

A Capacitive Sensor, Exploiting a YSZ Functional Layer, for Ammonia Detection

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Abstract—In different fields, the need for low-cost sensors allowing an early detection of (bio)quantities is emerging. This article deals with the design, realization, and characterization of a low-cost sensor for ammonia detection realized by fast prototyping techniques. The sensor is part of a more complex system aimed to assess the level of phenylalanine levels in biological fluids (blood, saliva, or urine), which would allow the development of a new approach for the self-monitoring of patients with the phenylketonuria (PKU) metabolic disorder. The obtained experimental results encourage the further development of the proposed approach. In particular, the sensor behavior has been investigated for an ammonia concentration in the range [0–2000] μM . The developed sensor prototype shows a responsivity of 3.53×10^{-14} F/ μM , a resolution of 0.15 μM , and a span-to-resolution of 1.3×10^4 .

Index Terms—Ammonia, characterization, interdigitated transducer (IDT) sensor, rapid prototyping, yttrium stabilized zirconium (YSZ) oxide.

I. INTRODUCTION

THIS study has been developed under the “PKU Smart Sensor” project, which aims at developing a point-of-care (PoC) platform, based on a disposable biosensor for the detection of phenylalanine (PHE) levels in biological fluids (blood, saliva, or urine), supported by an information communication technology (ICT) infrastructure facilitating communication with competent reference hub for remodeling and real-time data acquisition through mobile apps. The platform will allow the self-monitoring of PHE levels for phenylketonuria (PKU)’s

Manuscript received November 18, 2021; revised February 28, 2022; accepted March 25, 2022. Date of publication April 22, 2022; date of current version May 2, 2022. This work was supported by PKU Smart Sensor Project, under Grant Action 1.1.5 POR FESR 2014-2020 and Grant CUP G89J18000710007. The Associate Editor coordinating the review process was Tarikul Islam. (Corresponding author: Bruno Andò.)

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Digital Object Identifier 10.1109/TIM.2022.3167766

patients, without the assistance of clinicians or laboratory personnel.

PKU is an inherited autosomal recessive disorder of PHE metabolism. PHE is one of the essential aromatic amino acids found in protein-containing foods. PKU is mainly caused by a genetic mutation that alters the function of the enzyme PHE hydroxylase. Furthermore, PKU can be caused by deficiency of the enzymatic cofactor tetrahydrobiopterin (BH₄) [1]. The main findings presented by PKU patients are severe neurological damages that result in intellectual disability, microcephaly, motor deficits, autism, seizures, developmental problems, aberrant behavior, and psychiatric symptoms. Patients with PKU should have a diet therapy (which is low in PHE) and when the treatment is started early and well-maintained, individuals diagnosed with PKU can expect normal development and a normal life span [2], [3]. Therefore, early diagnosis and continuous monitoring of PHE levels in blood are crucial to avoid the abovementioned damages.

A. Approaches for the Measurement of NH₃ Associated With PHE: Main Motivations for This Work

The first simple test for the identification of PKU patients was developed in 1960, and PKU becomes the first inborn metabolic disorder to benefit from new-born screening. Since tandem mass spectrometry (MS/MS) was introduced to new-born screening laboratories during the late 1990s, it becomes the gold standard for PKU assay, thanks to the simultaneous, fully automated analysis of different analytes such as amino acids including PHE and acylcarnitine species [4].

Moreover, a huge set of analytical methods for PHE quantification are reported in the literature, including expensive analytical chemical methods like gravimetric methods (inorganic, organic precipitation agents, and electrodeposition) [5], volumetric methods (acid base titrations, deprecipitations, complexometry, and oxido-reduction) [5], or measuring instruments like spectroscopic method: Raman spectroscopy [6], [7], laser-assisted spectroscopy [8], and fluorescence [9].

Even though above methods are efficient, there are many disadvantages for patient’s care. These methods are very laborious and expensive, and the time required for test results is almost 4–5 days. It can delay the needed changes in treatment regimens.

The above statement highlights a unique need in developing biosensors which are accurate, portable, easy-to-use, and able to perform analysis in the sample-in-answer-out format with sensitivity comparable to the standard methods and with a fast response [10].

Examples of enzymatic-based biosensors for the evaluation of PHE levels in biological sample (blood, urine, and serum) are reported in the literature, including the biosensors based on PHE dehydrogenase (PDH) [11] and based on phenylalanine ammonia liase (PAL) [12], [13].

The PDH-based biosensors are mainly based on enzymatic reaction of PDH with PHE in the presence of NAD⁺ and provide a transduction of the cofactor nicotinamide adenine dinucleotide hydride (NADH) produced that can be colorimetric (fluorescence) [14] or electrochemical [15] or sensors adopting voltametric techniques [16], [17].

The PAL-based biosensors provide a fast deamination reaction of PHE to produce a proportional amount of trans-cinnamic acid and ammonia. Then, the level of PHE on biological sample can be monitored by the assessment of ammonia amount or by the increasing of pH value. In such case, the sensing principle would exploit the reaction of PHE with the PAL enzyme which, in turn, produces ammonia [18] or alternatively by the measurements of the pH values, as recently proposed in [19] for the development of a colorimetric paper-based lab-on-chip.

The enzymatic approaches imply a series of advantages such as the straightforward specificity, good sensitivity, fast response, and disposable format to be easily integrated in PoC system [20]. Since enzymatic reactions of PHE with PAL give NH₃ as by-product, which is proportional to the PHE quantity in the sample, the assessment of NH₃ could be quantitatively considered a convenient indirect approach for the PHE assessment.

B. Related Works on NH₃ Sensing Methodologies

Several approaches are proposed in the literature for the development of ammonia gas sensors, among which solid-state sensors are quite convenient for the realization of portable devices.

Recent reviews of ammonia gas sensors-based solid-state methods and carbon nanomaterials are presented in [21] and [22], respectively.

Examples of chemoresistive ammonia gas sensors are investigated in [23] and [24]. In [23], a conductive polyaniline (PANI) layer coated on interdigitated electrodes (IDEs) was tested for application in ammonia gas sensors. The development of a gas sensor in CMOS technology, exploiting a sensing layer of polypyrrole/reduced graphene oxide (PPy/RGO), to detect ammonia up to 10 ppm, is presented in [24].

Despite suitable performances of chemoresistive sensors, these solutions are not always convenient when a low-noise operation is required, such in the case of bio-chemical sensors aimed to detect low analyte concentration. Actually, conductance variations resulting from the charge transfer between the functional layers [e.g., carbon nanotubes (CNTs)] and the material under test (MUT) are affected by large $1/f$ noise

and sometimes incomplete sensor recovery [25]–[27]. Chemo-capacitive sensing, being dominated by dielectric effects and less sensitive to charge effects as in resistive sensors, performs better in terms of sensitivity and reliability especially for biochemical applications. Moreover, for the specific case of NH₃ detection, usually resistive polymeric sensors suffer for an irreversible reaction with NH₃ which increases the mass of the polymeric layer, thus producing a reduction of the sensor responsivity due to the continuative exposure to NH₃ [28].

An ammonia gas (NH₃) detection method using the porous SiC, as a dielectric in a capacitive sensing arrangement, is discussed in [29]. The devices response to NH₃ in the range 0–10-ppm NH₃ in dry N₂ carrier gas as well as the response to relative humidity between 10%RH and 90%RH is studied. The work presented in [25] developed a sandwich-structured gas sensor based on vertically aligned electrodes where the capacitance change of the sensor with target gas exposure was used as the sensing parameter. The sensor had relatively short response and recovery time. It was completely recovered within 6 min, without requiring any external stimuli. The fabrication and deployment of a select metal–organic framework (MOF) thin film as an advanced chemical capacitive sensor are focused in [28]. Purposely, the chemically stable naphthalene-based RE-fcu-MOF (NDC-Y-fcu-MOF) was elected and the fabricated MOF-based sensor showed a notable detection sensitivity for NH₃ at a concentrations down to 1 ppm, with a detection limit appraised to be around 100 ppb (at room temperature) even in the presence of humidity and/or CO₂. Performances of chemi-capacitance NH₃ sensors, exploiting functionalized SWNT arrays in two different configurations are investigated in [30]. The addressed operating range is up to 100 ppm, with a limit of detection lower than 0.5 ppm.

Other approaches exploit field-effect devices and surface acoustic wave structures. As an example, Winquist *et al.* [31] proposed a sensor based on a palladium MOS field-effect capacitor with a thin layer (3 nm) of iridium surrounding the palladium gate. The lower limit of detection for ammonia in air is 1 ppm (0.59 mg·kg⁻¹). Ammonia in aqueous solutions is determined by the use of a continuous flow system utilizing a gas-permeable membrane in combination with the sensor. An innovative, simple, and inexpensive Love-wave gas sensor based on Fe₂O₃@WO_{3-x} nanoneedles to detect the variation of ammonia at room temperature is described in [32]. The nanoneedles were successfully formed on Love-wave device via aerosol-assisted chemical vapor deposition (AACVD). The nanoneedles worked as guiding and functional layers detecting the changes of the elastic properties presented by ammonia interaction. The sensor was tested to ammonia concentrations between 25 and 90 ppm and showed large frequency shifts, high sensibility, short response time, and good reproducibility.

Despite the suitable performances of above illustrated sensors, such solutions are mainly adopted to detect gas targets, unless very complicate architecture is adopted, while the early detection of PHE requires low-cost sensors that can detect ammonia in liquid solutions.

The PKU Smart Sensor Project aims at the development of a low-cost PHE sensor, which relies on the exploitation of the indirect estimation of PHE by measuring the

TABLE I

BENCHMARK BETWEEN THE SENSOR INVESTIGATED THROUGH THIS ARTICLE AND SOLUTIONS AVAILABLE IN THE LITERATURE (1 ppm = 1 mg/L)

<i>Paper</i>	<i>Target</i>	<i>Sensing strategy</i>	<i>Functional Layer</i>	<i>Performances</i>
[23]	NH ₃ Gas Range: up to 100 ppm	Resistive (IDE, InkJet Printed)	PANI	Detection limit: 25 ppm
[24]	NH ₃ Gas Range: up to 10 ppm	Resistive (IDE, CMOS)	Polypyrrole/reduced graphene oxide (PPy/RGO)	Detection limit: 0.1 ppm
[25]	NH ₃ Gas Range: up to 15 ppm	Capacitive (Parallel electrodes)	Vertically aligned CNT	Relative variation of capacitance: 2% for 1 ppm of NH ₃ 10% for 15 ppm of NH ₃
[28]	NH ₃ Gas Range: up to 100 ppm	Capacitive (IDE, Physical Vapor Deposition)	Naphthalene-based RE-fcu-MOF (NDC-Y-fcu-MOF)	Detection limit: around 100 ppb
[29]	NH ₃ Gas Range: up to 10 ppm	Capacitive (IDE, standard deposition)	Porous SiC	Detection limit: 0.5 ppm
[30]	NH ₃ Gas Range: up to 100 ppm	Capacitive (2 Electrodes, standard lift-off)	SWNT	Detection limit: less than 0.5 ppm
[31]	NH ₃ in aqueous solution Range: up to 500 ppm	MOS field-effect capacitor	Thin layer of iridium	Detection limit: 1 ppm
[32]	NH ₃ Gas Range: up to 90 ppm	Surface acoustic wave	Fe ₂ O ₃ @WO ₃ -x nanoneedles	Detection limit: 1 ppm
This paper	NH ₃ in aqueous solution, Range: up to 34 ppm (2000 μM)	Capacitive (IDE, InkJet Printed)	Yttrium Stabilized Zirconium (YSZ)	Responsivity 3.53·10 ⁻¹⁴ F/μM Detection limit: 0.0025 ppm (0.15 μM) Span-to-resolution: 1.3·10 ⁴ .

NH₃ as a by-product of the enzymatic reactions of PHE with PAL. Under this framework, a preliminary theoretical study of a novel low-cost sensor for the detection of NH₃ in a liquid solution has been proposed in [33], mainly addressing the modeling and simulation of the sensing strategy.

As a follow up of [33], this article focuses on the development of the prototype of the low-cost capacitive biosensor for NH₃ measurement and its experimental characterization. The sensor is based on a stack structure made of IDEs inkjet printed on a polyethylene terephthalate (PET) substrate and the functional layer yttrium stabilized zirconium (YSZ) exposed to the MUT. The sensing principle exploits the variations produced by NH₃ concentration (in the MUT) on the dielectric permittivity of the YSZ functional layer.

Although features of the sensor investigated in this work will be substantiated in Section IV, a comparison between this sensor and solutions available in the literature is reported in Table I, in terms of sensing principle, functional layers, and performances. As it can be observed, the proposed solution shows interesting features, especially in terms of the simple fabrication process, the operation with liquid samples, as well as suitable performances (working range and limit of detection).

Although above considerations clearly demonstrate the unique value of this study, the main advantages and novel outcomes of the proposed approach are briefly summarized in the following.

1) The proposed sensing approach confers high reliability in NH₃ measurement.

2) The exploitation of YSZ in a planar sensing configuration as a sensitive layer for NH₃ in a liquid solution is a novel approach, which will enable the realization of PHE sensors. The indirect measurement of PHE, through PAL reaction, provides a very fast detection method as compared to other techniques.

3) The proposed sensing strategy adopts IDE electrodes, which maximizes the field interaction with the MUT. Moreover, IDE-based sensors can be easily miniaturized, thus offering the possibility to operate with a low volume of samples, to be integrated with electronics and to satisfy requirements of low-power sensing architectures (e.g., lab-on-chip) [28].

4) As above mentioned, the use of a capacitive readout strategy is convenient especially when the detection of low analyte concentration is addressed.

5) The adopted inkjet printing (IJP) technology allows for the rapid prototyping of low-cost sensors.

For the sake of completeness, in the following notes, examples of sensors developed by IJP technology are reported, with particular regards to the development of devices for biochemical sensing.

C. Related Works on IJP Technology for (Bio)Sensors

IJP is a rapid prototyping technology that can be used to conveniently develop sensors and biosensors, by exploiting multiple functional layers and a wide range of substrate and inks [34]. Andò *et al.* [35] highlight the need for low-cost mass-production of flexible and disposable devices, as well as the rapid prototyping of electronics and sensors. An overview

of main printing processes is given, along with examples of flexible transducers realized through low-cost IJP technology.

In [23], IDEs for gas sensor, fabricated with silver electrodes inkjet printed on Si/SiO₂ substrates, were tested for application in ammonia gas sensors. Conductive PANI layer was coated on the silver IDEs by drop-coating, and the conductivity of the PANI films is studied up on ammonia exposure.

The work in [36] describes IJP IDEs for chemiresistive sensors, based on a layer of single walled CNT (SWCNT) as sensing element deposited on a Kapton¹ substrate. IDEs were fabricated by IJP of a silver-based ink on top of the sensing elements.

Rosati *et al.* [37] studied IJP for the manufacturing IDEs arrays with a consumer-use inkjet printer with commercial AgNPs ink on flexible substrate, polyethylene (PET). The monitoring of *Lactococcus lactis* cultures and bacteriophage infection with this technique requires less than half the time of other traditional methods in terms of responsive time. The work presented in [38] is related to IDE sensors by IJP to monitor epithelial cell cultures with silver nanoparticles as a conductive element and SU-8 as the passivation layer. The focus is on the development of a real-time system and monitoring migration and detachment of keratinocytes by impedance spectroscopy. The impedance can track cellular migration over the surface of the sensors, thus showing a linear relationship with the standard method of image processing.

II. SENSING METHODOLOGY

The proposed approach consists of a capacitive sensor that can detect the NH₃ concentration in biological samples. The sensor structure is shown in Fig. 1. The first layer, whose thickness is h_1 and dielectric permittivity is ϵ_1 , is represented by the PET used as the substrate for the silver IDEs. On top of the IDEs, a functional layer of YSZ is considered. The thickness of such layer is indicated with h_2 and its dielectric permittivity as ϵ_2 . The capacitor transduction mechanism relies on the variation of the YSZ permittivity as a function of the NH₃ concentration in the MUT. The unique feature of IDEs-based sensor is the “one-side” exposition to the sensing target. The last layer, for the structure under investigation through this article, is the MUT, which, for the considered case, is represented by a solution of NH₃ in water, having thickness h_3 and dielectric permittivity ϵ_3 .

It must be observed that potential effect of exogenous quantities in the MUT (especially in the carrier fluid) could be compensated by adopting a differential approach.

A. Sensor Design, Modeling, and Simulation

The modeling of a multilayered sensor has been addressed in [33]. The conformal mapping technique, providing the expressions in closed form for the computation of the capacitance of interdigitated capacitor (IDC) electrodes based on the geometry of the sensor and dielectric properties of the materials used, and the partial capacitance methods, allowing to compute the capacitance between the IDC fingers and the fringing capacitance in the outer fingers [39]–[41], have been

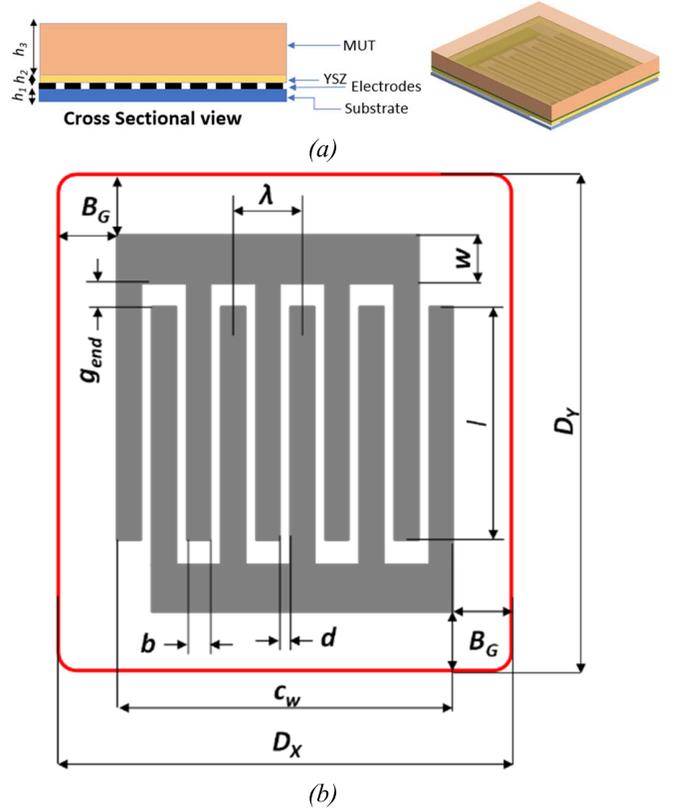


Fig. 1. NH₃ sensor: (a) cross section and (b) layout of the interdigitated electrodes.

used. As already presented in [33], main pillars bringing to the sensor design are summarized in the following notes.

As first, some considerations are reported to fix constraints provided by the application.

- 1) Device width, $D_x = 13.5$ mm.
- 2) Device length, $D_y = 9.8$ mm.
- 3) Guard bandwidth, $B_G = 1.5$ mm.
- 4) Requested size of the sensible area: 10.5 mm \times 6.1 mm.
- 5) MUT volume is 25 μ L. The latter will define the MUT thickness: $h_3 = 1$ mm.

Moreover, with reference to the IDE geometry, whose detailed features are shown in Fig. 1(b), the following values have been fixed to fulfill constraints given by the technology:

- 1) PET thickness: 140 μ m. PET dielectric constant: 1.93;
- 2) minimum finger spacing: $d = 280$ μ m;
- 3) finger end-gap: $g_{end} = 300$ μ m;
- 4) external strip width: $w = 300$ μ m.
- 5) thickness of the YSZ functional layer: $h_2 = 50$ μ m.

In the following, the thickness of the printed electrodes will be neglected, due to the thickness/width ratio of the IDE structure.

Given above quantities, the IDE cell width, c_w , can be expressed as: $c_w = D_x - 2B_G$.

The number of finger couples, n , can be calculated as

$$n = \text{floor}\left(\frac{c_w}{\lambda}\right) \quad (1)$$

where $\text{floor}(x)$ is the operator providing the greatest integer less than or equal to x , and λ is the IDE spatial period, which

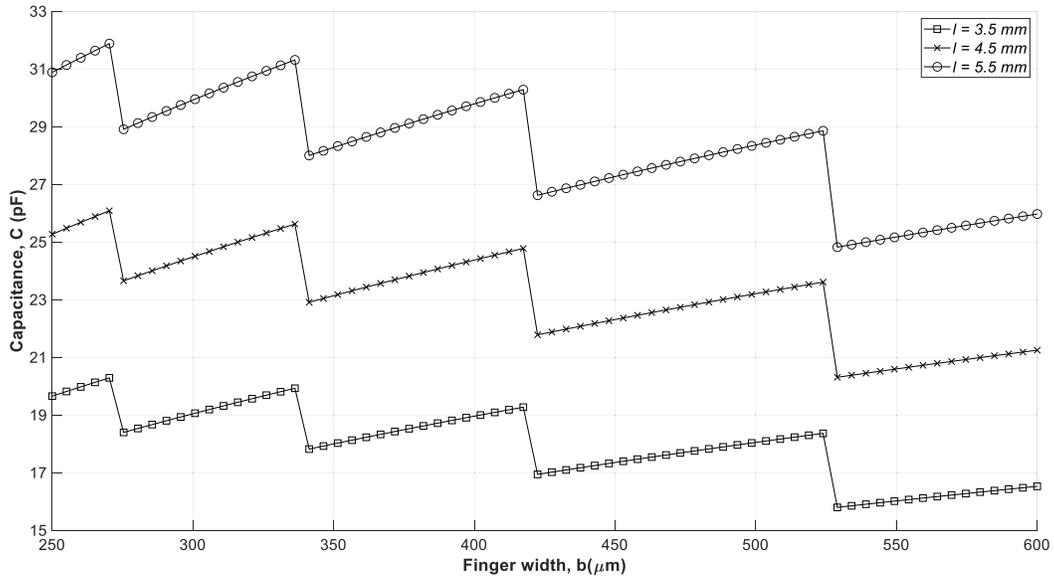


Fig. 2. Simulation results: the output capacitance as a function of interdigitated transducer (IDT) finger width, b , and finger length, l . Simulations have been performed by considering the following values for the functional and MUT layers: $h_2 = 50 \mu\text{m}$, $h_3 = 1.0 \text{ mm}$, $\epsilon_2 = 19$, and $\epsilon_3 = 75$.

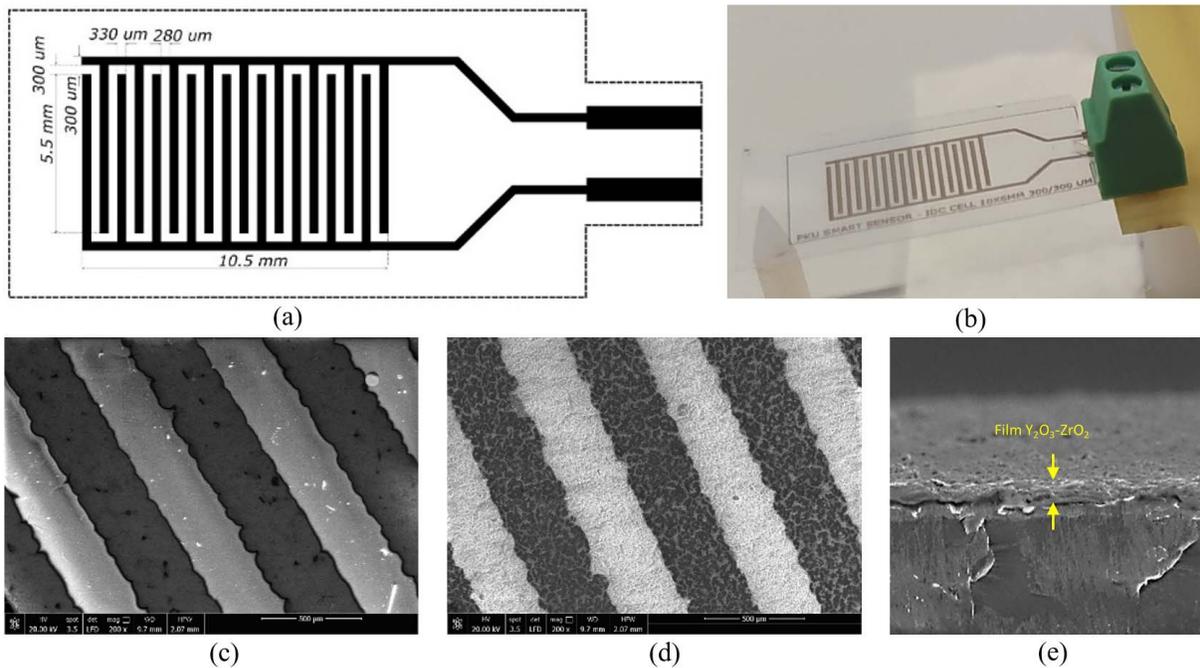


Fig. 3. (a) Electrodes layout; (b) Realized electrodes; (c) SEM micrograph of the interdigitated area; (d) SEM micrograph of the YSZ-modified electrode; (e) Cross-sectional SEM micrograph of the YSZ-modified electrode showing the thickness of the YSZ layer.

is given by

$$\lambda = 2(b + d). \quad (2)$$

By exploiting the model form discussed in [33] and considering above constraints, the device behavior has been simulated in MATLAB. The nominal dielectric constant of the functional layer of YSZ is 19. The MUT has been assumed to be made of deionized water with a nominal dielectric constant of 75.

From the simulation results, shown in Fig. 2, the following values of finger width, b , and length, l , have been fixed:

330 μm and 5.5 mm, respectively, which assure a suitable value of the sensor output capacitance.

III. SENSOR REALIZATION AND THE EXPERIMENTAL SETUP

On the basis of results provided by the device simulation, the sensor layout shown in Fig. 3(a) has been designed.

The sensor electrodes have been printed on the PET-based substrate Novele¹ IJ-220 by Novacentrix, Austin, TX, USA, with a thickness of 140 μm . This substrate is particularly

¹Trademarked.

convenient for low-cost and low-temperature applications and specifically engineered for Metalon¹ conductive inks.

The adopted printer is a low-cost EPSON piezo inkjet printer, while the conductive ink is the silver nanoparticle solution “Metalon JS-B25P” by Novacentrix. The adopted ink, which is formulated for compatibility and stability with piezo-inkjet printheads, shows the following characteristics: sheet resistance 60–70 m Ω /square, Ag content 25 wt%, viscosity 3–5 cP, and surface tension 28–32 dyne/cm.

The real device is shown in Fig. 3(b), while its SEM view is shown in Fig. 3(c).

Concerning the functional layer, ZrO₂-Y₂O₃ (8 mol% yttria, YSZ) with 5–10 nm (crystallite size) and surface area ≥ 100 m²/g was used as purchased from the manufacturer (Sigma-Aldrich, St. Louis, MO, USA). For preparing the suspension used for the deposition on the substrate by spray coating, YSZ was mixed with an acrylic binder (Max Meyer, Italy) in the wt/wt ratio of 2:1, respectively. In a typical preparation, 100 mg of YSZ with 50 mg of binder is dispersed in 25 mL of isopropanol and sonicated in ultrasonic bath at room temperature for 2 h. The tool used is an Airbrush with 7cc gravity feed cup and 0.25-mm nozzle/needle, working pressure 15–50 PSI, with heating plate. The sensor is placed on a plate heated to 100 °C and, through the help of a mask, the dispersed solution is deposited until the desired thickness is reached. The SEM view of the electrodes covered by the functional layer is shown in Fig. 3(d). A thickness $h_2 = 50$ μ m for the YSZ layer has been evaluated by the SEM analysis shown in Fig. 3(e).

In order to characterize the NH₃ sensor prototype, a GW-Instek LCR-6300 precision LCR meter has been used, which shows a basic accuracy of 0.05% in slow mode.

A dedicated printed circuit board (PCB) adaptor has also been developed to host the sensor and to convey out its electrical contacts, as well as to perform the calibration procedure allowing offsetting the stray capacitances of the connecting wires between the sensor and LCR meter.

With the aim of reducing undesired strain effects which could affect the sensor response, a rigid infrastructure, including a cantilever beam constraining the sensor, has been developed. The experimental setup is shown in Fig. 4.

IV. MEASUREMENT SURVEY

In the following, the measurement protocol adopted for the sake of the sensor characterization is summarized.

As first, the following electrical parameters of the sensor (resistance and capacitance), at zero NH₃ concentration, have been measured: 53.5 k Ω and 31.2 pF, respectively.

The obtained resistance value may highlight a loss effect, which will be addressed in the next release of the sensor, by introducing slight changes in the IDE layout and the use of additional layers in the sensor stack. In particular, the use of a hydrophobic layer between the printed electrodes and the YSZ layer may help to reduce the effect of the liquid carrier in the MUT, which penetrates into the porous structure of the YSZ layer, on the resistive behavior of the device.

The working range of interest for the NH₃ sensor is [0–2000] μ M, while the desired resolution should be

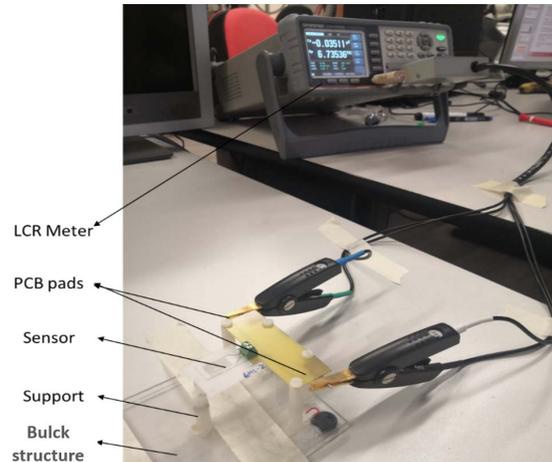


Fig. 4. Experimental setup for the sensor characterization.

around 100 μ M. To investigate the sensor behavior, reference samples of the MUT solution have been prepared by deionized water dispersed with different NH₃ concentrations: [0, 20, 200, 500, 1000, 2000] μ M.

The sensor has been exposed to the MUT at zero NH₃ concentration to get the zero bias output and for increasing values of the NH₃ concentration. For each NH₃ concentration, the time evolution of the sensor output has been observed up to the steady state regime was achieved. Among two consequent measurements, the following procedure has been implemented: 1) the MUT has been removed; 2) the sensor has been flushed with deionized water; and 3) the time evolution of the sensor during the restoring phase has been observed, until the sensor gets back to its rest condition.

The sensor response, C_{Sens} , against the excitation frequency, for different values of NH₃ concentration, is reported in Fig. 5. Mean values of the sensor output, estimated over 10 s of its steady state regime, are shown. The inspected range of frequency is [10–300 kHz] since below 10 kHz, the sensor response has not been considered reliable. As it emerges by the performed analysis, an optimal behavior, in terms of sensor responsivity and trends with increasing NH₃ concentration, has been observed at 20 kHz.

Time evolutions of the sensor output, observed at 20 kHz, for different NH₃ concentrations, are shown in Fig. 6, while the sensor output in the steady-state regime against the NH₃ concentration is shown in Fig. 7. As it can be observed, the sensor behavior shows a suitable trend.

In terms of repeatability, two main aspects in the behavior of the sensor have been investigated. The first one assesses the distribution of consecutive readings of the sensor output in the same operating conditions. In particular, fluctuations of the sensor output, estimated as the standard deviation over 10 s of the sensor output in its steady-state regime, for each considered value of the NH₃ concentration, range in the interval [0.032, 0.13] pF, which states for a good stability of the sensor response. The second analysis was aimed to the assessment of the system behavior in case of repeated experimental sessions. The adopted procedure, for each value of the investigated NH₃

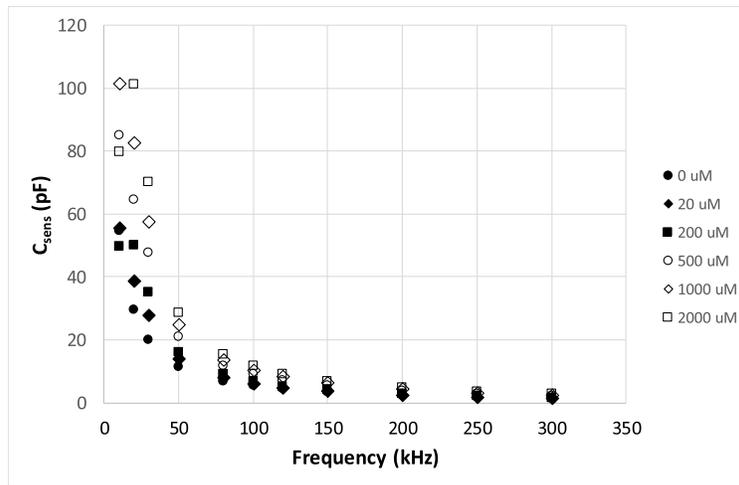


Fig. 5. Sensor response against the excitation frequency, for different values of NH₃ concentration.

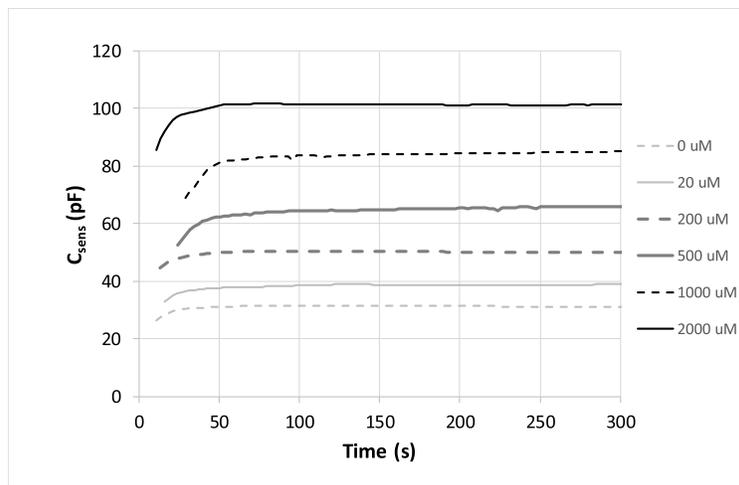


Fig. 6. Transient response of the sensor at 20 kHz for different NH₃ concentration.

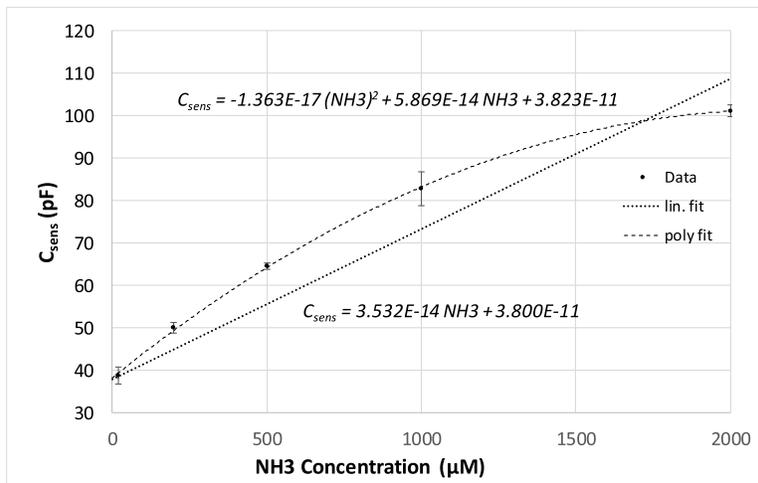


Fig. 7. Transduction diagram of the sensor at 20 kHz. Vertical bars state for the sensor repeatability, estimated for each value of the considered NH₃ concentration (the adopted procedure consists in repeated measurement sequences made of: 1) exposing the sensors to the MUT; 2) performing readings of the sensor output; 3) MUT removing; and 4) flushing with deionized water).

concentration, consists in three repeated sequences made of: 1) exposing the sensors to the MUT; 2) performing readings of the sensor output; 3) MUT removing; and 4) flushing with

deionized water. Results, in terms of the maximum scattering of obtained results, are graphically represented by vertical bars in Fig. 7.

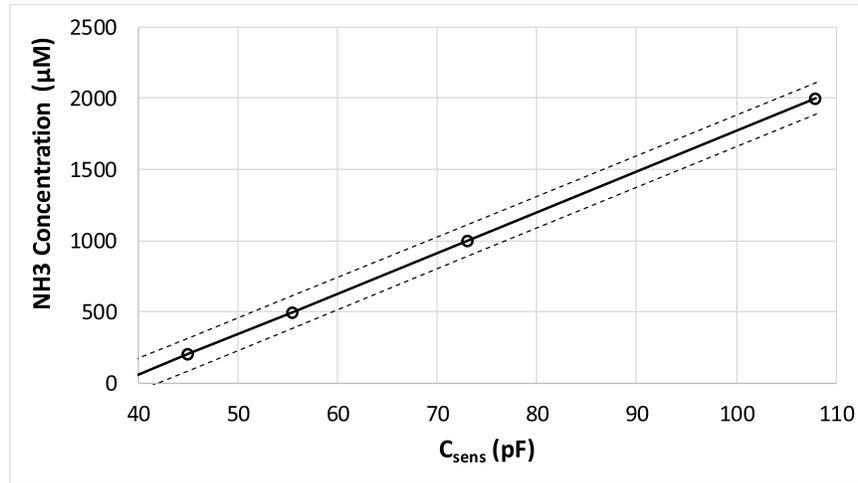


Fig. 8. Calibration diagram of the NH₃ sensor at 20 kHz, including the uncertainty bandwidth.

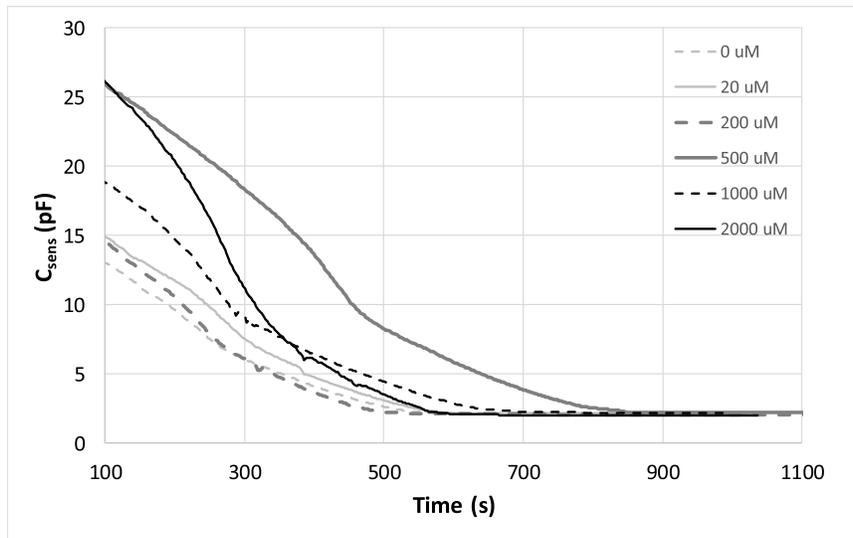


Fig. 9. Restoring behavior of the sensor after removing the MUT at 20 kHz.

Moreover, polynomial and linear interpolations are shown, being the latter more convenient from a practical point of view, although less performing. Fig. 8 shows the sensor calibration diagram, which has been obtained by considering the linear fitting of experimental data. The estimated uncertainty bandwidth is also shown. The sensor responsivity is 3.53×10^{-14} F/ μ M. The sensor resolution, estimated as the ratio between the standard deviation of the sensor output during the steady-state regime in case of a zero-ammonia concentration and the sensor responsivity, is 0.15μ M.

A span-to-resolution of 1.3×10^4 has also been estimated for the developed sensor prototype.

As the last investigation, Fig. 9 shows the restoring phase, implemented as above described. As it can be observed, independently on the steady-state value reached by the device (which depends on the NH₃ concentration), the sensor output in the absence of the MUT is always restored to its rest value of 2.18 pF. This result is worth of consideration because it demonstrates the restoring capability of the sensor.

The sensor characterization has been performed at room temperature (around 25 °C). The final version of the sensor, which will be used for the indirect estimation of PHE, will work at a fixed temperature around 37 °C by exploiting a temperature-controlled chamber. This is required by the enzymatic reaction ruling the conversion mechanism between PHE and ammonia. On the basis of such considerations, being the working condition investigated through this article not so far from the target thermal regime, meaningful effects of the latter against the sensor performances are not expected. However, future efforts will be dedicated to investigate the sensor behavior against temperature.

V. CONCLUSION

This article presents the development and the characterization of a multilayered IDE capacitive bio-sensor for the quantification of ammonia in biosamples. After the optimal design of the IDEs, achieved through the sensor model discussed in [33], the device has been realized by rapid prototyping

techniques, including the IJP of the sensor electrodes and the sputtering of the functional YSZ layer. An extended measurement survey has been performed for the sake of the sensor characterization. As remarked in Section IV, obtained results encourage the further development of the proposed sensing strategy, with the ultimate aim to implement a fast detection method for the estimation of PHE levels in bio-fluids, which turns out to be useful to support patients suffering for PKU disorder. Main outcomes of the proposed solution are related to the adopted capacitive sensing approach, which confers high reliability in NH_3 measurement due to the use of the YSZ-based functional layer in combination with IDEs. Such characteristic will enable the use of this sensor for the fast detection of PHE by exploiting the ammonia produced by the PHE–PAL reaction.

Further steps to be accomplished are related to the integration in the sensor of the enzyme layer allowing converting the PHE contents in corresponding ammonia concentrations. Moreover, the new sensor characterization will be performed to assess the abovementioned PHE detection mechanism.

Another aspect worthy of further investigation is the fringing effect occurring in IDT sensors. This effect, which could produce a deviation from the expected behavior, has been intrinsically considered by the experimental characterization of the sensor developed. However, the possibility to adopt strategies for its minimization, such as the use of a ground plate below the electrodes layer, will be investigated in view of the development of the final release of the sensor, aimed at the indirect estimation of PHE.

Finally, the development of a dedicated conditioning electronics will be addressed. Different solutions will be considered, e.g., by exploiting capacitance-to-digital converters showing resolution better than 0.5 fF. The latter value is compliant with the variation of the sensor output capacitance, corresponding to the expected resolution of the sensor in terms of NH_3 detection.

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