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ORIGINAL PAPER

Adsorptive stripping voltammetric determination of podophyllotoxin, an antitumour herbal drug, at multi-walled carbon nanotube paste electrode

Riyaz Ahmad Dar · Pradeep Kumar Brahman · Sweety Tiwari · Krishna Sadashiv Pitre

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Abstract Adsorption stripping voltammetry, a very sensitive electroanalytical method, was employed to determine podophyllotoxin, a kind of antitumour herbal drug at a multi-wall carbon nanotube (MWCNT)-modified carbon paste electrode (CPE) surface. In the following anodic sweep from 0.5 to 1.5 V, podophyllotoxin, adsorbed at the MWCNT-modified CPE surface, was oxidized and yielded a sensitive oxidation peak with $E_{1/2}/E_p$ approximately 1.16 V/1.18 V over the scan rates of 10–120 mV s^{-1}. From CV and SWV studies of podophyllotoxin in the acetate buffers of various pH values, it was found that protons were involved in the oxidation of the drug at the H^+/e^- ratio of one (ΔE_p/pH = 56 mV at 25 °C). Its electrochemical behaviour was irreversible. The experimental conditions, such as supporting electrolyte, pH value, accumulation time, ionic strength and scan rate, were optimized for the measurement of podophyllotoxin. The best results were obtained in 0.02 M acetate/acetic acid buffer (pH 4.6) containing 0.04 M KCl (1:49, v/v) for 60 s accumulation. The oxidation peak current varies linearly with the concentration of podophyllotoxin over the range of 199–1796 pg mL^{-1}. The limits of detection and quantification of the pure drug are 4.5 and 14.96 pg mL^{-1}, with the correlation coefficient, $r = 0.998$ and the relative standard deviation, RSD = 1.3% ($n = 5$). This new method was successfully applied to the determination of podophyllotoxin in a plant sample of the rhizome of *Podophyllum hexandrum*. Recoveries were 99.173–101.231%. The relative standard deviations of intraday and interday analyses for podophyllotoxin were 0.55 and 0.61%, respectively ($n = 3$).

Keywords Adsorptive stripping voltammetry · Cyclic voltammetry · Square wave voltammetry · Podophylotoxin · Irreversible wave · Determination in plant sample

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1 Introduction

Podophyllotoxin is the most abundant lignan isolated from Podophyllin, the resin obtained from species of the genera *Podophyllum* (Berberidaceae). There are different biological activities [1, 2] in lignans that make them interesting for several investigation groups, like reverse transcriptase inhibition and anti-HIV activity, immunomodulating activity, effects on cardiovascular system, properties against leishmaniosis, effects on high-density lipoproteins and hypolipemiant properties, 5-lipoxygenase inhibition, antifungic, antirheumatic, antipsoriasis, antimalarial and antiasmatic properties. But cytotoxicity and antiviral are the more important activities that maintain the interest in podophyllotoxin and its analogues [1, 2]. Podophyllotoxin is included in many Pharmacopoeias and used as an antiviral agent in the treatment of *Condyloma acuminatum* caused by human papilloma virus (HPV) and other venereal and perianal warts. The application of podophyllotoxin cured almost all the warts completely in less time than other strategies and with fewer side effects. Podophyllotoxin and analogue compounds are also active against cytomegalovirus and Sindbis virus. The compound has other uses in dermatology: it is a useful agent in psoriasis vulgaris. Antitumour activity is another outstanding property of podophyllotoxin. It is effective in the treatment of Wilms

tumours, different types of genital tumours (e.g. carcinoma verrucosus) and in non-Hodgkin's and other lymphomas. In combination with *cis*-platin, it is effective in treating neuroblastomas. The mechanism of action of podophyllotoxin as a drug is based on inhibiting the polymerization of tubulin and arresting the cell cycle in the metaphase [1, 2]. The determination of podophyllotoxin by HPLC, RP-HPLC, RP-HPTLC and LC/MS has been reported [3–6]. However, the voltammetric behaviour of podophyllotoxin and its determination have not been investigated so far. In this study, the voltammetric behaviour of podophyllotoxin was studied and a method for the determination of trace amounts of podophyllotoxin was developed. The developed method was applied for the determination of the compound in a plant sample of *Podophyllum hexandrum* and satisfactory results were obtained. The electrode reaction mechanism has been discussed. The electrochemical response of podophyllotoxin has been attributed to oxidation of an alcoholic group.

The most sensitive electrochemical procedures for the determination of trace concentrations of various pharmaceutical compounds have conventionally employed a two-step approach consisting of (i) an initial preconcentration step, during which the analyte is allowed to accumulate at the electrode surface under carefully controlled conditions and (ii) a subsequent measurement in which the accumulated analyte is then stripped-off and determined by a voltammetric method. This preconcentration/measurement sequence forms the basis of all of the so-called stripping techniques, which permits determination of electroactive compounds at very low concentration [7–9]. Square-wave voltammetry (SWV) provides several important analytical features for electrochemical studies. The applications of carbon nanotubes (CNTs) to biosensors has led to increased stability and enhanced biosensor response. The CNT modified biosensor exhibits approximately eightfold enhanced sensitivity [10]. Luo et al. [11] have investigated electrochemical behaviour of CNT modified glassy carbon electrode for the oxidation of biomolecules like dopamine, epinephrine and ascorbic acid resulting in considerable improvement in the electro oxidation of these biomolecules. Recent studies have reported that surface-confined CNTs can dramatically accelerate the electron transfer reactions of important analytes, including catecholamine neurotransmitters, hydrogen peroxide, cyctochrome C, NADH and hydrazine compounds [12, 13]. From an analytical point of view, these electrodes exhibit rather low background currents over a large range of potentials when compared with other solid electrodes, and after a renewability of their surface as well as a high versatility and simplicity of modification.

In the current study, a carbon paste electrode (CPE) modified with a multi-walled carbon nanotube paste (MWCNTP) is described for the determination of podophyllotoxin. The electrochemical behaviour of podophyllotoxin indicated that the MWCNT paste significantly increased the oxidation peak current of podophyllotoxin, and consequently remarkably improved the sensitivity for podophyllotoxin determination. All the experimental conditions were examined, and finally a voltammetric method was developed for the measurement of podophyllotoxin in biological samples using adsorptive stripping voltammetry. This newly proposed method has the following advantages: high sensitivity, rapid response, low cost and simplicity.

2 Experimental

2.1 Reagents and materials

Podophyllotoxin, Spectroscopic graphite powder and paraffin oil were purchased from Sigma Chemical Co. (USA). A standard stock solution of podophyllotoxin was prepared by dissolving 10 μg in 100 mL of pure ethanol. The supporting electrolyte was 0.2 M acetate buffer. All chemicals were of analytical-reagent grade. Triply distilled water was used throughout. Plant samples of rhizome of *P. hexandrum* was obtained from the hilly forest areas of village Sugan, District Budgam, Kashmir, Jammu and Kashmir (India).

2.2 Apparatus

All voltammetric experiments were performed with Ω Metrohm model 797 VA Computrace (ion analyzer, Switzerland) through electrochemical software version 3.1. A three-electrode cell was employed incorporating a hand-made working MWCNT-modified CPE, an Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode. Mass transport was achieved with a Teflon-coated bar at approximately 400 rpm using a magnetic stirrer (KIKA Labortechinik, Germany). A systronics digital μpH meter model-361 was used for pH measurements. All experiments were performed at room temperature and dissolved oxygen was removed by passing pure nitrogen through the solutions.

Carbon nanotube electrode was prepared in usual way by hand-mixing graphite powder (Aldrich, 1–2 mm), carbon nanotube powder (Sigma) and mineral oil (Sigma). The ratio of these three was 60:10:30. The prepared paste was filled into the Teflon well. A copper wire fixed to a graphite rod and inserted into the Teflon well serves to establish electrical contact with the external circuit. A good reproducibility of electrode response was achieved by simply renewing the surface of paste electrode. New electrode surface was formed by mechanically pressing

the paste from the top of the Teflon well. Smoothening of the electrode surface was done by rolling a smooth glass rod on the electrode surface and finally it was cleaned carefully by distilled water. Each measurement involved fresh carbon nanotube surface. Carbon paste electrode was prepared in the same way in which the graphite powder and mineral oil were mixed in the ratio of 70:30.

2.3 General analytical procedure

A 50-mL volume including 5 mL of 0.2 M acetate/acetic acid buffer pH 4.6, 1 mL ethanol, 1 mL of 2 M KCl and a specific amount of sample solution was added to the cell and purged with purified nitrogen for 5 min to remove oxygen. The DCV, DPV and CV voltammograms were recorded in Exploratory mode. The scanning potential was varied from 0 to +2 V in the positive potential direction. The preconcentration step was performed by immersing the electrode in a stirred 50 mL sample solution for a given period of time, at different potentials ranging from −0.5 to +1.2 V. The stirring was then stopped and after a delay period of 10 s to settle the solution and decrease the background current, cyclic or square-wave voltammogram was recorded in the positive potential direction. A renewed carbon nanotube paste surface was used for each measurement.

2.4 Isolation of podophyllotoxin from *P. hexandrum*

The literature reports the method of isolation of Podophyllotoxin from ground roots and rhizome of *P. peltatum* [14]. Ground roots and rhizomes of *P. hexandrum* were stirred at room temperature with chloroform for 1 h, the mixture was filtered with suction, and the residue was washed with chloroform. The yellow filtrate was concentrated in an air-current, filtered from flocculent material, and diluted with ligroin (petroleum ether). The precipitate was collected, redissolved in cold chloroform, and reprecipitated with ligroin, then dissolved again in chloroform, chromatographed on 17 parts of neutral alumina and eluted with chloroform. The yellow eluate was evaporated in an air-current, the oil was dissolved in dichloromethane, and the solution was diluted with pentane to incipient turbidity. The product was recrystallized three times from dichloromethane-pentane to yield rosettes of colourless prismatic needles, which when dried at room temperature for 24 h, melted at 160.4–161.6 °C. The melt resolidified on further slow heating, to melt again at 183–184 °C. When dried at 137 °C/0.01 mm of mercury for 24 h, the material melted at 183.3–184 °C.

2.5 Interday and intraday assay

Blank samples spiked with standard concentrations were used in the evaluation of interday and intraday assays. In this experiment, two standards 0.399 ng mL^{-1} each were spiked. Each spiked sample was determined in triplicate for three consecutive days. The interday and intraday precisions were evaluated using the relative standard deviation. Also, the same electrode was used for three consecutive days for evaluating the stability of the electrode.

3 Results and discussion

In 0.02 M acetate buffer (pH = 4.6) and at MWCNT-modified CPE, an anodic wave of podophyllotoxin (PTOX) was obtained by direct current voltammetry at $E_{1/2} = 1.16 \pm 0.01$ V and in differential pulse voltammetry a well defined sharp peak was obtained at $E_p = 1.18 \pm 0.01$ V. (Fig. 1a, b).

In order to show the unusual properties of MWCNTPE, the electrochemical properties of podophyllotoxin at two different working electrodes (i.e. bare CPE and MWCNT-modified CPE) were studied by differential pulse voltammetry (DPV). Figure 2 shows the differential pulse

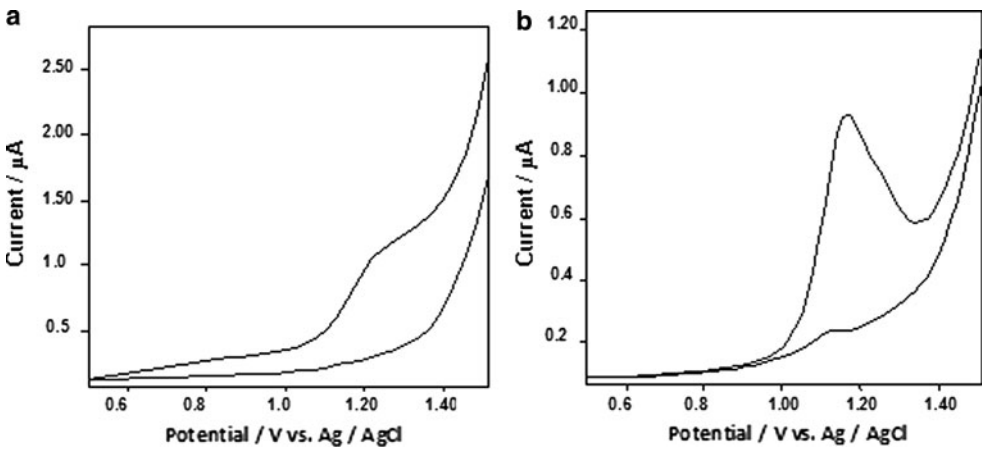


Fig. 1 **a** DC and **b** DP Voltammograms of podophyllotoxin. Conditions: 0.02 M acetate–acetic acid buffer/4% ethanol (pH = 4.6), 0.04 M KCl, 0.399 ng mL^{-1} PTOX, scan rate = 50 mV s^{-1}, $t = 60$ s

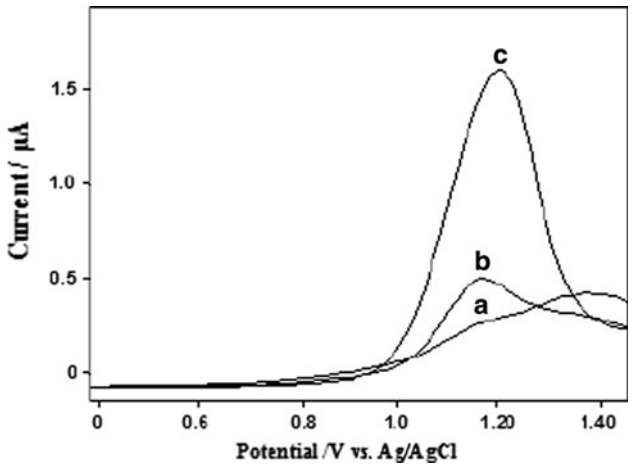


Fig. 2 Differential pulse voltammograms of 0.799 ng mL^{-1} podophyllotoxin in pH 4.6 acetate buffer at two different electrodes after 60 s accumulation. Curve (*a*) blank, curve (*b*) bare CPE, curve (*c*) MWCNT-modified CPE. Scan rate: 50 mV s^{-1}

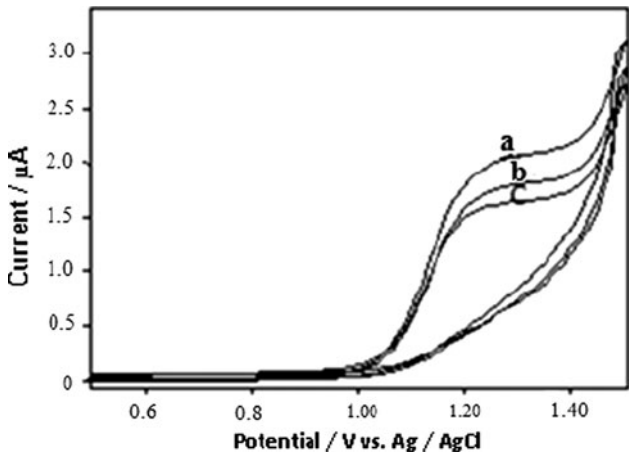


Fig. 3 Repetitive cyclic voltamperograms of 0.799 ng mL^{-1} PTOX. Conditions: 0.02 M acetate–acetic acid buffer/4% ethanol (pH = 4.6), 0.04 M KCl, scan rate = 50 mV s^{-1}, t = 60 s

voltammograms of 0.799 ng mL^{-1} podophyllotoxin in pH 4.6 acetate buffer at two different working electrodes. Curve a is the blank containing 0.02 M acetate buffer at pH 4.6. At bare CPE, 0.799 ng mL^{-1} podophyllotoxin yields a very low oxidation peak at 1.17 V after 60 s of accumulation (curve b). However, under identical conditions, the oxidation peak current of podophyllotoxin at an MWCNT-modified CPE increases about ten times in contrast to that at a bare CPE. The remarkable peak current enhancement can undoubtedly be attributed to the unique structure and properties of MWCNT (such as very large specific area, strong adsorptive ability, subtle electronic properties). In short, an MWCNT-modified CPE greatly improves the determination sensitivity for podophyllotoxin.

3.1 Adsorptive properties

3.1.1 Repetitive cyclic voltamperograms

Figure 3 shows repetitive cyclic voltamperograms for 0.799 ng mL^{-1} podophyllotoxin (PTOX) in sodium acetate–acetic acid buffer (pH 4.6) containing 0.04 M KCl, recorded after preconcentration at −0.4 V for 60 s. Only one anodic peak is observed in the first scan (curve a) at 1.18 V. Subsequent scans (curves b, c) exhibited a substantial decrease in the peak to a stable value after three scans at curve c, showing that podophyllotoxin has adsorptive characteristics at the carbon nanotube electrode. The decrease in peak current may be caused by the fact that the adsorption of podophyllotoxin or its oxidative product occurs at electrode surface. The adsorption fouls the electrode surface and retards the electro-oxidation of the drug. Also, in the forward scans (a, b, c), one anodic peak due to the oxidation of the secondary alcoholic group was observed and no peak was noticed in the reverse direction. The peak currents decrease with succeeding potential scans suggesting an adsorbed species formation on the electrode surface. This indicates that the oxidation process is an irreversible one.

3.2 Effect of deposition potential

The dependency of peak current on deposition potential is shown in Fig. 4. The maximum increase in peak height characterizes the formal potential of oxidation of podophyllotoxin (PTOX) in the electrolyte used. It is clear that at −0.4 V of deposition potential peak current assumes a value that does not grow any further. A further shift in the deposition potential would not improve the sensitivity of the PTOX determination. Thus, −0.4 V of deposition potential was applied in all measurements.

3.3 Effect of accumulation time

Figures 5a and 6a show the linear sweep voltammograms of 0.198 and 0.999 ng mL^{-1} of podophyllotoxin, respectively, and Figs. 5b and 6b show their plots of the anodic

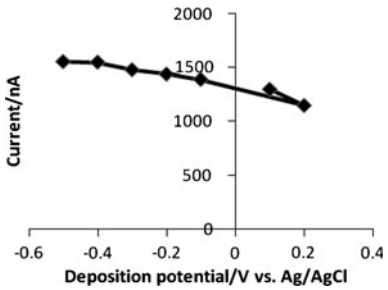


Fig. 4 Dependency of peak current in the oxidation of 0.399 ng mL^{-1} PTOX on deposition potential. Conditions: 0.02 M acetate–acetic acid buffer/4% ethanol (pH = 4.6), 0.04 M KCl, scan rate = 50 mV s^{-1}, t = 60 s

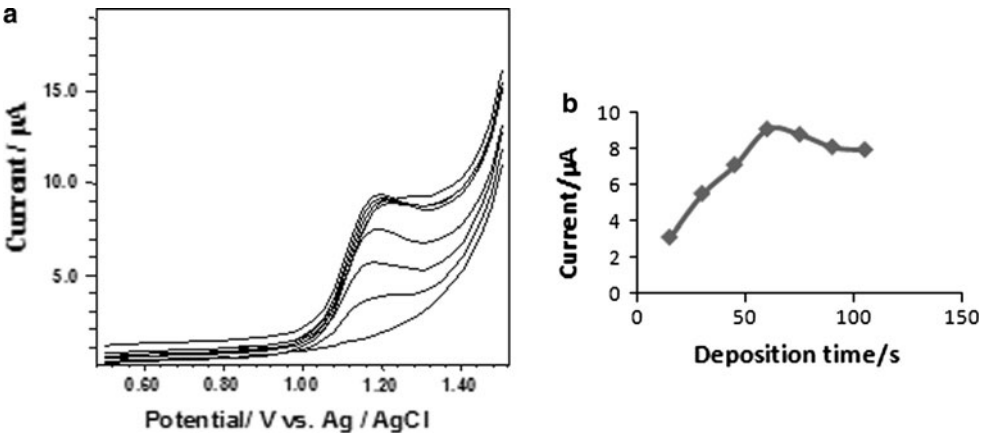


Fig. 5 **a** Linear sweep voltammograms of 0.198 ng mL^{-1} PTOX at different accumulation times. **b** Effect of accumulation time on peak current. Conditions: 0.02 M acetate–acetic acid buffer/4% ethanol (pH = 4.6), 0.04 M KCl, scan rate = 50 mV s^{-1}

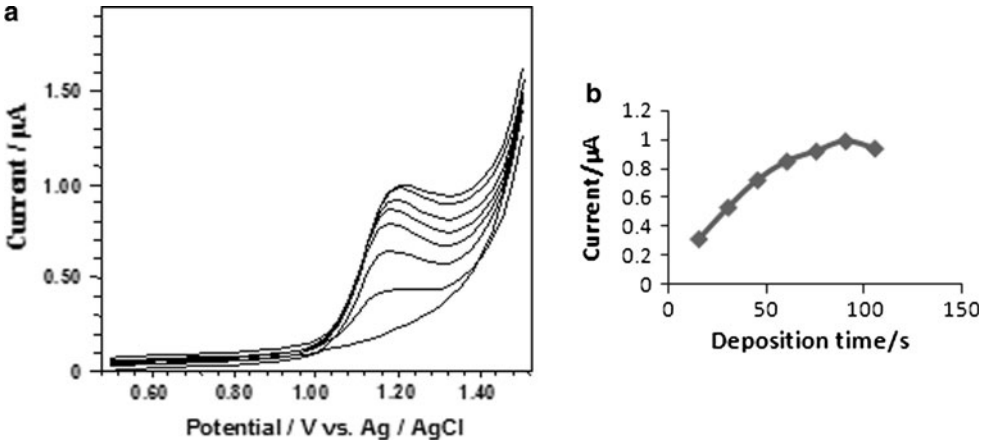


Fig. 6 **a** Linear sweep voltammograms of 0.999 ng mL^{-1}PTOX at different accumulation times. **b** Effect of accumulation time on peak current. Conditions: 0.02 M acetate–acetic acid buffer/4% ethanol (pH = 4.6), 0.04 M KCl, scan rate = 50 mV s^{-1}

peak currents (i_{pa}) of the peak in linear sweep voltammetry versus accumulation time (t). From these plots we see that at first, i_{pa} increased with the increase in accumulation time (t), indicating that before adsorptive equilibrium is reached, the longer the accumulation time, more the PTOX adsorbed and the larger was the peak current. However, after a specific period of accumulation time, the peak current tended to level off, illustrating that adsorptive equilibrium of PTOX on the electrode surface was achieved. The peak current increased with the increase in preconcentration time up to 90 and 60 s for 0.198 and 0.999 ng mL^{-1} PTOX, respectively. This also indicates that time to reach equilibrium varies for different concentrations.

3.4 Effect of scan rate

Figure 7 shows the effect of scan rate on the peak current. The peak currents i_{pa} increased with increasing scan rate (Fig. 7a). The experiments indicate further that when $t_{acc} = 0$ s, i_{pa} shows a linear relationship with $v^{1/2}$, illustrating that the oxidation of PTOX is diffusion controlled. A linear relationship was observed between log i_p and log v corresponding to the equation: log i_p (µA) = 0.451 log v + 1.408, where v is in mV s^{-1} (Fig. 7b). The slope of 0.451 is close to the theoretically expected value of 0.5 for a purely diffusion-controlled current [15]. When $t_{acc} = 60$ s, the i_{pa}–v curve became a straight line (Fig. 8a), suggesting that the electrode process was adsorption-controlled [16]. Also, the plot of $i_{pa}/v^{1/2}$ versus log v indicated an increase in peak current with an increase in sweep rate (Fig. 8b) confirming that the electrode surface has adsorption effects [17–19].

The E_{pa} of the oxidation peak was also dependent on scan rate. The plot of E_{pa} versus log v was linear having a correlation coefficient of 0.995 (Fig. 9) and this behaviour was consistent with the EC nature of the reaction in which the electrode reaction is coupled with an irreversible follow up chemical step [20]. The relation between E_{pa} and v can be expressed by the equation E_{pa} (V) = 0.085 log v + 1.054.

3.5 Effect of pH and ionic strength

The effect of pH on the oxidation of podophyllotoxin at the MWCNTPE was studied over the pH range 2–8 at the concentration 1.198 ng mL^{-1} by square-wave voltammetry (Fig. 10a). A small current was observed at pH 3.0, which

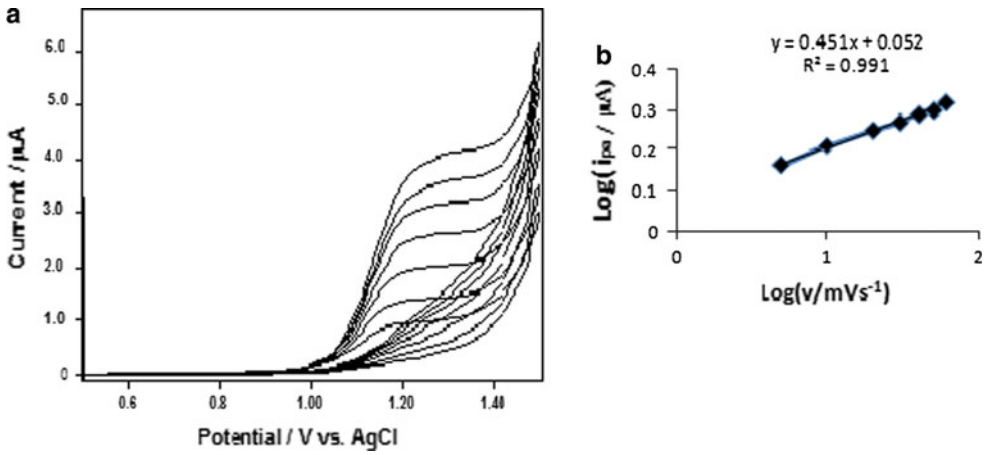


Fig. 7 **a** Effect of scan rate (v) on the anodic current of PTOX in 0.02 M acetate–acetic acid buffer/4% ethanol (pH = 4.6), 0.04 M KCl. Conditions: 0.799 ng mL^{-1} PTOX, $t_{acc} = 0$, scan rate 10, 20, 40, 60, 80, 100, 120 mV s^{-1}. **b** Variation of the logarithm of peak current with the logarithm of the sweep rate

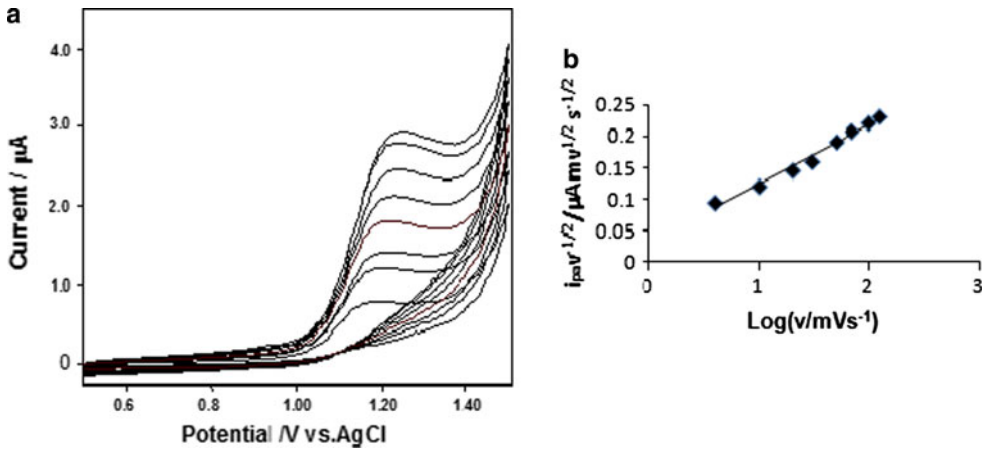


Fig. 8 **a** Effect of scan rate (v) on the anodic current of PTOX in 0.02 M acetate–acetic acid buffer/4% ethanol (pH = 4.6), 0.04 M KCl. Conditions: 0.799 ng mL^{-1} PTOX, $t_{acc} = 60$ s, scan rate 10, 20, 40, 60, 80, 100, 120 mV s^{-1}. **b** Dependency of $i_{pa}/v^{1/2}$ on log v

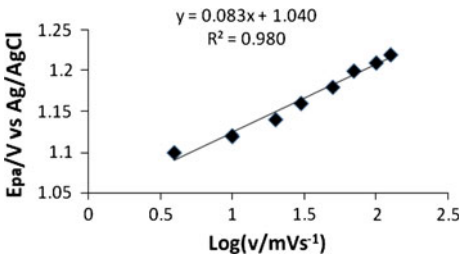


Fig. 9 Dependence of E_{pa} on log v for 0.799 ng mL^{-1} PTOX

increased gradually up to pH 4.6 and then decreased at higher pH. Thus, pH 4.6 was used in all measurements. Effect of pH on oxidation of PTOX can be attributed to the change of ionic strength and protonization ratio of its –OH group on CNTs which can affect the accumulation efficiency of the drug on the electrode surface. It can change the electrochemical reaction rate as it involves the proton transfer. Both the peak height and the peak shape were taken into consideration when choosing the supporting electrolyte. The results showed that sodium acetate–acetic acid buffer gave the best response. The influence of ionic strength on the efficiency of the accumulation of 0.399 ng mL^{-1} was studied for a 60 s preconcentration time. The ionic strength was varied by changing the KCl from 0.02 to 0.2 M, in 0.02 M sodium acetate–acetic acid (pH 4.6) (1:49, v/v). The results showed that increasing ionic strengths were found to be of great significance on the degree of accumulation. The stripping peak did not remain constant and this indicated that the process responsible for accumulation of the drug at the electrode surface was mainly electrostatic in nature and thus a change in ionic strength influences the peak current. The concentration effect of KCl was very important, as can be seen in Fig. 10b. The best accumulation was attained in 0.02 M sodium acetate–acetic acid buffer (pH 4.6) containing 0.04 M KCl (1:49, v/v).

4 Measurement of the number of electrons and protons transferred

Figure 10a shows square wave voltammetry of 1.198 ng mL^{-1} of podophyllotoxin in acetate buffer of different pH. It was observed that the peak potential of PTOX was

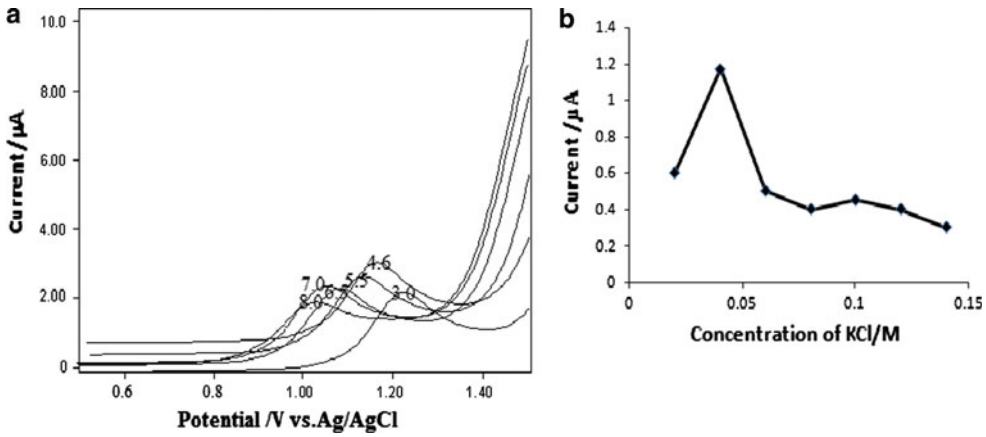


Fig. 10 **a** Effect of pH (3.0, 4.6, 5.5, 6.5, 7, 8 from *right* to *left*) on 1.198 ng mL^{-1} PTOX at 60 s using 0.02 M sodium acetate–acetic acid/4% ethanol buffer containing 0.04 M KCl (1:49, v/v) in square wave voltammetric mode of pulse amplitude 50 mV, scan rate 50 mV s^{-1}. **b** Effect of concentration of KCl on peak current of 0.399 ng mL^{-1} of PTOX at 60 s and acetate–acetic acid/4% ethanol buffer pH 4.6. Scan rate 50 mV s^{-1}

shifted towards negative potential with increase in pH. This reveals that the pH of the supporting electrolyte exerted a significant influence on electrooxidation of PTOX at MWCNTPE. The plot of peak potential versus pH gave a slope of 56 mV pH^{-1} which is close to the expected value of 59 mV pH^{-1} (Fig. 11).With this, we propose that the number of electrons and protons participating in the electrode process are equal i.e. during the reaction not only electrons, but also protons are released from the molecule [21–23]. The electron transfer coefficient 'α' is calculated from the difference between peak potential (E_p) and half wave potential ($E_{p/2}$) according to the Eq. 1 given below for the electrode process [22, 24].

$$\Delta E = E_p - E_{p/2} \ldots \quad (\text{for electrode process at } 298\ \text{K}) \qquad (1)$$

The value of α is calculated to be 0.6.

According to the Laviron equation [25] for the irreversible anodic wave:

$$W_{1/2} = 2.44RT/(\alpha n\text{F}) = 62.5/(\alpha n)\ (298\ \text{K})$$

where $W_{1/2}$ is the half-width of the peak. From the peak, $W_{1/2} = 59.2$ mV. From this αn was calculated to be 1.0557. The same process was repeated three times and gave $n = 1.759, 1.81, 2.01$.

Thus, we conclude that podophyllotoxin in 0.02 M acetate buffer undergoes two electron irreversible oxidation reaction. The expected oxidation product of PTOX on electrode surface is podophyllotoxone [26] (Scheme 1).

5 Selection of experimental conditions

In order to choose the optimum experimental conditions for the determination of PTOX by adsorptive stripping voltammetry, a series of experiments were carried out. Various supporting electrolytes, such as ammonium tartrate, tetramethyl ammonium bromide, NaOH, KCl, H_2SO_4, Phosphate buffer, NH_3–NH_4Cl and HOAc–NaOAc buffer solutions were tested (Fig. 12). HOAc–NaOAc was found to be the best because of the fairly well-defined voltamperogram and reasonably high sensitivity. The effect of supporting electrolyte concentration on anodic peak current was examined. The results showed that peak current increased with increasing acetate buffer concentrations from 0.002 to 0.02 M and remained almost constant at concentrations higher than 0.02. So 0.02 M acetate buffer was chosen for subsequent experiments. The effect of the deposition potential was also studied. Peak current increased with increasing deposition potential in the negative direction, then reached a constant value. A potential of −0.4 V was chosen as the optimum preconcentration

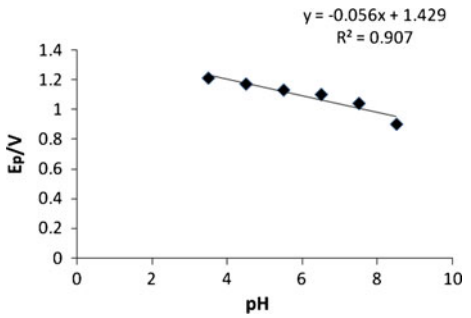
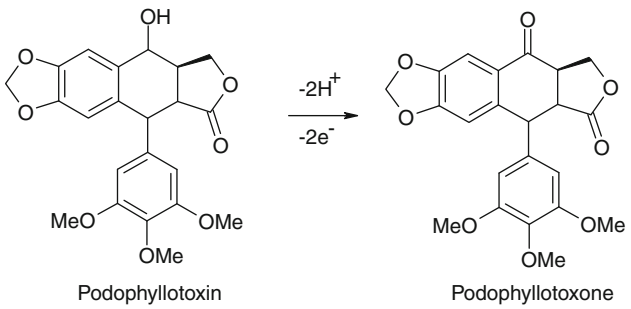


Fig. 11 The plot of peak potential (E_p) of podophyllotoxin versus pH values of the supporting electrolyte



Scheme 1 Probable oxidation mechanism of Podophyllotoxin

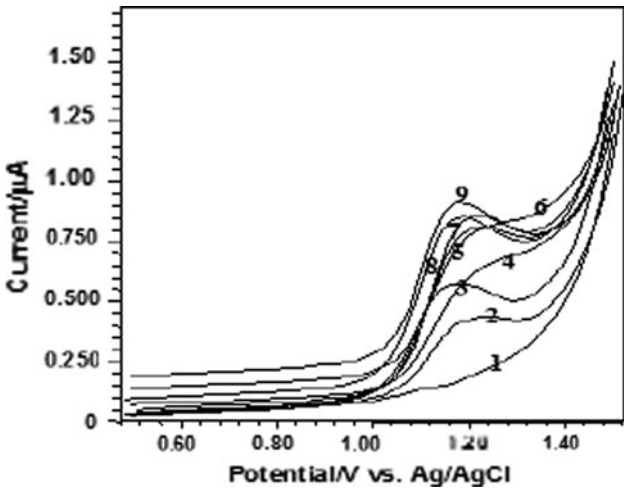


Fig. 12 Effect of supporting electrolytes on anodic oxidation of 0.399 ng mL^{-1} Podophyllotoxin; pH 4.6, 0.02 M each supporting electrolyte, scan rate 50 mV s^{-1}: *1* blank, *2* phosphate buffer, *3* NH_3–NH_4Cl, *4* ammonium tartrate, *5* tetramethyl ammonium bromide, *6* NaOH, *7* KCl, *8* H_2SO_4, *9* HOAc–NaOAc buffer

potential. A potential in positive direction showed no significant change. The stability of the system was fairly good.

6 Calibration graph and detection limit

The peak current increased with the increase in preconcentration time up to 90 and 60 s for 0.198 and 0.999 ng mL^{-1} PTOX, respectively. We selected 90 and 60 s deposition times for two calibration curves measured by DPV in determination mode. The characteristics of the two curves are shown in Table 1. Under the optimized conditions of 0.02 M sodium acetate buffer, pH = 4.6 and 0.04 M KCl a preconcentration potential of −0.4 V and a scan rate of 50 mV s^{-1}, the peak current of PTOX was found to be proportional to its concentration over the range 199–1796 pg mL^{-1} at deposition time of 60 s (Fig. 13). The results show positive deviations from linearity at concentrations higher than 1796 pg mL^{-1} PTOX at 60 s (with a slope of 2.4 ± 0.1) or from 1831 pg mL^{-1} PTOX at 90 s with a slope of 1.99 ± 0.4, respectively. This phenomenon and the change in the slope of the response might be attributable to surface effects of the investigated molecule [24].

The limits of detection and quantification were calculated according to IUPAC, the detection limit DL = $3s/k$ [27] and limit of quantification QL = $10s/k$ [28], where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte ($n = 5$); k is the sensitivity, namely the slope of the calibration graph.

7 Analytical figures of merit

The analytical figures of merit for podophyllotoxin determination are summarized in Table 2 by various reported methods and the proposed method. It is clear from the table that limit of detection for the PTOX determination using the developed voltammetric method is the least among all the other methods.

8 Analytical applications

8.1 Analysis of podophyllotoxin in a sample of rhizome of *P. hexandrum*

The present developed adsorptive stripping voltammetric method was used for the determination of podophyllotoxin in pharmaceutical dosage forms in a plant sample of rhizome of *P. hexandrum.*

Measurement of PTOX in *P. hexandrum* rhizome was performed in differential pulse voltammetric mode at deposition time of 60 s. Podophyllotoxin was isolated from the rhizome as discussed earlier. 40 μg of the isolated sample was dissolved in 100 mL of ethanol. 0.1 mL of the sample was diluted with the supporting electrolyte. The experimental conditions were set as discussed above. The peak potential of PTOX was set at 1.18 V in the determination mode analysis. The determination was accomplished by the standard addition calibration method (Fig. 14), and the results of a few analyses are given in Table 3. The relative standard deviation for intraday and interday assay was 0.55 and 0.61%, respectively. In addition, some recovery experiments were carried out and the recovery was 99.17–100.23%. This also proved the stability of electrode. The purity of isolated podophyllotoxin was

Table 1 Characteristics of linear regression of calibration curves for podophyllotoxin (PTOX) in sodium acetate buffer/4% ethanol, pH = 4.6 containing 0.04 M KCl using DPV in determination mode at different deposition times

Deposition time (s)	Linearity range (pg mL^{-1})	Correlation coefficient	Intercept ± SD	Slope ± SD	LOD (pg mL^{-1})	LOQ (pg mL^{-1})
60	199–1796	0.998	0.612 ± 3.6	2.4 ± 0.1	4.5	14.960
90	205.4–1831	0.978	0.571 ± 2.7	1.99 ± 0.4	4.07	13.567

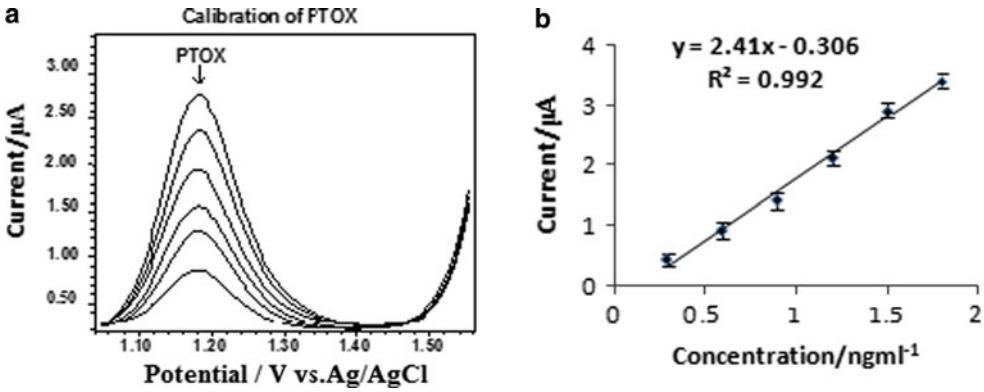


Fig. 13 The plot of peak current vs. concentration of podophyllotoxin in the acetate–acetic acid buffer/4% ethanol of pH 4.6 containing 0.04 M KCl, scan rate 50 mV s^{-1} and deposition time 60 s. Data in the calibration curve b represent the mean ± SD

Table 2 Analytical figures for comparison of results of podophyllotoxin by RP-HPLC, RP-HPTLC, LC–MS with proposed voltammetric method

Concentration (pg mL^{-1})			Regression line				Reference no.
Method	Linear range	LOD	Slope	Intercept	*r*(*n*)	% RSD	
RP-HPLC	3×10^4–3×10^6	30	0.979	0.0175	0.994(3)	Not reported	[5]
RP-HPTLC	3×10^4–3×10^6	51	0.564	1.294	0.988(3)	Not reported	[5]
LC/MS	10^6–10^8	2400	0.025	0.0464	0.99(3)	0.52–6.01	[6]
Proposed voltammetric method	199–1796	4.5	2.4	0.612	0.998(5)	1.3	Present study

LOD Limit of detection, *r* Correlation coefficient, *n* number of measurements

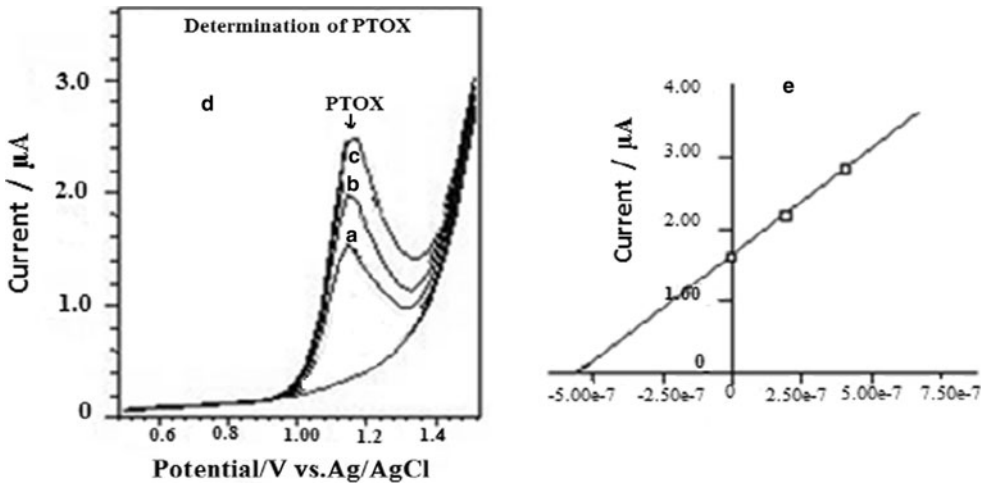


Fig. 14 Differential pulse voltammetric determination by standard addition calibration of podophyllotoxin at deposition time of 60 s in acetate–acetic acid buffer/4% ethanol of pH = 4.6 containing 0.04 M KCl (1:49, v/v). (*a*) 0.798 ng mL^{-1} sample, (*b*) addition-1 (0.399 ng mL^{-1}), (*c*) addition-2 (0.399 ng mL^{-1}), (*d*) standard additions, (*e*) calibration curve ($r = 0.989$). Scan rate 50 mV s^{-1}

found to be 92.95% and the amount of the drug in plant sample was found to be 6.08% which is in good agreement as reported by earlier workers [6–8]. The results confirm the usefulness of the proposed method for the determination of podophyllotoxin.

9 Interferences

For the possible analytical application of the proposed method, the effect of some common excipients used in pharmaceutical preparations were studied by analyzing sample solutions containing a fixed amount of podophyllotoxin (0.399 ng mL^{-1}) spiked with varying concentrations of each excipient under the similar experimental conditions. Some tested excipients, such as glucose, sucrose and lactose, were used. The interference studies were done by dissolving these excipients in ethanol/aqueous solution. However, no interference study has been done with starch and gelatin because they are not soluble in alcohol/aqueous medium and hence could not interfere. The determination of podophyllotoxin in the presence of

Table 3 Analytical results for podophyllotoxin in *P. hexandrum* samples (interday assay)

Day	Sample taken (ng mL^{-1})	Standard added (ng mL^{-1})	Found (ng mL^{-1})	Mean found (ng mL^{-1})	Correlation coefficient	RSD (%)	Recovery (%)
1	0.795	–	–	–	–	–	–
	–	0.399	–	–	–	–	–
	–	0.399	0.731	–	0.989	–	–
2	0.795	–	–	–	–	–	–
	–	0.199	–	–	–	–	–
	–	0.199	0.725	–	0.990	–	99.173
3	0.795	–	–	–	–	–	–
	–	0.133	–	–	–	–	–
	–	0.133	0.740	0.735	0.991	0.613	101.231

above mentioned excipients was evaluated and a recovery ranging from 99.18 to 99.59% was obtained. The results show that no serious interference occurred from the classical additives tested.

10 Accuracy and repeatability

The content of podophyllotoxin was determined by standard addition calibration method, and the results are shown in Table 3. The results obtained by this method are in good agreement with the results obtained by HPLC and LC/MS. Furthermore, in order to establish the suitability of the proposed method, known amounts of standard podophyllotoxin were added into the analytical solution, and the same procedure was applied. The recoveries indicate that the accuracy and repeatability of this proposed method are very good. The repeatability of the method was determined from multiple measurements at each of the studied samples ($n = 3$). An average deviation did not exceed 0.61%. The correlation coefficient of more than 0.991 and average relative standard deviation of 0.61% indicates adequate precision and accuracy of the method. From above experimental results, it is very clear that this novel method has great potential for practical sample analysis.

11 Conclusions

The voltammetric behaviour of podophyllotoxin has been investigated at the hand-made MWCNT-modified CPE in acetate buffer at pH 4.6. A mechanism of the oxidation of podophyllotoxin has been proposed which shows that two protons are involved in the oxidation of the drug at the H^+/e^- ratio of one ($\Delta E_p/pH = 55$ mV at 25 °C) and the process is two electron oxidation. Investigation on the various parameters has shown that oxidation is an adsorption-controlled process. The regression analysis revealed that the concentration of podophyllotoxin can be determined voltammetrically. The developed method has proved its accuracy in the determination of podophyllotoxin in the rhizome of *P. hexandrum.* The percentage recovery of podophyllotoxin using the developed electroanalytical method was found to vary from 99.173 to 101.231%. The limit of detection of the proposed method was found very low with respect to previous ones. The drug content in plant sample determined by this method is in good agreement with the data reported in literature. With its low cost, good stability, high sensitivity and the reproducibility of the voltammetric response make the prepared system very useful in the construction of simple devices for the determination of PTOX in clinical and pharmaceutical preparations. It can also be used to inspect various sources of podophyllin or *Podophyllum* species.

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