An Innovative Liquid Biosensor for the Detection of Lipid Molecules Involved in Diseases of the Nervous System [†]

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† Presented at the Eurosensors 2018 Conference, Graz, Austria, 9–12 September 2018.

Abstract: Growing evidence suggests that endocannabinoid levels are modulated during pathological conditions affecting both central and peripheral nervous system. In the present study, a novel approach (patent pending) based on an innovative liquid biosensor has been used to analyze two relevant endocannabinoid molecules with calibration purposes: *N*-arachidonoylethanolamine and 2-arachidonoylglycerol. The system was able to predict both compound concentrations with a Root Mean Square Error in Cross Validation (RMSECV) of 6.61 nM and 23.50 nM, respectively.

1. Introduction

The endocannabinoid system comprises endogenous lipid mediators (endocannabinoids, eCBs) like *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), along with their specific cannabinoid receptors, and the proteins responsible for eCB biosynthesis, inactivation, transport, and accumulation [1,2]. The understanding of the biological significance of eCBs would not be possible without the development of methods for the accurate, precise, and sensible detection and quantification of these molecules in cells, tissues and biological fluids. The currently available standard methods and instruments for eCB detection is liquid chromatography coupled with mass spectrometry (LC-MS), which is highly sensitive but in turn quite expensive and rather sophisticated [3]. In the present study, the feasibility of the innovative BIONOTE liquid sensor to analyze eCB molecules has been evaluated. The device is composed of a screen-printed electrode (SPE) probe and a dedicated electronic interface devoted to supply a variable input signal and to record the related output data [4]. The signal input consisted of a triangular waveform between -1V and 1V while the recorded output was the generated current response converted in voltage by a trans-impedance circuit. When the SPE is immersed in a solution, oxi-reduction phenomena depending on the specific voltage input

value and involving the analytes dissolved in the aqueous media are induced. The frequency of the input signal was set to 0.01 Hz while data was acquired with a sampling rate of 200 milliseconds, thus collecting 500 output values for each measuring cycle. The oxi-reductive profiles provided by the samples were treated as multidimensional electrochemical fingerprints (Figure 1). The complex data set was analyzed through multivariate data analysis techniques to provide a simplified representation of the multidimensional space and to highlight the most informative features.
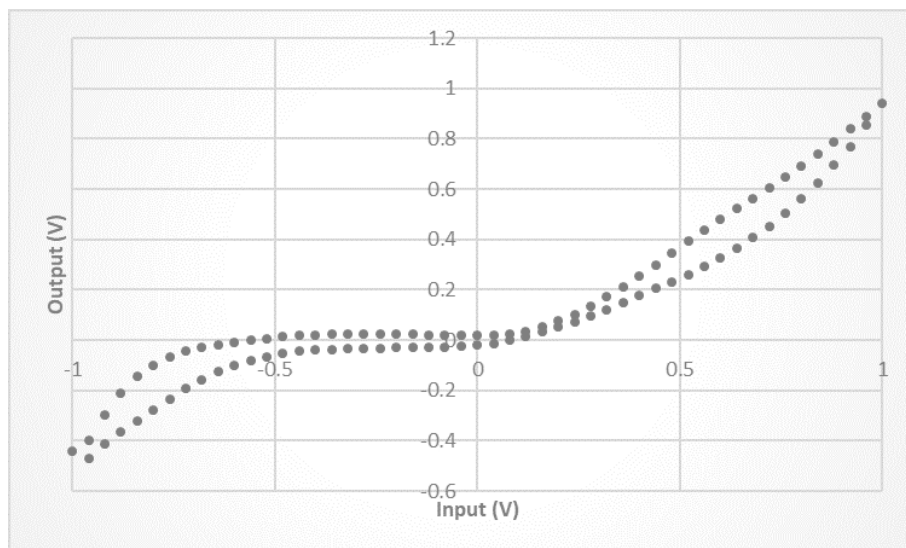

Figure 1. Example of a liquid sample's electrochemical fingerprint.

2. Materials and Methods

2.1. Materials

The calibration of the voltammetric sensor against typical eCBs was performed using arachidonylethanolamide (AEA; A0580, Sigma Aldrich, St. Louis, MO, USA) and 2-arachidonoyl glycerol (2-AG; A8973, Sigma Aldrich) chemical standards. To test the discriminating performance of the analytical system against eCBs related molecules, glycerol (G5516, Sigma Aldrich) and ethanolamine (ETA; 411000, Sigma Aldrich) were chosen. Bovine Serum Albumin (BSA, fatty acid free; A7030, Sigma Aldrich) and Ultra-low melting agarose (A2576, Sigma Aldrich) were exploited for probes functionalization.

2.2. Measure Setup

eCBs stock solutions were prepared dissolving all standards in ethanol at the concentration of 500 μM. Working solutions were fresh prepared diluting eCBs stock solutions in distilled water up to the concentration of interest. Voltammetric measurements were performed soaking the SPE probe for 10 min in the sample solution before triggering the electrochemical stimulation. Five independent analysis cycles were run for each sample.

2.3. Probe Functionalization

BSA solution at the concentration of 200 mg/mL was prepared in distilled water and was mixed with a hot 4% agarose solution with a volume ratio of 1:1. A volume of 10 μL (1 mg) of the prepared mixture was dispensed by drop casting onto the surface of SPE working electrode and it was let air drying until complete dehydration.

2.4. Data Analysis

Partial Least Square Discriminant Analysis (PLS-DA) coupled with the Leave-One-Out criterion as cross-validation method has been employed to obtain all the predictive models onto the calibration data. All the predicitve models were calculated using PLS-Toolbox (Eigenvector Research Inc., Manson, WA, USA) in the Matlab Environment (The MathWorks, Natick, MA, USA).

3. Results

As a preliminary approach to eCB quantification, standards of the two most representative members of this lipid family have been analyzed by the BIONOTE liquid sensor under controlled conditions. Aqueous solutions of AEA and 2-AG standards (Figure 2) at the concentrations of 100, 50, 25, 10, 5, 1, 0.50, 0.25 and 0.01 μM were measured and PLS-DA models on the obtained data set were calculated.
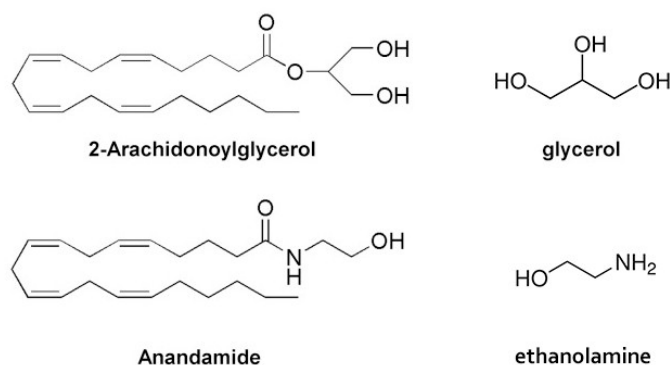

Figure 2. Chemical structures of the analyzed molecules.

Despite the device was able to detect qualitatively both molecules, the micromolar error associated with the calibration curves was too large to allow detection of these eCBs at physiological level. To improve system detecting performances, sensor functionalization has been exploited (Figure 3).
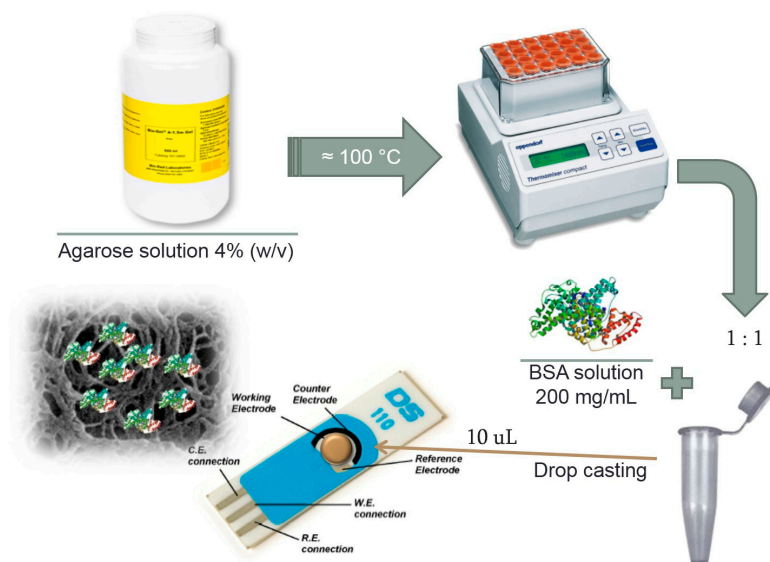

Figure 3. Graphical representation of the SPE functionalization.

Due to its ability to bind eCBs [5], BSA was selected as a candidate to attempt probe functionalization. A thin film of agarose gel containing 1 mg of BSA was deposited by drop casting technique onto the working electrode of the probes and eCB calibrations were repeated. By means of this experimental setup, the system was able to predict both AEA and 2-AG

concentrations with a Root Mean Square Error in Cross Validation (RMSECV) of 6.61 nM and 23.50 nM, respectively. Afterward, to evaluate BIONOTE discriminating efficiency, the system was challenged against two compounds sharing part of eCBs' chemical structure: ethanolamine and glycerol (Figure 2). These substances were measured at the same concentrations as AEA and 2-AG, replicating the experimental setup. The PLS-DA model calculated on the collected data highlighted a decrease of the system performance in the detection and quantification of ethanolamine and glycerol with an obtained RMSECV of about 5 μM and 20 μM, respectively. Finally, a comprehensive array containing the overall sensors' responses was built and the collected data were analyzed through multivariate data analysis techniques. The calculated PLS-DA models highlighted the ability of the system to distinguish between the 4 compounds with an efficiency of almost 100% in the classification.

4. Discussion

In the present work, we reported for the first time the development of an innovative lipid biosensor able to assess eCB content. Results has shown that employing BIONOTE device to efficiently detect eCB compounds was not effective until electrode functionalization was not achieved. The incubation of the modified SPEs within liquid samples has been demonstrated to improve sensibly the performance of the whole analytic system (this application is patent pending). Therefore, BSA layer deposited over the working electrode of the probes is able to pre-concentrate the target molecules during the re-hydration by specifically binding eCBs. The polarization of the sensor toward lipid compounds was confirmed by the higher error associated with the calibration against glycerol and ethanolamine in respect to the eCBs' one. Although the system needs to be further validated by comparing its outcomes with those obtained by LC-MS analysis, the exploitation of sensors for high-throughput screening of bioactive lipids has a diagnostic potential for medicine in the future.

Author Contributions: M.M. and S.G. conceived and designed the experiments; S.G. and T.B. performed the experiments; G.P., M.S. and A.S. analyzed the data; A.Z. and T.B. contributed reagents/materials/ analysis tools; G.P., M.S. and M.M. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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