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SHORT COMMUNICATION

ELECTROANALYSIS

Differential pulsed voltammetry of Δ9-Tetrahydrocannabinol (THC) on disposable screen-printed carbon electrodes: A potential in-field method to detect Δ^9-THC in saliva

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Abstract

Due to recent legalization of marijuana across many states in the U.S., there is an increased concern of users driving while impaired/intoxicated with Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive constituent of cannabis/marijuana. Hence, there is a need for a rapid roadside detection of this drug that can be used to accurately screen drivers. Current field sobriety tests rely on a series of physical and mental exercises administered during DUI investigations to help determine a driver's level of impairment. Due to their portability and effectiveness, screen printed carbon electrodes (SPCEs) are ideal to work with when it comes to devising a low-cost screening device for roadside testing. SPCE's can potentially detect low levels of Δ^9-THC in an individual's saliva via electrochemical oxidation of Δ^9-THC. Herein we report a fast, cheap, and accurate approach to electrochemically detect 1–20 μM Δ^9-THC in a 1 mL sample of artificial oral fluid (AF-OF) diluted to 50% with a buffer/electrolyte solution using differential pulse voltammetry (DPV) at the surface of a small SPCE. Implications for the use of this method to screen intoxicated drivers are discussed.

KEYWORDS
Δ^9-THC detection, differential pulse voltammetry, SPCEs

Currently, recreational marijuana is legal in 19 states in the United States. Cannabis contains over 500 different compounds, over 100 of which are cannabinoid derivatives [1]. Of these, 11-OH-Δ^9 tetrahydrocannabinol is the main psychoactive metabolite. THC can be tested in hair, urine, blood, breath, and saliva [2]. The most practical methods for rapid roadside detection are those that are the least invasive–saliva and breath. Importantly, the method of THC consumption can affect the retention of THC within saliva and the breath. Upon investigation, it was found that THC levels in the saliva of frequent smokers was on average, 9 μM, whereas for occasional smokers it was 3 μM [3]. For THC uptake via smoking, it took 0.17 h to detect maximum concentrations of salivary THC, and THC could still be detected 24.7–61.0 h after consumption. At the same time, when THC was consumed orally, the average salivary Δ^9-THC concentration was 944 nM in frequent users and 642 nM for occasional users [3]. For THC uptake via oral consumption, it took 0.33–0.41 h to detect maximum

salivary THC concentrations, and THC could still be detected 24.7–55.0 h after consumption. Δ^9-THC is retained within the breath for less time than in saliva, usually for up to 3 h, which presents challenges in ensuring that a driver is not impaired if they are tested 3 h after consumption. In contrast, THC can last much longer in oral fluid [3, 4]. Therefore, oral fluid is an ideal sample for monitoring THC for rapid roadside testing/screening of drivers. However, a recent review that compared the percentage of positive screening tests that were true positives against the percentage of negative screening tests that were true negatives, demonstrated that saliva-based THC screening was only warranted when reasonable belief of impairment was already established (i.e. smell of marijuana or impaired driving) [5].

For the purposes of this study, we present a simple method to rapidly detect salivary levels of THC (in the 500 nM to 10 μM range, typically found in saliva), as a quick and easy screening method of suspected DUI drivers prior to necessitating a confirmatory analytical blood-based screening method, where THC is typically present at much lower concentrations. Due to the presence of higher average salivary Δ^9-THC concentrations from smoking/vaporization ingestion routes [3], the SPCE-based THC detection method presented herein is best suited as a preliminary screening method to detect THC in those who consumed it via smoking or vaporization.

Many approaches towards THC detection have been reported in the literature in recent years. Some groups have utilized immunoassays to selectively detect THC molecules at nanomolar concentrations [6–10]. Others have used alternative analytical techniques like mass spectrometry or surface enhanced Raman scattering (SERS) to detect THC [12–14]. While the THC detection limits are quite low, these techniques rely on secondary instrumentation or modified electrodes (e.g., luminescence spectroscopy, electrochemical impedance spectroscopy, fluorescence spectroscopy, or modified SPCEs or electrode materials) that are often bulky, costly, non-portable, or non-scalable. In fact, the current on-market roadside test device – the Dräger DrugTest 5000 – uses an immunoassay approach to detect THC at levels as low as 16 nM concentrations. However, its widespread use is limited by the cost of the device (~$5500).

Several different electrochemical approaches for THC detection have been reported. The electrochemical induced single electron oxidation of the 1-hydroxyl group of THC is known to occur. The resulting hydroxy radical subsequently undergoes dimerization/coupling reactions similar to electrochemical phenol oxidation [15]. Two groups recently demonstrated the use of carbon nanotube (CNT) based sensors for THC detection [13, 15]. One study reported that THC could be detected in a 3-electrode cell set-up using a carbon-paper (strip) working electrode (WE) that has THC pre-adsorbed onto the WE. Detection of THC at 4 nM levels was reported using cyclic voltammetry (CV) and square wave voltammetry (SWV) techniques [15]. However, this requires that the THC is first adsorbed and dried onto the strip before the strip is dipped in electrolyte solution for measurement, and therefore is not representative of what would be desired for a true roadside testing of drivers.

Amongst SPCE based methods (and similar graphite/carbon-electrodes), numerous approaches have been reported in the literature. Several reports demonstrate THC detection at relatively high levels (>4 μM) using SPCE methods [16–19]. In a study similar to the one presented here, Nissim et al. reported detection of 0.5–16 μM THC using adsorptive stripping voltammetry with a fabricated carbon paste electrode [20]. In that study, THC was adsorbed onto a fabricated carbon-paste working electrode in both purely buffered solutions as well as AF-OF solutions. Importantly, THC was detected at as low as 0.5 μM in an AF-OF solution (practical LOD) with a theoretical LOD of 0.41 nM. However, the primary drawback of this THC detection method is the hand-fabrication of carbon paste electrodes coupled with the requirement for de-oxygenated solutions for detection. In a trial using human saliva samples, N-(4-amino-3-methoxyphenyl)-methanesulfonamide-mediated detection of 79.5–159 nM THC was demonstrated with SPCE 79.5–159 nM, though the sensors have a very small concentration range in which they accurately respond to THC [21]. Mishra et al. report a wearable electrochemical ring sensor that is capable of both THC and alcohol monitoring in saliva via SWV and chronoamperometry, and can detect as low as 0.5 μM THC levels [22]. Lastly, a comprehensive study from Ortega et al. explored multiple electrochemical oxidation techniques in aqueous solutions, simulated saliva, and real saliva samples using modified sensor electrodes [23]. They utilized a small amount of THC analyte in a pretreatment electrodeposition step, which then was able to remarkably enhance adsorption of analyte THC molecules in the test solution to the surface of the WE, resulting in THC detection between 0–32 nM. However, the authors note that the pretreatment/electrodeposition step makes the SPCEs air sensitive, reducing their shelf life to 24 h under ambient conditions, or alternatively requiring rigorous N_2 or vacuum storage after electrodeposition.

Electrochemical detection of THC via unmodified SPCEs has the potential to serve as an appealing means of compromising extremely low nM-pM detection limits as achieved by many of the approaches discussed above, with high nM – low μM detection ranges (in line with

typical salivary THC concentrations), portability, speed-of-detection, cost, and manufacturing scalability. Herein, we present a simple approach using screen-printed I-SENS carbon electrode strips to detect THC at as low as 500 nM concentrations in an aqueous solution and 1 μM concentrations in a 50:50 AF-OF:buffer matrix. This was achieved without any pretreatment process.

In initial studies the goal was to evaluate the optimal experimental conditions for THC detection in buffered solutions. We compared different electrochemical detection methods - LSV, DPV, and SWV – at both low (1 μM) and high (10 μM) concentrations of Δ^9-THC in a electrochemical cell containing 40 mM HEPES buffer (pH 7.4), 30% methanol, 0.1 M NaCl at a total volume of 1 mL. It was found that the DPV method with I-SENS strips gave consistently smaller background signals resulting in improved THC detection at low THC concentrations. Next, the effect of pH (and buffer) on the Δ^9-THC signal was examined. It was found that the THC signal was enhanced at elevated pH up to a maximum at pH 11 (Figure S1). Given the pKa of the Δ^9-THC phenol is 10.6, it is not surprising that enhanced electrochemical oxidation occurs at higher pH values. Additionally, at sequentially higher pH values the Δ^9-THC oxidation signal shifts to more negative potentials, due to a shift in the reaction quotient. Further, the efficacy of Δ^9-THC adsorption onto the carbon-ink SPCE working electrode was examined by incubating the SPCE in a solution containing 6 μM Δ^9-THC (with 0.1 M NaCl and 0.1 M phosphate buffer pH 11.07) at intervals between 1–15 min (Figure 1). It was found that the THC signal increases with greater incubation times, indicating that it adsorbs onto the carbon working electrode over time. Since significant THC signals were obtained after 10 min, we chose this as the pre-concentration period.

With the above parameters in hand, a Δ^9-THC calibration curve was constructed by obtaining five data points (for 0, 1, 3, 6, and 10 μM THC concentrations) in triplicate (Figure 2), resulting in a linear fit of $y = 3.06*10^{-8} \times + 1.846*10^{-8}$ and R^2 of 0.938.

The detection of THC in oral fluid comes with its own complications. To evaluate matrix effects associated with testing in a more viscous medium, simulated "artificial" oral fluid was used. The AF-OF tested in this study contained a mixture of salivary components, including sodium, ammonium, potassium, calcium, chloride, phosphate, thiocyanate, bicarbonate, and urea ions, as well as mucin, the primary salivary glycoprotein. Upon testing a spiked solution of AF-OF containing a 1:1 mixture of AF-OF and an aqueous solution consisting of pH 11 phosphate buffer (0.1 M), 0.1 M NaCl, and

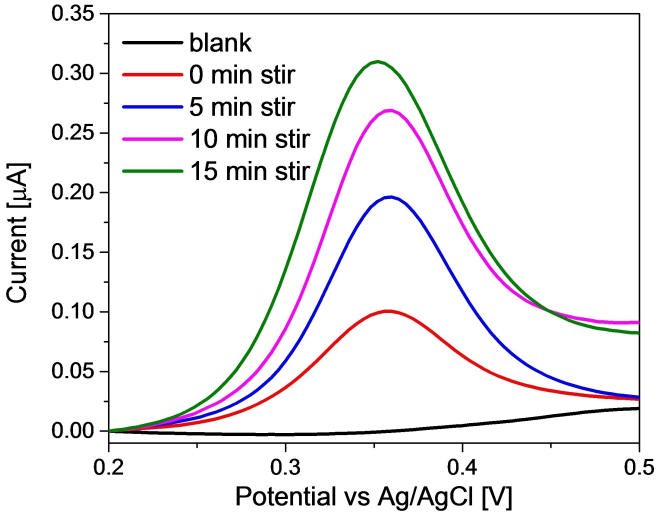

FIGURE 1 Comparison of how stir time effects THC absorption and corresponding detection via DPV (6 μM Δ^9-THC, 0.1 M NaCl, 0.1 M phosphate buffer pH 11).

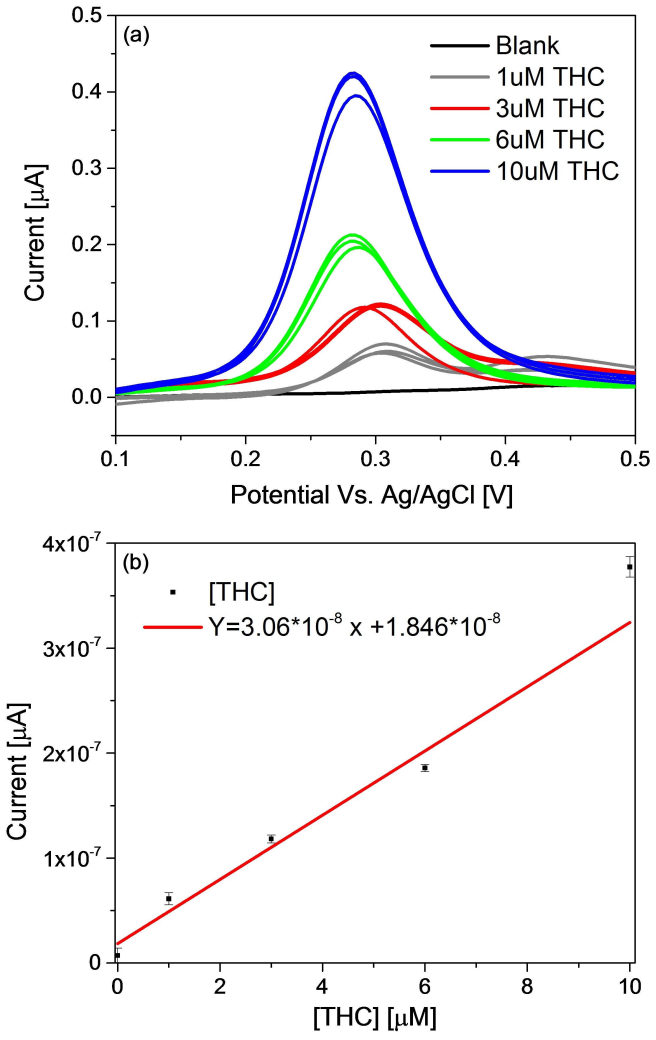

FIGURE 2 THC signals from triplicate experiments performed at 1, 3, 6, and 10 μM THC concentrations in a buffered solution (a) and linear fit of the THC data (b).

6 μM THC, it was found that the THC oxidation peak at 37.5 mV was occluded by background oxidative current due to the AF-OF additives. Since it was demonstrated that both pH 11 and 13 gave maximum THC signals in our aqueous studies and that at pH 13 the THC signal shifts by 30 mV (see Figure S2), further AF-OF testing was conducted at pH 13, where better signal separation between THC and salivary component oxidation event was observed.

Typical human saliva has kinematic viscosities around 1.40 $mm^2 s^{-1}$ (water = 1 $mm^2 s^{-1}$ at RT) [24], which can decrease the adsorption rate of electroactive species on the surface of the carbon working electrode. The AF-OF used in this study has a kinematic viscosity of 1.20 $mm^2 s^{-1}$. Varying ratios of AF-OF diluted in buffered solution (ranging from 50:50 to 95:5 AF-OF:aqueous solution) were tested to evaluate the magnitude of the matrix effect on our THC detection (see Figure S3). These results indicate that the matrix effect associated with working in a more viscous medium does decrease THC adsorption (and correspondingly, increases THC detection limit), though even at high ratios of AF-OF to buffered solution, a well resolved signal for 6 μM THC can be obtained.

Nevertheless, in order to measure THC concentrations aslow as 1 μM, the sample was partially diluted using AF-OF in a 50:50 AF-OF:aqueous media ratio. While this reduces the detection limits by a factor of two, this serves as a simple step to ensure that matrix effects are minimized. Given that the sample size is a 1 mL solution, for use in the field, the proposed method would require 0.5 mL of saliva to be collected and diluted with a 0.5 mL buffer/electrolyte solution at pH 13 before testing. A calibration curve under these conditions was generated at THC concentrations ranging from 0.5–10 μM in the diluted 1:1 solution (corresponding to an effective detection range of 1–20 μM in undiluted saliva) containing 0.1 M phosphate buffer at pH 13, and 0.1 M NaCl (Figure 3), giving a linear fit of $y = 1.822^{*10^{-8}} \times + 1.878^{*10^{-8}}$. The theoretical limit of THC detection was calculated by taking 3*(σ/S). The detection limit was determined to be 0.13 μM. Consequently, the limit of quantification (10 * σ/S) was calculated to be 0.43 μM. These results demonstrate THC detection in the range of THC concentrations typically found in saliva (0.5–10 μM), with an R^2 of 0.995.

In summary, a potentially simple and effective electrochemical DPV method is shown to detect low concentrations of THC (1–10 μM) in a sodium phosphate buffer and artificial oral fluid (0.5–10 μM) within 10 min at an elevated pH. This method allows for the third-lowest experimental THC detection limit reported to date compared to similar SPCE devices [20, 22–23], with the additional advantage that it does not require any pretreatment of the AF-OF sample and utilizes SPCE strips that are stable under ambient storage conditions. However, while the proposed electrochemical detection method is quantitative and reproducible in AF-OF and aqueous samples, real saliva samples may contain additional components that interfere with the oxidation signal for THC. To this end, additional studies are needed to identify what may cause interference with this simple approach to devising a reliable field test. For initial screening purposes, qualitative increases in the THC signal, irrespective of background interference, can still serve as a quick and simple screening method to identify whether the sample must be sent to a lab for more selective and sensitive testing. While the data presented was collected under lab conditions, it can be applied in the field with relative simplicity by dipping a SPCE strip in a vial of collected oral fluid (diluted in a small amount

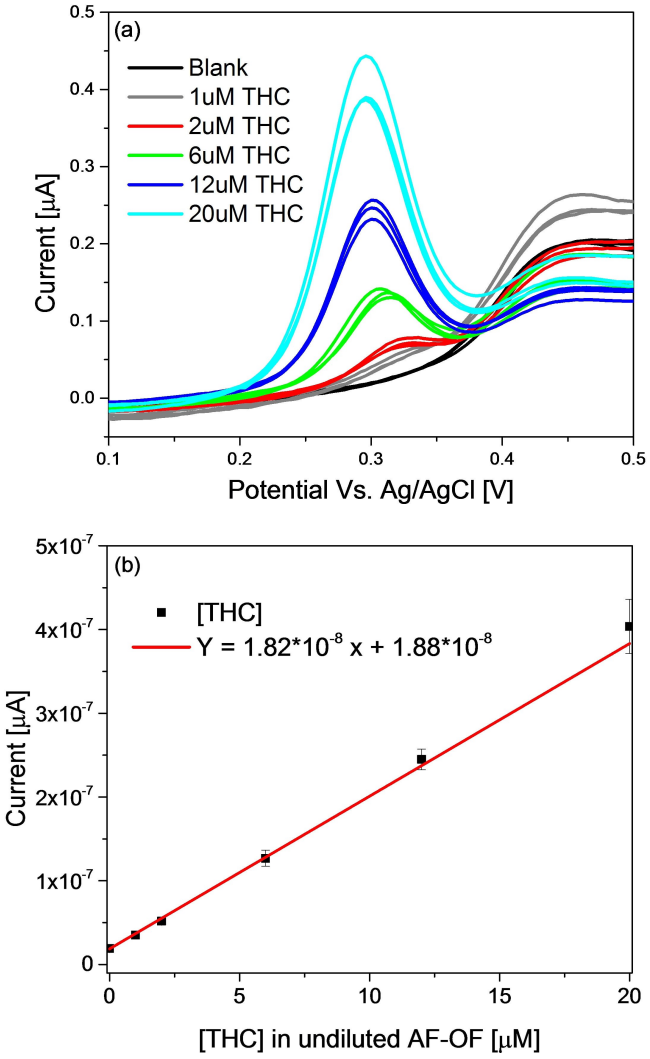

FIGURE 3 THC signals from triplicate experiments at 1, 2, 6, 12, and 20 μM THC concentrations in a 1:1 mixture of buffer and AF-OF solution (a) and linear fit of the THC data (b).

of buffer) and testing with a portable potentiostat. Alternatively, a pre-dried buffer coating could be adhered on top of the working electrode surface to dissolve upon contact with a saliva sample [25]. The proposed method has attractive features such as low cost, small sample size, and ease of use.

EXPERIMENTAL

All chemicals used were of analytical grade and used as received without any further purification. These included sodium phosphate tribasic dodecahydrate (> 98 %, Sigma-Aldrich), sodium phosphate dibasic (> 99 %, Sigma-Aldrich), HEPES buffer (> 99.5 % Sigma-Aldrich), sodium bicarbonate (> 99.5 %, Sigma-Aldrich), sodium carbonate anhydrous (> 99.5 % JT Baker), sodium chloride (> 99.5 %, Sigma-Aldrich), sodium hydroxide (> 97 %, Sigma-Aldrich), Δ^9-tetrahydrocannabinol (CRM, Cayman Chemical, stored at −20 °C), artificial saliva with mucin (pH 7, Pickering Laboratories, stored at 4 °C), and artificial saliva for pharmaceutical research (pH 7, Pickering Laboratories, stored at 4 °C). Screen-printed electrodes with conductive carbon-paste (DuPont BQ922) working and counter electrodes (4 mm^2 and 2 mm^2, area respectively) were obtained from I-SENS (Seoul, Korea). An Ag/AgCl (3 M KCl) reference electrode was purchased from CH Instruments (Austin, Texas).

General experimental conditions: All experiments were performed at room temperature under ambient conditions. Solutions were prepared with Millipore water from a Milli-Q Academic with a Millipak Express 20 filter, 220 nanometer filter, and Quantum Ex polishing cartridge.

General cell setup: Experiments were carried out on 1.0 mL solutions within a small glass cylinder (1.4 cm diameter by 1.0 cm height) containing 0.1 M NaCl, 0.5–10 μM Δ^9-THC, at pHs 7–13. An I-SENS strip was dipped into the cell and connected to the WE and CE potentiostat leads. An external Ag/AgCl electrode was also dipped into the cell to serve as a junction reference electrode.

Electrochemical measurements: Differential pulse voltametric measurements were performed using a CH model 760E electrochemistry workstation potentiostat. Prior to taking a measurement, the test-solution was stirred for 10 min to preconcentrate THC on the working electrode. Stirring was then shut off and the measurement was performed. The THC current at 0.3 V was used to generate linear fit data. The average current drawn and standard deviation from the triplicate set of experiments was plotted with a linear fit. The differential-pulse parameters used were as follows: potential step = 0.004 V, amplitude = 0.050 V, pulse width = 0.05 s, sampling width = 0.0167 s, pulse period = 2 s, quiet time = 2 s.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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