



Disposable 3D Printed Electrode for the Electrochemical Detection of Delta-9-Tetrahydrocannabinol in Aqueous Solution and 11-Nor-9-Carboxy-Tetrahydrocannabinol in Saliva

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Abstract. Currently, marijuana or cannabis is the most consumed drug worldwide, and is also the one with the highest number of seizures, with 354 tons seized in Brazil in 2017. The increase in its consumption has been called attention, not only by the authorities, due to accidents caused when driving under the influence of the drug, but also by companies that carry out toxicological analyzes on their employees. For this, rapid tests, such as colorimetric tests, are generally used to analyze the urine of users. The need for agile and proper identification of drugs of abuse has encouraged the scientific community to improve and to develop new methodologies. Electrochemical methods appear as a cheap, portable and easy to use alternative. The use of biodegradable materials for the development of disposable electrodes has become paramount today. The use of 3D printers emerges as an alternative for the manufacture of these electrodes and the use of saliva instead of urine, has also been a priority because it is a less invasive sample and difficult to adulterate. In this work, a three dimensional device was created in an innovative way containing the three electrodes, working, counter and reference, in conductive material. The device was then used to detect the active principle present in cannabis, delta-9-tetrahydrocannabinol, in aqueous solution, and its metabolite, 11-nor-9-carboxy-THC, in samples of real saliva, both through cyclic voltammetry. The positive results show that the proposed device can be used, as well as those already found on the market, for the identification of drug use and in the forensic field.

Keywords: Forensic electroanalysis; Delta-9-tetrahydrocannabinol; Saliva; Voltammetry; 3D-printed electrode.

1. Introduction

The history of mankind has been accompanied by the use of psychoactive substances, either for medicinal purposes or as an element of religious rites. Currently, the term drug, in its more restricted use, refers to illicit drugs in general, among which are cocaine, crack, LSD, ecstasy and marijuana, which is present in almost all related events with drug trafficking^{1,2}.

Marijuana, or cannabis, has the main psychoactive substance Δ 9-THC (delta 9-THC, short for delta-9-tetrahydrocannabinol). Its main forms of presentation are as herbs (in one piece or crushed) or resins (hashish), whose concentration of Δ 9-THC is higher. It can also be found in the form of a cigarette, ready to use, whose consumption can cause euphoria and hallucinations³⁻⁶.

According to the World Drug Report of 2019, cannabis trafficking is concentrated in the Americas, accounting for 60% of global seizures, with 38% being concentrated in South America⁷. In Brazil, the federal police seized more than 200 tons of marijuana per year between 2013 and 2018, with maximum seizures in 2017 at 354 tons⁸.

In addition to the analysis of the seized marijuana, there is also the need for the analysis of biological fluids, such as urine and saliva, to detect its metabolites, in order to confirm its consumption⁹. The highest concentrations of Δ 9-THC and its main metabolite, 11-nor-9-carboxy-THC (also known as 11-COOH-THC), can be found in biological fluids, as plasma and oral fluid, between 3 and 6 hours after consumption, but its stay in the human system for several days⁹⁻¹¹.

The use of saliva for the verification of drug use allows a less invasive sample collection and increases the difficulty of adulterating the sample¹²⁻¹⁴, being progressively more prioritized by government authorities, in cases of traffic accidents under the influence of such substances¹⁵, in monitoring of drug addicts¹⁶, as a clinical toxicology, to control the use of psychotropic substances in employees¹⁷, and even in sports, with anti-doping tests¹⁸.

Improving police officers' working conditions to identify the use of narcotics is crucial for driver control. Thus, the development of simple and specific drug detection methods, which can confirm at the scene of the occurrence if the illegal substance

was used, is a goal to be pursued to provide tools that facilitate the investigative work of the police.

Most of the preliminary tests used today, for field analysis, employ immunoassays, in which color changes are obtained for certain substances. These tests are easy to produce, cheap, fast and portable, but are mostly applied to urine^{14,16,19}.

Despite the practicality and low cost of colorimetric analysis, electrochemical sensors are a more versatile alternative^{20,21}, bringing together characteristics such as: 1) light and portable; 2) low cost; 3) have excellent reproducibility with high sensitivity and precision; and 4) it does not require the presence of a professional specialized in its use or instruments of complicated operation²¹.

Electrochemical techniques such as amperometric, potentiometric and voltammetric techniques have been frequently used in the last two decades for the detection and quantification of samples of forensic and pharmacological interest²²⁻²⁶. The literature involving voltammetric determination of $\Delta 9$ -THC comprehends the use of electrodes with and without chemical modification. There are methodologies for its detection when in seized samples²⁶⁻³⁰, and in saliva^{31,32}. However, it should be noted that in the literature only a few studies with artificial saliva have been reported for the electrochemical detection of $\Delta 9$ -THC³³.

When it comes to the development of fast, portable and low-cost chemical analysis devices, it is worth mentioning the use of disposable electrodes made in a 3D printer. These printers, initially created to supply needs in the field of engineering, have gained notoriety in other areas due to their speed of manufacture and the reduced use of raw materials, making it interesting for researchers and industry, especially due to its low cost and large-scale manufacturing³⁴⁻³⁶.

Despite the advantages of using electrochemical techniques, even considering this new possibility of using polymer as an electrode, there are few studies in the literature, involving the analysis of substances of forensic interest in biological matrix³⁷.

Taking this into account, the present work proposes a methodology for the detection of $\Delta 9$ -THC, in aqueous solution, and its metabolite, 11-COOH-THC, in saliva, using a polymeric material device (polylactic acid) printed on a 3D printer, containing the three electrodes, reference, auxiliary and working, and using the cyclic voltammetry methodology for voltammetric analyzes.

2. Materials and methods

2.1. Three-dimensional printed electrode (3DE)

The electrode design was developed using the Inventor (Autodesk) software and printed using a GTMax printer (model A1), with two extruder (0.45 mm nozzle size) at a temperature of 190 °C. The brown PLA filament was used as base and the black conductive PLA filament was used to draw the reference, counter and working electrodes. The printing established through the Simplify3D software.

The electrode design was based on the screen-printed electrodes already found on the market, maintaining the circular shape for the working electrode, while the reference and counter electrodes had their shape modified, Figure 1. After printing, the reference electrode was covered with a layer of silver ink, thus being considered a pseudo reference electrode. Also, to prevent the analytical solution from coming into contact with the electrical contacts, a barrier, in the form of a step, was added to the shape of the electrode.

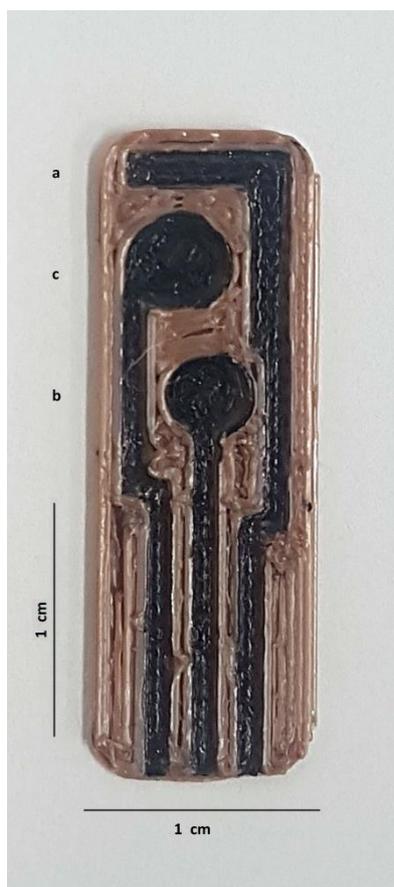


Figure 1. Three-dimensional printed electrode without the silver ink on the reference electrode; (a) counter electrode; (b) working electrode; (c) reference electrode.

2.2. Standard solutions and samples

For the analysis of Δ 9-THC, the solutions were prepared in aqueous medium of KNO_3 0.1 mol L^{-1} , in different concentrations, from a standard solution of Δ 9-THC (1 mg / mL - Cerilliant) in methanol.

Analyzes of the metabolite, 11-COOH-THC, were performed using samples of real saliva fortified with a standard solution of 11-COOH-THC (1 mg / mL - Cerilliant) in methanol. Solutions of saliva fortified with standard solution of Δ 9-THC were also tested.

Colleagues from this study provided aliquots of their saliva, being collected in a 5.0 mL falcon tube. It is worth mentioning that they had not ingested coffee or any type of food in the 2 hours prior to collection, to minimize any type of interference from the matrix.

For the measurements, 1.0 mL aliquots of saliva were collected, and the analyte was added to it. From this solution, 0.5 mL was collected and diluted with an additional 0.5 mL of 0.3 mol L^{-1} KNO_3 aqueous solution. After homogenization, an aliquot of this final solution, approximately 200 μL , was deposited on the electrode surface and the reading was performed.

It should be noted that the analysis of the "sample blank" was also carried out. This blank is the saliva sample diluted in the solvent used to prepare the analyte solution, in this case, methanol (Merck, HPLC grade).

Deionized water was used to prepare all aqueous solutions.

2.3. Voltammetric analysis

Voltammetric analyzes were performed on μ Autolab III (Metrohm), using NOVA 1.11 software. The cyclic voltammetry was performed for the analysis of Δ 9-THC and its metabolite 11-COOH-THC, with the objective of detecting it, that is, it focused only on the presence of voltammetric response for the analyte.

3. Results and discussion

As already mentioned, the scientific police and some companies have as priority only the identification of drug use, and not the quantification of the dosage administered by the user. Devices found on the market, such as immunochromatographic tests, have this purpose, that is, they do not have quantitative information for analysis^{14,19}. Since the highest concentrations of Δ 9-THC are found between 1 and 2 hours after

the use of marijuana, it is necessary to take this time into account when using saliva as a sample medium. There is an important economic approach for the device, once it reduces in 75% the cost of a screen printed electrode when compared to a commercial one.

The registered patent, PI 1104489-6, describes an oral fluid analysis methodology for detecting drug abuse, including $\Delta 9$ -THC. It is a micropotentiostat designated with more than 2 working electrodes whose purpose is the simultaneous detection of one or more drugs, using disposable electrodes. However, the results are not presented with the voltammogram records. The apparatus only confirms or not the presence of a drug³⁸.

Following the same principle, $\Delta 9$ -THC and 11-COOH-THC were detected using cyclic voltammetry.

3.1. $\Delta 9$ -THC analysis

After the 3DE has been printed and the silver ink has completely dried, it was connected to the potentiostat and 200 μL of solution was deposited on its surface, covering the three electrodes, for analysis. The analytical conditions applied were based on methods already used in the literature^{27,29}. The optimization of voltammetric parameters results in the application of a pre-concentration of 10 s at -0.1 V, with potential range from -0.4 V to 1.0 V, and scan rate of 0.15 V s^{-1} . The results will be presented only in the region of the voltammetric response and after the baseline of the voltammograms.

It is possible to observe that the analysis of $\Delta 9$ -THC, in aqueous solution of KNO_3 0.1 mol L^{-1} , results in an oxidation peak in approximately 0.1 V. The same potential region already reported in the literature for organic solutions used as support electrolyte, Figure 2^{27,29}.

Previous studies report that the presence of the phenolic group in the $\Delta 9$ -THC structure results in the observed anodic response^{26,29}. The reaction involved is represented in Figure 3.

Furthermore, it is still possible to observe the increase in the current response when the concentration of the analyte in the solution is increased, indicating that it may be possible to construct an analytical curve, relating the concentration of the analyte to the signal of the current response obtained. That is, it may be possible to

use the method for a quantitative analysis of marijuana seized samples by the scientific police. The signal can be obtained in cutoff concentration $1.5 \cdot 10^{-5} \text{ mol L}^{-1}$.

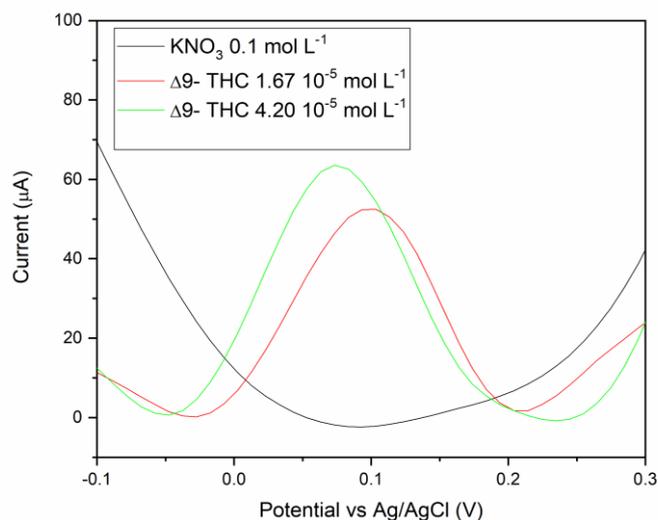


Figure 2. Voltammetric profile for $\Delta 9$ -THC using the 3 dimensional printed electrode; pre-concentration of 10 s at -0.1 V , and scan rate of 0.15 V s^{-1} .

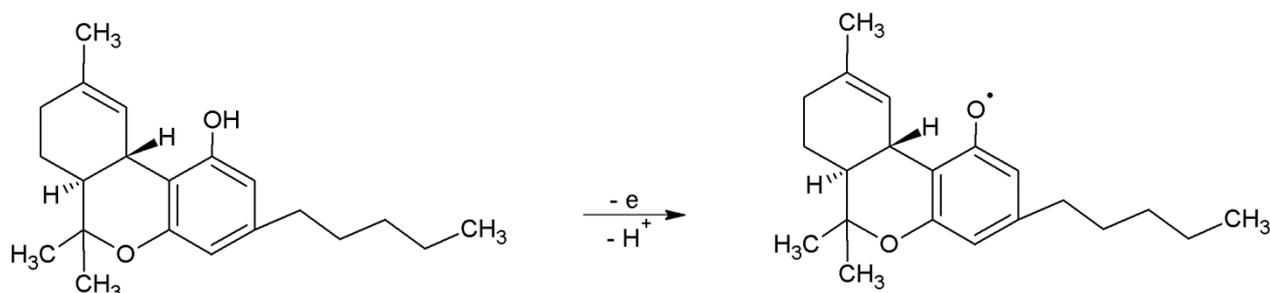


Figure 3. Oxidative reaction for the $\Delta 9$ -THC.

The analysis of $\Delta 9$ -THC added in saliva did not present any peak, so the application of the sensor is effective just for seized samples. The advantage is the low cost system applied in aqueous solution with low cost reagents.

3.2. Analysis of 11-COOH-THC

Since interest is the indication for the use of marijuana, the detection of its metabolite is essential. After a few hours of cannabis use, the concentration of its metabolite in saliva, 11-COOH-THC, is already high enough to be detected.⁹

To simulate a real sample, the use of real saliva fortified with the standard solution of the 11-COOH-THC was necessary to reproduce the condition of a

marijuana user as much as possible. We opted for real saliva instead of artificial saliva to give more veracity to the experiment.

The same experimental conditions performed in the detection of Δ 9-THC were then used to detect its metabolite. It is possible to observe the appearance of an oxidative peak again, but now in a region of different potential, approximately 0.55 V (Figure 4).

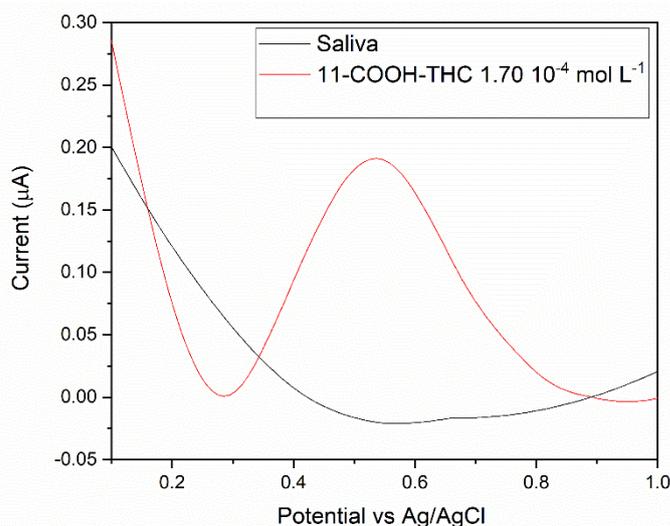


Figure 4. Voltammetric profile for the 11-COOH-THC using the 3 dimensional-printed electrode; pre-concentration of 10 s at -0.1 V, and scan rate of 0.15 V s⁻¹.

The structural difference between the two analytes is only in the carboxylic group, as shown in the Figure 5 below. Therefore, the oxidative reaction of the metabolite, observed in voltammetry, should probably occur in the phenolic group, just as it does for Δ 9-THC. The difference in peak potential could be explained due to this structural difference in the molecule.

The difference of approximately 0.45 V between the peak potentials for both analytes can also indicate that the proposed methodology is able to identify them when present in the same sample.

The analysis using several electrodes for the same solution showed a variation of approximately 8% in the experimental values obtained, showing the reproducibility achieved with the electrode and the proposed methodology. The substance 11-COOH-THC has a cutoff concentration 4.5 10⁻⁵ mol L⁻¹. This result can be explored for detecting minor concentrations, and the present work presents the possibility to expand the results in forensic area.

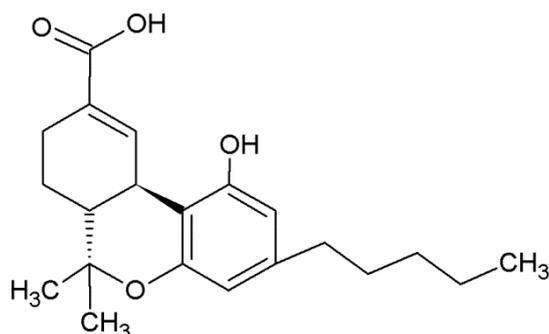


Figure 5. Structure of the metabolite, 11-COOH-THC.

4. Conclusions

The results presented showed that it is possible to build, in a simple and practical way, an analytical device with PLA material in a 3D printing, being biodegradable and low cost. Still, as an innovation, the device was built containing the working, reference and counter electrodes, format not yet found in the literature for this type of material.

With the proposed device, it was possible to apply it for the detection of Δ^9 -THC in aqueous media, that can be used for analysis of seized samples, and also for the analysis of its metabolite, 11-COOH-THC, in real saliva samples, being possible to indicate the cannabis use. Those results demonstrate that the proposed device and methodology can be used for the same goal as immunoassays for forensic purposes.

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