Electrochemical Study of the Antineoplastic Agent Etoposide at Carbon Paste Electrode and Its Determination in Spiked Human Serum by Differential Pulse Voltammetry

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The electrochemical oxidation of the antineoplastic agent etoposide was studied at carbon paste electrode in Britton–Robinson buffer solutions over the pH range 2.0—10.0 using cyclic, linear sweep and differential pulse voltammetry. Oxidation of the drug was effected in a single reversible, diffusion-controlled step within the pH range 2.0—4.0, a second oxidation process was produced above pH 4.0. Using differential pulse voltammetry (DPV), the drug yielded a well-defined voltammetric response in Britton–Robinson buffer, pH 3.0 at 0.500 V (*vs.* Ag/AgCl) on carbon paste electrode. This process could be used to determine etoposide concentrations in the range 2.5×10^{-7} to 2.5×10^{-5} M with a detection limit of 1.0×10^{-7} M. The method was successfully applied to the determination of the drug in spiked human serum.

Key words etoposide; differential pulse voltammetry; carbon paste electrode; electrochemical oxidation; pharmaceutical analysis

Etoposide (4′-demethylepipodophyllotoxin ethylidene-β-D-glucoside, Fig. 1) is a potent clinical anticancer agent.[1] It is active against several tumors including small lung cancers,[2,3] lymphoma,[4] leukemia[5] and Karposi's sarcoma associated with AIDS.[6] It is used as part of the preparatory regimen for bone marrow transplants in patients with advanced hematological malignancies.[7] The mechanism of action of etoposide is unknown but it is a cell-cycle, phasespecific drug that appears to act either by causing DNA breaks by an interaction with DNA-topoisomerase II or by forming free radicals.[8—13]

Methods used previously for the determination of etoposide in human plasma include, high-performance liquid chromatography (HPLC) with UV detection,[14—17] fluorescence detection[18—21] and electrochemical detection.[22—28] HPLC methods based on electrochemical detection also have been used for the analysis of etoposide catechol in human plasma.[27,28] Those assays using ultraviolet detection have sensitivities down to approximately 100 pg/l. Fluorescence detection improves the specificity and sensitivity such that concentrations as low as 50 pg/l can be measured. Electrochemical detection improves sensitivity further, allowing drug concentrations of 2 to 10 pg/l to be assayed. Radioimmunoassay (RIA) also has been used to measure etoposide.[29,30] but it is less specific than HPLC and less sensitive than HPLC using electrochemical detection. A method combining liquid chromatography and mass spectrometry[31] is both highly specific and sensitive that simultaneously quantifies etoposide and its catechol metabolite as protein-free and total concentrations in human plasma samples, but requires specialized facilities and is expensive

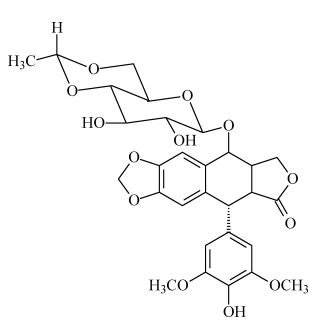

Fig. 1. Chemical Structure of Etoposide

The electroanalytical techniques are well known as excellent procedures for the determination of drug dosage forms and drugs in biological fluids, with the advantages that there is in most instances, no need for derivatization, and that these methods are less sensitive to matrix effects than other analytical techniques.[32] In recent years, the increasing use of carbon paste electrodes (CPEs) for electroanalytical measurement of a variety of organic species of biological and pharmaceutical importance has been reported.[33] The major advantages of carbon paste electrode are low background current; the ease of renewal of the whole electrode providing a fresh surface unaffected by electrode history and this electrode has a wide range of cathodic and anodic applicability.[34]

Experimental

Reagents Etoposide, kindly provided by Nippon Kayaku (Tokyo, Japan), was used without further purification. Stock solution (1.0×10^{-3} M) was prepared in methanol, and stored in a refrigerator at 4 °C. Standard solutions were prepared daily by diluting of the stock solution with a selected supporting electrolyte. Britton–Robinson (BR) buffer solutions (0.04 M each of acetic, *o*-phosphoric and boric acids, adjusted to the required pH with 0.2 M sodium hydroxide solution) were used as supporting electrolytes. High-purity reagents were employed in all experiments. Acetic acid and sodium hydroxide were from Sigma (Saint Louis, U.S.A.); boric and *o*-phosphoric acids were supplied from Merck (Darmstadt, Germany) and methanol from Carlo Erba (Milan, Italy). All solutions were prepared using doubly distilled water.

Apparatus The voltammetric measurements were carried out using a computer driven AEW2 Analytical Electrochemical Workstation with ECprog3 Electrochemistry software (Sycopel, England) in combination with C-2 cell stand with a three-electrode configuration: a carbon paste (BAS Model MF-2010, 3 mm diameter) working electrode, an Ag/AgCl/3 M KCl (BAS Model MF-2063) reference electrode, and a platinum wire (BAS Model MW-1032) counter electrode. OriginPro 7.0 software was used for the transformation of the initial signal. A CG 808 (Schott Gerate, Germany) digital pH-meter with glass combination electrode served to carry out the

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pH measurements.

Procedure The carbon paste was prepared in the usual way by hand-mixing of graphite powder (Aldrich, Milwaukee, WI, U.S.A.; ϕ=1—2 μm) and 1.8 ml of Nujol (Sigma; d=0.84 g ml^{-1}). The ratio of graphite powder to mineral oil was 70 : 30.

For voltammetric measurement, 5.0 ml of the electrolyte solution were transferred into the voltammetric cell. After measurement of the blank solution, the appropriate amount of etoposide solution was added and the anodic potential sweep was carried under different operational parameters. The electrodes were resurfaced before each voltammetric scan, thoroughly rinsed with doubly distilled water and gently dried with a tissue paper. All measurements were carried out at room temperature. Peak heights were evaluated as the differences between each voltammogram and the background electrolyte voltammogram.

Recovery Studies in Human Serum A liquid–liquid phase extraction procedure with chloroform was used for the extraction of etoposide from serum.[19] Known amounts of etoposide were added to the drug-free human serum (1.0 ml), then mixed with 2 ml chloroform. After shaking and centrifugation, the under phase was removed and evaporated under a stream of nitrogen gas. The residue was re-dissolved in 0.5 ml methanol solution, completed to 5.0 ml with BR buffer (pH 3.0, 0.02 M), transferred to a voltammetric cell and the procedure was continued as for pure drug. The anodic peak current was measured against the background current. Quantification was achieved by the standard addition method.

Results and Discussion

Preliminary differential pulse voltammetry (DPV) experiments for 1.25×10^{-6} M etoposide in Britton–Robinson buffer background solutions over the pH range 2.0—10.0 were carried out on carbon paste electrode. Figure 2 shows some representative differential pulse voltammograms obtained at carbon paste electrode. Oxidation of the drug was effected in a single step within the pH range 2.0—4.0 and a second oxidation process was produced at more positive potentials above pH 4.0. The first oxidation process was more pronounced, sharper and better-defined than the second process. Thus, the study was focused mainly on this oxidation peak. Figure 3 shows the effect of pH on peak potential and peak current of the first anodic peak using differential pulse voltammetry. The anodic peak potential is shifted to less positive values by increasing the pH with slope of -52 mV/pH in the range from pH 2.0 to 9.0. Thereafter, the peak potential remains practically pH independent (Fig. 3A). The pH-dependence of the anodic peak potential E_p at pH 2—9.0, indicates that the acid-base equilibrium between the phenolate and the phenol forms is established rapidly. The pH independence of E_{p1} at pH pK_a (9.7),[35] indicates the absence of a proton transfer between the two electron transfers, where the phenolate form predominates in the bulk of the solution. The effect of the solution pH on the peak enhancement is also shown in Fig. 3B. The best results with respect to signal enhancement accompanied by sharper response was obtained with Britton–Robinson buffer at pH 3.0. Moreover, it was found that the etoposide is stable at this pH value. This supporting electrolyte was chosen for subsequent measurement experiments.

A typical cyclic voltammogram of 1.0×10^{-4} M etoposide at carbon paste electrode in Britton–Robinson buffer at pH 3.0 is shown in Fig. 4. Etoposide has an anodic peak at 0.660 V with reduction peaks observed in the cathodic branch. Cyclic voltammograms were then recorded at different potential scan rates between 20 and 500 mV s^{-1}. The potential of anodic peak was found to be independent of the of the scan rate over the whole range studied, which confirms the reversibility of the process, with the simultaneous increase in peak current (i_{p1}) when the scan rate (ν) was increased. The linear relationship existing between peak current and the square root of the scan rate (correlation coefficient 0.998) gave a slope of 0.91, very close to the theoretical value of 1.0, which is expected for an ideal reaction of solution species,[36] so in this case the oxidation process is predominantly diffusion-controlled in the whole scan rate range studied. Investigations concerning the oxidation pathways of etoposide have been reported previously.[35] The electrochemical oxidation of the etoposide shows an overall two-electron transfer. At pH<4.0, the oxidation proceeds in one voltammetric oxidation step corresponds to an overall uptake of two electrons, At pH>4.0, the oxidation proceeds in two voltammetric oxidation steps. The first voltammetric oxidation step

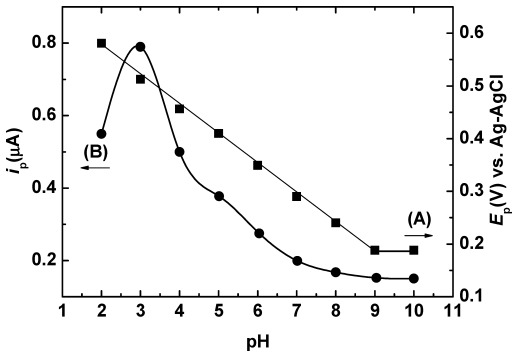

Fig. 3. Effect of pH on (A) Peak Potential and (B) Peak Current in Britton–Robinson Buffer Using Differential Pulse Voltammetry at Carbon Paste Electrode for 1.25×10^{-6} M Etoposide; Scan Rate, 10 mV s^{-1}; Pulse Amplitude, 50 mV; Pulse Width, 30 s

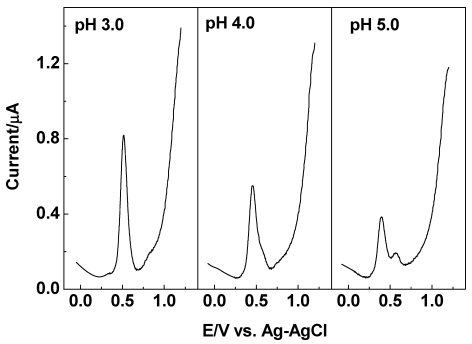

Fig. 2. Differential Pulse Voltammograms (CVs) for 1.25×10^{-6} M Etoposide on Carbon Paste Electrode in Britton–Robinson Buffer at Different pH Values; Using 50 mV Pulse Amplitude, 30 ms Pulse Width and 10 mV s^{-1} Scan Rate

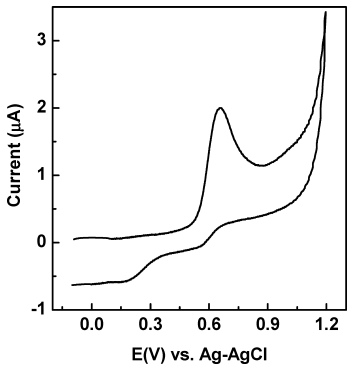

Fig. 4. Cyclic Voltammogram of 1.0×10^{-4} M Etoposide Solution on Carbon Paste Electrode in Britton–Robinson Buffer at pH 3.0 Scan Rate, 100 mV s^{-1}

is a reversible 1e-transfer resulting in a stable radical. The second step corresponds to the transfer of the second electron. The product formed after 2e-oxidation is an unstable cation which undergoes rapid conversion into the ortho-quinone. The irreversibility of the second electron-uptake is at least partly caused by the rapid conversions of the primary electrolysis product.

In order to develop a voltammetric methodology for determining the drug, we selected the differential pulse mode, since the peaks were sharper and better-defined at lower concentration of etoposide than those obtained by linear sweep voltammetry, with a lower background current, resulting in improved resolution. The optimum instrumental conditions were chosen from a study of the variation of the peak current with pulse amplitude, pulse width and scan rate. The peak current increased with increasing pulse amplitude from 20 to 100, but the peak became less sharp and ill defined. However, the peak current decreased as the pulse width increased from 30 to 90 ms. The peak current increased linearly with the scan rate up to 20 mV. Thus, the best peak definition was recorded when using 50 mV pulse amplitude, 30 ms pulse width and 10 mV s^{-1} scan rate. Using the optimum conditions described above, a calibration curve over a range of 2.5×10^{-7} to 1.0×10^{-5} M was obtained, which fitted the equation: i_p (μA)=0.29436+0.58165C (μM), with a correlation coefficient r=0.9983. Standard deviations for the intercept and slope of the calibration curve were 0.01462 μA and 0.00942 μA/μM, respectively. The limit of detection (LOD) of the procedure was calculated to be 1.0×10^{-7} M, estimated as: LOD=3$S_{y/x}/b$,[37] where $S_{y/x}$ is the standard deviation of y residuals and b is the slope of the calibration plot. The reproducibility of the measurement was calculated for five independent runs of 1.0×10^{-6} M etoposide solution. The relative standard deviations were calculated to be 0.56 and 2.77% for peak potential and peak current, respectively. For the determination of drug in spiked serum samples, five replicate samples at etoposide levels: 2.5×10^{-7}, 5.0×10^{-7}, 7.5×10^{-7} and 1.0×10^{-6} M (*i.e.*, 0.735, 1.471, 2.207 and 2.943 μg etoposide per ml of serum) were run through the procedure. The amount of etoposide was determined using the standard addition method. Figure 5 shows typical differential pulse voltammograms for etoposide extracted from serum samples and shows as inset the calibration plot. The average recovery was higher than 90% and the higher standard deviation was 5.5%, which indicated good recovery from serum samples

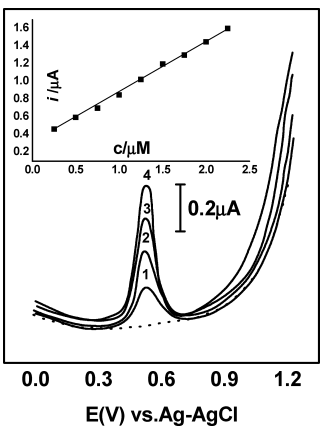

Fig. 5. Differential Pulse Voltammograms (DPVs) for Increasing Concentration of Etoposide: (1) 2.5×10^{-7}, (2) 5.0×10^{-7}, (3) 7.5×10^{-7} and (4) 1.0×10^{-6} M

Dotted line (.....) represents the blank in Britton–Robinson buffer pH 3.0 at carbon paste electrode. Scan rate, 10 mV s^{-1}; pulse amplitude, 50 mV; pulse width, 30 ms. Inset is the calibration plot.

Conclusion

Etoposide is electrochemically oxidized at carbon paste electrode. Application of the DPV method using carbon paste electrode to the determination of the etoposide drug in serum is possible after a simple extraction step. Convenient quantitation at the micromolar levels is feasible and the detection limit found for etoposide at the CPE for serum samples is low enough to reach the levels expected in serum after therapeutic doses. At an effective dosage of 250 mg per day, steady-state plasma concentrations are in the range, varying between 0.8 and 2.0×10^{-6} M.[38] The developed voltammetric measurement for serum samples after extraction step is, thus, sufficiently sensitive to determine the levels expected in serum after therapeutic doses. The proposed voltammetric technique has the advantages of being simpler, faster and less expensive than other methods described in literature.

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