrochemical investigation has so far not been reported for the study of the redox behaviour of WDL.

Boron-doped diamond electrode (BDDE) has been the subject of considerable interest as an electrode material for various electroanalytical applications, as it is more advantageous over the traditionally employed electrodes (e.g., glassy carbon electrode or the platinum electrode).^{31–37} This is because of its very wide potential window (upto 3 V) in aqueous solutions, low background currents, resistance to thermal shock, extreme electrochemical stability and corrosion

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Scheme 1. Chemical structure of wedelolactone.

resistant towards aggressive media.^{38–41} Moreover boron has low charge carrier activation energy of 0.37 eV,⁴² as a consequence of which it is by far the most widely used dopant to produce conducting diamond electrodes. Boron doping leads to a p-type semiconductor.⁴³

The present work reports a new and explicit electrochemical method for the study of WDL by square wave voltammetry (SWV) and cyclic voltammetry (CV) at BDDE, using Britton-Robinson buffer (pH 2.5) as a supporting electrolyte. BDDE unveiled the mechanistic aspects of the phytoconstituent in the solution system. Electrochemical impedance spectroscopy (EIS) was employed to scrutinize the charge transfer resistance of the BDDE and GCE detailing higher sensitivity of the boron-doped diamond sensor as compared to the GCE. This method was further successfully employed for the investigation of WDL in plant samples.

2. Experimental

2.1 Instrumentation

All electrochemical studies were performed at a PC-controlled AUTOLAB PGSTAT 302N (Eco-Chemie B.V., Utrecht, The Netherlands) potentiostat-galvanostat with IME663 and software NOVA 1.8. EIS was carried out using FRA 2 module. A standard three electrode electrochemical assembly was used in the study that contained BDDE and GCE as the working electrode, platinum wire as counter and Ag/AgCl (3M KCl) as reference electrode that were fitted in one compartment cell, connected with electrochemical workstation through Metrohm 663VA stand. The electrochemical cell was fitted with the nitrogen gas bubbler. All pH measurements were made on a Mettler Toledo pH meter fitted with a glass electrode and a Ag/AgCl electrode as reference which was pre-standardized with buffers of known pH. All measurements were carried out at room temperature.

2.2 Materials and reagents

WDL standard ($\geq 99\%$) was obtained from the Sigma Aldrich. Ultra-pure water (Milli-Q water with

resistivity 18 M Ω .cm) was obtained from ELGA purification system (U.K.). Standard solution of WDL (1 mg/mL) was prepared by dissolving pure compound in methanol and was further diluted with britton-robinson buffer to get the concentration in the working range. To investigate WDL in real samples, plant of *Eclipta alba* (authenticated by Department of Botany, Dayalbagh Educational Institute, Agra) was purchased from commercial source. Solutions at all the stages of the study were prepared by using analytical grade reagents and were used without further purification.

2.3 Working analytical procedure

BDDE and GCE were polished against the alumina slurry (particle size 0.01 μ m–0.3 μ m) spread over the Buehler cloth, and copiously rinsed with deionized water followed by gently blowing under the nitrogen stream to remove the residual alumina particles. After this mechanical treatment, electrodes were electrochemically activated against a suitable electrolyte by applying a potential of +1.3 V for 30 sec.

The stock solution of WDL (1 mg/mL) was prepared in methanol. Working solutions were prepared by further dilution with the supporting electrolyte to get the desired concentration range. Initially, a series of britton-robinson buffer (pH 2.5–12) were prepared in ultrapure water and used as supporting electrolytes. About 10 mL of electrolyte solution containing appropriate amount of standard WDL or sample was added to the electrolytic cell. Prior to all the electrochemical measurements, all the solutions were purged with the pure N₂ gas to remove the interference due to O₂. Then the electrodes were immersed and the voltammograms were recorded within the potential window of 0 to 1.4 V. Electrochemical impedance measurements were performed in the solution of 5mM K₃Fe(CN)₆ in 0.1 M phosphate buffer solution (PBS) by applying the AC voltage with 10 mV amplitude in a frequency range from 0.1Hz to 1000Hz. The results were plotted in the form of Nyquist plots. All voltammetric measurements were carried out at ambient temperature.

3. Results and Discussions

3.1 Electrochemical behaviour of WDL at BDDE and GCE

Electrochemical behaviour of WDL at BDDE and at the GCE was investigated by CV and SWV techniques. Figure 1 (A and B) represents the CV and SWV of WDL at the GCE (b) and at BDDE (c). At each of the

Figure 1. Square wave voltammogram (B, amplitude: 20 mV, frequency: 25 Hz, step potential: 5 mV, scan rate: 125 mV/s) and Cyclic voltammogram (A, scan rate 70 mV/s) comparing the peak potential and current values of WDL obtained at b) GCE and c) BDDE, a) blank in BR buffer pH 2.5 at a concentration of 500 mg/mL and 700 mg/mL respectively.

electrodes, WDL exhibited two oxidation peaks that can be ascribed owing to the presence of functional $-OH$ groups attached to ring structures.

The working surface area of the BDDE and the GCE were obtained by the CV using 1 mM $K_3Fe(CN)_6$ as a redox probe at different scan rate. The surface area can be calculated using Randles-Sevcik equation (298°K) as follows⁴⁴

$$I_p = (2.69 \times 10^5) n^{3/2} A C_0 D^{1/2} \nu^{1/2} \quad (1)$$

For $K_3Fe(CN)_6$, $n=1$ and $D=7.6 \times 10^{-6} \text{ cm}^2/\text{s}$. From slope of I_p (μA) vs. $\nu^{1/2}$ the area were determined to be 0.069 cm^2 for the BDDE and 0.028 cm^2 for the GCE electrodes. Thus, the current values for BDDE and GCE were observed to be $4.34 \mu\text{A}/\text{cm}^2$ and $3.52 \mu\text{A}/\text{cm}^2$ for CV, $14.49 \mu\text{A}/\text{cm}^2$ and $12.49 \mu\text{A}/\text{cm}^2$ for SWV.

3.2 Electrochemical Impedance Spectroscopy

The increase in the electron transfer at the BDDE was investigated using electrochemical impedance spectroscopy technique. For this AC amplitude of 10 mV at a frequency of 0.1–100 kHz was applied. The higher electrocatalytic behaviour of BDDE was confirmed by the reduction in the charge transfer resistance (figure 2A). The values observed for the charge transfer resistance, R_{CT} , fitting an appropriate equivalent circuit, were determined and found to be $174 \text{ k}\Omega/\text{cm}^2$ at BDDE and $643 \text{ k}\Omega/\text{cm}^2$ at GCE (figure 2B).

The electrical equivalent circuit compatible with the Nyquist plots comprises of R_s the solution resistance, Q_{dl} the double layer capacitance, R_{CT} the charge

Figure 2. Nyquist plots of 5 mM $K_3Fe(CN)_6$ in 0.1 M PBS at BDDE (A, a), GCE (A, b), and corresponding equivalent circuit (B).

transfer resistance and W the Warburg impedance. The semi-circle observed in the Nyquist plot reveals the lower charge transfer resistance at BDDE surface as compared to GCE which can be attributed to the higher sensitivity of the electrochemical sensor. A constant phase element was used instead of the pure capacitance because of the electrode surface inhomogeneities.⁴⁵

3.3 Effect of scan rates

To study the effect of scan rate on the oxidation peak of WDL, voltammograms were recorded for different scan rate from 10–220 mV/s at a fixed concentration (700 ng/mL) of WDL at BDDE. The information involving electrochemical mechanism can be obtained from the investigation of the response characteristics of CV on the electrooxidation process of WDL (figure 3). A linear relationship was obtained between the peak current intensity (I_p ; P_1 and P_2 anodic peak currents) and the square root of scan rate (ν)^{1/2}, suggesting the diffusion controlled process at the electrode surface. The linear relationship between I_p and (ν)^{1/2} for P_1 follows $I_p(\mu A) = 0.043(\nu)^{1/2} - 0.111$, $R^2 = 0.991$ and for P_2 , $I_p(\mu A) = 0.025(\nu)^{1/2} - 0.085$, $R^2 = 0.989$. The results

Figure 3. Cyclic voltammograms of WDL (700 ng/mL in Britton-Robinson buffer 2.5 pH) at scan rates 10–220 mV/sec (10, 30, 50, 90, 110, 130, 150, 200 and 220 mV/s, curves a – i respectively) and Inset figure represent plots of (ν)^{1/2} vs. I_p for P_1 and P_2 .

confirms the electrooxidation diffusion controlled process of WDL.

Moreover, from the literature,⁴⁶ for an anodic reaction, the relationship between E_p and ν can be described as:

$$E_p = E^\circ + \frac{RT}{\alpha n F} \left[0.780 + \ln \left(\frac{D^{1/2}}{k_s} \right) + \ln (\alpha n F \nu / RT)^{1/2} \right] \quad (2)$$

Where E° is the formal redox potential, R and F values are 8.314 J/K.mol and 96480 C/mol respectively, k_s is the standard heterogeneous rate constant, α is the charge transfer coefficient and D is the diffusion coefficient.

From the slope of the straight line of E_p against $\ln \nu$, αn was calculated to be 0.46 and 0.89 for P_1 and P_2 respectively. For a diffusion-controlled anodic behaviour, assuming α to be 0.5,⁴⁷ number of electrons participating (n) during the oxidation process of WDL was calculated to be one for the anodic peak P_1 and two for the anodic peak P_2 .

3.4 Effect of supporting electrolyte and its pH

To achieve the optimum electrolyte, the influence of various supporting electrolytes such as KCl, phosphate buffer, acetate buffer and Britton-Robinson buffer on the electrooxidation of WDL (700 ng/mL) was investigated. Britton-Robinson buffer was chosen as the optimum buffer with respect to the fairly well defined current responses and prominent peak shape. The effect of pH on the oxidation of WDL was examined over the pH range (2.5–12). The anodic peak currents (P_1 and P_2) and the peak potential for the oxidation of WDL were found to be sensitive towards the pH of the solution. It was observed that the current values (I_p) for anodic peaks P_1 and P_2 started to decrease with the increase in pH values and at higher pH (pH > 7.9), the peak current almost disappeared (figure 4A). Thereby considering the sensitivity and the peak shape, pH 2.5 was selected as the optimum pH for the entire electrochemical measurement. Moreover there was a sequential shift of the anodic peak potential with the augmentation of solution pH, indicating that the protons had participated in the electrode reaction process of WDL.^{48,49} Figure 4B explains the shifting of peak potential with the increase in pH values following the linear equation E_p (mV) = 700.3–88.05pH, $R^2 = 0.961$ (P_1) and E_p (mV) = 1092–45.64pH, $R^2 = 0.964$ (P_2). Furthermore, the peak potential and the pH of the solutions can be expressed by the following Nernst equation.⁵⁰

$$E_p = E^\circ - 2.303RTm \text{ pH} / (1 - \alpha) nF \quad (3)$$

Figure 4. Dependence of peak current (I_p , A) and peak potential (E_p , B) of WDL (700 ng/mL) on the pH (2.5–7.9) of supporting electrolyte (Britton-Robinson buffer).

where ‘m’ is the number of protons participating in the electrode reaction. Thus, from the slope of E_p vs. pH and the aforementioned Nernst equation ‘m’ was calculated to be one.

3.5 Probable oxidation mechanism of WDL

The voltammetric techniques can be utilized to elucidate the electroactivity and possible reaction mechanism of various compounds. Here in the present case, CV and SWV of WDL exhibited two anodic voltammetric peaks (P_1 and P_2), which can be attributed to the presence of phenol and dihydroxy moieties respectively. According to Corduneanu *et al.*⁵¹ and Janeiro *et al.*⁵² resorcinol group is found to be less electroactive than phenolic group, suggesting that the oxidative

peak P_1 appearing at lower potential corresponds to the phenol moiety whereas the peak P_2 appearing at higher corresponds to the dihydroxy group. Thus based on all the electrochemical measurements, one electron-proton transfer is observed in each step with a total of two proton-electron coupled reaction and a possible oxidation mechanism of WDL has been proposed as shown in scheme 2.

3.6 Calibration curve and detection limit

Under the optimized condition, response characteristics of SWV exhibited a linear relationship between the peak current values and the concentration of WDL within the range 50 ng/mL to 700 ng/mL (figure 5). The calibration plot of I_p (A) vs. concentration (ng/mL) can be described by the equations I_p (A) = 0.017(ng/mL) + 1.366; $R^2 = 0.994$ (P_1) and I_p (A) = 0.003(ng/mL) – 0.686; $R^2 = 0.991$ (P_2)

Limit of detection (LOD) and limit of quantification (LOQ) (estimated as 3 S/m and 10 S/m, respectively, ‘S’ being the standard deviation and ‘m’ being the slope of the calibration curve) were obtained as 43.87 ng/mL and 132.93 ng/mL. Various statistical parameters for the linear regression equation have been evaluated and reported in table 1.

Further to explore the analytical application and the specificity of the proposed method, the effect of some common excipients (such as glucose, sucrose and lactose) were studied by analyzing sample solution of WDL (100 ng/mL) spiked with varying concentration of each excipients under the optimized conditions. The determination of WDL in the presence of above-mentioned excipients was evaluated and a recovery range from 98.03% to 99.18% was obtained. Thus, the results

Scheme 2. Proposed oxidation mechanism for WDL.

Figure 5. Square wave voltammograms of WDL at different concentration levels, (50 ng/mL–700 ng/mL, b–j), blank (curve a) and Inset figure represents plot of concentration vs. current I_p for P_1 (50, 100, 200, 300, 400, 500, 600 and 700 ng/mL) and P_2 (200–700 ng/mL).

showed that there was no serious interferences occurred from the classical additives.

3.7 Determination of WDL in plant sample (*Eclipta alba*)

Isolation of the plant extract was attained by percolation of the methanol (48 h) through the bed of herbaceous material at room temperature to produce an extract having active principles contained in the herbaceous material. Before adding the solvent, plant material was washed, dried at room temperature and grinded to powder form. Then 5g of the dried plant material was weighed and soaked in 100 mL of solvent. Lustrous

Table 1. Square-wave voltammetric method validation parameters for standard linearity.

Linearity parameters	Results
Slope	0.0174
Standard deviation	0.0005
Intercept	1.3660
Standard deviation	0.2278
Correlation coefficient	0.994
Standard error of estimation	0.3385
Sum of squares of regression	113.6899
Sum of squares of residuals	0.6875
Limit of detection (ng mL ⁻¹)	43.87
Limit of quantification (ng mL ⁻¹)	132.93

Figure 6. (a) Square wave voltammograms of 100 μ l of plant extract; (b) and standard WDL additions 50ng/mL (c); 100ng/mL (d) with blank (curve a).

plant extract was obtained which was highly concentrated and contains a large proportion of the active principles, which can be used in lower unit doses for analytical experiments. The appropriate amount of the extract was taken in the voltammetric cell containing the supporting electrolyte and voltammogram was recorded (figure 6). In order to validate the veracity, WDL standard solution was added into the sample solution for recovery detection. The results are listed in table 2. The assay results indicate that the proposed voltammetric method can be applied for determination of WDL in plant extract effectively. The concentration of the unknown phytoconstituent (WDL) can be determined using the standard addition method by the following equation:⁵³

$$C_u = I_{p1} C_s V_s / [I_{p2} (V_u + V_s)] - I_{p1} V_u \quad (4)$$

After getting the voltammogram of the supporting electrolyte, known volume (V_u) of unknown concentration (C_u) of the investigated herbal drug is added and the resulting voltammogram is recorded and peak current (I_{p1}) is measured, then the known volume (V_s) of known concentration (C_s) of standard pure drug is

Table 2. Analytical results for WDL in *Eclipta alba* samples.

Standard added (ng/mL)	Standard found* (ng/mL)	(%) Accuracy ^a	(%) RSD
100	99.5	99.5	0.20
200	198.3	99.15	0.19
300	296.7	98.9	0.22

^a [standard found/standard added] $\times$ 100

*Amount found represents the average of six observations (n = 6).

Table 3. Comparison of the detection limit of the present work with other methods.

Method	Limit of detection/ quantification	Reference
HPLC-PDA	2 μ g/mL	54
HPLC	2.5 μ g/ml	55
Electroanal. Quant. (BDDE)	43.8 ng/mL	Present work

Abbreviations: HPLC-PDA, performance liquid chromatography-photo diode array; HPLC, high performance liquid chromatography; Electroanal. Quant., electroanalytical quantification; BDDE, boron doped diamond electrode.

added and peak current (I_{p2}) is measured. The amount of WDL content was found to contain 0.30% in the plant *Eclipta alba*.

3.8 Comparison of the sensitivity of the present work with other reported methods

Detection limit of the proposed method is compared with the other reported methods.^{54,55} Table 3 reveals that the present method is more sensitive and the detection limit of WDL is lower than check the reported methods.

4. Conclusions

A new method for the selective determination of WDL in Britton-Robinson buffer (pH 2.5) at BDDE has been presented in this paper. BDDE offered high sensitivity towards WDL and found to be appropriate and effective for the selective determination of the solution system. The sensitivity of the electrodes can be explained on the basis of electrochemical impedance spectra against the ferricyanide solution with lower charge transfer resistance at BDDE as compared to GCE. The voltammetric study of WDL at different scan rates revealed diffusion controlled reaction process. Electrode process dynamics parameters have been evaluated and based on this a plausible reaction mechanism has been deduced. The calibration plot reveals linearity within the range of 50–700 ng/mL with a correlation coefficient of 0.994 for P_1 and 0.991 for P_2 . The lower limit of detection (43.87 ng/mL) proved the sensitivity of the proposed method and the method was successfully employed for the determination of WDL in a real plant sample.

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