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New voltammetric strategy for determination and electrochemical behaviors of oxaliplatin by CPT-BDD electrode

Mehmet Aslan[a] and Abdulkadir Levent*[b]

[a]Department of Chemistry, Science Institute, Dicle University, 21280 Diyarbakir, Turkey

[b]Department of Chemistry, Faculty of Sciences and Arts, Batman University, 72100 Batman, Turkey

E-mail: leventkadir@hotmail.com

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In this study, a simple, fast and sensitive voltammetric method was developed for oxaliplatin (OxPt), which shows anticancer effects with cytotoxic properties. The boron-doped diamond (BDD) electrode was activated electrochemically in cathodic direction in 0.5 *M* H_2SO_4 medium. Electrochemical properties of OxPt were investigated on BDD electrode surface using square-wave (SW) and cyclic voltammetry techniques. OxPt in Britton-Robinson (BR) buffer (pH 5.0) gave a well-determined voltammetric response at +1.01 V (vs Ag/AgCl) using the SW voltammetry technique. The developed voltammetric technique was found to be linear with the concentration range of 1.0–3.5 μM in the BR (pH 5.0) medium and the limit of detection was 0.276 μM (0.109 μg mL^{-1}). Recommended method was successfully applied to drug forms of OxPt.

Keywords: Oxaliplatin, boron-doped diamond, voltammetry.

Introduction

Various drugs made of metal compounds have been used in many diseases including cancer[1]. OxPt, which is a platinum analogue with anti-cancer effect, was accentuated. OxPt is separated from cisplatin, one of analogues of platinum, by replacing 1,2-diaminocyclohexane with amine groups. Unlike cisplatin, OxPt in plasma rapidly undergoes non-enzymatic transformation with reactive compounds due to the replacement of the oxalate group, a process that complicates the pharmacokinetic profile. Most of the compounds are not pharmacologically active[2]. OxPt is an anti-cancer drug which is formed by the coupling the oxalate group and 1,2-diaminocyclohexane with the platinum atom, and which has a complex structure, and acts as an alkylation agent showing cytotoxic effect. Such drugs have been used against metastatic colorectal cancer for the first time.

Platinum-containing anti-cancer drugs are grouped as drugs that interact with DNA and the idea that the cancer cell is destroyed as a result of the binding these drugs to DNA and the preventing the transcription and replication mechanisms is dominant[3]. Because of this property of OxPt, it is very important to determine it in pharmaceutical and biological fluids. Mostly, researchers have used spectroscopic and chromatographic methods to perform the determination of OxPt. These methods include the techniques such as HPLC[4–6], capillary electrophoresis[7] and HPLC/ICP-MS[8–14] techniques. With the development of today's technology, MS technique is used successfully in combination with LC technique. However, even if these methods show high sensitivity and accuracy, it is a disadvantage that they need multi-expensive device, expert staff, organic solvent and complex processes.

Electrochemistry based studies about OxPt are quite limited in the literature. They are the DNA interaction studies of the OxPt compound using the DPV technique and dropping mercury electrode[15,16] and the SWCNTs-GE electrode[17]. Likewise, they can cause toxic and environmental pollution of solvents and reagents needed during the preparation of the electrodes used in these studies.

Electrochemical methods are mainly based for the determination of the target analyte in the biological and pharmaceutical environment, and it has significant advantages in terms of time, economy, and the use of various chemicals and the application of pre-separation processes requiring special skills. In the determination of the analyte present in

biological and pharmaceutical samples, these advantageous properties of electrochemical methods are further enhanced when a suitable and effective working electrode is selected.

The diamond in the structure of the BDD electrode used in this study; it provides a very important performance in electrochemical studies with its optical properties, chemical resistance, inertness against chemical reactions, hardness, high conductivity and electrical properties. BDD electrodes stand out with its features such as strength and durability in electrochemical applications, besides to its low noise signal and wide electrochemical potential range[18–20]. BDD electrodes are an excellent electrode that was discovered at the end of the 20th century. It has made that BDD is a preferred electrode in voltammetric analysis with its features such as wide potential range, ability to work with low current, to work in basic and acidic medium, being resistant to corrosion and surface contamination[21–23].

Even if BDD electrodes are used in a wide range of applications, it is seen that are mostly used in drug analysis[24–27].

It appears that the electrode is subjected to modification or activation to improve the response signal on the working electrode. Cleaning of the BDD electrodes are extremely important as are other solid electrodes to obtain stable and repeatable results in electrochemical studies. The cleaning and activation of BDD electrode is seen in the literature by applying anodic[24,25,28–30] and cathodic[31–33] potential in different solutions.

In this study, a new and alternative voltammetric method was developed on the BDD electrode surface as a result of cathodic activation of the BDD electrode in 0.5 *M* H_2SO_4 medium, it was observed that OxPt showed quite electro catalytic effect. With being this activation process is simple and fast, it is very important that the electrode is not exposed to any toxic chemicals and reagents during the modification process. The proposed method has been successfully applied to the drug form after a simple dilution without using any organic solvent, requiring long time separations or high cost devices.

Experimental

Chemicals and reagents:

Standard OxPt was purchased from Sigma-Aldrich firm. 1.25×10^{-3} *M* stock solution was prepared in methanol. In this study, Britton-Robinson buffer (BR, 0.04 *M*, pH 2.0–10.0), phosphate buffer (0.1 *M*, pH 3.0, 4.0, 7.4, 9.0) and acetate buffer (0.10 *M*, pH 4.8) were used as supporting electrolytes. In order to adjust buffers with desired pH values, 5.0 *M* NaOH or 5.0 *M* HCl. Milli-Q water (Millipore) was used in the preparation of all solutions. The solutions were stored in a +4°C refrigerator.

Apparatus and measurements:

AUTOLAB is a device for electrochemical measurements with PGSTAT128N (in combination with Nova 1.11 software) and a 10 mL electrochemical test cell (MR 1208) with a specially manufactured compartment with lid. In the electrochemical experiments, working, reference and auxiliary electrodes, BDD (Windsor Scientific Ltd., Ø: 3 mm diameter), (Ag/AgCl) (3 *M* NaCl) (MF 1063, BAS) and (MF 1032, BAS) were used respectively. The pH values were measured with Thermo Scientific Orion 3-star pH meter. Before starting the electrochemical analysis, BDD electrode was mechanically cleaned with Al_2O_3 solution and then electrochemically activated in the cathodic direction (–2.4 V/1200 s) in 0.5 *M* H_2SO_4 medium. Between each assay measurement, BDD electrode was subjected to 30 s activation process at –2.4 V potential in 0.5 *M* H_2SO_4 medium. Electrochemical measurements were carried out in the supporting electrolytes in the working environment.

Preparation of drug samples:

The commercially available Ploxal-S (100 mg/20 mL OxPt) drug was used to apply the developed voltammetric method to the commercial drug form. Analysis was carried out after adding a known amount of drug sample to the supporting electrolyte solution. Recovery calculations were made using the calibration curve. Each analysis was repeated three times.

Results and discussion

Cyclic voltammetric behavior:

The voltammetric behavior of OxPt at a cathodically pretreated BDD(here referred as CPT-BDD) electrode was studied by CV.

As shown in Fig. 1(A), two consecutive CVs were recorded for 125 μM OxPt in BR buffer (pH 5.0) at 100 mV s^{-1} scan rate. During the anodic sweep from 0.0 to +1.5 V, it is seen that it gives a very wide oxidation peak at around+1.225 V.

When the reverse scan continued, the reduction peak was not observed. The oxidation of OxPt shows that it is irreversible redox process under the experimental conditions. To better understand the nature of the oxidation process, the oxidation peak signals of 125 μM OxPt was studied at BR (pH 5.0) with CV at under different scan rates between 25–400 mV s^{-1} (Fig. 1(B)). It appears that as the scan rate gradually increased the oxidation peaks increase splayed.

On the other hand, as shown in Fig. 1(B); as the scan rate is gradually increased, the OxPt oxidation peak potentials shifted towards more positive. This phenomenon is characteristic of irreversible or semi reversible electrochemical reaction[34]. When the results obtained are evaluated, the oxidation peak current (i_p) of OxPt increased linearly with the increasing scan rate ($\sqrt{\nu}$), and can be expressed as following: i_p (μA)= 2.237$\sqrt{\nu}$ (mV s^{-1}) – 11.623, r = 0.9937; n = 6. It can be suggested that the OxPt oxidation peak is diffusion controlled at the CPT-BDD electrode.

In irreversible electrochemical processes, the correlation between oxidation peak potential and scan rate is given by $E_p = E_0 + (2.303RT/\alpha nF) \log (RTk_0/\alpha nF) + (2.303RT/\alpha nF) \log \nu$[35]. α is the charge transfer coefficient; and n is the number of electrons transferred in the redox reaction. R (8,314 J K^{-1} mol^{-1}), T (298 K) and F (96480 C mol^{-1}) are known as constants. The slope value in the E_p – log ν relationship is 0.0527. Using the equation above, αn is calculated as 1.12. For most systems in the case of a fully reversible electrode, α = 0.5 can be considered. Thus, n = 2.24 (≈2) is obtained. In the light of these data, the oxidation mechanism of OxPt can be shown as in Fig. 2.

In the proposed mechanism, most alkaline electrons belong to carbonyl oxygen since the non-centered electron pairs of the nitrogen atom in the OxPt compound are in coordination with Pt. Since the medium where the electrochemical process takes place is acidic (pH 5.0), the oxygen's are expected to be oxidized by one step transferring unconnected electron pairs of carbonyl oxygen to the proton. In the ongoing process, π electrons between carbon and oxygen are attracted by the oxygen atom to provide partial positive charge of carbonyl carbon. In fact, as this structure is expected to be positively charged carbon between two oxygen, the structure is a mesmerically stable carbocation. This reinforces

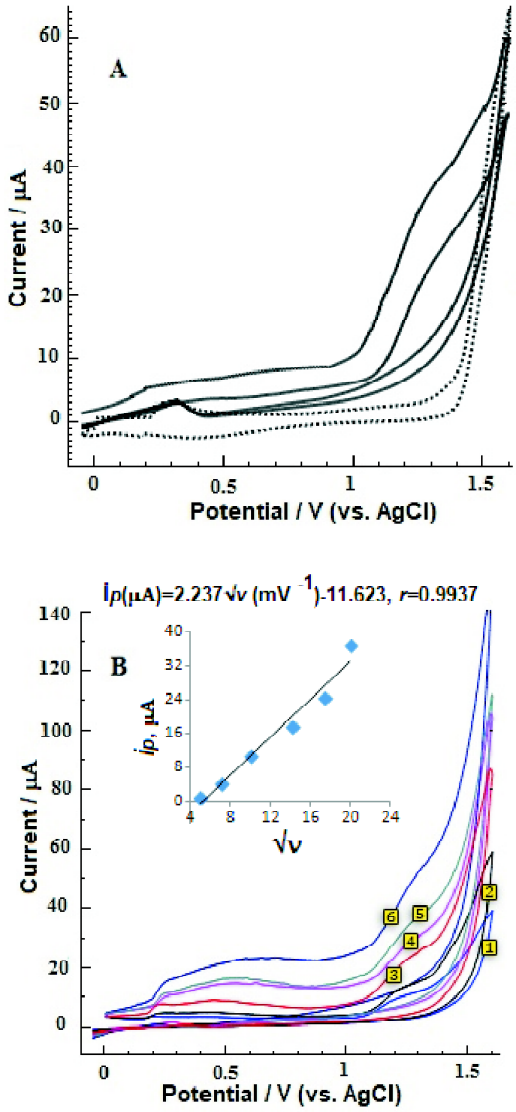

Fig. 1. The repetitive cyclic voltammograms on CPT-BDD electrode in BR buffer (pH 5.0) of 125 μM OxPt. A: Scan rate, 100 mV s^{-1}, B: Different scan rate (1) 25, (2) 50, (3) 100, (4) 200, (5) 300, (6) 400 mV s^{-1}. A; Supporting Electrolyte: Dashed line. B; Inset depicts the plot of peak current (i_p) vs scan rate.

that the recommended electrochemical mechanism process for OxPt on the CPT-BDD electrode surface in pH 5.0 medium can be so.

Effect of pH:

In the electrochemical studies, the supporting electrolyte ingredient and pH selection are extremely important to interpret the redox behavior of the analyte occurring on the electrode surface.

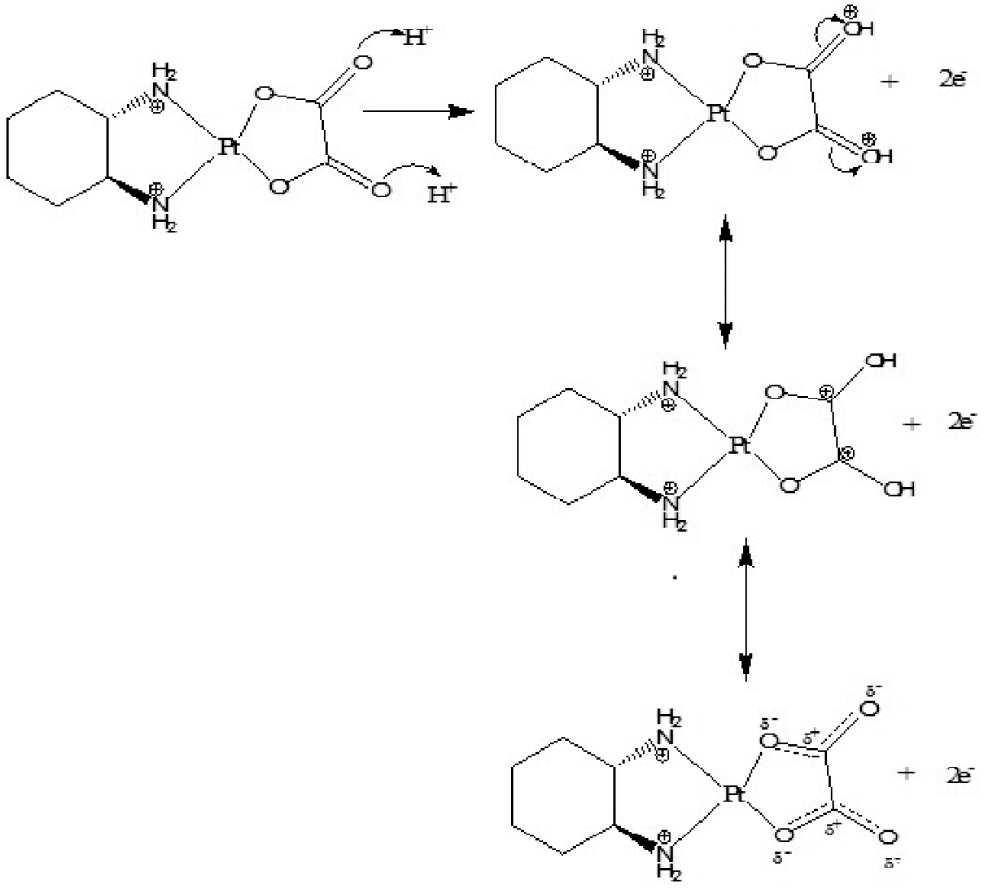

Fig. 2. Electrochemical oxidation pathway of OxPt.

Based on the above information; in this part of the study, voltammograms of 125 μM OxPt were recorded in BR (pH 2.0–10.0) buffer (Fig. 3B) and phosphate (pH 3.0, 4.0, 7.4, 9.0) and acetate (pH 4.8) buffers (Fig. 3A) using the SW technique on the CPT-BDD electrode. As can be clearly seen in Fig. 3(A)-(B), oxidation signals of OxPt were obtained more sensitive and well-evident in BR buffer (pH 5.0), as a result of working with different supporting electrolytes.

As seen in Fig. 3 and Fig. 4, when the pH value is increased, the oxidation peak potential shifts to more negative values. This result shows that proton transfer takes part in the oxidation process occurring on the CPT-BDD electrode surface.

When the E_p/pH relationship given in Fig. 4 is examined; it is possible to see that two linear regions are obtained, which is different slope values: E_p (mV) = 80.367 pH + 1354.4 (pH = 2.0–10.0 and r = 0.995), E_p (mV) = 88.8 pH + 1380.6 (pH = 2.0–6.0 and r = 0.998) and E_p (mV) = 97.5 pH + 1509.5 (pH = 7.0–10.0 and r = 0.997). When the E_p/pH relationship is evaluated in detail, it is observed that in a crossing sloping region, this slope (–80.367 mV/pH) is compatible with the value of pKa 7.23[36,37] of the OxPt.

Also, the obtained –80.367 mV/pH value is close to 59 mV/pH in nerst equation, and the number of electrons given per proton in the electrochemical process can be considered as equal.

Effect of square wave voltammetric parameters:

Investigation of the effect of SW parameters is extremely important (square wave frequency, f = 5–50 Hz; step potential, ΔE_s = 1–17 mV; square wave amplitude, ΔE_{sw} = 10–150 mV) in order to determine the optimum instrument variables for obtaining the most sensitive and symmetrical voltammograms. Electrochemical measurements were performed in an optimal BR (pH 5.0) supporting electrolyte con-

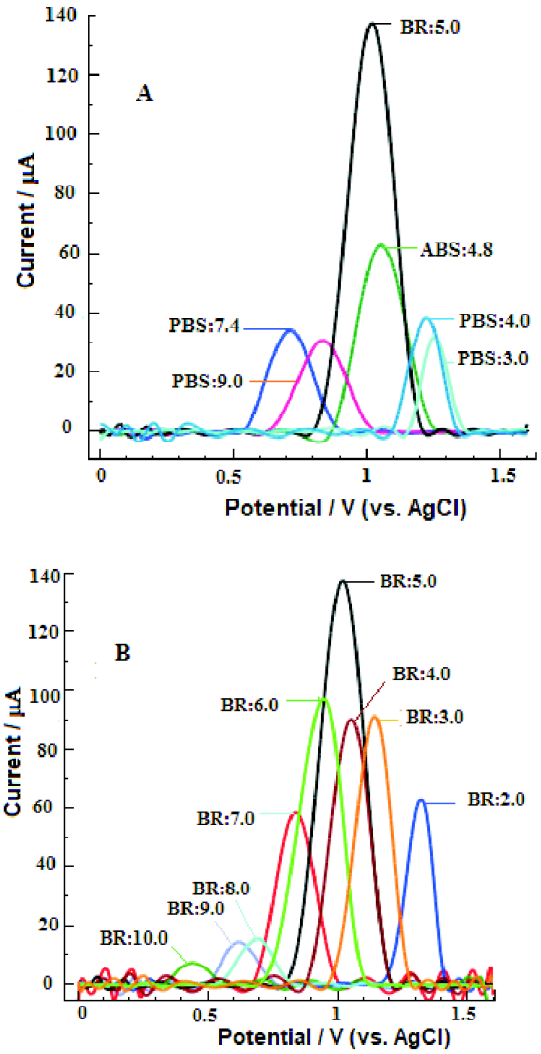

Fig. 3. SW voltammograms of 125 µM OxPt solutions in different supporting electrolytes and at different pHs. A: pHs of different supporting electrolytes; B: BR buffer pH 2–10. SW parameters: frequency, 30 Hz; step potential, 10 mV; amplitude, 90 mV.

taining 12.5 µM OxPt. SWV technique is related to amplitude, frequency and step potential. The increase up to f = 30 Hz and ΔE_s = 9 mV resulted in an increase in peak current values due to an increase in effective potential scan rate. However, after these values, current signal strength decreased and peak expansion and base line distortion were observed at f = 30 Hz and E_s = 15 mV. The analytical signal is also related to the pulse amplitude value (although not as much as the frequency). Peak heights increased to ΔE_{sw} = 90 mV, then decreased. SW variables: f, 30 Hz; ΔE_s, 10 mV; most uniform voltammograms were obtained when the ΔE_{sw}

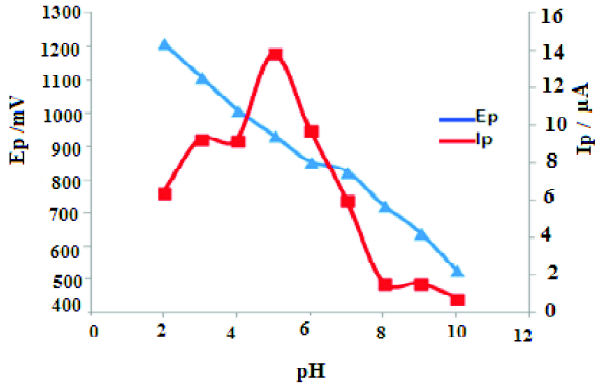

Fig. 4. Effect of the pH on the OxPt peak potential (E_p) and peak current (i_p) in the BR (pH 2–10) buffer solution. OxPt concertation, 125 µM. Other SW parameters as indicated in Fig. 3.

was 90 mV. These values are accepted as optimum in the following sections of the study.

Analytical application:

SWVs of OxPt solutions of different concentrations were recorded in the study carried out in order to investigate the effect of OxPt concentration on oxidation peak current in optimum experimental conditions. The SWVs of the standard OxPt solutions in the 1.0–3.5 µM range on the CPT-BDD electrode in BR buffer (pH 5.0) and the corresponding calibration curve graph are shown in Fig. 5.

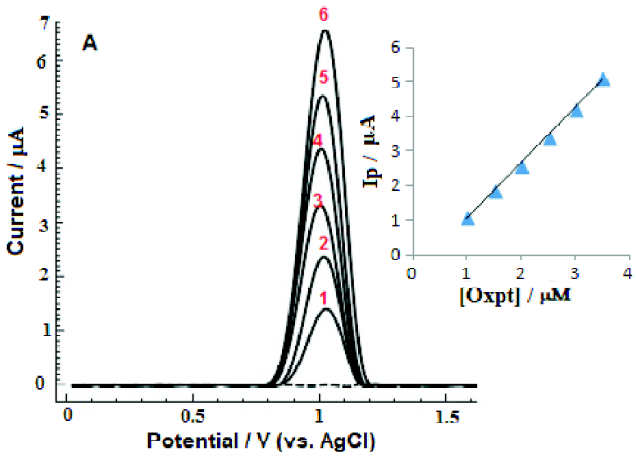

Fig . 5. (A) SWVs at CPT-BDD electrode in BR buffer (pH 5.0) containing different concentrations [(1) 1.0, (2) 1.5, (3) 2.0, (4) 2.5, (5) 3.0, (6) 3.5 µM] of OxPt. Dashed line, supporting electrolyte. Appendix (B): i_p/C OxPt calibration curve. Other SW parameters as indicated in Fig. 3.

The analytical curve presented in Fig. 5B give a linear response [I_p (μA) = 1.592 C (μM) 0.532 (r = 0.999, n = 6)] in the concentration range 1.0–3.5 μM. Limit of detection (LOD) and limit of quantitation (LOQ) values obtained by analytical curves were calculated according to LOD = 3 s/m; LOQ = 10 s/m. s in the equations is the standard deviation of the peak current (as the mean of 3 values) corresponding to the lowest concentration in the linearity range, and m is the slope value of the corresponding calibration equation. LOD and LOQ values were 0.276 μM (0.109 μg mL^{-1}) and 0.920 μM (0.365 μg mL^{-1}), respectively.

In order to determine the reproducibility level of the voltammetric method developed in BR (pH 5.0) medium on CPT-BDD electrode for OxPt compound, at 3 μM concentration were prepared under optimum experimental conditions and SWVs were recorded in these solutions 9 times in the same day. The oxidation peak current and potential values of these voltammograms were read and the results were evaluated as intraday precision. According to the obtained results, BSS % values for oxidation peak current and potential were determined as % 1.45 and % 0.43, respectively. As can be seen from the results obtained, it is observed that the repeatability of CPT-BDD electrode is very good under the studied conditions.

In order to verify the performance characteristics of the developed method in real samples, its applicability was applied to commercial drug forms. In order to check the precision and accuracy of the developed method, recovery studies were conducted. For this purpose, consecutive additions were made on the same injectable solution from the standard OxPt solution, provided that the linearity limits were not exceeded, and the SWVs were rerecorded after each addition. Peak currents were measured both in the original injectable solution and after successive additions. Then it was tried to be calculated how much of the pure substance added to the injectable solution sample could be determined. The results of the voltammetric analysis are given in Table 1. The results show that the accuracy of the voltammetric method developed on CPT-BDD electrode with recovery between %92–%107 is scientific validity.

Table 1. Voltammetric analysis results of OxPt in flacon

Sample	Determined (mg)[a,b]	Recovery[b] (%)±% RSD
1	94	94±3.28
2	105	105±4.11
3	106	106±3.96
4	92	92±4.33
5	107	107±3.31

[a]One flacon (20 mL) contains 100 mg OxPt.

[b]The results are the average of three independent analyses.

Conclusion

In this study, electrochemical properties of OxPt was first time examined with the CPT-BDD electrode, then a new SWV method was developed for determination.

To be more specific, this study presented a simple and low-cost effective voltammetric method for the determination of OxPt in the BR (pH 5.0) medium by CPT-BDD electrode. In this method, after pre-treatment in a 0.5 M H_2SO_4 medium in the cathodic direction, the electro catalytic and charge transfer rates in the activated CPT-BDD electrode were dramatically increased. This reflected that the electro catalytic property produced very sensitive results on oxidation of OxPt.

Thus, this newly proposed SWV method was successfully applied to the drug form after a simple dilution without using any organic solvent or waiting for long time separations and using high cost devices.

In addition, this electrochemical method was found to be fast and precise. This method does not require any pre-concentration, which is economical and sensitive. This method further works with a small amount of sample and can be analyzed without any time-consuming processes such as separation using more expensive devices mentioned in the literature. Therefore, this method may be an alternative to chromatographic and spectrophotometric methods, which have high operating costs.

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