INVESTIGATION ON READOUT STRATEGY FOR AQUEOUS NH_3 SENSOR DEVELOPED BY ADDITIVE TECHNOLOGY

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Abstract—Low-cost solutions for the detection of ammonia and its related compounds are of deep interest, due to several application contexts requiring suitable approaches for its detection, both as a direct product of industrial or biological processes, as well as a byproduct of complex reactions. This paper addresses the need of ammonia detection for the indirect estimation of phenylalanine, which is of interest in the context of Phenylketonuria disorder. The main idea behind the proposed approach is to exploit the ammonia produced by the enzymatic reaction phenylalanine-phenylalanine ammonia-lyase. The sensing methodology investigated through this paper is based on a chemo-capacitive readout strategy, implemented by a stack structure using interdigited electrodes and a functional layer of Yttria-Stabilized Zirconia. The sensor behavior is addressed, along with a dedicated signal processing allowing to retrieve a reliable information on the target media from the sensor output. The system shows a responsivity of 0.077 pF/μM, a resolution of 2.5 μM and an accuracy of 22.5 μM in the 3σ level.

Keywords—Ammonia detection, chemo-capacitive sensing, rapid prototyping, signal processing, sensor characterization.

INTRODUCTION

Chemical sensors are widely used in many applications, including healthcare, safety, industrial process control, food processing, agriculture and automotive. In particular, chemical sensors devoted to the detection of ammonia (NH_3) and its compounds have received great attention from the scientific community and various approaches have been proposed in the literature, being NH_3 a commonly involved reagent in many industrial sectors and, on the same time, a potential threat to human health and environment.

Several approaches, technologies and materials to develop NH_3 gas sensors have been reported in literature. Comprehensive reviews of the most widely adopted sensing techniques for NH_3 gas detection, including: semiconductor oxides-based sensors, which exploit the variations in conductivity/impedance of the material; electrochemical sensors exploiting an amperometric principle; optical sensors based on colorimetric or fluorescence effects; conductive polymers-based sensors; surface acoustic wave devices; field effect transistors-based sensors; carbon nanomaterials, are reported in [1], [2], [3] and [4]. Among these, solid state sensing techniques such as metal oxide and conductive polymer-based ones have been largely adopted thanks to their remarkable advantages over other approaches, including simple and cost-effective fabrication.

In particular, chemo-resistive NH_3 gas sensors, based on the variation of resistance of a sensitive material, have been investigated in [5] and [6].

Among conductive polymers-based approaches, those exploiting polyaniline (PANI) film as an active sensing layer have recently achieved enhanced sensing performance [7]. Such sensors exploit the variation in resistance of PANI film due to its exposure to NH_3 gas. In particular, the fabrication of a flexible NH_3 gas sensor based PANI film and operating at room temperature is reported in [8].

Although chemo-resistive sensors have demonstrated adequate performance in the detection of NH_3 gas, these are not suitable for use in noise-sensitive contexts, for example when the measurement of low analyte concentrations is required. In these cases, a chemo-capacitive approach, as dominated by dielectric type effects and less sensitive to charging effects than the resistive counterpart, exhibits better performance in terms of sensitivity and reliability. Furthermore, polymeric chemo-resistive sensors are affected by a non-reversible reaction with NH_3 which progressively degrades the sensor performance [9]. Examples of capacitive NH_3 sensors are reported in [9], [10] and [11].

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The above-mentioned solutions are mainly used to detect gas targets, while few convenient approaches to the detection of NH_3 in liquid solutions, (aqueous NH_3) have been reported. In [12] an aqueous NH_3 chemical sensor based on α-Fe_2O_3 metal oxide nanostructures, which exploits an I-V sensing technique, is discussed. Such sensor exhibits a high sensitivity to aqueous NH_3 in the low concentration range, but its response is highly non-linear and saturates at concentration values of 0.05 mM.

To summarize, the vast majority of ammonia sensors reported in the literature are dedicated to measurement in the gaseous phase. There are very few papers whose focus is the measurement of ammonia concentration in aqueous solution, in particular with integrated/miniaturized approaches.

With reference to the SOTA analysis proposed, only in [12] the development of a miniaturized sensor for aqueous ammonia is reported. Such sensor exhibits a very low response time of 10 s; however, it is not suitable for the application taken into consideration due to its highly nonlinear response that saturates at 50 µM, well below the upper limit of the detection range of interest (20 ÷ 2000 µM).

The development of an aqueous NH_3 capacitive sensor based on an interdigited capacitor (IDC) and on a functional layer of Yttria-Stabilized Zirconia (YSZ) is presented in [13] and [14], together with the optimal design of the sensor in terms of IDC layout and thicknesses of the functional layer and the Material Under Test (MUT), based on a dedicated model approach [15], [16], [17]. The experimental characterization of a sensor prototype has been investigated in [18]. The sensing principle exploited by such approach is based on the variations in the dielectric permittivity of the YSZ functional layer produced by the NH_3 in the MUT which, in turn, results in variations of the device capacitance. In [18]the effects of a surface treatment in UV/Ozone for the YSZ sensitive layer on the overall sensor efficiency have been discussed. An improvement of the sensitivity thanks to the surface treatment has been demonstrated through experiments.

The IDC in the above-mentioned sensor has been fabricated by means of a rapid-prototyping approach, by inkjet printing the conductive interdigited electrodes on a flexible polymer substrate, in order to satisfy the requirements for low-cost devices and fast approach to the development of sensors for both lab-scale and mass productions [19], [20] and [21].

The IDC sensor discussed in this paper has been fabricated by Aerosol Jet Printing (AJP) the conductive electrodes on a flexible polymer substrate. AJP is a mask less digital additive manufacturing technique originally developed by Optomec Inc. The Aerosol Jet deposition process starts with a carrier gas (nitrogen or compressed air) that causes the atomization of a liquid functional ink. The whole of the carrier gas with the functional ink particles in suspension is then convoyed to the print nozzle where a sheath gas focusses the stream on the substrate. The AJP process permits to selectively deposit functionals material (conductive or insulators) with trace size from 10 µm to 3 mm in width and from 100 nm to 10 µm in thickness. The AJP process permits to deposit custom designed microstructure with sensing capabilities directly on objects surfaces.

The ability to detect aqueous NH_3 is crucial for the research activity behind this paper, which is aimed at developing an easy-to-use, portable, sensitive, and accurate diagnostic assays called point-of-care (PoC) testing platform dedicated to people affected by Phenylketonuria (PKU).

Phenylketonuria (PKU) is a genetic metabolic disorder characterized by the lack of the enzyme phenylalanine hydroxylase (PAH) which converts phenylalanine (PHE) to tyrosine, resulting in a build-up of phenylalanine in the blood, which may lead to severe neurological impairments [22], [23].

In order to prevent developmental delays in newborns, an early diagnosis of the disease through a suitable screening performed in the first few days of life is crucial [24], [25].

At present, an appropriate diet and periodic checks of phenylalanine levels in the blood are the only tools available to ensure an acceptable quality of life for people with PKU.

With specific reference to the quantification of Phenylalanine in biological samples, in [26] the three methods traditionally used for blood phenylalanine monitoring are discussed and compared: the amino acid analyzer, high-performance liquid chromatography with fluorometric detection, and tandem mass spectrometry. Although very accurate, all these methods require both costly instruments and specialized personnel to be performed, thus they are typically made available only in few specialized laboratories and therefore analysis results may take up to four-six days to be delivered to patients. Taking this into account, a low-cost approach such as the one proposed in this work, capable of providing a result in times of the order of tens of minutes (and in this sense fast in the response), although less accurate than traditional methods of analysis lend itself well to daily monitoring of phenylalanine levels which can be carried out independently by the patients themselves.

For such reasons there is a strong interest in the development of portable, easy-to-use, fast, sensitive and accurate Point-of-Care testing devices for the regular monitoring of PHE levels. To this aim, enzymatic-based approaches exhibit several advantages, such as high specificity, good sensitivity, rapid response, and disposable format [27]. On the other hand, however, enzyme immobilization can occur which and cause a lack of enzyme activity and stability. In order to overcome this problem, anchoring technologies with nanomaterials [28] and engineering strategies based on the stabilization of biocatalysts have been successfully proposed [29].

The most widely used enzyme-based assays for the detection of PHE levels in biological samples exploit Phenylalanine dehydrogenase (PDH) [30] and phenylalanine ammonia-lyase (PAL) [24] enzymes. The enzymatic conversion of PHE to phenylpyruvate and NH_3 by PDH/NAD+ has been recently exploited in colorimetric [31], electrochemical [32], and voltametric [33] transduction approaches. The deamination reaction between PAL enzyme and PHE, which is converted into trans cinnamic acid and NH_3, allows for the indirect determination of PHE level by evaluating the amount of aqueous NH_3 or changes in the pH, as proposed in [34].

In this paper the research work aimed at developing a capacitive sensing approach for aqueous NH_3 detection is reported. The main outcome of this activity is the possibility to implement indirect measurement of PHE by detecting the amount of aqueous NH_3 produced by the PHE-PAL enzymatic reaction.

In particular, a chemo-capacitive sensor is investigated, which exploits a polymeric substrate hosting the sensing electrodes, a YSZ functional layer and a dedicated housing for the target media.

Main advantages of the proposed approach are related to:

- the capacitive sensing strategy, resulting in higher reliability in aqueous NH3 detection, due to the use of a YSZ functional layer and a planar sensing configuration, which maximizes the interaction between the functional layer and the MUT;

- the low-cost rapid prototyping technology adopted for the sensor realization;

- the indirect measurement of PHE, through the NH_3 produced by PHE-PAL reaction, which results in faster detection compared to other techniques.

Compared to device prototypes already discussed in [13], [14] and [18], main novelties introduced in this paper are related to:

i) new materials for the device substrate and conductive electrode;

ii) the additive technology adopted for the interdigited electrodes realization;

iii) a new set-up for the experimental measurements;

iv) the paradigm adopted to process the output signal of the sensor.

Within this paper the suitability of the developed device prototype for the detection of aqueous NH_3 in the concentration range of interest will be demonstrated through experimental measurements.

THE DEVELOPED SENSOR

With the aim of addressing the need for a fast PHE detection, the idea behind the proposed sensing strategy is the possibility to implement the indirect estimation of PHE amount in a biological sample, by exploiting a chemo-capacitive sensing strategy to detect the concentration of NH_3 as a byproduct of the deamination enzymatic reaction of PHE with PAL enzyme. The amount of NH_3 in the sample can be determined by exploiting the modifications induced in the dielectric permittivity of a YSZ sensitive layer deposited over IDC electrodes which, in turn, results in a variation of capacitance.

The electrodes of the IDC sensor have been realized with gold-based ink on a PEEK (Polyether Ether Ketone) substrate using AJP technique. PEEK substrate offers interesting physical and chemical characteristics, its melting point is343 °C [35], compatible with the gold ink thermal curing process. For IDC sensor realization, a 0.125 mm thick PEEKFilm (RS Pro) has been used as substrate. The conductive functionalization ink is the Metalon JG-014UA gold nanoparticle ink specially formulated for AJP. Its resistivity starts from 4.2x10-4 □·cm to 1.9x10-5 □·cm according to curing time and temperature. The realization process starts with the layout definition with a CAD software used to generate the printer part program. The printer, configured with the ultrasonic atomizer, deposits the gold ink drawing the electrodes with partially overlapped 150 □m traces. Then the realized sensors are placed in a static oven with the temperature set at 150 °C to promote gold nanoparticles aggregation.

The fabricated prototype is characterized by interdigitated electrodes with finger width of 500 µm and finger spacing of 300 µm, for a total of seven pairs of interdigitated electrodes. The IDC sensor active area is 13.5 ×9.8 mm^2.

The structure of the sensor is schematized in Fig. 1, where both the interdigited capacitive sensor and a rigid supporting frame, which also realizes the reaction chamber, are depicted. The flexible IDC sensor substrate with the sensitive material layer deposited over the electrodes is sandwiched between two rigid frames, with the aim of providing a robust mechanical structure while leaving empty areas on both sides of the IDC. The first frame is on the top side, while the other is the bottom side of the device. The empty zone on top realizes the reaction chamber for the MUT to be analyzed, while the support on the bottom side has been removed in order to reduce its effect on the capacitance of the IDC. Fig. 2 shows a picture of a real device, where the IDC sensor is assembled between the two rigid frames.

A layer of the sensitive dielectric material has been deposited over the printed IDC electrodes by spray coating ZrO_2-Y_2O_3 (8% mol Yttria, YSZ). The functional YSZ dielectric layer has been deposited over the printed IDC electrodes by means of an Airbrush spray-coater with 7cc Gravity Feed cup and 0.25 mm nozzle/needle, Operating pressure is 15-50 PSI, on heating plate. The thickness of each deposition layer has been varied controlling the total quantity of YSZ sprayed, while maintaining the other main operative parameters constant, i.e., the YSZ isopropanol suspension spray rate and the distance between the spray-coater nozzle and the IDC electrode. SEM cross-section analysis was used to measure experimentally the thickness of the coating obtained. The optimal YSZ thickness has been determined as a tradeoff between device responsivity and technology constraints, by considering the expected relative permittivity variations over the specified detection range for aqueous NH_3 (20 ÷ 2000 µM), starting from data available in literature.

Sensitivity to NH_3 has been achieved by exploiting the modifications induced to the dielectric permittivity of YSZ. The supporting rigid frames have been fabricated in 1.55 mm-thick FR4 (a woven glass-reinforced epoxy laminate material commonly used for Printed Circuit Boards fabrication) through a Protomat S103 Milling Machine by LPKF Laser & Electronics SE.

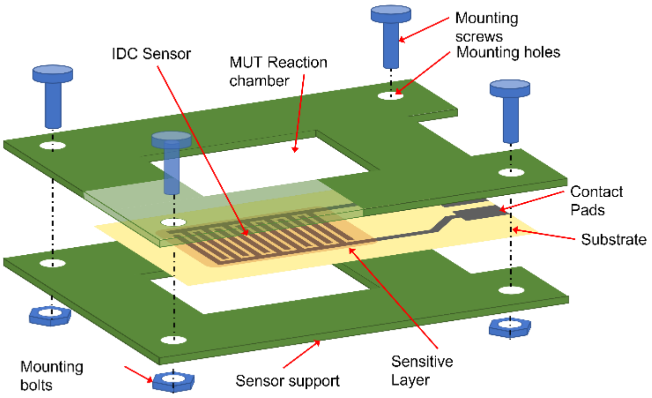

Fig. 1. Structure of the sensor (exploded 3D view) including the the supporting frame of the experimental set-up.

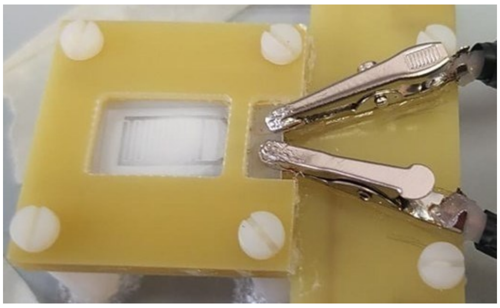

Fig. 2. Real view of a device prototype assembled with the supporting frame.

The IDC sensor substrate has been glued to the top rigid frame by using a 3M bi-adhesive gasket in order to avoid sample solution leaking during the test, then the two rigid frames have been fixed together with the IDC sandwiched in between by means of nylon screws and bolts.

THE EXPERIMENTAL BEHAVIOR OF THE SENSOR

In order to investigate the sensor behavior a dedicated experimental survey has been performed. The sensor has been exposed to different media (or MUT), consisting of aqueous NH_3. The sensor response to MUT samples with the following aqueous NH_3 concentration, [0, 50, 200, 500, 1000] μM, have been observed. The above investigation span has been fixed on the basis of constraints provided by the specific application addressed through this work. For each trial the sensing chamber has been completely filled up.

The measurement protocol can be summarized as it follows:

a) the sensor is firstly exposed to a clean MUT (0 μM of aqueous NH_3) and its time response has been observed;

b) successively the sensing chamber has been cleaned; the cleaning was carried out by means of two consecutive washings with deionized water. Each wash was performed by filling the sensing chamber with deionized water, waiting 30 minutes, and then emptying the chamber with a pipette.

c) the sensing chamber is then filled again by a MUT with 50 μM of aqueous NH_3 and the sensor time response is collected;

From now on steps b) and c) are repeated for all considered concentrations.

For each concentration two repetitions of the measurement protocol have been performed. Although the sensor is intended to be a disposable device, results related to different measurements performed on the same device, demonstrated that the sensor is reusable. Moreover, all the concentrations have been tested on the same sensor.

Experimental measurements have been performed through a GW Instek LCR-6300 Precision LCR Meter. A calibration procedure aimed at compensating the stray capacitive effects of the connecting cables has been performed on the instrument before the measurement survey.

For all trials an exposition time of 1300 s has been used, while the sampling time was of 2.66 s.

Raw time responses of the sensor are shown in Fig. 3-a for different MUTs. Data has been post-processed by using the dedicated procedure illustrated in Fig. 4, which aims to provide a unique relationship between aqueous NH_3 concentration in the MUT and the system output.

The first operation to be performed on raw data is a moving average filtering, which uses a 25 s windowing. Results of this process are illustrated in Fig. 3-b. As it clearly appears, time evolutions present a kind of trend which need to be removed. To such aim a “trend searching” algorithm has been developed, which uses the differentiated signal in order to split the signal in two parts. The right part is the one showing the trend to be removed. After the de-trend operator is applied to this part of the signal, the first part of the signal (not de-trended) and the de-trended signal are linked again. Results of this procedure are shown in Fig. 5-a. Obtained signal is then processed (by means of signal differentiation) to find the minimum time (indexing) from which on a flat trend is assured. For the case presented in Fig. 5-a, this procedure brought to a time indexing of 525 s, which is the minimum time from which the output signal is flat and so can be used to retrieve the information about the target quantity. Fig. 5-b shows values of the “processed output” versus the target concentration, while Fig. 5-c shows the output variation as respect to the clean MUT. The latter highlights the suitable relative variation of the sensor output for increasing values of the aqueous NH_3 concentration, which could be exploited by implementing a differential configuration.

Finally, Fig. 6 shows the calibration diagram obtained for aqueous NH_3 concentration in the range [0-500] μM. A linear trend has been used to interpolate experimental observations, which shows a regression index of 0.99. A sensor responsivity of 0.077 pF/μM has been obtained, while a resolution of 2.5 μM has been estimated. The latter has been obtained by computing the ratio between the standard deviation of the time evolution of sensor output (in case the sensor input is kept fixed) and the responsivity. The uncertainty bandwidth is also shown, which allows to define an accuracy of 22.5 μM in the 3σ level. The uncertainty has been estimated as $3\sigma/S$, where σ is the standard deviation of the residuals between the experimental data and the linear fitting model in the transduction diagram shown in Fig. 5-b restricted to [0, 500] μM, and S is the sensor responsivity.

CONCLUSIONS

Phenylketonuria (PKU) disorder is of primary interest due to its serious consequences, thus pushing the need for development of reliable, easy-to-use and fast sensing solutions for PHE detection. An interesting approach could rely in the indirect estimation of PHE by measuring NH_3 produced through enzymatic reactions.

This paper aims to investigate a chemo-capacitive sensor, which allows the measurement of ammonia in a liquid sample. The main advantage of the approach proposed are related to the low-cost and rapid-prototyping technology adopted for the sensor realization. A dedicated signal processing has been also developed.

Results obtained encourage further investigation and development of the proposed sensing approach. In particular, this study is aimed to the proof of concept of the proposed sensing methodology. The repeatability study of the realization process will be performed in the next future.

Future efforts will be dedicated to the implementation and testing of the whole sensing strategy, exploiting a dedicated enzyme layer for the direct detection of PHE in liquid samples.

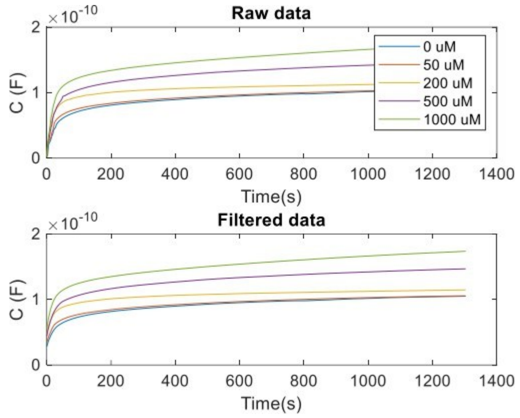

Fig. 3. (a) Time evolution of raw sensor output; (b) Filtered data.

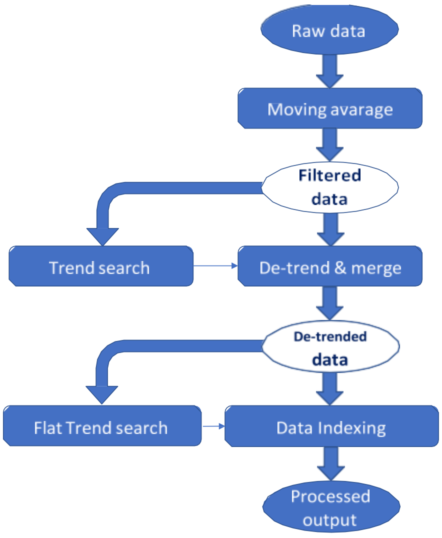

Fig. 4. Schematization of the signal processing procedure.

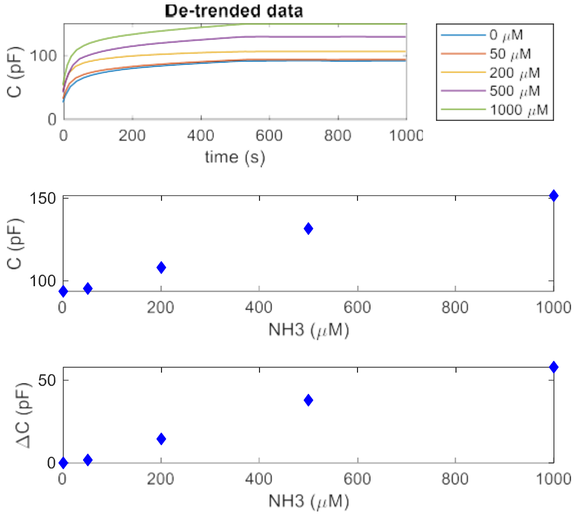

Fig. 5. (a) De-trended sensor output ; (b) processed output versus the target concentration estimated at 525 s; c) processed output variation as respect to the clean MUT.

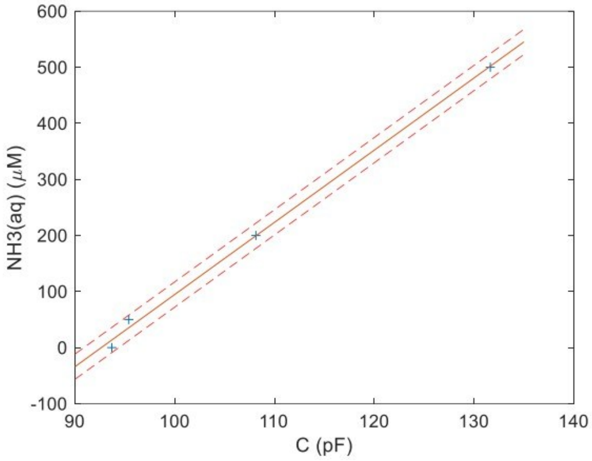

Fig. 6. Sensor response: calibration function and uncertainty bandwidth in the 3□ level.

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