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Short communication

Automated liquid–solid extraction of pyrene from soil on centrifugal microfluidic devices

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ABSTRACT

Organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) are present in the environment in increasing concentrations and so are of growing concern. Monitoring these species, particularly on-site, can be both difficult and expensive. This paper presents a novel miniaturised magnetically actuated liquid–solid extraction unit integrated in tandem with a filtration unit and a detection unit on a single centrifugal microfluidic device. A demonstration analyte, pyrene, was rapidly extracted and quantified by UV-absorbance from multiple soil samples. The system showed excellent performance for a system designed for field use. Characterization of two types of passive valves was performed along with an extraction time efficiency study. The system provides a factor of 150 reduction in sample weights and extraction solvent volumes and provides statistically similar recoveries to the conventional method with a pyrene detection limit of 1 ppm (0.03 μg absolute detection limit). The reduction in time and solvent and the potential for field use suggest that this device type may be valuable for environmental monitoring.

1. Introduction

Automating and miniaturizing analytical techniques to meet heightened environmental monitoring challenges is an important topic in analytical instrument design research. Microfluidic devices and, in particular, centrifugal microfluidic devices, sometimes known as "Lab on a CD" [1], offer the potential to meet many of these challenges with features including miniscule reagent consumption, low production costs, short analysis times and freedom from external connections such as pumps. These features provide compelling arguments for the development of these devices. Though centrifugal microfluidic systems are becoming well known [1,2], few centrifugal systems prepare and analyze environmental samples on the same integrated device and even fewer microfluidic devices of any type deal with solid samples such as soil [3].

Liquid–solid extraction is among the most common sample preparation techniques and is used to selectively remove analytes from a sample matrix. Unfortunately, common practice with this procedure involves large sample and solvent volumes and considerable time (labour) to process each sample. From an environmental point of view there are many drawbacks to using large solvent volumes. Microfluidic techniques have considerable advantage in that regard. As we will illustrate, the centrifugal microfluidic device also offers considerable advantage with respect to time.

Pyrene, a polycyclic aromatic hydrocarbon (PAH), is one of many PAHs being regularly monitored in aqueous and soil samples (EPA Method 610) since they are carcinogenic pollutants that are a public health risk. Regular monitoring represents large time expenditures and costs due to the sampling, shipping, storage requirements as well as an analysis by extraction and quantification by gas chromatography–mass spectrometry. With such a standard procedure, site assessment mapping of the contaminated areas is arduous. For these reasons, a smaller integrated device that could be field portable for fast and precise monitoring is desirable.

As Ducrée *et al.* [4] have described, a growing toolbox of "unit operations" such as valving [5], siphoning, liquid mixing and volume metering and splitting are now available for microfluidic systems. These "operations" can be sequentially integrated into total analysis systems and in particular centrifugal microfluidic devices. Recently we have demonstrated magnetically actuated solid sample preparation [3] and pre-concentration on miniaturised solid phase extraction columns [6] on centrifugal microfluidic devices. Using pyrene as a demonstration analyte, this paper adds magnetically actuated liquid–solid extraction of an environmental sample to that toolbox in tandem with sedimentation, filtration and detection, all on a single device.

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2. Experimental

2.1. Standards and reagents

From pyrene powder (84648-1G, purity >99%, Sigma–Aldrich, Oakville, ON) a 100 μg mL^{-1} pyrene primary standard solution was prepared in hexane (purity >99.9%, EMD, Gibbstown, NJ). Pyrene working solutions in hexane (2.0, 4.0, 6.0, 8.0, 10.0 and 20.0 μg mL^{-1}) were made daily from the primary standard solution in clean, dried 10 mL glass bottles. All solutions were stored in darkness at 4 °C. Soil samples were obtained from Saint-Jean-sur-Richelieu, Quebec, Canada [7]. Each 0.03 g soil sample was manually spiked with 200 μL of a pyrene working solution and allowed to air dry resulting in spiked soil samples with a pyrene concentration of 13–133 ppm. A series of blanks were also prepared spiking soil samples with 200 μL of pure hexane.

A 0.1 M sodium hydroxide (NaOH, Fisher Scientific, Fair Lawn, NJ) solution was prepared in distilled deionised water (DDW, 18 MΩ, Millipore Co., Bedford, MA) for pre-treating the fused silica capillaries. Ethyl alcohol (EtOH, Commercial Alcohols, Brampton, ON) was used for cleaning and pre-treatment of the capillaries.

2.2. Device fabrication

The centrifugal microfluidic devices were fabricated using a rapid prototyping technique similar to that developed by Kido *et al.* [8] Fabrication can be described in three steps: design, manufacture and assembly.

The multiple layers were first *designed* in the 3D computer-aided design (CAD) software "SolidWorks 2005" (SolidWorks Corp., Concord, MA).

Next, each layer was *manufactured* either by xurography as previously described by Bartholomeusz *et al.* [9] or by micro-milling. As seen in Fig. 1(a), Layers 2 and 4 consisted of 100 μm thick adhesive film (FLEXmount DFM 200 Clear V-95 150 poly H-9 V-95 4, FLEXcon, Spencer, MA) into which channels and vent lines including the passive valves (see valve section below) were cut by xurography with a cutting plotter (CE300Mk2-60, Graphtec America, Inc., Santa Ana, CA). The top, middle and bottom layers (Fig. 1(a)1, 3 and 5) consisted of 2.9 mm thick poly(methyl methacrylate) pieces (PMMA, Acrylite OP-1, Cyro Industries, Rockaway, NJ) which were milled to 120 mm diameter and into which chambers and center, loading and vent holes were made using a computer numerically controlled (CNC) micro-milling machine (QuickCircuit 5000, T-Tech, Inc. Norcross, GA). This particular PMMA was chosen for its good resistance to hexane and its optical properties being UV-transparent, since Layers 1 and 5 also served as the optical windows for the integrated detection cell (Fig. 1(d)3 and Fig. 2). At this point, the fused silica capillary (Fig. 1(d)5) was added (see valve section below).

Finally the devices were *assembled* by carefully aligning and firmly pressing together the layers using a hand cranked cold laminator (Jet Mounter ML25, Drytac, Concord, ON). Before laminating the final layer, 0.03 g of dried spiked soil sample and one "mobile magnet" (Fig. 1(d)7, 5.08 mm × 2.57 mm × 1.14 mm, nickel plated NdFeB rectangular magnet, magnetized parallel to width; NB045-35, Master Magnetics Inc., Castle Rock, CO) was added to each extraction chamber. The final device was a laminate of 5 layers and is illustrated in Fig. 1. Prior to extraction, all loading holes were sealed with microplate sealing tape (Nunc sealing tape T9571, Sigma–Aldrich Corp., St. Louis, MO) to avoid contamination.

2.3. Valves

Two types of passive capillary valves were integrated on the devices and their burst frequencies for hexane were studied.

The first type of valve was a rectangular passive capillary valve (Fig. 1(d)4) made by cutting fine features in the adhesive layer itself. These valves joined the extraction chamber (Fig. 1(d)1) and the filtration chamber (Fig. 1(d)2). As seen in the insert of Fig. 3(a), these valves had a fixed height of 100 μm (due to the adhesive used) while their widths were controlled by the design and xurographic cutting. Four different widths were studied (600, 800, 1000 and 1200 μm).

The second type of valve, a cylindrical passive capillary valve (Fig. 1(d)5), was made of fused silica capillaries (Polymicro Technologies, Phoenix, AZ), similar to those reported by LaCroix-Fralish *et al.* [10] These valves joined the filtration chamber (Fig. 1(d)2) to the detection cell (Fig. 1(d)3). As seen in the insert of Fig. 3(b), these valves had a fixed outer diameter (OD) of 360 μm, while three different inner diameters (ID) were studied (50, 75 and 100 μm). Before inclusion of the capillaries during the manufacturing step, the capillaries were pre-treated with 0.1 M NaOH, EtOH and air prior to being cut to 5 mm length. They were then glued into milled channels (1.0 mm wide by 400 μm deep) in the bottom side of the middle PMMA layer (Fig. 1(a)3) using commercially available 5 min epoxy.

2.4. Centrifugal system

A motorized stage, strobe, camera and fixed magnet base which has previously been described by Duford *et al.* [3] were used to spin the devices and acquire high speed still images (see Fig. 4(b)).

Each centrifugal device contained ten extraction chambers. To each of these chambers, which contained the dried spiked soil samples and one "mobile magnet" agitator, 200 μL of hexane was added. The device was then secured to the spindle of the motorized stage and spun at a series of different speeds as discussed below.

2.5. Detection system

After centrifugation, the device was moved from the motorized stage to a detection stage, though these could easily be merged into a single instrument in the future. The detection stage and the multi-wavelength ratiometric blank estimation technique are described in detail by LaCroix-Fralish *et al.* [11] and included a deuterium light source (DT-MINI-2-GS, Ocean Optics, Dunedin, FL) and a USB miniature spectrometer (USB4000-UV–VIS, Ocean Optics, Dunedin, FL). Pyrene was determined by absorbance using the 3.1 mm long integrated detection cells, as seen in Fig. 2. To account for the imprecision of these integrated detection cells, the multiwavelength ratiometric blank estimation technique required monitoring of the signal at 334 nm as the analyte's absorbing wavelength and the signal at 500 nm as the analyte's non-absorbing wavelength used to estimate the value of the blank at 334 nm.

Two different studies were completed with this configuration. First, an *extraction time efficiency study* was performed processing three replicate soil samples with the same pyrene concentration (33 ppm) at varying extraction times. With the ideal extraction time determined, the optimized automated extraction was performed as a *comparison to existing methods* processing replicate soil samples with varying pyrene concentrations at a fixed extraction time. The conventional method consisted of extracting from 5.0 g of spiked soil with 25 mL hexane in a separatory funnel, shaking for 20 min per sample, transferring the extractant to a 1 cm cuvette and determining pyrene by absorbance at 334 nm using a bench top spectrophotometer (Cary 5000 UV–Vis Near IR Spectrophotometer, Varian, Palo Alto, CA). No spectral correction (multiwavelength ratiometric blank estimation technique) was applied to the results from this double beam instrument.

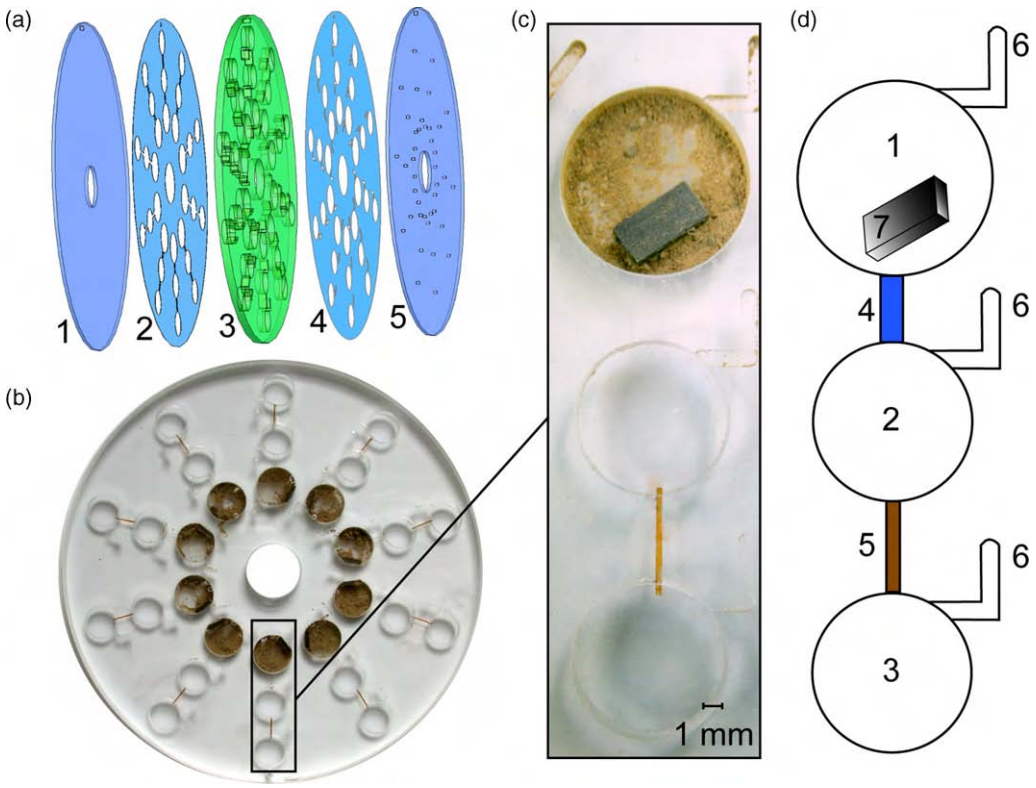

Fig. 1. (a) Exploded view of device showing following five layers 1) bottom PMMA, 2) adhesive layer including rectangular passive valves, 3) middle PMMA with chambers into which cylindrical capillaries are epoxied, 4) adhesive layer, 5) top PMMA with vent holes. (b) Photograph of device with 10 sets of chambers. (c) Enlarged view of one set of chambers. (d) Schematic of chambers including 1) magnetically actuated liquid–solid extraction unit, 2) filtration unit, 3) detection unit, 4) rectangular passive capillary valve, 5) cylindrical passive capillary valve, 6) vent holes and vent lines (0.1 mm deep × 1 mm wide), 7) "mobile magnet" agitator.

3. Results and discussion

In order to develop an effective automated controlled sequence of flows through the various stages of the analysis, it was vital to determine the *burst frequencies* using the solvent, hexane, for the two types of passive capillary valves. Typically only one type of valve is used on a device but the interaction of the two types of valves complemented themselves and allowed for greater control. As seen in Fig. 3(a), the burst frequencies for the rectangular valves ranged from 200 to 500 RPM (3–8 Hz) depending on the width. Similarly, as seen in Fig. 3(b), the burst frequencies for the cylindrical valves ranged from 300 to 900 RPM (5–15 Hz). The high repro-

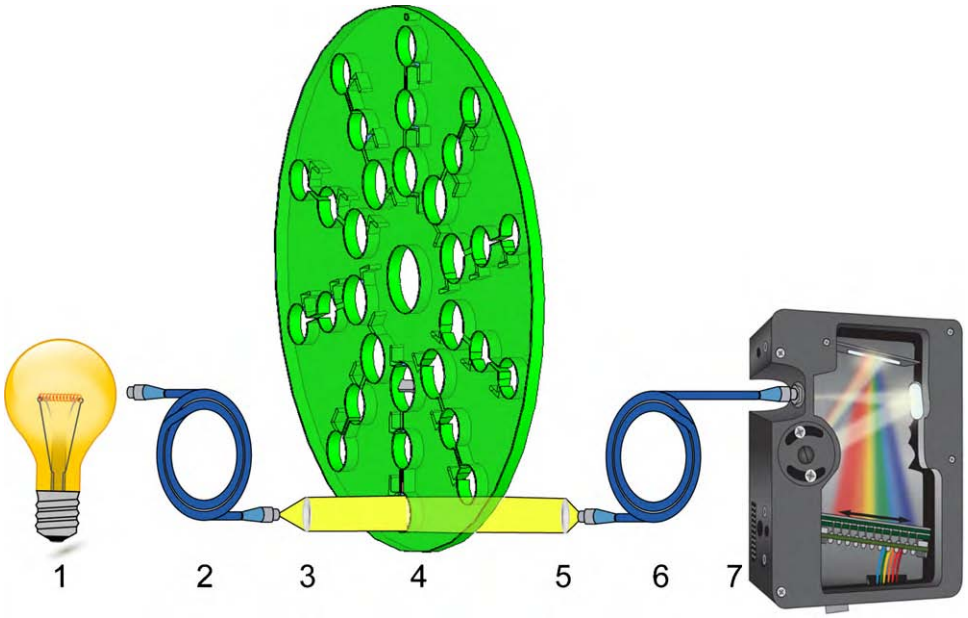

Fig. 2. Schematic of detection system: 1) light source, 2) fiber optic, 3) collimating lens, 4) integrated detection cell, 5) focusing lens, 6) fiber optic, 7) photodiode array spectrometer for detection at 334 nm.

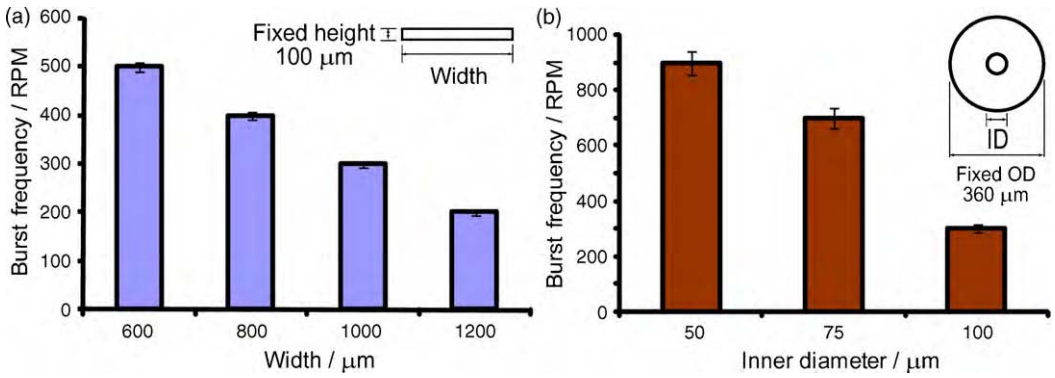

Fig. 3. (a) Burst frequency of hexane as a function of different channel widths in the adhesive. (b) Burst frequency of hexane as a function of different inner diameter capillaries. Inserts of both (a) and (b) show cross-sectional geometry of channels.

ducibility for both types of valves meant that implementation of multiple simultaneous runs on a single device could be achieved with the same spin sequence. From these results, the rotational speeds were chosen to be sufficiently above the burst frequency of the desired valve and below that of the subsequent ones to insure a controlled, reproducible transfer of liquid. The following parameters were chosen for the automated liquid–solid extraction: 1) 1200 μm wide (fixed 100 μm height) rectangular valve with a 200 RPM burst frequency, 2) 75 μm ID cylindrical valve with a 700 RPM burst frequency and 3) the following spin sequence:

First, the device was rotated at 50 RPM, well below the burst frequency of the first valve. The solvent and soil were in the inner *magnetically actuated liquid–solid extraction unit* (Fig. 1(d)1). At this point the magnetic field of the "mobile magnet" interacted with magnetic fields of the magnets in the fixed base of the motorized stage, inducing an efficient and effective movement of the magnet, mixing the liquid and solid. Without this magnetically induced mechanical motion, the soil samples would simply sediment to the bottom of the chambers by centrifugal force with minimal extraction of pyrene.

Next, the device was spun at 400 RPM (greater than the burst frequency of the first valve but less than the second) without the fixed magnetic base of the motorized stage. At this point, sedimentation of the soil in the extraction chamber was achieved by the inherent centrifugal forces and the extractant and some particles were transferred to the *filtration unit* (Fig. 1(d)2).

Once the extractant was completely transferred to this middle chamber, the device was spun at 1200 RPM at which point a secondary sedimentation process took place and only clear extractant was transferred into the *detection* cell (Fig. 1(d)3) for pyrene determination on the device by absorbance at 334 nm. This final transfer could also have been achieved using a 50 μm capillary instead of a 75 μm capillary; however, no advantage is obtained and a lower flow rate results from the smaller diameter.

The extractant was not directly transferred from the extraction chamber to the detection chamber since either the smaller capillaries would have clogged with soil particles or the larger capillaries would have allowed the transfer of soil to the detection cell, increasing undesirable scatter.

The results for the *extraction time efficiency study* are presented in Fig. 4(a). A pyrene extraction plateau is obtaining in 10 min with an efficiency very similar to that of the conventional method.

Extracting all subsequent soil samples for 10 min, a *comparison to an existing method* was made (Table 1). The two methods show linearity over the concentration range.

The Instrumental RSD is for the measurement of pure 5.0 μg mL^{-1} pyrene standards either injected into multiple integrated detection cells on the centrifugal device (*i.e.* no extraction) or in a single standard cuvette. As would be expected, very small variations are observed using a single cuvette with alignment fixed by the double beam bench top instrument (0.2% Instrument RSD). Of interest is the 6% Instrument RSD for the multiple cells on the device. This variation is due to slight variations in path length and in surface quality of the different cells, which would affect the light path and the amount of scattering and reflection. An attempt was made to account for this using the multiwavelength ratiometric blank estimation technique. This technique significantly improved the precision but not to the level of the bench top instrument.

The Analysis RSD is for the measurement of pyrene extracted from soil samples. An *F*-test confirmed that the centrifugal

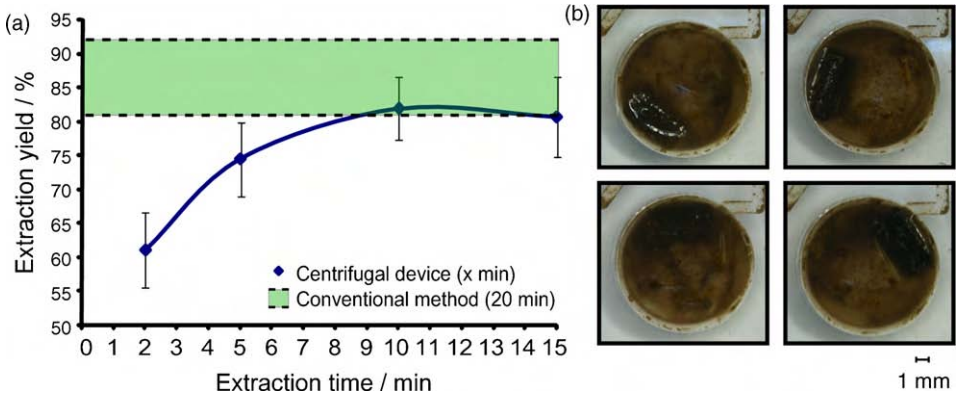

Fig. 4. (a) Extraction time efficiency study and comparison to conventional method. A pyrene extraction plateau equivalent to conventional method is obtained in 10 min. (b) High speed images of spinning device showing the "mobile magnet" movement in top inner extraction chamber. The "mobile magnet" spins upon its own axis as well as back-and-forth in the extraction chamber.

Table 1
Comparison of extraction methods.

	Centrifugal microfluidic device	Conventional separatory funnel
R^2	0.99	0.99
LOD[a,b] (n = 11)	1 ppm (0.03 μg)	0.3 ppm (1.5 μg)
Linear range[b]	1–130 ppm (0.03–4 μg)	0.3–40 ppm (1.5–200 μg)
Path length/mm	3.1	10.0
Instrument RSD[c] (n = 8)	6% (multiple cells)	0.2% (single cuvette)
Extraction yield	78–84%	81–92%
Analysis RSD[d] (n = 7)	5%	8%
Processing time	10 min for 10 samples	20 min for 1 sample
Sample weight/g	0.03	5.0
Solvent volume/mL	0.20	25

[a] Limit of detection as 3× standard deviation of the blank measured on two different devices.
[b] Absolute mass detection limits and linear range are expressed in parentheses for 0.03 and 5.0 g soil samples for the centrifugal and conventional methods respectively.
[c] Instrument RSD based on analysis of pure pyrene standard.
[d] Analysis RSD based on analysis of pyrene extracted from soil samples.

microfluidic device method was statistically more precise that the conventional method, even with a 150 times reduction in sample weights and extraction solvent volumes. The extraction yields of the two methods were also compared. A second *F*-test confirmed that there was no significant difference in the precision of the yields and a *t*-test confirmed that the yields of the two methods were statistically similar to a 99% confidence limit. This demonstrates that the techniques are comparable with the exception of the limit of detection (LOD), which is one order of magnitude higher for the device as compared to the bench top instrument. The higher LOD is to be expected given the differences in path lengths though future integration of methods to increase path length on a disk such as those developed by Grumann *et al.* [12] is to be envisioned. It should be emphasized that the present performance of the centrifugal method is obtained with one order of magnitude improvement in throughput and two orders of magnitude reduction in both sample and solvent consumption. This reduction in sample size is reflected in the one order of magnitude lower absolute detection limit. As is, this device is capable of detecting below the Canadian government limit of 8 ppm pyrene in agricultural, residential and parkland soil [13].

Pyrene, a PAH, was used as a demonstration analyte. Due to the similar spectral characteristics, we expect that this device could be used for the detection of high to moderate levels polychlorinated biphenyls (PCBs) in, for example, soil in the area of a transformer spill. For the detection of the more noxious benzo(a)pyrene and many other PAHs, a lower detection limit may be required. A variety of alternatives are envisioned to achieve a lower LOD including: 1) using more reproducible cells, 2) integrating a pre-concentration step as demonstrated by Lafleur *et al.* [6], 3) using a more sensitive detection technique such as fluorescence spectrometry or 4) using longer path length cells as described by Grumann *et al.* [12]. Alternatively, the sample preparation stage can be used as a sample preparation stage for a technique like gas chromatography–mass spectrometry. The reduction in time and solvent use makes this device an advancement and sustainable alternative of great interest for environmental monitoring.

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