Development of an electrochemical biosensor for Δ9-tetrahydrocannabinol detection based on gold surfaces functionalized with self-assembled monolayers

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Abstract:

In this work, we have developed a new alternative for Δ9-THC detection: an electrochemical immunosensor. To the very best of our knowledge, this is the first study concerning cannabinoid detection with a biosensor. Here, gold electrodes were first functionalized with a self-assembled monolayer (SAM) of mercaptoundecanoid acid (MUDA), a long-chain carboxylic acid-terminating alkanethiol. The acid groups were then activated with the 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) before further grafting of anti-cannabinoid monoclonal antibodies. Electrochemical results showed a wide dynamic range varying between 10^{-15} and 1 mg/ml. The limit of detection of the designed sensor, of order of 10^{-15} mg/ml, is largely inferior to that obtained with conventional techniques.

1. Introduction

Cannabis is one of the most widely-consumed drugs in the world. According to the United Nations' Word drug report, the number of cannabis consumers worldwide was of order of 238 million in 2017 and this number is in constant increase. Certainly, cannabis can be administrated for therapeutic use, and in several medicines; in this case, the administered doses take into account both medical and psychic states of the patients. When consumed as a drug, and depending upon the dose, method of administration and prior experience, cannabis can cause an altered state of consciousness, heart rate increase, and impaired coordination / concentration. For an equivalent dose of 15 mg of Δ9-tetrahydrocannabinol, the active ingredient of cannabis, the consumer can get attacks of anxiety, hallucinations, delusions and impression of knowing nothing[1]. France has recorded 7 % of fatal accidents involving cannabis' user's drivers. The Δ9-THC, as an example of cannabis, can be detected with its metabolites in the blood, sweat and saliva by chromatographic methods with a limit of detection in the order of 0.4 ng/ml. But, these methods are very expensive and need a high

technicality. This study concerns the design and realization of a sensitive biosensor for quick and efficient specific detection of cannabis

2. Results and Discussion

2.1. Sensor construction and electrochemical characterization

The gold surfaces were first cleaned with a piranha solution (H_2SO_4/H_2O_2; 97%/33%) during 7 min to obtain a clean and activated surface. The electrodes were then immersed in a MUDA solution (20 mM) during 18 h at 4°C. An acid self-assembled monolayer is thus formed through the affinity between sulfur and gold[2]. The acidic group were after that activated by EDC/NHS solution (75 mM/15 mM) during 1 h to form a more reactive NHS-ester group[4]. 20 µl of 1 mg/ml anti-cannabinoïde monoclonal antibody were then deposited on the functionalized surfaces. After 15 h, the electrodes were rinsed with PBS (0.16 M) to eliminate the excess of the antibody. To block the non-reactive sites, the electrodes were incubated during 1 h in a BSA solution (10%). A schematic representation of these steps is presented in Figure 1.

The different steps of surface modification, were characterized by electrochemical impedance spectrometry (in the presence of Fe $(CN)_6^{3-/4-}$(5 mM)and PBS) and by angle of contact measurements.

Electrochemical results gathered in Figure 2.A show an increase in the resistance of charge transfer R_{tc} after SAM's formation which may be attributed to the deprotonation of acidic groups, which partially block the electron transfer towards the surface. Contact angle values gathered in Table. 1 confirm this result as the angles decreased from 72.3° to 54.2°. In fact, after deprotonation, the surface became extremely polar which causes the spreading of the drop on the plate.

Activation with EDC/NHS leads to the decrease of the charge transfer resistance. This is probably due to the fact that after activation, the terminal carboxylic groups of MUDA were converted to neutral NHS-esters, which facilitates the charge transfer. Replacing the group NHS-ester with antibodies leads to a decrease in the R_{tc} and contact angle values due to "favorable" antibodies orientation. The small increase of R_{tc} after incubation in BSA may be attributed to the blocking character of this protein. The further antibodies-antigen recognition leads to R_{tc} increase.

2.2. Δ9-THC detection

Δ9-THC antigen concentrations were varied from 10^{-15} to 1 mg/ml. Figure. 2B shows that an increase in Δ9-THC concentration leads to an R_{tc} augmentation. This may be attributed to the steric hindrance related to the complex antibodies- antigen formation.The limit of detection of the designed sensor, of order of 10^{-15} mg/ml, is largely inferior to that obtained with conventional techniques.

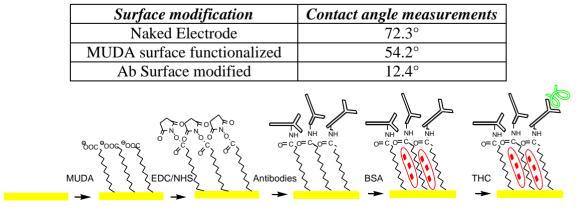**Table1. Contact angle measurement after each modification of the surface**

Surface modification	Contact angle measurements
Naked Electrode	72.3°
MUDA surface functionalized	54.2°
Ab Surface modified	12.4°

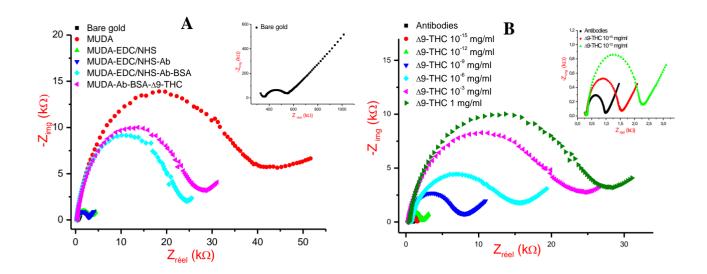**Figure1. Schematic of biosensor construction**

Figure2. Impedance analysisfollow up after A) the different functionalization steps of gold electrode B)
Δ9-THC detection by the designed immunosensor

3. Materials and methods

The Δ9-THC antigens and antibodies were purchased from. 11-Mercaptoundecanoic acid (MUDA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-Hydroxysuccimide (NHS), phosphate buffer solution (PBS) and bovine albumin serum (BSA) were purchased from sigma Aldrich. All chemical measurements were determinate with the voltalab using the calomelelectrode as reference electrode and palatine electrode as auxiliary electrode.

4. Conclusion

A MUDA-SAM electrochemical biosensor was designed and realized for rapid Δ9-THC detection.Electrochemical impedance spectrometry and contact angle measurements were investigated to follow up the different functionalization steps of the gold electrodes. The designed immunosensor permits to detectΔ9-THC antigen in a range of concentration varying from 10^{-15} to 1 mg/ml.This study paves the way for the development of new analytical devices able to detect cannabis traces in few minutes and in humanbody fluids.

5. References

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