



A Simple and Rapid Spectro-electrochemical Analysis of delta-9 Tetrahydrocannabinol (Δ^9 -THC) using Carbon and Platinum Screen Printed Electrodes

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ABSTARCT

This research introduces a new voltammetric method for detecting delta-9-tetrahydrocannabinol (THC), addressing the widespread concern over marijuana's illegal use. Utilizing methanol, potassium nitrate as a supporting electrolyte, and a screen-printed carbon electrode, the study developed a precise approach to identify THC and its functional groups via spectral analysis. Experiments yielded a linear THC detection range from 1.875 to 30 $\mu\text{mol L}^{-1}$, with a high correlation coefficient of 0.995 and a detection limit of $0.57 \times 10^{-5} \mu\text{mol L}^{-1}$. FTIR analysis highlighted a phenol group, undergoing oxidation, as a key indicator of THC presence. To confirm THC in seized cannabis samples, the study applied a spectroelectrochemical method alongside preparative thin layer chromatography for sample purification, using a hexane:acetone mix. This approach efficiently isolates THC, proving the method's effectiveness for forensic and analytical applications in drug control.

Keywords: Delta-9-THC, Marijuana, Thin layer chromatography, Spectroelectrochemical method.

INTRODUCTION

Cannabis is a commonly used prohibited drug which consist delta-9-tetrahydrocannabinol (Δ^9 -THC)^{1,2}. THC is a principle terpenophenolic compound that can cause psychosis and cognitive decline^{3,4}. When an unknown substance suspected for cannabis is seized and received in forensic laboratory the focus of examination is detection of cannabinoids including THC. The examination starts with preliminary testing which includes

colorimetric identification by Duquenois Levine, Fast Blue B or Fast Blue BB test⁵⁻⁷. Additional examination includes thin layer chromatography for further specificity⁸. Preliminary examination is followed by confirmatory analysis that includes analysis by high end instrumental techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC), Mass spectrometry (MS), GC-MS, LC-MS, Fourier transform infrared spectroscopy (FTIR), etc. All the examination is carried out under Scientific Working Group for the



Analysis of Seized Drugs (SWGDRUG) guidelines⁹. These guidelines classify different techniques in three groups i.e. A, B and C depending on their sensitivity. Nuclear magnetic resonance, MS, and other highly conclusive procedures are found in category A, whereas GC, LC, and other focused tests are found in category B, and less focused techniques like ultraviolet spectrophotometry and colour tests are found in category C¹⁰.

Recently, more advanced and affordable techniques for analyzing illicit drugs have been created among which electrochemical techniques are one of them. These techniques are widely used in pharmaceutical, chemical, battery and more industries¹¹⁻¹⁶. These techniques focus on chemical processes that occur in solution between an electrode and an electrolyte at a solid/liquid interface¹⁷. They are quite specific and selective best applicable for qualitative analysis¹⁸. The cost-effective instrumentation along with its potential application in providing determining electrochemical reaction mechanisms¹⁹ has motivated several groups to develop methods for detecting illicit drugs. Different voltammetric techniques were used for analysis of various illicit drugs including narcotic, psychotropic, designer as well as new psychoactive substances²⁰⁻²³. Due to their high sensitivity, they provide identification of such drugs at trace level quantity. As known, cannabis is widely abused drug worldwide; researchers focused on electrochemical analysis of cannabis. Primarily detection was performed by using conventional electrodes which later on replaced by screen printed electrodes (SPE) due to their more sensitivity and simplicity²⁴⁻²⁷.

There are numerous studies on the electrochemical analysis of chemicals of forensic significance, but there are no studies on the spectroelectrochemical analysis of THC using screen printed electrodes. Additionally, there is relatively little study on the analysis of cannabis samples that have been confiscated utilizing voltammetric methods in addition to spectroscopic methods. These days, there is a requirement for study on the spectroelectrochemical examination of many drugs. As a result, we have investigated the electrochemical behaviour of THC while directly analysing it with cyclic voltammetry utilising a carbon SPE and then performing an ATR FTIR spectroscopy analysis.

MATERIAL AND METHODS

Δ^9 -THC was purchased from Cerilliant (Sigma Aldrich, Bangalore), prepared in methanol having concentration of 1 mg/mL. Methanol ($\geq 99.9\%$) and Potassium nitrate (99.99%, crystalline form) was also obtained from Sigma Aldrich, Bangalore.

General Procedure

For electrochemical analysis, 1mg/mL Δ^9 -THC certified reference standard (CRM) from Cerilliant (Sigma-Aldrich) was used for preparation of working standard solutions. Working standards were prepared in methanol used as a diluent. 90 μ L from 1mg/mL Δ^9 -THC CRM was taken by using micropipette and 910 μ L methanol was added followed by ultrasonication for preparation of 30 μ M stock solution. The stock solution of 30 μ M was further diluted to half serially in the range of 15, 7.5, 3.75, 1.875 μ M to plot a calibration graph. As mentioned above, no sample preparation was required for ATR-FTIR analysis.

53 seized samples suspected to cannabis were collected from CoENDPS, NFSU, Gandhinagar. 5 mg from each seized sample was weighed using weighing balance and transferred to 2 mL eppendorf tube. 1 mL methanol was added in each vial and was kept in ultrasonicator for 5 minutes. Later, the extract was filtered by with the help of syringe filter and green colour filtrate was collected and stored in eppendorf tube. For electrochemical analysis of Δ^9 -THC it was necessary to separate the specific cannabinoid i.e., Δ^9 -THC from other cannabinoids present in cannabis. The pre-purification was achieved by preparative thin layer chromatography (TLC). In preparative TLC, instead of small spots the band of each sample was spotted on a separate plate and allowed to run in hexane: acetone mobile phase (80:20) for 15-20 minutes. A fast blue B reagent was sprayed on left part of the TLC plate by covering its right area to locate Δ^9 -THC. The scrapping was performed from the cover part of TLC plate. The scrapping silica containing analyte was diluted with methanol and the resulting solution was filtered to remove the silica particles. The filtrate was then analyzed on the potentiostat/galvanostat. Before voltammetry, some portion of scrapped silica powder was removed for FTIR analysis for functional group study.

Cyclic voltammetry analysis was performed by the setup mentioned above. By using drop coating method 10 μL quantity of $\Delta^9\text{-THC}$ CRM was applied on working electrode area and kept for 2 min for drying. Settlement of analyte was followed by addition of 1000 μL 0.1 mol/L potassium nitrate. Initially the potential scan is performed by covering full range of -0.8V to +0.8V, at the scan rate of 100 mVs^{-1} . Later, the window is minimized and kept having range of -0.5V to +0.6V with similar scan rate. The range was same for solvent, supporting electrolyte as well as analyte of interest.

Detection method

Identification of $\Delta^9\text{-THC}$ was achieved by using a multichannel potentiostat/galvanostat Autolab model PGSTAT204 (Metrohm, Switzerland) coupled with Nova 2.1.5 Software. Screen-Printed Carbon Electrodes (SPCE) and platinum SPCE were purchase from Metrohm DropSens (model 110). The working and auxiliary electrode of SPCE is constitutes carbon whereas reference electrode is made of silver. The diameter of working electrode is 4mm. All the three electrodes are fixed on a ceramic plate representing a small setup of electrochemical cell. Functional group study was carried out on a Fourier Transform Infrared Spectrophotometer (FTIR) an Invenio X Fourier Transform Infrared Spectroscopy (Bruker, Massachusetts, United States) was used for analysis in addition with OPUS software. The Attenuated Total Reflection (ATR) mode was used in FTIR which does not require any sample preparation for sample analysis. Weighing balance (UniBloc, Shimadzu), micropipettes (Labquest, Borosil), ultrasonicator (Labman) was used for sample preparation.

Analytical discussion

An electrochemical cell consists of working electrode, reference electrode and counter electrode which are miniaturize on a single ceramic platform called as screen-printed electrode. As known electrode selection plays an important role in voltametric analysis. Electrode which gives better peak current response will be more suitable for that analyte. For electrode selection, voltammograms of standard solution of THC was recorded for different concentrations on carbon and platinum screen printed electrodes²⁸. The selection of solvent and supporting electrolyte was performed and the CV of supporting electrolyte was studied. Preconcentration

time which is also known as accumulation time was analyzed and enhanced by using lowest concentration i.e. 1.875 $\mu\text{mol L}^{-1}$ of the standard. Before applying the voltametric potential cycle, preconcentration step is employed for accumulation of the analyte on working electrode. This fixes the analyte's molecules on electrode surface by creating attraction between them²⁹. Cyclic voltammograms were obtained at four different time intervals starting from 60 to 120 seconds to know the intensity of anodic peak current. The influence of scan rate on anodic peak current was investigated from 40 mVs^{-1} to 100 mVs^{-1} .

The calibration curve was created in the range of 30, 15, 7.5, 3.75, 1.875 $\mu\text{mol L}^{-1}$. The specificity study for THC was conducted against four different drugs among which opium and atropine are naturally occurring substances whereas phenobarbitone and phenytoine are synthetic drugs. For the study of functional group study, the FTIR analysis was performed in the range of 400 $^{-1}$ to 4000 cm^{-1} . Sixty-four scans of each sample were taken and average of the data points of all scans was plotted as one FTIR spectra for a single sample. In FTIR analysis no sample preparation was required as the analysis was conducted in ATR mode. The voltametric detection of $\Delta^9\text{-THC}$ in the seized samples suspected for cannabis was performed by the developed method.

RESULTS AND DISCUSSION

Method Development and Optimization

Electrochemical response showing presence of anodic peak for the analyte was comparatively better on carbon screen printed electrode as shown in Figure 1.

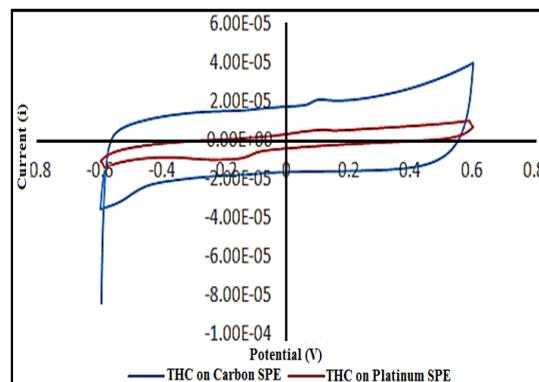


Fig. 1. Electrochemical response of THC on two different electrodes

The selection of methanol as solvent and 0.1 mol/L potassium nitrate as supporting electrolyte gives reliable outcome in the fixed range of -0.5V to +0.6V (vs. Ag/AgCl). The supporting electrolyte did not show any peak current in the applied range of potential cycle whereas $\Delta 9$ -THC show an anodic peak current at 0.15V indicating irreversible oxidation reaction as shown in Fig. 2. The oxidation mechanism includes transfer of one proton from phenolic group of $\Delta 9$ -THC molecule as shown in Figure 3.

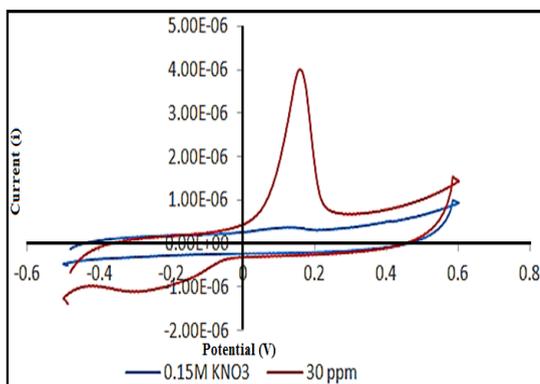


Fig. 2. Cyclic voltammogram of supporting electrolyte

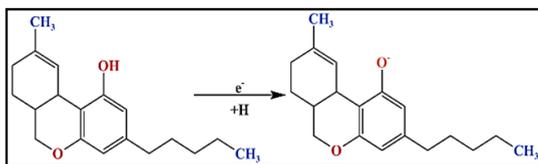


Fig. 3. Oxidation mechanism of $\Delta 9$ -THC

The highest peak current shows at 60 seconds of accumulation time and as the time was increased decrease in peak current was observed and presented in Figure 4.

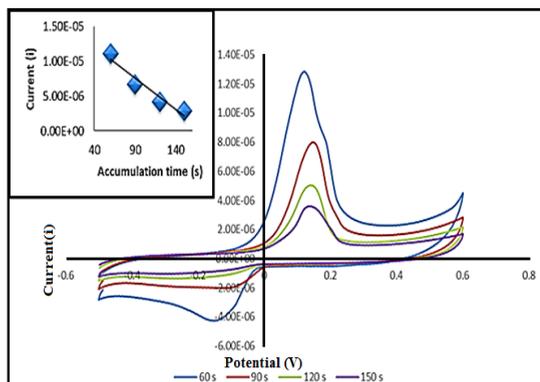


Fig. 4. Cyclic voltammogram after studying effect of preconcentration time with decrease in peak current intensity from 60s to 120s

As shown in Fig. 4 the anodic peak current is directly proportional to the applied scan rate.

Further increase in scan rate shows saturation of $\Delta 9$ -THC on working electrode which results in an unknown polymeric product formation²⁶. The shift in anodic peak revealed an irreversible process and revealed that the adsorption of 9-THC regulates mass transport. A scan rate of 100mVxs⁻¹ was taken into consideration for the advance experimental study. It is presented in Figure 5.

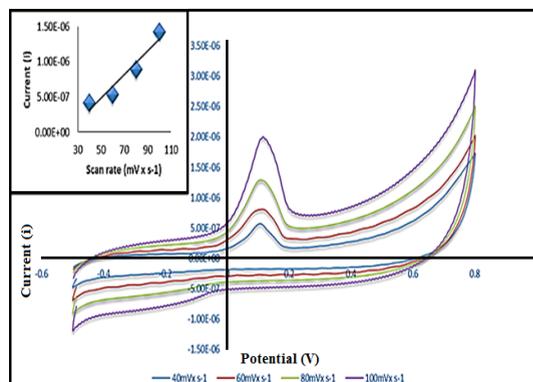


Fig. 5. Cyclic voltammogram after studying effect of scan rate with increase in peak current intensity from 40mVs⁻¹ to 100mVs⁻¹

The voltametric response with respective concentration of $\Delta 9$ -THC was examined on the electrode surface by applying a fix potential range from -0.5V to +0.6V at 100100mVxs⁻¹ scan rate followed by preconcentration for 60s. As mentioned above the lowest concentration shows an anodic peak and the anodic current linearly increases with increase in analyte's concentration. The calibration curve produces a correlation coefficient of 0.995 Fig. 6 and standard deviation of 0.0129 μ A. The limit of detection (LOD) and limit of quantification (LOQ) was calculated as 0.57 $\times 10^{-5}$ and 1.72 $\times 10^{-5}$ mol L⁻¹, respectively by employing the relationship 3SD/m and 10SD/m, where m represents amperometric sensitivity of the curve²⁵.

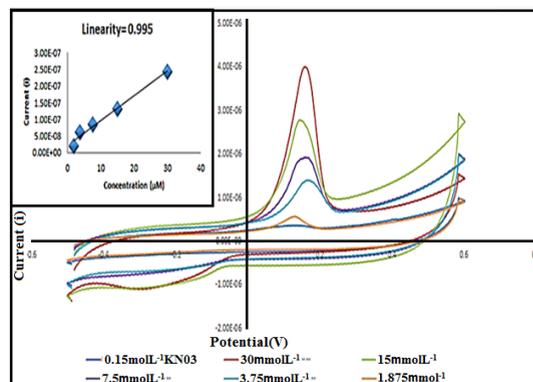


Fig. 6. Cyclic voltammogram after successive addition of $\Delta 9$ -THC with increase in peak current w.r.t increase in concentration

Method Specificity Study

The establishment of electroanalytical procedures for the identification of drug active substances addresses specificity as the validation parameter^{30,31}. It can be described as “an electroanalytical method because of its capacity to measure the analyte precisely and specifically in the presence of components that might be anticipated in the sample matrix.” When using electroanalytical techniques, when just one parameter may be assessed, specificity is appropriately used. Analyte, supporting electrolyte, matrix with and/or without analyte, contaminants of the starting material, degradation products, or metabolites should all be studied to demonstrate the specificity of the suggested electro-analytical procedures³¹. The electroanalytical approaches have the benefit of typically being unaffected by the inactive components of pharmacological dose forms.

Opium extract prepared from crude opium resin shows presence of anodic and cathodic peak with different amplitude. It represents a quasi-reversible reaction when opium was analyzed by similar method developed to study the voltametric nature of THC. Similarly, atropine undergoes oxidation reaction on carbon screen printed electrode having different peak position compared to the anodic peak position of THC. Formation of two different oxidation products followed by single reduction was observed in phenobarbitone whereas phenytoine shows anodic peak with different peak position in similar fashion to atropine. The results are presented in Figure 7.

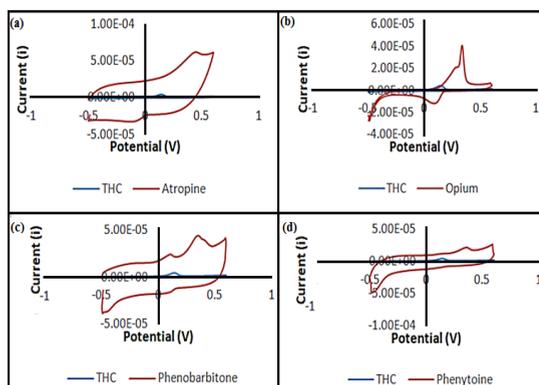


Fig. 7(a). CV of THC vs Atropine. (b) CV of THC vs opium. (c) CV of THC vs phenobarbitone. (d) CV of THC vs phenytoine

FTIR Spectrum in transmission mode of the $\Delta 9$ -THC standard was obtained in ATR mode.

It was observed from Fig. 5, the strong band at 1017 cm^{-1} corresponds to stretching vibrations for C-F group. A medium peak at 3294 cm^{-1} corresponds to strong and broad stretching vibrations for carboxylic OH group followed by the presence of two more medium bands at 2918 cm^{-1} and 2850 cm^{-1} showing resemblance with medium C-H stretching vibrations³². The peak at 1732 cm^{-1} was due mainly to weak C-H bending found in aromatic compounds. Occurrence of the sharp peak at 1609 cm^{-1} is due to C=C stretching vibrations found in conjugated alkanes. The small bands at 1415 cm^{-1} and 1378 cm^{-1} indicates presence of medium O-H bending for alcohol and phenol, respectively³³. Strong C-O stretching are responsible for the peak at 1247 cm^{-1} . The peaks observed between 800 cm^{-1} and 440 cm^{-1} can be assigned to strong C-H bending vibrations at different substitutions. The number of hydrogen atoms adjacent to the C-H bond causes different peaks to be observed³⁴. The spectrum is presented in Figure 8.

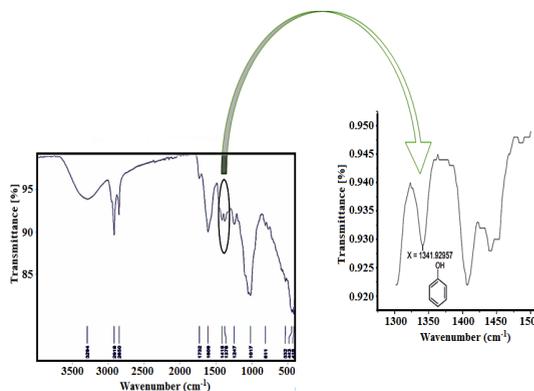


Fig. 8. FTIR spectrum of $\Delta 9$ -THC standard

Analysis of Real Seized Samples

Samples were prepared by following the protocol as described in this paper. Since other extract constituents also include phenolic groups, the electro-oxidation of the phenol group is not limited to $\Delta 9$ -THC. When cannabis extracts were analyzed by CV without pre-purification, shifting and broadening of anodic peak was observed. Separation by TLC was therefore crucial to run proposed methodology successfully²⁵. After separation and extraction of THC, cyclic voltammogram shows an increase in anodic peak current followed by peak sharpening. Using the drop coating technique along with standard addition method, it was possible to detect THC in all seized cannabis samples. Fig. 9 shows the graphical representation of all cannabis samples where they show an anodic peak current response at a particular potential value.

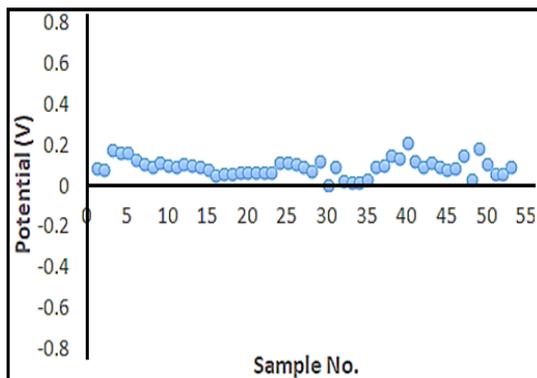


Fig. 9. Anodic peak potential response of seized cannabis samples

FTIR analysis of all the seized cannabis samples was performed along with cyclic voltammetry to understand the chemical processes occur on the electrode area. Such can be studies performed in a combined FTIR Spectro-electrochemical setup or separate analysis can be carried out. Examination of all samples in FTIR shows presence of different functional groups which shows resemblance with functional groups present in THC standard. Presence of band from 1390-1 to 1310 cm^{-1} indicates existence of phenol group in THC which is responsible for giving anodic peak in cyclic voltammogram due to its oxidation. Apart from phenol, band at different positions are also observed after purification of THC which is due to interference of silica material. Such interferences can be avoided by analyzing the sample in combined FTIR Spectro-electrochemical setup. The diagrammatic representation of systematic analysis of seized samples suspected for cannabis by FTIR Spectro-electrochemical technique is shown below in Figure 10.

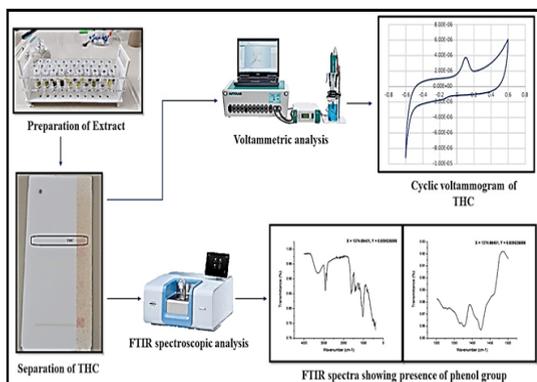


Fig. 10. Systematic analysis of seized cannabis sample by FTIR Spectro-electrochemical approach

CONCLUSION

Using the electrochemical method of cyclic voltammetry in conjunction with FTIR, it was discovered that the spectroelectrochemical detection of THC was achievable for the first time. Using carbon SPE, a procedure for the analysis of 9-THC was created and improved. A 60-second accumulation period is applied, followed by a potential application range of -0.5 to +0.6V at a scan rate of 100mV s^{-1} . 9-THC displayed an anodic peak current at 0.15V, indicating an irreversible oxidation reaction caused by the presence of the phenol group, which was determined by FTIR to be present between 1390 cm^{-1} and 1310 cm^{-1} . For THC-controlled samples, in a potassium nitrate-supporting electrolyte, the resulting measurement revealed a linear relationship between concentration and peak current with a LOD of $0.57 \cdot 10^{-5}\text{ mol L}^{-1}$ and LOQ of $1.72 \cdot 10^{-5}\text{ mol L}^{-1}$. With this combined analysis approach, it is possible to investigate the functional group that determines how this substance behaves voltametrically depending on the applied electrochemical potential. The monitoring of electrochemical processes is made simple and dependable by the technical implementation of the combination of ATR FTIR spectroscopy and electrochemical investigations. The utilization of these techniques, which show to be dependable, quick, and straightforward, are significant for the creation of a portable in-field sensor.

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Conflict of interests

The authors declare that there is no conflict of interest.

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