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Short communication

Miniaturised centrifugal solid phase extraction platforms for in-field sampling, pre-concentration and spectrometric detection of organic pollutants in aqueous samples

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ABSTRACT

Great variations in pollutant concentrations are observed in the environment and pre-concentration is often required to detect trace contaminants in water samples. This paper presents a novel solid phase-extraction device integrated onto a centrifugal microfluidic platform for rapid on-site pre-concentration and screening of organic contaminants in aqueous samples. In-column fluorescence and absorbance measurements are obtained directly from an analyte trapped on the top of a solid phase extraction microcolumn. Results are presented for the representative fluorophore fluorescein and the polycyclic aromatic hydrocarbon anthracene. An absolute detection limit of 20 ng was obtained for anthracene using a simple light emitting diode for fluorescence excitation. One of the main advantages of this device is that only a simple motor is needed to induce liquid flow, making simultaneous on-site extraction and measurement of multiple samples easy while minimizing sample losses and contamination.

1. Introduction

Many toxic or potentially harmful pollutants are released into the environment every day as a direct consequence of human activity. Polycyclic aromatic hydrocarbons (PAHs), which are widespread by-products of incomplete combustion, are an example of organic pollutants ubiquitous in the environment. Their presence in our environment predates the industrial era due to such natural causes as forest fires and volcanic eruptions. Their concentrations have increased dramatically as a consequence of the burning of fossil fuels, resulting in increased contamination of water resources. Many PAHs are toxic to aquatic life and several have carcinogenic properties. Anthracene is one of the 16 PAHs selected by the US Environmental Protection Agency (EPA) as priority pollutants [1].

New analytical tools that can rapidly screen organic pollutants with minimal sample handling are required in order to assess and monitor their fate and impact. A recent review by Li and Lin [2] demonstrates the growing interest in applying microfluidic technologies to environmental analysis. Microfluidic systems are a tool of choice for the analysis of pollutants in the environment because several steps of a chemical analysis can be performed rapidly and directly on the microfluidic platforms. Sample transportation to the laboratory becomes unnecessary, minimizing analysis time, sample losses and contamination.

Microfluidic devices that use centrifugal force to drive the flow of liquids have recently been developed for environmental analyses. LaCroix-Fralish et al. [3] recently developed a micro-analytical system for the detection of nitrite and Cr(VI) to demonstrate the potential of centrifugal microfluidic systems for on-site (field) analysis of water samples. Lafleur and Salin [4] also recently introduced a miniature centrifugal solid phase-extraction (SPE) device for the rapid determination of trace metals in water by Laser Ablation (LA) Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

SPE is one of the most commonly used sample preparation techniques for the extraction and pre-concentration of analytes in environmental samples [5]. Since the concentration of PAHs can range from less than 1 ppt in pure groundwater to greater than 1 ppm in heavily contaminated sewage, extraction and pre-concentration are often necessary [6]. PAHs can be extracted by SPE using a reversed phase C_{18} stationary phase column followed by elution with a toluene–methanol mixture [7]. The collected eluate is then analyzed using the appropriate method. However, fluorescence and absorbance measurements could be performed directly on the sorbent material. This has the advantage of reducing the number of sample preparation steps, thereby minimizing risks of sample loss and contamination and reducing analysis time. The elimination of the elution steps also reduces the use of organic solvents which are detrimental to health and the environment.

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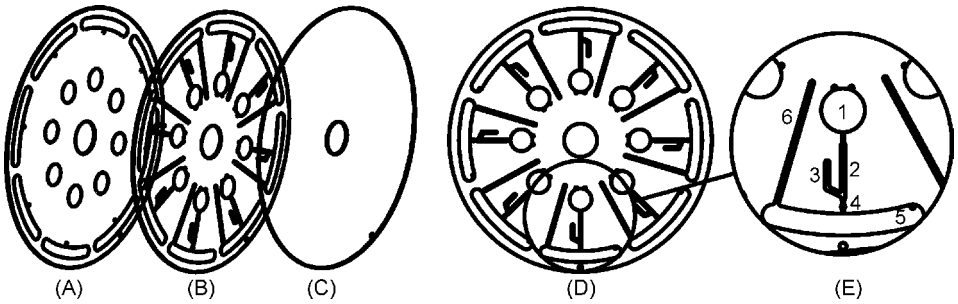

Fig. 1. (Left) Device component layers: (A) PMMA or PC base (the reservoirs are not milled completely through the material). (B) Adhesive layer. (C) Quartz or PMMA top layer. (Right) Device layout: (D) assembly. (E) Detailed view of the extraction unit: (1) sample reservoir; (2) extraction column; (3) packing channel; (4) quartz wool frit; (5) waste reservoir; (6) vent.

Several methods have been reported for the direct optical analysis of organic compounds pre-concentrated on stationary phases. In 1984, Guthrie and Jorgenson [8] proposed that a sizable sensitivity advantage could be gained by detecting analyte fluorescence directly on the stationary phase (in-column) in open-tubular capillary liquid chromatography. This increased sensitivity arises from the minimization of dilution effects. Using perylene as a representative fluorophore, they obtained a detection limit of 10 pg. Walbroehl and Jorgenson [9] extended the method to non-fluorescing compounds by designing a UV absorption detector specifically for open-tubular capillary electrophoresis, obtaining detection limits of 15 pg for isoquinoline and 250 pg for lysozyme. Carr and Harris [10] obtained detection limits as low as 0.17 ppt for the in situ detection of the PAH pyrene in 0.96 mm i.d. quartz tubes filled with C_{18} silica by laser fluorescence spectroscopy. More recently, the direct determination of ethylbenzene by Raman spectroscopy directly through the quartz wall of an SPE cartridge has been reported [11]. Laser induced fluorescence (LIF) measurements can also be performed directly on solid phase microextraction fibres (SPME) after 1–140 h exposure allowing determination of PAHs (as total PAH-34) with a detection limit of 2 ng/mL [12]. Methods have also been developed for the direct spectrophotometric detection of benzenic pollutants on polydimethylsiloxane (PDMS) sorbents [13].

According to Lamotte et al. [13], direct determination methods cannot be expected to give results as precise as those given by chromatographic methods, but they may have valuable applications, particularly for on-site pollution monitoring. We present here a method for obtaining analysis results directly in the field. Fluorescein and anthracene are extracted and pre-concentrated from water samples on miniature SPE devices where absorbance and fluorescence measurements are performed directly on the sorbent material.

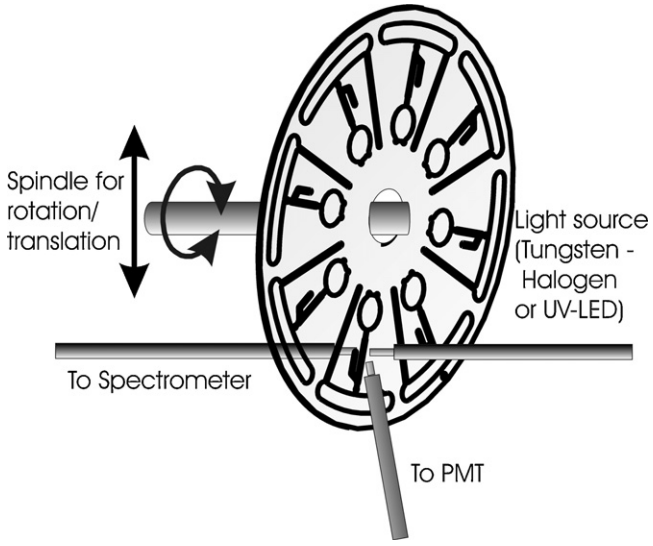

Fig. 2. Optical detection configuration used for fluorescence and absorbance measurements.

2. Experimental

2.1. Standards and reagents

Octadecylsilane silica gel (Nucleosil C_{18} silica, 100 Å pore size and 10 μm mean particle size) was obtained from Macherey-Nagel (Düren, Germany). Fluorescein (Fluka, Ronkonkoma, NY, USA) was prepared in pH 9.8 buffer. Anthracene (200 μg/mL, 1 mL ampoules in methanol, Supelco, Bellefonte, PA, USA) was prepared in 0.05% Triton X-100 (TX-100, AccusSpec, Baie D'Urfé, Qc, Canada). TX-100 was prepared with distilled deionised water (DDW, 18 MΩ, Millipore Co., Bedford, MA, USA).

2.2. Device fabrication

Devices were fabricated using a rapid prototyping technique based on a combination of thin film lamination [14] and xurography [15] developed by Kido et al. [16]. High aspect ratio features such as reservoirs were created using conventional Computerized Numerically Controlled (CNC) milling (QuickCircuit 5000, T-Tech, Inc., Norcross, GA) of polycarbonate discs (PC) (Blank uncoated CDs and DVDs, 120 mm diameter, 1.2 and 0.6 mm thickness, respectively, U-Tech Media Corp., Taiwan) or poly(methylmethacrylate) (PMMA) discs (6.35 mm thick sheets cut to 120 mm diameter discs, Acrylite OP-1, Cyro Industries, Rockaway, NJ, USA). Low aspect ratio features such as channels and vent lines were cut in 100 μm thick adhesive film (FLEXmount DFM-200-Clear V-95 150 poly V-95 400 poly, FLEXcon, Spencer, MA, USA) with a cutting plotter (CE3000MK2-60, Graphtec America, Inc., Santa Ana, CA, USA). Quartz discs (120 mm diameter, Prism Research Glass, Raleigh, NC, USA) were used as top layers in experiments requiring UV excitation. All layers were carefully aligned and pressed firmly together using a hand crank cold laminator (Jet Mounter ML25, Drytac, Concord, ON, Canada). For the fluorescein determination, the centrifugal devices were constructed entirely out of PMMA. For the anthracene determination, the base of the device was made of polycarbonate while the top layer of the device was made out of quartz to ensure maximum transmission in the UV region of the spectrum. The device is illustrated schematically in Fig. 1.

2.3. Column packing

The sorbent material was introduced in the columns as a methanol slurry using a micropipette. The column volumes

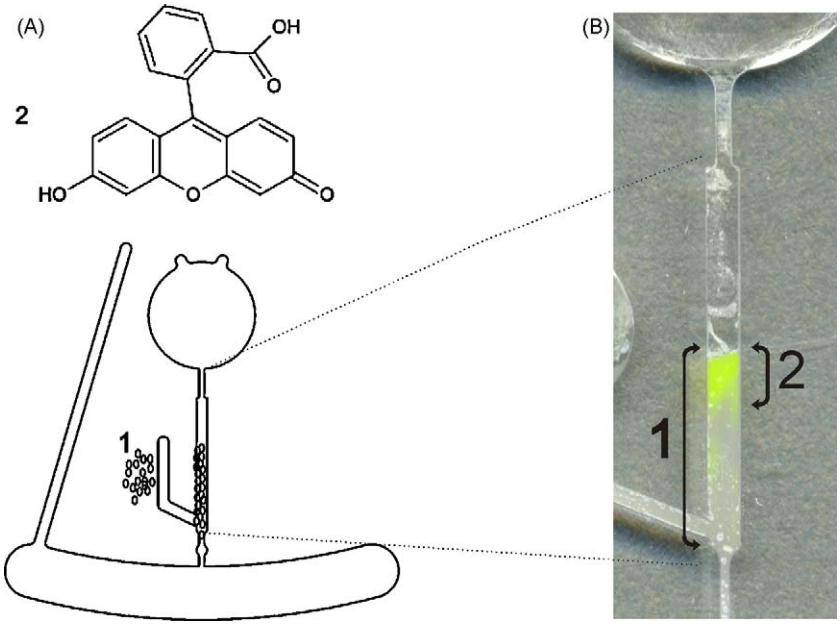

Fig. 3. (A) Simplified experimental procedure: (1) the column is packed with C_{18} functionalized silica gel and (2) fluorescein is added to the column. (B) Fluorescein trapped on a centrifugal solid phase extraction unit: (1) packed column and (2) fluorescein.

ranged between 1 and 1.5 μL. All slurries were carefully homogenized using a vortex mixer (Maxi-Mix, Barnstead International, Dubuque, IA, USA). Column consolidation was achieved by applying centrifugal force (2000 rpm) using a device built in-house consisting of a permanent magnet type 90 V DC motor (Baldor Motor and Drives, Fort Smith, AR, USA) driven with a motor drive (Penta KB Power, KBPC-240D, TechnoMotion, Montréal, Qc, Canada).

2.4. Solid phase extraction

Prior to the extraction, the packing was conditioned with 10 μL of methanol to allow resolvation of the sorbent. The column was conditioned for the introduction of the aqueous sample by slowly changing the mobile phase carrier from methanol to pH 9.8 buffer (fluorescein) or 0.05% TX-100 (anthracene). The samples (10 μL aliquots, 4–9 ppm fluorescein or 140 μL aliquots, 100–300 ppb anthracene) were percolated through the columns at 1800 rpm.

All calibration curves were obtained using the method of external standards with three to five calibration standards for each calibration curve. Replicates were obtained by running 5–8 aliquots of the same sample on different SPE columns. Each column was used only once and contained sufficient packing material to ensure that the maximum loading capacity was not reached.

2.5. Spectroscopic analysis

The discs were mounted on a stage built in-house which allowed scanning along the length of the columns at intervals of 1 mm. Alternately, the discs were mounted on a motorized translation stage (MTS-50 with TDC001 controller, Thorlabs, Newton, NJ, USA) to provide higher resolution scanning of the column. A 600 μm diameter UV–vis optical fibre (QP600-2-SR, Ocean Optics, Dunedin, FL, USA) was used to illuminate the column with a deuterium tungsten halogen light source (DT mini, Ocean Optics, Dunedin, FL, USA). Alternately, a UV light emitting diode (255 ± 10 nm LED, Seoul semiconductors, Roithner Lasertechnik Gmbh, Vienna, Austria) equipped with a ball lens was used to illuminate the column directly without a fibre optic. Fluorescent light was collected on the front face of the microfluidic device with a 600 μm diameter UV–vis optical fibre and recorded with a photomultiplier tube (PMT, H5784-04, Hamamatsu, Bridgewater, NJ, USA) equipped with a 320 nm or 400 nm longpass filter. Absorbance was measured simultaneously by placing a third 600 μm diameter UV–vis optical fibre directly facing the illumination fibre optic, behind the SPE column. Transmission was recorded with a diode array spectropho-

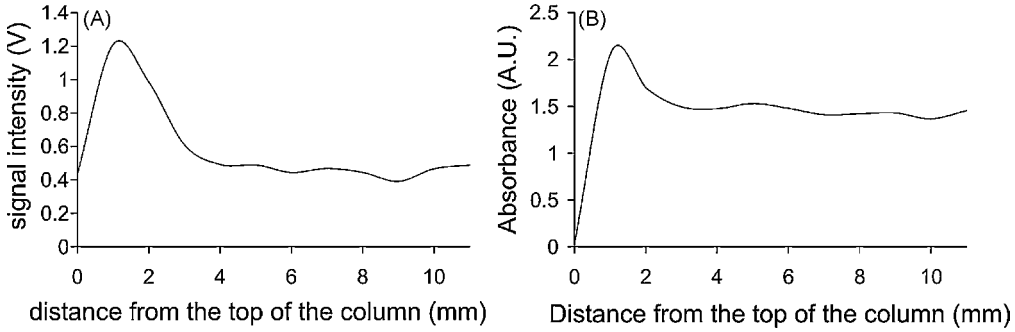

Fig. 4. (A) Fluorescein fluorescence with halogen lamp excitation recorded with a PMT equipped with a 400 nm longpass filter. (B) Fluorescein absorbance with halogen lamp excitation recorded at 494.13 nm with a photodiode array spectrometer.

Table 1
Calibration results for the direct determination of organic molecules trapped on a miniature centrifugal SPE platform.

	Fluorescein		Anthracene
	Absorbance	Fluorescence	Fluorescence
R^2	0.99[a]	0.99[a]	0.94[b]
Least square equation	$y = 13.2x - 0.03$	$y = 13.8x + 0.03$	$y = 0.014x - 0.20$
Precision	19–26%[c]	13–35%[c]	7–15%[d]
Limit of detection[e,f]	5 mg L^{-1} (50 ng)	5 mg L^{-1} (50 ng)	1 mg L^{-1} (20 ng)
Limit of linearity[f]	9 mg L^{-1} (0.09 μg)	9 mg L^{-1} (0.09 μg)	140 μg L^{-1} (20 ng)

[a] $N = 4$ standards.
[b] $N = 3$ standards.
[c] $M = 5–7$ replicates.
[d] $M = 3–7$ replicates.
[e] LOD calculated as 3 × standard deviation of the blank.
[f] Absolute mass detection limits and limits of linearity are expressed in parentheses for 10 and 140 μL samples of fluorescein and anthracene, respectively.

tometer (USB 4000, Ocean Optics, Dunedin, FL, USA). The detection system layout is illustrated in Fig. 2.

For the recovery studies, the content of the waste reservoirs were collected after sample extraction/pre-concentration and analyzed with a Fluormax-2 spectrofluorometer (Horiba Jobin Yvon, Edison, NJ, USA).

3. Results and discussion

Two practical problems are encountered when analysing PAHs. The first is the low solubility of PAHs in water. The second problem is the adsorption losses encountered during storage. For these reasons, Certified Reference Materials (CRMs) are not available for PAHs in water [17]. Hertz et al. [18] showed that adsorption losses in 1 ppb PAH solutions could reach 80% after 4 h of stirring in a glass container. Solubility is often increased by the addition of organic modifiers to the solvent. Ratios ranging from 2:98 to 0.3:99.7 acetonitrile–water can be used during pre-concentration [19,20]. Alternatively, it has been demonstrated that non-ionic surfactants (such as Brij-35) at concentrations greater than the critical micelle concentration can be used to both increase the solubility and diminish the loss of PAH by adsorption on polymeric material [21]. Guha et al. [22] also showed that PAH solubility could be enhanced in micellar solutions of TX-100 and that solubility increased with surfactant concentration. In this study, we have used the non-ionic surfactant TX-100 to increase anthracene solubility in water and to reduce the losses by adsorption on the polymeric disc material. A longpass filter was used to reduce the broad emission peak of Triton X-100 centered at 300 nm.

Simultaneous front-face fluorimetry and absorbance measurements were obtained for fluorescein using the optical set-up illustrated in Fig. 2 while only fluorescence was monitored for anthracene. The experimental procedure is illustrated schematically in Fig. 3(A) for fluorescein. The resulting extracted fluorescein is visible to the naked eye as a bright yellow slug concentrated on the top of the column pictured in Fig. 3(B). The resulting fluorescein fluorescence signal recorded along the length of the column is shown in Fig. 4(A). The signal is markedly higher at the top of the column, where the fluorescein is concentrated. The fluorescein absorbance signal recorded along the length of the column is shown in Fig. 4(B), again exhibiting a marked increase in absorbance at the top of the column, where the fluorescein is concentrated.

Recovery studies showed a 98% trapping efficiency when a 300 ppb anthracene sample is percolated through the miniature SPE column. As shown in Fig. 3(B), some of the analyte travels along the walls of the column. This is due to some inhomogeneities in the packing along the column walls.

Calibration results are listed in Table 1. Although it is expected that fluorescence would result in a lower limit of detection than absorbance, the autofluorescence of PMMA, affected the precision of the fluorescein fluorescence measurements due to high blank fluorescence. This is a common drawback with using polymeric materials in spectrometric analyses.

For anthracene, an in-column fluorescence detection limit of 140 μg L^{-1} (absolute LOD of 20 ng for a 140 μL sample) was obtained. The system behaves as predicted by a theoretical model with a detection limit only one order of magnitude greater than calculated for the ideal model [23]. Although this technique obviously cannot compete in sensitivity and selectivity with conventional SPE used in conjunction with chromatographic separation, it should find its use for the rapid monitoring of some pollutants. Label free deep-UV fluorescence detection in microfluidic devices is the object of considerable interest as demonstrated by a 2009 review by Schulze and Belder [24]. UV-LEDs provide relatively good sensitivity, as demonstrated above, but their power is too low to provide excellent detection limits for the analysis of PAHs. The UV-LED used for the determination of anthracene above has a maximal 0.150 mW power output. A laser source, which can emit monochromatic light with a high intensity could be used to increase the linear range of the method and lower the detection limits achievable. For example, Kuijt et al. [25] used the light from a quadrupled Nd–YAG NanoUV laser emitting at 266 nm with an average power of 5.4 mW to detect PAHs in capillary electrophoresis with 2–6 ppb detection limits. Using this more powerful light source, detection limits should be reduced by a factor of approximately 40, matching those obtained by Kuijt et al. [25] More powerful lasers are available but may not be suitable for field work.

As for selectivity issues, many PAHs have very distinctive fluorescent bands and multivariate calibration techniques can be used in the cases of overlapping spectra. Vilchez et al. [26] developed an auxiliary equation system in which the overlapping fluorescence contribution of the PAHs benzo[a]pyrene, benzo[a]anthracene and pyrene could be obtained, allowing their determination by synchronous solid-phase spectrofluorimetry. Algarra et al. [27] have also shown that it is possible to determine several PAHs by synchronous direct fluorescence analysis and by front-face fluorimetry on a sorbent using partial least square analysis [20]. Also, in a more sophisticated design, separations should be possible on the SPE column after pre-concentration by addition of an appropriate amount of eluent. In this case, analytes would be separated physically on the column, without being eluted and scanning along the column would allow the quantification of the analytes.

4. Conclusions

It has been demonstrated that organic molecules, such as fluorescein and the PAH anthracene, can be trapped on miniature centrifugal chromatographic devices for rapid on-site extraction and detection. Fluorescence and absorbance measurements can be

carried out directly and simultaneously on the sorbent material for rapid screening. The system performed as predicted by a theoretical model. The precision and sensitivity cannot compete with laboratory based methods of analysis, but the method has potential for the rapid on-site screening of contaminants.

Acknowledgements

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