



A fully integrated passive microfluidic Lab-on-a-Chip for real-time electrochemical detection of ammonium: Sewage applications

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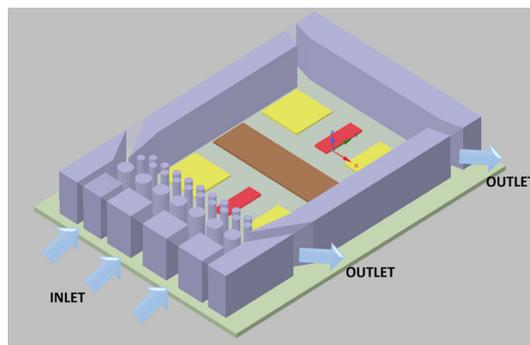
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HIGHLIGHTS

- Lab-on-a-Chips are consolidated as powerful analytical tools
- They are especially useful for the real-time and in-situ detection of biomarkers within environmental analysis of waters.
- The key feature of this miniaturized Lab-on-a-Chip is its ability to perform electrochemical measurements combined with passive microfluidics.
- The passive flow of sample created removes the need of using external pumps and valves what extremely reduces the power consumption.

GRAPHICAL ABSTRACT

The assembly PDMS-microfluidic structure and silicon transducer lead to the fabrication of a passive-microfluidic Lab-on-a-Chip to perform in-situ and real time electrochemical analysis in aquatic environments.



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ABSTRACT

The present work reports on the development of a new generation of Lab-on-a-chip (LOC) to perform in-situ and real-time potentiometric measurements in flowing water. The device consisted of two differentiated parts: a poly (dimethylsiloxane) (PDMS) microfluidic structure obtained by soft lithography and a fully integrated chemical sensing platform including four working microelectrodes, two reference microelectrodes and one counter microelectrode for detecting ammonium in a continuous mode. The performance of the device was evaluated following its potentiometric response when analyzing ammonium containing samples. As a key parameter, its time of response was compared to that of a commercially available electrical conductivity sensor used as reference sensor during tests in laboratory using flowing tap water and technical scale using flowing wastewater. As a result, the LOC showed a slope of 55 mV/decade, a limit of detection of $4 \cdot 10^{-5}$ M and a time of full response between 10 and 12 s. It was demonstrated that the device can provide fast and reliable data at real time when immersed in a laminar flow of water. Moreover, the test of robustness showed that it was still functional after immersion in sewage for at least 15 min. Besides, the LOC reported here can be helpful for a wide variety of flowing-water applications such as aqua culture outlets control, in-situ and continuous analysis of rivers effluents and sea waters monitoring among others.

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1. Introduction

Waste discharges from industrial sewage, agriculture, animal and human activities have hugely increased since the worldwide population and economy grow (Wang et al., 2014). Accordingly, wastewater quality assessment has become an essential tool for environmental monitoring on the last decades. Since the European Union implemented the Urban Waste Water Treatment Directive in 1991 to regulate the quality of wastewater, the control of pollutants discharged has significantly increased (European Commission, 2016; European Environment Agency, 2015). However, the presence of emerging biological and chemical contaminants in wastewater is still alarming (Awasthi et al., 2016; Bougnom and Piddock, 2017; Gaw and Glover, 2016; He et al., 2016; López-Serna et al., 2013; Petrie et al., 2015; Rizzo et al., 2013; Zabinski et al., 2017). Yet, sewage monitoring is not only helpful to improve wastewater quality from an environmental point of view, but also to perform epidemiology studies in communities. Such is the case of profiling illicit drug intake by population. Collecting information concerning illicit-drug uses plays a vital role in not only helping law enforcement agencies in prevention and fight against criminal organizations, but also to estimate illegal stimulating substances production and consumption. At present, the chemical analysis of wastewater is a powerful tool to monitor the patterns and trends of illicit drug consumption traditionally carried out in surveys (Castiglioni et al., 2014; Xu et al., 2017). In one of the latest studies in sewage epidemiology, Zuccato et al. used SPE and HPLC-MS/MS to obtain the profile of drug consumption in several Italian school populations. They found cannabis and cocaine intake up to 6 g/day for 1000 people to be the most used drugs followed by amphetamine-type stimulants (ATS) such as ecstasy or methamphetamine where the consumption can rise up to 0.2 g/day for 1000 people depending on the school and season (Zuccato et al., 2017).

Commonly, most extended analytical techniques used to analyse wastewater are sample-based studies like spectroscopic measurements and especially capillary electrophoresis and chromatographic analyses coupled with mass spectrometry techniques (HPLC and GC-MS). Although these techniques are highly selective and sensitive, they are still limited by the high costs and also they are time consuming. Moreover, these sophisticated equipment cannot provide results at real time neither to be used for on-site analyses. Thus, it is important to develop new techniques that produce reliable data in-situ, are easy-to-handle, inexpensive and provide fast and accurate results. In contrast to conventional analytical methods, chemical sensors are an attractive alternative or supplement to detect contaminants in a continuous mode. In the recent years, the presence of Lab-on-a-Chip (LOC) has emerged to monitor water quality (Nurani et al., 2017; Wade et al., 2012). Microfluidic LOC platforms have been extensively studied due to their possibility of replacing a fully equipped conventional laboratory. Great efforts have been devoted in downscaling the instrumentation, and electrochemical devices that enable the real-time monitoring of water effluents have appeared in the last decade (Jang et al., 2011). D. Geon et al. have recently reported on the development of a LOC to determine the total phosphorous (TP) concentration for water quality assessment (Geon et al., 2018). Here, the device consisted of integrated mixing and pretreatment chambers and was based on photocatalytic reaction for phosphorous detection in a time interval of 20 min. The instrumentation's size has been highly minimized and its performance was comparable to conventional TP analyzers. Similarly, a microfluidic multisensorial LOC that was able to operate in all natural waters has been reported. It used colorimetric measurements to assess nitrate and nitrite concentration with a limit of detection of $0.02 \mu\text{mol}/\text{dm}^3$ for both inorganic anions (Beaton et al., 2012). However, this was time consuming as it requires multiple steps for a chemical assay (sample pre-treatment, transport, time for reaction and conditioning). Contrarily, electrochemical LOCs are described as an attractive and alternative chemical analyses tool as they present compactness, low sample consumption, low-cost production, easy process control, real time analyses and are able to provide fast response

(Gabriel et al., 2017; Mao et al., 2015; Ning et al., 2017; Wu et al., 2013; Zhang et al., 2017; Zhao et al., 2018).

In this context, we report on the development of a passive microfluidic LOC for in-situ and real time potentiometric measurements in flowing water. The device was made of two parts: a transducer holding an array of gold working (WE), silver/silver-chloride reference (RE) and platinum auxiliary (AE) microelectrodes; and a PDMS microfluidic structure that consisted of internal passive micromixers, microfilters and a detection microchamber. Both subunits (PDMS/transducer) were covalently bonded by surface activation using O_2 plasma. Gold WEs were modified as ammonium-selective electrodes (ASEs) and potentiometric measurements were carried out to monitor ammonium-containing samples as a control experiment to validate the principle of concept. A passive and continuous flow was created inside the microfluidic LOC by diffusion when it was immersed in a laminar flow of tap water. This was verified by the quick response time of the LOC. Furthermore, the microfluidic LOC was subject of different immersions in a sewage pipe for different exposure times in order to evaluate its robustness and performance against clogging and its further application in sewage analysis. The manufacturing process was a challenge for rapid prototyping and our ambition is to produce reliable, low-cost, easy-to-operate and miniaturized sensing devices. Moreover, the LOC was compact for in-situ and real time detection of chemical markers in flowing water application such as sewage epidemiology, sea water or aqua culture outlets monitoring among others.

2. Materials and methods

2.1. Chemicals

Poly (dimethylsiloxane) (PDMS) (Sylgard 184) was purchased from Dow Corning, France. Carbon tetrachloride, heptane, octadecyltrichlorosilane (OTS), polyvinyl chloride carboxylated (PVC-COOH), di-octyl sebacate (DOS), pyrrole, sulfuric acid, ammonium chloride, hydrogen peroxide, potassium ferrocyanide (II), potassium ferricyanide (III), nonactine (ammonium ionophore) and acetonitrile were purchased from Sigma & Aldrich. Cesium Cosane ($\text{Cs}[3,3'\text{-Co}(1,2\text{-C}_2\text{B}_9\text{H}_{11})_2]$) was purchased from Katchem spol. s.r.o. It was used as doping agent for the preparation of a conducting-polymer intermediated layer between the ammonium-sensitive membrane and gold transducer. As reference, an electrical conductivity electrode "TetraCon 925" distributed by Xylem Analytics Germany Sales GmbH & Co.KG was used.

2.2. Microelectrodes fabrication

In a first stage, a batch of transducers was manufactured at the Barcelona Microelectronics Institute IMB-CNM (CSIC), Spain. They were made-up in silicon-substrate wafers of 100 mm diameter which contain 230 devices. The final device size is 4×7 mm and it integrates four gold WEs of 0.64 mm^2 each, two Ag/AgCl REs of 0.13 mm^2 and one Pt AE of 1.37 mm^2 as shown in Fig. 1(a). The transducer fabrication process starts by growing a silicon dioxide layer of 800 nm thickness using thermal oxidation to isolate the metallic microelectrodes from the silicon substrate. Afterward, gold microelectrodes were fabricated by physical vapor deposition (PVD) of a tri-layer of Ti (50 nm), Ni (50 nm), and Au (200 nm).

The Ti layer ensures good adhesion to the SiO_2 and the Ni layer avoids intermixing of Ti and Au. The geometry of the microelectrodes was defined by photolithography and wet chemical etching. The platinum microelectrodes were fabricated by PVD of a bilayer of Ti (15 nm) and Pt (150 nm), followed by photolithography and lift-off. Silver for the reference electrodes was deposited by PVD as a bilayer of Ti (15 nm) and Ag (150 nm), and patterned by photolithography and lift-off. A dielectric passivation layer was deposited over the microelectrodes by plasma enhanced chemical vapor deposition (PECVD) of

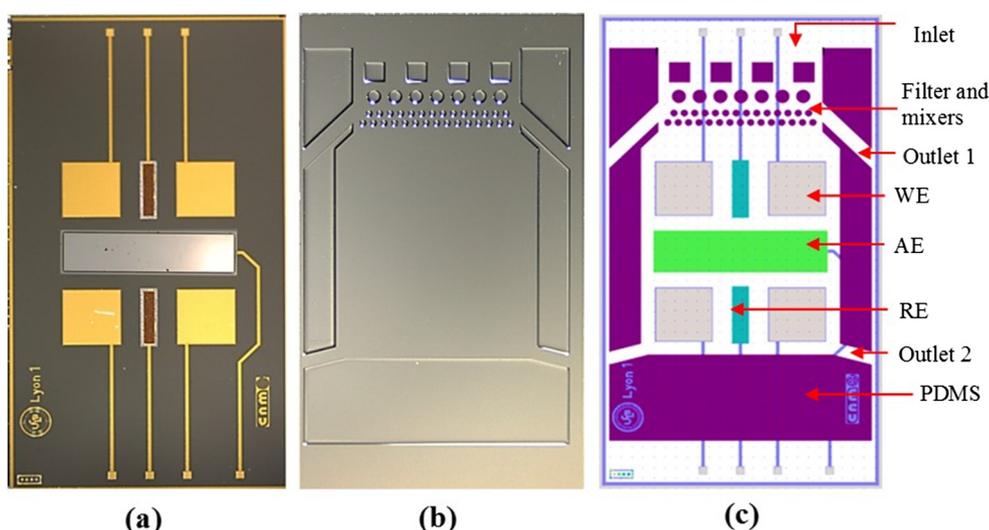


Fig. 1. In (a), a micrograph of the transducer manufactured. In (b), a micrograph of the negative-shaped silicon mold manufactured. In (c), the layout for the production of transducers and negative-shaped silicon molds bearing the microfluidic elements.

SiO₂ (400 nm) plus Si₃N₄ (400 nm). The passivation was removed then from the active microelectrode areas and the microelectrodes' pads by photolithography and dry reactive ion etching. Afterwards, the transducer was glued to a printed circuit board (PCB) using an epoxy resin (Ep-Tek H70E-2LC, from Epoxy Technology). Finally, all microelectrodes' pads were wire-bonded (using Kulicke and Soffa equipment 4523A) to the gold tracks of PCB and then the electrical connections were insulated using the same epoxy resin (Ep-Tek H70E-2LC).

2.3. Silicon master manufacturing process

A batch of negative-shaped silicon molds was fabricated at the IMB-CNM (CSIC), Barcelona, using a design that included microstructures such as microfilters, passive micromixers and microchannels as shown in Fig. 1 (b) and (c). The heights of the structures were etched to a depth of 100 μm. The microstructures were defined in the silicon surface to produce PDMS microfluidic systems with positive-shaped superficial structures by soft lithography replica molding. Silicon molds were fabricated by patterning the surface with a photoresist resin using established photolithography techniques. Then, the silicon wafer with (100) orientation was etched to a depth of 100 μm using deep reactive ion etching (601 DRIE, Alcatel, France). DRIE was preferred over other techniques, such as reactive ion etching (RIE) and inductively coupled plasma (ICP), since DRIE conditions were well optimized for etching these multilayer systems (Caballero et al., 2009; Mills et al., 2007; Prats-alfonso et al., 2012), and the technique provided good definition of the side walls to depths of hundreds of nanometres. After wafer dicing, the silicon masters were immersed in piranha (H₂SO₄/H₂O₂ ratio 2:1) solution for 2 h. Thus, Si-OH groups are formed on the Si surface. Subsequently, the activated surface is put in contact with a solution of OTS (5 μM) and carbon tetrachloride (0.4 mM) in heptane creating therefore, an octadecyl silane monolayer at the Si surface through nucleophilic reaction between Cl groups from OTS and Si-OH. Finally, the silicon molds were then rinsed with heptane and placed into the oven for 1 h.

2.4. Preparation of ammonium sensitive layer

In order to evaluate the performance of the passive microfluidic device, WE included in the LOC were functionalized as ammonium-selective microelectrodes to analyse ammonium during control experiments. The fabrication process started by growing a solid contact layer of conducting polymer polypyrrole[3,3'-Co(1,2-C₂B₉H₁₁)₂], (called

from now PpyCOSANE) by electrochemical polymerization onto gold microelectrodes using cyclic voltammetry as we have reported previously in the literature (Bilovan et al., 2007, 2006; Gallardo-Gonzalez et al., 2018a; Gallardo-González et al., 2018b; Marques de Oliveira et al., 2008, 2006; Saini et al., 2018). Subsequently, a combination of 33 wt% of PVC-COOH, 66 wt% DOS and 2 wt% of nonactine as ammonium ionophore was dissolved in 1.5 mL of THF. Afterwards, 2 μL of the mixture were drop-cast on top of each WE already modified with the PpyCOSANE layer. The membrane composition used has been obtained from the literature and adapted to fabricate the miniaturized ammonium-selective microsensors (de Beer and Van Den Heuvel, 1988). The device was left at room temperature overnight for total solvent evaporation. At this stage, the chemical sensor was ready to be joined to the microfluidic system.

2.5. Replica molding

The microfluidic systems based on PDMS were manufactured by soft lithography replica-molding. As indicated by its name, this method enables to obtain a replicate polymeric structure from the Si mold (Xia and Whitesides, 1998). For this purpose, a pre-polymer (PDMS) was mixed with the curing agent at the ratio of 10:3. The PDMS pre-polymer mixture was degassed in order to remove all air bubbles trapped in the polymer mixture. This step was very important to prevent mechanical defects in the microfluidic system. The mixture was then poured onto the silicon mold (previously silanized with OTS) and the ensemble mold/PDMS was left in the oven at 90 °C for 1 h. After PDMS curing, the elastomeric microfluidic system was peeled off from the silicon mold bearing the microstructures on its surface.

2.6. LOC assembly

The irreversible bonding between PDMS and transducer surfaces was achieved by surfaces activation through O₂ plasma in order to create hydroxyl groups Si-OH on the surfaces. Oxygen plasma was applied at 100 mTorr of pressure and 0.4 W/cm² during 30 s for PDMS and 2 min for silicon transducer. An inert and opaque mask was manufactured in epoxy resin to protect the microelectrodes area from the plasma source. Immediately after the activation process, both subunits (PDMS/transducer) were placed in a conformal contact and immediately an irreversible bonding O-Si-O was formed providing the sealing of the final microfluidic LOC.

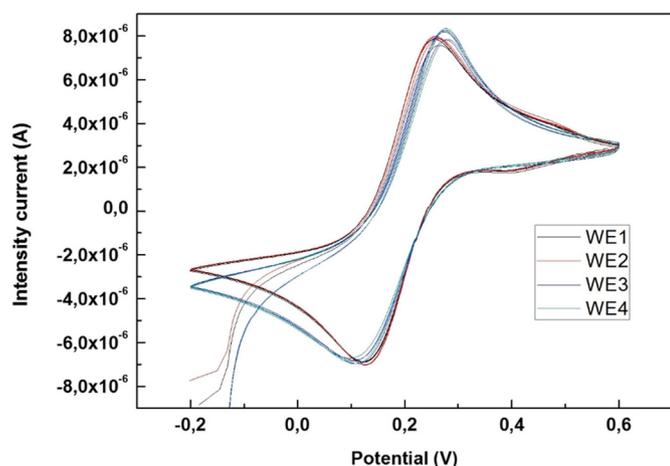


Fig. 2. Cyclic voltammogram of four gold working microelectrodes included in the transducer. Measurements performed in redox probe $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ 5 mM in phosphate buffer solution. Potential scanned from -0.2 to 0.6 V vs internal Ag/AgCl reference microelectrode at scan rate of 100 mV/s. Measurements were carried out simultaneously using a single-device electrochemical cell.

2.7. Electrochemical measurements

Cyclic Voltammetry (CV) was used for electrode characterization and electrochemical polymerization of the PpyCOSANE conducting polymer using a multichannel potentiostat (Biologic-EC-Lab VMP3) analyzer. Potentiometric measurements were made relative to internal Ag/AgCl RE and were carried out at room temperature using a multi-channel data acquisition system with four working microelectrodes connected simultaneously. Once the LOC was assembled, the calibration was performed in laboratory by titration of standard solution of ammonium chloride from 10^{-8} M up to 1 M prepared in the ionic strength buffer *tris*-HCl/*tris* with a ratio 9.5:1 in 25 mL of buffer to maintain a constant $pH = 7.1$. The LOC was calibrated using the generalized standard addition method (GSAM) (Saxberg and Kowalski, 1979). A solution of $(NH_4)_2CO_3$ 2 M (denoted as ammonium solution from now) was used for control tests in flowing water at technical scale with the electrical conductivity (EC) sensor used as time-of-response reference sensor.

3. Results and discussion

3.1. Transducer characterization

In order to validate the electrodes manufacturing process, gold WEs were characterized by CV. Measurements were made in redox

probe $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (5 mM) in phosphate buffered saline (PBS, pH 7.4). The potential was scanned from -0.2 to 0.6 V vs Ag/AgCl internal reference microelectrode at scan rate of 100 mV/s. Cyclic voltammograms were recorded simultaneously for each WE as shown in Fig. 2. The redox peaks observed at $E = 0.12$ V and 0.27 V as well as the symmetry of the intensity current peaks recorded at $I = \pm 8 \mu A$ were uniform and stable over the four microelectrodes. This confirms the normal behavior of the redox reaction; $Fe^{2+} \leftrightarrow Fe^{3+} + e^-$, at the gold interface which validates the good microelectrodes manufacturing process.

3.2. Microfluidic LOC assembly

The performance of the LOC was evaluated following its potentiometric response to different ammonium-containing samples. Therefore, all gold WEs were chemically functionalized to obtain four ammonium-selective microelectrodes as described in the experimental section. Fig. 3 illustrates the steps for the fabrication. First, a conducting layer of PpyCOSANE polymer was electrodeposited simultaneously onto all gold WEs to improve the mechanical and electrical contact between the sensitive membrane and gold microelectrode as described above. The optimized parameters of electrodeposition have provided a homogeneous PpyCOSANE layer (black layer in Fig. 3 (b)). This layer served to establish a formal contact between the polymeric membrane and the gold microelectrode enhancing the overall transduction process. Afterwards, the polymeric membrane that contained a mixture of PVC, plasticizer and nonactine as ammonium ionophore was drop-cast on top of each PpyCOSANE-modified WE (see Fig. 3 (c)) to obtain four ASEs. At this stage the chemical sensor platform was ready to be joined to the microfluidic system.

As it was mentioned previously, the PDMS microfluidic structure was obtained by replica molding of the silicon mold. This soft-lithography technique enables to obtain a positive-shaped elastomer bearing the desired microstructures. In Fig. 4 (a) a micrograph of one of the PDMS microfluidic systems obtained is shown. The system presented a combination of pillars and microchannel acting as microfilters and passive micromixers. The high resolution of the PDMS structure obtained using the replica molding technique was confirmed by its interferometry image shown in Fig. 4 (c).

The microfluidic system has been designed to present a depth of $100 \mu m$ when assembled with the electrochemical microsensor creating therefore, a combination of microchannels. Thus, the sample reaches the detection microchamber by capillarity in a first stage then diffuses through the microfluidic system. The covalent bonding between the silicon-based transducer and PDMS microfluidic structure was made through O—Si—O bond formation between Me_2SiO_2 group from PDMS and SiO_2 from the silicon transducer (Baraket et al., 2016, 2013; Lee et al., 2013; Tang and Lee, 2010). Both surfaces (PDMS and transducer) are chemically activated via Si-OH formation using O_2 plasma. Thus, an irreversible bond occurs through condensation reaction when both

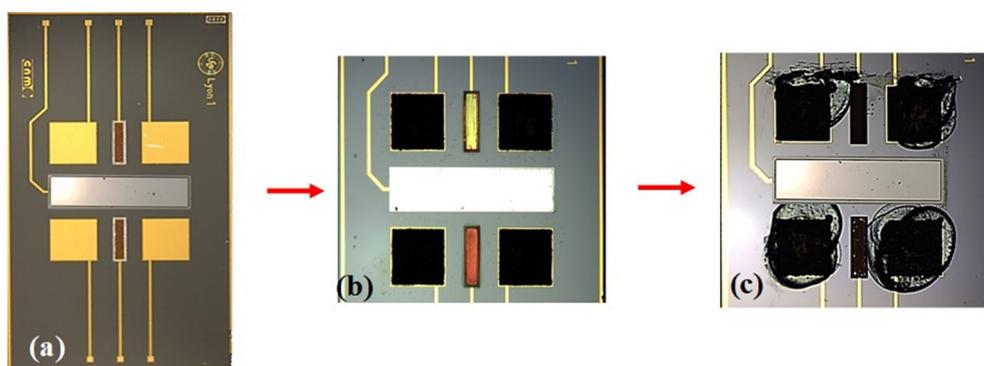


Fig. 3. Micrographs of (a) transducer with bare electrodes. In (b) PpyCOSANE-modified microelectrodes (c) transducer holding four ammonium-selective microelectrodes.

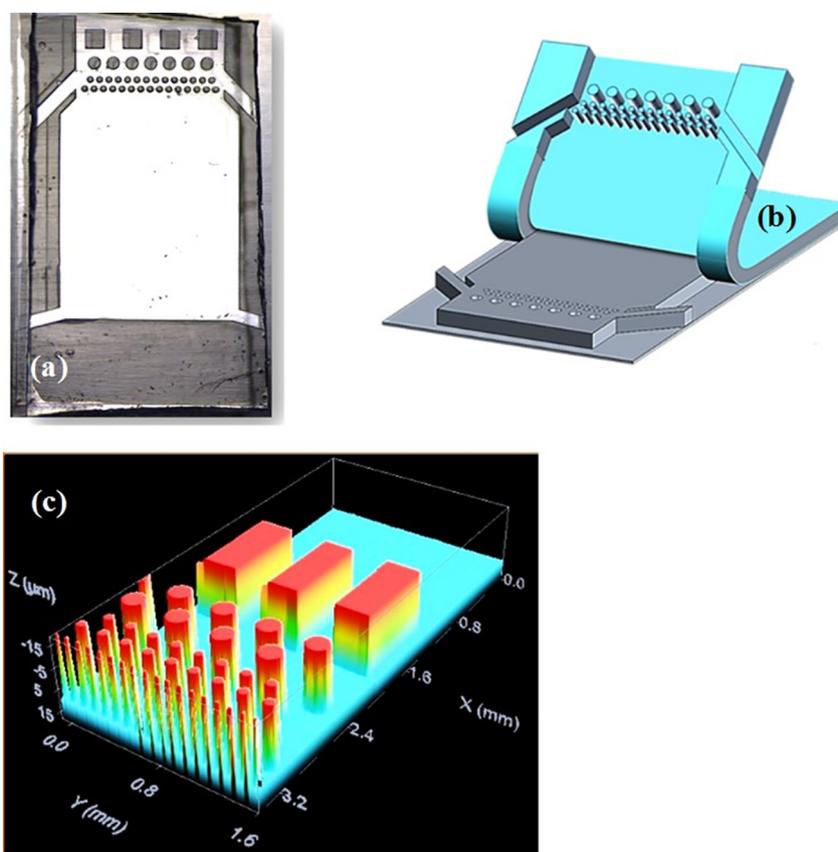


Fig. 4. (a) Micrograph of PDMS-microfluidic system obtained by replica molding. (b) Replica molding illustration (c) Interferometry image of a part of the PDMS microfluidic structure.

surfaces are put in contact. Fig. 5 shows the final device. The microfluidic system was perfectly aligned to the transducer so that the microelectrodes were inside the microchamber of analyse. Thus, they are surrounded by the microfluidic elements in such a way so the microfluidic system automatically controls the amount of sample reaching the detection chamber. Therefore, the exposure of the microelectrodes to the harsh environment is highly reduced.

For this interest, the inlet of the microfluidic system was placed in the front side and it formed a combination of pillars with different

sizes and geometries acting as microfilters and passive micromixers (see Fig. 5). The four ASEs were hosted in the detection microchamber and therefore, ammonium can be monitored in continuous. The outlets 1 allowed to evacuate the non-filtered particles so that clogging is highly minimized at the main inlet of the LOC. Afterwards, the filtered sample reached the detection microchamber where it was analyzed then automatically evacuated from the outlets 2.

3.3. Potentiometric measurements

It is well-known that for any ion-selective electrode (ISE), the most sought parameters are the slope, the limit of detection (LOD), the selectivity in the presence of a complex matrix and the time of response. The slope provides information about the electrical-charge interaction between target-compound and membrane. Thus, in the case of a monovalent cation such as ammonium, the ideal value should be close to 59 mV/decade of ammonium concentration (concentration referred to the total ammonium concentration (TAC) as an equilibrium $\text{NH}_3/\text{NH}_4^+$). The ISE's sensitivity is given by the LOD and the time of response is defined as the time needed by the sensor to achieve the 95% of the response expected when a detection event occurred. Fig. 6 present the dynamic response of potentiometric measurements carried out using the LOC for ammonium detection following the GSAM (Saxberg and Kowalski, 1979). The initial volume used for the calibration of the LOC was $V_0 = 25$ mL of buffer *tris*-HCl/*tris* (9.5:1 ratio).

Its performance was found to be comparable to those previously reported in the literature (de Beer and Van Den Heuvel, 1988) with a slope of 55 mV/decade, a limit of detection of $4 \cdot 10^{-5}$ M and a time of full response between 10 and 12 s. Results summarized in Table 1 show the ASEs performance.

As the LOC includes four working microelectrodes that can measure at the same time, the repeatability and reproducibility of the

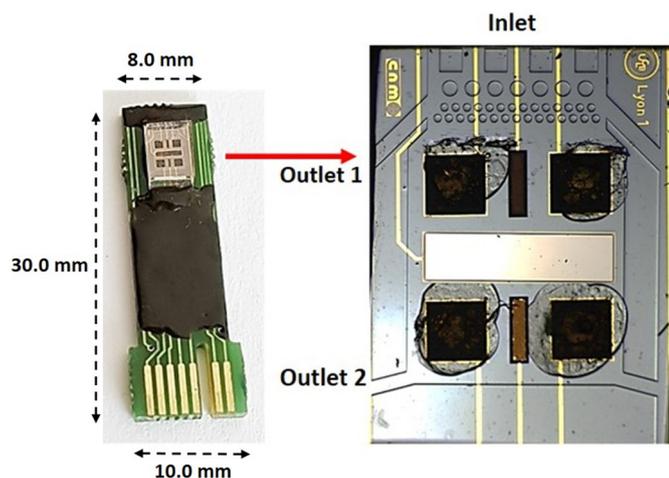


Fig. 5. Final microfluidic LOC made of transducer holding four ammonium-selective microelectrodes and on top of it the PDMS microfluidic system has been glued. The microelectrodes fit perfectly inside the detection microchamber and are surrounded by the passive microfluidic elements.

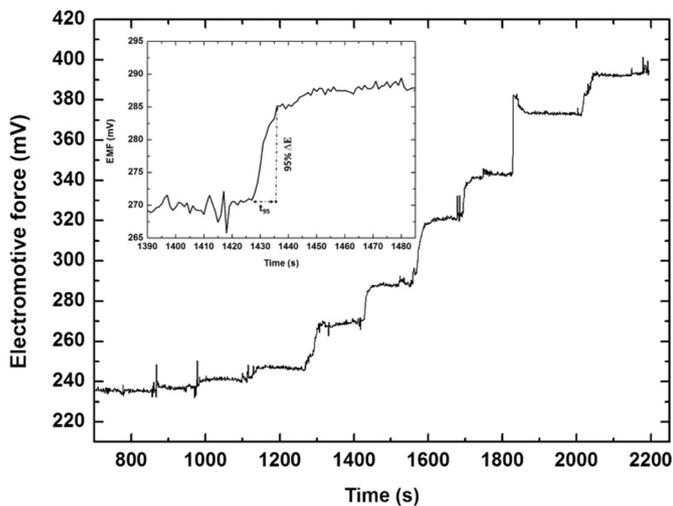


Fig. 6. Dynamic response of one of the ASES included in the microfluidic LOC for step changes in the concentration of NH_4Cl from 1 μM to 40 mM in buffer *tris*-HCl/*tris*.

measurements was also evaluated. The negligible deviations obtained (see Table 1) confirmed the good reproducibility of the ammonium-selective microelectrodes prepared.

3.4. Evaluation under real conditions

The main purpose of the LOC was to be able to perform continuous and real-time analysis in flowing water applications. For this purpose, the time of response has been assessed as a key parameter since we needed to demonstrate that the presence of the microfluidic structure does not affect the microsensors' response. Thus, the main objective was to evaluate the overall performance of the passive microfluidic LOC as a device that is able to perform in-situ and real time analysis in flowing water applications. In this context, ammonium-selective microelectrodes were chosen as control microsensors to validate the proof of concept of the passive microfluidic device. For this purpose, the LOC time of response was compared to that of a commercial EC sensor used as reference sensor during the analysis of ammonium-containing samples. Firstly, both devices (LOC and EC sensor) were placed in a beaker that contained 200 mL of tap water under stirring and the baselines were recorded until signal stabilization was reached. Afterwards, 20 mL of ammonium solution were added at $t = 10$ s. As it can be observed from Fig. 7, the LOC time of response was delayed by 15 s when compared to the EC sensor response. Subsequently, a second addition of 20 mL of ammonium solution were added once the sensors signals were stable again (at $t = 130$ s). Again, the LOC time of response was delayed by 15 s when compared to the EC sensor response.

Secondly, the device was evaluated under flowing tap water. Here, a testbed made of roof gutter (pipe diameter of 75 mm; length: 2 m; slope ~2%) with a partly dammed outlet and supplied with flowing tap water was set up. The flow-rate was set at 2 L/min using a peristaltic pump.

Table 1

Potentiometric response of the four ASES included in the LOC. Calibration carried out at initial volume, $V_0 = 25$ mL of buffer *tris*-HCl/*tris* at pH = 7.1. Standard deviation values referred to the measurements obtained with the four microelectrodes included in the LOC.

Parameter	ASE
Slope (mV/decade)	55 ± 0.7
Lower limit of detection (M)	$4 \cdot 10^{-5} \pm 8.1 \cdot 10^{-6}$
Lower limit of linear range (M)	$1.0 \cdot 10^{-4}$
Time of full response (s)	10–12

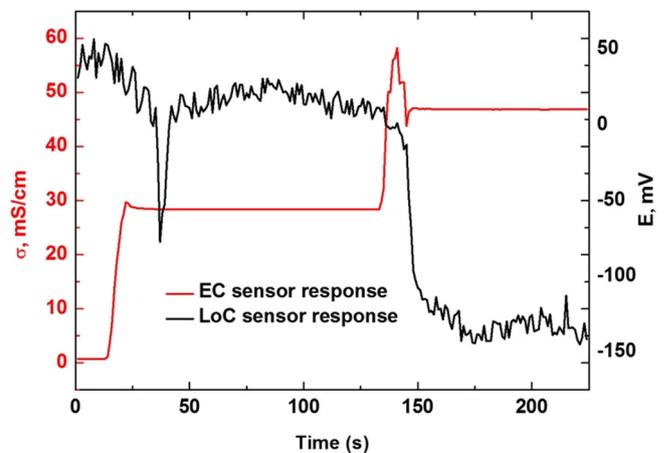


Fig. 7. LOC and EC sensor's responses to ammonium containing samples. Initial volume, $V_0 = 200$ mL of tap water.

After reaching stable conditions with tap water, 500 mL of the ammonium solution were pumped into the testbed.

In this case, no delay was observed when the time of response of both the LOC and EC sensor were compared. Since the microfluidic-system's inlet was oriented to the laminar flow applied in the pipe, diffusion is favored and therefore, the sample reaches the LOC's detection microchamber faster when compared to the previous test performed in a beaker and under stirring. Moreover, as the LOC's signal returns to the baseline right after the peak related to the ammonium detection (see Fig. 8), it was confirmed that the liquid flows ceaselessly through the microfluidic system creating a continuous stream. These findings appear to be very promising as they confirm that the presence of the microfluidic structure on top of the microelectrodes does not affect its time of response. Moreover, the results suggest that in a first stage, the LOC is filled with the sample through capillarity when it was immersed in the liquid medium. Subsequently, as the device is oriented to a laminar flow, diffusion governs the dynamic flow created throughout the microfluidic system.

Additionally, the robustness of the device was evaluated by immersion of the microfluidic LOC in a real municipal sewage pipe at the Berlin Center for Competence and Water, Germany to assess its future usefulness under extreme conditions such as sewage monitoring. The wastewater used in the test facility was pumped from a combined sewer of the Berlin sewer-network into a storage tank which was placed before the

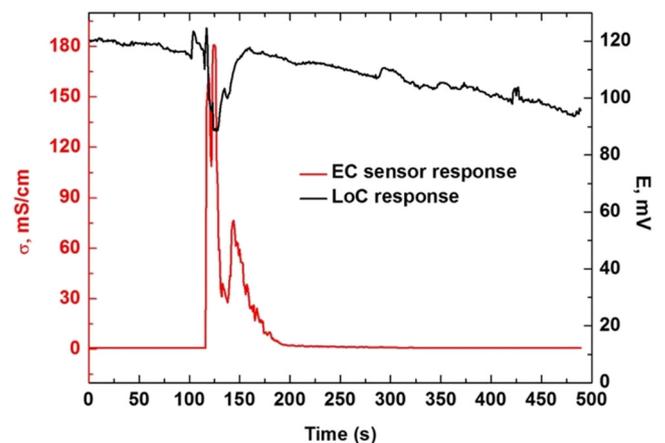


Fig. 8. LOC's and EC sensor's responses recorded when 500 mL of ammonium solution were pumped into the testbed. No delay is observed when both the LOC and EC sensor responses were compared.

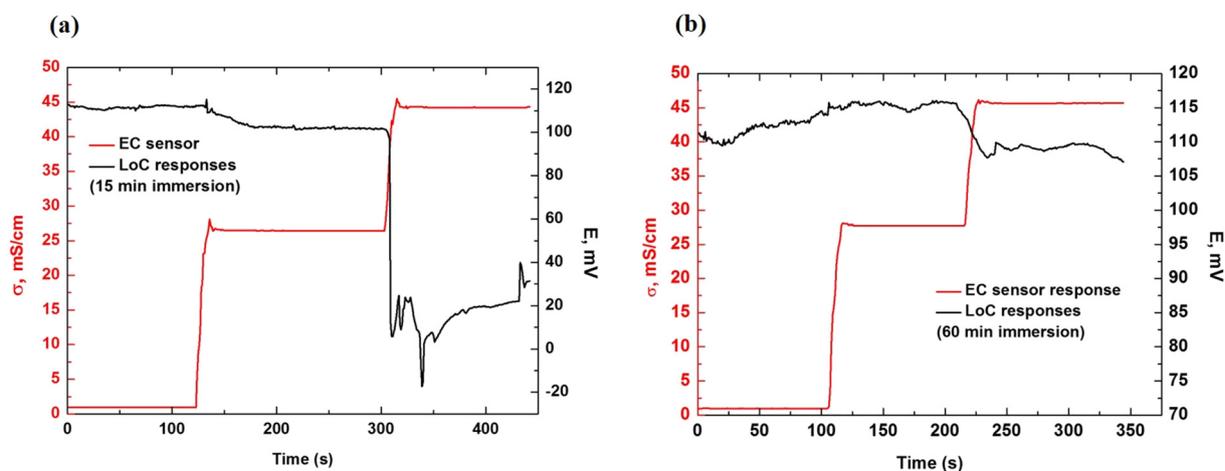


Fig. 9. LOC and EC sensor's responses to two additions of 20 mL of ammonium solution after LOC immersion in flowing wastewater for (a) 15 min and (b) 60 min respectively. Measurements carried out in tap water as the LOC was removed from the sewage.

testbed (pipe-diameter of 350 mm) at a higher elevation. A flow of wastewater up to 10 L/s could be provided continuously. The respective velocity in the pipe ranged from 0 to 0.6 m/s (and max. 0.8 m/s at 15 L/s).

In this context, the LOC was set in a plastic module especially designed for sewage application and the ensemble was immersed in the pipe to evaluate two exposure times: 15 and 60 min respectively. Immediately after the exposure to the sewage, the microfluidic LOC was removed from the pipe, it was immersed into a beaker with 200 mL of tap water under stirring together with the commercial EC sensor. The objective was to check again the LOC response but this time after being submerged into the wastewater stream for 15 min. Firstly, the sensors baselines were recorded. Once LOC and EC sensor reached stable conditions (at $t = 125$ s) 20 mL of ammonium solution were added. Here both EC and LOC had the same response time. Subsequently, once the LOC and EC sensor signals were stable again, a second addition of 20 mL of the ammonium solution was added at $t = 300$ s. Once again, it was observed that the response time seen for both the LOC and EC sensors was the same. Fig. 9 (a) shows the LOC and EC sensor responses to ammonium after being immersed in the wastewater stream for 15 min. For both injections, the LOC time of response remains in the same order when compared to the EC sensor's response. This confirmed that the LOC was still functional after 15 min immersion in sewage and therefore no clogging phenomenon was observed.

The same previous experience was repeated with 60 min immersion of the LOC in sewage. In this case, the LOC only responded to the second addition of 20 mL of the ammonium solution (see Fig. 9 (b) $t = 180$ s) which might be a sign of partial clogging. Moreover, the time of response of the LOC to the second injection was about 20 s delayed, when compared to the previous test where the response was immediate. Therefore, even if the LOC was still functional, the results confirmed the partial clogging at the inlet of the LOC when it was immersed in the wastewater for 60 min. Partial clogging consisted on fibres and particles acting as a porous dam at the inlet of the microfluidic device. Consequently, a decrease of the flow-rate inside the microfluidic system occurred as the sample started to diffuse more slowly through the LOC and therefore the response time increased. As it can be observed in Fig. 9 (b), the LOC response start at 180 s, after the second addition of ammonium and finish at 220 s. A time of response of 40 s approx. is a sign of sample diffusing through the partially clogged inlet of the LOC. To minimize clogging and so to perform real-time analysis for longer exposure times, a combination of passive and active microfluidics is under development. For this purpose, we are currently working on the design of a microfluidic manifold that will contain a piezoelectric micropump connected to the outlet of the LOC. Therefore, when the device will show signs of clogging, a small amount of buffer will be

injected into the LOC using the micropump in order to flush the system out of particles.

4. Conclusions

A microfluidic Lab-on-a-Chip for real-time potentiometric measurements in flowing water has been developed. The device was manufactured by irreversible bonding of two subunits: a PDMS-flexible passive microfluidic structure and a transducer holding an array of four gold working microelectrodes, two Ag/AgCl reference microelectrodes and one Pt auxiliary microelectrode. As a proof of concept, the LOC was chemically functionalized to incorporate four ammonium-selective microelectrodes and therefore, ammonium-containing samples were analyzed in flowing water successfully. As a result, it was demonstrated that a continuous water stream is naturally created through the microfluidic LOC when it was immersed in a laminar flow of water and, what is more important, without the need of an external pump or actuator. Thus, the power consumption is reduced to zero and therefore, it is extremely helpful for applications in inaccessible environment. The test of robustness confirmed that the LOC was still functional and responded to ammonium-containing samples after being immersed in sewage for at least 15 min. Therefore, the low-cost, low-power consumption, easy-to-operate and miniaturized LOC presented here can be used for in-situ and real-time potentiometric measurements in flowing water locations. Using the same strategy, one can include almost any kind of electrochemical sensor in the LOC and perform water quality assessment in situ and real time for environmental applications such as sewage epidemiology, quality controls in a river, aqua culture outlets or "off shore" fish cages among other.

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