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Electrochemical Study of Delta-9-Tetrahydrocannabinol by Cyclic Voltammetry Using Screen Printed Electrode, Improvements in Forensic Analysis

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Abstract: Rapid screening of seized drugs is a continuing problem for governmental laboratories and customs agents. Recently new and cheaper methods based on electrochemical sensing have been developed for the detection of illicit drugs. Screen printed electrodes are particularly useful in this regard and can provide excellent sensitivity. In this study, a carbon screen printed electrode for the voltammetric analysis of Δ^9-THC was developed. The analysis was performed using cyclic voltammetry with 0.15 $mol \times L^{-1}$ potassium nitrate as a supporting electrolyte. In the analysis, a Δ^9-THC standard solution was added to the surface electrode by a drop coating method. A study of scan rate, time of pre-concentration, and concentration influence parameters showed versatility during the investigation. The high sensitivity, quantitative capability and low limit of detection (1.0 $\mu mol \times L^{-1}$) demonstrate that this electrochemical method should be an attractive alternative in forensic investigations of seized samples.

Keywords: Detection, Cyclic voltammetry, Screen printed electrodes, Delta-9-tetrahydrocannabinol, Versatility.

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1. Introduction

Cannabis, a widely used illicit drug [1-4], contains delta-9-tetrahydrocannabinol (Δ^9-THC), a primary terpenophenolic substance which can induce psychosis, and reduction of cognition [2-4]. When a marijuana sample is seized, the sample is first extracted in organic media and then a presumptive test is performed. Typically, colorimetric reagents such as Fast Blue B salt or Duquenois–Levine are used. [5]. These tests produce a change of color when Δ^9-THC and other cannabinoids (such as Cannabinol or Cannabidiol) are present. This test can also be combined with thin layer chromatography for additional specificity [6]. However, these tests can produce false positive and false negative results due to a variety of interfering substances [7]. For confirmatory results, many countries follow the recommendations of The Scientific Working Group for the Analysis of Seized Drugs – SWGDRUG

[8-9]. The SWGDRUG guidelines divide analytical methods into a set of three groups based on specificity. In general, colorimetric and other less specific tests are defined as group C, more specific tests such as chromatography are placed in group B, and highly specific spectroscopic and spectrometric tests are placed in group A. Conclusive identification usually involves a presumptive test such as C or B followed by a more confirmatory type A test. (See Table 1) Unfortunately, more specific analytical techniques (classified as A category) are not always available in smaller laboratories. Thus, one complementary type C procedure and two chemical analyses in category B may be carried out as an alternative [8-13].

Table 1. Recommendations purposed for forensic test illegal drugs [6-11, 21].

Category A	Category B	Category C
Raman	GC, LC, TLC	Color tests
NMR	Capillary Electrophoresis	Fluorescence spectroscopy
Infrared Spectroscopy	Pharmaceutical identifiers	UV Spectroscopy
MS	Micro \ macro tests	Immunoassay
X-ray Diffractometry	For Cannabis only	Melting point

1.1. The Advances of Electrochemical Methods in Forensic Analysis

Recently, newer and cheaper methods have been developed for analyzing illicit drugs. For example, the detection of illegal drugs at low concentrations can be carried out using voltammetric techniques. These procedures can be quite selective and quantitative [13]. The low cost of the electrochemical instrumentation, as well as its potential application in providing determining electrochemical reaction mechanisms, [14-16] has motivated several groups to develop methods for detecting illicit drugs. For example voltammetric analysis has been used in the determination of cocaine [10, 13, 17-21], as well as 3,4-methylenedioxy-methamphetamine (*ecstasy*) [22-24]. For the electrochemical detection of Δ^9-THC, there are methods for both indirect [25-26] and direct analysis using voltammetry. Carbon paste electrodes (with or without chemical modification) have shown reliable results for cannabinoid detection [2, 27]. Voltammetric analysis of Δ^9-THC (following extraction of seized marijuana samples) using glassy carbon electrode in organic media produced limits of detection between 1×10^{-9} to 1×10^{-7} mol×L^{-1}. The novel electrochemical procedure could easily differentiate between five different plant species which produced false positives during colorimetric testing [6].

1.2. Screen Printed Electrodes

Screen-printed electrodes (SPEs) present an easily accessible platform for voltammetric measurements. These devices have 3 electrodes printed on a flat ceramic or plastic surface and are designated for working with microvolumes of analytes. The low volume requirement and facile surface access permit rapid measurements [28]. There are a number of publications on the application of voltammetric analysis in forensic science [13]. Aribas, *et al.*, studied the determination of cocaine using homemade SPE`s [29], and carbon screen printed electrodes (CSPE) modified with the enzyme cytochrome P450 [30-31]. Commercially manufactured SPE`s have proven useful in the determination of cocaine following modification of the working electrode surface by electrodeposition of $CoCl_2$ and $K_3Fe[(CN)_6]$ in an aqueous solution [13] or after chemical modification by drop coating using Schiff Base films [10].

3,4-Methylenedioxymethamphetamine and 4-methoxyamphetamine, have been detected by electrochemistry using graphite screen printed electrode [23]. CSPE devices have also proved useful in the detection of Δ^9-THC in marijuana in seized sized samples [13], and in an undiluted saliva, using N-(4-amino-3-methoxyphenyl)-methanesulfonamide as a mediator [32]. Cyclic voltammetry permits the study of electroactive substances given by the potentials at which oxidation or reduction occurs, and the intensity of anodic peak current, I_{pa} or cathodic peak current, I_{pc} [33-35]. In this paper, the goal was to examine the voltammetric behavior of Δ^9-THC using a supporting electrolyte in aqueous solution [2, 27] by CSPE.

2. Experimental

2.1. Reactants and Solutions

A standard solution of Δ9-THC 1 mg/mL (Cerilliant) and potassium nitrate (Acros Organics) were employed to prepare the solutions in this study. Δ^9-THC was diluted in methanol. 0.15 mol×L^{-1} potassium nitrate was prepared in deionized water and used as a supporting electrolyte. This solution was purged with nitrogen gas for 15 minutes prior to use.

2.2. Cyclic Voltammetry

CV experiments were performed on a potentiostat Autolab model 128 N coupled to a microcomputer controlled by Nova 2.0 software. Carbon screen printed electrodes from DropSens (model DRP-110) is constituted by working (4 mm in diameter) and counter electrode of carbon, and reference electrode of Ag. All voltammetric measurements were carried out in triplicate under optimized parameters. A drop coating method was used to add a small quantity of Δ^9-THC standard solution onto the surface of the working electrode. This was allowed to dry at room temperature for few minutes [27].

The influence of concentration was determined using an applied potential from −0.8 V to 0.9 V (vs. Ag - SPE) at a scan rate of 100 $mV\times s^{-1}$. A potential of −0.5 V for 60 seconds was used to pre-concentrate Δ^9-THC species on the electrode surface.

3. Results and Discussion

3.1. Voltammetric Behavior of Supporting Electrolyte

The Voltammetric response to 10 µL of the supporting electrolyte solution on the surface electrode is illustrated in Fig. 1. A potential of −0.8 to 0.8 V vs. Ag-SPE, was applied at a scan rate of 100 $mV\times s^{-1}$. The cyclic voltammogram indicates the expected behavior with an absence of faradaic (cathodic and anodic) peaks.

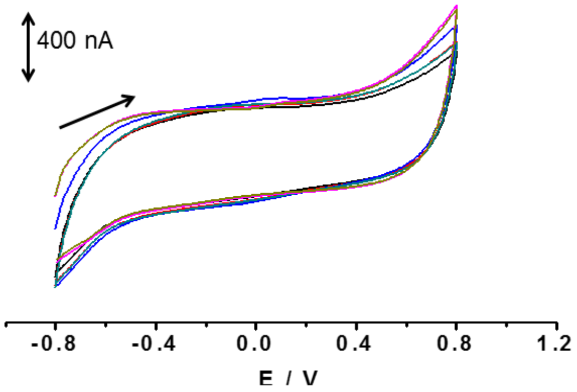

Fig. 1. Cyclic voltammogram of the 0.1 $mol\times L^{-1}$ KNO_3 supporting electrolyte solution.

3.2. Accumulation Time

An 18 $\mu mol\times L^{-1}$ Δ^9-THC standard was used to optimize the accumulation time. This step occurs prior to the voltammetric scan and is utilized to preconcentrate the sample on the surface of the working electrode due to an attraction between the molecule and surface charges. As shown in Fig. 2, a proportional increase in the anodic peak current (I_{pa} at 0.13 V vs. Ag-SPE) occurs during the time of accumulation, reaching a plateau after that 80 seconds (See Fig. 3). Fig. 4 illustrates the oxidation of the Δ^9-THC phenol group which involves the loss of one electron and one proton generating a radical phenoxy group [2, 5-6, 9, 13] and subsequent polymeric products [2]. This process passivates the electrode due to saturation of the molecule on the electrode surface. [26-27, 35-36].

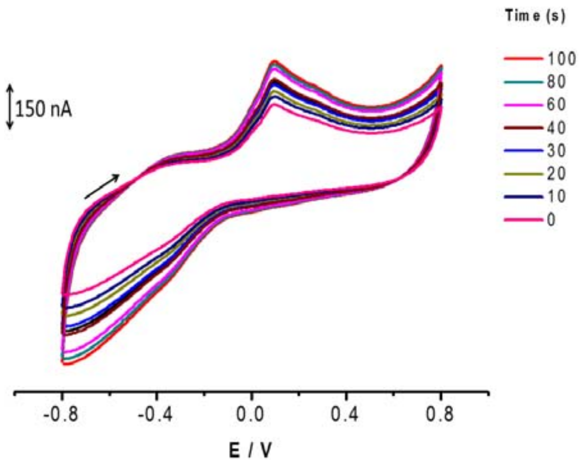

Fig. 2. Cyclic voltammogram of 18 $\mu mol\times L^{-1}$ of Δ^9-THC standard solution at different accumulation times. Supporting electrolyte: 0.15 $mol\times L^{-1}$ of KNO_3, scan rate: 100 $mV\times s^{-1}$.

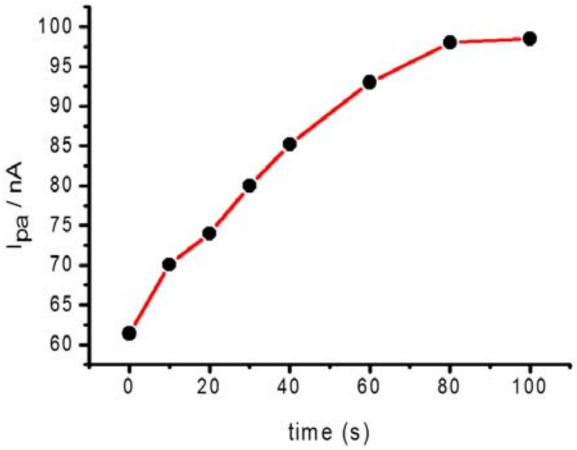

Fig. 3. Influence of pre-concentration time.

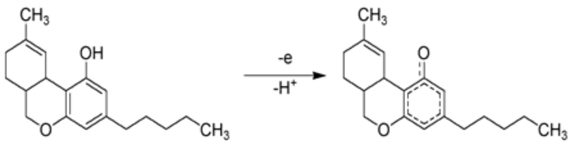

Fig. 4. Purposed electrooxidation of Δ^9-THC molecule [2, 6, 9, 13].

3.3. Influence of Scan Rate

The effect of scan rates from 20 to 150 $mV\times s^{-1}$ on the voltammmetric response was next investigated. (Fig. 5). The anodic peak potential increased proportionally with the applied scan rate. The change of anodic peak potential (E_{pa}) indicated that an irreversible process is occurring and that mass transport is controlled by adsorption of Δ^9-THC. A scan rate of 100 $mV\times s^{-1}$ was adopted for future study of the influence of concentration. A linear dependence of I_{pa} against the scan rate is reported in the Fig. 6.

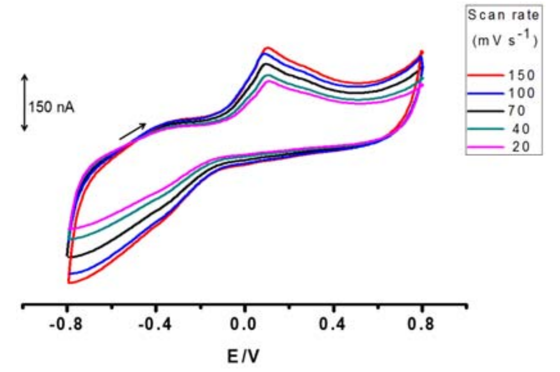

Fig. 5. An examination of the influence of scan rate on the cyclic voltammogram of an 18 µmol×L^{-1} Δ^9-THC standard solution. Time of accumulation: 60 s. Supporting electrolyte solution, 0.15 mol×L^{-1} potassium nitrate.

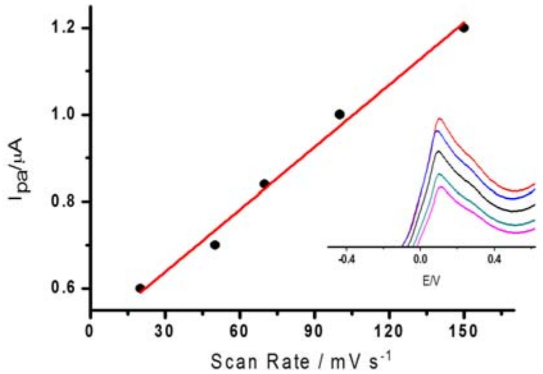

Fig. 6. Linear dependence of I$_{pa}$ vs. scan rate.

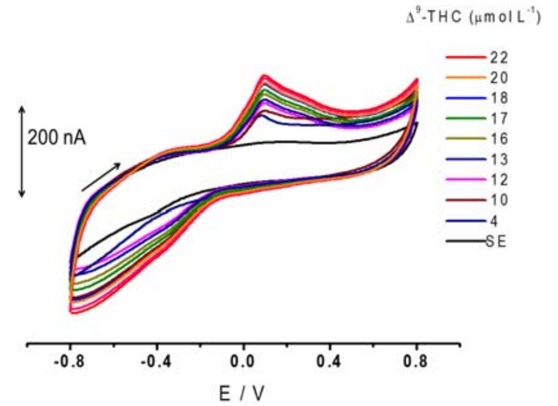

Fig. 7. Cyclic voltammogram referring to successive additions of Δ^9-THC standard solution. Time of accumulation: 60 s. Scan rate: 100 mV×s^{-1}.

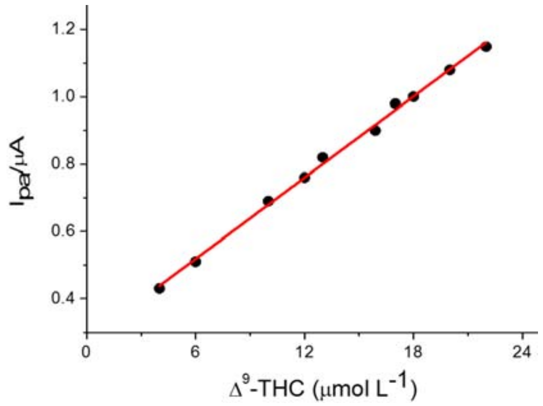

Fig. 8. Analytical curve I$_{pa}$ vs. concentration of Δ^9- THC standard solution (µmol L^{-1}).

3.4. Influence of Concentration

The voltammetric response to increasing concentrations of Δ^9-THC applied to the electrode surface was next measured at 0.13 V vs. Ag-SPE. Fig. 7 shows the resultant cyclic voltammograms. The I$_{pa}$ values increase linearly in proportion to concentration (4 - 20 µmol×L^{-1}). The analytical curve (Fig. 8) produced a correlation coefficient (r^2) of 0.997 and a standard deviation (SD) of 0.01 µA. The equation of the line was:

$$I_{pa} = 0.3\ \mu A + 0.04\ \mu A/\mu mol{\times}L^{-1}\ \Delta^9\text{-}THC \quad (1)$$

These results demonstrate a limit of detection (LOD) of 1.0 µmol×L^{-1} and a limit of quantification (LOQ) of 3.3 µmol×L^{-1} using the relationship 3SD/m and 10SD/m respectively, where m is the amperometric sensitivity of the line.

7. Conclusions

A method for the analysis of Δ^9-THC was optimized using carbon screen printed electrodes. The process involves a 60 second pre-accumulation step followed by measurement of a peak current at 0.13 V vs. Ag-SPE. The resultant measurement produced a linear relationship between concentration and peak current with a limit of detection of 1.0 µmol×L^{-1}. This method should prove useful in the quantitative analysis of seized marijuana samples as well as in presumptive testing. Overall, the application of carbon screen printed electrodes is rapid, efficient and uses minimal chemical reagents, indicating that this procedure should be a useful alternative in forensic investigation.

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