

Applications of autonomous microfluidic systems in environmental monitoring

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Concern about the environment is increasing and so is the search for analytical methods that make continuous monitoring possible. Microfluidic devices such as lab-on-a-chip emerge as an alternative to the laboratory-based conventional techniques, making possible the development of unmanned monitoring tools. This review covers the last five years on the application of autonomous microfluidic devices for continuous environmental monitoring and addresses the existing demands in this field.

1. Introduction

A quick look in the newspapers is enough to realize how concern about the environment is increasing. And the more important the environment is, the more important environmental analysis and monitoring become.

Environmental monitoring is one of the main instruments in pollution control and is of paramount importance for human health, as demonstrated by the examples reviewed by Delattre *et al.*¹ However, while investigative analytical demands are completely satisfied by the available technology, predictive evaluations still need more effective, continuous, autonomous and on site analysis systems, in order to make possible the permanent monitoring and instant detection of problems, in contrast to the prevalence of laboratory-based analyses using traditional techniques, making them dependent on sample collection, transport and storage. Each additional step has a negative effect on the time needed to obtain results and mitigate damages, as well as the risks of contamination and degradation.^{2–4} These steps may be easily avoided by *in situ* analysis strategies, resulting in more effective monitoring. Among the large number of technologies available for this purpose, microfluidic devices offer the advantage of being field deployable, using small samples and reagent volumes, making waste management easier, as well as being simple to assemble.

The first microfluidic tool for analytical purposes was probably the gas chromatography presented in 1979 built on silicon.⁵ Notwithstanding, microtechnology has gained interest in the last two decades with the first commercial device being available only in 2001 (Agilent, EUA). Microdevices are advantageous for many applications due to the small amount of sample and reagent consumption and consequent waste

production, fast analysis, ease of integration and high degree of automation.³ The latter two aspects make miniaturization particularly attractive for environmental analyses. Ease of integration results in the development of Lab-on-a-chip (LOC) able to perform multiple sample processing steps, while the high degree of automation avoids external contact, preserving both sample and operator from contamination.

Nowadays such systems find uses in many different areas, from analysis to drug delivery. In environmental monitoring, they are commonly employed for water quality evaluation, with less publications related to other matrices.⁶ As an example of *in situ* environmental monitoring instrument, it is possible to mention the apparatus proposed by Salin's group.^{7,8} The device for nitrate and nitrite monitoring in water samples is disposable and uses the Griess reaction, the same one used on a mesoscale as the official method. In these analyzers solid reactants are placed on the bottom of a detection chamber during the fabrication steps, remaining confined during use and avoiding contamination. The device is built onto an audio compact disk (CD) polymeric layer and a flow can be pumped by centrifugation, avoiding external pumps. A conventional photometer is used. It can be used in field-analysis, decreasing sample transport and storage and consequently lowering damage to the samples.

Despite the large number of disposable microchips described in literature, like the one previously discussed,^{7–9} the highlight is probably the development of field deployable systems (FDS). This review focused on microfluidic strategies based deployable devices, and FDS will herein refer to such systems. Such systems consist of one or more LOC assembled and linked to electronic parts and sample collection apparatuses. FDS can be used *in situ* and left unmanned during long periods. This makes the detection of spikes in pollutant concentration possible allowing faster responses. This change in paradigm can be exemplified by examining the works of Ahn's group.^{9–13} Their first paper presented a disposable heavy

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metal detector, but three years later they published an FDS for the same application, using similar strategies.

The choice for long-term devices, instead of observing the premise of “cheaper, smaller, faster” that rules most of the development in microfluidics, involve adjustments to the design, material and operation of LOC. In this review paper we discuss some of the main issues involved in environmental monitoring applications made by FDS. We also discuss some successful examples, focusing on chemical contaminants. Pathogen detection is another important aspect of environmental pollutants, but the use of microdevices for their analysis was recently revised by Moon *et al.*¹⁴ In addition, this paper concentrates on unmanned continuous monitoring, while the general environmental applications of microfluidics was recently reviewed by Jokerst *et al.*¹⁵

2. Modifications demanded for the use of microfluidic devices in field deployable systems

Many groups involved in the production of microfluidics devices for FDS investigated microfabrication techniques^{16,17} or developed support structures, such as pumps^{18–21} and valves.²² These papers show the importance of adjustments. In this section we present solutions found in literature for one or more problems faced in unmanned environmental monitoring. Not all devices mentioned here are FDS, but all of them represent a way to reach this goal.

2.1 Adjustments to make long-term use possible

Due to long-term use of these microfluidic systems, the long-term stability of both the FDS components and the process is crucial. Detection strategies, materials, fabrication methods and on-chip reactions need to be planned having this demand in mind. The changes demanded in detection strategies and device design to achieve long-term uses will be discussed.

2.1.2 Adaptations in detection strategies. Detection for long term uses cannot depend on human handling or large equipment structures. Due to this most devices use photometric, fluorescence or electrochemical detection. These strategies have the advantage of using simple electronic circuitry that may profit from microelectronic advances. The former became possible when Light Emitting Diodes (LED) became popular. LEDs are used as light sources while a photodiode on the opposite side receives the non-absorbed or emitted radiation. It is possible to couple both of them to the chip in different positions: alignment of the beam on the channel direction, as done by the group from the National Oceanography Centre (NOC),^{2,23–25} results in longer optical paths, although perpendicular to the channels, more often found in literature (Fig. 1), may result in higher limits of detection due to the shorter optical path.

Electrodes are another common option, since they can be easily fabricated in small dimensions using traditional techniques, which are also compatible with other microfabrication process. When applied to long term and field

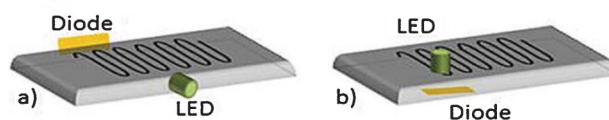


Fig. 1 Illustration of two possible alignments for detection coupling. (a) Along the channel direction, (b) perpendicular to the channel.

deployable devices all miniaturized detection techniques need to undergo modifications to be adapted to environmental conditions beyond those implicit in downscaling.

In the case of photometric detection, long term unmanned operations demand storage of all reactants and products involved in color formation reactions. For that purpose, reservoirs need to be coupled to reaction reservoirs and chambers. The larger their size, the larger the unmanned operation time, but increasing reservoir dimensions may imply increasing the final size of the FDS. Some authors developed independent external storage structures, which may be replaced without affecting LOC operation.² They may be changed once a week while the system remains deployed for longer periods. When positioned in places with abundant particulate matter, filters used to prevent occlusion may need frequent replacement too.²⁶ Another solution to size problems is the design of recycling structures resulting in lower amounts of consumed reactants. This change in design is particularly critical when toxic reactants are used, but, despite being mentioned,² it has not been used in any of the evaluated papers. The insertion of these structures may result in larger LOCs, but the increment in operational time would be reasonable in the particular case of unmanned environmental monitoring.

Storage also means solution stability. They need to endure temperature, pH, acidity, light incidence and concentrations present in the environment, among other factors. Some sample collectors used nowadays face temperature and pressure variations of 30 °C and 200 atm, respectively, each 15 days.²⁷ During unmanned operation the whole FDS must withstand these conditions. Large gradients also demand stable materials and methods for analysis, or systems suitable for reconditioning or recalibration. Therefore careful choices have to be made, mainly in methods based on enzymes and antibodies.²⁸

An emblematic example of changes needed due to long term use may be seen in the publications of Diamond and co-workers.^{18,26,29–33} The final objective was phosphate detection in estuarine water. Phosphorous containing compounds are usually detected using molybdenum blue based photometric techniques. In this method, the sample reacts with ammonium molybdate producing an intermediate molecule, which can be reduced by ascorbic acid, hydrazine or bisulfite to a blue complex, which can be detected photometrically. However, ascorbic acid, commonly used in the reaction is quite unstable and can be easily deteriorated. Additionally, its presence induces precipitate formation, initially finely dispersed, but resulting in channel clogging after long periods. Both problems may be solved by substituting the second step

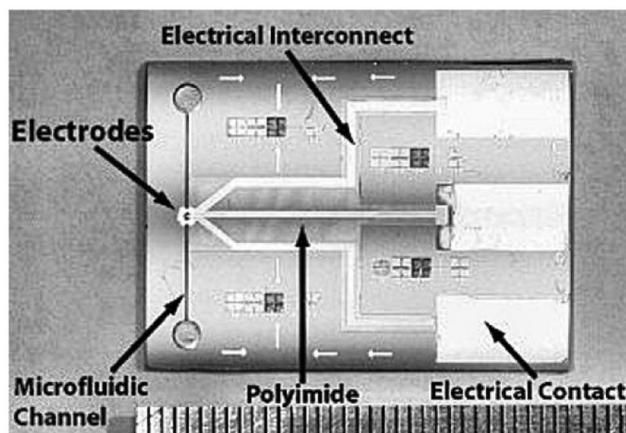


Fig. 2 General design of a microfluidic device using electrochemical detection extracted from Kim *et al.*,³⁵ reprinted with permission. Basically, microchips contain a microfluidic channel for electrode surface regeneration and sample injection and three electrodes with contact pads. These structures are indicated by arrows in the figure.

by a different reaction, resulting in vanadomolybdophosphoric acid, detected at 400–470 nm. The negative aspect of this technique is the need of inert polymers, since the acid formed during the reaction is very aggressive and may affect metallic parts.³⁴ Thus, the microsystem should be planned so that all electrical components remain protected. Stability of reagents also affected the project described by VanderNoot *et al.*,²¹ detailed later. In this case, a more stable fluorophore was used, instead of one that would result in lower detection limits.

Cleaning and calibration are also challenging aspects related to detection. Many devices depend on water or standard solution circulation for this, demanding additional valves, channels and reservoirs and increasing design complexity. The issue is clarified when comparing the LOC published by Kim *et al.*³⁵ and Li *et al.*³⁶ for chronoamperometric/chronocoulometric detection of nitrate. In these kinds of electrochemical detection, microfluidics can be beneficial to the electrode surface regeneration, avoiding the current variations due to interfacial concentration changes that may occur in stationary analysis. For this reason, many LOCs based on electrochemical detection have similar designs (Fig. 2).

The Kim *et al.* approach is very simple, while the Li *et al.* approach, which makes use of copper electrodes (more sensitive but less stable), provides a calibration step and has additional channels and a reservoir for the reconditioning solution. The circulation of cleaning solutions restores the electrode to a non-passivated condition by removing its superficial layer. In the first case, where no cleaning process was planned, some spikes were identified when thin film silver electrodes were used. Instead of cleaning the electrode, Martinez-Cisneros *et al.*³⁷ developed a removable one. Thereby the passivated electrode can be replaced without modifying the entire chip. To avoid leaking in the electrode access point, a seal was used, representing an additional design complexity and demanding periodic access to the system. Besides, calibration of every electrode before its use is

needed to guarantee its reproducibility. The last examples illustrate also the importance of the materials in fabricated LOCs.

The impact of electrode material on prototype development can be evaluated by papers published by the Ahn and Bishop groups.^{9–13} These authors decided to replace conventional, efficient but pollutant mercury by more ecological bismuth electrodes in the device used for square wave anodic stripping voltammetry. Usually bismuth electrodes are produced *in situ*, i.e. metal is mixed into the sample and simultaneously deposited into carbon electrodes. In continuous use devices, this technique may lead to metal leakage and, in pH above 5, may induce undesirable composites resulting in channel clogging. Due to these problems, an electron beam was used as an alternative deposition technique. Other microfabrication techniques could be used, such as sputtering³⁸ or electrochemical deposition, prior to chip sealing, eliminating metal excesses and induced precipitation. Additional modifications may be used to improve electrode sensitivity. Liu *et al.*,³⁹ for example, covered electrodes with carbon functionalized nanotubes for trinitrotoluene detection in water samples, with limits of detection close to 1 ppb.

Another difficulty found in the miniaturization of electrochemical detectors is the reference electrode. In conventional systems it usually remains immersed in KCl solutions, a condition hard to achieve in autonomous microfluidic autonomous devices. To replace these solutions, polymeric membranes containing chloride ions can be used. Another alternative is the use of immobilized particles in channel walls, isolating the solution from the main channel.¹¹ A difficulty with these particles is the risk of displacement in case of mechanical shock. On the other hand, membranes can hardly be replaced *in situ*.

Finally, another detection form that is beginning to be miniaturized is surface enhanced raman spectroscopy (SERS). Like photometry, LEDs are used as excitation sources. Photons are collected by optical fibers, being directed to a portable spectrometer, placed close to the device. Optical fiber communication still represents a physical limit for device independence, but this setup makes possible the use of SERS in estuarine water analysis, or marginal installations.⁴⁰

2.1.2 Improving the lifetime of the devices. The long and continuous working time in non-controlled conditions influences the type of material used in the device, since it is necessary to develop resistant structures, compatible with the environment, so the LOC does not lose efficiency or become hazardous to the ecosystem. The main parts of microfluidic systems used nowadays are made in poly(methyl methacrylate), PMMA, poly(dimethylsiloxane), PDMS, and cyclic olefin copolymer, COC. These materials presented good thermal, chemical and mechanical resistance, biocompatibility and low cost. However, they are naturally transparent, increasing backlight problems in photometric detection, quite common in nutrient analysis.^{2,7,8,18,23,24,26,27,29–34,41} Among the solutions described in the literature is the use of dark covers around the detector zone²⁵ or the whole LOC.^{18,29–33} The devices developed by the NOC and the Nano Group, both from Southampton University,^{2,23,24,27,34} were built in tinted polymer. As colour is uniform in the whole structure, the light

absorbed in the regions between the detection cell and the light source and the detection cell and the photodiode need to be considered in the calculations. The authors estimated 51.5% losses in each region.²⁴

When compared to other polymers usually employed in microfabrication, PDMS absorbs close to 10 times more water, which can result in matrix compound absorption, resulting in cross contamination and efficiency loss. This effect is even more pronounced with some organic solvents.^{3,42,43} Due to these characteristics, PDMS is not commonly used in FDS systems.

Which of the needed apparatuses will be integrated to the LOC is another important decision to take. As was already mentioned, some facilities, such as filters or reservoirs, need to be changed more often than others and it would be appropriate to keep them external to the microchip, making frequent access easier. Additionally, building many components in a single structure may increase its complexity, making the fabrication process expensive or not viable, whereas connections are more likely to produce leakages, instabilities and errors, and might receive special attention.⁴⁴

Whenever sealing is necessary, it must be carefully chosen, having in mind the need to resist environment conditions. Among traditional techniques, thermal methods are the ones resulting in less mechanical resistance of the bond. The use of solvents usually results in better sealing, but processes need more precise control. An example is the paper published by NOC and the Nano group about a pre-treatment attempt to obtain less roughness in the channel and better sealing in the final device. Each layer is exposed to chloroform vapour before pressure and temperature application.⁴¹

Another problem resulting from the attempt to join many functions in the same chip, shared with other microfluidic areas, is the incidence of bubbles, generated or trapped, despite laminar flow. While negligible in one-use devices, this drawback is a limiting factor for FDS, producing anomalous results, as reported by McGraw *et al.*,²⁶ in which the working system was interrupted due to bubbles each 12 h. Some design characteristics aggravate the problem, such as sharp angles, in whose edge bubbles can be trapped, while others have the opposite effect, like serpentine. Roughed surfaces are also prone to bubbles,⁴⁵ it being interesting to use polishing techniques like the one presented by Ogilvie *et al.*⁴¹

Finally, it is necessary to plan the autonomous operation time according to energy sources. The most common solution is the use of 12 V lithium batteries. Some authors report autonomous periods of 7 days using this source.²⁶ When an FDS is carried by vessel and remains connected to it, it is possible to use the connection cables to deliver energy and feed the device as observed in the sensor designed by Fukuba *et al.*⁴⁶ The system was fed by a 24 V power supply placed in the research ship and sent the data by the same cable group.

It is already possible to find in the scientific literature the design and testing of alternative energy sources, planned for use in microscale devices.^{47–49} However, to our knowledge, no actual use in environmental FDS has been reported, probably because most of the works on sources is still incipient. Many are in the first stages of application, and others have presented

just proof of concept, not yet having sufficient maturity for a real LOC integration.

2.2 Strategies to improve device's autonomy

Since most analyzed matrices are complex, with a huge number of components, environmental samples may demand some pre-treatment. Integration decrease risks and contamination during such processes⁴ but, as samples cannot be handled out of the microchip, it is necessary to develop strategies to speed up the processes, since separations, reactions, and mixing should occur in a confined space, during a short time.

Concentration of the analyte can be obtained statically (extraction, adsorption or filtration) or dynamically (electrophoretic stacking, focusing, among others).¹⁵ In the particular case of bacterial contamination monitoring, it may be necessary to grow cultures.¹⁵ This objective can be reached by the selective immobilization on the system walls.¹⁵ In this case, the material should be chosen having in mind the need for biocompatibility and the retention of antibodies and nutrients needed. Among the most popular strategies found in the literature for the different sample pre-treatments that may be demanded, it is possible to find nanoparticles and channel modification.⁴

Autonomy is strongly dependent on the time and space consumed by each sample processing step. So, any modification able to speed up pre-treatment or analysis is helpful. In this section the strategies used to increase the mixing rates or efficiency in the separation process and particular tools like membranes and particles that can be employed with different functions will be discussed.

2.2.1 Increasing mixing rates. Concerning compound mixing, one of the main microfluidic disadvantages is the predominance of laminar flow with low Reynolds numbers. In these systems, diffusion is the main mass transport process, resulting in longer mixing and reaction times. A simple solution is the use of passive structures inside the micro-channels, such as wings,²⁵ among others. Bhagat and Papautsky⁵⁰ evaluated different passive mixing geometries in order to reach good particle dispersions.

Another way to solve the same problem is the use of active tools, based on valves, pumps and actuators. An example of active mixing is multi-commutation.^{44,47,48} This system alternates inputs from different flows in a high frequency, creating small zones of each one, more easily mixed.

2.2.2 Separation step. Capillary electrophoresis (CE) is probably the most common dynamic separation technique used in microchips,^{4,15} but rarely found in FDS applications. However, Ding *et al.*⁵¹ applied CE to the separation of phenols, using different surfactants added to the background electrolyte. When absorbed by channel walls, they cause changes in electroosmotic flow, increasing process efficiency. Systems based on capillary electrophoresis also represent a large number of commercial devices used in one-use environmental analysis, probably due to the ease of production. Shen *et al.*⁵² used a Hitachi (Japan) system to evaluate contamination in some Japanese rivers.

An exception is made by the Lab-on-a-Robot developed by Garcia's group^{53,54} for gas sample analyses. They are one of the

FDS to use capillary electrophoresis. The robots are controlled by electronic systems, whose complexity is much greater than the ones involved in electrophoresis control. In this way, the increase in complexity added to the device by the separation is negligible and analysis efficiency increases significantly.

2.2.3 Particles. Micro and nanoparticles are mentioned in the literature as a way to increase efficiency in sample preparation, usually in separation and concentration steps. A good example of the use of larger particles are the results reported by Wei *et al.*⁵⁵ These authors used 400 μm silica particles functionalized with C30 chains in the concentration of herbicides. This avoids salt and debris being transferred to the following steps.

Yazdi and White⁴⁰ reported the use of silver nanoparticles in order to trap and concentrate herbicides in a channel region, where they were measured. A LOD of 0.05 ppb was obtained for the fungicide thiram and 63 ppb for melamine using this process.

Magnetic particles offer the additional advantage of being easily controlled by magnetic field application, being easily positioned and displaced. Martinez *et al.*⁵⁶ used this property to immobilize particles into the desired channel region, using them for both separation and concentration of the sample. The same method was used by Ramadan and Gijs⁵⁷ to purify samples. By applying and removing the field over a sample–nanoparticle mix that flows through the channel it is possible to form and destroy particle agglomerations that trap, carry and release the analyte in a cleaner area in each cycle, increasing purity.

2.2.4 Membranes. Membranes are usually used in the literature as a way to filter samples, avoiding anomalous behaviour associated with channel clogging due to trapped particles. A paramount aspect in the use of membranes is the pressure drop caused, which may compromise the performance of pumps and actuators. Noblitt *et al.*⁵⁸ described microchips used for capillary electrophoresis separation in which they used the pressure drop induced by a membrane as a way to control the pressure applied in the electrophoresis sample input, responsible for changes in flow profile. However, they also observed that compounds retained in the membrane may cause cross contamination, being necessary to clean it after each analysis.

2.3 Adaptations to make continuous monitoring possible and efficient

Some authors indicate that stopped flow with multiple parallel reaction chambers may be more efficient in achieving high sampling frequencies, because new samples can be collected before the previous one has flowed through the detector. The use of additional valves and a more elaborated flow control to implement stopped flow is necessary, but analytical frequency may increase significantly.^{2,29,34} A good alternative to this process, when the LOC involved is simple, is using multiple LOC for parallel processing.

Finally, it is necessary to plan data handling. The electronic apparatuses in the FDS should have data acquisition and communication systems, to send data to the lab in real time. In some cases the electronic system is packed with the LOC.²⁶ The most advanced device uses GSM communication technol-

ogy to receive commands and send data, being controlled by a computer placed in the laboratory.²⁶ Other papers present cable connection, similar to internet protocols, limiting sensor freedom² and makes it useful only in wastewater plants or small lakes and rivers. Radio communication has also been used.³³ In this case, the antenna has to be placed above water level, which may become a problem in deep water analysis.

2.4 Adaptations to make system locomotion easier

Nowadays, the main part of FDS remains static during the whole operation time, or is transported from one analysis site to another by ships. A possible step, in order to increase autonomy is using Lab-on-a-Robot technology. To our knowledge, robots seem to be used only for gas samples^{53,54} but may be adapted. In rough waters it is possible to find an equivalent structure: the autonomous underwater vehicle (AUV), described by Johnson and Needoba.⁵⁹ These carriers are still very large, since they support all their own operational components and the ones needed by the FDS. While dry vehicles were adapted from motorized tricycles, the AUV reaches 3 m in length. Their displacement of water may unbalance the ambient around it, being useful mainly in hard to access or dangerous places and operations, like explosive detection.⁵⁹

3 Examples of microfluidic systems applied to environmental monitoring

Despite many challenges involved in the design, development and fabrication of FDS, it is possible to find in the literature some examples of successful devices used in water monitoring. The following examples still deal with common analytes, but represent the application of the advanced concept and technology developed for LOC. The examples presented in this section were chosen in order to give a good perspective of the state of art and the alternatives available in literature. Based on the class and function of the compounds, examples were separated in five sections: nutrients, which include phosphorous compounds and nitrite; heavy metals, focused on metal ions in its free forms; physical-chemical properties, when a parameter is measured instead a compound determination; warfare agents, usually more complex molecules, which detection is based on different strategies, and finally air samples, that demand different handling and pre-treatment.

3.1 Nutrients

Excesses of nutrients may result in eutrophication, *i.e.* an excessive increase in the algae population. Some micronutrients may be found in water, like zinc, copper and manganese, but phosphate, nitrate and nitrite represent the main risks. Usually associated with human activity, these species accumulate in water due to lixiviation and as residues of farming activity or failures in water treatments.²⁶ These three principal nutrients are usually detected by colorimetric devices. Particularly in the case of nitrite, easily degraded during transport and storage, microfluidic based FDS represent good alternatives to traditional techniques.²⁴

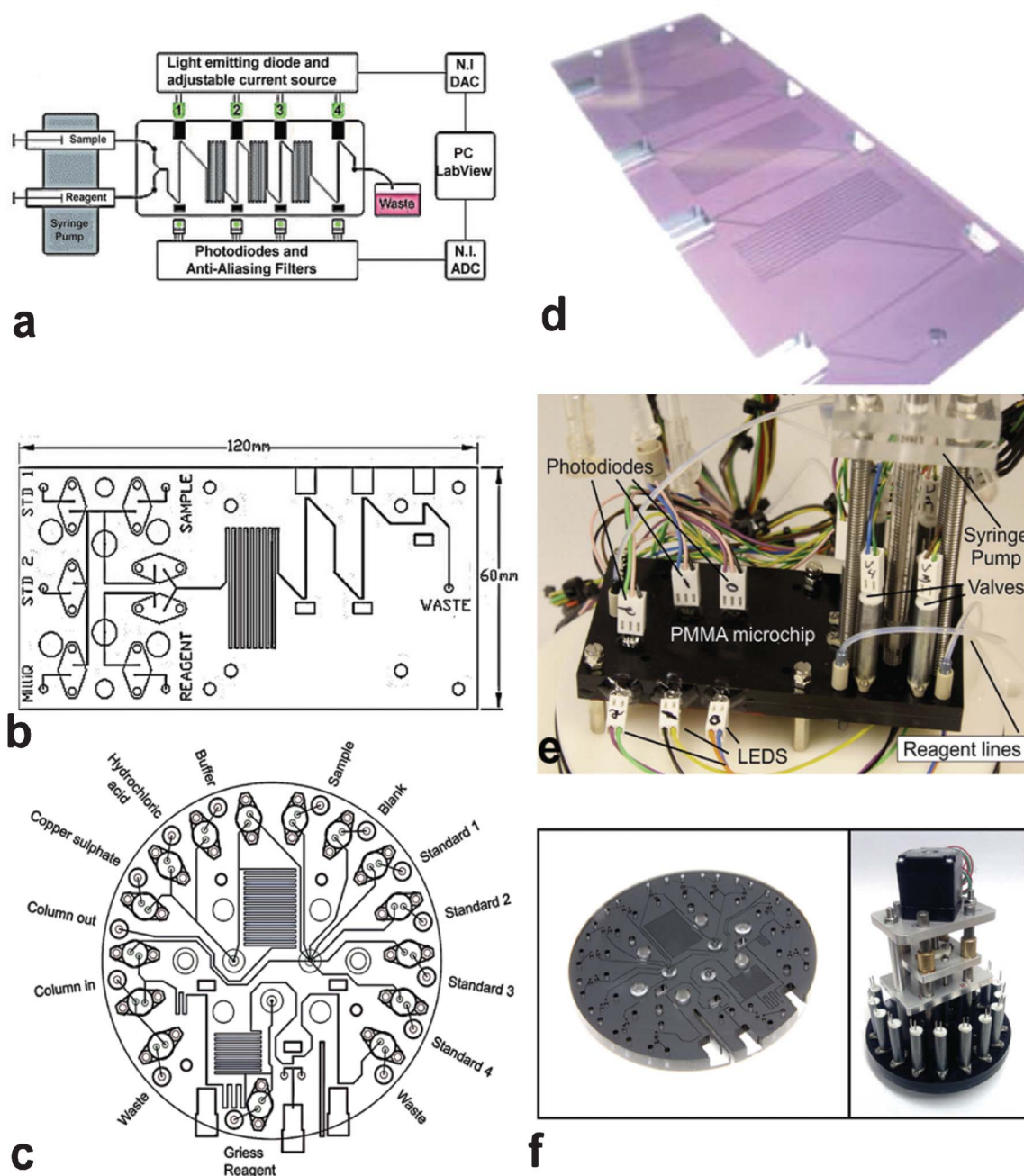


Fig. 3 Diagrams (a–c) and final device photographs (d–f) from the first (a, d), second (b, e) and last (c, f) devices produced by the Southampton group.^{2,23,24,27} Adapted from refs. 2, 23, 24 and 27, with permission.

A good example of microfluidic techniques applied to this case are the devices fabricated by NOC and the Nano Group, both from Southampton University, whose evolution can be seen in the papers describing their development.^{2,23,24,27,34} The final device was used in real estuarine water analysis. The first prototype presented (Fig. 3a and 3d) focused only on nitrite detection, using the Griess reaction to obtain a photometrically detectable product. In this method, the anion reacts with sulfonyl amide and the resulting product with *N*-naphthyl-ethylenediamine (NED), resulting in an azo compound detected at 543 nm. Miniaturization increased the

frequency of analysis.²⁴ The device was built in tinted PMMA, as described previously. The simplified design had only a $300 \times 300 \mu\text{m}$ (width \times depth) channel, divided into two sections. A larger and winding region, in which the sample was mixed, and a straighter one, 2.5 cm long, used as a detection cell. A green 525 nm LED was used as the light source. This prototype was used only for artificial samples, reaching a LOD of 14 nmol L^{-1} .

The second design (Fig. 3b and 3e) added the possibility to choose the input between samples and standards, which were injected into the channel together with the Griess reactants.

This strategy makes possible the periodic recalibration of the LOC. Another modification was the addition of a smaller detection cell, 0.5 cm long, more suitable for the detection of concentrated solutions. Channel width was changed to 150 μm in the mixing regions. Most of the control system - valves and connectors - were then built into the LOC.²³

In order to understand system behavior in real conditions, the device was combined with reactant, standards, blank, and waste storage bags and submersed in a estuary near Southampton for 57 h, taking 375 measurements that were compared with values obtained from manually collected samples. The control was made by a standalone system making possible unmanned operation. At higher concentrations the LOC presented a negative bias, justified by the difference in collection points. The authors reported an LOD of 15 nmol L^{-1} .²⁷

In the final prototype (Fig. 3c and 3f), nitrate detection was included. The high concentration detection cell was reduced once again, this time to 0.25 mm, being used for samples with concentrations above 30 mmol L^{-1} . In this device, water samples were filtered in the input and then driven to the desired analysis pathway. For nitrite analysis the sample flows through a reference cell and is then mixed with Griess reactant, before passing through a 25 cm long channel, being measured at the end. For nitrate detection, the samples are passed through a cadmium tube for reduction before entering into a process similar to the one already described. The authors mentioned the possibility to integrate the cadmium tube into the LOC.²

The device, with 100 mm diameter and 200 mm depth and consuming 1.5 W was placed for 26 days in the estuary. During this period, storage bags were brought to the surface and changed. Added to salinity and temperature data, the results obtained by this FDS were used to understand estuarine behavior as a function of environmental parameters, being one of the few cases in which the sensors developed were really converted into a practical tool.²

Despite this success, authors considered some optimizations were still needed, in order to make the device more attractive. The main changes proposed were the addition of a valve to make possible the reuse of Griess reactant, and the use of multiple parallel stopped flow, increasing analytical sampling, as described earlier. Additionally, in other published papers,^{2,27,34} authors indicated a microchip to which phosphate, manganese and iron detection would be added, but no results were described for these analytes.

Griess reaction-based photometry is the most popular technique for nitrate and nitrite detection. The same strategy was used by Diamond *et al.*,³³ who also tried their system under real conditions, with an estimated LOD of 65 nmol L^{-1} for nitrite.

They also proposed a multinutrient analyser, but presented results for differently dedicated LOC, without presenting a single FDS with multiple analyses. Da Rocha *et al.*⁴⁴ proposed the same photometric detection. This device differs from others published in the literature due to the use of green tape instead of traditional polymeric materials. Green tape is a low temperature co-fired ceramic (LTCC) structure composed by 45% alumina, 40% glass and 15% organic compounds. This

material allows for the easy fabrication of electronic controls by screen printing and presents good chemical and mechanical resistance. On the other hand, it is more difficult to create a transparent area for optical detection, which should be made through the positioning of a glass window. Another innovation in this LOC is the use of 7 LED with different wavelengths, making the system more flexible to adaptations, which was demonstrated by its use to detect phenol and Cr(VI).

As alternatives to photometric detectors, Kim *et al.*³⁵ and Li *et al.*³⁶ reported electrochemical reduction of nitrate using chronoamperometry and chronocoulometry, with LOD in the range of 3–75 $\mu\text{mol L}^{-1}$ (chip-to-chip variation) and 10 $\mu\text{mol L}^{-1}$, respectively. These limits are superior to those achieved by photometry. However, ease of fabrication may justify the option for electrochemical detection in cases where pollutants are abundant. Conversely, Jang *et al.*¹¹ used ion-selective electrodes for the potentiometric detection of nitrate and also pH. Usually, these electrodes have membranes for ion selection. Instead of this, polystyrene beads were trapped into the microchannel, restricting the flow and allowing just the target ions to reach the electrode. Target ions are helped by an ionophore solution, which is contained near the electrode by the same spheres. A similar strategy was used to isolate a KCl solution at the reference electrode. The nitrate sensor showed linear response from 10^{-5} to 10^{-1} mol L^{-1} .

3.2 Heavy metals

Heavy metals are of concern because they are not biodegradable, remaining in the environment for a long time.³ The first FDS proposed by the Ahn and Bishop groups¹⁰ used bismuth electrodes to detect Pb(II). The device was built in COC and the working electrode was deposited by electron beam. The reference electrode was covered with a polymeric membrane containing chloride ions. After Pb(II) deposition with a -1.2 V potential, detection was made by varying the applied potential between -1.2 and -0.45 V. LOD was 8 ppb, using a deposition time of 60 s. Microfluidic channels drive and confine the flow close to the electrodes and minimize bubble problems. Each FDS has seven LOC, with three electrode groups each. In each analysis, only one LOC with three groups of electrodes is used and only the valves linked to this LOC remain open, resulting in triplicate analysis for each collection. This way, the system works similarly to an interrupted flow, leading to shorter sampling times and avoiding cross-contamination.

The second device was an adaptation to detect Cd(II).¹¹ The device has a similar aspect to the one previously described, but has just two groups of three electrodes. LOD was 9.3 ppb, using deposition at -1.2 V for 90 s, and detection between -1.2 and -0.5 V. The authors evaluated Pb(II) interference and observed that the peak current is -0.85 and -0.58 V for Cd(II) and Pb(II), respectively. This difference is enough for the simultaneous quantification of both ions.

The last prototype used silver as the working electrode, reducing memory effect (Fig. 4).¹² The new electrode also presented good selectivity between the two metals ions, with LOD of 1 ppb for Pb(II). At the end of each analysis cycle, a -0.2 V potential was applied during 20 s to eliminate residual deposits. However, the paper does not report actual application of the FDS.



Fig. 4 Final prototype developed by the Ahn and Bishop groups¹² for the analysis of Pb(II) and Cd(II). Reprinted from ref. 12, with permission.

Chen *et al.*⁶⁰ presented a similar design for Hg(II) detection by differential pulse voltammetry. Using gold, silver and gold for working, reference and counter electrodes, respectively, they reached a LOD of 3 ppb. While most of the papers used electron beam to deposit metals, the authors used vapour deposition. The LOC was not coupled to a FDS, being tried only in the laboratory.

Alves-Segundo *et al.*⁶¹ presented a colorimetric strategy to detect Cr(VI) in water. Built in LTCC, the system used the reaction with 1,5-diphenylcarbazide to produce a purple product, detected at 543 nm. The LOD was estimated as 50 ppb for real samples, enriched with the target metal.

The photonic LOC presented by Ibarlucea *et al.*⁶² is an alternative to the previously mentioned strategies for the detection of Pb(II) and Hg(II), and is based on selective complexation with ligands derived from ferrocene. The resulting complexes produce new absorbance bands and wavelength shifts. In this particular case, the use of photonic strategies, like air mirrors, allow an increase in the optical path, improving detection. However, even with these adaptations, the LOD observed were 490 and 868 ppb for Hg(II) and Pb(II), respectively, higher than those achieved using voltammetry.

3.3 Determination of physical-chemical properties

Sensors for pH and oxygen demand using microfluidic techniques have also been described in the literature. These parameters are important because they indicate global changes, resulting from different factors, not always well detected by specific concentration sensors. Rérolle *et al.*⁴⁵ developed sensors to observe pH changes in oceanic waters, based on photometric detection. Determination is based on the addition of an indicator dye to a seawater sample. Accuracy was estimated in 0.001 units for short-term use. Long-term use is still under evaluation. The device was used on a cruise boat for ocean analysis during a month and made it possible to map the pH of the seawater near the Irish coast.

Zhang *et al.*⁶³ presented a device used to measure the chemical oxygen demand (COD) in waste waters. COD is a way to evaluate water's capacity in consuming oxygen during organic matter degradation. This may be the device that is farthest from a FDS and illustrates how much work is done to

optimize the LOC before coupling it in a FDS. Detection was based on the photocatalytic oxidation of the evaluated compounds. Under UV radiation, in a thin layer of water produced in microfluidic channels, organic matter is stoichiometrically converted on the surface of a TiO₂ electrode. The amount of electrons transferred to the electrode (or the charge, Q) can be measured during degradation. These authors found the relation between Q and COD as $\text{COD (ppm)} = Q/(8000 FV)$, where F is the Faraday constant and V is the applied potential. The device was tested with real samples that were brought to the laboratory without any field testing. The microfluidic sensor presented good results for pH between 4 and 10, with LOD of 0.2 ppm.

3.4 Warfare agents

Under this category, we may include biological (such as anthrax) or chemical agents, such as explosives and nervous system agents among others. Relating to environmental monitoring, explosives are probably the most evaluated substances. Veitch *et al.*^{64,65} developed a system used to detect trinitroglycerin (TNT) in water samples, using immobilized antibodies and indirect detection. Detection is achieved by competitive assay using a fluorescent probe linked to the antibody. When a sample flows into the chamber, TNT molecules displace the probes, which reach the detector and promote a fluorescent signal. As the antibody was immobilized on the LOC walls, when it loses its activity the entire system needs to be cleared. The authors observed a 300 s bleeding at the beginning of the tests, so it is necessary to consider this time during operation. Tests made in lakes resulted in efficient detection.

3.5 Air analysis

Despite water being the most common matrix evaluated in environmental analyses using microfluidics, it is possible to find some examples of air monitoring. Additional challenges may be encountered, such as increased photodegradation,⁶⁶ and gas absorption and transfer. When compared to the previously mentioned devices for water analysis, the air-based devices present increased sample collection frequency and reduced analysis time. Two examples are described to permit illustrating the complexity demanded.

Sameenoi *et al.*⁶⁶ presented a FDS for air analysis and evaluation of the oxidative activity of aerosol components. Resulting from generation of reactive oxygen, it may cause damage to tissues, DNA and proteins, mainly in the lung⁶⁷ and is aggravated by air particulate matter containing metals, such as Fe, V, Cr, Mn, Co, Ni, Cu, Zn, and Ti. In the planned system, the collection is made using a vacuum pump and the air is driven to mix with dithiothreitol (DTT) and lithium fluoride, used as internal standard. DTT is oxidized by the analytes and the unreacted DTT is driven to the electrode, where it is oxidized and analyzed by cyclic voltammetry. The less DTT oxidized in the second step, the higher the oxidative activity observed (Fig. 5).

The electrode is built in carbon and modified with Co(II) phthalocyanine (Co(II)-PC). The modified electrode presented good selectivity for catalytic oxidation of thiol compounds, such as DTT, avoiding overpotential. The microfluidic system

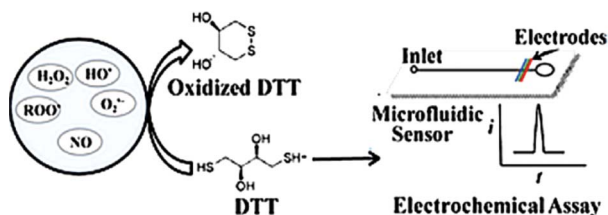


Fig. 5 Scheme of DTT analysis by Sameenoi's device.⁶⁶ Reprinted from ref. 66 with permission.

was able to measure a sample every 10 min. The electrode should be changed once a month, and washed once a day. The assays required from 7 to 214 ng of particulate mass and it was found that the DTT consumption rate correlates well with the aerosol concentration.

Another example is the system designed by Shen *et al.*⁶⁸ (Fig. 6) to detect microorganisms in air samples.

The authors used a microfluidic system, followed by field effect transistors (FET) with silicon nanowires covered with antibodies for influenza virus detection. This FDS can be described as the integration of three subsystems, responsible for collecting samples, their analysis and data handling. Microfluidics is used for sampling, in order to transfer the collected samples to the FET and then to waste, using a vacuum pump. Silicon nanowires fabricated by CVD were

connected to electrodes and used as substrate for antibody immobilization. When a virus is trapped by the antibody, a discrete shift in the operational current is observed. This prototype still has poor detection when compared to PCR, but is much faster, being a valuable alternative for continuous monitoring. The main problem in this case may be the repeatability in transistor fabrication.

Fang *et al.*^{69–71} analysed nitroaromatic explosives in air samples based on fluorescence detection. But instead of using analogous molecules, they used a fluorescent film as the substrate for detection. The fluorescent film was prepared by the dispersion of poly(diphenylsilane) in tetrahydrofuran, followed by the addition of *n*-butyllithium drops. After reaction, the solution was poured onto substrates with reactive anchors. After coupling, the structure is cleaned. When an explosive is present in the sample, the fluorescence is reduced due to interaction of the fluorescent material and the analyte.

4 Future challenges

In addition to the technical difficulties mentioned before, due to the established objective of obtaining unmanned long term use devices, environmental monitoring shares some challenges common to all LOC.

Rios *et al.*⁶ presented some data for the biochips market. In 2014, marketing should reach US\$ 5.9 billion, more than twice

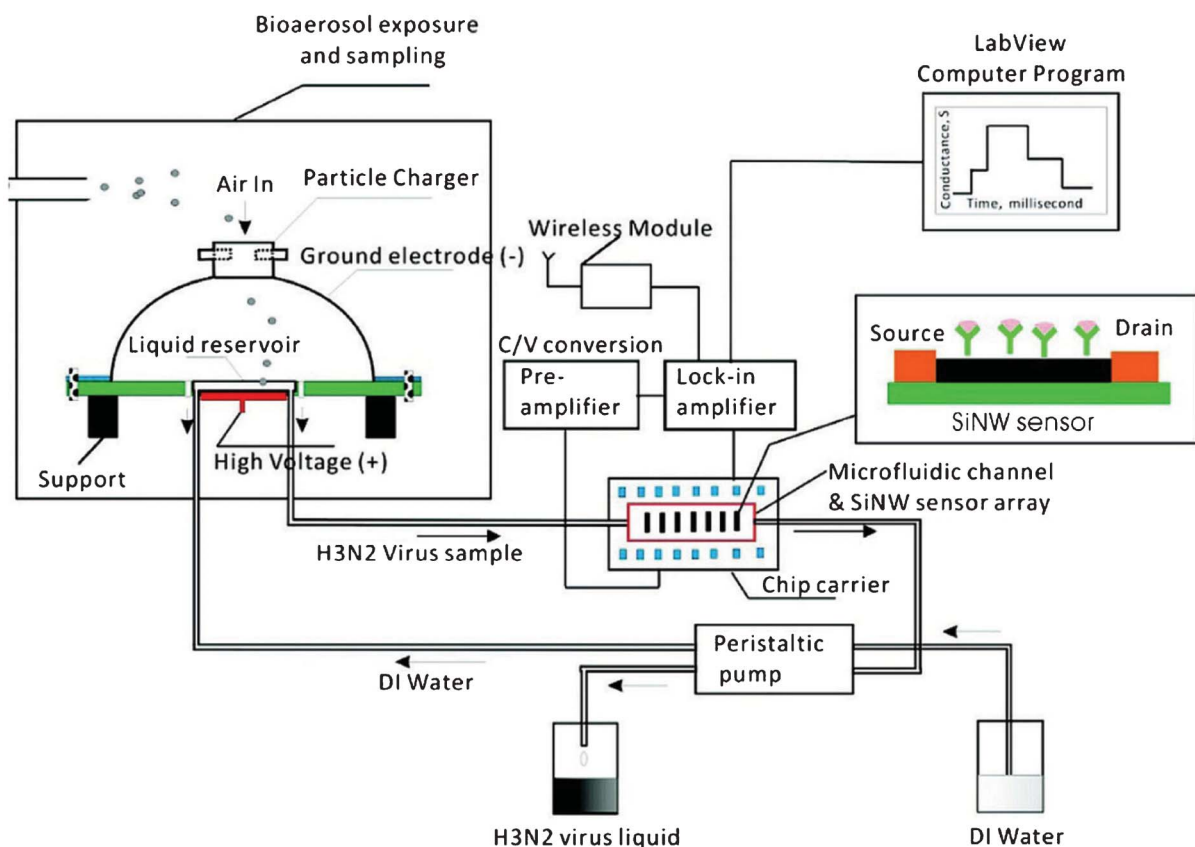


Fig. 6 Scheme of the enzymatic device developed by Shen *et al.*⁶⁸ Reprinted from ref. 68 with permission.

compared to 2009, and LOC may represent 35% of this market. Increases in daily use may increase demand for reproducibility and more efficient fabrication techniques, similar to that occurred with microelectronics. This will lead to new techniques, materials and designs, but also to a larger number of products in use, that will be disposed of at the end of their lifetime.

As mentioned by Jang *et al.*³ it is a paradox that when environmental monitoring demand increases, the fabrication of the needed devices represents a pollution source. Environmental friendly techniques should be developed, independent of toxic or pollutant reactants. Some papers mention the concern about correct disposal of the device, replacing toxic materials, such as mercury by greener ones, like bismuth. We will probably observe an increased concern on this subject in the next few years. With the spread in nanoparticle use “nanogarbage” will need to be faced. Some authors have reported contamination of water and fish due to the incorrect disposal of nanoparticles, and their effect on human organisms is not well-known yet.⁷²

At the end, it is necessary to understand that miniaturization is not only scaling down. With changes in dimensions, a significant change occurs in force ratios. This new ratio may be kept in mind during device design. The differences in force balance among *meso* and *microscale* is clear in the paper presented by Torrents *et al.*⁷³ During device development, mathematical models for electrodes used in biochemical oxygen demand were made and, based on these models, the authors report a significant impact of oxygen consumption by the electrode, which is not significant in mesoscale devices. This kind of variation, occurring in many parameters, must be considered in analytical calculations by using the original equations instead of simplified ones, by considering it in calibration curves or by treating it as an error, which may or may not be ignored. Crevillen *et al.*⁷⁴ also mentioned the small Reynolds number in which viscous forces predominate over inertia. Overlooking these aspects may compromise all the development process.

5 Conclusions

While the majority of applications are guided by the “simpler, cheaper and smaller” principle, environmental monitoring devices focus on the continuous unmanned use for multiple applications, resulting in increased complexity and final size. Changes demand the increase in robustness, mechanical resistance and the decrease in power consumption so the system could be used without an external source. In order to achieve these features the importance of fabrication costs is minimized. Many changes in design, fabrication, materials and operation are needed to reach these objectives. Monolithic LOC must be replaced by modular structures with coupling of the LOC and its auxiliary devices.

Many groups present FDS able to evaluate a single analyte, but no article introduced devices for the simultaneous analysis

of multiple analytes, although many authors mentioned this possibility and presented versatile LOC mainly based on colorimetric techniques. However, many projects represent perspectives for a new generation of LOC, which will lead to multiple-analyte FDS. They have presented new fabrication techniques, new miniaturized detectors and more efficient mixing and reaction strategies. Although water is the main evaluated matrix, we expect that applications on air and even soil analysis will grow in the near future.

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