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Full Paper

Carbon Paste Sensor for the Determination of an Anticancer Drug Paclitaxel in Pharmaceuticals and Biological Fluids

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Abstract- A sensitive determination of paclitaxel (PAC) at physiological pH was developed by carbon paste electrode using cyclic, linear sweep and differential pulse voltammetric techniques. The voltammetric response of paclitaxel was excellent with carbon paste electrode at sweep rate of 50 mV/s in the potential range from 0.8 V to 1.4 V. The voltammogram of paclitaxel was irreversible. The effect of scan rate showed that the electrode process was diffusion controlled. The concentration and pH effects on the voltammogram of paclitaxel were studied. Under optimized conditions, the concentration range and detection limit were 2.0×10^{-6} to 2.0×10^{-5} M and 4.6×10^{-9} M, respectively. The analytical performance of this electrode was evaluated for the detection of paclitaxel in pharmaceutical and real samples.

Keywords- Voltammetry, Paclitaxel, Carbon paste electrode, Pharmaceutical dosage, Human biological fluids

1. INTRODUCTION

Electrochemical detection of analyte is a very elegant method in analytical chemistry [1]. The interest in developing electrochemical-sensing devices for use in environmental monitoring, clinical assays or process control is growing rapidly. Electrochemical sensors satisfy many of the requirements for such tasks particularly owing to their inherent

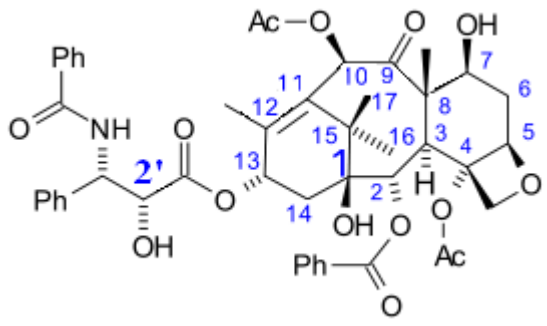
specificity, rapid response, sensitivity and simplicity of preparation for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids [2,3]. Carbon electrodes, especially glassy and paste electrodes are widely used in electrochemical investigations.

Carbon paste electrodes (CPEs) are very popular due to their wide anodic potential range, low residual current, ease of fabrication, easy surface renewal and low cost. Nearly a half of a century, carbon pastes made of carbon powder and a liquid binder belong among the most popular materials for preparation of various electrodes and sensors [4,5]. Hand-made carbon paste mixtures are a soft and non-compact material that has to be packed into a special electrode holder; the whole setup representing – in traditional point of view – carbon paste electrode (CPE) [6].

The most frequent holders for carbon paste are bodies made of glass and plastic tubes [7], compact rods with a well drilled in [6,8], or piston-driven constructions [9,10]. These fundamental types usually offer a simple and quick renewal of the electrode material, which is one of the most distinct advantages of CPEs [4-6].

Paclitaxel is an anticancer drug with the structure shown in Scheme 1. It interacts with microtubule proteins in a manner that catalyzes the formation of microtubules from tubulin and stabilizes the resulting structures [11]. Its antitumor activity is based on increasing the stability of microtubules and preventing mitosis [12–14]. In the cell, this phenomenon leads to an altered morphology, with microtubules forming stable bundles and the cell being unable to assemble a normal mitotic spindle [15]. In cells treated with Paclitaxel, the transition between interphase and mitosis is normally arrested and the cells die. The elucidation of this unique mechanism during the late 1970 s and 1980 s promoted

The development of Paclitaxel as an anticancer drug. Paclitaxel has shown unusual efficacy as a clinical agent for the treatment of breast, ovarian, skin, lung, head, and neck cancers. In 1993, Paclitaxel was approved by the U.S. Food and Drug Administration for use in the treatment of breast and ovarian cancers.



Scheme 1. Chemical structure of paclitaxel

Different methods have been reported for the determination of paclitaxel, including square wave voltammetry at Cysteamine/DNA/SWNTs-Film modified Au electrode [16], liquid chromatography with anodic amperometric detection [17] and different HPLC methods for the determination of PAC [18-22]. The main problems encountered in using such methods are time consuming extraction and separation procedures.

The main objective of this work is the development of a simple, rapid, and sensitive voltammetric method for the determination of PAC by CPE and applying it to the pharmaceutical and real samples. There are no sample preparation and time-consuming extraction steps other than sonication for the determination of PAC in injection form by the proposed voltammetric method. The obtained results by the proposed methods have been compared with the labeled values of injection sample, and the proposed method yields accurate, fast, and reproducible results.

2. EXPERIMENTAL

2.1. Materials and methods

Pure PAC in powdered form was obtained from Reddy's Laboratory, Hyderabad, India and was used without further purification. A stock solution (1.0 mM) of PAC was prepared in methanol. Paclitaxel containing injections marketed by NEON Lab. LTD. were purchased from the local pharmacy. Phosphate buffer solutions (Ionic strength=0.2 M) were prepared according to the literature method [23]. All other chemicals were of analytical grade and were used without further purification. All solutions were prepared with deionized water treated in a Millipore water purification system (Millipore Corp.). All experiments were carried out at room temperature.

2.2. Instrumentation and analytical procedure

Voltammetric measurements were performed with a CHI 630 D electrochemical analyzer (CH Instruments Inc., USA). A conventional three-electrode cell was used, including a saturated calomel electrode (SCE) as reference electrode, a platinum wire counter electrode and a carbon paste working electrode (CPE). All the potentials are given against the Ag/AgCl (3.0 M KCl). PH measurements were performed with an Elico LI 120 pH meter (Elico Ltd., India).

The parameters for differential pulse voltammetry (DPV) were initial potential: 1.0 V; final potential: 1.3 V; increase potential: 0.004 V; amplitude: 0.05 V; pulse period: 0.5; accumulation time: 60 s; sensitivity: 1×10^{-6} A/ V. Selectivity of the electrode was carried out by taking different electrodes such as glassy carbon electrode, gold electrode and carbon paste electrode. As we obtained high peak intensity for carbon paste electrode, we selected carbon paste electrode for our study.

2.3. Preparation of carbon paste electrode

The carbon paste electrode was prepared by mixing graphite powder with appropriate amount of silicon oil and through hand mixing in a mortar and pestle (70:30) to produce a homogeneous carbon paste. The portion of the carbon paste was then packed into the cavity of a Teflon rod (3 mm i.d) and then smoothened on a weighing paper. Electrical contact was made by a copper wire provided at the end of the tube. The carbon paste electrode surface was renewed by extrusion of approximately 0.5 mm carbon paste from the cavity of the Teflon rod and replacing it with a new paste.

2.4. Plasma sample preparation

Human blood samples were collected in dry and evacuated tubes (which contained saline and sodium citrate solution) from a healthy volunteer. The samples were handled at room temperature and were centrifuged for 10 min at 1500 rpm for the separation of plasma within 1 h of collection. The samples were then transferred to polypropylene tubes and stored at -20 ^{0}C until analysis. The plasma samples, 0.2 mL, were deprotonised with 2 mL of methanol. After vortexing for 15 min, the mixture was then centrifuged for 15 min at 6000 rpm, and supernatants were collected. The supernatants were spiked with known amounts of PAC. Appropriate volumes of this solution were added to phosphate buffer pH 7.0 as supporting electrolyte and the voltammograms were then recorded.

2.5. Injection sample preparation

Taxeleon (paclitaxel) injection is a clear, colorless to slightly yellow viscous solution. It is supplied as a non-aqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Taxeleon is available in 100 mg in 16.67 mL. Accurately 1.42 mL of Taxeleon was pipetted and was dissolved in 100 mL methanol. The mixture was allowed to stand for a few minutes with intermittent sonication to ensure complete solubility of the drug. An aliquot of this solution was transferred to a voltammetric cell and analyzed under same conditions as were used to obtain the calibration graph. Voltammograms were recorded as described for pure PAC.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetric behaviour of paclitaxel

Cyclic voltammograms of 1.0 mM on the CPE were recorded in pH 7.0 phosphate buffer solution with the result shown in Fig. 1. It can be seen that a well-defined oxidation peak appeared at 1.126 V without the reduction peak, indicating an irreversible electrochemical process. In multiple sweeps cyclic voltammograms the oxidation peak current decreased

gradually with the further increase of the scan (Fig. 1 inset), which indicated the adsorptive behavior of the product and the reactant.

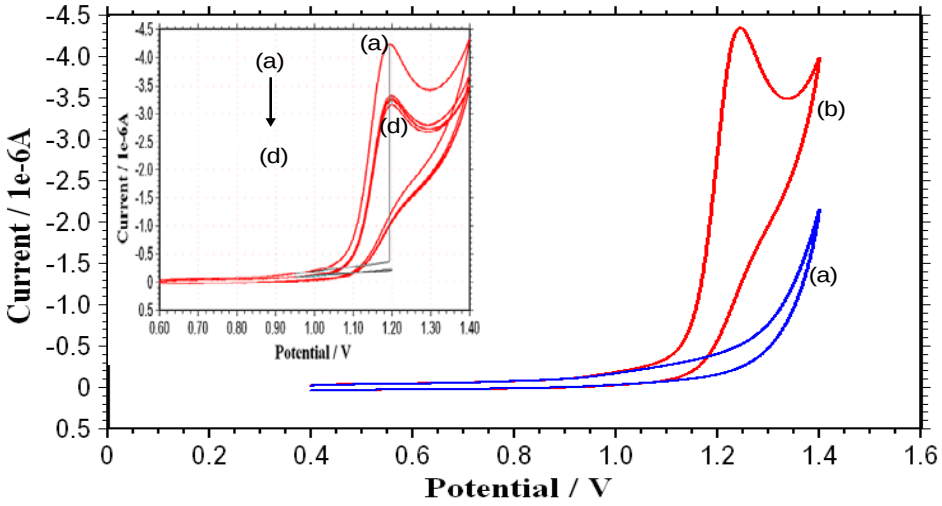


Fig. 1. Cyclic voltammograms of 1.0 mM PAC on carbon paste electrode, bare CPE(a); CPE+1.0 mM PAC (b); scan rate 50 mVs^{-1}; phosphate buffer of pH 7.0 as supporting electrolyte; accumulation time: 60 s (at open circuit). Inset: Successive cyclic voltammograms obtained for 1.0 mM PAC at CPE

3.2. Effect of accumulation conditions

The two parameters of accumulation step, accumulation time and potential were examined. Open circuit accumulation is widely used in electro-analytical chemistry to accumulate analyte and improve the sensitivity. The influence of accumulation time ranging from 0 to 120 s on the oxidation of PAC at CPE was as shown in Fig. 2.

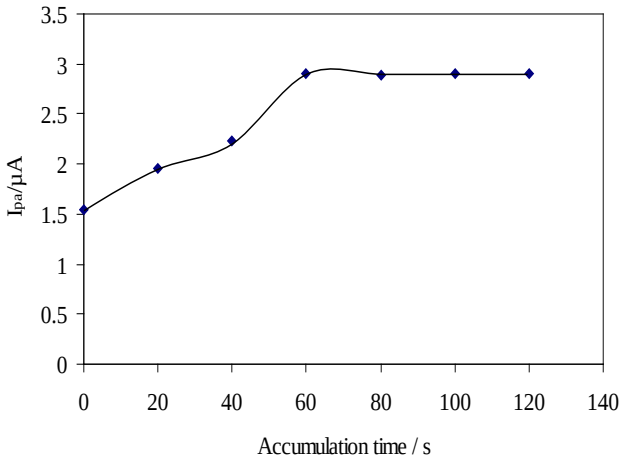


Fig. 2. Variation of the anodic peak current I_{pa}(µA) with accumulation time t (s). Other conditions are as in Fig. 1

The current increased gradually as accumulation time increased from 0 to 60 s. However, with further increasing, accumulation time beyond 60 s, the peak current tends to be almost stable. Therefore, optimal accumulation time of 60 s was employed in further experiments.

With the change of accumulation potential, the peak current of PAC varied slightly. So, the accumulation potential has practically no effect on the peak current of PAC.

3.3. Influence of buffer pH

The influence of buffer pH on the electrochemical responses of cytosine was investigated in the pH range from 4.2 to 10.4 with the results shown in Fig. 3A. With the increase of buffer pH the oxidation peak moved to the negative potential, indicating that proton take part in the electrode reaction.

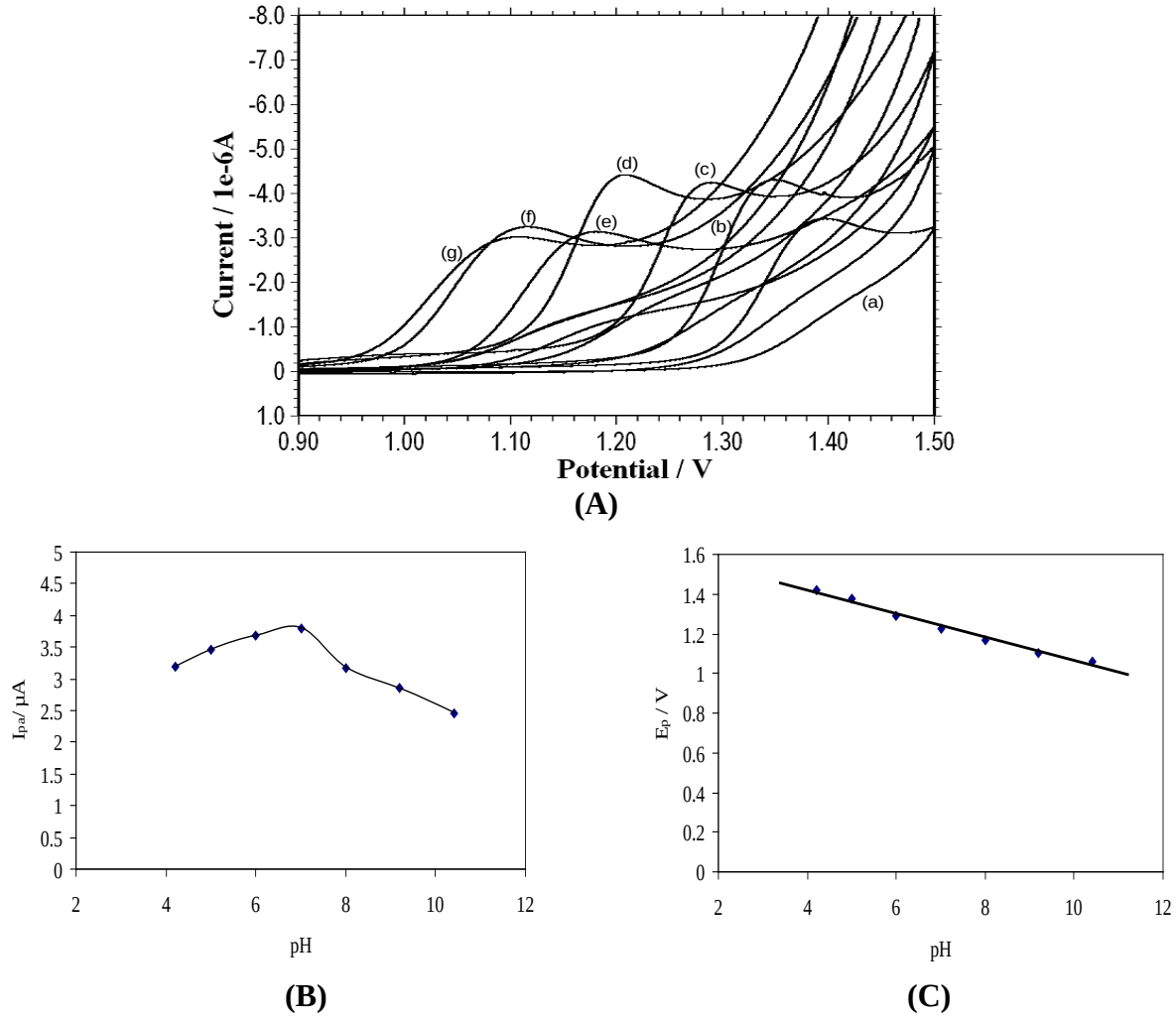


Fig. 3. (A) Influence of pH on the shape of the anodic peak, pH: (a) 4.2; (b) 5.0; (c) 6.0; (d) 7.0; (e) 8.0; (f) 9.2; (g) 10.4; **(B)** Variations of peak currents I_{pa}(μA) of PAC with pH. Other conditions are as in Fig. 1; **(C)** Influence of pH on the peak potential E_p (V) of PAC. Other conditions are as in Fig. 1

The pH dependence of the peak potential and peak current obtained when cyclic voltammetry was used is shown in Fig. 3B and Fig. 3C. With the increase in pH of the solution, peak potential shifted to less positive values, (Fig. 3B), and obeys the following equation:

$$E_p\,(V)=1.6606 - 0.0597\ pH\ (R^2=0.9876) \quad (1)$$

The slope of this equation is found to be -59.7 mV /pH. This closeness of the slope to the expected theoretical value [24] of -59 mV/pH suggests that the number of electrons transferred is equal to that of the hydrogen ions taking part in the electrode reaction.

From the plot of I_{pa} *vs.* pH (Fig. 3C) it is clear that the intensity was increased to a high value at pH 7.0, then the peak intensity decreases. Because the best result with respect to sensitivity accompanied with sharper response was obtained with pH 7.0, it was selected for further experiments.

3.4. Effect of scan rate

The effect of scan rate on the electro-oxidation of PAC was examined by linear sweep voltammetry (Fig. 4A). The influence of the square root of scan rate on the peak current showed a linear relationship in the range of 25 to 225 mV s^{-1}, which is typical of a diffusion controlled process [25], and the equation can be expressed as I_{pa} (μA)=0.0914 $\upsilon^{1/2}$ ($V^{1/2}s^{-1/2}$) – 0.0422; R^2=0.9928

A linear relationship was observed between log I_{pa} and log υ, and the corresponding equation can be expressed log I_{pa} (μA)=0.4232 log υ (Vs^{-1})−1.0212; R^2=0.9906. The slope of 0.4232 was close to the theoretically expected value of 0.5 for a purely diffusion controlled process [25], which in turn further confirms that the electro-oxidation of PAC was diffusion controlled.

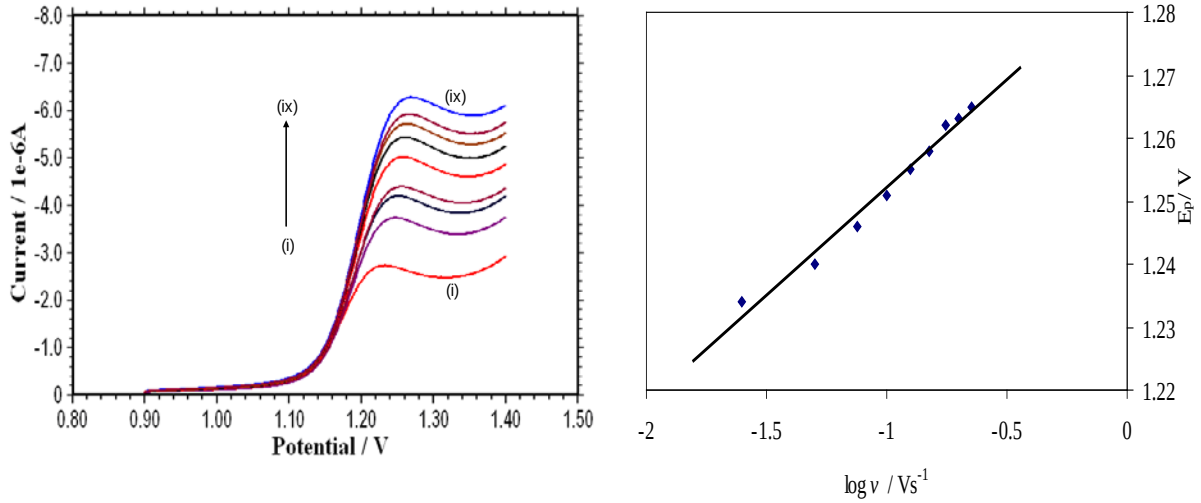
Also the oxidation peak potential moved to the positive direction with the increase of the scan rate. The relationship of the oxidation peak potential was proportional to the value of log I_{pa} with the equation as E_p (V)=0.0384 log υ (Vs^{-1})+1.287; R^2=0.9831(Fig. 4B). Based on the Laviron's equation [26] for an irreversible electrode process;

$$E_P = E^{0'} + \left(\frac{2.303RT}{\alpha nF}\right) log\left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) log v \quad (2)$$

Where α is the transfer coefficient, k^0 the standard heterogeneous rate constant of the reaction, n the number of electrons transferred, *v* the scan rate and $E^{0'}$ is the formal standard redox potential. Other symbols have their usual meaning. Thus value of αn can be easily calculated from the slope of E_p *vs.* log *v* plot. In this system, the slope is 0.0348, taking T=298 K, R=8.314 $JK^{-1}mol^{-1}$, and F=96480 C mol^{-1}, the αn value was calculated to be 1.6994. According to Bard and Faulkner [27], α can be given as

$$\alpha = \frac{47.7}{E_P - E_{P/2}} mV \quad (3)$$

Where $E_{p/2}$ is the potential when the current is at half the peak value. From this, the value of α was calculated to be 0.883. Further, the number of electrons (n) transferred in the electro-oxidation of PAC was calculated to be 1.92~2. The value of k^0 can be determined from the intercept of the previous plot, if the value of $E^{0'}$ is known. The value of $E^{0'}$ in eqn. (1) can be obtained from the intercept of E_p *vs*. *v* curve by extrapolating to the vertical axis at *v*=0 [28]. In our system, the intercept for E_p *vs*. log *v* plot was 1.287 and $E^{0'}$ was obtained to be 1.2343 and the k^0 was calculated to be 2139.79 s^{-1}.

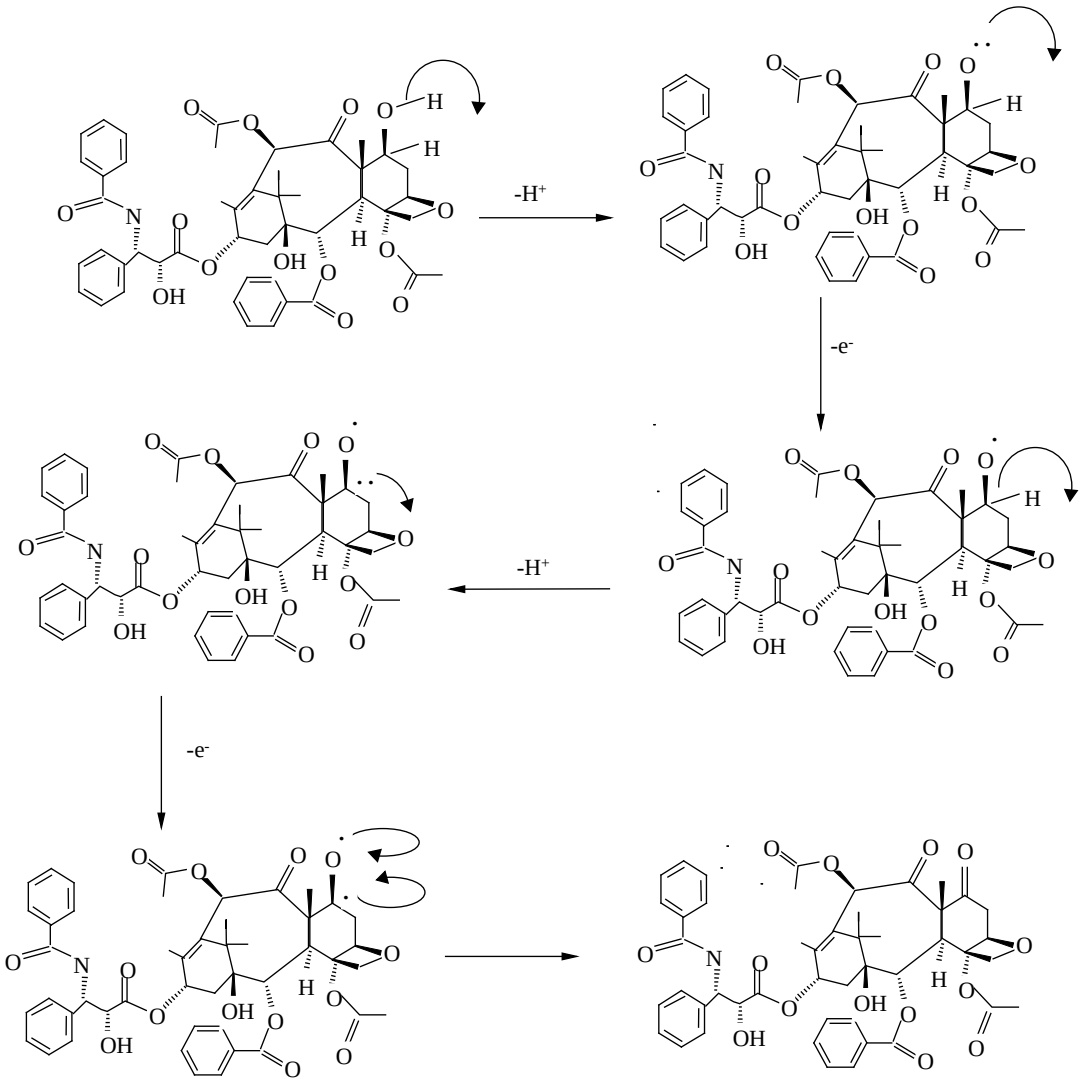


(A) **(B)**

Fig. 4. (A) Linear sweep voltammograms for the oxidation of PAC at CPE at different scan rates (i) 0.025 (ii) 0.05 (iii) 0.075 (iv) 0.10 (v) 0.125 (vi) 0.150 (vii) 0.175 (viii) 0.2 (ix) 0.225; **(B)** Dependence of oxidation peak potential on the logarithm of scan rate

3.5. Mechanism

In the proposed method, the electro-oxidation of PAC involves two electrons and two proton transfer process. Literature survey reveals that C-7 hydroxyl group of PAC is easily oxidized than C-2' hydroxyl group [29,30]. Therefore PAC undergoes oxidation at C-7 hydroxyl group forms 7-oxo paclitaxel. The product is also confirmed by LC- MS spectrum which shows molecular ion peak at 851.88 m/z. The probable mechanism is as shown in Scheme 2.



Scheme 2. Probable electrode oxidation mechanism of PAC

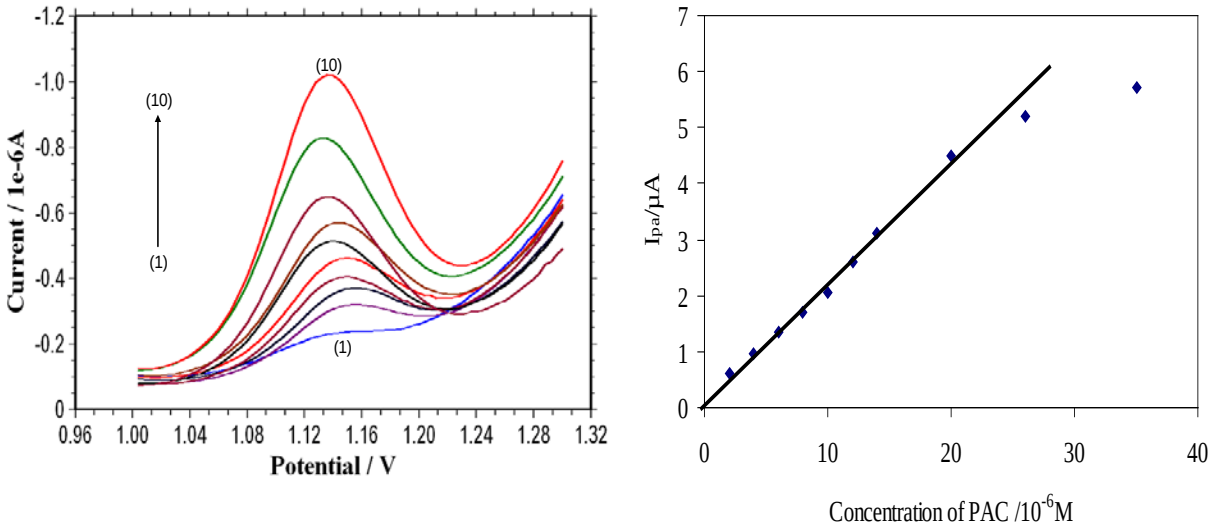
Here the hydroxyl group (-OH) is attached to the carbon atom(C-7) of the cyclohexane ring of the paclitaxel. During the electrolysis when the first proton is removed, oxygen gets a negative charge and anionic form of paclitaxel is formed. To stabilize the anionic form of the paclitaxel the hydrogen atom attached to the carbon (C-7) of the cyclohexane has undergone further electro oxidation and stable product 7-oxo paclitaxel is formed.

3.6. Calibration curve

In order to develop a voltammetric method for determining the drug, we selected the differential-pulse voltammetric mode, because the peaks are sharper and better defined at lower concentrations of PAC than those obtained by cyclic and linear sweep voltammetry, with a lower background current, resulting in improved resolution. The analytical characteristics of the calibration plot are summarized in Table 1. According to the obtained

results, it was possible to apply this technique to the quantitative analysis of PAC. The phosphate buffer solution of pH 7.0 was selected as the supporting electrolyte for the quantification as PAC gave maximum peak current at pH 7.0. Differential pulse voltammograms obtained with increasing amounts of PAC showed that the peak current increased linearly with increasing concentrations, as shown in Fig. 5(A) and Fig. 5(B). Using the optimum conditions described above, linear calibration curves were obtained for PAC in the range of 2.0×10^{-6} M to 2.0×10^{-5} M. The linear equation was I_{pa} (μA)=0.216629 [PAC] (μM)+0.052 (R^2=0.9940). Deviation from linearity was observed for more concentrated solutions, due to the adsorption of PAC or its oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from five different determinations. The limit of detection (LOD) and quantification (LOQ) were 4.6×10^{-9} and 1.53×10^{-8} M, respectively. The LOD and LOQ were calculated using the following equations: LOD=3s/m; LOQ=10s/m.

Where s is the standard deviation of the peak currents of the blank (five runs), and m is the slope of the calibration curve [31]. The LOD and LOQ values calculated by the present method are better compared to the reported work [16,19,22]. The detection limits reported at different electrodes are tabulated in Table 2.



(A) **(B)**

Fig. 5. (A) Differential pulse voltammograms for increasing concentration of PAC at CPE: (1) 2.0 (2) 4.0 (3) 6.0 (4) 8.0 (5) 10.0 (6) 12.0 (7) 14.0 (8) 20.0 (9) 26.0 (10) $35.0/10^{-6}$ M. other conditions are same as in Fig. 1; **(B)** Plot of current against the concentration of PAC

The precision of the method was investigated by intra-day and inter-day determination of PAC at two different concentrations (number of measurements=5) within the linear range.

Accuracy of the methods expressed as bias% and RSD% for intra and inter days as shown in Table 3, which indicates the high precision of the proposed method.

In order to study the reproducibility of the electrode preparation procedure, a 1.0×10^{-5} M PAC solution was measured with the same electrode for every several hours within a day, the R.S.D. of the peak current was 4.18% (number of measurements=5). As to the between day reproducibility, it was similar to that of within a day if the temperature was kept almost constant. Owing to the adsorption of oxidative product of the PAC on the electrode surface, the current response of the electrode would decrease after successive use. In this case, the electrode should be reconstructed again.

Table 1. Characteristics of PAC calibration plot using differential pulse voltammetry at carbon paste electrode

Linearity range (M)	2.0×10^{-6}- 2.0×10^{-5}
Slope of the calibration plot	0.2166
Intercept	0.052
Correlation coefficient (r)	0.9940
RSD of slope (%)	0.153
RSD of intercept (%)	5.63
Number of data points	5
LOD (M)	4.6×10^{-9}
LOQ (M)	1.53×10^{-8}
Repeatability (RSD %)	2.97
Reproducibility (RSD %)	4.18

Table 2. Comparison of linear range and detection limits for PAC to different classical methods

Linear range (μg/mL)	LOD (μ M)	Reference
9.9-8.6	8.86	[17]
15-180	0.0351	[20]
48-72	1.96	[23]
1.707 -17.078 (= 2.0×10^{-6} –2.0×10^{-5} M)	0.0046	Present work

Table 3. Analytical precision and accuracy of PAC determination by DPV

	Added ($\times10^{-5}$ M)	Found[a] ($\times10^{-5}$ M)	S.D	R.S.D (%)	Bias (%)
Intraday	3.0	2.992	0.057	1.905	-0.24
	5.0	4.998	0.018	0.366	-0.04
Interday	3.0	3.003	0.016	0.536	0.12
	5.0	4.973	0.037	0.763	-0.53

[a] Average of five determinations

3.7. Effect of excipients

For the possible analytical application of the proposed method, the effect of some common excipients used in pharmaceutical preparations was examined. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 5% for determination of PAC. The effects of these excipients on the voltammetric response was carried by analyzing sample solutions containing a fixed amount of PAC (1.0×10^{-5} M) spiked with various excess amounts of each excipient under the same experimental conditions.

Table 4. Influence of potential excipients on the voltammetric response of 1.0×10^{-5} M PAC

Excipients(1.0 mM)+Drug (1.0×10^{-5})	Potential observed (V)	Signal change (%)
Only Paclitaxel	1.161	0
Citric acid+PAC	1.170	+0.77
Dextrose+PAC	1.163	+0.17
Glucose+PAC	1.160	-0.08
Gum acacia+PAC	1.163	+0.17
Lactose+PAC	1.160	-0.08
Sucrose+PAC	1.158	-0.25
Tartaric acid+PAC	1.171	+0.86
Starch+PAC	1.158	-0.25

The experimental results (Table 4) showed that hundredfold excess of citric acid, dextrose, glucose, gum acacia, lactose, starch, tartaric acid and sucrose did not interfere with the voltammetric signal of PAC. Thus, the procedures were able to assay PAC in the presence of excipients, and hence it can be considered specific.

3.8. Injection analysis and recovery test

In order to evaluate the applicability of the proposed method in the pharmaceutical sample analysis, it was used to detect PAC in "Taxeleon" injection (100 mg in 16.67 ml) as a real sample by applying DPV using the standard addition method. The procedure for the injection analysis was followed as described in the procedure section. The results are in good agreement with the content marked on the label (Table 5).

The recovery test of PAC ranging from 1.0×10^{-6} to 7.0×10^{-6} M was performed using differential-pulse voltammetry. Recovery studies were carried out after the addition of known amounts of the drug to various pre-analyzed formulations of PAC. The recoveries in different samples were found to lie in the range from 99.2% to 101.7%.

Table 5. Analysis of Taxeleon injection by DPV and recovery studies

Taxeleon	DPV
Labeled claim (mg)	100
Amount found (mg)[a]	99.81
R.S.D. (%)	1.35
Bias (%)	-0.19
Added (mg)[a]	3.0
Found (mg)	2.98
Recovered (%)	99.33
R.S.D. (%)	1.54
Bias (%)	-0.62

[a]Average of five determinations

3.9. Detection of PAC in urine samples and human serum

The developed differential pulse voltammetric method for the PAC determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of PAC. The urine samples were diluted 100 times with the phosphate buffer solution. A quantitative analysis can be carried out by adding the standard solution of PAC into the detect system of urine samples. The calibration graph was used for the

determination of spiked PAC in urine samples. The detection results of five urine samples obtained with recovery percentage and RSD are listed in Table 6.

The applicability of the DPV to the determination of PAC in spiked human plasma sample was investigated. The recoveries from human plasma were measured by spiking drug free plasma with known amounts of PAC. The plasma sample was prepared as described in plasma sample preparation section. A quantitative analysis can be carried out by adding the standard solution of PAC in the detect system of plasma sample. The calibration graph was used for the determination of spiked PAC in plasma samples. The detection results obtained for four plasma samples with recovery percentage and RSD are listed in Table 6.

Table 6. Application of DPV to the determination of PAC in spiked human urine and blood plasma sample

Sample	Added ($\times 10^{-5}$ M)	Found[a] ($\times 10^{-5}$ M)	Recovery (%)	R.S.D (%)	Bias (%)
Urine sample1	2.0	1.988	99.43	0.46	-0.57
Urine sample 2	5.0	4.953	99.07	0.71	-0.924
Urine sample 3	6.0	6.130	102.17	0.32	-2.17
Urine sample 4	8.0	7.985	99.81	0.21	-0.18
Plasma sample 1	1.5	1.502	100.16	0.111	0.16
Plasma sample 2	3.0	2.985	99.52	0.553	-0.47
Plasma sample 3	4.5	4.671	103.80	5.068	3.80
Plasma sample 4	6.0	6.069	101.15	0.741	1.15

[a]Average of five determinations

4. CONCLUSIONS

A simple and rapid differential pulse voltammetric method for the determination of PAC was demonstrated by CPE at phosphate buffer solution of pH 7. Based on the study, the influence of several physico–chemical parameters like potential scan rate, pH and concentration were investigated. A suitable electrochemical oxidation mechanism for PAC was proposed and the product was identified by LC-MS study. The electrode has been used to determine PAC in pharmaceutical samples. The proposed method offered the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. In addition, the results obtained in the analysis of PAC in spiked urine and also in blood serum samples demonstrated the applicability of the method for real sample analysis.

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