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Practical Aspects of Biogenic Amines Detection †

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Abstract: This paper presents a method for monitoring food freshness and quality through the early detection of biogenic amines, which are indicators of food spoilage/degradation. The urge to monitor food quality has led to a growing interest in the detection of toxic compounds, such as biogenic amines (BAs), as chemical indicators of food degradation by using different bioanalytical tools. The bioanalytical platform includes several parts and modules useful for food hazard evaluation. This paper presents how electrochemical and spectrometric detection works for biogenic amine monitoring in food and how the communication interface provides useful data.

Keywords: electrochemistry; 2D printing; sensors; functionalization

1. Introduction

The most important goal for BA monitoring in food is the development of cost-effective and robust miniaturized opto-electrosensitive platforms. The designed sensitive platforms are based on modifications of different commercial sensor templates with nanocomposite materials and specific bioreceptors, showing enhanced electrochemical, optical, and mechanical properties. Through functionalization of carbon nanomaterials with metallic nanoparticles or polymeric dots, new opto-electroactive platforms with improved catalytic activity, high charge transfer capacity, and enhanced optical properties for sensor applications are obtained.

Electrochemical detection is realized using specialized microcontrollers and embedded firmware capable of implementing specific methods, while spectrometric detection uses an optical fiber and an UV–VIS image CMOS sensor capable of a very good resolution. The common element is an electrochemical sensor capable of providing enhanced selectivity to biogenic amines and a very good sensitivity to determine low concentrations.

2. Measuring Method

The innovation of the new sensors results from the combination of tailored functionalized carbon nanomaterials with a highly sensitive opto-electrochemical detection method, electrochemiluminescence (self-ECL) [1]. The originality and complexity of this solution consist in the design of new versatile biosensors based on the synergistic combination of carbon nanomaterials and luminescent-based nanomaterials with specific bioreceptors. In this way, the proposed system represents the first integrated opto-electrosensitive platform for miniaturized bioanalytical tools to be used for sensitive and selective detection of BAs in food. Nanomaterial-based (bio)sensors provide reagent-free, low-limit detection, one-step analysis, and sometimes label-free assays with high sensitivity and a small sample volume required. New carbon-based materials, such as fullerene, graphene oxide, and single- and multi-walled carbon nanotubes, have attracted great interest due to their solubility in water (and polar solvents) and stability. Therefore, carbon nanomaterials are considered good candidates for the development of sensing materials based on enzymes or aptamers [2–4]. The

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bioanalytical platform includes a biosensor, an electrochemical detector, an optical detector, and a control unit. Figure 1 presents a schematic configuration of the detection system.

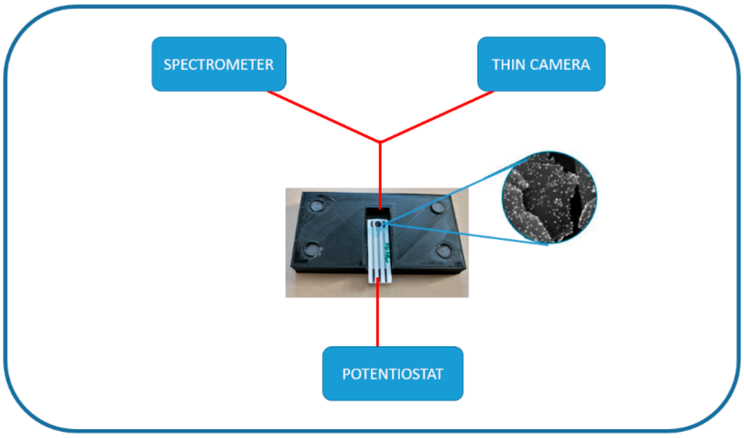

Figure 1. Modules of the detection platform.

The carbon paste screen-printed electrodes were modified with single-walled carbon nanotubes (SWCNTs), Prussian blue (PB) redox mediator and gold(AuNPs), respectively, and silver nanoparticles (AgNPs) for sensitive electrochemiluminescence detection of hydrogen peroxide using luminol as a luminescent species. The spectrometer uses optical fiber and is capable of recording several spectra over time or at certain polarizations. A thin camera allows for the analysis of the optical response just for a viewport and represents a low-cost alternative to a fiber optic spectrometer.

3. Sensor Functionalization

The platform will be developed in several stages according to the characteristics of each component. SPE (screen-printed electrode) sensor preparation starts with the functionalization of SWCNTs with redox mediators and metallic nanoparticles by directly precipitating the mediator precursors ferry/ferrocyanide and directly depositing the gold/silver nanoparticles or polymer dots onto nanotubes. The next stage of sensor preparation continues with the immobilization of specific bioreceptors for histamine and histidine onto the SWCNT-PB-AuNP-based sensors, using the entrapment in the sol–gel or polymeric matrix [5]. The optimization of the bioreceptor concentrations for further immobilization was performed through spectrophotometric measurements. The developed biosensors are characterized through electrochemical techniques (voltammetry and EIS) using miniaturized portable detectors. The development of electrochemiluminescent (ECL)-based biosensors for specific BAs detection is based on the construction of a self-ECL system based on metallic nanoparticles and luminol as luminophore species. Figure 2 explains generically how the detection chain works for this type of experiment with food samples.

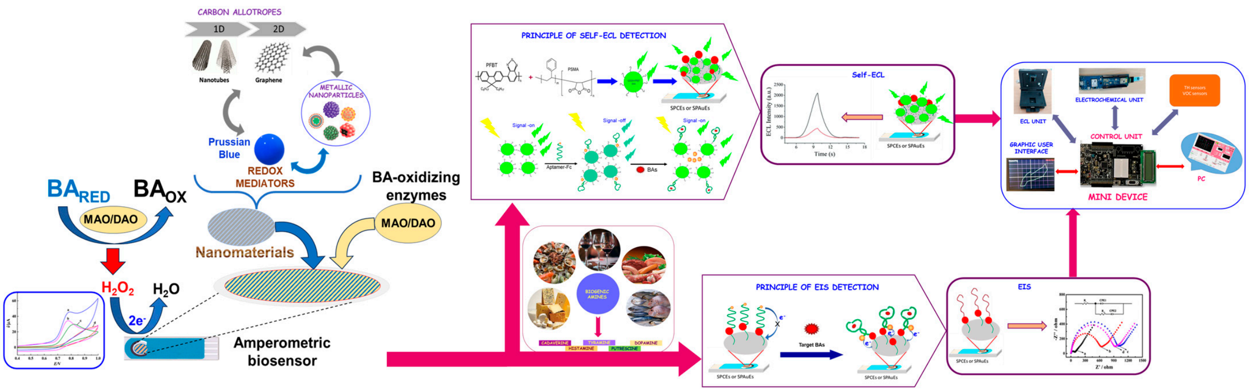

Figure 2. Proposed detection chain for biogenic amines- (BA) determination in food.

Functionalization of the sensor surfaces with nanocomposite materials based on metallic nanoparticles or polymeric dots is expected to enhance the ECL signal. The carboxyl-functionalized polyfluorene dots are used for modification of the SWCNT-PB-based sensors in order to obtain the self-ECL luminophore [6]. These polymeric dots exhibit a remarkable brightness and a high emission rate, serving at the same time as a matrix for the immobilization of the bioreceptors. The platform also includes specific detection cell prototypes made via 3D design adapted to the developed biosensors and then 3D-printed using various materials and shapes, which will be optimized in order to obtain the best results for BAs detection. Figure 3 shows a comparison between a commercial SPE with an alumina substrate and a 2D-printed electrode made on paper.

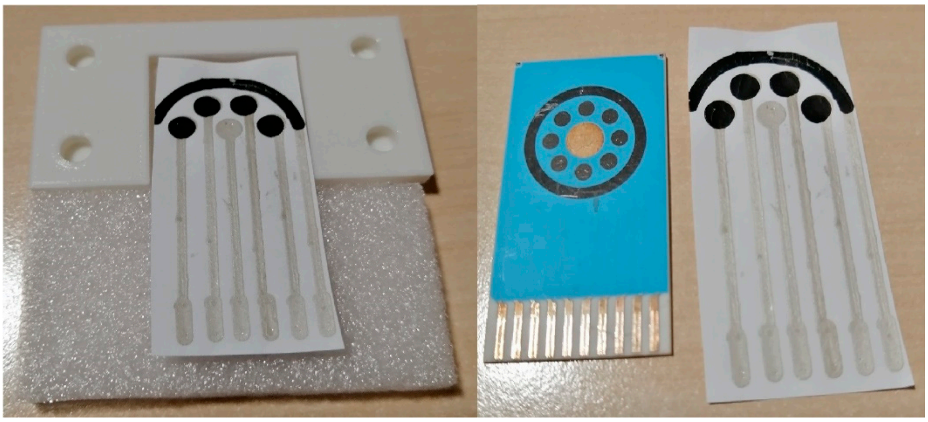

Figure 3. Customized functionalization of multi-SPE using 2D printing.

The advantage of 2D printing is that the SPE can be functionalized with a specific sensitive ink, and the shape can be changed. The customized cell also has an upper cover that can accommodate a minicamera or an optical fiber. The minicamera and the specific software can extract not only the light intensity for a designated area but also the spectral response. The optical fiber goes to a spectrometer and measures the entire spectrum. Using sensors based on functionalized materials and integrated with a portable detection system using PSOC technologies, the system includes reconfigurable hardware and parametric software.

The schematic representation of the miniaturized opto-electrosensitive based biosensors for BAs determination in food is given in Figure 2, while the base architecture of the electrochemical detection system is given in Figure 4.

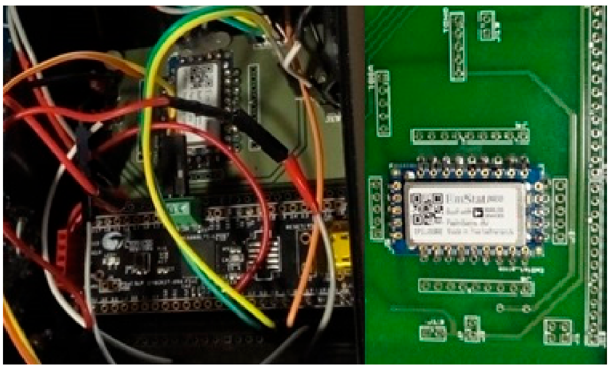

Figure 4. Electrochemical module prototype using PSOC 5 and ADuCM355.

4. Hardware Configuration

The proposed platform includes a microcontroller specialized for electrochemical measurements and impedance characterization, as well as another microcontroller capable of handling LORA communication [7]. This type of communication module operates at 868

MHz and is capable of sending data up to 700 m between nodes and mesh. Electrochemical detection provides voltametric and amperometric detection based on screen-printed electrodes (SPE). The electrochemical detection module is supported by an ARM Cortex-M3 microcontroller ADuCM355 from Analog Devices [8] and upgraded with firmware developed by Palmsens [9]. Method parametrization of the electrochemical module is made by a PSOC 5 microcontroller configured like in Figure 5, as well as an ARM Cortex-M3 architecture [10], using an UART interface to communicate between them.

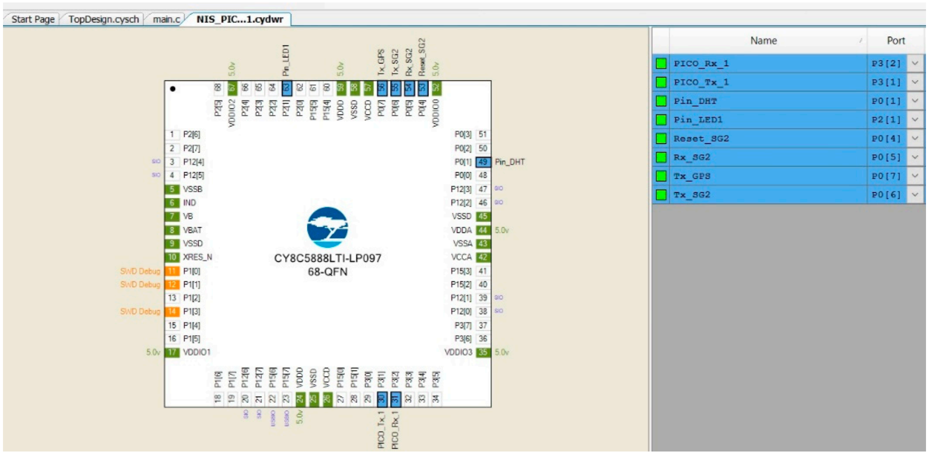

Figure 5. Details regarding the PSOC5 configuration.

Functionalization of SPE is partially achieved using a 2D printing technique in order to ensure superior repeatability. Performing 2D printing is possible using Voltera V-ONE equipment, which is capable of using different types of inks based on carbon, silver, or platinum [11].

Figure 6 presents a setup using a carbon SPE made on alumina. The cover of the cell can be connected to a flow system or can be used like a batch system. All these variants can also be 3D-printed, and the join is made with magnetic cylinders.

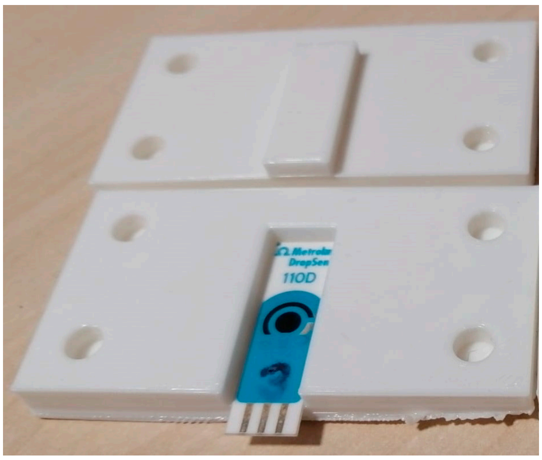

Figure 6. Setup for electrochemical measurements.

5. Software Developments

The most important part of the software is developed in C to implement basic measurement functions and communication routines. Firmware development is based on the PSOC Creator development package from Cypress–Infineon and embeds advanced functions for the PSOC 5 microcontroller. Communications routines are able to transfer data upon request over LORA IQRF modules [12]. To be able to manage the interface between PSOC 5 and Microrisc controllers, a custom SPI interface was implemented. The master GUI placed on a PC sends requests over 868 MHz to the PSOC 5, and the response provides important information regarding the measured data.

The PSOC 5 functions perform electrochemical or optical measurements and store data in the local RAM memory. All data can be gathered via 868 MHz to the PC and then graphically represented via LabWindows/CVI technology [13] on a bigger LCD display. For monitoring purposes, it is also possible to use serial communication via the USB plug of the computer.

In order to obtain relevant measurements, the goal is to obtain samples of food, condition them, and then apply a certain volume to the surface of the electrode or electrochemical sensor. An innovative cell for combined measurements was used, and in Figure 7, there are some details of the configuration. In Figure 8, the spectrometric response obtained with a fiber optic spectrometer has good sensitivity. Both signals must be correlated, and the response will indicate the presence of BA.

Figure 7. Innovative cell combined measurements.

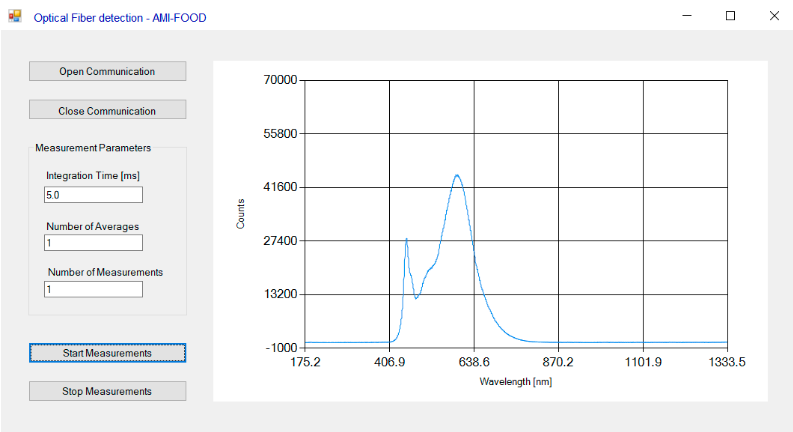

Figure 8. Spectrometric detection on the electrode surface using optical fiber.

For specific purposes, the custom-designed setup accommodates SPE electrodes on a PVC substrate, as shown in Figure 9, and on the top cover is a micro-camera capable of identifying the spectra.

Figure 9. Combined electrochemical and optical setup initial prototype.

The optical response can be performed from a specific area of the camera view. In this situation, the analysis of the optical response can be focused on specific areas of the cell, mainly on the working electrode. For electrochemical detection, the setup can also accommodate SPE on a PVC substrate, which has improved sensitivity and provides a better detection limit. The carbon material for the working electrode is functionalized with bioreceptors and other nanomaterials. In Figure 10, SPE electrodes on PVC are connected to a potentiostat and accommodated in a 3D-printed setup.

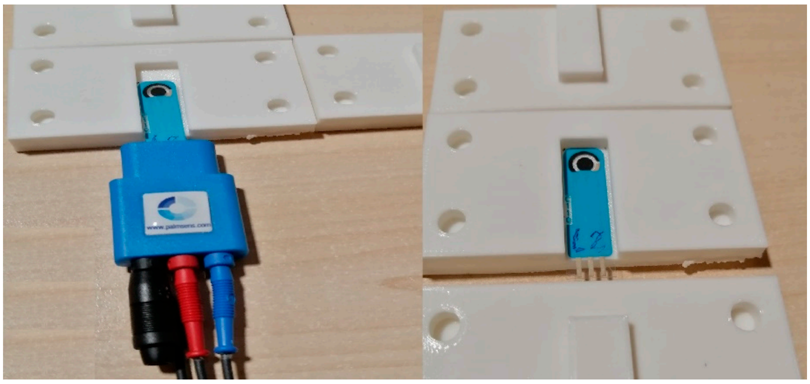

Figure 10. Example of an electrochemical setup.

Using optimized parameters and performing an amperometric detection at a polarization of about 0.2–0.3 V, measurements of the response current in time can be useful to determine the biogenic amines in food or in body fluids.

The determination of the polarization potential can be made using cyclic voltammetry between −0.8 volts and +0.8 volts and is undertaken for each type of electrode, as shown in Figure 11. The maximum oxidation current is about 160 microamperes. Depending on the type of measurement and the sensitivity of the SPE electrode, a calibration curve will be performed and implemented in the PC software. In this situation, the graphical interface is also capable of displaying a concentration indication. Taking into account the various numbers of possible measurement types, there will be an option to select the proper calibration curve.

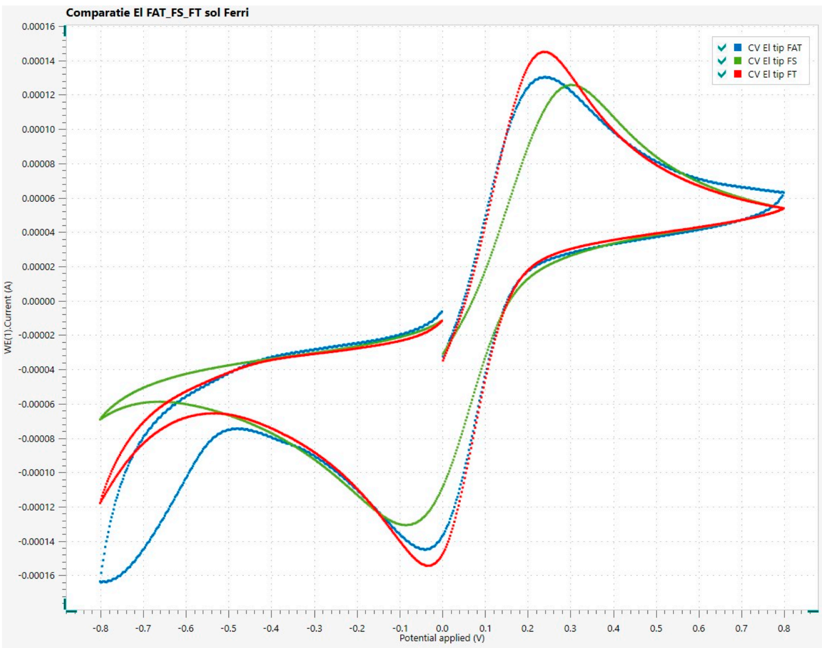

Figure 11. Measurements made on different types of functionalization.

6. Results

The great advantage of the designed biosensors is that the biorecognition process is monitored by two different detection techniques: electrochemical—following the modification of the electron transfer resistance at the surface of the biosensors (Nyquist spectra)—and an optical method. The assessment of BAs in food is performed with enhanced sensitivity and specificity, lowering the detection limits in the nanomolar range of concentration. Thus, the early and fast evaluation of food spoilage/contamination based on two complementary analytical signals is more precise and reliable. The anticipated outcomes brought by the proposed platform are as follows: (a) an understanding of how detection systems work; (b) a prototype of the opto-electrosensitive platform for highly sensitive and specific detection of BAs in food; (c) a method to obtain functionalized nanocomposite materials with advanced optical and electrochemical properties; and (d) the development of selective and sensitive biomolecular sensors for food applications. The proposed platform contributes to the improvement of life quality and to significant research and educational progress in the field of functional materials for sensing applications, responding to several scientific and socio-economic issues of great interest. The educational impact on food safety and health quality consists of teaching how we can reduce and eliminate contaminated food from the market and to use microcontroller platforms and communication techniques.

7. Conclusions

The main purpose of this paper is to present a custom combined platform using electrochemical-based biosensors for sensitive and selective detection of biogenic amines in food. Efforts were made to synthesize and characterize novel nanomaterials for biosensors and also to synthesize, characterize, and optimize luminophore nanodot materials and metallic nanoparticles using hydro/solvothermal techniques. In short, the development of BA-based biosensors involves the modification of the sensor surface with carbon nanomaterials functionalized with redox mediators and luminophores, metallic nanoparticles, or synthesized polymer nanodots. Several measurements were made using sensors and an electrochemical platform based on some 3D-printed accessories to accommodate all the modules and integrate the hardware and software components.

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