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Abstract

A Phenylalanine Ammonia Lyase Capacitive Sensor for Phenylalanine Detection †

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Abstract: In this paper, an easy-to-use and fast biosensor for phenylalanine quantification in patients affected by phenylketonuria is investigated. The phenylalanine concentration was indirectly estimated through the ammonia released as a by-product of an enzymatic reaction, which was then detected by exploiting an yttria-stabilized zirconia layer deposited over an interdigitated capacitive sensor. The latter was manufactured by rapid-prototyping technologies. A sensor limit of detection higher than 1.25 μM was estimated, along with an accuracy better than 18.31 μM.

Keywords: phenylalanine; ammonia; biosensor; interdigitated capacitor; rapid prototyping

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1. Introduction

Phenylketonuria (PKU) is a genetic disorder of phenylalanine (Phe) metabolism that may potentially lead to severe neurological damages. To date, a specific diet and monitoring the Phe levels in the blood is the unique therapy available [1]. Commonly, techniques for Phe quantification are complex, expensive and time-consuming [2]. This research work addresses the development of a low-cost and fast-responsive capacitive sensor for Phe quantification, which offers an accuracy compliant with early warning tasks. The sensing methodology exploits the indirect estimation of Phe by detecting aqueous ammonia (NH_3(aq)) as a by-product of a specific enzymatic reaction with Phenylalanine Ammonia Lyase (PAL). Most NH_3 sensors are aimed at gas detection [3], while few approaches address the quantification of NH_3(aq) [4], none of which provides solutions for measurements in small volumes. The modeling and design flow of the sensor are discussed in [5], while the ability of YSZ to detect NH_3(aq) is demonstrated in [6]. In this paper, the behavior of the complete sensor is investigated, including the Phe-PAL enzymatic reaction. The main outcomes of this approach include: (i) a capacitive readout strategy, which is convenient for low analyte concentrations; (ii) high specificity to the target analyte, thanks to the enzymatic reaction; (iii) faster response times compared to those of traditional Phe quantification methods; (iv) low costs, also provided by the adopted rapid-prototyping technologies.

2. The Developed Device

The structure and real view of the capacitive sensor are shown in Figure 1a,b. The IDC electrodes were realized by aerosol jet printing a gold-based conductive ink over a 125 µm thick Polyether Ether Ketone (PEEK) substrate. The sensor active area was 13.5 × 9.8 mm^2, and the IDC electrodes were 500 µm wide, with a spacing of 300 µm. A 50 µm thick yttria-stabilized zirconia (YSZ) dielectric functional layer, sensitive to NH_3(aq), was spray-coated over the IDC electrodes. The YSZ layer was subjected at room temperature, for 45 min, to an O_3/UV_{254nm} process performed by BioForce equipment (Nanosciences) to increase its surface hydrophilicity (126.6° ± 3°). The proposed enzymatic method exploits the deamination of Phe catalyzed by the PAL enzyme to produce trans-cinnamic acid and NH_3. The yield of the latter is proportional to the Phe concentration [7]. PAL showed optimal sensitivity at temperatures ranging from 32 to 40 °C and pH values ranging from 7 to 8. A rigid supporting frame for the IDC sensor was milled in 1.55 mm thick FR4. The frame also provided the reaction chamber hosting the MUT.

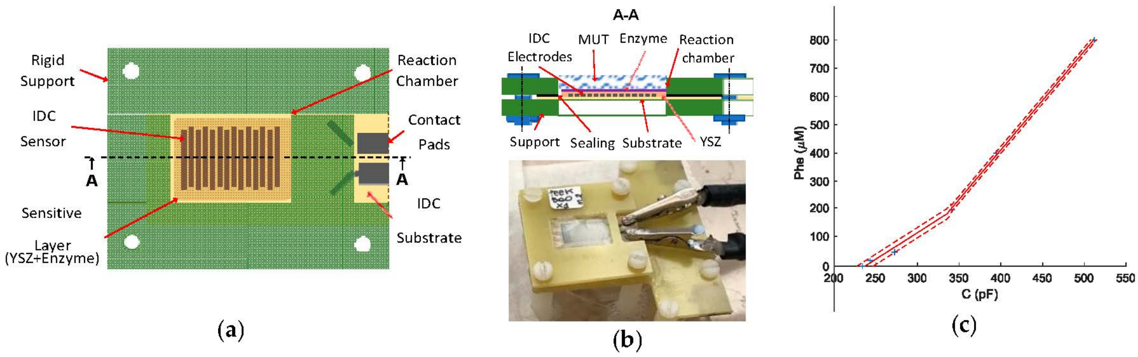

Figure 1. The sensor: (**a**) top view (layout), (**b**) cross section and real view, (**c**) calibration diagram.

3. Experimental Results

The sensor behavior was investigated by observing its response to standard test solutions with Phe concentrations in the range of [0, 800] µM. The test solutions were prepared by mixing Phe with 10 µL of PAL (containing 0.156 units in 200 µL of 10 mM sodium phosphate buffer at pH 8.31) and 1 mL of 2 mM sodium phosphate buffer at pH 7.5. A volume of 500 µL of these solutions was dropped on the YSZ sensing layer, and the temperature was set to 37 °C (±2 °C) for 30 min to realize the enzymatic reaction. In each trial, the sensing chamber was completely filled. Time evolution of the sensor capacitance was acquired by a GW Instek LCR-6300 precision LCR meter. The obtained calibration diagram is shown in Figure 1c. A piecewise linear model was used to interpolate the data. The sensor responsivity was 5.31×10^{-1} pF/µM for Phe concentrations in the [0–200] µM range and 2.86×10^{-1} pF/µM for Phe concentrations in the [200–800] µM range. The accuracy in the 3σ limit, for the two considered intervals, resulted to be 18.31 µM and 12.34 µM, while the estimated limit of detection was 0.98 µM and 1.25 µM, respectively.

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